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Aspects of the breeding ecology of the house sparrow, *Passer domesticus*

Thesis submitted for the degree of Doctor of
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by

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ProQuest

a — Large-badged male

b — Small-badged male

c — Asymmetric badge

d — Female

Abstract

Aspects of the breeding ecology of the house sparrow, <u>Passer domesticus.</u>
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1) This study examined the breeding ecology of the house sparrow, <u>Passer domesticus,</u> in Kentucky, USA, with particular regard to sexual selection, infidelity and parasitism. These aspects were also examined, to a lesser extent, in a archipelago population in Helgeland, Norway.

2) Male badge size, a character posited to be under sexual selection, did not appear to influence reproductive success. Large-badged males produced more fledglings within a season than small-badged males, although this was not significant after controlling for time of breeding. Large-badged males did not commence breeding earlier than small-badged males, they were not paired to higher quality, more fecund females, and their young did not fledge in better condition. Badge size was not related to an individual's age or condition, and although badges varied in their degree of asymmetry, this was not related to any measures of reproductive success. Badge size did not influence reproductive success in Helgeland.

3) The level of extra-pair paternity in both populations was relatively low [10.3% of young in Kentucky, 4% of young in Helgeland (based on retrospective identification of parents)]. No extra-pair fathers were assigned, although there were no obvious phenotypic differences between males which were cuckolded and those with complete paternity within their broods. There was no association between cuckoldry and either infertility, breeding synchrony or density. Males appeared to rely upon frequent copulation as opposed to mate guarding as their main means of paternity protection. Copulation rates were unrelated to male sperm reserves as measured by the size of their cloacal protuberance.

4) Females did not adjust the sex ratio of their brood in response to their own physical condition or the attractivness or quality of their mate.

5) Hatching asynchrony and brood reduction were both common in Kentucky, although the two phenomena were not associated. Within-female variation in egg size did not influence their occurrence, nor did it affect other life history parameters.

6) Parasites did not appear to influence sexual selection, and exerted only a minor reduction in adult fitness. However, parasites were not common, with several taxa being absent completely (e.g. haematozoa). Most parasites detected were haematophagous nest-dwellers (principally mites and carnid flies), such that, in the Kentucky population, nestlings reared in parasite-free nests fledged in better condition than those in infested nests. There was some evidence that badge size reflects individual immune activity, since in a sample of males collected soon after their moult, badge size was negatively related to the size of the spleen. This result was not significant, however, when age classes were analysed separately.

7) The genetic variation at three microsatellite loci did not vary between the different populations within the Helgeland archipelago, contrary to predictions derived from their route of colonization and the presumed differential extent of inbreeding.

Acknowledgements

Tirez le rideau, le farce est joué.

Table of contents

Chapter 1. General introduction

" .....the advantage which certain individuals have over others of the same sex and species solely in respect of reproduction."

Darwin (1871) *The Descent of Man and Selection in Relation to Sex*

1.1 Sexual selection

1.1.1 The history of sexual selection

In *The Origin of Species* (1859), Charles Darwin outlined his precipitous theory of natural selection by the preservation of favoured races. Although Darwin stoutly defended his theory, he was quietly troubled by many animal features which appeared to hinder their bearers and thus could not have evolved through natural selection. The peacock's lengthy, patterned tail, and the huge, weighty antlers of the extinct Irish elk represent classic examples of such encumbrances.

12 years after the publication of *The Origin*, Darwin proposed a new theory to account for the evolution of such characters: sexual selection. In *The Descent* of *Man* (1871), Darwin recognized that traits which would be opposed by natural selection could still be favoured by evolution if they imbued their bearer with a reproductive advantage. To use the traits cited above, peacocks possessing elaborate tails are more able to charm females, while deer stags with large antlers are more likely to win physical contests with rival males and thereby gain access to does.

Darwin's theory was heavily attacked (J. Huxley 1938, cited in Mayr 1972). Although his contemporaries were willing to believe that combative structures such as stag antlers served to resolve male-male disputes, the notion of female choice for functionless male adornments was vilified. In Victorian England, females were not credited with an aesthetic sense, and male beauty had only theological significance. Even the co-discoverer of natural selection, Alfred Russell Wallace, parted company with Darwin on the matter of female choice, attributing gaudy plumage to the males' 'excess vitality' (a theory which later reappeared under the guise of 'good genes').

Darwin's few supporters faded, and the theory of sexual selection remained virtually moribund for almost a century (reviewed in Cronin 1991). The mathematical validation of female choice (Fisher 1915, detailed in Fisher 1930), passed largely unnoticed. Even in the post-war heyday of ethology, sexual selection was merely considered as a means of maintaining the reproductive isolation of species (Cronin 1991).

It is difficult to identify the precise stimulus which refocused interest in sexual selection, although George Williams' (1966) book was surely a significant publication. Mathematical modellers rediscovered and refined Fisher's work in the 1970s and early 80s, leading to a rapid accumulation of new theories (Zahavi 1975, Maynard Smith 1976, Lande 1981). Laboratory and field studies provided empirical tests of these hypotheses, resulting in some classic experiments and further theories (Weatherhead & Robertson 1979, Andersson 1982, Hamilton & Zuk 1982, Heywood 1989). As judged by publication rates and conference biases, sexual selection is now the dominant topic of current research in behavioural ecology (Gross 1993).

1.1.2 Parental investment and sexual selection

To paraphrase Darwin, sexual selection is usually seen as a struggle between males for the possession of females. Two entomologists (Richards 1927, and particularly, Bateman 1948) demonstrated why females are usually the coy sex, actively courted by males. The variation in male reproductive success is considerable larger than that of females, as a simple consequence of anisogamy (differences in gamete size). Males produce large numbers of small gametes (sperm), whereas females produce relatively few, energetically expensive gametes (ova). In theory, a male's reproductive success is limited only by the number of inseminations he can achieve, whereas a female's reproductive success is limited by the number of eggs she produces (Bateman 1948). Consequently, females are under strong selection to maximise the quality of the resultant offspring, and have evolved to become the more discriminating sex. The different reproductive intentions of each sex were neatly summarized by Williams (1966): males seek to maximize, whereas females seek to optimize.

Trivers (1972) extended Bateman's findings to propose that sexual selection is governed by sexual differences in parental investment (the allocation of resources per gamete). Males which desert following fertilization suffer only

minimal losses. Since sperm are relatively cheap, little prior investment has been made, and moreover, males can initiate a new breeding attempt immediately. By contrast, deserting females sacrifice a substantial prior investment, and cannot commence breeding again until they obtain the nutrients needed to produce their relatively large gametes. This asymmetry predisposes females to invest more heavily. Taken together, the work of Bateman and Trivers illustrates why sexual selection usually involves a struggle between males. Females are the choosy sex since they contribute the vast majority of the zygote, and are also more likely to invest afterwards.

(Note : the decision of whether to prolong care does not depend upon prior investment [described as the 'Concorde fallacy' by Dawkins & Carlisle 1976] but is determined by the further investment necessary to rear offspring to independence, Coleman & Gross 1991).

1.1.3 Theories of sexual selection

The field of sexual selection is vast and controversial, and is only briefly summarized here. Several recent books contain excellent reviews of its history and theories, including Cronin (1991), Møller (1994b) and, outstandingly, Andersson (1994).

There are two forms of sexual selection, which are not mutually exclusive; male-male competition and female choice. Extreme examples of each are seen in, respectively, the rutting of red deer stags (Clutton-Brock 1982) and the fanning of the peacock's tail (actually the upper-tail coverts) (Petrie *et al.* 1991). Both forms have evolved through females attempting to gain either direct, tangible benefits [such as access to food, superior territories etc.] or indirect, genetic benefits (or both) by mating with a particular male. Although antlers and peacock's tails are generally acknowledged to be epigamic characters (i.e. those concerned with sexual selection), their evolution and maintenance is contentious. Two differing philosophies account for the existence of such traits, which have been bifurcated by Cronin (1991) into 'good taste' and 'good sense'.

The first theories to be considered represent female choice for indirect benefits. These have attracted the most attention, probably because many

studies of sexual selection involve lekking species in which females receive only sperm (Andersson 1994).

The Fisher process of sexual selection (= 'good taste')

Fisher (1930) reasoned that the extravagant tail of the peacock is the product of continued directional selection, and peahens must therefore have similar preferences. If there was a genotypic association between high fitness and a large ornament, then a female's preference for such an ornament would be adaptive (*cf.* Darwin's 'aesthetic appeal' [1871]). Sons of these females would inherit the large, attractive ornament, and daughters would inherit the preference (provided the ornament and preference gene were in linkage disequilibrium). This frequency-dependent, positive feedback (occasionally termed 'runaway selection') would cause ornaments to escalate in size until their reproductive advantage was overwhelmed by opposing natural selection. Several mathematical analyses have confirmed the plausibility of the Fisher process (e.g. O'Donald 1980), although the circumstances which initiate runaway selection are unclear. Several authors have suggested that Fisherian traits were originally favoured through natural selection (i.e. males with slightly longer tails were more successful in the pursuit of flying insects), but became ensnared and enlarged through runaway selection (Ridley 1994). Irrespective of their original function however, the ornaments invoked by the Darwin-Fisher theory are arbitrary and provide no information concerning the quality of their bearer.

The Fisher process was later inveigled into Weatherhead & Robertson's (1979) 'sexy son' model, which proposed an indirect benefit for females settling on territories of males which are already paired (and thus provide no rearing assistance). Although these secondary females consequently rear smaller broods, their fecundity cost would be offset if their sons inherited the male's attractive trait.

The 'good genes' theories of sexual selection (= 'good sense')

The Fisherian models are opposed by the large suite of theories collectively known as 'good genes' (Wallace 1889, Williams 1966). These suggest that ornaments such as the peacock's tail have evolved through sexual selection since they reveal the 'quality' or 'vigour' of their bearer. Females which mate with males possessing elaborate ornaments obtain 'viability' genes for their

offspring, a decision which Cronin (1991) terms 'good sense'. Compared to the arbitrary nature of Fisherian traits, good genes traits are therefore utilitarian.

In 1975, Zahavi proposed that large ornaments have evolved as 'handicaps'. Male peacocks which can survive despite being encumbered by their long tail signal their high quality. Zahavi's theory was initially dismissed as unworkable and 'maddeningly contradictory' (Dawkins 1976), largely because it assumed no link between genotype and trait (merely that low quality birds which happened to possess large handicaps would be unlikely to survive). In response, Zahavi modified his theory by assuming epistasis between the male trait and general viability. Under his 'conditional handicap' model (Zahavi 1977), the hindrance of a long tail would be disproportionately costly for low quality males, and could only be borne by males of a genuinely superior constitution. This honesty maintains the stability of conditional handicaps, as has been confirmed by several simulations (e.g. Pomiankowski 1987).

A specific application of Zahavi's theory is the 'revealing handicap' of Hamilton & Zuk (1982) (discussed more fully in Chapter 11). Ornaments such as pheasant tails have been favoured through sexual selection since they honestly reveal the parasite infection and/or resistance of their bearer: only males free from disease can produce and maintain a large tail. The Hamilton & Zuk model is notable in that it is one of the few theories of sexual selection which makes an interspecific prediction at all.

The direct benefits hypotheses (= 'good sense')

The direct benefits hypotheses assume that variation in female reproductive success is mostly determined by her mate's parenting abilities rather than his genetic quality. Females attempt to obtain a tangible advantage (such as more, well-fed offspring) by preferentially pairing with males whose traits indicate them to be 'good parents' (Heywood 1989, Hoelzer 1989). This restricts the good parent process to monogamous birds with biparental care. Although 'parenting' encompasses a wide range of activities (e.g. defence against predators, nest sanitation etc.) it usually refers to the quality of the territory the male provides, or the amount of the amount of feeds he delivers. Males which are in good physical condition, adjudged by their plumage colouration, display frequency, or general vigour, are predicted to be good parents since they have demonstrated their feeding efficiency.

1.1.4 Sexual selection in monogamous birds

In birds at least, it is a truism that elaborate male ornamentation is usually associated with polygyny (e.g. birds of paradise, most pheasants) (Selander 1972). However, males are also showy in many monogamous species, such as the British finches (Newton 1972), or more exuberantly, the resplendent quetzal (Alcock 1988). This troubled Darwin's vision of a 'struggle between males', since the adult sex ratio in most birds is equal, and consequently all males would become paired, irrespective of their appearance. Sexual selection in these birds was mostly ignored, and as late as 1972, no less a figure than Ernst Mayr wrote "the sexual dimorphism of monogamous species has little if anything to do with sexual selection, and can be explained in terms of natural selection".

Although only 25 years have elapsed since Mayr's pronouncement, it is now widely accepted that sexual selection occurs in monogamous birds (Andersson 1994). Fecundity differences of females, coupled with variation in male quality, would select for the exaggeration of indicator traits in males (reviewed in Møller 1994b). Moreover, since males may gain additional fertilizations outside of their pair-bond (Birkhead & Møller 1992), the variation in male reproductive success may increase, exacerbating the strength of sexual selection on a favoured trait.

1.1.5 Evidence for the theories of sexual selection

Many studies of mate choice, both observational and experimental, have demonstrated a female 'preference' for males with larger, brighter or more elaborate traits than their competitors. These diverse traits include the length of widowbird tails, the song repertoire of sedge warblers, the display vigour of black grouse, the number of white spots on great snipe tails, the length of pheasant spurs, and the stone carrying ability of black wheatears (reviewed in Andersson 1994). However, to demonstrate that a trait is under sexual selection requires there to be a positive relationship between male reproductive success and trait size (i.e. the chosen male must gain a reproductive advantage).

Several studies have managed to associate female choice with reproductive success gains (Table 1). All of these studies, with the ironic exception of the peacock, refer to monogamous species. Furthermore, all of these examples represent what Cronin (1991) termed 'good sense'. There is no empirical

evidence for the Darwin-Fisher theory of sexual selection ('good taste'), beyond theoretical confirmations of its stability providing certain conditions are fulfilled (Ridley 1994).

Table 1. Benefits of female choice in birds.

Criterion for choice	Species	Benefit	Reference
nest site / territory	pied flycatcher	direct	Alatalo *et al.* 1986
nest defence	dark-eyed junco	direct	Enstrom *et al.* 1997
chick rearing ability	house finch	direct	Hill 1991
courtship gifts	common tern	direct	Nisbet 1973
incubation assistance	moorhen	direct	Petrie 1983
increased fecundity	barn swallow	direct	Møller 1994b
parasite resistance	barn swallow	indirect	Møller 1990c
heritable ornament	great tit	indirect	Norris 1993
offspring viability	peacock	indirect	Petrie 1994

1.2 Aims of this thesis

This thesis describes a study of the breeding ecology of the house sparrow, *Passer domesticus*, undertaken at two separate locations. The specific aims of this study were as follows;

1. The male house sparrow possesses a conspicuous throat patch of black feathers, which is thought to have evolved through directional sexual selection. If this is true, then one would predict a positive association between badge size and reproductive success. Fundamentally, the study assesses reproductive success of individual males in the population by considering the number and quality of offspring produced. However, to further address sexual selection in the house sparrow, the study also attempts to elucidate other factors which govern the variation in reproductive success between individuals, and considers the roles of parental age and quality in addition to purely eugenic characters.

2. As an extension of the sexual selection paradigm, the study considers whether house sparrows pursue a mixed mating strategy by attempting to obtain fertilizations outside of their pair bond. The use of modern genetic techniques enables a direct analysis of nestling parentage, and consequently

7

reveals whether extra-pair paternity occurs in the populations monitored, and if so, which demographic parameters influence its occurrence. As a corollary, the work also examines the prevalence of any adult behaviours which have evolved to either promote or restrict these infidelitous activities.

3. The study also examines the development, in the house sparrow, of three specific parental mechanisms which are thought to have evolved to maximise the fitness gain which can be expected from a given initial reproductive investment. These mechanisms (hatching asynchrony, brood reduction and variation in egg size) are important components of the breeding ecology of many bird species, and should be considered in any study of differential reproductive success, since sexual selection alone need not dictate parental fitness.

4. Although parasites have been traditionally viewed as exerting only a minor influence upon their hosts, recent research indicates that this view may be misleading. Hence, one of the key aims of the present work is to survey the parasite communities present in both study populations, elucidate their ecology, and assess whether their presence invokes a fitness cost in house sparrows. Indirectly, this also addresses a specific form of sexual selection, in which females select males on the basis of a sexual trait (in the house sparrow, the male badge) which reveals either his genetic resistance to parasitism or his current load of potentially contagious disease.

1.2.1 Organisation of the thesis

The present chapter (Chapter 1) introduces the theoretical background to the study, and describes the study species and main field site, in Kentucky, USA, and the basic aims of the research. The remainder of the thesis is grouped into four blocks, each of which contains one or more inter-related chapters.

The first block (Chapters 2 - 8) describes the variance in reproductive success which occurred between individuals at the main field site, together with a consideration of its causal factors with particular regard to sexual selection. The extent of extra-pair paternity at this site is described, and discussed in the context of factors reported from other bird species which affect its incidence, including the behaviour of either sex.

The second block (Chapters 9-10) describes the patterns of hatching, within-brood mortality, and egg size variation at the main field site, and relates these to parental reproductive success estimated from the number and quality of the surviving offspring.

The third block (Chapters 11-12) describes the incidence, prevalence, and ecology of the range of parasite types encountered in the main house sparrow population, together with an assessment of their effects upon both parent and nestling birds. The defensive capabilities of individual males are also considered by examining the variation in the size of their spleen, the major avian disease resistance organ, with particular regard to the expression of their secondary sexual character.

The fourth and final block (Chapter 13) catalogues the work undertaken at a second field site, in Helgeland, Norway, which was similar to that undertaken in Kentucky. This chapter also contains a full description of the laboratory techniques used to assess the genetic relationships between house sparrow families collected from both the Norwegian and American study populations.

The thesis is then summarised in an overall discussion chapter (Chapter 14).

1.2.2 History of the thesis

This thesis has a slightly convoluted history, although its underlying theme - sexual selection in the house sparrow - has been conserved. I was originally to have studied this subject at a colony in Leicestershire, England. However, since this colony failed to become established, the study was relocated to a Norwegian archipelago to link up with another house sparrow project (see Preface, Chapter 13). For logistic reasons, several aspects of the site were not suitable for my purposes, and hence another site was sought. Professor David Westneat, of the University of Kentucky, graciously allowed me access to a house sparrow colony he had recently established at a farm near his university. I therefore worked at the Kentucky site for two years, in collaboration with two of his own graduate students. Since most of the fieldwork for this thesis was undertaken in Kentucky, I have presented the American work first, and the results of the Norwegian collaboration are actually discussed in the final chapter of this thesis (Chapter 13).

1.3 The study species

The house sparrow (*Passer domesticus*) is an abundant small passerine related to the African weaver finches (Ploceidae). The species is of Afrotropical origin, and is thought to have spread initially into the middle east during the Pleistocene (Summers-Smith 1963). With the aid of several deliberate and accidental introductions it has since become promulgated throughout Europe and thence most of the inhabited world (Summers-Smith 1988). The house sparrow is associated with man throughout most of its range, and is a familiar sight in both urban and agricultural landscapes.

The species is sexually dimorphic, with the males possessing chestnut upperparts streaked with white and black, and a contrasting head pattern of pale cheeks, black eye stripes and a grey crown. Males are also distinguished by a throat patch or 'bib' of black feathers, which becomes particularly noticeable during the breeding season (Møller 1987a). Female house sparrows are the archetypal small brown bird, with their only clear feature being a pale supercilium. Males are also slightly larger in most dimensions (Johnston & Fleischer 1981).

The breeding ecology of house sparrows has been well documented in several field studies (e.g. Weaver 1943, Murphy 1978a,b, Anderson 1994). They are socially monogamous (Veiga 1992), and usually remain faithful to both their partner and nest site in successive field seasons (but see Sappington 1977). Nests are untidy balls of straw, and are preferentially constructed within any available crevices or cavities, often in proximity to several other pairs. Although incubation and brooding duties are only undertaken by the female, both sexes feed the nestlings and provide post-fledging care (Summers-Smith 1963). Clutches average around five eggs, which are incubated for about 10 days. Nestlings are fed on a range of insects and soft grain, and fledge at around 15 days of age (Weaver 1942). Over most of their range, sparrows are multibrooded with a relatively long breeding season (Seel 1968a). Adults are granivorous, particularly outside the breeding season, where they often achieve pest status by feeding on stored grain in large flocks.

The house sparrow is an ideal study species for several reasons. It will readily use nest-boxes, which allows convenient access to both eggs and nestlings. It

is reasonably easy to trap and observe, and is remarkably sedentary, allowing individuals to be followed throughout the year.

1.4   The study site

Fieldwork was conducted between April - August in 1995 and 1996. Most of the fieldwork upon which this thesis is based was undertaken in central Kentucky, USA. This state lies in the eastern half on North America, and straddles the median line of latitude. The climate is fairly mild throughout the year, with stormy springs and warm, dry summers. The study population was located at an agricultural station owned by the University of Kentucky, and used mostly for crop trials and horse breeding. The whole farm was fairly flat.

In early 1992, around 40 wooden nestboxes had distributed between the barns (by Professor Westneat), which had been occupied successfully from that season onwards. Occasional pairs bred also bred in inaccessible cracks and crevices at each barn, and the farm itself had a network of approximately 100 free standing boxes erected for bluebirds of which several were utilised instead by house sparrows. During the 1995 season, all of the 34 boxes distributed over the three main barns were studied. In the 1996 season, the number of boxes at one of the barns was increased from 8 to 18 boxes, of which most were used at least once during the season. Since more detailed methodology is given with each chapter, only a brief description of field methods is given here.

Catching methods

Adults were fairly difficult to catch during the breeding season. Mist netting was occasionally successful, although the exposed nature of the site meant that suitably sheltered sites were limited. Whilst the sparrows frequently enter the main barn during the winter period to avoid inclement weather, they were noticeably wary of entry during the summer, presumably because of the resident breeding population of cats. Modified Potter traps baited with seed, bread, and occasional live insect baits, were reasonably successful, particularly during the early stages of the summer. After a few months of continuous operation, the birds showed little interest in the traps, despite several alternative baits being tried.

To counter this, birds were trapped at the nest, either when feeding or brooding nestlings. Brooding females were gently taken from the nest before dawn, although this was restricted to the period when the nestlings were between three and six days old. Older nestlings are not always brooded overnight (*pers. obs.*), and females occasionally deserted if nestlings were younger (and invariably deserted if taken whilst incubating eggs). Most adults were caught using a modified pied flycatcher trap (Ross 1997) in which a triangle of card attached to a piece of angling line was thumbtacked within the box, and the line pulled when the bird had entered, covering the hole until the bird could be extracted. Several birds escaped from the boxes during trapping or left the nest as it was being approached before dawn. To minimize the risk of desertion, trapping of these birds was postponed until the following breeding attempt.

Nest and clutch monitoring

Non-operational nests were visited every two or three days unless parental behaviour (such as copulation or frequent female attendance) implied clutch initiation was immininent, whereupon daily visists were commenced. As the clutch was produced, eggs were numbered in sequence with an indelible marker pen. When the clutch was complete the nest was not checked again until three or four days later, to minimize disturbance.

Nestling processing

Nestlings were individually marked whilst too young to ring by gently clipping their toenails in a predetermined sequence. Wherever possible, hatchlings were clipped in order of emergence, although the synchrony of hatching, particularly of the early eggs of a clutch, meant that this was rarely achieved completely. To permit individual recognition, toenails occasionally had to be reclipped as the nestling developed. Nestlings were ringed when most members of the brood were 10 days old Each individual was fitted with a combination of a single metal numbered ring (issued by the United States Fish and Wildlife service, Maryland) and three split plastic colour rings (purchased as commercial 'play-beads'). There were eight colour variants, of which one ('hot orange') was found to fade badly in strong sunlight between years, but was still sufficiently distinct to merit inclusion. In addition to

weight, the lengths of both tarsi (to the nearest 0.1mm) and both wings (to the nearest mm) were measured, using Vernier callipers and a zero-stopped ruler respectively.

Adult processing

Adults were weighed to the nearest 0.1g using either a 30g or 50g spring balance (regularly checked against objects of known weight). Wing lengths (flattened chord method) and the lengths of both tarsi were measured to the nearest mm and 0.1mm respectively. Bill depth (at base) and culmen length (from the bill tip to the feathering) were measured to the nearest 0.1mm. As described for nestlings, adults were individually marked using the same combination of one metal and three plastic rings, and a blood sample was obtained.

Blood sampling and storage

Approximately 50µl of blood was obtained from adults and nestlings following brachial venipuncture, and stored in 100µl of 1 x TNE buffer (Tris, NaCl, EDTA). Tubes of blood/buffer mix were kept during the day in a coolbox with frozen gel refrigerant and stored permanently at -70°C upon return to the laboratory. Blood samples were transported to Leicester by a commercial courier. The 1995 season samples were transported on dry ice, and aliquots of the 1996 samples were sent in a coolbox with refrigerant (50µl blood/buffer mix plus 250µl lysis buffer).

1.5   Statistical methods

All of the statistical methods used are described in Sokal & Rohlf (1981) and Fowler & Cohen (1996). The software package Systat 5.2 for the MacIntosh was used throughout the thesis (Wilkinson 1992). All tests are two-tailed unless otherwise stated, and the data were checked for normality by performing the Lilliefors test (Crawley 1993). Data were normalised wherever possible to allow the use of parametric tests. A probability of $p \leq 0.05$ was taken to indicate biological significance. The p-value for each test is give in full, unless it exceeds 0.1, in which case it is merely denoted as NS (not significant).

Chapter 2. Phenotypic correlates of reproductive success in the house sparrow

"In the winter the bramble-finches came into a wire enclosure where the waterfowl were fed. I caught a cock bird, and it seemed to me that with a pair of scissors, by carefully cutting off the fringe of each feather on the head and neck, the bird could be turned into breeding plumage".

Grey (1927) *The charm of birds*

2.1 Introduction

2.1.1 Compromise plumages in birds

This excerpt from Edward Grey's classic work describes the relatively rare phenomenon known as 'compromise' plumage, by which individuals can attain two distinct appearances from a single moult (Ginn & Melville 1995, Veiga 1996b). When freshly moulted, certain feathers are obscured by pale tips, to produce a relatively dull appearance during autumn and winter. During late winter, these soft tips become abraded to reveal the full breeding plumage, which is usually considerably more dramatic.

Considering only British birds, cock reed buntings and bramblings (referred to in the above quote) both have nondescript, brownish heads during the winter, which are revealed as jet black during the breeding season as the buff tips wear away (Newton 1972). By the same process, the buffy winter coat of the male snow bunting is replaced with a striking white summer plumage.

A more restricted compromise plumage is displayed by male house sparrows, where the winter tips are limited to the black throat feathers, and to a lesser extent, those of the grey crown (Ritchison 1985). Although the black throat patch or 'bib' is small in winter, and large in the breeding season (Summers-Smith 1963), the male plumages are otherwise identical.

2.1.2 Bibs and winter dominance

There is considerable variation in plumage patterning and colouration in many bird species, particularly among the males (Darwin 1871). To account for this variation, Rohwer (1975) proposed the 'status-signalling' hypothesis.

14

This is a reformulation of the 'pecking order' effect famously displayed by domestic hens, and suggests that birds have evolved visible status signals (described by Rohwer (1975) as 'badges') because they allow individuals to assess each other's rank at a distance, and consequently settle dominance interactions with minimum dispute. These status signals are likely to be favoured in any species where several individuals compete for a patchily distributed resource (Johnstone & Norris 1993). Many passerines, including the house sparrow, are ideal candidates for Rohwer's (1975) scenario, since they aggregate in tight flocks outside of the breeding season and compete for ephemeral food items such as berries or seeds (Perrins 1979).

Møller (1987a, 1988) confirmed that the black bib of the male house sparrow acts as a badge of status. During winter flocks, and in captive populations, males with large bibs were dominant at feeding sites over males with small bibs, irrespective of their age, size and physical condition. This linear dominance hierarchy was confirmed in a captive population of house sparrows in Norway (E.J. Solberg & T.H. Ringsby *pers. comm.*).

2.1.3   The maintenance of honesty

The stability of the conventional badges of status model has been questioned however (Owens & Hartley 1991, Johnstone & Norris 1993), since the system is open to deception by 'cheats' which bear large badges and thereby gain automatic access to food by virtue of their apparent dominance. Møller (1987b, 1988) surmised that the honesty of the house sparrow badge was socially controlled through frequent challenges, since these were common between males of similar bib sizes, particularly if the combatants had large bibs.

If badges are an honest indicator of male aggressiveness and fighting ability, 'cheat' males which possessed large badges but were not aggressive would be punished by frequent attacks. Veiga (1995) demonstrated that 'cheating' incurs a fitness cost by experimentally enlarging the badges of yearlings. This caused a reduction in their survival probability, presumably as a result of frequent attacks by genuinely dominant males. Using a similar approach, Møller (1987b) found that low ranking males with experimentally enlarged badges were unable to maintain a high dominance rank unless the treatment was accompanied by an injection of testosterone. Since testosterone is often

correlated positively with aggression (Keeler *et al.* 1970, Ketterson & Nolan 1982), badges of status would be honest if high levels of testosterone were costly. This cost may be manifested for example, by lowered disease resistance, since it has been established in several birds that androgens suppress immune defence (Zuk *et al.* 1990, Folstad & Karter 1992). Moreover, the analysis of a simple evolutionarily stable strategy (ESS) model revealed that frequency-dependent challenges could only maintain honest signalling of aggressiveness if the most aggressive individuals incur a proportionately high cost independent of any fights (Johnstone & Norris 1993).

2.1.4 Badges and sexual selection

In male house sparrows, the size of the winter badge remains constant until just before spring, when the buff tips are lost relatively rapidly to reveal the greater extent of the black badge (Summers-Smith 1963, Bogliani & Brangi 1990). Although this is partly due to natural abrasion, most of the loss results from active preening of the badge area by the birds themselves. The darker, basal region remains, since the strengthening presence of the melanin deposits resists abrasion (Bonser 1995). Males which ultimately have large badges preen the breast area more often than those with small badges, and thereby assume their breeding plumage earlier (Møller 1992a).

In aviary experiments involving a range of stuffed males, females implanted with oestradiol displayed most frequently before males with large badges (Møller 1988). However, this result could not be repeated by Kimball (1995) when using live males placed in a Maltese cross apparatus, and indeed, there was no conclusive evidence of a preference for any male trait (Kimball 1996). Moreover, neither study could have distinguished whether females desired certain males as social mates or merely extra-pair partners.

Møller (1989b) produced indirect evidence that badge size acts as a sexual character when large-badged males in a free-living population of house sparrows acquired mates earlier, and reared more fledglings than small badged males. Furthermore, those males which remained unmated had small badges (Møller 1989b). In an equally intensive study however, Veiga (1993) found that badge size conferred no reproductive advantage in terms of mate attraction and offspring production, although males with larger badges did have a higher probability of acquiring a nest site. As the breeding density was

much higher in his population than in Møller's (Veiga 1996a), he considered that badge size functioned in male-male competition rather than female choice. Hence, both authors agreed that the badge was a sexually selected character, although their opinions differed concerning the nature of choice.

2.1.5   What does the badge signal?

Although Møller (1989b) originally assumed that badges revealed their bearer's genetic quality, Veiga (1993a) has since argued that badges are a phenotypic signal which honestly reflect a male's condition (*sensu* the carotenoid dependent plumage of male house finches, Hill 1994). Møller (1989b) supported his genotypic argument with the observation that badges did not change with age, and were heritable (as revealed by a significant father-son regression). However, badges increased with age in the Spanish population (Veiga 1993a), and a recent cross-fostering experiment found no evidence of a heritable component to badge size (Griffith 1998).

Veiga (1993a) has marshalled considerable support for his argument that badges are condition-dependent. Firstly, badge size was positively correlated with physical condition, irrespective of age (Veiga 1993). Secondly, yearling males which had fledged early in the previous season, and were presumably in better condition before the autumn moult, produced larger badges than yearlings which had fledged towards the end of the season (Veiga 1993). More conclusive evidence came from a simple aviary study, where males were maintained during the moult and provided with food *ad libitum* (Veiga & Puerta 1996). Although adults had significantly larger badges than yearlings in his free-living population (Veiga 1993), there was no age-related difference in badge sizes of captive birds. However, the levels of blood proteins dropped during the moult in juveniles but not adults, which suggests that, in the wild, young birds cannot bear the costs of producing a large badge (Veiga & Puerta 1996). Although the physiological costs involved in producing the badge are not yet known, these must be significant for the badge to function as an honest signal of condition.

2.1.6   The annual significance of badge size

In most species with compromise plumage, individuals probably benefit from a dull winter appearance through crypsis (Grey 1927). In the house sparrow,

however, each plumage phase has a distinct function subject to a different selective pressure (Møller 1989b). During the winter, *natural* selection favours males with larger badges, as large-badged males obtain greater access to ephemeral food supplies (Møller 1987a). However, natural selection also opposes badge size through differential predation, as large-badged males were more frequently taken by sparrowhawks (although it is doubtful whether this was due to badge size *per se*) (Møller 1989a). During the breeding season, *sexual* selection favours large badges through increased mating success and other reproductive advantages (Møller 1988, Veiga 1993a).

2.1.7   Badge size and reproductive success

Several predictions arise from Møller and Veiga' s work, which were tested in the population of house sparrows established at Maine Chance farm. If large-badged males are adjudged to be of higher quality, irrespective of whether this is genetic or conditional, females would benefit by pairing preferentially with these males.

Males with large badges should therefore obtain mates and begin breeding earlier than males with small badges. They should also attract higher quality females, and during the course of the season, produce more fledglings.

2.1.8   Parental condition and reproductive success

The physical condition of each male will also affect their reproductive success, irrespective of any plumage characteristics. This is also true of females, since, all else being equal, birds in better condition can invest proportionately more into reproduction than self-maintenance activities and sustenance. The hypothesized effects of physical condition were also tested in the Maine Chance population of sparrows.

Adults in good condition are predicted to be better parents, and therefore produce more fledglings than those in poor condition. Furthermore, as the survival of these fledglings is strongly influenced by their condition when they leave the nest (Perrins 1979), better parents should produce nestlings in better condition.

2.1.9 Adult age

In short lived passerines such as the house sparrow, reproductive success generally improves with age and experience (Saether 1990, Pärt 1991). Older individuals have greater experience with foraging and other survival factors such as predator avoidance. I therefore predicted that older individuals of either sex would have higher reproductive success than birds in their first breeding season (i.e. yearlings).

2.1.10 Additional measures of parental phenotype

In 1996 I examined a range of male and female phenotypic characters which may affect sexual selection and reproductive success.

Badge abrasion

Møller (1992a) found that males with large badges preened their general breast area at a higher rate than males with small badges, and thereby revealed the full extent of their ornament earlier. The degree of badge abrasion may thus serve as an index of male dominance, since non-aggressive birds may not reveal the full extent of their badge to avoid challenges. I thus predicted that the degree of abrasion is proportionately greater in large-badged males.

Beak blackness

Although the production of the black badge feathers is not dependent upon testosterone (Keck 1934), the black pigmentation of the beak does not develop in the absence of androgens. If beak blackness served as a crude reflection of testosterone levels, several related hypotheses concerning the interaction of hormones and dominance could be tested without recourse to expensive endocrinological methods. I therefore investigated whether beak blackness could be used as an indirect cue of testosterone levels.

Song rate

House sparrows have a notoriously simple 'song' comprised of repeated, throaty chirrups supplemented occasionally with more tuneful vocalisations (Summers-Smith 1963). Despite the simplicity of this song, the high frequency with which it is produced (several hundred times per hour) is presumably

Two measures of badge size were therefore available for each male: 'black badge size' (BBS) and 'white badge size' (WBS). As a more crude measure, badges were allocated into size categories following Møller (1987a) (Figure 1). Males which were not caught were allocated into badge size categories according to the same scale, which allowed the trapped and untrapped males to be included in the same analyses.

Deviations slightly to either side of a recognized category were given a plus or minus score (i.e. a badge slightly smaller than a '4' was scored as '4 -'). The scale used in 1995 was taken directly from Møller (1987a), although in 1996 it was modified to make the intergrading more gradual (Figure 2), as Møller's scale categorized almost all birds as being either a '3' or a '4'.

Figure 1. The range of badge size categories used in 1995 (Møller 1987a)

1   2   3   4   5
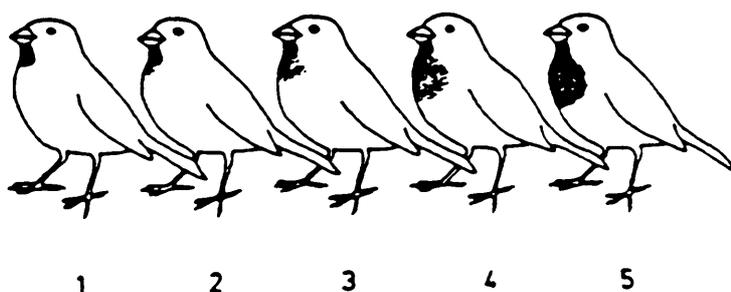
Figure 2. The range of badge size categories used in 1996.

1   2   3   4   5
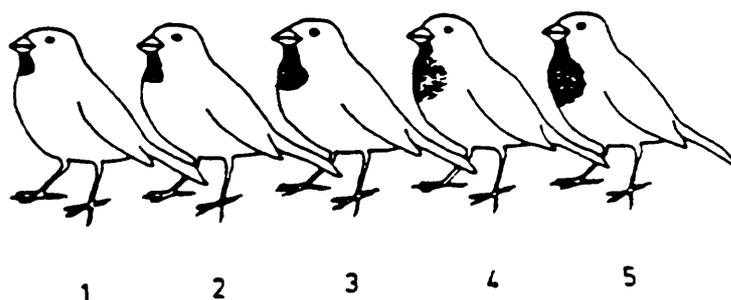
The repeatability of badge measurements was assessed using a one-way ANOVA (Harper 1994). In 1995, data was available for eight males which were recaptured at least once, and in 1996, data was available for seven recaptured males.

21

energetically expensive (Andersson 1982). More importantly, the frequency with which males sing is a more immediate reflection of their current health than dead feathers produced many months previously. If song rate is an index of male quality, males which call at the highest rates should obtain a mate and begin breeding soonest.

Female dominance

Although aggression in house sparrows (and indeed most birds) is usually associated with males, agonistic encounters are occasionally witnessed between females (Summers-Smith 1963, Veiga 1990). During these events, and in other stressful situations such as handling (*pers. obs.*), females flare their pale supercilial feathers to produce a frontally-directed 'arrow' exacerbated by the slope of the bill, which presumably acts as a form of threat. If a larger supercilium is associated with dominance and therefore quality, females with larger supercilia should have access to the highest quality males, and produce more fledglings during a season than females with small supercilia.

2.2   Methods

The methods used in this section were all concerned with measuring various aspects of parental phenotype.

2.2.1   Badge size of the bird in hand

Badge sizes were measured using the method described in Møller's (1987a) original study. Males were held in a natural 'sitting' position, and the maximum length and width of the badge of black feathers was measured to the nearest 0.1mm with Vernier callipers. Four measurements were taken from each badge: the length and width of the inner region of pure black feathers (the 'black badge'), and the length and width of the outer region of black feathers which still retained their pale buff tips (the 'white badge'). All badge sizes were calculated using the formula specifically derived by Møller (1987a):

badge size (mm^2) = 166.7 + 0.45 (badge length x badge width)

2.2.2 Badge tracings

In 1995, as a secondary measure of badge size, each male was laid on its back with a thin sheet of plastic over its breast, and the outline of both the black and white badge was traced using a fine tipped marker pen. Traces were obtained from 26 males, three of which were traced on two separate occasions. As the plastic was very thin and pliant, the tracing theoretically represents an exact copy of the badge.

To test this supposition, the four badge dimensions (i.e. black badge length and width, white badge length and width) were recorded from the tracing using a clear plastic ruler graded in millimetres, and compared against the equivalent data taken from the bird *in situ* with callipers. On the three occasions where two sets of measurements had been obtained from a bird, care was taken to match the relevant mensural data to each trace.

Tracings were scanned into a Power Macintosh computer, and analysed using NIH Image. The areas encompassed by both the black badge and the white badge were obtained, and a 'blackness percentage' was calculated for each badge by dividing the black area by the area of the whole badge, and multiplying by one hundred.

2.2.3 Badge photography

In 1996, frontal photographs of trapped males were obtained using a Nikon SLR camera fitted with 400 ASA colour film. Each bird was photographed at a standard distance (arm's length). Colour slides were scanned into a Power Macintosh computer using a SpeedScan 3000 peripheral routed through Adobe Photoshop 4.0, and analysed using NIH Image. As no scale bar was available on the photographs, they were individually calibrated by fitting a horizontal chord between the beak flanges, and designating this distance as 8 mm (a reasonable average of bill width *unpub. data.*). The maximum length and breadth of the white badge was measured according to this calibration. (The extent of the white badge can be determined on photographs since the buff tips are noticeably 'spiky'). Finally, the entire perimeter of the white badge was carefully delineated using a tracing tool, and the area enclosed was calculated by the computer.

2.2.4 Supplementary measurement of badge size in 1996

In addition to recording the badge dimensions of 'sitting' birds, in 1996 the length and width of the black and white badge was measured while the bird was held flat on its back with its bill pointing directly upwards. This method was intended to minimize badge distortion and elongation which occasionally resulted from postural differences of 'sitting' birds (*pers. obs.*).

2.2.5 General measures of adult phenotype

Adults were weighed to the nearest 0.1g using a 30g spring balance. Wing length (flattened chord method) and tarsal length were measured to the nearest millimetre and 0.1mm respectively. Bill depth and culmen length were measured to the nearest 0.1mm. Tail length was recorded by sliding the central rod of Vernier callipers, held in reverse position, along the underside of the central rectrices until the end of the rod butted against the point of feather insertion. A body condition index was derived for each adult as the residual of regressing body mass on tarsal length. Age data was available on several adults which were ringed in previous seasons.

House sparrows cannot be aged during the breeding season, as the skulls of both yearlings and adults are completely ossified (Nero 1951). To counter this restriction, I attempted to classify sparrows as either adults or yearlings on the basis of their leg colour, since the legs of several other passerines darken with age (e.g. the dunnock, Davies 1992). The upper tarsal region of birds trapped was matched to one of six shades of brown shown on a 'test-strip' obtained from a commercial paint shop (LexArt, Lexington). Several of these birds were already colour-ringed, and thus their minimum age was known as a reference.

2.2.6 Additional measures of parental phenotype

Badge abrasion

Three central feathers were selected from the periphery of the white badge, and the length of their 'buff tips' was measured *in situ* to the nearest 0.1 mm using Vernier callipers.

Bill blackness

The beak of each male handled was wiped clean, and its blackness was directly compared against a beak which had been dissected from a breeding male found dead on the farm turnpike.

Song rate

The song frequencies of seven males were measured in the first two weeks of the field season, with song rate being denoted as the average number of calls emitted during each 30-second bout of my half-hour observation periods. The occurrence of two common call-types was measured (the throaty chirrup, and the less frequent high-pitched trill).

Female dominance

The feathers of the left supercilium of each female processed were gently smoothed backwards, and its length and maximum depth were measured to the nearest 0.1 mm with Vernier callipers.

2.3 Results

2.3.1 Badge size of the bird in hand in 1995

Unfortunately, of the four badge measurements taken in 1995, only black badge length (BBL) was repeatable ($F_{7,9}$ = 6.46 p = 0.01). The black badge width (BBW) was sufficiently erratic to render black badge size (BBS), calculated using the product of BBL and BBW, similarly unrepeatable ($F_{7,9}$ = 2.59 p = 0.093). Badges could not be placed into size categories with significant repeatability ($F_{7,9}$ = 1.20 NS), and were summarily discarded. Therefore, 'badge size' used in all of the 1995 analyses refers to a single dimension, black badge length or BBL.

This situation probably arose because badges in the first month of the 1995 season were measured by a co-worker, whereas I measured badges for the remainder of the summer. Five of the badges had been originally measured by my co-worker, and were remeasured late in the summer by myself when the birds were recaptured. Slight differences in our technique would have led to discrepancies between our badge size measurements.

2.3.2 Badge size of the bird in hand in 1996

Only one badge dimension was repeatable when the bird was measured while held flat on its back (BBL $F_{6,8}$ = 7.5 p = 0.006, BBW $F_{6,8}$ = 2.61 NS, WBL $F_{6,8}$ = 1.15 NS, WBW $F_{6,8}$ = 1.87 NS). Using these data gave repeatable values for black badge size ($F_{6,8}$ = 4.86 p = 0.022) but not white badge size ($F_{6,8}$ = 2.6 NS).

All four badge dimensions were individually repeatable when the bird was measured in a 'sitting' position (BBL $F_{6,8}$ = 30.63 p = 0.001, BBW $F_{6,8}$ = 6.87 p = 0.008, WBL $F_{6,8}$ = 3.74 p = 0.045, WBW $F_{6,8}$ = 4.02 p = 0.037). Using these data gave repeatable values for both black badge size ($F_{6,8}$ = 4.75 p = 0.024) and white badge size ($F_{6,8}$ = 9.44 p = 0.003).

I therefore calculated the badge sizes of all other males using the data obtained when measuring birds held in the sitting position.

Although the white badge size of each bird was significantly larger than the black badge size (Paired t = 16.16 df = 46 p = 0.01), the two measures were strongly correlated (r = 0.619 n = 47 p < 0.01). Hence, males with large black badges also had large white badges. I therefore use white badge size throughout this thesis as the single measure of 'badge size', in accordance with other house sparrow studies (Møller 1987a, Veiga 1993a, Kimball 1995, Griffith 1998).

The measured size of each badge correlated with its size category (r = 0.397 n = 31 p < 0.05) and also its refined size category involving plus and minus adjustments (r = 0.407 n = 31 p < 0.05). As this refinement did not markedly improve the correlation, it was discarded. In truth, the badges of birds in the hand were only allocated into size categories as a reserve measure in case the linear dimensions were not repeatable (as had happened in the previous season). The repeatability of all four badge dimensions in 1996 rendered the size categories redundant, beyond their usefulness in estimating badge size categories of untrapped males from a distance.

2.3.3 Validity of the badge size tracings

Although the tracing and direct measures of both the BBL and BBW were significantly correlated (r = 0.407 and r = 0.669 respectively, n = 26 P < 0.05),

further analysis revealed that only BBW did not differ between the two measures (Wilcoxon's test for matched pairs $z = -0.127$ NS). An examination of the means of the two measures for each dimension revealed that the tracings exaggerate the real size of the badge (Table 1).

Table 1. Mean badge dimensions taken from the bird in hand and tracings.

Character	Real size (mm)	Traced size (mm)	% Real / Trace
BBL	11.96	16.89	71
WBL	18.71	22.52	83
BBW	14.17	20.52	69
WBW	21.69	27.48	79

This discrepancy is probably due to the badge becoming flattened and distorted when the plastic is overlaid. Although this was unfortunate, the situation would be remedied if the degree of distortion was constant for each badge. Repeatability tests were performed on the trace dimensions of the three birds measured twice. Of these measures, only black badge length and blackness percentage were significantly repeatable ($F_{2,3} = 36$ p $= 0.008$; $F_{2,3} = 9.48$ p $= 0.049$). Furthermore, badge sizes taken from traces could not be placed into basic categories without 'mediums' being hugely over-represented. Thus, the badge size data from the tracings had to be discarded. It is worth noting however, that this method has been used elsewhere with 67% repeatability (S. Norris *pers. comm.*). With practice, it may have eventually proved useful as a quick means of obtaining an exact replica of each badge.

2.3.4   Validity of the badge size photographs

Both of the badge size dimensions obtained using the computer were significantly smaller than those obtained from the bird in hand (Paired $t = 6.10$ df $= 22$ p $= 0.001$ for WBL, Paired $t = 7.15$ df $= 22$ p $= 0.001$ for WBW). Consequently, badge areas calculated using the computed values were significantly smaller than those calculated using the field dimensions (Paired $t = 8.78$ df $= 22$ p $= 0.001$). The consistent size differences probably result from an underestimate of bill width being used for the calibration.

Fortunately, although the field and computer measurement techniques produced clear size differences, the correlations at each dimension showed

that these differences were consistent (WBL $r = 0.480$ $n = 23$ p < 0.05, WBW $r = 0.608$ $n = 23$ p < 0.01). Consequently, the badge area generated using the NIH Image dimensions was strongly correlated with that generated using the field dimensions ($r = 0.597$ $n = 23$ p < 0.01). Relative badge size is conserved, irrespective of the measurement used.

The validity of Møller's (1987a) badge size calculation was assessed by using NIH Image to determine the lengths and widths of the 17 photographed badges. Møller's (1987a) equation was used to calculate an expected area for each badge on the basis of the two dimensions, which was then compared against the badge area calculated by Image.

The badge areas derived using Møller's (1987a) equation were significantly larger than the actual areas generated by Image (Paired $t = -1\ 7.1$ df $= 22$ p $= 0.001$). However, the two measurements were highly correlated ($r = 0.937$ $n = 23$ p < 0.001), which suggests that shape differences are minimal and vindicates the use of Møller's (1987a) equation (Figure 3).

Figure 1. Calculated badge area against real area.
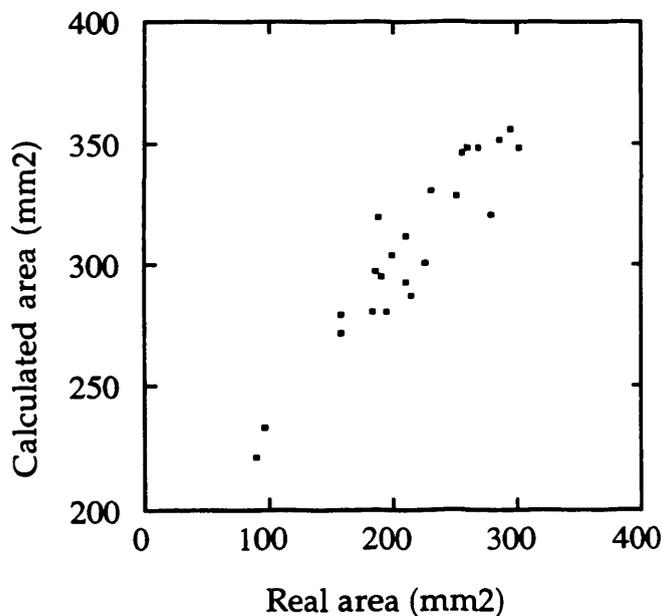
In a comparable study of house sparrow sexual selection, Kimball (1995) derived badge areas from photographs in preference to calculating badge sizes from linear measurements. Møller's (1987a) regression equation (described above) was unsatisfactory in her population, which could have been caused by greater variation in badge shape (Kimball 1995). At Maine

Chance, badge shape was not uniform, with some badges appearing wider and flatter than others. However, this variation was not particularly marked, nor is it clear why badge shape would vary between populations.

2.3.5 Discounted measures of parental phenotype

Several of the measurements taken in the field are not discussed in the remainder of this thesis, for a variety of reasons.

Badge abrasion

The length of the buff tips could not be measured satisfactorily, as this varied considerably on each bird, even between adjacent feathers. Nor could I find a reliable criterion for classifying birds as having either short, medium, or long tips.

Beak blackness

The beaks of live males did not appear to differ from the test beak with respect to blackness, although some were obviously more glossy than others. The negligible variation in beak blackness suggests that the testosterone-dependent darkening merely advertises the reproductive readiness of the bearer.

Female dominance

Practical difficulties rendered it impossible to assess the significance of size variation in the female supercilium. The measurements of supercilium length and depth were not significantly repeatable ($F_{10,14} = 1.24$ and $F_{10,14} = 1.59$ respectively), which was attributed to the problems posed by supercilial curvature.

Song rate

It proved too difficult to measure the song rates of individual males, as the density of boxes at the main observation site (Barn B) meant that several males were calling to attract a mate simultaneously. Moreover, males appeared to increase their own call frequency in response to other calling males, and would also chirrup furiously if a female sparrow merely flew past

the barn. Although males issue chirrup calls at later stages of the season, particularly during the fertile period of their mate (*pers. obs.*), the intensity of these is very low. Nevertheless, these observations suggest that song functions in sexual selection, at least during the early stages of the season when males are attempting to attract mates.

Age assessment

House sparrows could not be placed into age categories according to their leg darkness. Although the legs did indeed vary in colour between 'rice puff' and 'oak brown', this was not associated with age, as the older birds at the site did not have darker legs than the known yearlings. All unringed birds trapped were thus assigned a minimum age of one year.

2.3.6   Badge size measurements used throughout the thesis

To reiterate, analyses from the 1995 season involving 'badge size' refer to the length of the black badge. However, analyses from the 1996 season involving 'badge size' refer to the calculated area of the white badge.

Although other methods for assessing badge size were investigated (such as computer analysis of photographs), these were discarded as they did not prove any more informative. However, since both forms of selection work on differences (Darwin 1859, 1871), the methods finally chosen were adequate to consistently rank individuals according to the size of their traits.

For two reasons, the phenotypic components of reproductive success in 1995 and 1996 were analysed separately. Firstly, the index used to describe 'badge size' differed between seasons, which made pooling results invalid. Secondly, the 1995 data set was considerably smaller, and was blighted with confounding variables such as partner changes.

2.3.7   The determinants of reproductive success in 1995

Badge size and reproductive success

Male with large badges did not commence breeding earlier ($r = -0.59$ $n = 8$ NS), although only a small sample size was available for this test as several of

the males present for first broods were usurped before they could be caught. Large-badged males were not paired to large females (mass $r = - 0.240$, tarsal length $r = - 0.52$, n = 13 both NS), and females paired to large-badged males did not lay more eggs during the season ($r = 0.212$ n =13 NS), nor larger clutches ($r = 0.539$ n = 12 NS).

The critical measure of reproductive success is the number of offspring which recruit into the breeding population in the following season(s) (see Newton 1989). As these are difficult data to obtain in a free-living population, the usual correlate of reproductive success is the number of fledglings produced during the season.

Within-season fledgling production was independent of badge size (r = 0.147 n = 16 NS) (Figure 2). This lack of numerical advantage was not countered by any tendency to produce chicks which were heavier (as measured by nestling day 10 mass,r = -0.311 n = 16 NS) or structurally larger (as measured by average tarsal length r = -0.148 n = 16 NS).

Overall, badge size in 1995 did not affect fledging production or any of the usual factors which contribute to reproductive success.

Figure 2. Fledgling production in relation to badge size.
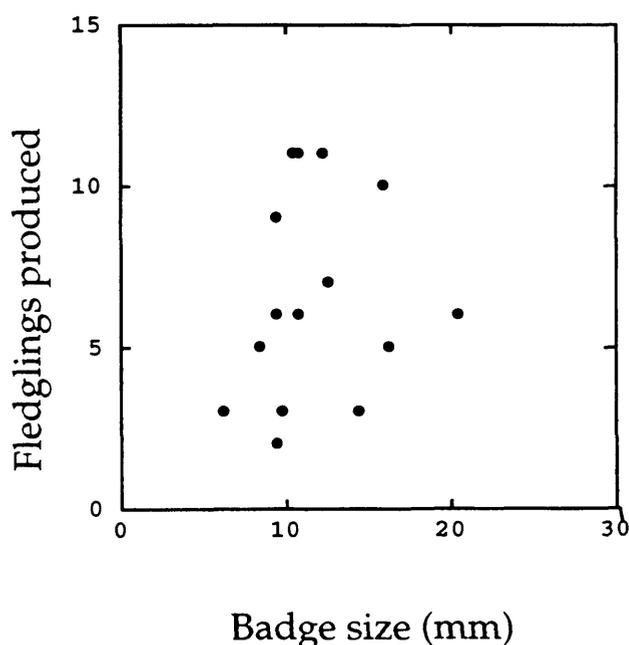
Badge size (mm)

A more parsimonious explanation for reproductive success differences is variation in the physical attributes of one or both parents. Although badge size did not appear to be related to reproductive success, body size varies independently of badge size (mass r = -0.217 n = 33 NS ; tarsal length r = -0.100 n = 35 NS), and may be having a more significant impact on fitness parameters. This is particularly important with regard to female quality, as the number of eggs which are laid during a season sets the upper limit to within-pair reproductive success.

Female size had a positive effect upon one breeding parameter, as heavier females produced larger clutches (r = 0.581 n = 13 p < 0.05) (Figure 3). Clutch size was independent of tarsal length however (r = 0. 273 n = 13 NS), and seasonal egg output was independent of both measures of female size (mass r = - 0.359, tarsus r = 0.194 , both n = 18 NS). Despite the influence of female mass upon clutch size, the overall reproductive success of either parent during the season was independent of their size (males ; mass r = 0.173, tarsus r = 0.326, both n = 16 NS : females ; mass r = 0.162, tarsus r = 0.063, n = 18 NS). There was no evidence of assortative mating for size (male mass vs female mass r = -0.333 n = 10 NS, male tarsus vs female tarsus r = 0.401 n = 12 both NS).

Figure 3. Average clutch size in relation to female mass.

Female mass (g)
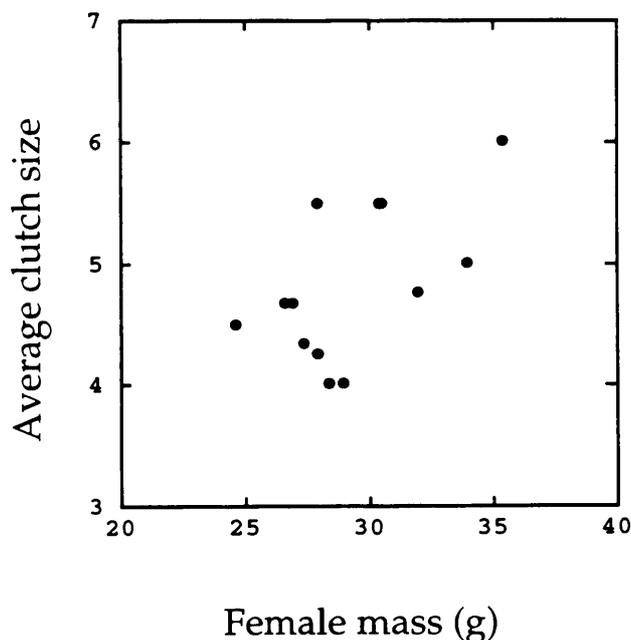
2.3.8   The determinants of reproductive success in 1996

Badge size and reproductive success

Although large-badged males commenced breeding earlier, this was not significant (r = -0.134 n = 25 NS). Females paired to large-badged males did not lay more eggs during the season (Spearman rank r = 0.103 n = 23 NS), nor did they lay larger clutches (r = -0.028 n = 23 NS). Males with large badges did produce significantly more fledglings (r = 0.434 n = 23 p < 0.05) (Figure 4). When badge size was entered into a multiple regression to control for timing of breeding, large-badged males did not produce significantly more fledglings ($F_{2,20}$ = 2.33 NS, for FED p = 0.90, for badge size p = 0.057).

Figure 4. Large-badged males produce more fledglings.
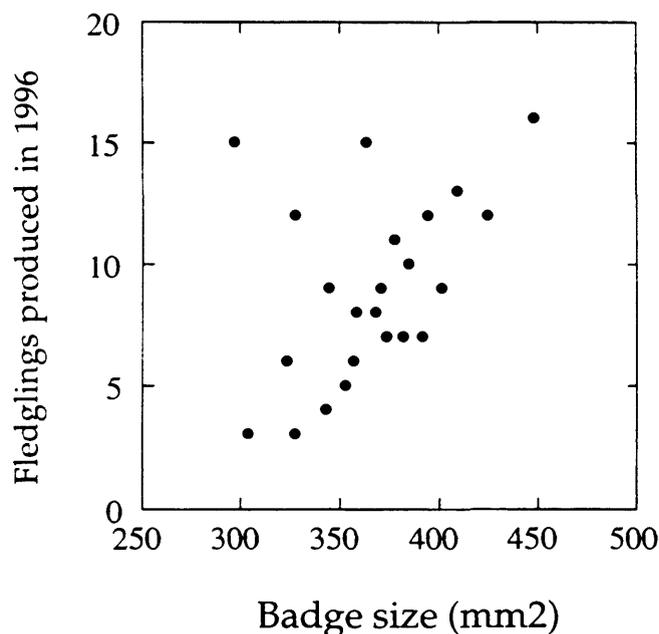
Parental phenotype and reproductive success

Fledging success was independent of male size (Table 3). By contrast, females which were structurally large (as measured by their tarsal length) and in good physical condition produced significantly more fledglings (Pearson correlation coefficients reported in Table 3).

Table 3. The influence of parental size upon fledgling production.

(* = p < 0.05)

Parental variable	Male ($n = 26$)	Female ($n = 21$)
Mass	0.151	0.067
Tarsal length	0.097	0.433 *
Body condition index	0.017	0.415 *

The physical characteristics of nestlings were compared against the equivalent parameters from either parent (e.g. the average mass of nestlings from sequential broods reared in the same nest-box was compared against the mass of the attendant male and female). There were no paternal or maternal influences upon nestling size (Table 4).

Table 4. The influence of parental size upon nestling size.

Nestling Character	Paternal effect ($n = 25$)	Maternal effect ($n = 23$)
Average mass	0.337	0.233
Average tarsus length	0.336	0.357
Average wing length	0.115	0.203
Average BCI	-0.192	-0.023

Age and fledging success

Although several birds of known age were not caught, sufficient numbers of yearlings and adults were present to allow certain aspects of fledgling production and phenotype to be compared between the two age classes. Older males did not produce more fledglings than yearlings ($t = -0.027$ df = 18 NS), nor was there any age-related difference in fledgling production in females ($t = 0.348$ df = 16 NS). There was no difference in badge size between yearling males and adults ($t = - 1.59$ df = 11 NS).

Badge size and physical size

There were significant, positive correlations between badge size and tarsal length ($r = 0.301$ $n = 46$ p < 0.05), and badge size and body mass ($r = 0.349$ $n = 46$ p < 0.05). Badge size was not correlated with body condition however ($r = 0.161$ $n = 46$ NS).

In addition to the obvious potential index of male attractiveness (badge size), I also considered whether several fairly disparate morphometric measures bore any relationship to male reproductive success.

Two captive mate choice trials have reported a female preference for males with deeper bills (Møller 1988, Kimball 1995). If this is an indicator of male attractiveness, these birds should obtain a mate and commence breeding earlier. This prediction was not upheld ($r = 0.250$ $n = 25$ NS). If bill size is an index of male quality, then all else being equal, males with larger bills should produce more fledglings. However , there was no relationship between bill size and fledging success (bill length, $r = -0.042$ $n = 26$ NS, bill depth $r = 0.280$ $n = 26$ NS).

Indices of female quality are less frequently reported in birds, although Kimball (1995) found that females with longer bills provisioned nestlings at a higher rate. Bill size did not correlate with female fledging success at Maine Chance however (bill length, $r = 0.073$ $n = 22$ NS, bill depth $r = 0.111$ $n = 22$ NS). In a British population of house sparrows, females with longer tails produced more fledglings (Burke 1984). This was also true at Maine Chance ($r = 0.519$ $n = 18$ p < 0.05).

2.4 Discussion

As discussed earlier, the classification of badge size and several of the key breeding parameters were not comparable between years. To aid clarity, the results from each season are briefly summarized in sequence before the main discussion.

2.4.1 Findings of the 1995 season

Large-badged males did not begin breeding earlier than small-badged males, nor were they paired to larger, more fecund females. Large-badged males did not have higher fledging success than small-badged males, nor were their young likely to fledge in better condition. Badge size was independent of male size and condition. Although heavier females laid larger clutches, their seasonal fledging success was not greater than that of lighter females. The

number of fledglings produced, and their average size, was not related to the physical size of either parent.

Overall, there were no detectable influences of reproductive success in 1995.

2.4.2 Findings of the 1996 season

Large-badged males did not commence breeding earlier than small-badged males, and were not paired to more fecund females. Although males with large badges produced more fledglings than males with small badges, this was not significant after controlling for the timing of breeding. Badge size was positively correlated with both mass and tarsal length, although it was independent of body condition.

Male size had no influence on fledging success, although large females produced more fledglings than small females. Offspring size and therefore survival probability was unrelated to the physical size of either parent, and any reproductive success differences probably result from the numbers of fledglings produced, not their quality.

2.4.3 Comparison with other populations

The relationship between badge size and fledging production varies markedly between studies (Table 10). In the initial study undertaken in Denmark (Møller 1989b), large-badged males produced significantly more fledglings each season. However, this was apparently due to differences in territory quality rather than paternal quality. Whereas large-badged males tended to occupy relatively safe hole-nests, small-badged males were restricted to relatively vulnerable open nests, from which nestlings were more frequently lost (Møller 1989b).

Large-badged males in a New Mexico nest-box population commenced breeding earlier, and also produced significantly more fledglings during the season (Kimball 1995). However, after controlling for the time of first breeding , fledging success was independent of badge size. Kimball (1995) reported that the symmetry of the badge was a more important determinant of breeding success than badge size *per se*, although this was not the case at Maine Chance (Chapter 3).

Table 10. The influence of badge size on fledgling production.

Badge size influence	Country	Author
Positive[1]	Denmark	Møller 1989b
Equivocal[2]	Spain	Veiga 1993a
Positive[3]	USA	Kimball 1995
Negative	England	Griffith 1998
Positive[3]	USA	This study

([1] confounded by territory quality, [2] experimental , [3] confounded by time of breeding)

In a Spanish nest-box population, the badge size of several males was either reduced or enlarged by experimental manipulation (Veiga 1993a). These males did not produce significantly fewer or more fledglings than control males, although curiously, males with reduced badges produced more fledglings than males with enlarged badges.

The most paradoxical results were reported from Lundy Island, England (Griffith 1998). Males with small badges commenced breeding earliest, and were paired to more fecund females (with regard to their average clutch size). Large-badged males produced significantly fewer fledglings during a season and, most importantly, fewer recruits into the breeding population. However, males with large badges had higher overwinter survival and thus, on average, may have achieved higher lifetime reproductive success (Newton 1989).

Note that the Kentucky result is listed in Table 1 as 'Positive', although in 1995, badge size actually had no influence upon fledging success. This is because the 1995 result was subsequently 'over-ruled' by the findings of the following season, as the 1996 data set was more rigorous, particularly concerning the measurement of badge size.

This study concurs with that of Kimball (1995) in that large-badged males produced more fledglings during the season than small-badged males, but this was not significant after controlling for the time of breeding initiation.

2.4.4 Preferred traits and reproductive success

Demonstrating a female preference is difficult in a free-living population, and consequently most field studies (including this one) attempt to detect indirect correlates of attractiveness (Hill 1990, Petrie *et al.* 1991, Møller 1994b).

A classic ornithological correlate of male attractiveness is 'timing of pairing', or more precisely, the date on which the first egg of the season appears in his nest (the first egg date (FED)) (Newton 1989). All else being equal, high quality females should be ready to breed before low quality females. These preferentially pair with the most attractive males who consequently begin breeding earliest (Ross 1997). Early breeding pairs gain a two-fold advantage. Firstly, nestlings which fledge earlier in the season have higher survival than those which fledge later (Perrins 1979, Newton 1989), since the abundance and quality of food declines as the season progresses. Secondly, pairs which commence breeding early can potentially rear more broods within the same season (Perrins 1979, Møller 1994b).

Although both Møller (1989b) and Kimball (1995) found that large-badged males began breeding earlier, there was no relationship between badge size and FED at Maine Chance. House sparrows in Kentucky have a long breeding season, with the first clutches initiated in the last week of March, and the final broods fledging in the last week of August. The selective pressure upon early breeding is unlikely to be intense, and indeed, there was evidence in both years of the study that 'premature' breeding attempts were abandoned due to adverse weather conditions.

2.4.5 Fledging success and territory quality

In a Danish population of house sparrows, variation in territory quality was an important determinant of fledging success (and presumably female choice) (Møller 1989). Differences in territory quality were unlikely to have been important at Maine Chance however, since all nest-boxes were of the same design, and appeared equally vulnerable to predators and parasites. Variation in nest-box quality is almost certainly exceeded by variation in male quality.

2.4.6 The significance of female size

Although heavier birds laid larger clutches in 1995, female size did not have any consistent effect upon reproductive components (although both Murphy (1978) and Kimball (1995) reported that larger females laid more eggs during the season). The maximum number of fledglings which a pair can achieve is limited by the number of eggs the female lays during the season. In the house sparrow, the energetic cost of egg production is relatively low (estimated as an extra 15% of basal metabolic rate (Krementz & Ankney 1986)). Hence, egg production is unlikely to be constrained by female quality (as illustrated by the unfortunate female induced to lay eggs on 50 consecutive days (Cole 1917). Similarly, clutch size is not constrained by energetic requirements, but is adjusted to optimize fledgling production (Lack 1947).

2.4.7 Parental influences upon nestling size

Nestlings which fledge in good physical condition are more likely to survive to become breeding adults (Perrins 1979, Griffith 1998, but see Thompson *et al.* 1993). An indirect index of expected parental reproductive success is therefore the average size and body condition of their offspring. However, no phenotypic traits of either parent (including the badge size of the father) were correlated with nestling size. The attributes of 'parental quality' could not be inferred from nestling quality.

The average mass of each nestling represents the outcome of parent-offspring conflict (Mock & Forbes 1994). A point is reached at which adults benefit relatively less from further provisioning, as the survival probabilities of nestlings do not increase proportionately (Dawkins 1976). Seel (1970) proposed that house sparrow nestlings which fledge beneath a 'threshold' mass (*c*21g at Oxford) are unlikely to survive. If this is correct, then once nestlings exceed threshold mass, their survival may depend upon other qualities beyond mere size, such as mental alertness or thermal tolerance (Perrins 1979, Newton 1989). Therefore, one may not expect a direct correlation between parental size and nestling size.

2.4.8 Parental size and fledging success

Although fledging success for males was independent of their size, large females in good physical condition produced more fledglings than small females. This difference between the sexes was understandable, given their respective roles in the reproductive cycle (Summers-Smith 1963). Although the costs incurred at each basic stage may appear minimal (egg production, incubation, brooding and rearing), the male is only involved in the latter, whereas females are heavily involved in all four (Seel 1968b). The cumulative effects of these activities may be disproportionately costly for small females, whereas large females in better condition can afford to devote less time to feeding and more time to nest attendance.

2.4.9 Age, badge size and reproductive success

As found by Møller (1989b), badge size was independent of age, whereas both Veiga (1993a) and Griffith (1998) found that older males have larger badges. Under Veiga's (1993) scenario, the size difference is a result of older males being in better condition. As a corollary of this, females preferentially pairing with large-badged males would be more likely to obtain an old male. This mechanism for female choice would not be tenable at Maine Chance.

Moreover, Veiga (1993a) demonstrated that the badge size of *individual* males increased between seasons (although the difference was only significant in males making the transition between yearlings and adults). Unfortunately, this could not be assessed at Maine Chance, since relatively few males were trapped in both seasons.

In many birds, age (and therefore experience) has a much-vaunted, positive effect upon various breeding parameters and overall reproductive success (Saether 1990, Pärt 1991). At Maine Chance however, adult house sparrows of either sex did not produce more fledglings than yearlings.

2.4.10 A cautionary note on statistical power

This chapter has occasionally relied upon a simultaneous assessment of the effects of several phenotypic variables taken from the same individual upon single measures of fitness (e.g. first egg date, clutch size, reproductive

success). These phenotypic variables are unlikely to be statistically independent within a particular individual (e.g. birds with large tarsi would also be expected to possess long wings, because of simple allometric patterns of development, Birkhead 1993). Moreover, although other variables may be statistically independent (e.g. female beak length and clutch size), these were also compared against the same fitness measures taken. Performing multiple correlations against fitness measures taken from the same individuals inevitably increases the likelihood of making a Type I error (i.e. the false rejection of the null hypothesis, Sokal & Rohlf 1981). If 20 correlations are performed, one of these is likely to give a significant result purely by chance, which is consistent with the 95% confidence limits accepted as standard around most probability distributions (Fowler & Cohen 1996).

Overall, however, there were very few significant correlations detected between the phentotypic variables of either parent and the measures of fitness used. Consequently, the problems discussed above are not considered to have seriously affected the conclusions of the analysis.

2.4.11 Badge size and sexual selection at Maine Chance

Although Møller (1989b) and Veiga (1993a) dispute the qualities which are signalled by the house sparrow badge, both authors predict higher mating success for males with large badges. Large-badged males did indeed produce more fledglings at Maine Chance, although this result was weakened after controlling for timing of breeding. This study could not distinguish between Møller and Veiga's theories of what the badge signals. However, the failure to relate badge size to body condition favoured the former. Although larger males had larger badges, badge size is unlikely to have evolved merely to indicate body size, as this would be tautonymic.

To summarize, male fledging success was influenced by badge size, while female fledging success was influenced by physical size. The evidence was consistent with the badge being a sexually selected trait, although the data presented here could not resolve whether large-badged males gain their reproductive advantage through female choice or male-male competition.

Other manifestations of sexual selection could also have been present in the Maine Chance house sparrows, although these were beyond the scope of this work (e.g. whether females have a pre-existing sensory bias, Basolo 1990,

attempt to mate assortatively to produce heterozygous offspring, Brown 1997, or whether they use mate choice cues which are invisible to humans, Bennett *et al.* 1994, Andersson *et al.* 1998).

2.4.12 The house sparrow badge : past and future research

Although the house sparrow badge is generally acknowledged to function as a sexual character (but see Kimball 1996), the conclusive experimental study has yet to be performed. Møller's (1988) original study relied upon stuffed males, and hence important behavioural cues used in mate choice may have been lacking. Furthermore, the females involved in the trials may have been affected by the hormonal implants used to induce sexual activity.

Distinguishing between female choice and male-male competition is difficult in any taxon (Cronin 1991), and as this is particularly apparent in the house sparrow, the scope of captive trials is limited. Both Kimball (1995) and Veiga (1996b) readily accept that the badge may function in male-male competition, as the breeding density in their populations was considerably higher than that in Møller's (1989b), leading to more nest disputes and displacements.

The house sparrow is a remarkably sedentary species (Summers-Smith 1963), and in most populations (including that at Maine Chance (E. E. Walker, *pers. comm.*)) the birds which feed together in interactive winter flocks settle to breed locally. Females could plausibly assess male qualities outwith the breeding season by subtly observing the outcome of dominance interactions, and later settle with the highest ranking male. This hypothesis could be tested without experiment by interpolating between the winter dominance of each male and their breeding success.

If mate choice in spring was 'decided' during the previous winter, then the significance of badge size variation during different periods of the year would become blurred. Evidence from the great tit suggests that some pairing occurs during the winter (Perrins 1979), and there is no reason why this could not occur in the house sparrow.

Chapter 3. Fluctuating asymmetry in the house sparrow

3.1 Introduction

The fluctuating asymmetry (hereafter FA) of a trait refers to small deviations from perfect, bilateral symmetry which arise during its development (Ludwig 1932). These deviations are thought to arise because of 'stress' caused by environmental and internal factors, such as parasitism, poor nutritional status, or adverse weather conditions (Møller 1994). Since perfect symmetry is the ideal state, a high level of FA is assumed to reflect a poor quality individual (or, perhaps tautologically, an individual which was in poor condition while the trait was developing).

The early studies of FA remained in relative obscurity (Ludwig 1932, van Valen 1962) until Møller & Pomianowski (1993) recognized the potential of trait asymmetry as an indicator of individual quality, and hence a criterion for mate choice. Theoretically, if the symmetry of a trait reflects the genetic quality of its bearer, and this is heritable, females would receive good-genes benefits by preferentially mating with these males. FA should be a particularly reliable indicator of quality as it is uncheatable, and if coupled with a biotic stressor such as a parasite, continual co-evolution would result in the persistence of FA and hence its value as a signal.

Empirical evidence supports the hypothesis that FA is associated with mate choice. Firstly, the degree of asymmetry is considerably higher in sexual traits than in ordinary morphological characters (Møller & Pomianowski 1993). Secondly, within a species, the levels of FA are usually negatively correlated with the size of these characters (Møller 1994b). This indicates that high quality individuals can invest heavily into ornament production, whilst resisting the stresses associated with their development.

The earlier studies of FA focused upon elongate, paired traits, such as earwig forceps and swallow tail streamers (Møller & Pomianowski 1993). Taking advantage of the ease with which these can be manipulated, Møller (1992) indirectly demonstrated a female preference for barn swallows with symmetrical tails. The twinned nature of these structures allows their relative length and thus symmetry to be directly compared, particularly as they are often prominently advertised during sexual display (Møller 1994b).

However, ornaments do not have to be paired to be asymmetric. The chest plumage of zebra finches is comprised of short feathers, patterned with speckles and horizontal bars. The natural overlap of these feathers is such that the horizontal bars form a continuous line across the chest. Swaddle & Cuthill (1994) generated males with asymmetric plumage following the selective removal of certain feather tips. In an aviary experiment, female finches displayed more frequently and for longer periods before males with symmetric barring.

Although the house sparrow badge is devoid of any patterning, it is similarly comprised of short, overlapping feathers. The symmetry with which the black feathers of the badge are produced may provide information about the quality of their bearer in addition to badge size *per se*. Kimball (1995) examined the significance of badge FA in both captive and free-living populations of house sparrows in New Mexico. Although the degree of FA was not correlated with female choice in the aviary trial, it was related to several components of fitness in the free living population (Kimball 1995).

If badge FA is an indicator of quality, I thus predict that males with more symmetrical badges commence breeding earlier, pair with higher quality females, and rear significantly more nestlings during the season. The level of FA was also investigated in two non-sexual traits, the length of the tarsi and wings.

3.2   Methods

3.2.1   Photography

Details of field photography and the acquisition of computerised images are described in Chapter 2. Usable photographs were available for 17 males. The symmetry of each badge was scored by hand, after using the computer application NIH Image to process the original photographs. The ' threshold ' function was used to delineate the black outline of the badge while on the screen, which was then converted to a binary file, enlarged to A4 size, and a hard copy obtained.

Using this hard copy, the vertical axis of the badge was designated by a perpendicular line between the crown and the body centre. The badge was

then divided by 10 equidistant horizontal lines, and the width of both halves was measured at each division. The relative symmetry at each division was designated as the absolute difference between the right and left hand sides, divided by half the width of the badge at that division. This controls for width variation arising from the general light bulb shape of the badge. The relative symmetries at all 10 divisions were summed to give a FA score for each of the 17 badges.

The use of absolute asymmetry measures avoids the possibility that positive- and negative-signed values cancel each other out to produce apparently symmetrical badges. However, to ensure that the vertical axes had not been seriously misplaced, the *relative* asymmetries were summed for all 17 badges. FA scores using this method were normally distributed, and ranged between - 0.61 and + 1.54, with an average of 0.53. Thus, some badges tended to be consistently skewed to the left, and some tended to be skewed to the right, but most were fairly symmetrical overall.

3.3   Results

3.3.1   Morphological asymmetry

The length of both wings was known for 84 adults (42 males, 42 females). Only nine of these birds had asymmetric wings, with the difference being one millimetre in seven cases. The length of both tarsi was known for the same 84 adults. 20 of these birds had asymmetric tarsi (9 males, 11 females), with the difference being 0.1 mm in 16 cases.

The length of each tarsus and wing were re-measured in 19 birds trapped more than once. In 13 cases, tarsal symmetry (or asymmetry) was consistent between sampling events (11 birds with symmetrical tarsi, two with the right tarsus longer than the left). Wings of all birds measured more than once were symmetrical on both occasions.

Males with symmetrical tarsi produced significantly more fledglings than males with tarsi of different lengths ($t = 2.25$ df = 25 p = 0.033) (Figure 1).

44

Figure 1. Tarsal asymmetry and fledging success.

(mean + SE)
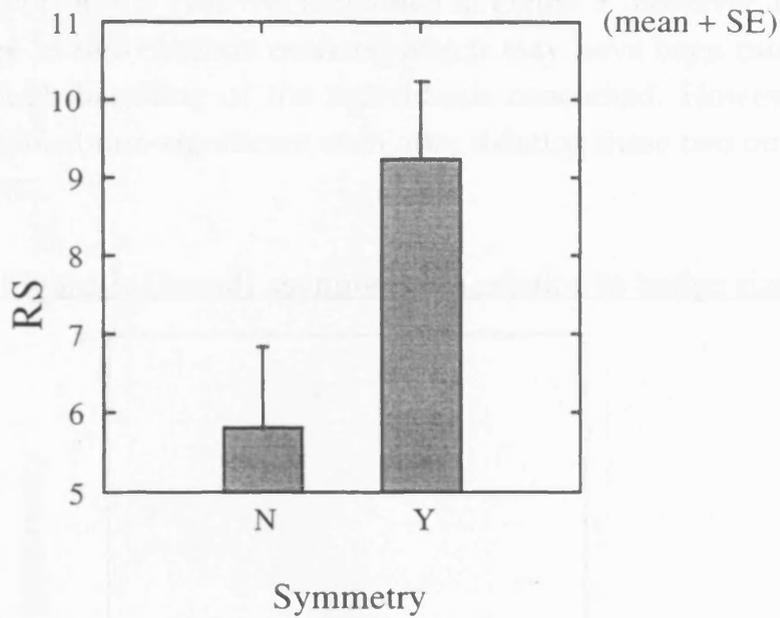
3.3.2 Badge asymmetry

The average FA score for the 17 males photographed was 1.86 (range 1.25 - 3.60). FA distribution among males showed a negative binomial distribution, with most males having relatively low FA, and occasional birds having markedly asymmetric badges (Figure 2).

Figure 2. Distribution of asymmetry classes.
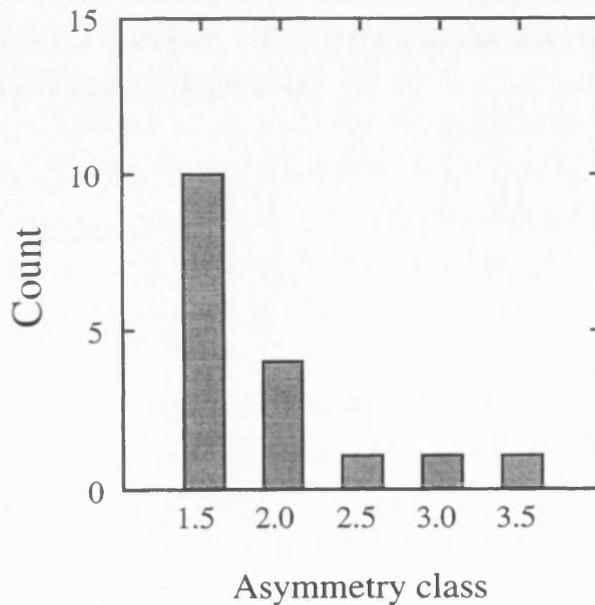
FA scores were independent of badge size ($r = 0.038$ $n = 17$ NS), and male condition ($r = 0.197$ $n = 17$ NS). As illustrated in Figure 3, however, the plot is distorted due to two obvious outliers, which may have been caused by particularly rough handling of the individuals concerned. However, the correlation remained non-significant even after deleting these two outliers ($r = 0.270$ $n = 15$ NS).

Figure 3. Overall asymmetry in relation to badge size.

Black badge size (mm2)
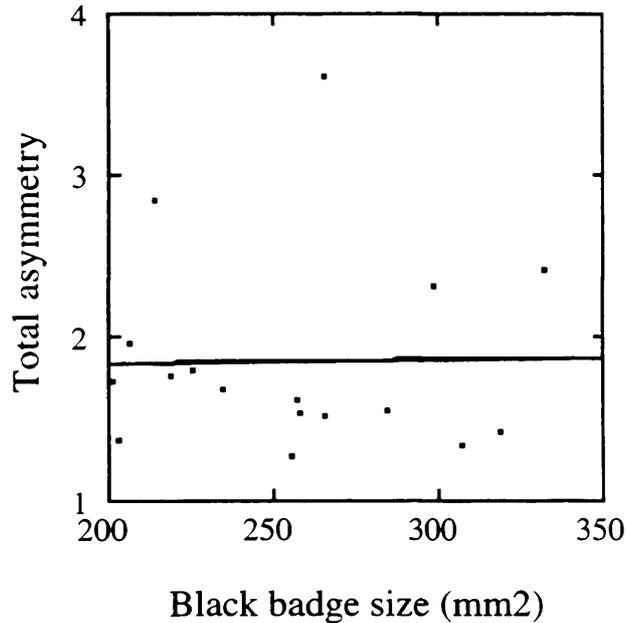
The total fledging success was only known for 12 of these 17 males. Five of the birds were either captured as transients and never resighted, or nested within inaccessible crevices. Fledging success was not correlated with FA score ($r = 0.23$ $n = 12$ NS) (Figure 4).

46

Figure 4. Badge asymmetry and fledging success.
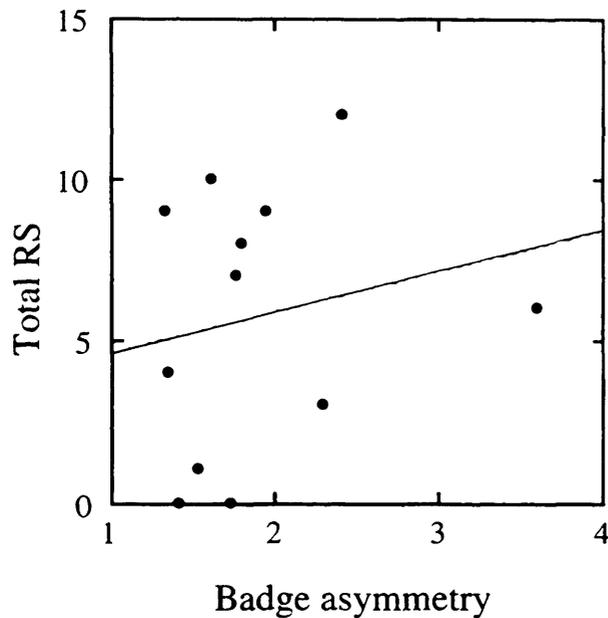
3.4 Discussion

3.4.1 Morphological symmetry

Most of the house sparrows which were examined had symmetrical wings, which was not surprising. Natural selection would act strongly against those birds with asymmetric wings, as their flight would be impaired. Consequently, they would become more susceptible to predation and, to a lesser extent, starvation. Tarsal asymmetry was more common, occurring in around a quarter of the birds measured. Males with asymmetric tarsi had lower fledging success, which is consistent with the prediction of FA theory. The asymmetric tarsi are unlikely to be the cause of lower fledging success *per se* , but their asymmetry reflects the general low quality or condition of the bird. The low fledging success is an indirect manifestation of the low quality.

A similar effect was described in black grouse by Rintamäki *et al.* (1997). Blackcocks with asymmetric tarsi were found at the periphery of leks, and obtained fewer matings. However, greyhens do not select mates on the basis of their tarsal asymmetry, but rely upon condition-dependent behavioural traits such as display vigour and fighting ability. Blackcock tarsal asymmetry is merely a corollary of poor condition , as are these displays. The mean tarsal

asymmetry reported by Rintamäki *et al.* (1997) was only 0.37 mm, which corresponds to 0.6 % of average male tarsal length. This figure is close to the mean tarsal FA found at Maine Chance (0.1 mm, corresponding to 0.5 % of house sparrow tarsus length).

However, as the variability in repeated measures illustrates, asymmetries at this scale have to be interpreted with caution. Indeed, consistent directional asymmetry was only achieved in 13 of the 19 birds remeasured. Although tarsal asymmetries may often be genuine, and tarsal length itself is a repeatable measure (I.R.K. Stewart, unpublished data), observer error could easily account for the asymmetries reported here (a tenth of a millimetre), since this is the smallest division available on standard Vernier callipers. Consequently, measurements have to be 'rounded up' to the nearest tenth of a millimetre, which reduces the stringency of repeated measures even further. Nevertheless, this is a problem encountered by all field ornithologists.

3.4.2   Badge asymmetry

Whereas the length of either tarsus is fixed at an early age, the badge feathers are renewed annually (Ginn & Melville 1994), and should thus provide a more accurate reflection of individual condition. There was no evidence of this at Maine Chance. For the 17 males photographed, FA scores were wholly independent of badge size. If FA reflects male quality, this relationship should have been negative, with large badges being relative symmetrical. This relationship was reported from a separate population by Kimball (1995), although the correlation was not significant.

FA theory also predicts a positive relationship between symmetry and fledging success, as males with the most symmetrical traits have access to the highest quality females, and are themselves in better condition. However, symmetry was not correlated with fledging success at Maine Chance, and indeed, the relationship tended to oppose this prediction, suggesting that symmetrical males actually rear fewer nestlings per season.

There was no compelling evidence to suggest that badge FA was important at Maine Chance. This contrasts with the New Mexico population studied by Kimball (1995), in which females paired to males with symmetrical badges began breeding earlier and laid more eggs during the season. This translated

into greater fledgling production for males with symmetrical badges. However, females paired to these males did not undertake a greater share of nestling provisioning, and as chicks were not measured on standard days, it is not known whether the offspring of symmetrical males fledge in better condition. Hence, the benefits of pairing with a symmetrical male may actually be indirect. Heritability of FA has been demonstrated elsewhere (Thornhill & Sauer 1992, Møller 1994b), and it is thus possible that females preferentially pair with symmetrically badged males in order to produce sons with symmetrical badges.

To confound the issue further, there was no female preference for males with symmetrical badges in captive mate choice trials (Kimball 1995). FA may thus function in male-male competition rather than female choice, and may be merely an indirect corollary of male condition.

3.4.3   Are house sparrow badges really asymmetric?

Unfortunately, the conclusions reported here remain tentative due to a methodological failing. Males should have been photographed on different occasions to assess the repeatability of the FA scores. This may be a critical point, as badges tend to assume an irregular appearance when the bird is in the hand. Upon their release, males usually preen the bib area to such an extent that the badges of perched birds appear symmetrical. Thus females may not perceive badges as being asymmetrical.

3.4.4   Future directions

Any further investigation of badge FA and female choice should utilize an experimental approach. If the badge asymmetry reflects developmental stresses which occur during the moult, individuals which are maintained in a favourable environment (e.g. abundant food, mild temperatures) should produce more symmetrical badges than matched birds kept in relatively poor conditions.

Chapter 4. Extra-pair paternity in the house sparrow

"Some cocks regularly go through other territories, moving silently and cryptically, keeping in dense cover. This is particularly true of cocks whose mates begin to incubate early, while neighbouring hens are still soliciting for copulation."

Newton (1972) *The Finches*

4.1 Introduction

Newton's description of chaffinch behaviour echoes that recorded by Marler (1956), also in this species, and unwittingly illustrates what is now a recognised phenomenon in birds: infidelity (Gowaty 1996a).

These observations were difficult to interpret in 1972. Birds were considered to be faithful to their mates, and hence males had no reason to make these surreptitious visits to other females. However, within a few years of *The Finches* being published, evidence emerged from a rather unexpected source that these visits may result in fertilization. During a government-funded attempt to reduce local numbers of the ubiquitous red-winged blackbird, a recognized pest, Bray *et al.* (1975) vasectomized a sample of males. Curiously, females paired to these males still produced fertile eggs. Since it was unlikely that all vasectomies had failed, the authors suggested that the eggs had been fertilized by an intruding male from a neighbouring territory. The existence of these events (termed extra-pair copulations [EPCs]) was subsequently confirmed in a range of intensive field studies (see Birkhead & Møller 1992). Concurrently, relatively simple biochemical techniques demonstrated that these matings can result in extra-pair fertilizations (EPFs) (e.g. Manwell & Baker 1975, Westneat 1987).

Unfortunately, the proteins used in these early electrophoretic techniques showed only minor variation, and consequently, their resolving power was poor (i.e. if many individuals within the population share the same genotype, then the probability of detecting an offspring gene derived from an extra-pair male is low, since the resident male is also likely to possess this gene). Moreover, to detect a satisfactory number of allozyme loci required a considerable amount of tissue (usually breast muscle, Baker 1981), which limited their use in field studies (Romagnano *et al.* 1989).

50

This problem was circumvented by the application of 'DNA fingerprinting'; a technique which had recently been serendipitously discovered by mammalian geneticists (Jeffreys *et al.* 1985). This powerful method of paternity assessment involved isolating nuclear DNA from a very small blood sample (< 0.1 millilitres), obtained without having to sacrifice specimens.

Two British research groups incorporated DNA fingerprinting into a long-running field study of house sparrows, and identified several nestlings which were genetically mismatched with their social father (Wetton *et al.* 1987; Burke & Bruford 1987). The convenience of the techniques described prompted a flurry of similar studies, producing further reports of extra-pair paternity (EPP) in other species. Within five years, infidelity was assumed to be widespread in birds (Gowaty 1996a), causing Lack's (1968) verdict of the preponderance of monogamy in birds to be refined: 90% of bird species are indeed monogamous, but only socially. In birds, (as in humans, Ridley 1994) social and sexual monogamy are not synonymous.

4.1.1 Why do birds seek extra-pair copulations?

Many adaptive theories attempt to explain avian infidelity by considering its specific costs and benefits (reviewed in Birkhead & Møller 1992). Most of these theories are without supporting data, and hence the following account is restricted to those for which evidence exists.

The male perspective

Since sperm are relatively cheap, the reproductive success of males is limited by the number of females they can inseminate. Males which can achieve EPCs therefore benefit by increasing their offspring production with only minimal expenditure. There are few recognized costs which males incur through the pursuit of extra-pair matings, the most widely cited being the risk of physical attack by the pair-male, and the risk of acquiring a sexually-transmitted disease (Sheldon 1993).

The female perspective

In most birds, females obtain no direct benefit from copulating with more than one male. Exceptional examples of direct benefits gained through EPCs include copulation feeding in polygynous red-winged blackbirds

(Gray 1997), and ensuring male assistance in the comparatively rare polyandrous mating system (Davies 1992). In contrast, there are several indirect, genetic benefits which could be accrued from multiple mating, of which the most parsimonious is offspring diversity (Birkhead & Møller 1992). However, it is questionable whether the genetic diversity obtained from EPFs would provide the brood with a net benefit (T. Burke, *pers. comm.*). Moreover, under this hypothesis, all females should attempt to mate randomly in an attempt to produce genetically diverse broods, a pattern which has been only rarely observed in nature (see Double 1995). Other theoretical objections make it unlikely that EPCs are an attempt to improve offspring genetic diversity (Gowaty 1996a).

Consequently, females are presumed to seek EPCs to accrue one of the two genetic benefits described by Cronin (1991) as 'good taste' and 'good sense'. Females of a species exhibiting 'good taste' sexual selection want their sons to inherit the extra-pair father's attractive Darwin-Fisher trait. In contrast, females of a species exhibiting 'good sense' sexual selection want their offspring to inherit the extra-pair male's superior genes for viability or disease resistance (Williams 1966, Hamilton & Zuk 1982). A non-genetic, indirect benefit to EPCs (which could also be interpreted as a direct benefit) is posited by the fertility insurance hypothesis (Wetton & Parkin 1991). Under this scenario, females benefit from EPCs by obtaining sufficient sperm to ensure all their eggs are fertilized.

The pursuit of EPCs by females is potentially more costly than it is for males, since their mate may retaliate to perceived cuckoldry by deserting the current breeding attempt (Trivers 1972). In a less extreme situation, males may respond to the absence of their mate by reducing their level of care, thereby minimizing misdirected parental effort. Females pursuing EPCs also risk contracting a sexually transmitted disease (Sheldon 1993).

Invoking William's (1966) sexual dichotomy of reproductive success, males seek to maximize, whereas females seek to optimize. This difference can be crudely summarized with respect to EPCs: males attempt to produce *more* offspring whereas females attempt to produce *better* offspring.

4.1.2   Interspecific variation in extra-pair paternity

Extra-pair paternity (EPP) levels have now been reported for almost 100 species of bird, which are listed in Table 1. The majority of these species are monogamous, in which case EPP refers to any offspring fathered by a male other than the social male. A small number of the passerines are either polygamous or exhibit cooperative breeding, in which case EPP refers to any offspring fathered by a male outside of the social group (described as extra-bond paternity by Owens & Hartley (1998)). Data were compiled using Gowaty (1996), Westneat & Sherman (1997), Owens & Hartley (1998) and personal communication. Studies which relied upon allozyme markers are indicated, as are EPP estimates based on fewer than 50 sampled offspring. This arbitrary figure is not intended as a criticism, but merely an acknowledgement of the difficulties involved in studying the birds in question, which are mostly large, k-selected species with dispersed nesting distributions (e.g. vultures, divers). Small samples are also prone to statistical error. Where more than one population of the same species has been studied, the upper and lower rates of EPP are given. A mean EPP rate was calculated for these species before they could be included in a simple statistical comparison.

Although these rates are not directly comparable, because the molecular techniques used to elucidate infidelity varied between studies, two main conclusions emerge. Firstly, EPP is considerably more prevalent among passerines than non-passerines (occurring in 54 of 64 species studied at the time of writing, compared to only 16 of 35 non-passerine species). Secondly, the incidence of EPP is higher and more variable among passerines than non-passerines (18.4% σ = 16.35 versus 7.5% σ = 7.18 Mann-Whitney U-test z = 21.35 p < 0.01).

Table 1. Frequency of extra-pair paternity in different species of birds.
(* = allozyme study, § = less than 50 young sampled, r = range of several studies)

Non-passerines	Percentage	Reference
Common loon	0	Piper et al. 1997§
Shag	17.9	Graves et al. 1992§
Spotted sandpiper	10.8	Oring et al. 1992
Oystercatcher	1.5	Heg et al. 1993
Eurasian dotterel	4.6	Owens et al. 1995§
Pukeko	0	Jamieson et al. 1994

Tasmanian native hen	0	Gibbs *et al.* 1994§
Eurasian moorhen	0	McRae & Burke 1996
Lesser snow goose	2.4	Lank *et al.* 1989*
Barnacle goose	0	Larsson *et al.* 1995
Blue duck	0	Triggs *et al.* 1991§
Leach's storm petrel	0	Mauck *et al.* 1995
Northern fulmar	0	Hunter *et al.* 1992
Short-tailed shearwater	11	Austin & Parkin 1996
Cory's shearwater	0	Swatschek *et al.* 1994
Brown skua	0	Miller *et al.* 1994
Western gull	0	Gilbert *et al.* 1998
Least auklet	0	F. M. Hunter unpub.
Whiskered auklet	11	F. M. Hunter unpub.
Adelie penguin	3	F. M. Hunter unpub.
Erect-crested penguin	3.9	F. M. Hunter unpub.
Willow ptarmigan	9.4	Freeland *et al.* 1995
Australian brush turkey	27.7	S. Birks unpub.
Red-cockaded woodpecker	1.3	Haig *et al.* 1994§
Acorn woodpecker	2.2	Mumme *et al.* 1985*
Eleonora's falcon	0	Swatschek *et al.* 1993
Merlin	0	Warkentin *et al.* 1994
American kestrel	8.3	Villarroel unpub.
Lesser kestrel	3.4	Negro *et al.* 1996
Eurasian kestrel	1.9	Korpimaki *et al.* 1996
Galapagos hawk	0	Faaborg *et al.* 1995
Black vulture	0	Decker *et al.* 1993§
Screech owl	0	Lawless *et al.* 1997
Feral pigeon	0	Lovell-Mansbridge 1995
European Swift	4.5	J. Blakey unpub.

Passerines

European bee-eater	1	Jones *et al.* 1991
House martin	14.5 - 19	r
Barn swallow	22.2 - 30	r§
Purple martin	18.8 - 23.9	r
Pied flycatcher	4.4 - 24	r
Collared flycatcher	15	B. Sheldon unpub.
Tree swallow	51.5 - 68.5	r
Cliff swallow	2	Brown & Brown 1988
Silvereye	0	Robertson 1996
Dusky antbird	0	Fleischer *et al.* 1997
Willow tit	0.9	Orell *et al.* 1997
Blue tit	6 - 11	r
Great tit	3.5 - 18.4	r
Black-capped chickadee	17	Otter *et al.* 1994
Crested tit	12	Lens *et al.* 1997
Bearded tit	14	Hoi & Hoi-Leitner 1997
Black-eared bush tit	0	Bruce *et al.* 1996

Great reed warbler	3.1	Hasselquist *et al.* 1995
Aquatic warbler	36	Schulze-Hagen *et al.* 1993
Henderson reed warbler	6.9	Brooke & Hartley 1995
Wood warbler	0	Gyllensten *et al.* 1990
Hooded warbler	29	Stutchbury *et al.* 1994
Wilson's warbler	32	P. Bereson unpub.
Yellow warbler	37	Yezerinac *et al.* 1995
Sedge warbler	8.3	K. Buchanan unpub.
Superb fairy wren	76	Mulder *et al.* 1994
Splendid fairy-wren	65	Brooker *et al.* 1990*
White browed scrubwren	12	Whittingham *et al.* 1997
Stripe-backed wren	10	Piper *et al.* 1995
Bicolored wren	2.3	Haydock *et al.* 1996
Wheatear	11	Currie *et al.* 1998
Bluethroat	20	Krokene *et al.* 1996
Eastern bluebird	8.4 - 20	r
Western bluebird	16.5	J. Dickinson unpub.
European robin	4	J. Tobias unpub.
New Zealand robin	0	Ardern *et al.* 1997
Jackdaw	0	I.G. Henderson unpub.
Black-billed magpie	5	D. Parrott, 1995
Scrub jay	0	Quinn *et al.* 1990a
Alpine accentor	0	Hartley *et al.* 1995
Dunnock	1	Burke *et al.* 1989
Bull-headed shrike	10.1	Yamagishi *et al.* 1992
Red-backed shrike	5.2	Fornasari *et al.* 1994
Starling	1.1 - 10.2	r§
Noisy miner	6	Poldmaa *et al.* 1995
Chaffinch	17	Sheldon & Burke 1994§
House finch	8.3	Hill *et al.* 1994
Red-winged blackbird	23.7 - 35	r
Northern oriole	32.2	Richardson 1997
Cardinal	14	Ritchison *et al.* 1994§
Bobolink	14.6	Bollinger & Gavin 1991*
Indigo bunting	35	Westneat 1990
Corn bunting	0	Hartley *et al.* 1993*
Smith's longspur	0	J. Briskie unpub.
Reed bunting	52.5	Dixon *et al.* 1994
Yellowhammer	37	Sundberg & Dixon 1996
Savannah sparrow	23	Freeman-Gallant 1996
Field sparrow	14.5	J. Carey unpub.
White-throated sparrow	11	M. Tuttle unpub.
White-crowned sparrow	34	Sherman & Morton 1988*
House sparrow	0.9 - 19	r
Zebra finch	2.4	Birkhead *et al.* 1990*

Table 2. Duplicate studies of EPP used to derive the means used in Table 1.

House martin	19	Whittingham & Lifjeld 1995
	14.5	Riley *et al.* 1995
Barn swallow	22.2	Smith *et al.* 1991§
	30	Primmer *et al.* 1995
Tree swallow	68.5	Dunn & Robertson 1993
	51.5	Lifjeld *et al.* 1993
Purple martin	18.8	Wagner *et al.* 1996
	23.9	Morton *et al.* 1990
Pied flycatcher	24	Gelter & Tegelstrom 1992
	4.4	Lifjeld *et al.* 1991
	7.1	Ellegren *et al.* 1995
	10.8	Ratti *et al.* 1995
	6.1	Ross 1997
Starling	8.7	Smith & von Schantz 1993
	9.7	Pinxten *et al.* 1993
	1.1	Hoffenberg *et al.* 1988
	6.7	Double 1995
	4.9	Double 1995 §
	10.2	Double 1995
Red-winged blackbird	35	Gray 1996
	23.7	Westneat 1993
	25.1	Westneat 1995
	28	Gibbs *et al.* 1990
Eastern bluebird	8.4	Meek *et al.* 1994
	20	Gowaty unpub
Blue tit	11	Kempenaers *et al.* 1992
	6	Gullberg *et al.* 1992
Great tit	14	Blakey 1994
	15	Gullberg *et al.* 1992
	18.4	Lubjuhn *et al.* 1993
	3.5	Verboven & Mateman 1997
Willow warbler	0	Gyllensten *et al.* 1990
	33	Bjørnstad & Lifjeld 1997

4.1.3 Intraspecific variation in EPP

Several of the species listed in Table 1 are represented by an average EPP level, since infidelity rates have been reported from more than one population. The data used to calculate these averages (Table 2) showed a highly significant repeatability ($F_{9,27} = 3.53$ p = 0.005) using a one-way ANOVA after Harper (1994). Hence, there is less variation in EPP rates

within a species than between species, and the use of an average value to represent a species' EPP is valid. (The house sparrow data are not presented since they are discussed in more detail later). It is important to realise, however, that this does not imply each species is genetically programmed to exhibit a specific rate of infidelity. The most parsimonious explanation for the apparent consistency is merely that each species has a conserved nesting ecology with regard to influential parameters such as density, synchrony, and the nature of their mating system, irrespective of geographic location.

4.1.4 Why does the level of EPP vary so much between species?

Several comparative analyses have used this rapidly accumulating data set to unravel the causal factors of interspecific variation in EPP rates (Westneat & Sherman 1997, Owens & Hartley 1998). These techniques test for associations between a species' EPP rate and the biotic and abiotic components of its breeding ecology (e.g. breeding density, habitat features etc) as well as various aspects of the species' life history (e.g. body size, longevity, pair stability, clutch size). Phylogenetic controls are used to remove confounding effects caused by shared ancestry.

Although an exhaustive summary of reviews and comparative analyses is unwarranted here, most authors conclude that a relatively small suite of key parameters account for most of the interspecific variation. The most prominent of these is breeding density (Westneat & Sherman 1997), which itself covaries with other influential factors such as breeding synchrony and territoriality.

4.1.5 The effect of breeding density upon extra-pair paternity

Breeding density is thought to exert a major influence upon the frequency of EPP at both the interspecific and intraspecific level. This follows from the basic assumption that the frequency of conspecific interactions is strongly dependent upon spatial proximity. Since these are more common at high densities, EPCs should also increase with density (Hoogland & Sherman 1976, Hatchwell 1988).

Intensive observational studies of colonial species such as auks and hirundines have generally supported this prediction (reviewed in Birkhead & Møller 1992), although several notable exceptions exist (e.g.

white ibis, Frederick 1987). Furthermore, in an interspecific comparison, EPCs were more frequent in colonial birds that those with a dispersed nesting pattern (Birkhead & Møller 1993).

However, observational work is prone to sampling biases (as discussed above), and this correlation could easily be spurious. For example, colonial species tend to live in open habitats (where EPCs may be more visible), whereas solitary species nest in relatively occluded environments.

In the light of these concerns, Westneat & Sherman (1997) performed a simple pair-wise comparison of local breeding density and EPFs. Although this method differed from that of Birkhead & Møller (1993), and relied upon a different phylogeny, the two analyses were fundamentally similar. The result was unexpectedly ambiguous: density and EPFs were *not* correlated, nor was the relationship even positive. Density appears to affect EPCs and EPFs differently.

4.1.6 The use of EPC data versus EPF data

The conflicting conclusions of these studies probably derive from a different measure of infidelity. Whereas the early analyses used EPCs as their criterion, more recent authors have taken advantage of the burgeoning data on EPFs. In evolutionary terms, it is only EPFs which contribute (positively or negatively) to fitness, and are therefore more accurate measures of infidelity. Unfortunately, the analyses which involve either EPCs or EPFs are difficult to reconcile, since the relationship between the external and internal events is not simple.

In their major review, Birkhead & Møller (1992) reported a positive correlation between the proportion of EPCs and EPFs. However, Dunn & Lifjeld (1994) argued that this correlation was an artefact caused by two outlying data points, corresponding with the dunnock and the splendid fairy wren, neither of which had been correctly represented. Their analysis of the revised rates, supplemented with more recent data, failed to find a significant correlation between the proportion of EPCs and EPFs (Dunn & Lifjeld 1994).

Birkhead & Møller (1995) partly rebuffed these criticisms, and justified their inclusion of polygynous species, since multiple mating promotes

sperm competition irrespective of any social bonds. They nevertheless re-analysed the existing data, after controlling for phylogeny (which had been neglected in both of the previous papers). The significant correlation was upheld, although the authors conceded that it was too weak to have any predictive power. More importantly, there was no correlation between the proportion of EPCs and EPFs when the analysis was restricted to socially monogamous species (Birkhead & Møller 1995).

The discrepancies are caused by several sampling problems, particularly those concerned with habitat features. Because of the potential costs of their partner suspecting that he has been cuckolded, female-solicited EPCs are likely to be surreptitious, and any local cover may be used as a trysting-ground. For this reason, all copulations (within-pair, forced extra-pair and female-solicited extra-pair) will be more noticeable where the breeding habitat is open (e.g. large colonial seabirds nesting on a flat, featureless landscape) rather than occluded (e.g. a passerine bush-nest in a dense woodland). Females often leave their territory to solicit EPCs (Dickinson 1997), in which case focal watches of the nest area would underestimate infidelity.

For EPCs to be adaptive , they should result in at least *some* fertilizations. However, several notable incongruities underline the difficulty in interpreting observational data. The frequency of EPFs was high in the reed bunting (55%), and yet no EPCs were witnessed (Dixon *et al.* 1994). No illegitimate young were detected in the western gull, despite several EPCs being witnessed (L. Gilbert *pers. comm.*).

4.1.7 Female control of paternity

When first documented, EPCs tended to be viewed from the male's perspective. Females were assumed to be relatively passive, with sperm transferred during EPCs and within-pair copulations (WPCs) having an equal chance of fertilizing an egg. Only more recently has it been recognized that females possess a range of anatomical attributes and physiological adaptations which allow them to exert more subtle control over fertilization (Birkhead & Møller 1993, Lifjeld *et al.* 1994, Eberhard 1996).

In a disparate range of birds (most famously waterfowl, but also screamers and ostriches), males possess an intromittent organ which allows sperm to be introduced directly into the vagina (Birkhead & Møller 1992). This ancestral feature has been lost in most species, however (Briskie & Montgomerie 1997), and consequently, a behaviourally successful EPC does not automatically deposit sperm into the female vagina (Hunter *et al.* 1996). To allow sperm uptake, females must evert their vagina into the cloaca. Since this is likely to be under conscious control, females could accept an EPC without eversion, condemning sperm to remain within the cloaca until lost in subsequent defecation. Consequently, successful insemination in most birds probably requires female co-operation.

Even post-insemination, a battery of physio-chemical defences continue to influence 'sperm selection' (Birkhead *et al.* 1993b). The female tract is remarkably hostile to male ejaculates, partly to prevent the spread of contagious diseases (Birkhead *et al.* 1993b), but also to ensure that only the fittest, most motile sperm reach the site of fertilization.

It is this possibility of cryptic female choice (Eberhard 1996), set against a background of internal fertilization, which causes uncertainty of paternity and its associated evolutionary strategies. Ultimately therefore, it is the female which controls extra-pair paternity in birds (Birkhead 1996).

4.1.8 Predictions

The hypotheses discussed above lead to several simple predictions. With regard to the basic breeding parameters, if the opportunity for EPCs depends upon the frequency of conspecific interactions, EPP should be more prevalent at higher densities, and in asynchronous populations.

Male house sparrows possess a 'badge' of black throat feathers which appears to function in mate choice. In captive trials, females preferred to display in front of male mounts with large badges (Møller 1988a, but see Kimball 1996). In free-living populations, large-badged males commence breeding earlier (Møller 1989, Veiga 1993a, Kimball 1995) and rear more offspring (Møller 1989, Kimball 1995, this thesis). This suggests that large-badged males are in some way more attractive or of higher quality than small-badged males.

If females seek genetic benefits by obtaining EPFs from males of a higher quality than their mate, then two predictions emerge concerning badge size and EPP. Large-badged males should be cuckolded less frequently than small-badged males, and large-badged males should be more likely to obtain EPFs. Furthermore, under the original fertility insurance hypothesis (Wetton & Parkin 1991), if females seek EPFs to guard against their partners infertility, there should be a positive association between the incidence of infertile eggs and the presence of EPY.

All of these hypotheses were tested in the house sparrow population established at Maine Chance farms.

4.2   Methods

4.2.1   Number of families analysed in each year

In 1995, blood samples were obtained from 176 nestlings, representing 57 families (although for nine 'families', only the male was known). In 1996, blood samples were available for 359 nestlings, from 98 families (although for 15 'families', only the male was known).

For the paternity analysis, the families were grouped according to their barn of origin and divided between myself and co-workers. My own allocation represented all of the broods collected from barn B in 1995 (n = 26), and all of those collected from barn D in 1996 (n = 28). A single brood from barn D was inadvertently included with my allocation of the 1995 samples. As the male was known at the box of origin, the blood samples from a second brood raised in this box in 1995 were also obtained. The 1995 sample therefore comprised 85 nestlings from barn B, and nine nestlings from barn D. All of the 121 nestlings which formed the 1996 sample were from barn D.

4.2.2   Sample transport and paternity analysis

The samples from the 1995 field season, still in their original TNE buffer, were transported to Leicester on dry ice. Unfortunately, due to a delivery problem, they were completely thawed upon receipt. Nevertheless, DNA

was extracted from around 50 µl of each blood/buffer mix using the phenol/chloroform technique described in Bruford *et al.* (1992), digested with *Mbo*I, and transferred to two nylon membranes by Southern blotting of the electrophoresed fragments (Bruford *et al.* 1992). These were fixed using an UV transilluminator, then radiolabelled using the most reliable of the available single locus probes (c*Pdo*MS14 Hanotte *et al.* 1992). Autoradiography produced scoreable bands in the nestling lanes but, unfortunately, most of the adult lanes were blank or smeary, suggesting that their DNA had become degraded. Unlike nestling blood samples, those taken from adults were not placed into buffer in the field, as plasma samples were required for other analysis. Although all blood samples were held in a coolbox while in the field, several hours often elapsed before they were returned to the laboratory and frozen, during which time the whole blood samples of the adults could conceivably have undergone autolysis.

To circumvent any problems which may have arisen from poor quality DNA, the paternity of all subsequent samples was assessed using microsatellite markers, since this technique requires only a tiny fraction of intact DNA (Ellegren 1992). Microsatellites are short, tandem stretches of nucleotide sequences (usually of only two or three nucleotides), which can be amplified using the polymerase chain reaction (PCR) (Ellegren 1992). Two oligonucleotide 'primers' are designed, which flank the stretch of DNA to be amplified. Double stranded DNA is denatured by heating, and each primer anneals to its complementary strand of DNA. DNA synthesis proceeds in the direction dictated by the primer, and since these are opposite, a complete copy of the target section of DNA is produced. This becomes denatured in the following PCR cycle, and thus serves as its own template for further amplification (Ellegren 1992). Repeated cycling leads to an exponential increase in the amount of DNA present, which eventually becomes sufficient for visualization using a range of methods (Primmer *et al.* 1995, Griffiths *et al.* 1998). The variability in the products visualized is due to differences in the number of repeat units present (Ellegren 1992).

Fortunately, three primer sets which amplify repeat units in house sparrows were already available. Two of these were published (Neumann and Wetton 1996), and a third had recently been designed at Leicester

University (by Mr S. Griffith). These primers had been used during a similar study of parentage in the house sparrow population studied in Norway, (see Chapter 13), and since the PCR conditions are identical, they are not repeated here. PCR products were visualized by the silver staining technique (Bassam *et al.* 1991), which is also discussed fully in Chapter 13.

To avoid any repetition of the 1995 incident, lysis buffer was added to an aliquot of the samples from the 1996 season, which were then transported at room temperature. As the concentration of blood in each sample varied, DNA was extracted using either the phenol/chloroform method, or a chelating resin ('Chelex') (Walsh *et al.* 1991). Although the latter method produced DNA of inferior quality, it proved adequate for PCR.

Samples were run in family groups within individual gels.

4.3 Results

4.3.1 Primer variability and the fallacy of mismatches

A primer pair's utility is limited by the variability of the locus at which it amplifies. For example, if a particular primer amplifies only two alleles, an illegitimate nestling would be relatively difficult to detect, since the extra-pair father would have a probability of one half of also having that allele. If the hypothetical locus was extremely variable however, with over 100 alleles recognized, an illegitimate nestling would be relatively easy to detect, since the probability of the extra-pair father sharing an allele with the social father would be negligible.

This empirical observation was expressed mathematically by Gundel & Reetz (1981), who formulated a series of exclusion probabilities for parentage studies of dogs, based upon the number of alleles detectable at a particular locus, and their spread. The exclusion probability increases with larger litters between the range 1 - 5 (Gundel & Reetz 1981), which is conveniently comparable to the size of house sparrow broods (average brood size c3.5). Gundel & Reetz's (1981) formulae were translated (courtesy of Mr E. Bell) into a user-friendly computer program which generated the probabilities of false paternal inclusion from a given frequency distribution of alleles. The number of alleles detectable at each

locus was determined by electrophoresis of 14 random adults, to produce the probabilities shown in Table 3.

The probability of false paternal inclusion (*fpati*) is thus the probability of assigning a nestling as within-pair when it has actually been sired by an extra-pair male. The inverse probability, false paternal exclusion (calculated as 1 - *fpati*), is thus the probability of designating a nestling as resulting from an extra-pair fertilization, when in fact the mating was legitimate.

Although more advanced statistical packages are available for these calculations (e.g. Cervus), these are primarily used in larger studies where the gentotypes of most, if not all, adults in the population are known. These packages calculate the probability of a particular individual being the correct parent, based on their genotype and the proportion of other individuals in the population sharing one or both of their alleles. Consequently, these packages are more useful for assigning parentage, rather than the simple exclusion required in this study (T. Burke, *pers. comm.*).

Table 3. Characteristics of the three microsatellite loci used in this study.

Locus	Alleles detected	P (*fpati*)
*Pdo*μ3	10	0.233
*Pdo*μ4	18	0.146
*Pdo*μ5	16	0.129
combined		0.004

The number of alleles detected at each locus is similar to that detected in other house sparrow populations (S. Griffith, *pers. comm.*), although the number of alleles at*Pdo*μ4 is considerably less than reported in the original paper (number of alleles = 37, P(*fpati*) = 0.087) (Neumann & Wetton 1996). However, the degree of amplification at this locus is very sensitive to changes in PCR conditions (Neumann & Wetton 1996), with several alleles failing to amplify if the annealing step is altered by only a single degree celsius (Neumann & Wetton 1996). Since the conditions which had been optimized for *Pdo*μ4 at Leicester were different to those reported in

the original paper (Neumann & Wetton 1996), this presumably resulted in the loss of several alleles.

Unfortunately, loci which are more variable are by definition more unstable and, as expected, $Pdo\mu4$ has a high frequency of mutation (> 2%) (Neumann & Wetton 1996). A nestling may thus possess a mutant allele at this locus which is not present in one or both parents. Because of this, nestlings should not be designated as extra-pair based on genetic incompatibility at this locus alone. Therefore, nestlings had to be mismatched at at least two of the three loci to be classed as illegitimate. This pattern was usually evidenced by a mismatch at the two variable loci ($Pdo\mu4$ and $Pdo\mu5$), and apparent inclusion at the least variable locus ($Pdo\mu3$). The probability of incorrectly detecting an extra-pair offspring (EPO) with this sequence of mismatches is given by $1 \times 0.146 \times 0.129 = 0.018$ (i.e. under 2% of nestlings assumed to be illegitimate are in fact within-pair). The probability of incorrectly assigning a nestling as an EPO based on a mismatch at all three loci is extremely low (given by $0.233 \times 0.146 \times 0.129 = 0.004$). The EPO which were detected at only two loci should ideally be confirmed by using a fourth microsatellite locus.

4.3.2 Extra-pair paternity in 1995

Sixteen of the 94 nestlings sampled mismatched with one or both of their parents at one or more loci. One nestling mismatched with the mother at the least variable locus, and did not match either parent at the other two loci. This nestlings was therefore assumed to have resulted from intra-specific brood parasitism (ISBP) (Petrie & Møller 1991), and as this does not represent infidelity *per se*, it was deleted from further calculations. This level of ISBP was also too low to allow further investigation.

Nine nestlings were mismatched at all three loci and were thus almost certainly extra-pair offspring. This gives a minimum estimate of the EPF rate as 9.7% (compared to the maximal estimate of 16.1%). However, using the pre-ordained criterion of mismatches at at least two loci, 13 of 93 nestlings were classified as illegitimate, which gives an overall EPF rate of 14.0%. Illegitimate young occurred in 11 of 28 broods (39.3%).

4.3.3 Extra-pair paternity in 1996

In 1996, the data set included 11 samples taken from hatchlings which later died (seven attributed to brood reduction, and an entire brood of four nestlings taken by a predator).

Seven of the broods collected in 1996 (29 nestlings) were totally mismatched with their presumed father (but not their mother) and had to be discarded. One of these broods was attributed to misread colour rings. The six other discarded broods had been collected from two nests where the previously unringed males were not trapped until late in the season. Although each of these males was the genetic father of the nestlings in the final brood, they were mismatched with those in the prior broods. It is not impossible that these males had been completely cuckolded in their earlier broods, but the more parsimonious explanation is simply that the male had changed, particularly since divorces and disappearances were more prevalent near the end of the season (*pers. obs.*). At the other nests studied, most of the males involved were caught and colour ringed near the start of the season.

Using the criteria of at least two mismatches to designate infidelity, six of the remaining 92 nestlings were classified as illegitimate, giving an overall figure of 6.5 % extra-pair offspring. These occurred in five of 28 broods (17.9 %).

4.3.4 Extra-pair paternity levels pooled from both years

Combining results from both seasons, 19 of 185 nestlings were illegitimate, which corresponds with 10.3% EPO. Illegitimate young occurred in 16 of 56 broods sampled (28.6%).

If, however, the 29 mismatched nestlings in 1996 had indeed resulted from complete cuckoldry, then the upper limit of EPP would be 25.9% of nestlings (48/185) and 41% of broods (23/56). As explained above however, this circumstance is considered unlikely, although not impossible.

4.3.5 Breeding density and extra-pair paternity

The average nearest neighbour distance was calculated differently at each barn. At barn B, the boxes were distributed evenly over all four sides, with two boxes being slightly offset (Chapter 1). Since paternity data were available from all boxes, the breeding density was simply expressed as the combined length of all four sides divided by the number of boxes. The average nearest neighbour distance at barn B was calculated as 15 metres.

At barn D, most of the boxes used during the season were evenly spaced along the front side, with a smaller cluster at the rear. Only one successful brood was produced from the three sporadically placed side boxes, at which the male was not caught. Therefore, only the front and rear were used when calculating nearest neighbour distances, producing an average figure of 1.5m.

The EPP rates used in the comparison were restricted to the barn B samples in 1995, and the barn D samples in 1996. The two broods taken from barn D in 1995 were discounted to avoid any confounding effect of year (although in actuality, as this would have contributed only one EPO from nine nestlings sampled, the overall barn D rate would not have been altered significantly).

The EPF rate at barn B was higher than that at barn D, although this was not significant (χ^2 = 2.79 df = 1 p = 0.095). There was again no significant difference when the comparison was repeated on a per-brood basis (χ^2 = 3.15 df = 1 p = 0.076), although more broods contained EPFs at the lower density.

4.3.6 Extra-pair paternity and breeding synchrony

A breeding synchrony index (BSI) was calculated for the date of initiation of each successful brood (i.e. the FED) using the equation given by Kempenaers (1993):

BSI = number of females fertile on day i / total number of females

The fertile period of house sparrows was assumed to commence on day -5 (with day 0 = FED) and extend until the day on which the penultimate egg was laid. This is a conservative estimate of the fertile period of a typical passerine (Birkhead & Møller 1992), and was used by Møller (1987c) in a previous study of house sparrows. Calculation of the synchrony index at barn D required particular care, since the number of breeding pairs declined steadily throughout the season.

The BSI at barn B in 1995 varied between 0.13 and 0.63, and averaged 0.26. The degree of breeding synchrony at clutch initiation did not differ between broods containing extra-pair young and broods fathered entirely by the social male ($t = 0.449$ df = 24 NS).

The BSI at barn D in 1996 ranged between 0.08 and 0.67, and averaged 0.36. Again, the degree of breeding synchrony was unrelated to the presence of extra-pair young ($t = 1.04$ df = 27 NS).

Breeding synchrony was higher at barn D than barn B (Figure 1), although this was not significant (Mann-Whitney U -test $z = 3.39$ p = 0.066). However, since the overall breeding density was considerably higher at barn D, this difference was not unexpected. Table 4 summarizes the synchrony, density and extra-pair paternity at each barn (NND = nearest neighbour distance, BSI = breeding synchrony index, EPP = extra-pair paternity).

Figure 1. Comparison of breeding synchrony between barns.

(mean + SE)
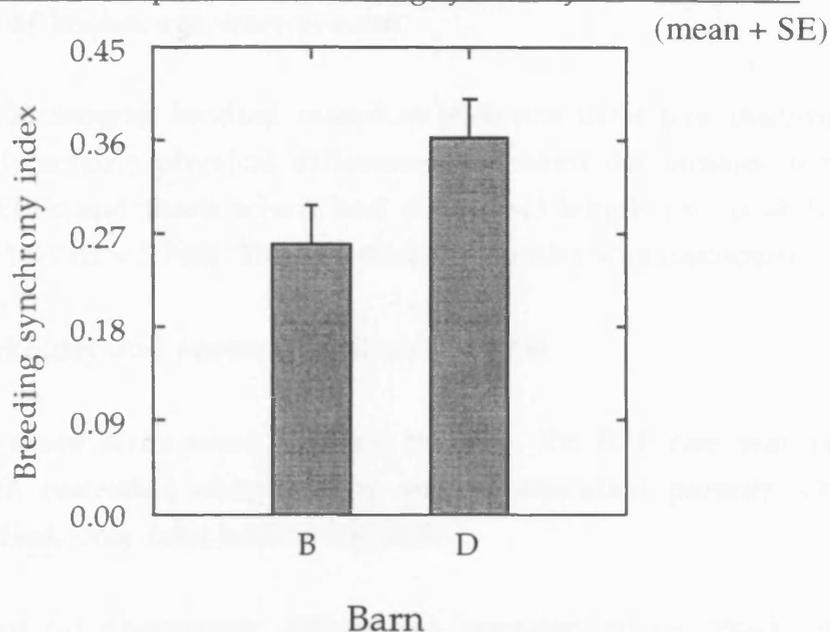
Table 4. Nearest-neighbour distance (NND), breeding synchrony index (BSI) and extra-pair paternity (EPP) between the two study barns

	Year	NND	BSI	% EPP
Barn B	1995	15	0.260	14.3
Barn D	1996	1.5	0.364	6.5

4.3.7 Cuckoldry and parental attributes in 1995

Eight of the 12 males studied in 1995 had been cuckolded at least once. Although all birds at the barn were colour ringed, three had been ringed in a previous season but were not retrapped in 1995. Hence, although they were individually recognizable, their physical dimensions and badge size were not known. This meant that only limited phenotypic comparisons could be made between the eight cuckolded males and the four which had complete paternity within their nests.

Cuckoldry was independent of the social male's badge size and structural size as measured by tarsal length ($t = 1.31$ df = 6 NS, $t = 0.458$ df = 6 NS). Males which were cuckolded were significantly heavier than those with complete paternity ($t = - 2.66$ df = 6 p = 0.045), although this was not a convincing result since the mass of only two birds from the latter category

was known. The effect of age upon cuckoldry could not be investigated as few males of known age were present.

Six of the 11 females studied reared at least one extra-pair nestling. There were no detectable physical differences between the females which had obtained EPFs and those which had not (tarsal length t = - 0.34 df = 6 NS, mass t = - 1.61 df = 5 NS). The age of most females was unknown.

4.3.8 Cuckoldry and parental attributes in 1996

Although more birds were trapped in 1996, the EPF rate was relatively low, which restricted comparisons among attendant parents. Of the 12 males studied, only four were cuckolded.

There were no phenotypic differences between males which had been cuckolded and those which had achieved complete paternity within their nests (badge size t = 0.865, tarsal length Mann-Whitney U = 10.0, mass t = 0.318, body condition t = 0.551 , all df = 6 NS). There was slight evidence of an age-related component to cuckoldry. Three of the four definite adults had been cuckolded, compared to only one of the three juveniles, although these sample sizes are very low.

Four of the twelve females had sought extra-pair matings as adjudged by the presence of extra-pair offspring in their nests. There were no phenotypic differences between females of either category (tarsal length t = - 0.278, mass t = 0.855, body condition t = - 0.817 df = 5 NS). Only four females of known age were present at barn D in 1996 (two adults and two juveniles), although coincidentally, these were the four females which had sought EPFs.

4.3.9 Perceived versus realized fledging success

In both years, merely counting the number of fledglings each male produced from his own nest represented a significant underestimate of his actual reproductive success (1995 one-tailed t = 3.55 df = 10 p = 0.001; 1996 one-tailed t = 1.82 df = 11 p = 0.045, one-tailed tests used since reproductive success cannot be overestimated by this method). Unfortunately, the sires

of the extra-pair offspring have yet to be identified and thus the true range of reproductive success is unknown.

4.3.10 Infertility and cuckoldry

There was no significant association, in either year of the study, between the presence of extra-pair young in a brood and the occurrence of infertile egg(s) (1995 $\chi^2 = 0.088$ df = 1 NS ; 1996 $\chi^2 = 0.004$ df = 1 NS,).

4.4   Discussion

4.4.1   EPP in the house sparrow : a comparison with other species

As shown in Figure 2, most passerine populations contain between 0 - 20 % extra-pair young (EPY), with an average of 18.4%. The EPP level at Maine Chance (10.3%) was thus slightly lower than the overall average, but similar to that observed in many other passerines. EPP levels of non-passerines have not been included in Figure 2, since several aspects of their biology may either preclude infidelity (such as the extremely low breeding densities of vultures (Decker *et al.* 1993), or misrepresent 'female choice' (such as forced EPCs by drake waterfowl, McKinney *et al.* 1983).

Figure 2. The incidence of EPP in passerine birds.

71
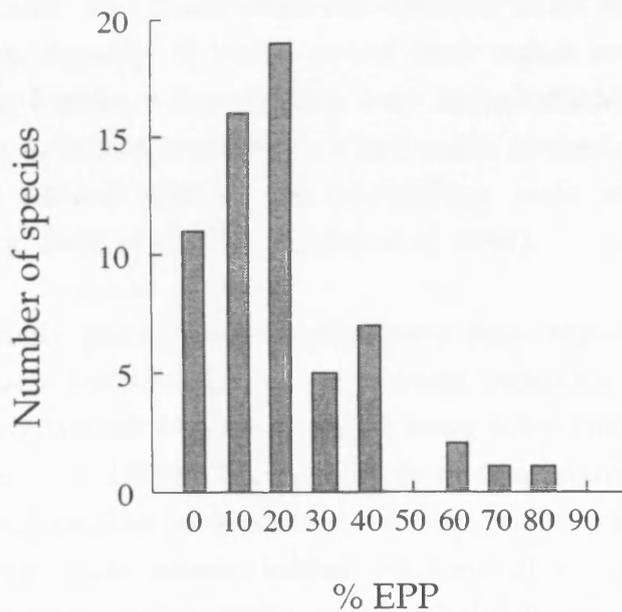
4.4.2 Cuckoldry and parental attributes

No phenotypic differences were detected between cuckolded and non-cuckolded males (although the sample sizes for many tests were small). Males with either small or large badges were equally likely to be cuckolded, as was found by R. Whitekiller & D. F. Westneat (*pers. comm.*) and Cordero *et al.* (MS). Cuckoldry was independent of the host male's age, as reported in a British house sparrow population (Wetton *et al.* 1995).

Few studies have attributed cuckoldry to male phenotype unambiguously, since phenotype may be age-related. Many of the clearest patterns of cuckoldry have been reported from species exhibiting delayed plumage maturation (DPM), in which yearling males have a female-like subadult plumage (Richardson 1997). These subadult males were more likely to be cuckolded than old males in northern orioles (Richardson 1997) and purple martins (Morton *et al.* 1990).

The likelihood of cuckoldry may be more strongly influenced by the proximity of rivals rather than a male's phenotype since, in several territorial passerines, males were most frequently cuckolded by their neighbours (e.g. Kempenaers *et al.* 1992, Venier *et al.* 1993, Ross 1997). Neighbouring males are more likely to be aware of a temporary absence of the resident male, and could enter his territory in an attempt to gain EPCs with his mate. Equally, if males guard their mates intensively with only short foraging breaks, a female may only have sufficient time to solicit an EPC from an adjacent male before her mate returns. Despite these two scenarios, in several species the cuckolding male was not the nearest neighbour (e.g. Ratti *et al.* 1995, Currie *et al.* 1998).

Several distinctly paradoxical results have been reported. Male northern cardinals which were bright red (an honest indicator of superior dietary carotenoid acquisition) were cuckolded more often than those which were dull (Linville *et al.* 1998). Black pied flycatcher males, which have been shown to be attractive to females in aviary trials, were cuckolded more frequently than pale brown males (Lifjeld *et al.* 1997). In the indigo bunting, younger males were more likely to be cuckolded in one population, whereas older males were cuckolded more frequently in another (Westneat 1987).

Arguably the more important result, which has yet to be elucidated at Maine Chance, is the characteristic(s) of the males which gain from EPP. Although direct benefits of EPCs have been recorded in other species (Gray 1997), female house sparrows presumably receive only sperm from extra-pair matings. Unless females pursue EPFs from random males to increase the genetic diversity of their brood (which seems unlikely), the males from which EPFs are solicited must be of high genetic quality or fertility. The physical attributes of these preferred males would identify the direction of sexual selection in the species concerned.

Only a small proportion of the studies documenting EPP have managed to identify the cuckolding males. This is not surprising, since the technique usually used (multi-locus DNA fingerprinting) relies upon parental *exclusion*, and is therefore of limited use in identifying true fathers. Few unambiguous correlates of the successful males' characteristics have emerged, even in well studied birds such as the pied flycatcher (Lifjeld *et al.* 1997). In several species, older males were more likely to achieve EPFs, such as the purple martin (Morton *et al.* 1990), house sparrow (Wetton *et al.* 1995), yellowhammer (Sundberg & Dixon 1996) and northern oriole (Richardson 1997). However, the martin and oriole examples are confounded by DPM, in which two phenotypic categories of males are present (Richardson 1997). In other species, older males were not more successful in gaining EPFs, such as in the tree swallow (Dunn *et al.* 1994), reed bunting (Dixon *et al.* 1994), red-winged blackbird (Westneat 1995), great reed warbler (Hasselquist *et al.* 1996) and pied flycatcher (Ross 1997).

Since age (i.e. survival) represents an unusually unambiguous demonstration of genetic quality, a female preference for older males as extra-pair sires is not surprising. Males of several species possess secondary sexual characters which appear to increase with age, and could thus act as signals of quality. Adult house sparrows have larger badges than yearlings (Veiga 1993a, Griffith 1998, although not in this study), since nutritional constraints limit the younger males from expressing a full badge (Veiga & Puerta 1996). A similar effect may account for the subadult plumage of yearling males in species displaying DPM (Richardson 1997). Pied flycatcher males darken with age (Ross 1997), and yellowhammer cocks become increasingly yellow as they age due to the accumulation of dietary

carotenoids (Sundberg 1995). Plumage redness does not change with age in house finches however (Hill 1990), and other classic secondary sexual characters, such as the peacock's train (Petrie & Halliday 1994) and the barn swallows tail (Møller 1994b), do not increase with age. The characteristics of successful sires in these species are thus far unknown, although age may not therefore be important.

No studies have managed to associate female phenotype with cuckoldry (see Ross 1997), although the predictions for this sex are more convoluted. All male birds presumably seek EPFs to a variable extent, although only some are successful. In contrast, not all females are expected to solicit EPFs, since only those which are paired to low quality males, or are of low quality themselves, would benefit. Female quality is difficult to define, however, since plumage variation is minimal in most hen birds, and does not change with age.

Although I have assumed throughout this chapter that all EPFs were actively solicited, this may be overstating female control. Multi-male 'communal chases' did occur at Maine Chance (*pers. obs.*), and although I did not witness any of these chases resulting in successful copulation, a co-worker observed a single female being inseminated by at least two males (T. B. Rambo *pers. comm.*). EPFs which result from forced copulations clearly do not represent female 'choice', since the extra-pair sires may merely be the most aggressive males rather than the most attractive (Møller 1987b, c). As was discussed earlier however, it is difficult to believe that female sparrows could be inseminated unwillingly through a forced EPC.

Nevertheless, it seems reasonable to expect that cuckolded male sparrows were of relatively low quality. Even if the EPFs had resulted from forced copulations, the male has obviously failed to defend his mate from the unwelcome attention of other males (Møller 1987c). Equally, the 'constrained female' hypothesis (Gowaty 1996b) suggests that females would only seek EPFs if paired to an inferior male. However, this study found no phenotypic differences between the cuckolded and non-cuckolded males.

4.4.3 Breeding density and EPP

The most interesting finding from the genetic data, albeit not significant, was an unexpected *inverse* relationship between breeding density and EPP. Although density had no effect upon EPP in tree swallows (Dunn *et al.* 1994) or in a Spanish house sparrow population (Cordero *et al.* MS), a positive relationship between density and EPP has been found in red-winged blackbirds (Gibbs *et al.* 1990), eastern bluebirds (Gowaty & Bridges 1991), European starlings (Double 1995), bearded tits (Hoi & Hoi-Leitner 1997) and northern orioles (Richardson 1997).

These studies followed the logic of interspecific comparisons in assuming that conspecific interactions increase as density increases (Birkhead & Møller 1992), thereby providing greater opportunities for males to achieve EPCs. However, one could argue that EPCs/EPFs would actually be *lower* at high densities, since males would respond to the increased threat of cuckoldry by intensifying their paternity assurance behaviours (mate guarding and/or frequent copulation). Unfortunately, insufficient data were available to compare the intensities of these behaviours at each density. Nevertheless, this illustrates the difficulty of making predictions without knowing the balance of power exerted by each sex (Parrott 1995).

One of the first reports of a positive association between density and EPCs concerned the guillemot (Hatchwell 1988). Ironically, a study of the closely related razorbill stimulated Wagner (1993) to consider breeding colonies from a fresh perspective: that of the female. Hence, the 'EPC hypothesis' proposes that colonial nesting is a female-driven phenomenon (Wagner 1993) since it increases the opportunities for EPCs with local males. Northern orioles exhibit two nesting distributions, 'clumped' and 'solitary' (Richardson 1997). Average EPP was significantly higher in pairs nesting in clumps than pairs which nested in relative isolation (Richardson 1997). The EPC hypothesis (Wagner 1993), which also predicts a positive relationship between density and EPP, was not supported at Maine Chance. Female house sparrows did not achieve more EPFs at higher densities, although it is unknown whether this reflects fewer solicitations or more attentive paternity guards.

4.4.4 Breeding synchrony and extra-pair paternity

At Maine Chance, the frequency of EPFs was unrelated to local breeding synchrony. This was unexpected, since synchrony (i.e. the overlap of the females' fertile periods) is theoretically an important determinant of EPP since it regulates the operational sex ratio (Emlen & Oring 1977).

However, as is true of theories positing a relationship between the frequency of EPP and breeding density (to which synchrony is surely contingent), one could make opposite predictions. Synchrony was originally thought to decrease opportunities for EPCs (Birkhead & Biggins 1987), since males cannot simultaneously guard their mates and pursue EPCs. Indeed, by breeding synchronously, females could impose monogamy on their mates by 'forcing' them to guard (Ims 1990, Birkhead & Møller 1992). More recently, however, Stutchbury & Morton (1994) have argued that in species where mate guarding is weak, synchronous breeding may actually *increase* the opportunities for EPP, since many females are fertile simultaneously.

Data which relate EPP to clear differences in synchrony are relatively sparse (Westneat *et al.* 1990). Synchrony did not affect EPP in the northern oriole (Richardson 1997), pied flycatcher (Ross 1997) or house sparrow (Cordero *et al.* MS), although EPP tended to increase with synchrony in the black-billed magpie (Parrott 1995). In a comparative analysis, Stutchbury & Morton (1995) concluded that EPP in songbirds was more prevalent (on a per-brood basis) in synchronous populations. Their correlation was not significant once the tropical songbirds which breed year-round were removed, although a positive trend remained.

4.4.5 Unhatched eggs and extra-pair fertilizations

There was no association between infertility and cuckoldry in both years of the study, and hence no support for the fertility insurance hypothesis (Wetton & Parkin 1991). There was no such association in a separate population of house sparrows (Cordero *et al.* 1998), and to my knowledge, the findings of the original study have not been repeated in any species. Birkhead & Møller (1992) have argued convincingly that if females seek EPCs to guard against the risk of their partner's infidelity, then one would

predict a *negative* association between infertile eggs and the presence of extra-pair young.

Infertility *per se* is probably rare in birds (Birkhead & Møller 1992) since it would be under strong negative selection. However, infertile eggs could result from low sperm counts or sperm depletion, particularly in birds such as the house sparrow, where pairs may perform over 50 copulations in a single day (Chapter 7). If sperm reserves become depleted through frequent copulation, then one would predict that eggs laid later in the sequence are more likely to be infertile. Veiga (1990) and Cordero *et al.* (1998) actually found the opposite pattern in house sparrows, with the first-laid eggs most likely to be infertile (although there was no positional bias at Maine Chance [Chapter 5]). These first-laid eggs were assumed to be inviable because of delayed incubation (Veiga & Viñuela 1993).

Male house sparrows have fewer sperm in their seminal glomera as the day proceeds, which suggests that frequent copulation may result in sperm depletion (Birkhead *et al.* 1994). However, since the seminal glomera apparently function as temporary sperm stores which are replenished each night, sperm depletion, if it occurs at all, would only occur on a daily basis. Moreover, microscopic techniques are required to designate eggs as being genuinely infertile. By staining sperm nuclei present on the ovum wall of 'infertile' house sparrow eggs, Birkhead *et al.* (1995) found that most (67%) had actually been fertilized, but had suffered from very early embryonic mortality. The reason for this was unclear, and it was unknown whether the embryo had resulted from a within-pair fertilization or an EPF. (This may be determined in future studies by utilizing the sensitivity of the polymerase chain reaction).

As found in other house sparrow populations (e.g. Seel 1968b, Dawson 1972), a relatively large proportion of eggs failed to hatch (*c*12%, Chapter 1). High failure rates tend to be associated with colonial nesting and/or a complex mating system (e.g. co-operative breeding, polygynandry) (Koenig 1982). Lifjeld (1994) argued that these patterns were the result of forced mating attempts and harassment, which are relatively frequent in both of these situations. With regard to the house sparrow in particular, 'communal chases' (involving the pursuit of a fertile female by several males) are a well-described aspect of the species' biology (Summers-Smith

1963) (Chapter 7). Lifjeld (1994) suggested that females suffer (undefined) internal stresses during these frequent chases, particularly if they are followed by forced EPCs, and that these stresses result in the production of infertile eggs (or, in retrospect, the early death of fertilized embryos). Although this is a plausible explanation, communal chases were not common at Maine Chance (Chapter 7), whereas many clutches contained at least one unhatched egg (Chapter 2). A similar inequality concerning the seasonal distribution of chases and unhatched eggs had been observed in the population studied by Wetton & Parkin (quoted in Birkhead *et al.* 1995). As a more general concern, Lifjeld's (1995) hypothesis suggests that communal chases would be maladaptive, since they would not result in a fertilized egg (on the day of insemination at least).

Birds' eggs fail to hatch for a variety of reasons other than infertility and early embryonic mortality (Koenig 1982). The underlying causes of the failures are poorly understood, particularly in the case of embryos which die only a few days before hatching (although this may be caused by bacterial invasion [Pinowski *et al.* 1994]). The use of multi-locus DNA fingerprinting in paternity analyses enabled researchers to test a long-standing hypothesis : egg failure results from the deleterious effects of inbreeding (Romanoff 1960). Although this was not supported in blue tits (Kempenaers *et al.* 1996), in other species closely related pairs produced a higher proportion of unhatched eggs than unrelated pairs (e.g. great reed warbler, Bensch *et al.* 1994). Although the relatedness of house sparrow pairs at Maine Chance was not assessed, they were unlikely to have been inbred, since a very low proportion of natal birds returned to breed at the study site (10 / 423 nestlings ringed = 2.3 %).

To conclude, a relatively large proportion of house sparrow eggs failed to hatch (*c*12%), for unknown reasons. Most unhatched eggs showed no signs of development, although it was impossible to know whether these had been fertilized. There was no support for Wetton & Parkin's (1991) fertility insurance hypothesis, nor for Lifjeld's (1994) notion of maternal harassment.

4.4.6   Is the EPP rate an artefact of the sampling regime?

In several broods, the small, last-hatched nestling died before blood samples could be obtained. This may have led to an underestimate of the frequency of EPP, since in the house martin, extra-pair young tended to hatch from the final egg (Riley *et al.* 1995). A similar effect was reported (albeit from a very low sample size) in cattle egrets (J. Geig, *pers. comm.*) where frequent fratricide reduces the fledgling probability of late-hatched young.

Observational evidence from other species suggests that extra-pair young are more likely to occur in the later eggs of a clutch, since males appear to relax their paternity assurance behaviours while their partners are still fertile (Birkhead & Møller 1994). Mate guarding stops completely once laying commences in several species (Birkhead & Møller 1992) including the house martin cited above (Riley *et al.* 1995). The curious decline in mate guarding intensity and/or copulation frequency suggests that the outcome of sperm competition will become biased towards EPCs as the breeding cycle progresses. However, although red-winged blackbirds showed a similar pattern of decline, this was not associated with the pattern of cuckoldry (Westneat 1995).

Unfortunately, a positional bias in EPFs could not be demonstrated in this study, as although the laying sequence of most clutches was known, hatchlings could not be matched to their eggs. There was no *a priori* reason to expect EPFs to show a positional bias at Maine Chance, since the twin paternity assurance behaviours did not exhibit the marked patterns described in other species. Mate guarding was only weakly developed throughout the female cycle, and the copulation frequency remained relatively high, even during the laying period (Chapter 6; also Møller 1987c).

In this study, and those of all other passerines, the major obstacle in determining whether EPFs have a positional bias is the synchrony of hatching (particularly of the nestlings which survive until processing) (Stenning 1997). To circumvent this, Cordero *et al.* (1998) numbered house sparrow eggs as they were laid, then removed the entire clutches mid-incubation. Paternity analysis using embryonic DNA uncovered an

obvious positional bias, with extra-pair young tending to occur in the earlier eggs of a clutch (Cordero *et al.* 1998).

Cordero *et al.* (1998) did not witness any EPCs, and hence their timing relative to EPFs is unknown. Although no EPCs were observed at Maine Chance, the frequency of within-pair copulations peaked on the day the first egg was laid (Chapter 6), implying that extra-pair copulations might be less frequent at this time. If the EPFs detected at Maine Chance were also derived from the early eggs of the clutch (which would not be unexpected), then this would present a paradoxical pattern of cuckoldry.

The peak in copulation frequency on the first egg date is probably in response to the first reliable cue that females have commenced their laying cycle. On the preceding evening, this egg is known to weigh around 70% of its eventual mass (Schifferli 1979), and presumably impairs the flight performance of its bearer. This would alert a female's mate and any rival male(s) that she was in her laying cycle, and result in a flurry of coition the following morning to coincide with her insemination window (Beecher & Beecher 1979). Rival males are not wholly reliant upon this cue, however, since several first-laid eggs have been shown to result from EPFs (Cordero *et al.* 1998).

Females which have solicited extra-pair matings to gain a fitness benefit would gain most if these fertilized the earlier eggs in the clutch. Hatching asynchrony and brood reduction are common in house sparrows (Chapter 2) and, consequently, the later laid eggs have a lower probability of translating into a successful fledgling (Cordero *et al.* 1998). In contrast, nestling losses are relatively minor in the house martin (O'Connor 1978), and extra-pair young would still have a high probability of survival despite their apparent preponderance in last-laid eggs (Riley *et al.* 1995).

To conclude, it seems unlikely that sampling biases affected the EPP estimates at Maine Chance. If extra-pair young were clustered among the first hatched young, as reported by Cordero *et al.* (1998), then they would have been detected, irrespective of brood reduction.

4.4.7   EPP at Maine Chance : a comparison with other studies

Population	n	% EPY	Reference
Barcelona	106	10.4	Cordero et al. 1998
Helgeland	208	3.9	this thesis
Kentucky	185	10.3	this thesis
Lundy Island	305	0.9	Griffith 1998
New Mexico	55	12.7	Kimball 1995
Nottingham	536	13.6	Wetton & Parkin 1991
Oklahoma	56	19.0	R. Whitekiller pers. comm.
Villalba	171	7.0	J. Veiga & L. Boto pers.

The house sparrow is unquestionably one of the world's most thoroughly studied birds (Summers-Smith 1988). It is therefore unsurprising that EPP rates are now known from more populations of house sparrows than any other species, with over 1,500 nestlings analysed. The results from these eight populations (listed in Table 5) were all obtained using DNA techniques (mini- or microsatellites), and are thus directly comparable.

The overall level of EPP at Maine Chance (10.3%) is similar to that reported from the other populations. Even if the results from different years were used separately (14% and 6.5%), the congruence would persist. EPP levels do not vary substantially between the different populations, with the exception of the remarkably low incidence reported from Lundy Island (Griffith 1998). The relatively high level of EPP recorded in the Oklahoma population may be an artefact of the small sample size.

Table 5. Levels of EPP in eight populations of the house sparrow.

4.4.8   Differences in EPP rates between populations

Relatively little   data are available on intraspecific variation in EPP (Gowaty 1996b), since (understandably) studies of a particular species' infidelity are rarely repeated elsewhere. The comparative data which do exist have often resulted from parallel population studies carried out by

unknowing research groups, or transient ornithologists with an inordinate fondness for a particular bird (e.g. Westneat 1993, 1995).

In most species which have been studied in more than one population, the extent of infidelity appears to be conserved. Møller (MS) reported that EPP rates from 18 such species were highly repeatable (68%), although the data were not presented. In several birds, however, the level of EPP obviously does differ between populations (e.g. 0% vs 33% in two populations of the willow warbler (Gyllensten *et al.* 1990, Bjørnstad & Lifjeld 1997)).

The factors which underlie intraspecific variation in EPP are poorly understood (Petrie & Kempenaers 1998). Emlen & Oring's (1977) classic paper on the evolution of mating systems considered that the environmental potential for polygyny varied between sites due to the spatial distribution of environmental factors. These abiotic and biotic factors (e.g. nest sites, food) may also apply to the potential for extra-pair paternity. For example, the natural distribution of suitable nest sites dictates breeding density (and indirectly, synchrony). The scarcity or abundance of food influences the trade-off between foraging and mate guarding (Westneat 1994).

Several of Emlen & Oring's (1977) environmental factors do not apply to nest-box populations of house sparrows. Practically all pairs are monogamous, territories are not defended, and the conditions within one nest-box are probably much the same as in another. Since house sparrows are remarkably adaptable, it is difficult to believe that the food sources are distributed differently at each site. Several other factors have been proposed which are applicable to house sparrows, however.

(i)   Differences in population structure

Identifying demographic differences between the house sparrow populations listed in Table 3 would be extremely difficult. Immigration, dispersal, predation and overwinter mortality are probably extensive in all of these populations (except Lundy and, to a lesser extent, Helgeland). Since breeding adults cannot be aged satisfactorily (Nero 1951), it would be difficult to know the population age-structure and pair fidelity without intensive monitoring of surrounding sites.

(ii)   Different manifestations of sexual selection

Since the pursuit of EPFs is a component of sexual selection, intraspecific variation in EPP may result from the focus of sexual selection differing between populations. Female pied flycatchers prefer male characteristics in Norway (Lifjeld *et al.* 1997, Lampe & Sætre 1995) whereas they prefer territory characteristics in Sweden (Alatalo *et al.* 1986). In the latter situation, female reproductive success may largely depend upon physical benefits such as food availability, and there would be no incentive to pursue EPFs.

Females may seek EPFs to obtain disease resistance genes for their offspring (Hamilton & Zuk 1982). In the pied flycatcher and the house sparrow, the incidence of blood parasitism varies between locations (Bennett *et al.* 1995, Chapter 11). Disease resistance genes will be more valuable in a population where parasitism is rife than in a population where parasites are rare or absent. Several island species are considerably less bright than their mainland equivalent, such as the Azores bullfinch (Lack 1976). One theory suggests that parasites are rare on the island and hence the intensity of sexual selection has decreased (M. Cant *pers. comm.*).

(iii)   Intraspecific variation in breeding density

Extra-pair paternity tends to increase with density within a species (Westneat & Sherman 1997, Section 4.1). Unfortunately, the breeding density was not known for half of the studies listed in Table 3, and thus a simple comparison was not possible. However, since none of these reports made explicit reference to the local density being particularly high or low, each population probably contained the spread of densities which arise as an automatic consequence of distributing boxes around a variety of farm buildings.

In practice, uncovering a relationship between population densities and EPP would be difficult, since many subtleties would be obscured in the comparison. For example, the EPP level at Maine Chance differed according to the local breeding density. If similar differences exist elsewhere, then representing each site with a single, average figure for

both density and EPP would be misleading. This is particularly pertinent in the current example, since the EPP levels did not differ dramatically between sites, other than being rare on Lundy Island. The range of densities in the Lundy population (S. Griffith *pers. comm.*) was comparable to that experienced in Kentucky, which suggests that density *per se* does not account for the intraspecific variation in EPP in house sparrows.

(iv)   Differences in genetic variation

If females are gaining only genetic benefits from EPFs (Chapter 1), several authors have suggested that the extent of EPP depends upon a female's ability to obtain genes of a higher quality than those of her mate (Birkhead & Møller 1992, Petrie & Kempenaers 1998). This in turn depends upon the genetic differences within the population. If males vary only marginally in their genetic quality (as is often presumed to happen in real or habitat islands) females have no incentive to pursue EPFs.

This hypothesis predicts that the level of EPP in a particular population will correlate positively with the magnitude of genetic variation. Møller (MS) supported this prediction in seven passerine species where data were available on both EPP and genetic variability in two populations. (Variability was defined as the band sharing coefficient of DNA fingerprints derived from multi-locus minisatellite probes). Although Møller's result was based on a small sample size, it illustrated the importance of minimizing confounding factors, particularly in the assessment of variability. In a more rigorous assessment, a single researcher used randomly amplified polymorphic DNA (RAPDs) under standard conditions to determine the variability of several individuals of a particular population or species. (This assumes that the variation observed in RAPDs reflects overall genotypic variation). Using a pair-wise comparison, species or populations with high EPP were more variable than sister-species or populations with low EPP (Doums *et al.* MS), although the differences were not significant.

(v)   Genetic differences between house sparrow populations

Gross differences in genetic variability may exist between these populations. House sparrows spread across Europe in association with the Roman conquest, and have been in England for around 2,000 years (Summers-Smith 1963). The North American population stems from a subset of the European birds (Anderson 1978, Chapter 1), and the Lundy birds derive from an even smaller subset of the British mainland sparrows. Sparrows in North America, and particularly those on Lundy, must therefore have passed through a 'bottleneck' (but see Carson 1990, Marin *et al.* 1994).   Klitz (1972; cited in Baker 1980) found more polymorphic loci in Continental house sparrows than those from Britain, which in turn had more than those from North America (although admittedly, the loci were relatively invariant (Parkin 1987)).

If the North American populations suffered from low genetic variance as a consequence of this bottleneck, then one would have expected their EPP levels to be correspondingly low. This pattern was not found, however (Table 4), and the highest level of EPP was actually recorded in central continental America (Oklahoma). The outstandingly low incidence of EPP occurred on Lundy Island, England. This is potentially consistent with the 'genetic variation' theory of EPP differences, since the island was only recently colonized by the current population of house sparrows (1972) and consequently may be genetically impoverished due to inbreeding and/or founder effects. In a cross-specific comparison, island populations of birds had significantly lower levels of genetic variation than their mainland counterparts (Frankham 1997).

To test the genetic variation hypothesis, Griffith *et al.* (MS) scored the variability at four polymorphic microsatellite loci of 20 adults drawn at random from each of the Lundy, Kentucky, and Nottingham populations. The number of alleles detected at each locus was not significantly different in adults from each population, and thus the genetic variation hypothesis was not supported.

(vi)   Sample sizes and EPP

It is important to control for sample size differences when interpreting EPP levels (Griffith *et al.* MS). Since extra-pair young tend to be distributed non-randomly in 'clusters' (Double 1995), the level of EPP may be severely overestimated or underestimated if relatively few broods are sampled. For example, the 95% confidence limits of the EPP estimate from the New Mexico population are broad (5.5 - 26 %) because of the small sample size. The detection of differences in EPP levels between populations probably requires larger sample sizes than those usually used.

4.4.9   Future analyses

Several tests are currently restricted by small sample sizes (particularly at barn D in 1996, where both trapping success and EPP levels were low). However, once the paternity analysis is completed on the remaining broods (particularly those at barn B in 1996), the data will be more comprehensive. Several birds of known age were present at barns B and C in 1996, most of which were trapped and measured. The sires of the extra-pair young are currently being elucidated.

Thus far, it is unknown whether there is a between-year effect on EPP, since different barns were analysed in different seasons. Indeed, since the breeding density was increased at barn B in 1996, even a comparison of EPP levels between years may not prove instructive. Fortunately, barn C should serve as a 'control' for assessing annual differences in EPP rate, since the box placements remained unchanged.

Chapter 5.
Paternity protection in the house sparrow : Frequent copulation

5.1 Introduction

5.1.1 Paternity protection behaviour in birds

Anisogamy (the sexual asymmetry in gamete size), represents one of the cornerstones of sexual selection theory (Trivers 1972). Males tend to produce large numbers of small gametes (sperm), whereas females tend to produce small numbers of large gametes (ova) (Bateman 1948). A male's reproductive success is therefore limited by the number of matings he can achieve, while a female's reproductive success is limited by the quality of these matings (Bateman 1948). Because of this difference in reproductive potential, males are expected to be the promiscuous sex, whereas females are expected to be coy (Trivers 1972).

In most birds, biparental care (concerning food provisioning in particular) markedly improves the size and condition of nestlings (Perrins 1979, see Black 1996). This increases their probability of surviving to recruitment (Perrins 1979) and, consequently, the reproductive success of each adult is increased as a corollary of care. However, adults do not gain fitness benefits from parental care if this is misdirected into offspring which are not genetic progeny (Trivers 1972).

In birds, misdirected parental care can result from three circumstances, none of which are mutually exclusive. The first of these, conspecific brood parasitism ('egg dumping'), occurs when a female surreptitiously lays a fertilized egg in the nest of a rival (Kendra et al. 1988). This 'parasitizes' the care of both of the resident parents, which unwittingly rear a nestling to which neither are related (Petrie & Møller 1991). In the second circumstance, known as quasi-parasitism, females solicit copulations from an extra-pair male, but then lay the resulting egg in *his* nest rather than her own (Wrege & Emlen 1987, McRae & Burke 1996). Since the offspring are related to the resident male but not his cuckolded mate, only the female's parental care is misdirected. However, both egg dumping and quasi-parasitism are relatively rare in altricial birds (MacWhirter 1989, Riley et al. 1995), and will not be discussed further.

In the third and most common circumstance (Gowaty 1997), the resident female solicits copulations from an extra-pair male (Westneat *et al.* 1990). Consequently, the social male devotes parental care into rearing one or more offspring to which he is unrelated. Since parental care is costly (Askenmo 1979), males are under strong selection to restrict its provision. Males therefore attempt to ensure paternity within their own brood with the selective use of two behavioural strategies: mate guarding and frequent copulation (Birkhead & Møller 1992). These are traditionally viewed as alternatives, with some species copulating rarely but showing intensive mate guarding (such as the reed bunting; Dixon *et al.* 1994), while others copulate frequently in lieu of mate guarding (such as the osprey; Birkhead & Lessells 1988). This section examines whether males attempt to protect their paternity through frequent copulation.

5.1.2 The frequency of copulation in birds

In most birds, a single copulation introduces sufficient spermatozoa to fertilize several hundred thousand eggs (Birkhead & Møller 1992). In many species however, males exacerbate this surplus by copulating with their mates repeatedly during a single breeding attempt (Birkhead & Lessells 1988, Petrie 1992), with over 300 copulations per clutch recorded in the Smith's longspur (Briskie 1993).

This paradoxical excess results from the conflict of interest between three individuals: the female, the pair male, and the extra-pair male (Dunn *et al.* 1994). Firstly, females wish to ensure that their eggs are fertilized by the highest quality sperm, which often involves seeking copulations with a male superior to her own (Kempenaers *et al.* 1992). In this circumstance, a suite of behavioural and physiological adaptations dictate which male's sperm are most likely to fertilize the egg.

The sequence in which the within and extra-pair males inseminate the female exerts a major influence upon the outcome of sperm competition. Males are much more likely to achieve fertilization if they copulate last in the sequence (an effect referred to as 'last-male precedence') (Birkhead 1996). Female birds of all species thus far examined possess sperm storage tubules (SSTs) located at the utero-vaginal junction (Birkhead 1996). A

small proportion of the sperm transferred in each ejaculate is retained in these tubules and released upon ovulation the following morning to coincide with the brief 'fertilization window' (Cheng *et al.* 1983). Last male precedence was initially attributed to a sequential layering of the sperm within these SSTs, with the final male gaining an advantage through a 'last in-first out' effect (Birkhead & Hunter 1990). Further experiments have shown that the last-male effect is contingent upon the timing of inseminations relative to egg-laying (Birkhead 1996), since the advantage is nullified if the copulations occur several hours after the laying of the previous egg.

Less than 1% of the sperm from each ejaculate manage to reach the SSTs, since the female vagina has a battery of defences which allow only the highest quality sperm to penetrate, irrespective of whether they result from a within or extra-pair mating (Birkhead *et al.* 1993b). In this 'hostile tract', sperm which are of low quality, or are otherwise undesirable (e.g. if accompanied by pathogens), are filtered out by a suite of physiological and chemical responses (Birkhead *et al.* 1993b). Males, regardless of their social attachment to the female, consequently must inseminate a vast excess of sperm to ensure that they are represented at the site of fertilization.

Moreover, each male must trade-off the frequency of within-pair copulations against his own attempts to achieve extra-pair copulations (EPCs) (Westneat *et al.* 1990). However, these attempts at parasitizing the parental care of other males are open to reciprocation (e.g. Wetton *et al.* 1995). Thus, intraspecific copulation rates are predicted to vary in accordance with the threat a male perceives from other males.

5.1.3 Copulation frequency in the house sparrow

Female house sparrows appear to choose males as both social and sexual partners according to the size of their throat patch of black feathers known as the 'badge' (Møller 1990b, Veiga 1993a). A female preference for males with large badges was demonstrated in captive trials (Møller 1987a, but see Kimball 1996), and females in a free living population preferred to solicit EPCs from large-badged males (Møller 1990b).

Although the benefits of this preference are unclear, one suggestion is that large-badged males are more fertile (Møller & Erritzoe 1988, Sheldon 1994b). Since hatching failure is relatively common in house sparrows (Seel 1968b), females paired with large-badged males may receive higher quantities of sperm, which is also sufficiently robust to penetrate their physio-chemical obstacles (Birkhead *et al.* 1993b). In support of this 'increased fertility' hypothesis, Møller (1990b) found that large-badged males copulate at a higher rate than small-badged males.

Alternatively, if large badges reflect the high genetic quality of their bearer, females may prefer to mate with these males to obtain viability genes for their offspring (Williams 1966, Hamilton & Zuk 1982). If frequent copulation serves to ensure within-pair paternity, then males which perceive their risk of cuckoldry as being high should copulate at the highest rates (Ross 1997). Females paired to large-badged males are less likely to pursue EPCs (Møller 1990b), and hence their 'confident' mates would copulate at relatively low rates.

The two conflicting hypotheses were addressed in the Maine Chance population of house sparrows. The 'increased fertility' hypothesis would be supported if large-badged males copulate at high frequencies. By contrast, the 'confident male' hypothesis would be supported if large-badged males copulate at low frequencies.

5.2   Methods

Copulations were observed using binoculars or a telescope, either from within a vehicle or, more usually, hidden inside army surplus signal boxes. Due to the clustered box sitings, as many as four pairs could be observed simultaneously. Most observations were taken in the morning, although this was supplemented later in the season during afternoon and early evening periods. In each 30-minute observation period, the number of behaviourally successful copulations was recorded (i.e. those in which successful cloacal contact was achieved), as well as those which failed. From this, an hourly copulation rate was calculated.

5.3   Results

5.3.1   A brief description of copulatory behaviour

Most pairs copulated on, or near, their nest-box, although copulations were occasionally witnessed on the ground or upon fence posts. Females solicited copulations from their mates by squatting with their cloacal region slightly raised, and shivering their wings (occasionally giving a soft *seep* call) (Summers-Smith 1963). As in most passerines, coitus lasted only a few seconds (Birkhead *et al.* 1987), and was terminated when one or both birds flew away, or when both sexes settled down for ritualised preening at the nest (Summers-Smith 1963).

5.3.2   The general frequency of copulations at Maine Chance

Copulation frequency was monitored in 125 observational periods, each lasting 30 minutes. Most observational work (98 periods) was carried out before noon, since copulation rates in most birds peak during the morning to coincide with the fertilization window (Birkhead *et al.* 1987). Sixteen of the remaining 27 periods were during the afternoon, and 11 were during the evening (later than 1600).

All copulations witnessed were within-pair. On average, each pair copulated six times during an observation period, up to a maximum of 38 copulations in 30 minutes. Pairs tended to copulate three or four times in quick succession, although these 'bouts' occasionally involved as many as 10-15 copulations (cf. Møller 1990b). Since the volume of sperm transferred in two bouts of triple copulations is presumably similar to the volume transferred in three bouts of double copulations, the 'copulation rate' used in the analyses merely referred to the total number recorded during an observation period.

The total number of copulations which occurred during each clutch was unknown, as no complete female cycles were followed. However, as several pairs were observed to copulate more than 50 times during only five hours of observation, the total copulations per clutch probably regularly exceeds one hundred.

5.3.3 Diurnal variation in copulation rates

When grouping all observational data into two-hour blocks (i.e 0600 - 0759, 0800-0959 etc.), the frequency of copulation showed a distinct bimodal pattern (Figure 1). The average copulation rate peaked first in mid-morning, with a second, higher peak in the late afternoon (note that the rates at 1800 and 2000 have large error bars, since most pairs were not observed to copulate at all during these periods).

Figure 1. Variation in copulation rate with time of day.
(columns refer to mean + SE)
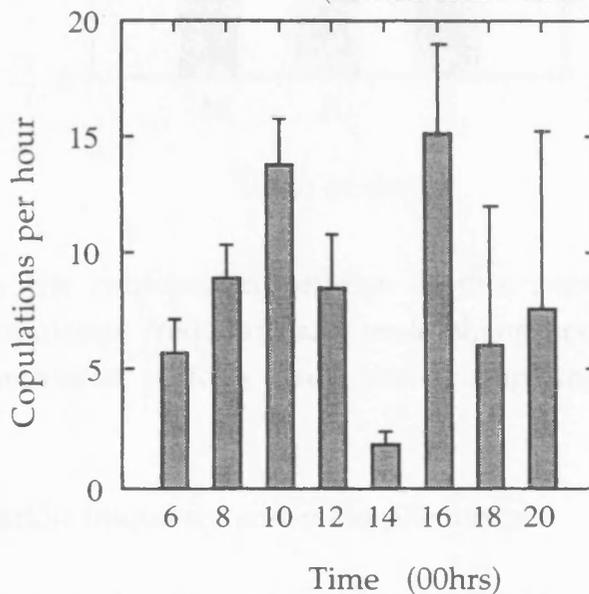
This pattern was clarified when the data were pooled into three arbitrary categories: morning (before 1200), afternoon (between 1200 and 1600), and evening (after 1600) (abbreviated to M, A and E in Figure 2). Mean copulation rates did not vary significantly between these three periods (Kruskal Wallis $H = 4.79$ $p = 0.09$), nor between the morning and evening (Mann-Whitney U-test $z = 0.245$ NS).

The copulation rates in the morning and evening of the same day were known for five pairs. In these five pairs, copulation rates were significantly higher in the evening than in the morning (Wilcoxon's signed rank test, $z = 2.03$ $p = 0.04$).

Figure 2. Variation in copulation rate according to diurnal category.

(columns refer to mean + SE)

Time of day
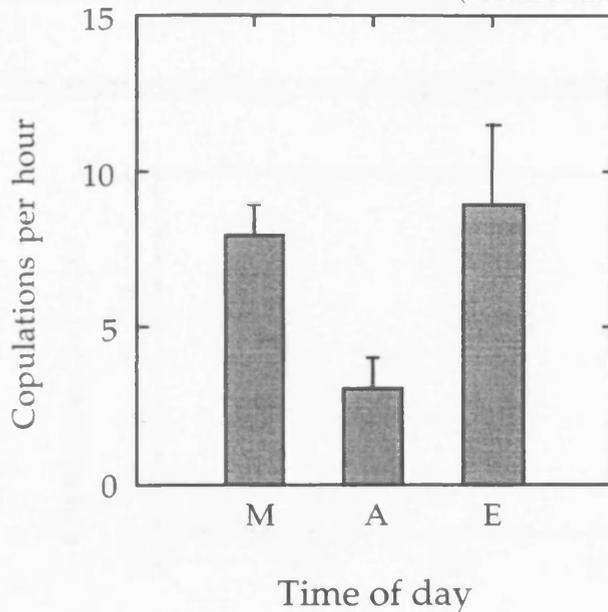
To minimise the confounding effects of this periodicity, the primary analysis of copulation frequency and male phenotype was restricted to the morning observation periods, since these comprised the majority of the data.

5.3.4 Copulation frequency and male phenotype

Nests were only included in the analyses if data were available from at least four observational periods (equal to two hours). Adequate data on copulation rates were available for nine males, although only six of these had been trapped and their tarsal length, mass and badge size measured. The three untrapped males were observed at a distance, and their badges were placed into a size category according to the reference sketches given in Møller (1987).

For the six trapped males, copulation frequency was positively related to body size and condition, although though none of the correlations was significant (tarsal length $r = 0.486$, body mass $r = -0.086$, body condition = 0.600, all $n = 6$, NS). Large-badged males copulated at a higher rate, although this was not significant ($r = 0.636$ $n = 6$ p < 0.1) (Figure 3). When the analysis was repeated using badge size categories instead of mensural

data (allowing the three untrapped males to be included), copulation rate was independent of badge size ($r = - 0.034$ $n = 9$ NS).

Figure 3. Variation in morning copulation rate in relation to male badge size.
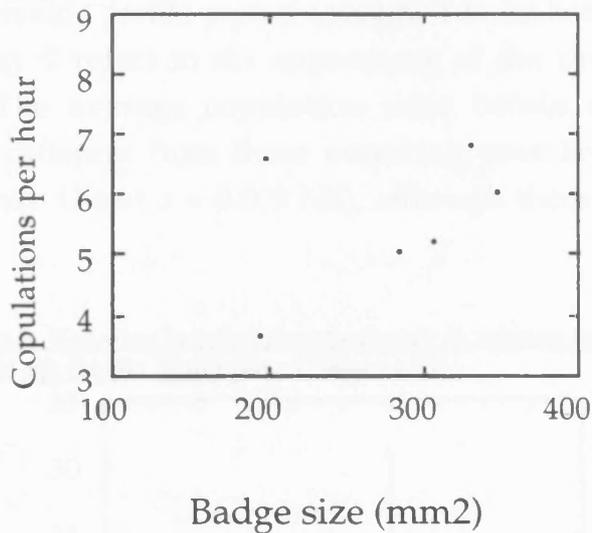
Badge size (mm2)

Only around half of the study pairs were observed to copulate during the late afternoon/evening period. However, there was no significant difference between the badge sizes of the males which continued to copulate late in the day, and those which appeared to cease (Mann-Whitney U-test z= 0.03 NS, Figure 4).

Figure 4. Comparison of badge sizes of males which did or did not copulate late in the evening (mean + SE).

Observed to copulate?
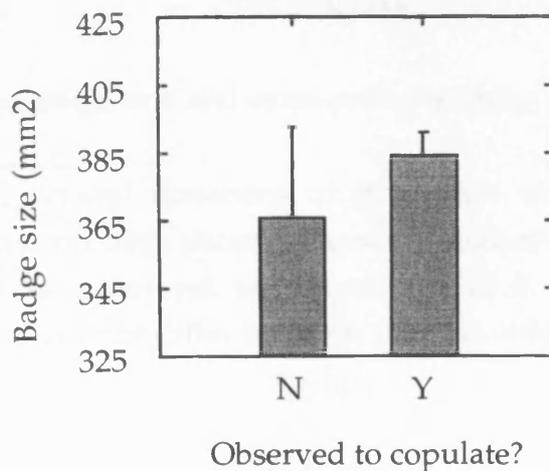
5.3.5   Copulation frequency and the female cycle

The earliest copulation was witnessed nine days before the first egg was laid, and the latest copulation occurred three days into incubation. These were relatively unusual events however, with most copulations occurring within the female's fertile period (assumed to be between day -5 and day +3, where day 0 refers to the appearance of the first egg, Møller 1990b) (Figure 5). The average copulation rates before egg laying were not significantly different from those occurring once laying had commenced (Mann-Whitney U-test $z = 0.003$ NS), although there was an obvious peak on day 0).

Figure 5. Variation in copulation frequency in relation to the time in the female fertile period (mean + SE).
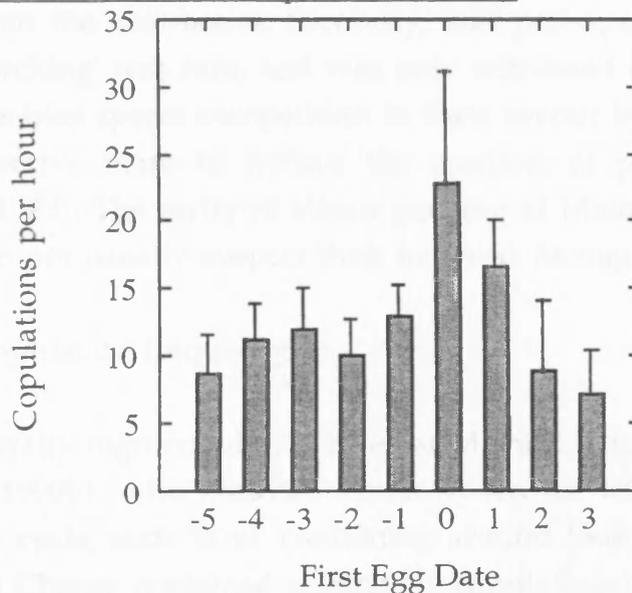
5.3.6 Copulation frequency and extra-pair paternity

Unfortunately, several instances of predation reduced the number of broods with data on both paternity and copulation frequency to only ten, five of which have not yet been analysed (T.B. Rambo, *pers. comm.*). Copulation rates did not differ between the cuckolded and intact broods ($t = 0.018$ df = 3 NS).

5.4 Discussion

5.4.1 Copulatory behaviour

House sparrows at Maine Chance, as expected, showed several of the stereotypical behaviours associated with copulation which were reported initially by Summers-Smith (1954), and later by Møller (1990b). However, several important features which had been described previously were either never witnessed, or occurred only rarely.

Firstly, although EPCs were presumed to occur regularly in the study population (as several broods contained extra-pair young) these were never witnessed, and were assumed to take place either surreptitiously, or away from the nest-boxes. Secondly, and perhaps as a corollary of this, 'cloaca-pecking' was rare, and was only witnessed on two occasions. Male dunnocks bias sperm competition in their favour by pecking the cloaca of a prospective mate to induce the ejection of previous males' sperm (Davies 1983). The rarity of cloaca pecking at Maine Chance suggests that males do not usually suspect their mates as having copulated elsewhere.

5.4.2 Copulation frequency

The generally high copulation rates at Maine Chance were consistent with Møller (1990b), who credited house sparrows with about 47 bouts per breeding cycle, each 'bout' containing around four copulations (each bout at Maine Chance contained around six copulations).

In birds, copulation frequency follows a bimodal distribution, with the majority of species copulating fewer than five times per clutch, or between 20 and several hundred times (Birkhead et al. 1987). The house sparrow falls within the upper range of the latter category. Of the two paternity guards which birds regularly employ (mate guarding and frequent copulation, Birkhead & Møller 1992), house sparrows at Maine Chance appear to favour the latter strategy. In accordance with this, a cross-specific survey revealed that house sparrows have relatively large testes for their size (and hence relatively large ejaculates) (Birkhead et al. 1993b).

5.4.3 Diurnal variation

Copulation frequency at Maine Chance did not show the morning peak reported by Møller (1987c). Data collection in this study generally encompassed the early morning emergence of the females, and it is unlikely that any dramatic surge of copulations would have been missed. Admittedly, evening observation periods were scanty, and hence the average copulation rate could be artefactually high. However, the direct comparison between morning and afternoon rates of specific pairs, although based on a small sample size, suggests that for some pairs at least, copulation rates are genuinely higher in the evening.

Although this does not accord with the general reproductive biology of birds (Birkhead 1996), it is not an inconceivable result considering the biology of house sparrows at Maine Chance. Most birds, including the house sparrow, lay their eggs in the morning (Schifferli 1979). About an hour after an egg is laid, the ovum from the largest remaining follicle is shed from the ovary. It rapidly moves into the infundibulum, where there is a short 'fertilization window' of around 30 minutes during which it is capable of being fertilized (Cheng *et al.* 1983). Although the exact time at which the eggs were laid at Maine Chance was unknown, house sparrows generally lay between 0500 - 0600 (Summers-Smith 1963). Female house sparrows did not emerge from their boxes, on average, until about 0700 (*pers. obs.*). Consequently, the egg may have already been fertilized by the time the female emerged.

Female birds of all species thus far examined possess sperm storage tubules (SSTs) located at the utero-vaginal junction (Birkhead 1996). A proportion of the sperm transferred in each ejaculate are retained in these tubules, and are released upon ovulation the following morning. Although sperm competition near to egg-laying is strongly influenced by the sequence of insemination, with the last males to copulate having an advantage ('last-male precedence'), this effect disappears within hours (Birkhead 1996). Hence, males which can maintain a high frequency of copulation in the evening before ovulation do *not* gain a temporal advantage, but would still bias sperm competition in their favour by increasing the proportion of their sperm present in the SSTs (Birkhead 1994).

97

5.4.4   Male phenotype and copulation frequency

As found in previous intra-and interspecific studies, copulation frequency was independent of body size (Møller 1990b, Birkhead *et al.* 1993b). Although my data were not sufficient to compare copulation rates of yearlings and adults, no age-related difference was found by Møller (1987c).

At Maine Chance, large-badged males tended to copulate at higher rates. Although this result was not significant, the sample size involved was restrictively small. Møller (1990b) found a significant positive relationship between copulation frequency and badge size in house sparrows, and argued that, *prima facie*, large-badged males would have a greater certainty of paternity. Furthermore, badge size correlated with mean testis size in a sample of sparrows collected in suburban Copenhagen (Møller & Erritzøe 1988), suggesting that large-badged males would have a dual advantage if ejaculate size correlates with testis size.

In a separate population of house sparrows however, testis size was not significantly correlated with badge size (although the relationship was positive) (Birkhead *et al.* 1994). Moreover, Birkhead *et al.* (1994) caution that ejaculate sizes are inherently variable, even under standard conditions, and hence testis size is not an automatic predictor of ejaculate size. Nevertheless, even if ejaculate sizes were not consistently different between individuals, large-badged males would still have an advantage in sperm competition if they performed a greater number of copulations.

Large-badged males did not continue to copulate later in the day than small-badged males. The variation in evening copulation frequency may, however, be meaningless, as evidence taken from house sparrows suggests that copulations become less valuable as the day progresses. Birkhead *et al.* (1994) counted the number of sperm in the seminal glomera of males taken at different hours of the day, and found a significant decline with time. Using an average estimate of ejaculate volume taken from Møller & Erritzøe (1988), and assuming an average of 16 copulations per day, Birkhead *et al.* (1994) calculated that male sperm reserves would be depleted before the end of the day. It seems reasonable to assume, therefore, that the Maine Chance males which copulated over 50 times

during a single morning would have exhausted their sperm reserves by the evening.

5.4.5 Copulation frequency in relation to the female cycle

Although temporal fluctuations in copulation frequency are undoubtedly confounded by variation between males, the obvious peak in copulation rate coincided with the day on which the first egg was laid (the First Egg Date or FED). This contrasts with the population of house sparrows studied by Møller (1987c), in which a high frequency of copulation was maintained from day -4 until day 0 (the FED). Møller (1987c) also found that copulations became less frequent during egg laying, and were rare during incubation. At Maine Chance, copulations were equally frequent both before and during egg laying, although in common with Møller (1987), copulations were rare when the clutch was complete. Copulations which occur while the female is incubating are superficially maladaptive, as they are extremely unlikely to result in fertilization (Cooke et al. 1995). They have, however, been documented in other birds (Cooke et al. 1995, Ross 1997), and may result from misdirected paternal care, or may function to maintain the social bond at a time when the male is a peripheral figure.

It has been demonstrated experimentally that male house sparrows can distinguish between fertile and non-fertile females (Møller 1987c). This is probably achieved using subtle behavioural cues associated with a female's status. Fertile females were heard to issue distinctive calls by Møller (1987), although these were not noted at Maine Chance. Despite the existence of these cues, it is not surprising that copulation rates peak on the day the first egg is laid. In house sparrows, the fertilized egg passes slowly down the oviduct during the day, and is around two-thirds of its eventual mass before the female goes to roost (Schifferli 1979). The resulting imbalance presumably affects the flight performance of females, which provides observant males with a more reliable cue that she is fertile. Once their mates have established that the first egg has been laid (i.e. by brief, daily inspections of the nest contents), the female is assuredly fertile, leading to a surge in the within-pair copulation rate.

5.4.6 Copulation frequency and extra-pair paternity

Although there was no difference in average copulation frequency between the broods which contained extra-pair young (EPY) and those which did not, the sample size involved was small. However, even with a large sample size, the direction of this relationship is difficult to predict *a priori* because of the cryptic nature of female choice (Eberhard 1996).

Superficially, a high rate of copulation should correlate with a low level of cuckoldry (Birkhead & Lessells 1988), and EPY should be more common in nests where the male copulates only a few times during each cycle. However, this view of paternity behaviour is inherently male-biased, whereas it is ultimately the female who controls fertilization (Dunn *et al.* 1994). This is particularly true in birds such as the house sparrow, where the male does not have an intromittent organ, and females unwilling to copulate could simply adjust their posture. Even when copulation does occur, the sperm is merely deposited into the cloacal region, and unless the female uses muscular 'pulses' to draw the sperm into her everted vagina, it would merely be ejected upon defecation (Birkhead 1996).

Assuming this level of control, one could predict that frequent copulation would be associated with *high* levels of cuckoldry, not low. Males which copulate at a high rate may do so because they suspect their mate has been unfaithful (referred to as 'retaliatory copulations', Gowaty 1997). If this fear is justified, then despite these dilution measures, the egg may still be fertilized by the rival's sperm, according to the differential timing and volume of the inseminations (Birkhead 1996). By contrast, males which copulate at a low rate may perceive their risk of cuckoldry as low (the 'confident male' hypothesis, Gowaty 1996a), which would be justified if they possessed desirable qualities such as good genes' (Williams 1966).

If one assumes that EPCs in the house sparrow are solicited entirely by the female (even if not overtly), then females should perform this act surreptitiously, and not risk invoking any compensatory measures by their partner (Dunn *et al.* 1994). A female paired to a small-badged male should secretly obtain a copulation from a male of higher quality, then subtly resist all copulation attempts by her social mate. These copulations would dilute the sperm of the preferred male, and thus neutralize the

original purpose of seeking EPCs. Admittedly, female resistance of WPCs was rare at Maine Chance, although this may be a subtle tactic of the female to placate her mate (Dunn *et al.* 1994). Any sperm transferred into the cloaca from an undesirable copulation could be ejected out of view of the male within minutes. Unfortunately, this 'strategic defecation' hypothesis would be difficult to test, because even after a behaviourally successful copulation, a proportion of the ejaculate is merely ejected with the faeces (Birkhead 1996).

5.4.7 Badge size, infertility, and unhatched eggs

Wetton & Parkin (1991) were the first to describe a possible direct female benefit of indulging in EPCs. In a contentious paper, they reported a positive association between cuckoldry and infertility in the house sparrow; broods containing EPY were significantly more likely to contain an unhatched egg than those with no EPY. Under their scenario, a female paired to a low quality male with relatively small sperm reserves, sought extra-pair matings with a higher quality, more fertile male, to guard against the risk of laying infertile eggs (Koenig 1982). If badge size was related to testis size (Møller & Erritzoe 1988), and if testis size was related to ejaculate volume, large badged males would be preferred as copulation partners both within- and extra-pair (Møller 1990b).

This version of Trivers' (1972) 'sexual competence' hypothesis was extended by Sheldon (1994a), who proposed that females selected males on the basis of secondary sexual characters which indicated their fertility.

Although Wetton & Parkin's (1991) theory of EPCs as a form of 'fertility insurance' is entirely plausible, it has not been widely accepted, for several theoretical and empirical reasons. Firstly, the 'fertility insurance' hypothesis would predict *fewer* infertile eggs in broods containing EPY, rather than more (Lifjeld 1994). Secondly, Birkhead *et al.* (1994) indirectly demonstrated that male house sparrows replenish their sperm reserves overnight, and thus the presence of infertile eggs is unlikely to be the result of sperm shortage. Thirdly, it is unclear whether badge size is indeed correlated with testis size and, moreover, whether larger testes produce larger ejaculates (Birkhead *et al.* 1994). As a more general point, a link between testis size, testosterone and the expression of the male badge has

yet to be demonstrated and currently, the experimental evidence suggests that there is no link (Keck 1934 , but see Evans *et al.* MS).

SPECIAL NOTE

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Chapter 6.
Paternity protection in the house sparrow : Mate guarding

6.1   Introduction

6.1.1   Paternity protection mechanisms in birds

Infidelity is widespread among birds (Gowaty 1996a, Chapter 4). Males benefit from extra-pair fertilizations (EPFs) by increasing their reproductive success at the expense of another male, who may devote considerable effort into rearing offspring to which he is unrelated. This cost of cuckoldry has led to the evolution of counter-measures which males employ to ensure paternity within their broods (Birkhead & Møller 1992). These measures take three main forms: territoriality (Møller 1994b), frequent copulation (Birkhead & Lessells 1988), and close following (more commonly referred to as 'mate guarding') (Birkhead 1979).

In the first scenario, males maintain and defend distinct territories within which the female nests (and often forages) (Howard 1920, Hinde 1956). Any rival males which intrude upon the territory (irrespective of their purpose) are aggressively repelled, particularly during the female's fertile period (e.g. in red-backed shrikes, Jakober & Stauber 1989).

Frequent copulation may generate a volumetric advantage, if males can flood their mate's reproductive tract with their own sperm (Birkhead 1996). This biases sperm competition in their favour, simply by diluting any rival sperm which may be present from an extra-pair copulation (EPC) (cf. Martin & Dzuik 1977). The advantage is enhanced if the copulations are timed to coincide with the period of maximum uptake of sperm by the female (Birkhead 1988).

'Mate guarding' is a conspicuous feature of the breeding behaviour of many birds (Birkhead 1979), in which a male endeavours to remain in close proximity to his mate by following her. This association has a dual function. Firstly, rival males approach females less frequently if their mate is present (Møller 1985). Secondly, the prolonged attention of her mate would minimize a female's opportunities to seek EPCs elsewhere (Møller 1985). The importance of mate guarding has been demonstrated experimentally by the

detention of the male during his mate's fertile period (Currie 1995). This almost invariably increases the intrusion rate of rival males (usually neighbours) into the 'vacant' territory, and also the rate at which these males attempt EPCs with unaccompanied females (Dickinson 1997).

All three of these paternity protection devices have been recognized in a plethora of both observational and experimental field studies (reviewed in Birkhead & Møller 1992). Although in theory the three strategies are not mutually exclusive, they appear to act as alternatives rather than complement one another (e.g. species which copulate frequently do not mate guard, and *vice versa*). However, several species such as the chaffinch (Sheldon 1994a) combine both mate guarding and frequent copulation.

Which strategy is employed probably depends upon the species' life history (Birkhead & Møller 1992). For example, male seabirds and large raptors cannot effectively guard their mates, as their feeding trips are necessarily prolonged and wide ranging, and the females usually remain at the nest to incubate the first egg(s) (Fisher & Lockley 1953, Brown 1976). To compensate, males copulate with their mates at remarkably high rates, particularly after returning from these feeding trips (Birkhead & Lessells 1988). Ecological constraints, such as the degree of habitat occlusion, may also dictate the preferred option (Birkhead *et al.* 1987). Males of species which nest in open habitats can guard their mates with relative ease; an interloping rival would be visible at some distance. This should be particularly true at low nesting densities (e.g. black vulture, Decker *et al.* 1993), and when the operational sex ratio (OSR, the ratio of males to fertile females) was low (Møller 1985).

As the high incidence of EPFs in some species testifies, these defence mechanisms are hardly flawless (Owens & Hartley 1998). Whatever strategy is utilized, males vary in both their ability and their volition. The success of each individual male depends upon a composite of several factors, which may be innate (his quality and desirability, and that of his mate) or labile (OSR, breeding density, desirability of rivals) (Birkhead & Møller 1992). In this study, the relative strength of these factors was examined in the house sparrow colony at Maine Chance in 1996.

6.1.2   Mate guarding and the house sparrow

Although frequent copulation (Møller 1987c) and mate guarding (Hegner & Wingfield 1986) have both been described in the house sparrow, the development of territoriality appears to be minimal (Summers-Smith 1963). The house sparrow is not a territorial species, and will tolerate the presence of conspecifics beyond a *c*30cm radius of the nest (Summers-Smith 1958, *pers. obs.*). This study was therefore restricted to frequent copulation and mate guarding, of which the former is discussed in more detail elsewhere (Chapter 3). Mate guarding data were collected to test a specific hypothesis: house sparrows at Maine Chance guard their mates according to their perceived threat of cuckoldry.

6.2   Methods

Most studies of mate guarding involve tracking the movements of one member of a focal pair (almost always the female) and observing the relative proximity of their mate. This approach is practical in species which occupy finite, delineated territories (Howard 1920), as the movements of the pair are usually confined within a relatively small area. Unlike most passerines, however, house sparrows are not markedly territorial, and may have a home range of several hundred metres (*pers. obs.*). Thus, an indirect approach was needed to investigate mate guarding at Maine Chance, which used the nest area as the focus of observation (*cf*. Hegner & Wingfield 1986). The inherent weakness in this approach is that the position and behaviour of the birds which are not in view is unknown. The strength of the indirect method is that data can be collected simultaneously at more than one nest.

6.2.1   Mate guarding

Nest watches were undertaken in the morning and late afternoon, to encompass the peak intensities of mate guarding reported in other studies (Parrott 1995). Each 30-minute observation period was subdivided into sixty 30-second bouts. At the end of each bout, the position and identity of any birds present at the box was recorded. If a female had not been seen during an observation period, but was strongly suspected to be inside the box (e.g. during the terminal stages of the fertile period), observations were continued for a further five minutes. If a female had not emerged after this time, I

approached the box to within three metres, which stimulated the sitting bird, if present, to emerge. This level of disturbance was equivalent to that caused regularly throughout the day by farm workers, and females readily returned to their boxes soon afterwards.

For each box, the data were summarized to represent, firstly, the proportion of bouts in which the male *was* present (i.e. sitting inside the box), and secondly, the proportion of bouts in which he *was seen* to be present. Female presence, and female visible presence, were tallied in the same way, as was the proportion of time during which both sexes were present at the nest. This was divided further into 'male awareness time' (MAT). MAT encompassed both the period when the male could see the female and, also, when the female was not in view but the male was aware that she was inside the box (having witnessed her entering).

Any other conspecific interactions which occurred during observational periods were recorded (e.g. fights, communal chases). When one member of a pair left the nest area in a definite 'flight' (as opposed to merely hopping around the nest area) I noted whether their mate followed.

6.2.2 Data presentation and analysis

The mate guarding data obtained from each box on a given day were pooled for analysis and graphical representation. Most data could not be normalized, and hence statistical tests were non-parametric unless otherwise stated. The fertile period was conservatively assumed to be between days -5 and +3 for a clutch of five eggs (with day 0 = First Egg Date), as used by Møller (1987c).

6.3 Results

6.3.1 General patterns of nest presence

Seventy seven hours of behavioural observations were obtained, from 14 nests. Males spent less time near the nest as the breeding cycle progressed (Figure 1), although this was not significant (Spearman Rank $r = -0.497$ $n = 12$ $p < 0.1$). The proportion of time when males were present at the nest did not differ between their mate's pre-fertile and fertile periods (Mann-Whitney U-

test z = 0.911 NS), and most of this time was spent perched outside of the box (Figure 2), since males rarely hid inside.

Figure 1. Male nest presence in relation to the female cycle.

(mean + SE)

Day relative to the first egg
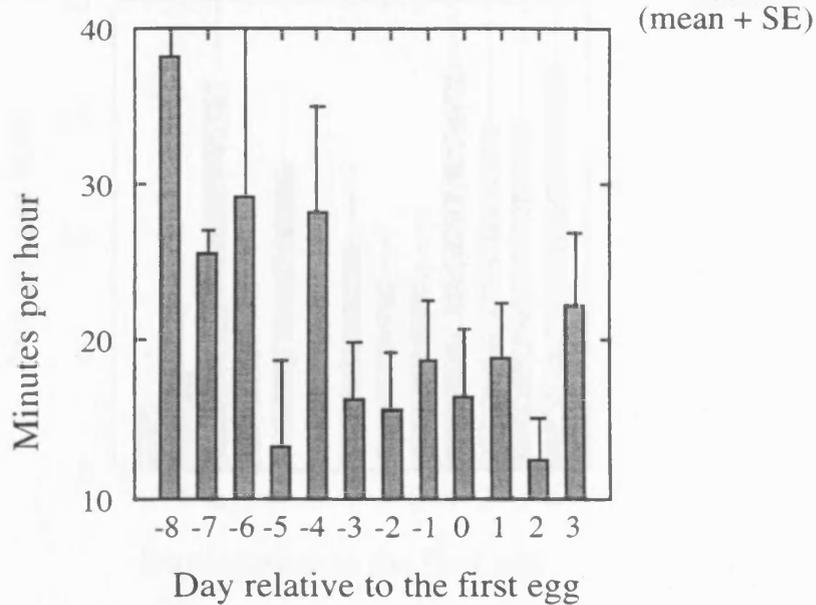
Figure 2. Allocation of male nest presence spent either in view or hidden.

Day relative to first egg
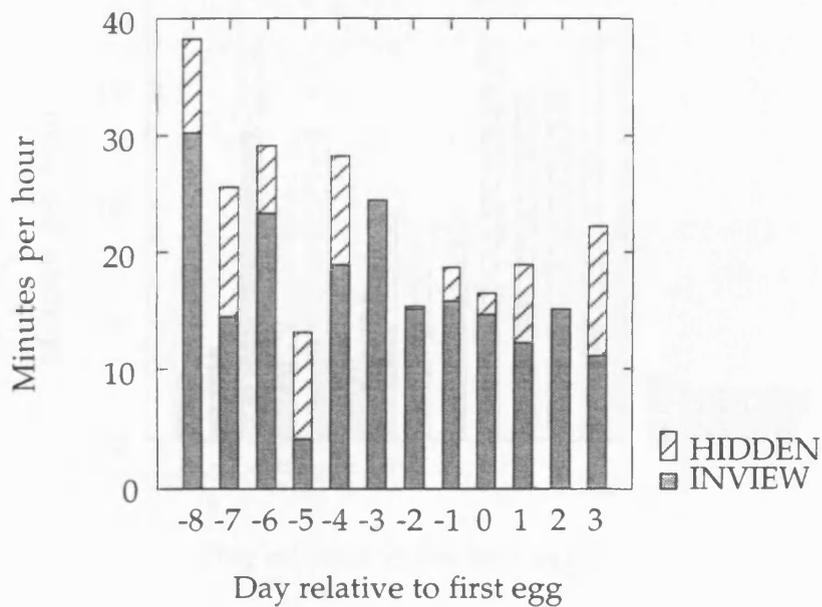
In contrast, females spent proportionately more time at the nest as the breeding cycle progressed (Spearman Rank r = 0.703 n = 12 p < 0.01) (Figure 3). Consequently , females spent significantly more time at the nest during their fertile period (Mann-Whitney U = 17.35 p = 0.001) compared to their pre-fertile period. Most of the this time was passed while hidden inside the box (Figure 4).

Figure 3. Female nest presence in relation to the breeding cycle.

(mean + SE)

Day relative to the first egg
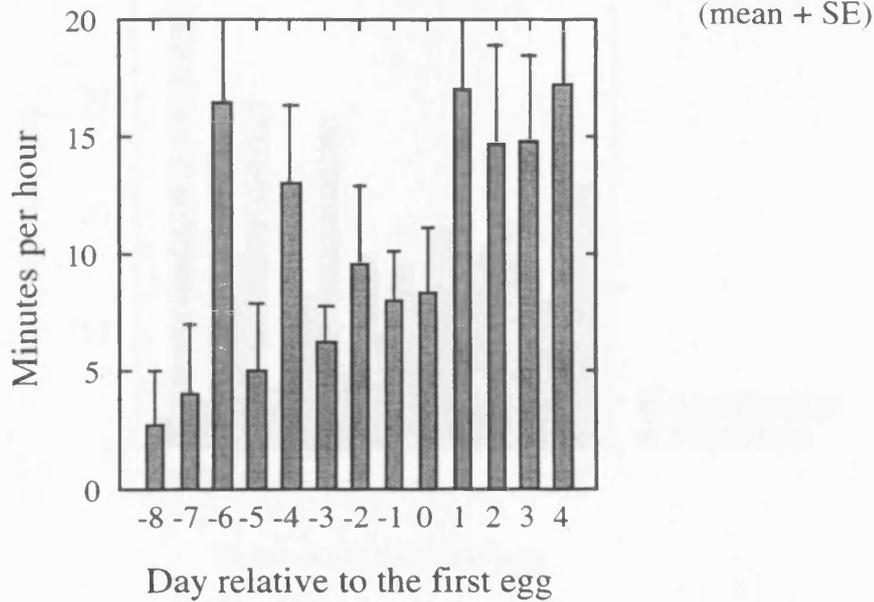
Figure 4. Allocation of female nest presence spent in view or hidden.

Day relative to the first egg
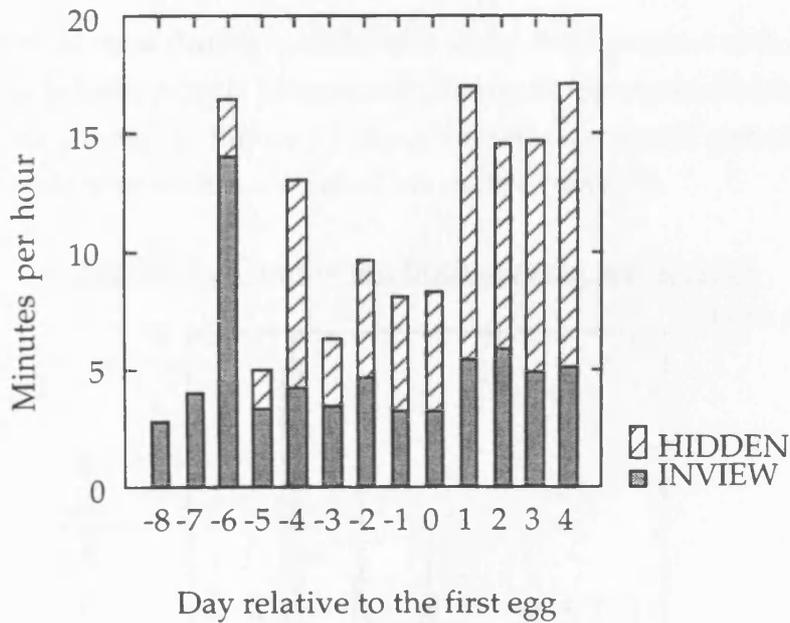
Figure 5 shows how the presence of each sex changes in accordance with the breeding cycle. In the course of the entire cycle, males spend significantly more time around the nest than females (Paired $t = -4.00$ df $= 11$ p < 0.01).

Figure 5. Presence of each parent in relation to the breeding cycle.

Day relative to first egg
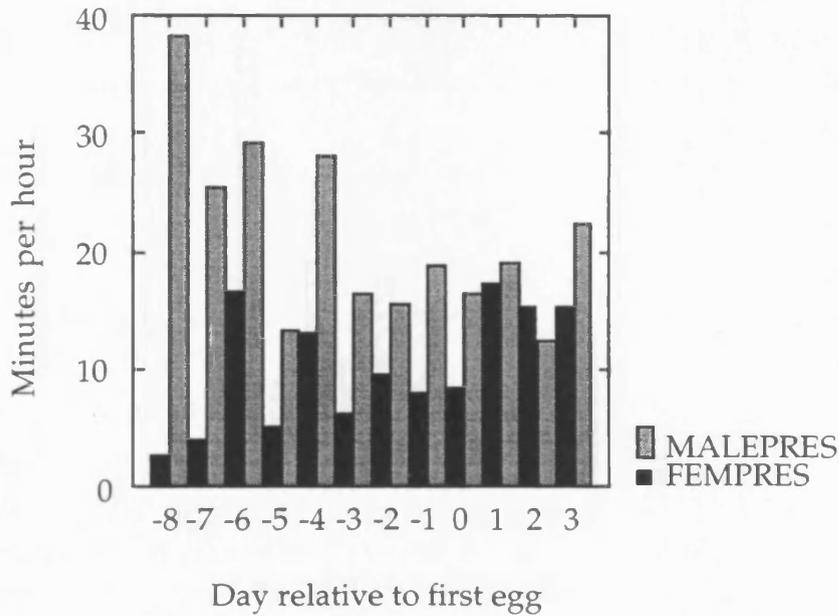
6.3.2 Time in close proximity at the nest

The proportion of time during which both sexes were present at the nest did not vary as the breeding cycle progressed (Figure 6) (Spearman Rank $r = 0.181$ $n = 13$ NS). As shown in Figure 7, when the pair were both present around the nest, the male was usually aware of his mate's presence.

Figure 6. Time when both parents are present.

(mean + SE)

Day in relation to first egg
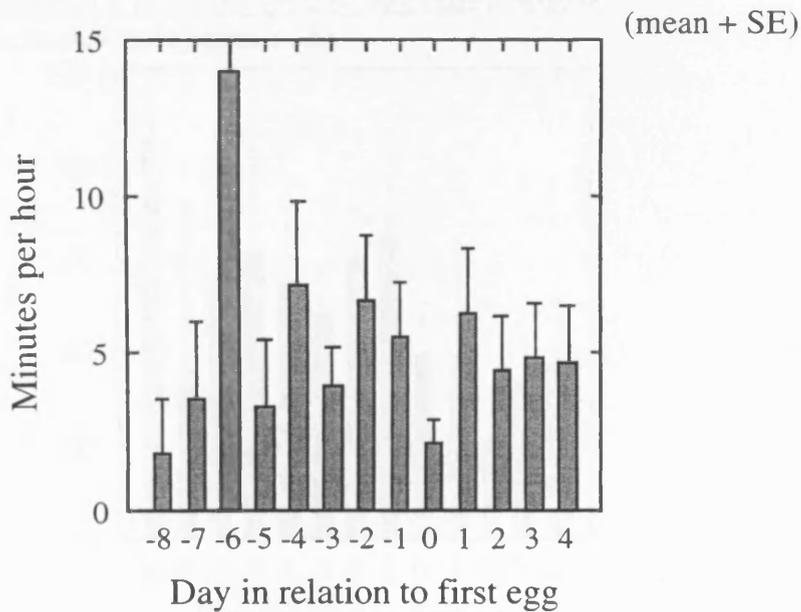
Figure 7. Allocation of time spent 'in awareness' during combined nest presence.
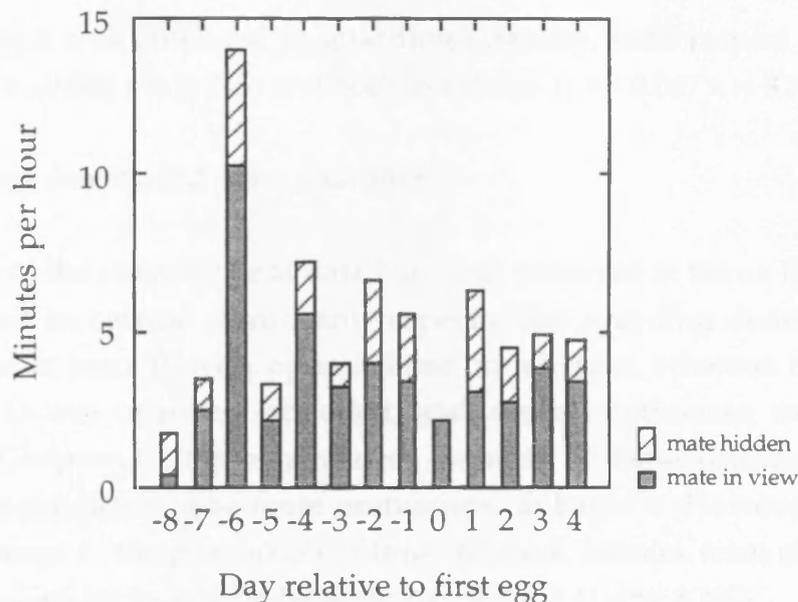
The most pertinent index of mate guarding, however, is the proportion of time which the male spends in association with his mate (i.e. when the female is present, how often the male is with her). As shown in Figure 8, females were accompanied by their mate significantly less frequently as the breeding cycle progressed (Spearman Rank $r = -0.593$ $n = 13$ p < 0.05). Females were equally likely to be accompanied by their mate during the pre-fertile and fertile periods, however (Mann-Whitney U-test $z = 0.06$ NS).

Figure 8. Proportion of time that females were observed to be accompanied by their mate in relation to the nest cycle (mean + SE).
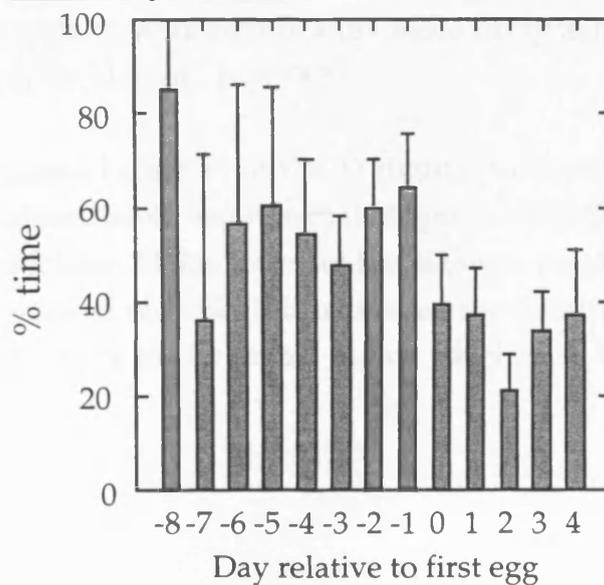
6.3.3 Male phenotype and mate guarding

Male phenotype was unrelated to guarding intensity, with respect to both badge size ($r = - 0.046$ $n = 8$ NS) and body condition ($r = - 0.067$ $n = 8$ NS).

6.3.4 Breeding density and mate guarding

The majority of the observational data had been collected at barns B and D, which differed in several significant respects. The breeding density was slightly lower at barn B, with open habitat on all sides, whereas the area around barn D was relatively occluded, with several outhouses, trees and bushes (see Chapter 1.1 for plan views). Because of these features, mate guarding was predicted to be more pronounced at barn D. However, there was no difference in the proportion of time in which females were observed to be accompanied by their mate between barns ($t = 0.41$ df = 8 NS).

6.3.5 Flight following behaviour

Forty-four definite flights were witnessed. Thirty of these were initiated by the female, nine were initiated by the male, and in the remainder, both pairs appeared to fly simultaneously. Females initiated significantly more flights than would have been expected by chance ($\chi^2 = 11.3$ 1df p = 0.005).

Of the 30 flights which were initiated by the female, the male followed on 22 occasions (73%). Of the nine male-initiated flights, four were followed by their mate (44%). Males were significantly more likely to follow female flights than vice versa ($\chi^2 = 12.5$ 1df p =0.005).

Males had copulated before 11 of the 22 flights that were followed. The pair had copulated immediately before female flight in six of the eight cases where the male did *not* follow. Males were not less likely to pursue their mate if they had recently copulated with her (Fisher's exact test p = 0.407). Copulation did not occur before any of the five male flights which were not followed by the female.

6.4 Discussion

6.4.1 General patterns of mate guarding at Maine Chance

As the breeding cycle progressed, males spent proportionately less time near the nest, whereas females increased their presence. Females presumably spend most of their pre-fertile period away from the barns, to obtain the nutrients required for egg production (Summers-Smith 1963, Murphy 1978a). Whereas males only rarely entered the nest-box, females often remained within the box for prolonged periods, particularly when eggs were present. Since incubation does not commence until the fertile period is completed, this may minimize the risk of infanticide or egg-dumping (although both were rare at Maine Chance). The most likely benefit, however, is the avoidance of unwanted harassment from other males (Summers-Smith 1963). Fertile females perched outside their box were occasionally subjected to multi-male 'communal chases', which may present an injury risk (Summers-Smith 1954). Moreover, Lifjeld (1994) proposed a causal link between infertile eggs in the house sparrow, and the occurrence of these stressful chases (but see Birkhead et al. [1995] for the preponderance of infertility, and also Westneat et al. [1990] for an alternative view of multi-male chases).

The most informative results, and the closest approximation of 'mate guarding', concerned the proportion of female presence in which the males were also present. Mate guarding would have been confirmed if, firstly, females present at the nest were often accompanied by the male and, secondly, the frequency with which males were in attendance increased during the fertile period (Double 1995). Both of these predictions have been upheld in other species (e.g. Møller 1994b).

In the first instance, males were only present for about 50% of the time that females were present, a figure which was used by Møller (1994b) to indicate the termination of mate guarding in barn swallows. Secondly, a male was not more likely to be associated with the female during her fertile period. These results suggest that mate guarding by physical proximity occurs only weakly, if at all, in house sparrows at Maine Chance.

6.4.2 Mate guarding versus male phenotype

Theoretically, males which suffer from the greatest threat of cuckoldry should mate guard most intensively (Møller 1994b). In a separate population of house sparrows, males with large badges were more likely to gain both solicited and forced EPCs from females paired with small-badged males (Møller 1990b). If this bias also occurred at Maine Chance, then small-badged males would have been expected to guard their mates more intensively than males with large badges.

This prediction was not upheld, since the intensity of mate guarding was independent of badge size. However, in this and other populations, cuckoldry was also independent of badge size (Cordero *et al.* 1998, R. Whitekiller & D. F. Westneat *pers. comm.*, Chapter 4), which queries the assumption upon which this prediction was based.

Moreover, studies relating mate guarding to male phenotype are equivocal. In the barn swallow and pied flycatcher there was no relationship between male attractiveness and mate guarding intensity (Møller 1994b, Lifjeld *et al.* 1997). However, whereas attractive male barn swallows were cuckolded *less* frequently (Møller 1994b), attractive male pied flycatchers were cuckolded *more* frequently (Lifjeld *et al.* 1997). This illustrates the generally confounded triumvirate of mate guarding intensity, male phenotype and cuckoldry.

6.4.3 Density

Contrary to expectation, males breeding at higher densities did not guard their mates more intensively. However, density appears to have a variable influence upon mate guarding (Westneat & Sherman 1997). In the starling, breeding density has either a positive (Pinxten *et al.* 1987), negative (Wright & Cotton 1994) or negligible (Double 1995) effect upon mate guarding.

6.4.4 Flight following

Elements of mate guarding were apparent in the proportion of partner flights which were followed, since significantly more males followed female flights than vice versa. In most cases where female flights were not followed, the male had copulated several times with the female only minutes before the

flight, and may have adjudged his paternity as (temporarily at least) assured (Birkhead & Møller 1992). However, half of the female flights which *were* followed had also been preceded by copulation, and hence this explanation of assured paternity seems unlikely.

As expected, it was unusual for females to pursue male flights. Theoretically, the direct cost of their mate's infidelity is lower for females than males (Birkhead & Møller 1992), and female mate guarding is consequently less common or well developed. As an example, females of the facultatively polygynous European starling guard their males by a combination of close proximity and frequent copulation (Eens & Pinxten 1995), apparently to prevent them from attracting a secondary female.

6.4.5   How useful is mate guarding?

The fact that male house sparrows only weakly guard their mates implies that they do not perceive a threat of cuckoldry (Wright & Cotton 1994), or that an unknown factor(s) precludes efficient guarding. In support of the former statement, extra-pair paternity (EPP) at Maine Chance was relatively infrequent compared with most passerines ($c10$ %). Similarly, mate guarding and EPP were both low in a British population of pied flycatchers (Ross 1997).

The evidence that mate guarding reduces the risk of cuckoldry is itself equivocal (reviewed in Birkhead & Møller 1992). Empirical evidence (mostly from removal experiments) suggests that it can function as an effective paternity guard (Dickinson 1997), although several counter-intuitive results have been reported. In the eastern bluebird, for example, males which had guarded their mates intensely were more likely to be cuckolded (Gowaty & Bridges 1991). The 'confident male' hypothesis (Gowaty 1996a) posits that males may relax mate guarding, in favour of the pursuit of EPCs, if they perceive their quality as being higher than that of their neighbours. This situation (which has been alluded to in blue tits [Kempenaers *et al.* 1992]) would generate an apparently paradoxical result, since males which only guarded weakly would not be cuckolded.

The success of mate guarding probably depends upon the nature of EPCs in the species concerned (Birkhead & Møller 1992). In the barn swallow, for instance, rival males attempted to gain EPCs by approaching females directly

(Møller 1994b). These EPC attempts were highly visible, and were rapidly thwarted by the attendant pair male. Mate guarding may be less effective, however, in species where EPCs are solicited surreptitiously by the female. As is now recognized, females may exert fine control over paternity (Lifjeld *et al.* 1994). Since males cannot always guard their mates because of the need to forage (Westneat 1994) females have opportunities to subtly seek EPCs in their absence. Their success in this venture will be determined by ecological constraints such as the distance to the nearest male, and the degree of cover afforded by the local habitat (Birkhead & Møller 1992).

The intensity of mate guarding may be further confounded by variables associated with male quality and experience (Birkhead & Møller 1992). This may be particularly important in species such as the house sparrow, where males use the same nest box in successive years (Summers-Smith 1963), and thereby acquire knowledge of the local topography and optimal vantage points. However, although older male house sparrows were more likely to gain EPFs, they were cuckolded at an equal rate to young males (Wetton *et al.* 1995), which suggests that paternity protection behaviours do not become more efficient with age.

6.4.6 Limitations of the data

Admittedly, the data collected were not sufficiently complete to perform a rigorous investigation into the determinants of mate guarding. On average, each sex spent only 20 minutes of each hour around the nest, and their behaviour and associations during the remaining 40 minutes were unknown. Following individual birds during the course of an observation period would have produced more precise data, but would not have been practical due to the breadth and frequency of their flights. It is also possible that males were 'guarding at a distance', from an unobserved position (e.g. perched in trees above the observation points). This is particularly true at barn D, where several habitat features would have provided an ideal vantage point for males to observe their mate over a wide area. Despite the uncertainties of the data, paternity assurance behaviours are expected to be most pronounced around the nest area in response to the concentrated presence of the other breeding males (Wright & Cotton 1994, Double 1995). The patterns of mate guarding which were observed around the nest-box are therefore assumed to reflect the broader pattern.

Chapter 7. Cloacal protuberance size in the house sparrow

7.1 Introduction

7.1.1 Morphology of the cloacal protuberance

The reproductive system of male birds is comprised of four paired regions; the testis, epididymis, ductus deferens, and a terminal storage region (Birkhead & Møller 1992). In most non-passerines, this storage region is a small and relatively uncomplicated canal, although in passerines, the canal becomes convoluted into a densely packed mass of folds known as the seminal glomus (Birkhead *et al.* 1993b).

At the onset of sexual activity, each glomus becomes enlarged, and forms a visible projection known as the cloacal promontory, or more commonly, cloacal protuberance (hereafter CP) (Quay 1987). In house sparrows, this appears as a firm, bulbous swelling, often with a rostrum of stiff feathers. Both sexes have CPs in the house sparrow, although that of the females is considerably less pronounced (Svensson 1984, *pers. obs.*). The function of the female CP swelling is unclear, although it may help facilitate sperm transfer (Birkhead *et al.* 1993).

7.1.2 Function of the male cloacal protuberance

The size of the CP varies considerably between species. Birkhead *et al.* (1993a) used a cross-specific analysis to test the four main hypotheses proposed to account for the function of the CP.

The first of these asserts that sperm remain viable for longer periods if they are held in the CP, because the local temperature is significantly lower than the core body temperature (Wolfson 1954) (analogous to the external testes of most mammals). Although it is true that non-passerines, which generally have lower core temperatures (McNab 1966), do not have CPs, this finding is not exclusive to the sperm viability hypothesis. Moreover, it is not obvious why sperm maturation, which also occurs in the CP, would be constrained at the higher core temperature.

Alternatively, the CP may aid successful sperm transfer, by acting as a pseudo-phallus (M. Winterbottom *pers. comm.*). As this function is difficult to assess *in situ*, this hypothesis predicts that copulation should be

115

brief in species with large CPs. No relationship between copulation brevity and CP size was found, although as Birkhead *et al.* (1993b) admit, the important variable in determining coital duration is more likely to be the volume of sperm transferred. The third hypothesis, that some species require larger CPs because their sperm are larger, was supported but was generally inconclusive.

The most convincing evidence was found for the sperm competition hypothesis (Birkhead *et al.* 1993a). CPs are particularly well developed in species with complex mating systems such as the polygamous accentors (Nakamura 1990, Davies 1992) and co-operatively breeding Australian fairy wrens (Mulder & Cockburn 1993), and also species with high levels of extra-pair paternity such as the reed bunting (Dixon & Birkhead 1997).

Interspecifically, the intensity of sperm competition (estimated by average copulation frequency and relative testis size) correlated positively with CP size. Hence, in species where males copulate frequently, sperm production is high, and the storage of sperm in the CP becomes more advantageous (Birkead *et al.* 1993b, Birkhead *et al.* 1994). Although this conclusion is confounded by several variables regarding fluctuations in ejaculate size and sperm density, CP size generally appears to reflect the intensity of sperm competition.

7.1.3 The house sparrow cloacal protuberance : aims and predictions

Birkhead *et al.* (1993b) credited house sparrows with relatively large testes and a high frequency of copulation (> 20 per clutch). Consistent with their hypothesis, house sparrows are known to have relatively large CPs (Quay 1987). Theoretically, Birkhead *et al.*'s (1993) sperm competition hypothesis extends to the intraspecific level, so that variation in CP size between males house sparrows should reflect differential sperm storage and utilization. In house sparrows, large-badged males copulate at a higher rate than small-badged males (Møller 1990b). The sperm competition hypothesis therefore predicts that males with large badges and/or a high frequency of copulation should have larger CPs than small-badged males which copulate at lower rates. These hypotheses were tested in the population of house sparrows established at Maine Chance.

7.2 Methods

Males were held in the 'ringers grip', and the hemispherical CP was fully exposed by smoothing away any surrounding abdominal feathers. Vernier callipers were used to measure the length and breadth of the CP at its base to the nearest 0.1 mm ('breadth' = perpendicular to the body axis). The CP height was recorded using reversed callipers. As a novel means of categorizing CP height, a series of C-shaped pieces of card had been previously cut, with inner depths ranging from two to eight millimetres (graded in millimetres). These were placed vertically over the CP until the closest fit was found.

The repeatability of CP measurements was assessed 'blindly' (i.e. the calliper reading was shown to an assistant, before being reset to zero, and the CP measured again without knowledge of the first reading). This could not be done satisfactorily using the C-shaped cards, since they were also colour coded.

7.3 Results

7.3.1 Cloacal protuberance size and the repeatability of measures

Cloacal protuberance measurements were obtained from 46 males. Although Birkhead *et al.* (1993b) state that CPs are circular in cross-section, this was not the case in house sparrows. Almost all of those examined at Maine Chance (42/46) resembled an ellipse, with breadth exceeding length.

Unfortunately, the measurements of CP length and width were not significantly repeatable ($F_{3,4} = 0.191$ NS and $F_{3,4} = 0.142$ NS respectively) and had to be discarded. Both of these had been difficult to measure, as there was no clear demarcation between the abdomen and the base of the CP. The measurements of the CP height were repeatable however ($F_{3,4} = 12.42$ $p = 0.013$). This was convenient, because height is the most widely used index of CP size (Birkhead *et al.* 1993b).

7.3.2 Male phenotype and cloacal protuberance height

Cloacal protuberance height varied between 2.4 and 6.8 mm, and averaged 4.7 mm. CP height was independent of physical traits (tarsal length $r = 0.001$, mass $r = 0.097$ $n = 46$ NS). Male badge size was negatively related to

CP height (Figure 1), although this was not significant ($r = -0.177$ $n = 46$ NS). There was no age-related variation in CP height (adults in at least their second year vs yearlings $t = -0.41$ df $= 13$ NS).

Figure 1. Cloacal protruberance height and badge size.

Badge size (mm2)
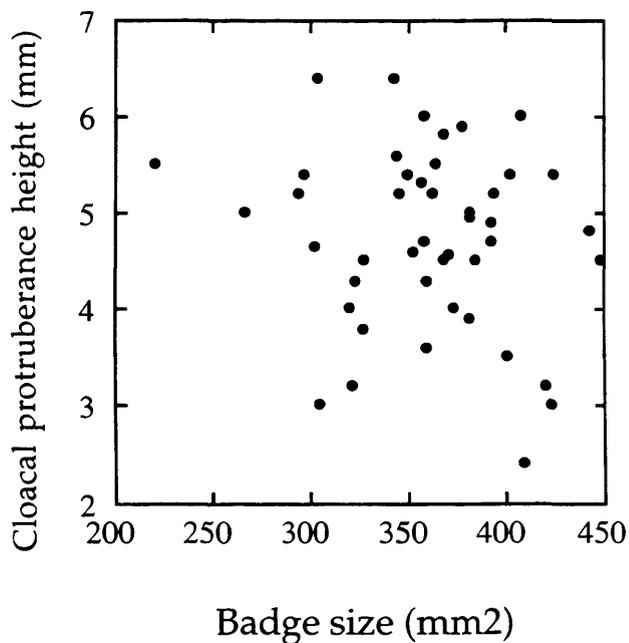
These results may have been confounded by the diurnal pattern of sperm loss. Sperm reserves become progressively depleted throughout the day in house sparrows (Birkhead *et al.* 1994), and need to be replenished during the evening. The seminal gloma, which comprise the bulk of the CP, are used as temporary sperm stores, and it is therefore possible that CP height declines with sperm loss. To control for this possibility, the analysis was repeated after all males which had been trapped and measured after 12 noon were discounted. CP height was still unrelated to morphology (tarsal length $r = -0.023$, mass $r = -0.031$, $n = 21$ NS), and badge size ($r = -0.161$ $n = 21$ NS).

The reliance upon a single CP dimension (height) as an estimate of size may also have been misleading. To counter this, the analyses were repeated with CP volume as the index of size rather than height. The volume of the CP was calculated as though it was a hemisphere (which was a reasonable assumption), with a volume given by the formula $2/3\pi r^3$ (where r = CP height). The use of CP volume as opposed to merely height

had only a minimal effect upon the previous correlations however, and was thus discounted (e.g. badge size r = -0.206 n = 46 NS). All further analyses thus relied on CP height as the measure of size.

The scores from the C-shaped cards were not used in any comparisons, as their repeatability had not been assured. However, as guesses were strongly correlated with measured height (Spearman rank r = 0.380 n = 42 p < 0.05), they probably were reliable.

7.3.3 Relative cloacal protuberance size as a cross-specific comparison

'Volume index' is a standard measure, albeit crude, which relates CP size to body size for use in interspecific comparisons (Dixon & Birkhead 1997). It is calculated by dividing the product of CP length, breadth and height by average body mass. The volume index of 46 Maine Chance males ranged between 1.5 and 10, and averaged 6.4.

Using a large, cross-specific data set, Birkhead et al.(1993b) generated the following regression equation which relates CP height to body size ;

$$\log \text{CP height} = 0.67 * \text{body mass}$$

The mean mass of 50 males caught during the breeding season at Maine Chance was 28.3g, which predicts a CP height of 8.22 mm when fitted to the above equation. The average CP height of 46 males was actually only 4.74 mm, substantially lower than Quay's (1987) reported average of 8 mm.

7.3.4 Copulation frequency and cloacal protuberance height

Unfortunately, several males with adequately detailed copulation rates were never caught, and hence their CP size was unknown. Data from the six available males produced a negative correlation between cloacal protuberance height and copulation rate (Figure 2), although this was not significant $(r$ = -0.540 n = 6 NS).

Figure 2. Copulation frequency and cloacal protruberance height.

Cloacal protruberance height (mm)
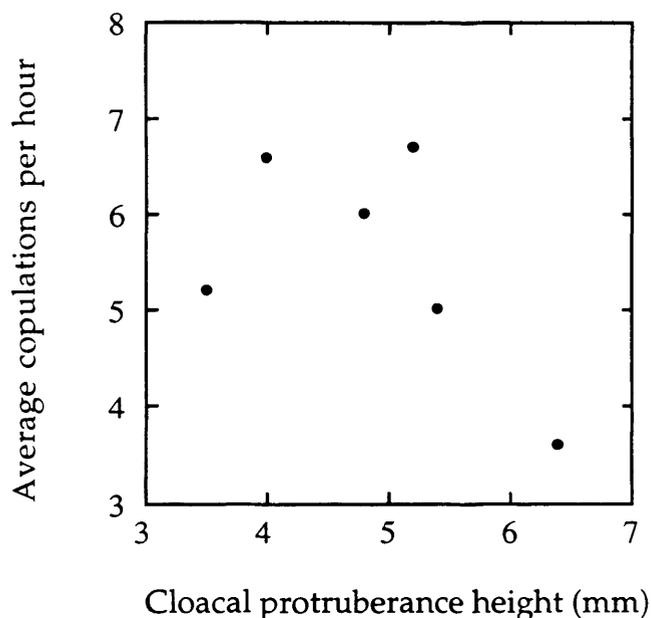
7.4 Discussion

7.4.1   The measurement of cloacal protuberance height

Obtaining repeatable CP measurements in the field proved to be difficult, which may be limiting the power of the analyses. Ideally, the precise volume of the CP would be known, as opposed to the single linear dimension which was used in this and other studies. As has been found in other species however, the actual shape of the house sparrow CP varied little between individuals (Mulder & Cockburn 1993, Dixon & Birkhead 1997), and CP height remains constant once the breeding season has started in earnest (Hegner & Wingfield 1986). Furthermore, although sperm depletion is known to occur on a daily basis in the house sparrow, most of the seminal gloma which underlies the CP is actually tissue rather than sperm, and hence CP height is unlikely to change during short periods. Theoretically, a simple formula involving length, breadth and width should suffice for calculating the volume of each CP, provided that the dimensions could be recorded accurately.

7.4.2   Cloacal protuberance height and its influences

Although the variation in CP height between males was by no means dramatic, it was substantial, with the largest CPs being almost three times

the size of the smallest. Despite this variation, no correlates of CP height were apparent. CP height was independent of body size, which suggests that the protuberance is unlikely to have any somatic function (as an allometric relationship would have been expected).

Moreover, there was no evidence to suggest that CP height was linked to sperm competition. Indeed, badge size was actually negatively correlated with CP height, which is contrary to the prediction of the sperm competition hypothesis. Although the data set was small, CP height was negatively correlated with copulation frequency. Although no data on copulation frequency and CP height is available from other intraspecifc studies, this finding also contradicts the sperm competition hypothesis.

7.4.3 Cloacal protuberance or cloacal 'pizza' ?

House sparrow CPs are relatively small compared to their body size. Their mean volume index of 6.4 is considerably less than that of the reed bunting (26.7), dunnock (22.9) and chaffinch (28.0) (Sheldon & Birkhead 1994, Dixon & Birkhead 1997). Of these three examples, the reed bunting exhibits intense sperm competition, and the dunnock has a complex mating system (Davies 1992). Although an ability to store large quantities of sperm would obviously be beneficial in both of these circumstances, this is not true of the chaffinch. Like the house sparrow, the chaffinch is socially monogamous, with a relatively low level of extra-pair paternity (EPP) (17%) (Sheldon 1994b). If CP size is related to the intensity of sperm competition, then it is unclear why, firstly, the chaffinch would have such a large CP, and secondly, why the house sparrow has such a small CP.

The average CP height reported from Maine Chance was about half of the average reported by Quay (1987), which is puzzling. Although Quay (1987) was vastly experienced in recording CP dimensions from a variety of birds, there is no reason to doubt my own measurements. House sparrows at Maine Chance do have relatively small CPs.

7.4.4 Is the cloacal protuberance really related to sperm competition?

Although reproductive adaptations such as CP height vary in tandem with the intensity of sperm competition (Birkhead *et al.* 1993a), the

molecular revelations of infidelity suggest that this interaction may be less obvious than realized. Splendid fairy wrens have pronounced CPs, and exhibit the highest level of extra-bond paternity thus far recorded (76%). Paradoxically however, their copulation rates are relatively low (Mulder *et al.* 1994). Furthermore, the reed bunting, which has a remarkably high incidence of EPP (55%, Dixon *et al.* 1994), has a large, cylindrical CP, but also copulates at a surprisingly low rate (Dixon & Birkhead 1997).

These results imply that CP size is indeed related to the intensity of sperm competition, but is primarily governed by the frequency of EPCs rather than WPCs (Dixon & Birkhead 1997). Almost by definition, EPCs may be restricted to a single mating. It is important, therefore, that males intent on cuckoldry have a sizeable store of sperm available should an EPC opportunity arise. For example, male zebra finches produce larger ejaculates during EPCs than WPCs (Birkhead *et al.* 1991), which would rely upon readily available sperm. The discrepancy between copulation rate and CP size noted in the fairy wren and reed bunting may also arise through an observational bias, since in most birds, EPCs are rarely observed in comparison with WPCs (Birkhead & Møller 1992).

If CP size has evolved in relation to a species' predilection towards infidelity, one would still have expected a positive correlation between CP height and badge size. Although no EPCs were witnessed at Maine Chance, large-badged males in a separate population were more likely to achieve EPCs than small-badged males (Møller 1990b).

In conclusion, despite the large sample sizes involved, no correlates of CP height variation were found. This may be occluded by the differential investment between males in EPCs and WPCs. However, a more parsimonious conclusion is that variables such as the timing of copulation and sperm quality are more important in regulating sperm competition at Maine Chance than the capacity to store sperm *per se*.

Sex ratio variation in nestling house sparrows

8.1 Introduction

8.1.1 Sex ratios in Nature

Most vertebrate populations contain a roughly equal number of males and females. With characteristic prescience, Fisher (1930) explained why this parity is so commonly observed. Individuals which are genetically predisposed into producing a surplus of males lose their advantage as this sex becomes progressively more common. Individuals predisposed into producing females then gain the ascendancy, until the surfeit of females allows male-producers to regain the initiative. Consequently, the advantage of producing biased broods of either sex perpetually fluctuates.

Although Fisher's rule is robust, several of its rather restrictive assumptions have led to refinements. As Hamilton (1967) noted, Fisher's rule assumes that the competition for mates is population-wide, whereas in Nature, sex differences in dispersal enhance sibling rivalry and lead to Local Mate Competition (LMC), in which case sexual parity is not the ideal state.

Nor does Fisher's rule make any prediction about the most effective parental strategy concerning the allocation of resources as opposed to gender (Clutton-Brock 1982), particularly in species where the offspring vary in size. In a hypothetical species where sons are twice the size of daughters, sexual parity in a brood of six represents an equal *gametic* investment but a biased *resource* investment. In this case, a propensity for 'equal resources' (two sons and four daughters) is more likely to be evolutionarily stable.

8.1.2 The determination of sex in birds

Although in several groups of birds, the adults show marked sexual dimorphism (e.g. ducks, many passerine families), in others (e.g. many seabirds, gulls, corvids) the adults cannot be identified at any distance save for a limited range of behavioural cues. Until recently, these species could only be sexed using surgical techniques (laparatomy), cloacal eversion (waterfowl only), flow cytometry, or chromosomal analysis (particularly

useful in species such as woodpeckers (Piciformes) which have unusually enlarged sex chromosomes (Shields *et al.* 1982).

This is particularly true of nestling birds, as most are monomorphic in both size and appearance. Even in species where a size difference becomes apparent at an early age (e.g. red-winged blackbirds, Falconiformes (Gowaty 1994)) the overlap is considerable, and may often be confounded by hatching order. This uncertainty has restricted investigations of sex-related differences in life history components such as growth, dispersal and survival.

In the wake of the DNA fingerprinting revolution of the late 1980s (Burke 1994), several workers discovered (fortuitously) that the minisatellite probes used in paternity analyses simultaneously detected regions of the W-chromosome to produce a female-specific autoradiograph band (Graves *et al.* 1993). In birds, unlike most vertebrates, the females are the heterogametic sex, and possess a W-chromosome analogous to the Y-chromosome of human males (consequently, female birds are represented in genetic 'shorthand' by Z/W, and males denoted by Z/Z).

Quinn *et al.* (1990b) could restrict probing to sex identification in snow geese, after managing to clone a sequence derived from the W-chromosome. Despite this narrow focus however, their technique still involved lengthy and hazardous autoradiography, and required relatively large volumes of blood. The critical advance in molecular sex typing occurred when Griffiths *et al.* (1992) serendipitously amplified a W-linked marker from a minute drop of starling blood using the Polymerase Chain Reaction (PCR). Although this particular marker appeared to be restricted to the Sturnidae, others have since been developed which have amplified sequences from all non-ratites thus far tested (Griffiths *et al.* 1998).

The target gene (known as *CHD*) is remarkably conserved among birds, and actually exists in two copies. One copy (*CHD-W*) is restricted to the female W-chromosome, and the other occurs elsewhere in the genome of both sexes (*CHD-NW*). Although both amplify equally, minor size differences in the products generated allow them to be distinguished through electrophoresis (Ellegren & Sheldon 1997).

The ease with which this can be accomplished, and the minimal volumes of blood required, has led to a resurgence of interest in nestling sex ratios (Ellegren & Sheldon 1997). This examines the intrinsic and extrinsic circumstances in which overproduction of a particular sex represents a maternal adaptation to maximize lifetime reproductive success. According to the two leading hypotheses, females exhibit facultative sex ratio bias in response to different criteria.

8.1.3 The Maternal Condition model

In most vertebrates, reproductive success varies more widely between males than between females (Trivers 1972). Low quality males often fail to gain any matings, whereas females rarely remain umated, irrespective of their quality or rank. From this simple observation, Trivers & Willard (1973) hypothesized that the offspring sex ratio depends upon maternal condition : females in good condition should produce more sons, whereas females in poor condition should produce more daughters.

The maternal condition model has been supported in several rigorous experimental studies, particularly of mammals. Mice, opossums, coypus and hamsters all tended to produce daughters when fed on poor quality diets, and sons when fed on high quality diets (Ridley 1995). Red-winged blackbird broods which are produced early in the season contain more sons than those produced later (Fiala 1981), when the females' condition has presumably declined.

However, other studies have produced results which are ambiguous or even contradictory. White-tailed deer fed on low protein diets produced more sons than daughters, and early season broods of the common grackle contained more females than those produced later (Howe 1977). In red deer, offspring sex ratio was independent of female condition, although high ranking does produced more sons (Clutton-Brock 1982).

8.1.4 The Mate Attractiveness model

In a captive population of zebra finches, Burley (1981) artificially created 'attractive' males by fitting them with plastic rings of a 'preferred' colour. Female finches produced more sons when paired to attractive males.

Although the result was not widely accepted (because of the small sample sizes involved), it was nevertheless the first experimental demonstration of a facultative sex ratio bias (Clutton-Brock 1982).

Recent evidence from two nest-box studies has supported Burley's theory. Female blue tits produced more sons when paired to males with a high probability of overwinter survival (Svensson & Nilsson 1996), although the phenotypic cue of quality (and hence attractiveness) was unknown. Collared flycatcher broods contained more male nestlings if the father's forehead patch (a secondary sexual character) was large (Ellegren *et al.* 1996).

8.1.5   Offspring sex allocation in birds

The first evidence of non-random offspring sex determination was indirect , when the sex ratio was reported to vary with laying sequence in the ring-billed gull (Ryder 1979), lesser snow goose (Ankney 1982), and a single passerine, the common grackle (Howe 1977). The evidence from the two precocial birds in particular provided compelling support for the Trivers-Willard maternal condition hypothesis (1973).

Ring-billed gulls almost invariably lay three-egg clutches, and each brood must therefore contain an excess of one sex. In the population studied by Ryder (1979), there was a marked surplus of females, such that many homosexual pairs were formed. After sexing entire broods at necrospsy, Ryder (1979) failed to uphold his prediction that this bias was due to an overproduction of females at the nestling stage. Although the overall brood sex-ratio was equal, the first egg was more likely to produce a male chick , and the second egg was more likely to produce a female chick. Since the chick which hatches from the first egg usually gains a size advantage, Ryder (1979) interpreted this as an adaptive strategy by the female to imbue sons with greater initial resources.

The last-laid egg (the C-egg) is significantly smaller than the other two, and the chick which hatches from this egg is relatively unlikely to fledge unless food is particularly abundant (Parsons 1970). As expected, the sex ratio of gulls which hatch from this 'insurance' egg was 1 : 1.

On a similar theme, Ankney (1982) reported that of the four eggs laid by most lesser snow geese, the first two eggs tended to produce male goslings, whereas the final two tended to produce females. Moreover, the two earlier eggs were significantly heavier than those laid later in the sequence, and produced goslings which were physically larger and grew more rapidly. As a result, male goslings appear to have an immediate selective advantage. This was strong evidence for the Trivers-Willard hypothesis, since laying female geese draw heavily on stored food reserves acquired on spring staging grounds (Cooke *et al.* 1994). In addition to their greater size, eggs laid earlier in the sequence may thus be of better quality (i.e. higher yolk content) than the late-laid eggs which are produced by females in worse condition. However, Ankney's result could not be repeated in a separate snow goose study involving considerably larger sample sizes (Cooke & Harmsen 1983). Whether his result was genuine, or merely a statistical artefact of small sample sizes , is debatable (Cooke *et al.* 1995)

8.1.6 How can birds adjust the sex of their offspring?

Although the exact mechanism of sex-determination in birds is still unknown, three possible methods have been proposed;

    i)   non-random segregation of sex chromosomes
    ii)   differential growth of follicles of either sex
    iii)   temperature induced sexual differentiation

Although the evidence for each is equivocal, the first mechanism is most widely invoked (e.g. Howe 1977, Ryder 1979). The third mechanism, temperature dependence, is perhaps the least plausible. This phenomenon has been reported most famously in reptiles (Bull 1983), and the traditional (though now disputed, Thomas 1997) view of birds as ' glorified reptiles ' no doubt adds to the appeal of this theory. However, since the avian egg is 'incubated' before it is laid (Romanoff 1960), thousands of cells have already developed before the nascent embryo is exposed to any environmental stimuli. While avian sex is by no means 'fixed', it seems reasonable to assume that it is established before laying.

8.1.7 How should sex ratios be biased in house sparrows?

Both competing hypotheses make clear predictions about the direction of the sex ratio bias. Under the Trivers-Willard hypothesis, females house sparrows in better physical condition should produce broods containing proportionately more males. Furthermore, if condition declines during the season as a consequence of prior reproductive effort, the proportion of males in all broods should consequently show a decline. Under the male attraction model (Burley 1981), females paired to large-badged males should produce a higher proportion of sons.

8.1.8 Sex allocation in house sparrows

To assess the costs and benefits of offspring sex allocation, it is important to know whether sons and daughters differ in size (and hence their rearing requirements). Fortunately, size data had already been collected from nestlings of a standard age (ND_{10}) as part of the field protocol, and this could be analysed retrospectively once the sex of processed nestlings was known.

8.2 Methods

Blood sampling and DNA extraction were carried out using the methods described in Chapter 2. In 1995, blood samples were only obtained from nestlings ringed at ND_{10}, whereas in 1996 blood was obtained from several hatchlings which later died.

The general PCR protocol followed that of the paternity analysis described in Chapter 13. The primers used (P2 and P8) were taken from Griffiths *et al.* (1998), and have the following nucleotide sequences ;

$$P2 : \quad 5' - TCT \; gCA \; TCg \; CTA \; AAT \; CCT \; TT - 3'$$
$$P8 : \quad 5' - CTC \; CCA \; AGG \; ATG \; AGr \; AAy \; TG - 3'$$
$$(r = A / g \; , \; y = C / T)$$

The PCR conditions involved an initial denaturing step of 94 $^\circ$ C for 90 secs, and then 40 replicates of the following cycle ;

$$94 \,^\circ C/15 \, s, 50 \,^\circ C/20 \, s, 72 \,^\circ C/25 \, s$$

The PCR products were distinguished using single stranded conformation polymorphism (SSCP). The denatured DNA strands from either sex form slightly different three-dimensional shapes according to their size. These move through the gel matrix at different rates, and since females have products from both the *CHD-W* and *CHD N-W* genes, two bands are produced. Males only produce the band corresponding to the latter gene. Once the reaction was complete , samples were heated at 94° C for a further 180s, and electrophoresed through a denaturing polyacrylamide gel for at least 30 minutes. Samples were usually loaded in ' piggy-back ' style (i.e. into partly-electrophoresed paternity gels), allowing PCR products from each locus to be visualized simultaneously using the silver staining technique (Bassam *et al.* 1991).

House sparrows of each age class were measured as described in the general field protocol (Chapter 1).

8.3 Results

8.3.1 Sexual size differences of nestlings in 1995

Ninety four nestlings were sexed in 1995. There were no significant size differences between nestlings of each sex (tarsal length Mann-Whitney $U = 1165$, ND_{10} mass Mann-Whitney $U = 949$, both df = 94 NS).

8.3.2 Sexual size differences of nestlings in 1996

Nest monitoring was considerably more intensive in 1996. ND_{10} masses were collected after acounting for the intra-brood age spread which usually persisted following asynchronous hatching. A further measure of nestling size (wing length) was recorded.

As shown in Table 1, there were no sex-related differences in either mass or wing length (Mann-Whitney $U = 870$, Mann-Whitney $U = 867$ respectively, both df = 89 NS), male nestlings had significantly longer tarsi than females (Mann-Whitney $U = 779$ df = 89 p = 0.024). Furthermore, male nestlings were in better condition on ND_{10} than females (Mann-Whitney $U = 711$ df = 87 p = 0.026).

129

Table 1. A comparison of nestling morphometrics in 1996.

(* = p < 0.05)

	Male	Female
Tarsal length	19.02	18.73 *
Wing length	51.52	50.71
Mass	25.11	24.41
BCI	0.118	- 0.097 *

In two similar studies, both Lowther (1979) and Schifferli (1980) found that male nestlings were slightly larger than females with respect to most traits , although there was no significant dimorphism. Despite the latter result, male nestlings may not actually *fledge* in better condition. Nestling mass decreases significantly after ND_{10} , and fledging weights were not known (and indeed are notoriously difficult to obtain from any passerine). In fact, tarsal length is the only truly credible nestling morphometric contrasted here, as it is immutable once fully developed (> ND_7).

As a partial validation of the use of ND_{10} mass, data was available from 38 birds which were ringed as nestlings, and later recaptured and re-weighed as juveniles. Individuals showed a significant increase in mass with age (nestling average = 25.17 g, juvenile average = 27.21g, Paired t = 6.05 df = 37 p < 0.001) However, this difference was consistent, as the two weights were strongly and positively correlated (r = 0.419 n = 38 p < 0.01).

These results contrasted with those reported for adults (Table 2), in which tarsal lengths did not differ between the sexes (t = 0.973 df = 85 NS), and males had significantly longer wings (Mann-Whitney U = 1729 p = 0.001). As was found in nestlings, there was no weight difference between adults of either sex (t = 1.38 df = 82 NS). There were no significant differences in beak dimensions between adults of either sex, although these measures were not recorded from nestlings and cannot be compared. Beaks are not fully developed until several months after fledging (*pers. obs.*).

Previous studies have also found that males are slightly larger than females (about 2% on average for each trait), but not significantly so (Lowther 1979)

Table 2. A comparison of adult morphometrics in 1996.

($¥$ = p < 0.1, *** = p < 0.001)

	Male	Female
Tarsal length	19.19	19.06
Wing length	78.99	75.75 ***
Mass	28.32	27.77
Beak length	12.52	12.74
Beak depth	8.35	8.27 $¥$

A cursory examination of the tables indicates that females with smaller tarsi become lost before the breeding season commences, and that the wing length difference present in adults is not manifested while the birds are in the nest.

8.3.3 Sexual size differences of juveniles in 1996

Juveniles were trapped in an attempt to measure the intensity of natural selection. These were sexed according to the presence or absence of a dusky throat patch (Johnston 1967), as only males exhibit this precursor of the black 'badge'. Of the 59 juveniles which could be definitely aged, 31 had been originally ringed as nestlings at the study barns. Around four weeks had elapsed between ringing and recapture, and although the beaks were not fully developed, primary emargination was complete, allowing final wing length to be measured.

There were no significant morphometric differences between juveniles of either sex, although several trends were apparent (Table 3). Male juveniles had longer wings and were heavier than their female counterparts, although this was not significant (t = - 1.92 df = 27p = 0.066, t = 1.77 df = 57 p = 0.083 respectively). Tarsal length did not differ between the sexes (t = 1.10 df = 57 NS). Lowther (1979) also found that recaptured male juveniles were slightly heavier than females, although were otherwise equal.

131

Table 3. A comparison of juvenile morphometrics in 1996.

($¥$ = p < 0.1)

	Male	Female
Tarsal length	19.18	19.03
Wing length	76.70	75.32 ¥
Mass	27.57	26.74 ¥

8.3.4 Sex ratios in 1995

Ninety four nestlings were sexed in 1995, of which 52 (= 55 %) were males. This ratio does not differ significantly from unity (χ^2 = 1.08 1 df NS). Most females in 1995 (and also in the following year) produced several broods. These were pooled to give an average within-season sex ratio for each female.

8.3.5 Maternal condition in 1995

The proportion of males produced by each female during the course of a season was independent of their body condition (r = - 0.199 n = 8 NS) and also of its two morphometric components (tarsus r = - 0.202, mass r = 0.081, both n = 8 NS). Considering each brood as an independent data point, the proportion of males produced actually increased as the season progressed, although this was not significant (Spearman rank r = 0.324 n = 28 p < 0.1).

Figure 1. Proportion of males produced versus time of season.
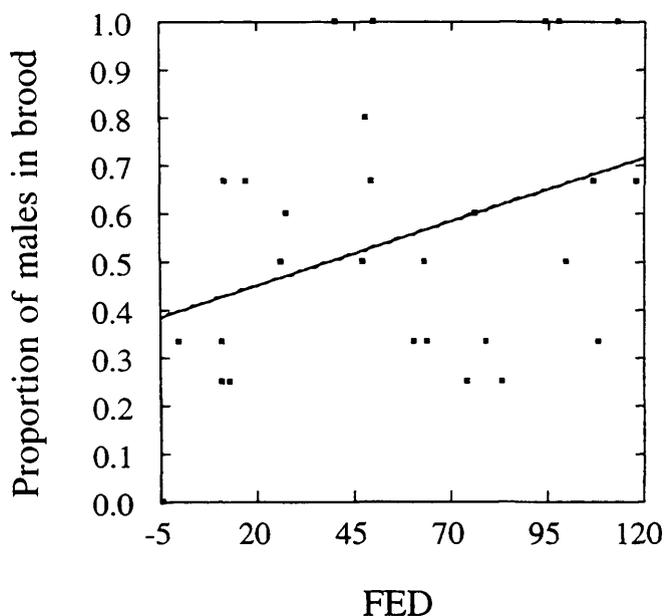
Considering sequential broods of the same female, the proportion of sons produced was not consistent ($F_{6,13}$ = 1.38 NS). Eight of the monitored females produced at least one brood during the season, and I thus compared the sex ratios of their first and last broods. Since there was a period of around two months between the production of these broods, the maternal condition hypothesis predicts that the proportion of males should be lower in the later broods. However, in three of these pairings the ratio remained constant, and in the remaining five cases the proportion of males was actually higher in the final broods.

8.3.6 Mate attractiveness in 1995

Females paired to large-badged males did not produce relatively more sons (r = 0.195 n = 8 NS). However, when the analysis was restricted to the five intact broods (i.e. all eggs which hatched produced a sampled nestling) where the male had been trapped there was a strong negative relationship between badge size and the proportion of males (r = - 0.581 n =5 NS), although this was not significant.

8.3.7 Sex ratios in 1996

One hundred and one nestlings were sexed in 1996, of which 43 (= 43 %) were male. This is not significantly different from a 1: 1 ratio (χ^2 = 2.24 1 df NS). Trapping success was relatively low at barn D, and although productivity was higher than in the previous year, the parental sample size is comparably small.

8.3.8 Maternal condition in 1996

Females in better condition did not produce more sons (r = 0.174 n = 7 NS). Although offspring sex ratio was independent of maternal structural size (tarsus length r = 0.432 n = 7 NS), heavier females produced more sons (r = 0.735 n = 7 NS) (Figure 2)

Considering each brood as an independent data point, the sex ratio remained constant throughout the season (r = 0.222 n = 25 NS). When this analysis was restricted to intact broods, there was still no relationship (r = 0.239 n = 12 NS). Females did not produce a consistent ratio of sons throughout the season ($F_{5, 11}$ = 0.233 NS). Coincidentally, the sex ratios of first and last broods of the season could again be compared for eight females. In most of these (six), the

133

proportion of sons was actually higher in the final broods, in contradiction to the maternal condition hypothesis).

Figure 2. Proportion of male nestlings and maternal mass.
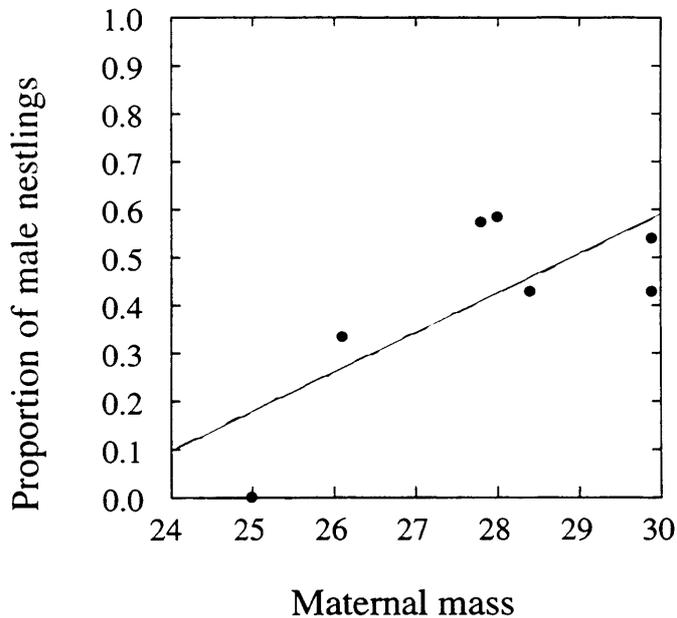
8.3.9 Mate attractiveness in 1996

Although not significant, there was a clear positive relationship between the proportion of sons a female produced and the badge size of her partner ($r = 0.640$ $n = 6$ NS) (Figure 3).

134

Figure 3. Proportion of male offspring and badge size of mate.

Figure 3. Proportion of male offspring and badge size of mate.
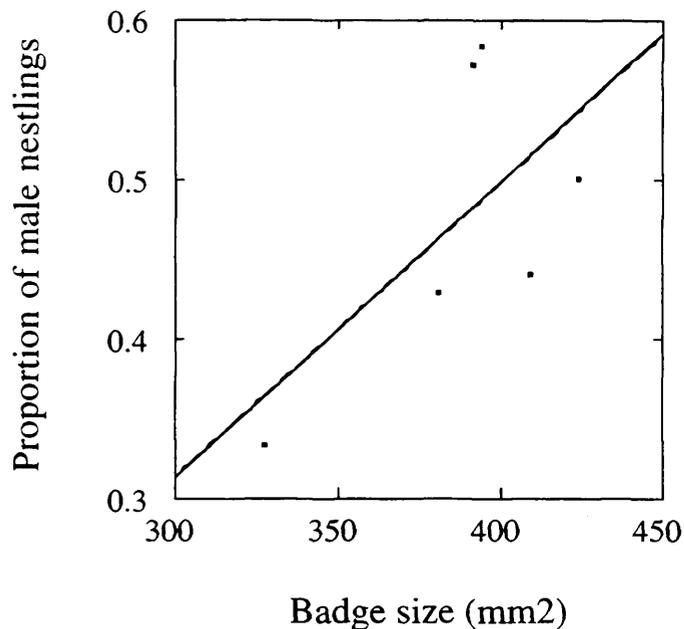
8.3.10 Sex of extra-pair nestlings

Eleven of the sexed nestlings in 1995 resulted from extra-pair matings.
Six of these were male, and five were female. Only five of the nestlings sexed
in 1996 were illegitimate, of which three were male and two female.

8.3.11 Sex of nestlings lost through brood reduction

Tissue samples were obtained from 31 nestlings which were found dead in
the nest with an otherwise successful brood. Blood samples are also available
from 30 hatchlings which presumably died and were ejected by their parents.
Unfortunately, these have not yet been sexed, and it is currently unknown
whether there was a sex-bias in nestling mortality.

8.3.12 Nestling sex in relation to laying sequence

Although the precise laying sequence was known for around a hundred
clutches, the majority of eggs in each clutch tended to hatch synchronously,
such that individual hatchlings could not be assigned to particular eggs.
Exact hatching sequences are notoriously difficult to determine in most
passerines, primarily because of this synchrony. A destructive approach was

utilised in the house sparrow by Cordero *et al.* (1998), who numbered eggs as they were laid, then merely collected the completed clutches midway through incubation. Using DNA from the semi-developed embryos, the authors established that both infertile eggs and extra-pair young tended to occur early in the laying sequence. This technique could be used to determine whether nestling sex varied with clutch position.

8.4 Discussion

8.4.1 Nestling size variation

Although male nestlings had significantly longer tarsi, and tended to be slightly larger in all dimensions, sons and daughters were of similar sizes. This accorded with two previous studies of the house sparrow (Lowther 1979, Schifferli 1980), which found male nestlings to be around 2% larger than females, although the differences were not significant. This suggests that the costs of rearing sons and daughters are similar.

8.4.2 Juvenile size variation

If these juveniles were representative of the local population, these data suggest that natural selection has removed the female fledglings with small tarsi. The wing length difference between juveniles of either sex was apparent, but not significant, suggesting that selection removes males with smaller wings during the winter.

These results contrast with the direction of overwinter selection reported in a northern population of sparrows (Johnston & Fleischer 1981, Fleischer & Johnston 1982). The distribution of size categories changed differently in each sex as a result of selection pressures caused by severe winters. Post-winter males were larger than pre-winter males, whereas post-winter females were smaller than pre-winter females (Johnston & Fleischer 1981).

In a more detailed analysis of selection upon body size proportions as opposed to overall size, Allen's biogeograhic rule was supported for females but not males (Fleischer & Johnston 1982). Females were under strong selection for smaller limbs, whereas males with larger cores were favoured. Presumably, the selective advantage for males derives from food acquisition,

since larger males are more dominant over winter food sources (Cink 1977). Since males are generally dominant over females at feeding sites (Cink 1977), selection favours females with high core:limb ratios which minimize heat loss. However, Johnston & Fleischer's study population was located in Calgary, Canada, where the average winter temperature was under - 40 °C. The winters in Kentucky are mild (rarely below zero), and although competition for food will be an important determinant of survival and hence selection, the remarkable similarity between the tarsal lengths of juveniles and adults of both sexes suggests that the disruptive selection demonstrated in Calgary does not occur at Maine Chance.

Selection does not act upon sexual size dimorphism as such, but depends upon the advantages for each sex of being a particular size (McGillivray & Johnston 1987). The only morphometric differences between adults at Maine Chance were seen in wing length and beak depth (which was, moreover, only a trend). It is not clear why over winter selection appears to favour males with longer wings, although there is evidence that male beak depth is a sexually selected character (Møller 1989, Kimball 1996).

8.4.3   Nestling sex and dispersal

Rather than being caused by post-fledging dispersal, these results may merely reflect sex-biased dispersal. In two separate populations, female sparrows dispersed more readily than males (Lowther 1979, Fleischer *et al.* 1984). The female fledglings with small tarsi, which were assumed above to have died, may have merely dispersed longer distances to avoid competition.

However, of the nestlings which were ringed at the study barns and were later recaptured upon returning, most were females (24 of 31 juvenile 'recaps'). Although this does not demonstrate that females are more philopatric at Maine Chance, as it is not known where they eventually bred, it does suggest that they do not disperse more readily. Thus, independent of dispersal, female fledglings with small tarsi appear to be at an immediate selective disadvantage.

8.4.4 Nestling sex ratios

The sex ratio did not differ from unity in either year of the study, although this is largely attributable to the small sample sizes involved. To detect a significant departure from a 1 : 1 ratio requires either a considerable skew, or large sample size. Since avian sex ratios are rarely dramatic, several hundred nestlings need to be sampled. For example, in Schifferli's (1980) study of house sparrow sex ratios, he quoted an adult sex ratio of 52% males. To demonstrate that this was statistically significant, with β set at the usual level of 0.80, would have required a sample size of 2, 438 birds (β being the probability of making a Type II error, and accepting the null hypothesis when in fact it should be rejected, Sokal & Rohlf 1981).

8.4.5 Maternal condition and offspring sex ratio

There was no convincing evidence of a condition-dependent sex-ratio. In 1996, heavier females produced significantly more males, although their body condition *per se* did not affect offspring sex ratio. Furthermore, the critical variable is the condition of the female at the time of ovulation. Since females were usually only caught and weighed once or twice per season, the overall figure of body condition used here is necessarily subject to error. Pooling the data from successive broods may also have confounded any relationship.

The sex ratio did not become biased towards daughters as the season progressed in either year. This was strong evidence against the maternal condition hypothesis, as it seems reasonable to assume that all females suffer a decline in physical condition towards the end of the season, irrespective of their initial condition.

8.4.6 Mate attractiveness and offspring sex ratio

Females did not appear to produce more sons when paired to more attractive males, although again, the data were few. However, the crux of this hypothesis when applied to house sparrows is the assumption that males with large badges are in some way 'attractive'.

Although large-badged males did indeed have higher reproductive success at Maine Chance, this is only an indirect demonstration of attractiveness. After

controlling for confounding effects such as territory quality (Møller 1989) and timing of reproduction (Kimball 1996), this relationship has not been found in other populations (Chapter 2). More importantly, captive trials have yet to demonstrate conclusively that a female preference for large-badged males exists (Chapter 2).

8.4.7 Limitations of the study

A recurrent problem with these analyses was the paucity of 'intact' broods (i.e. those with no nestling mortality). Since brood reduction was common in both years of the study, the sex ratios were mostly recorded from incomplete broods. However, once the samples taken from hatchlings which later died have been sexed, considerably more broods will be completed.

Of the 12 broods classed as 'intact' in 1996, only three were genuinely intact, since nine also contained an egg which failed to hatch. Although all of these eggs appeared to be infertile, they may have contained embryos which died at a very early stage (Birkhead *et al.* 1995). The true primary sex ratio can therefore only be established once the gender is known of both perished embryos and unfertilised eggs. The sensitivity of the PCR makes this a theoretical possibility (Ellegren & Sheldon 1997).

8.4.8 Should one expect a sex-biased mortality?

It is difficult to imagine that the sex ratio of unfertilised eggs or dead embryos would be anything other than 1 : 1. Eggs fail to develop or hatch for a multitude of reasons (Koenig 1982), and failure can never be adaptive, irrespective of a bird's condition or status.

Sex allocation theory would predict that the nestlings lost through brood reduction are more likely to be female. At Maine Chance, and in other house sparrow populations, the last hatched nestling is usually at an immediate competitive disadvantage (mediated through asynchronous hatching) and often starves to death. If the conditions at hatching are favourable, then this final nestling may fledge successfully, but in relatively poor condition. A daughter in poor condition is more likely to realize at least some offspring than an undersized son.

139

8.4.9   Sex ratio or sex allocation?

Theoretically, parents could influence the fledging sex ratio by preferential feeding of a particular sex. For instance, if a brood composed of two sons and two daughters hatched synchronously at a time when food was abundant, the parents would benefit by directing a greater proportion of the feeds towards the sons. If the food supply at hatching was poor, parents should compensate by preferentially feeding daughters. Such circumstances probably arise in many passerines where the delay between fertilisation and hatching is substantial, and there are few predictors of food availability.

The unpredictability of the food supply may cause stabilising selection for an equal sex ratio. Under the extreme scenario, females which produce a clutch of five female eggs would not gain a large reproductive benefit should food be abundant at hatching (as their daughter's reproductive success is limited by the number of eggs they lay). Females which laid five male eggs would face a fearsome dilemma should conditions be poor : either risk rearing five poor males, or direct all feeding efforts into rearing a single high quality male.

Although parent birds apparently cannot detect the presence of extra-pair young in their nests, they can distinguish nestling sex in at least one species. In the red- winged blackbird, both parents feed sons at a higher rate than daughters (Westneat *et al.* 1995), although since male nestlings are significantly heavier than females, parents may be merely following a rule of ' feed the largest chick ' (Ellegren & Sheldon 1997).

This phenomenon is unlikely to extend to house sparrows however. In sparrows, there is no significant size difference between nestlings of either sex. Moreover, any visual cues would be of limited use, since the nest interior is dark (particularly in nest boxes). For these reasons, brood reduction in house sparrows is unlikely to be caused (indirectly or directly) through neglect of nestlings of a particular sex. Most of the offspring lost through brood reduction die within days of hatching , when the nestlings appear (to the human eye at least) entirely monomorphic.

A lack of nestling sex advertisement may be a facet of parent-offspring conflict. If parents tended to neglect a particular sex of offspring, and the

direction of this neglect fluctuates with variable ecological circumstances, then neither nestling would benefit by disclosing its sex.

8.4.10 Future work

Only around a third of the broods sampled in 1996 have been sexed thus far. Once the gender of the remaining broods is known, most of the analyses performed here can be repeated more rigorously. Several of these unsexed broods are genuinely intact, most have data on paternal phenotype (i.e. badge size) and many (18) were produced by females of known age. Age may be the important variable which the previous analysis has lacked. In red-winged blackbirds, offspring sex ratio varies with maternal age, with older females producing proportionately more males (in support of the maternal condition hypothesis). Although the current data is insufficient, a rigorous analysis would need to simultaneously control for female quality, seasonality, and male phenotype.

8.4.11 Should one expect house sparrows to show a sex ratio bias?

At Maine Chance, there was no evidence that the (secondary) sex ratio deviated from that expected by random meiosis. However, given the life history of the house sparrow at this site, one may not have expected a biased sex ratio. Male nestlings are only slightly larger than females, and consequently, there is probably little difference in the costs of rearing either sex.

At Maine Chance, the extra-pair paternity rate was low (especially at barn D in 1996) and hence the variance in male reproductive success may not be markedly larger than that of females. Wetton *et al.* (1995) found that older males were more successful in gaining EPFs, but were equally likely to be cuckolded. Although the sires of the extra-pair nestlings have not yet been elucidated at Maine Chance, cuckoldry was independent of badge size. This suggests that differences in fledgling production would not be exacerbated even if, as theory predicts, large-badged males are more likely to gain EPFs. However, the evidence that male badge size is an 'attractive' trait is equivocal at Maine Chance and in other populations (Møller 1989, Veiga 1993, Kimball 1996).

Large-badged males did not provision nestlings at a higher rate, and thus their mates should not produce more sons in the expectation of receiving more assistance with rearing. Badge size is not heritable (Griffith 1998) , and thus females paired to large-badged males would not produce male-biased broods in an attempt to produce 'sexy sons' (Weatherhead & Robertson 1979). It is possible that females paired to large-badged males receive 'good genes', which may be manifested, for example, by improved offspring survival. On average, sons with good genes will achieve higher lifetime reproductive success than daughters with good genes. Female sparrows, under this assumption, should produce more sons if paired to a male with good genes. Since the badge size of their partner did not affect offspring sex ratio, either females cannot determine offspring sex, or genetic quality is expressed through a character other than badge size. Unfortunately, my data were too few to test for good genes effects, since nestlings were rarely resighted as adults. Of all the nestlings ringed, a very low proportion returned to breed at the barns (c 2 %), which suggests that sparrows should not bias their sex ratio to minimize local mate competition (LMC) (Hamilton 1967).

To conclude, although there was no evidence of any facultative offspring sex ratio bias in either season, several aspects of house sparrow life history suggest that this would only be adaptive in certain circumstances.

9.1 Introduction

9.1.1 The phenomenon of hatching asynchrony

As naturalists have known for centuries, clutches of birds' eggs rarely hatch simultaneously (e.g. White 1759). David Lack (1947), in a compelling insight, proposed that staggered hatching had evolved as a parental mechanism to optimize reproductive output. Hatching asynchrony (hereafter HA), where the eggs within a single clutch hatch out of phase with one another, has since been recorded in most avian families (Mock 1994, Stenning 1997).

Birds exert unusually direct control over the hatching pattern of their eggs. Because each egg requires an equal period of development, a difference in the advent of incubation would theoretically produce a parallel difference in hatching (Lack 1947). Incubation is initiated in many passerines (including the house sparrow [Summers-Smith 1963]) when the penultimate egg is laid (Clark & Wilson 1981). The final egg is thus incubated a day behind the remainder of the clutch, and as a consequence of this lag, usually hatches a day later (Veiga & Viñuela 1993). Compared to the its nest-mates, the individual hatching from the final egg is unavoidably disadvantaged in both size and motor function. Consequently, the threat of starvation is especially acute for this nestling, which often dies within a few days (Zach 1982). This apparently maladaptive waste of resources is known as 'the paradox of HA' (Mock 1994).

Lack (1947) suggested that HA was an adaptive response to the vagaries of food availability. If the food supply was unpredictable at the time of clutch initiation, females should lay an optimistic clutch size and incubate it asynchronously. If resources are scarce when the hatchlings emerge ($c10$ days later in the house sparrow; Seel 1968b), asynchrony concentrates selective pressure upon the last-hatched individual, and hence brood size would be quickly reduced (Magrath 1992). This improves the growth and survival probabilities of the remainder of the brood, and theoretically maximizes the fledging success of the parents (Skagen 1988).

Many experimental tests of Lack's hypothesis have been performed in which eggs or hatchlings were transferred between nests to create synchronous and asynchronous broods (see Magrath 1990). Some of these have supported

Lack's hypothesis (e.g. Hahn 1981, Husby 1986), while others have reported either equivocal (Skagen 1988) or even contradictory evidence (Hillstöm & Olsson 1994). This has led to several refinements of the Lack hypothesis, and a suite of new theories purporting to explain the preponderance of HA (all 16 are reviewed in Stoleson & Beissinger 1995, see also Stenning 1997).

Not all of these are adaptive. Non-adaptive hypotheses argue that HA is an inevitable consequence of constraints on other activities such as feeding and incubation (Slagsvold 1986). Other theories argue that the focus of selection pressure is actually the early onset of incubation, and the eventual hatching patterns are merely consequential (Clark & Wilson 1981). For example, the 'egg viability' hypothesis assumes that eggs which are left unincubated for several days suffer decreased viability, and hence birds commence incubation before the clutch is complete to improve the hatchability of these eggs (Arnold *et al.* 1987).

Despite the conflicting data from experimental tests, Lack's original HA hypothesis (1947) persists to this day as the most credible and general adaptive explanation. HA benefits the parents because it facilitates the reduction of brood size if hatchling food is scarce.

9.1.2 The phenomenon of brood reduction

Ricklefs (1965) coined the phrase 'brood reduction' (hereafter BR) to describe the adaptive loss of one or more nestlings from an otherwise successful brood. BR refers classically to the starvation of the smallest and weakest individual, and can arise following either active neglect by the parent(s), or competition with nest-mates (Clark & Wilson 1981, Stenning 1997). Parental care (usually food provisioning) is diverted away from the smallest individual and apportioned to the remainder of the brood, whose growth and condition improve at the expense of their dead sibling (Stouffer & Power 1991). For this selective mortality to be adaptive, the proportional increase in fitness these nestlings accrue must exceed that lost when a whole chick dies (Mock & Forbes 1994). The usual (although not essential) assumption of BR is that all nestlings are of equal genetic quality (Mock 1994).

BR is often (incorrectly) equated with HA (Lack 1947), presumably because most hypotheses for each phenomena involve an unpredictable food supply. It is important to realize, however, that BR is not an automatic consequence

of HA, and that the two events are distinct (Mock 1994). BR via nestling hierarchies can occur without HA, and HA need not result in BR (Mock 1994). Furthermore, while hatching asynchrony is entirely the dictate of the female, BR is the result of two conflicts: parent-offspring and nestling-nestling. Parents benefit from HA, whereas each surviving nestling benefits more from BR than do their parents (Dawkins 1976).

Despite these dichotomies, there is little doubt that HA facilitates BR by creating immediate size differences (Zach 1982). Blind hatchlings show the stereotypical begging posture when only *c2/3* days old, and hence even at this early nestling stage, size asymmetries would be significant and potentially fatal (Magrath 1990). Small nestlings are hampered in the struggle to be fed, irrespective of whether they fail to beg so intensively or so loftily as their nest-mates, or whether the parents preferentially feed the largest nestlings (Mock 1994). BR usually occurs within these first few days, as models show that parents derive most eventual benefit from BR when the chicks are still small (i.e. the investment lost is minimized) (Stoleson & Beissinger 1995).

9.1.3   Aims and predictions

The basic hypotheses of HA and BR were tested in the population of house sparrows established at Maine Chance farm.

Hatching   asynchrony

If HA is an adaptive response to facilitate the reduction of brood size in the event of food shortage, then three automatic predictions follow. Firstly, larger clutches should be more likely to hatch asynchronously, since a food shortage would be exacerbated in large broods. Secondly, since food shortages are presumably more likely to occur towards the end of the summer (Perrins 1979, Lowther 1990), the proportion of broods which hatch asynchronously should increase as the season progresses. Thirdly, if HA maximizes nestling condition, nestlings from asynchronously hatching broods should be heavier, on average, than those from synchronous broods.

If HA is a non-adaptive consequence of early incubation aimed at ensuring the viability of first-laid eggs, then large clutches should be more likely to hatch asynchronously (i.e. females which delay incubation until large clutches are completed have a greater risk of the first-laid egg being inviable).

145

Brood reduction

Firstly, if HA facilitates the reduction of brood size, then one would expect BR to be more common in clutches which hatched asynchronously. Secondly, if BR occurs in response to an unfavourable food supply, then the proportion of broods in which one or more nestlings are lost through starvation should increase towards the end of the season. Thirdly, the BR hypothesis predicts that survivors in asynchronously hatching broods where some mortality has occurred, should be in better condition than those in asynchronous broods with no mortality.

9.2   Methods

9.2.1   Classifying hatching asynchrony

Because *all* clutches of birds' eggs hatch asynchronously to some extent (Lack 1947), HA has been defined as a hatching spread of 24 hours or more (Clark & Wilson 1981).

In the 1995 breeding season, nests were visited daily in the midday/afternoon period. Following Clark & Wilson's (1981) definition, hatching was classed as asynchronous if hatchlings plus at least one egg were present on the first afternoon, and then fresh hatchlings were present the following afternoon. Hatchlings which had emerged on a particular morning ('day hatchlings') were easily identified in the afternoon nest visits because of their size and colour. 'Recent' hatchlings (i.e. those less than three hours old) were also easily distinguished by their damp appearance and burgundy colour, which pales fairly rapidly. If 'recent nestlings' were present on the first inspection, and 'day' hatchlings were found on the second inspection, then this was classified as a synchronous brood (i.e. a hatching spread of less than twenty four hours).

In 1996, the nest visitation schedule was more intensive. Wherever possible, three visits were made per day (at approximately 0900, 1300 and 1700). The toenails of any fresh hatchlings were clipped (in the sequence described in the general field methods) and the number of the remaining egg(s) was recorded. Repeating the criterion used in 1995, clutches were classed as asynchronous if their hatching spread was at least 24 hours. Any eggs which were still present in the nest 48 hours after the remainder of the clutch had hatched were

146

opened to determine whether they were undeveloped or had suffered from embryonic mortality).

9.2.2 Classifying brood reduction

The shrivelled bodies of apparently starved nestlings were occasionally found on the ground beneath their nest-boxes or pushed to the front lip of active nests. In most cases however, BR had to be inferred following the disappearance of one or more nestlings from an otherwise intact brood. Dead chicks were usually removed by the parents, presumably for hygienic reasons, and dropped some distance from the nest (*pers. obs.*).

9.2.3 Ambiguous incidences of HA or BR

In 1995 (and in 1996 to a lesser extent), the visitation schedule led to several ambiguous disparities between the numbers of eggs in a nest on one visit, and the number of hatchlings which were found on the subsequent visit (e.g. four hatchlings present from a clutch of five eggs). In these cases it was impossible to distinguish whether one of the eggs had failed to hatch and had been ejected, or whether the egg had hatched but the hatchling had died within hours and also been ejected. These clutches were deleted from the analysis.

9.2.4 Nestling data

Nestlings were weighed to the nearest 0.1g using a 30g spring balance. All weights used in the analysis were obtained on nestling day 10 (ND_{10}, where day 0 refers to the day of hatching). Any age spread within a brood which had been induced by HA was taken into account when obtaining ND_{10} weights.

9.3 Results

9.3.1 The occurrence of hatching asynchrony

Adequate data on hatching patterns were obtained from 32 clutches in 1995, and 88 clutches in 1996. Tables 1 and 2 show, for each year, the frequency distribution of each clutch size, and the proportion of these clutches which hatch either synchronously or asynchronously.

Table 1.   Clutch sizes and hatching synchrony in 1995.

Clutch size	Frequency	Synchronous	Asynchronous
3	4	3	1
4	12	2	10
5	11	2	9
6	3	1	2

Table 2.   Clutch sizes and hatching synchrony in 1996.

Clutch size	Frequency	Synchnronous	Asynchronous
3	10	8	2
4	27	7	20
5	43	8	35
6	6	1	5

The last-laid egg in asynchronous clutches almost always hatched between 24 and 36 hours after the first. The identity of the last-hatched egg was known in 29 of the asynchronous clutches in 1996. In all cases, this was also the last-laid egg, and would usually hatch a day out of phase with the other eggs.

In all analyses, synchronous clutches were scored as '0', and asynchronous clutches were scored as '1'. The proportion of asynchronous clutches in a particular category (e.g. June), was thus an average of the June '0's and '1's. In the two extreme circumstances, an overall proportion of '1' indicates that all clutches hatched asynchronously, whereas an overall proportion of '0' indicates that all clutches were synchronous.

HA was common in both years (24 of 32 clutches in 1995; 63 of 88 clutches in 1996). HA varied significantly with clutch size (Kruskal-Wallis $H = 21.1$ df = 3 $p = 0.001$) (Figure 1), and there was a significant positive correlation between clutch size and HA (Spearman rank $r = 0.251$ $n = 120$ $p < 0.01$). As shown in Figure 1 however, this result was an artefact of the low proportion of 3-egg clutches which hatched asynchronously. When the 3-egg clutches were deleted from the analysis, HA was independent of clutch size (Kruskal-Wallis $H = 1.24$ df = 2 NS), and there was no correlation between HA and clutch size ($r = 0.00$ $n = 106$ NS)

Figure 1. The frequency of hatching asynchrony in relation to clutch size.

(mean + SE)
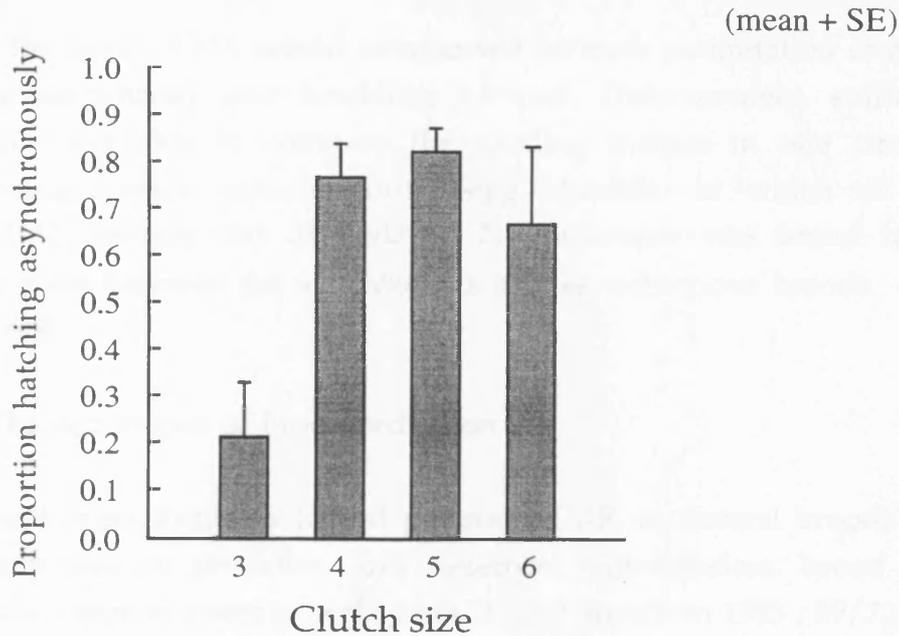
Although the proportion of clutches which hatched asynchronously did not differ according to their month of eclosion (Kruskal-Wallis H = 6.94 df = 3 p = 0.074), HA became significantly more common as the season progressed (Spearman rank r = 0.206 n= 120 NS) (Figure 2).

Figure 2. The frequency of hatching asynchrony in relation to time of season.

(mean + SE)
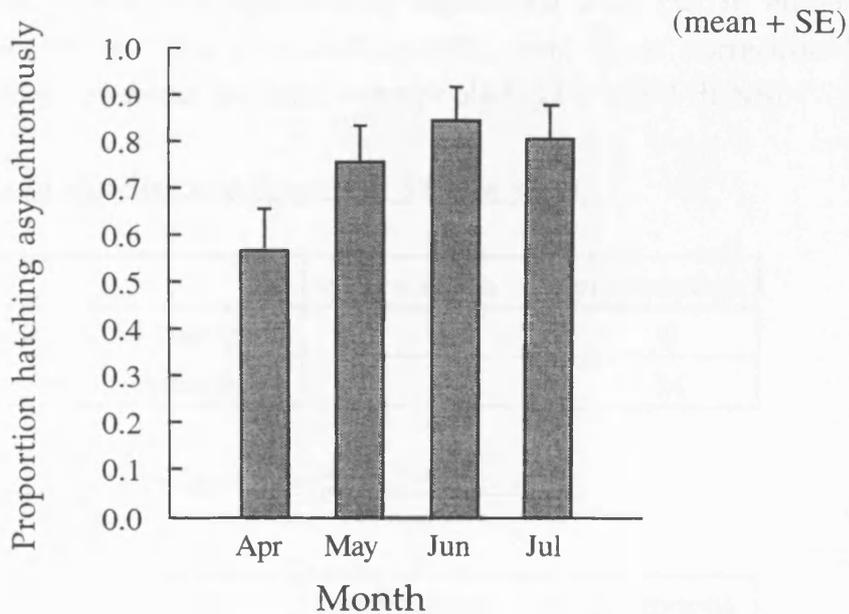
9.3.2 The influence of hatching asynchrony on nestling size

Ideally, the effect of HA would be assessed for each permutation of clutch size, hatching asynchrony and hatchling survival. Unfortunately, sufficient data were only available to compare the nestling masses in one circumstance: synchronous versus asynchronous 5-egg clutches, in which all nestlings survived to nestling day 10 (ND_{10}). No difference was found in average nestling mass between the synchronous and asynchronous broods ($t = -1.573$ df = 18 NS).

9.3.3 The occurrence of brood reduction

Fewer data were available to test patterns of BR, as several broods were lost completely due to predation and desertion. Nevertheless, brood reduction was also a common event in both years (17/29 broods in 1995 ; 39/72 broods in 1996). Usually only a single nestling was lost through BR (19/23 cases in 1995; 34/39 cases in 1996). Of the nine other instances of BR noted over the two years, seven broods lost two chicks, and two broods (of five) lost three chicks. There was an overwhelming trend for the single nestling lost to be the last-hatched individual (14/16 documented broods in 1995, 20/22 documented broods in 1996).

Brood reduction was not significantly associated with HA in either breeding season ($\chi^2 = 1.05$ in 1995, $\chi^2 = 0.632$ in 1996, 1 df, Yates' correction, both NS). (Tables 3 and 4), or when the data were pooled ($\chi^2 = 1.39$ 1 df NS).

Table 3. Fate of clutches and broods in 1995 ($n = 29$).

	synchronous	asynchronous
no brood reduction	4	8
brood reduction	3	14

Table 4. Fate of clutches and broods in 1996 ($n = 72$).

	synchronous	asynchronous
no brood reduction	13	20
brood reduction	12	27

The proportion of broods in which one or more nestlings starved showed a significant increase as the summer progressed ($F_{1,41}$ = 7.63 p = 0.009, when grouping data into months (Figure 3; A , M ... = April, May etc).

Figure 3. Seasonal frequency of brood reduction.

(mean + SE)
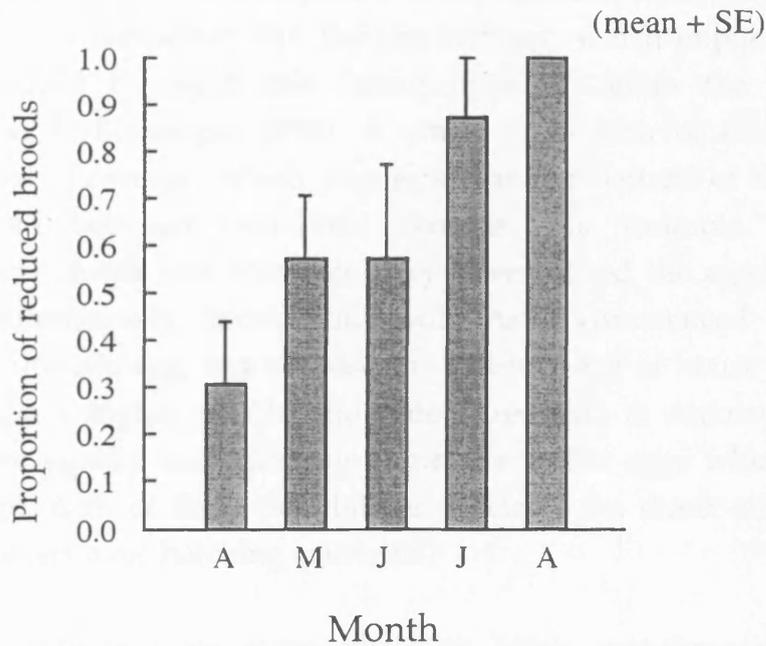
9.3.4 The influence of brood reduction on nestling size

Data were few for this analysis, since broods which hatched synchronously could not be justifiably compared against those that were asynchronous. Nevertheless, sufficient data was available to examine the fate of nestlings resulting from asynchronous 4- and 5-egg clutches.

Broods from 22 asynchronous 5-egg clutches survived until ND_{10}. Brood reduction occurred in nine of these, and 13 remained intact with five nestlings ringed. There was no difference between the two groups in mean nestling mass (reduced broods = 22.76g, intact broods = 23.46g ; t = - 0.543 df = 20 NS). Broods from 18 four-egg clutches survived until ringing age. Brood reduction occurred in nine of these, and nine remained intact with four nestlings ringed. Again, mean nestling mass did not differ between the two groups (reduced broods = 23.86g , intact broods = 22.99g t = 0.521 df = 16 NS).

9.4 Discussion

9.4.1 Hatching asynchrony

Most house sparrow clutches hatched asynchronously at Maine Chance, as has been found in other populations (Seel 1968b, Anderson 1986, Veiga 1990a). The final egg to hatch was invariably the last-laid egg, which implies that the asynchrony is caused by incubation being initiated before the clutch is complete (Stoleson & Beissinger 1995). A small proportion of clutches did hatch synchronously however, which suggests that the pattern of incubation or laying differed between individual females. For example, delaying incubation until the clutch was complete may have caused the eggs to hatch synchronously. Alternatively, incubation could have commenced with the laying of the penultimate egg, but because the last-laid egg of house sparrows is provisioned with a higher yolk/white ratio (Krementz & Ankney 1986) it may develop more rapidly and 'catch up' with the earlier eggs which have a 24 hour advantage. Both of these possibilities illustrate the direct control that female birds can exert over hatching patterns.

Although larger clutches were more likely to hatch asynchronously than small clutches, this was considered an artefact of the high proportion of synchronous 3-egg clutches. When these relatively uncommon small clutches were deleted from the analysis, HA was independent of clutch size. The proportion of clutches which hatched asynchronously increased as the season progressed, consistent with Lack's (1947) hypothesis that HA is an adaptive response to the likelihood of food shortage. Asynchrony also increased with laying date in a Spanish population of house sparrows (Veiga & Viñuela 1993). HA did not confer any detectable benefits in terms of nestling mass (and therefore survival) when contrasting paired synchronous and asynchronous broods.

The 'egg viability' hypothesis (Arnold et al. 1987) was rejected, since HA was independent of clutch size. In contrast, Veiga & Viñuela (1993) supported this hypothesis in house sparrows, since large clutches were more likely to hatch asynchronously, and moreover, the first-laid egg was less likely to hatch in synchronous than asynchronous nests. The first-laid eggs were also the least likely to hatch in a Kansas population of house sparrows (Murphy 1978),

although there was no positional bias in the occurrence of unhatched eggs at Maine Chance (I.R.K. Stewart, unpublished data).

9.4.2 Brood reduction

Brood reduction was also common in both breeding seasons, and was consistent with the sequence of events described in other studies (Zach 1982, Skagen 1988, Magrath 1989). The nestling which starved was almost always the last to hatch, and had therefore usually emerged from the last-laid egg (Magrath 1989, Veiga 1990). Despite this obvious pattern, BR was not facilitated by HA, since it was not more likely to occur in clutches which had hatched asynchronously (Magrath 1990).

One prediction of the classical brood reduction hypothesis (Ricklefs 1965, Lack 1968) was supported, since the proportion of broods in which one or more nestlings were lost increased with advancing hatching date (Amundsen & Stokland 1988, Mock 1994). This was presumably in response to a seasonal decline in the quality and quantity of the food supply (Perrins 1979), although admittedly, no data on this were obtained at Maine Chance. The surviving nestlings did not appear to benefit from BR however, since there was no significant difference in average mass between matched broods which had either lost a nestling or remained intact (but see Thompson *et al.* 1993 for a comment on the validity of using mass as a measure of fat reserves).

9.4.3 Synthesis

The patterns of hatching sequence and selective mortality which were observed at Maine Chance were consistent with several tenets of hatching asynchrony and brood reduction theory. Generally however, the results were equivocal, since neither strategy appeared to improve the mass (and therefore survival probability) of the remaining nestlings.

However, observational investigations into both phenomena are difficult to interpret (Skagen 1988, Mock 1994, Stenning 1997). For example, although the average nestling mass did not increase in broods from which a single hatchling was lost, one can never know what mass the nestlings would have achieved had the hatchling survived (Magrath 1989). More pertinently, since both phenomena are usually assumed to be adaptive

means by which parents maximize their reproductive success, the benefits of each strategy may be manifested in improved parental survival rather than nestling growth (Gustafsson & Sutherland 1988, Mock & Forbes 1994). Without an experimental approach, it is premature to dismiss the significance of hatching asynchrony and brood reduction (Stoleson & Beissinger 1995, Stenning 1997).

Chapter 10. Egg size variation in the house sparrow

10.1 Introduction

10.1.1 The study of egg size variation

In 1947, David Lack proposed that clutch size in birds had evolved to correspond to the maximum number of young which the parent(s) can adequately rear. Lack's theory of an optimal clutch size stimulated a plethora of both natural and experimental field studies, many of which have supported the hypothesis (e.g. Perrins 1965), although others have found ambiguous or even contradictory results (e.g. Skagen 1988, Ward 1973). Although Lack's hypothesis still represents a classic example of stabilising selection, several conditions and refinements have been added. The most important of these has been the acceptance that individuals may trade-off current investment against future reproductive success (Gustafsson & Sutherland 1988, Monaghan & Nager 1997). Consequently, measures of reproductive investment became more accurate, as fieldworkers began to measure eggs as well as count them (Birkhead 1991). In plovers for example, four large eggs represent a greater energetic investment than four small eggs.

Differences in egg size do not only exist *between* birds however. It was well established, even in Lack's day, that eggs vary in size within the same clutch (Lack 1968). Extreme examples of this include barn owl eggs dwindling in size with laying sequence, the small first egg of *Eudyptes* penguins, and most famously, the runt last egg (the C-egg) of herring gulls (Parsons 1970). These 'insurance eggs' function as adaptive modifications against unpredictable breeding conditions (Williams 1994). If conditions are bad, then the small egg (or hatchling) is lost at relatively little cost. Although relatively few birds lay genuine 'insurance eggs', in most species, the maternal influence over egg size may have subtle implications for the ontogeny and eventual fledging success of the embryo within (Slagsvold *et al.* 1984).

Most studies of the significance of egg size variation have focused on non-passerines, most commonly geese and seabirds (Williams 1994). Egg size differences are visibly more profound in birds such as geese (Cooke *et al.*

1995), and when the transition between egg and active chick is brief, as in most precocial birds, the advantage of hatching from a large egg is obvious. Although much relevant data is available for such birds (summarized in Williams 1994) most examples used hereafter are from passerines.

10.1.2 Egg size, hatching asynchrony and brood reduction

Eggs of most altricial birds, including the house sparrow, hatch asynchronously (Lack 1954, Clark & Wilson 1981). Slagsvold *et al.* (1984) recognized that egg size variation during laying could act in concert with maternally induced hatching spread to maximise reproductive success under an unpredictable food supply. Their simple dichotomy represents two theoretical extremes of an associative breeding strategy.

'brood survival' strategy   =   synchronous hatching and large last egg
'brood reduction' strategy   =   asynchronous hatching and small last egg

In the 'brood survival' strategy, if the last laid egg is relatively large, then the hatchling it produces will consequently be relatively large (O' Connor 1978). This reduces the likelihood of brood reduction by avoiding an initial size asymmetry, and ultimately, increases the probability of this final chick fledging. Females utilising the 'brood reduction' strategy handicap the final hatchling with a temporal disadvantage (Lack 1954), and then exacerbate its plight further by reducing its initial size relative to that of their nestmates. If the conditions at hatching are good, then this final nestling will fledge successfully. If, however, the conditions are bad, then this doubly-handicapped hatchling will be lost rapidly (Ricklefs 1966). The parents thereby minimize resources invested into a nestling destined to either starve before fledging, or fledge with negligible survival prospects.

The following section describes an investigation into the causes and consequences of egg size variation in the house sparrow, carried out in the population of house sparrows established at Maine Chance farm. Hatching asynchrony is common in this population (Chapter 9), and becomes more frequent as the breeding season progresses (Chapter 9). If egg size is regulated according to the brood reduction strategy (Slagsvold *et al.* 1984), then two predictions arise. Firstly, the final egg will be relatively small compared to the previous eggs in the clutch. Secondly, the magnitude of

this difference should increase as the season progresses, to parallel the increase in frequency of hatching asynchrony.

10.2 Methods

10.2.1 Field data

Non-operational nests were visited every two or three days unless parental behaviour (such as copulation or frequent female attendance) implied clutch initiation was imminent, whereupon daily visits were commenced. As the clutch was produced, eggs were numbered in sequence with an indelible felt-tip marker pen. When the clutch was complete (incubation day zero or ID_0) the nest was not checked again until ID_5, to minimize disturbance. The length and breadth of each egg to the nearest 0.1mm was measured using Vernier callipers.

In 1995, eight eggs were dropped or cracked during handling and measuring, and these clutches were excluded from the analysis. In 1996, eggs were measured after about five days of incubation, so that in the event of accidental breakage, an embryo could be salvaged for DNA analysis. Breakage was rare in 1996 however (three eggs of over 600 measured).

A sample of fresh egg masses was obtained by visiting nests between 0700 hrs and 1000 hrs, and weighing new eggs to the nearest 0.025g using a 5g spring balance. House sparrows lay at around 6am (Summers-Smith 1963) and thus any loss of mass through incubation would have been negligible. Nests were visited late on ID_9 and early on ID_{10} to coincide with hatching (Seel 1968a), and the same balance was used to obtain masses of several fresh hatchlings (known from the nest visitation schedule to be less than three hours old).

Several studies have commented that the final egg in clutches of the house sparrow is often relatively pale and/or lightly patterned (Dawson 1972), and Lowther (1988) produced deductive evidence of a distinct last laid egg in over 90% of clutches. In this study, eggs were numbered daily, and several precise laying sequences were obtained. When complete, each clutch was scored 'blind' for the presence of visibly odd eggs (i.e. a farm

worker unfamiliar with the objective arranged the eggs so as to obscure their numbers).

10.2.2 Calculating egg volume

Several classic field studies of egg size variation (e.g. geese, auks) have used mass as their index of egg size (Williams 1994). For small passerines however, the increased handling required may lead to breakage, and hence an approximation of volume was used in this study, based on the length and breadth of each egg. Preston (1974) used trigonometric methods to produce a basic equation for calculating egg volume, whilst lamenting that 'it cannot be done with any real accuracy on the basis of only two measurements'. Tatum (1975) used mathematical theory to extend this equation, and finally Hoyt (1979) used Archimedes' water displacement method to accurately determine the volume of measured eggs, and thus derive the equation which best related this to the two linear dimensions. This equation, now universally accepted and employed, is;

$$\text{Volume (mm}^3) = 0.51 \times \text{Length} \times \text{Breadth}^2$$

All eggs were assumed to be uniform in shape when calculating egg volume. Although clutches of noticeably elongate or rounded eggs did occur, almost all resembled the usual avian blunt-ended ellipsoid.

10.3 Results

10.3.1 Egg morphometrics

Egg size data was obtained from 84 clutches (388 eggs) in 1995, and 134 clutches (625 eggs) in 1996.

Egg volume varied substantially. The smallest egg recorded was 2005 mm^3, and the largest was around 70% larger at 3389 mm^3. Volume and mass pairings were obtained for 54 eggs, of which 29 were from separate clutches. In common with previous studies, the fresh mass of each egg was strongly and positively correlated with its volume ($r = 0.958$ $n = 53$, p < 0.001 when including more than one egg from certain clutches; $r = 0.956$ $n = 28$ p < 0.001 when considering only eggs from individual clutches). Both volume and mass are therefore equally valid indices of 'egg size'.

158

House sparrows laid lighter eggs than predicted by the logistic regression of Rahn *et al.* (1975). Assuming a female mass of 27.2g (averaged from 26 breeding females caught during the 1996 season) egg mass should be 3.25g, whereas the real figure is around 2.85g (using an average of 29 eggs from separate clutches). This represented about 10 % of female body mass (an average of 10 egg/female comparisons, which ranged between 8 - 12 %). A clutch of five eggs therefore represents about half of the body weight of a laying female.

10.3.2 Hatchling mass

Hatchling assignations were obtained from 35 eggs. Fresh hatchling mass was strongly and positively correlated with egg volume ($r = 0.523$ $n = 35$ p < 0.01) (Figure 1).

Figure 1. Hatchling mass versus egg volume.
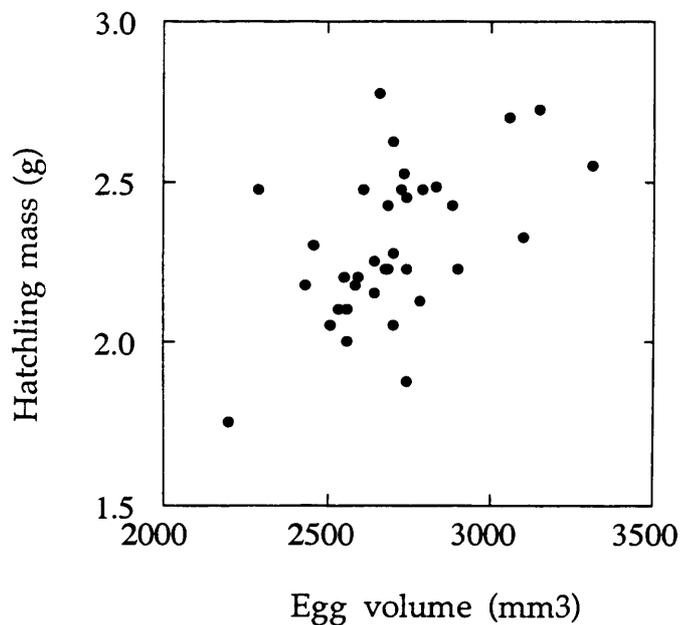
Several of these assignations were from eggs of the same clutch, which introduced a potential bias. However, hatchling mass was still strongly correlated with egg volume even when a single assignation was selected randomly from separate clutches (Spearman rank $r = 0.496$ $n = 28$ p < 0.01).

10.3.3 Egg/hatchling/fledgling comparison

Because house sparrow clutches at Maine Chance generally hatched asynchronously (Chapter 9), the most common hatchling assignation was from the final egg. Many of these final hatchlings starved (Chapter 9), and additional broods were either abandoned or lost to predators. Only 15 hatchlings survived until ringing age (at nestling day 10, or ND_{10}), and of these, four were from the final egg and were obviously undernourished. Thus, the eventual correlation between egg size and ringing mass was restricted to 11 cases unconfounded by the last egg effect. No correlation was found ($r = - 0.284$ $n=11$ NS).

A slightly larger sample was available for the average egg volumes of successful clutches. Data on average ringing mass was available for 15 'perfect' broods (ie. those in which all eggs hatched and produced a healthy nestling). No correlations were found between average egg volume and either average ND_{10} mass ($r = - 0.009$ $n = 15$ NS) or average tarsal length ($r = - 0.016$ $n = 15$ NS).

10.3.4 Colouration of the last-laid egg

The laying sequence was known for 76 clutches in 1996 (from 43 nests). The final egg was distinct (lighter in colour and less densely patterned) in 72 of these clutches (= 95%) (see Table 1). The four clutches which did not follow this pattern were all comprised of eggs which were unusually pale and only lightly spotted, reducing the likelihood of a particular egg appearing distinct.

Table 1. Distribution of clutches containing distinct last-laid eggs.

Clutch size	N (number)	N with distinct last egg
3	4	4
4	18	17
5	43	40
6	11	11

10.3.5 Causes of egg size variation

Three main factors are reported to affect intraspecific egg size variation (Birkhead 1991), none of which are mutually exclusive: parentage, laying sequence, and environmental conditions.

Parentage

Considering the first clutches of 10 females selected at random, most variation in egg size occurs between females (F $_{9,\ 40}$ = 16.36 p < 0.01) (Lessels & Boag 1987, Harper 1994). A comparison of sequential clutches laid by the same females throughout the season found that egg size was highly repeatable (F $_{24,\ 52}$ = 5.44 p < 0.001). Eight females bred in both seasons. There was no difference in the average size of eggs which each female laid in either year (t = -1.73 df = 8 NS).

There was no obvious cause for the consistency. Structurally large birds did not lay large eggs in 1995 (r = 0.338 n = 15 NS) (when correlating average volume of all eggs laid with mean tarsus size), but they did in 1996 (r = 0.664 n = 27 p < 0.05). Heavier birds did not lay larger (and thus heavier) eggs in 1995 (r = - 0.158 n = 16 NS) , but they did in 1996 (r = 0.410 n = 26 p < 0.05). Older birds (in at least their second breeding season) did not lay larger eggs than yearlings (t = 0.276 df = 12 NS).

Although females were expected to lay proportionately small eggs in large clutches (and vice versa), egg size was independent of clutch size in both years (1995 Spearman rank r = -0.053 n = 33 NS ; 1996 Spearman Rank r = 0.009 n = 83 NS ; treating clutches as independent data points).

Laying   sequence

Egg size increased with laying sequence. Pooling data from both years, the last laid egg was significantly larger than the first laid egg in all clutch sizes (4-egg clutches t = - 3.54 df = 21 p = 0.002 , 5-egg clutches t = - 8.78 df = 56 p = 0.001 , 6-egg clutches t = -3.23 df = 9 p = 0.002).

The increase in volume between the first and last egg of each clutch ranged between - 8% and + 33%, and averaged + 8%. As reported by

Lowther (1990), the volume increase during laying was primarily due to an increase in egg width, not length. Egg width is determined by the contraction or relaxation of the appropriate region of the oviduct, which presumably does not affect egg length. The width increase may merely result from passive loosening of the oviduct walls as the laying sequence progresses (P. Lowther *pers. comm.*). Figure 2 shows the change in mean volume with laying sequence for 78 clutches (pooled from both years) where the exact laying order was known (i.e. for each clutch size, the plot shows the mean volume of first laid egg, the mean volume of second laid egg etc). The curves suggest that egg volume increases steadily in the small clutch size (four eggs), increases throughout but eventually levels out in the modal clutch size (five eggs), and increases throughout but declines with the final egg in the large clutch size (six).

Figure 2. Egg volume changes with laying sequence
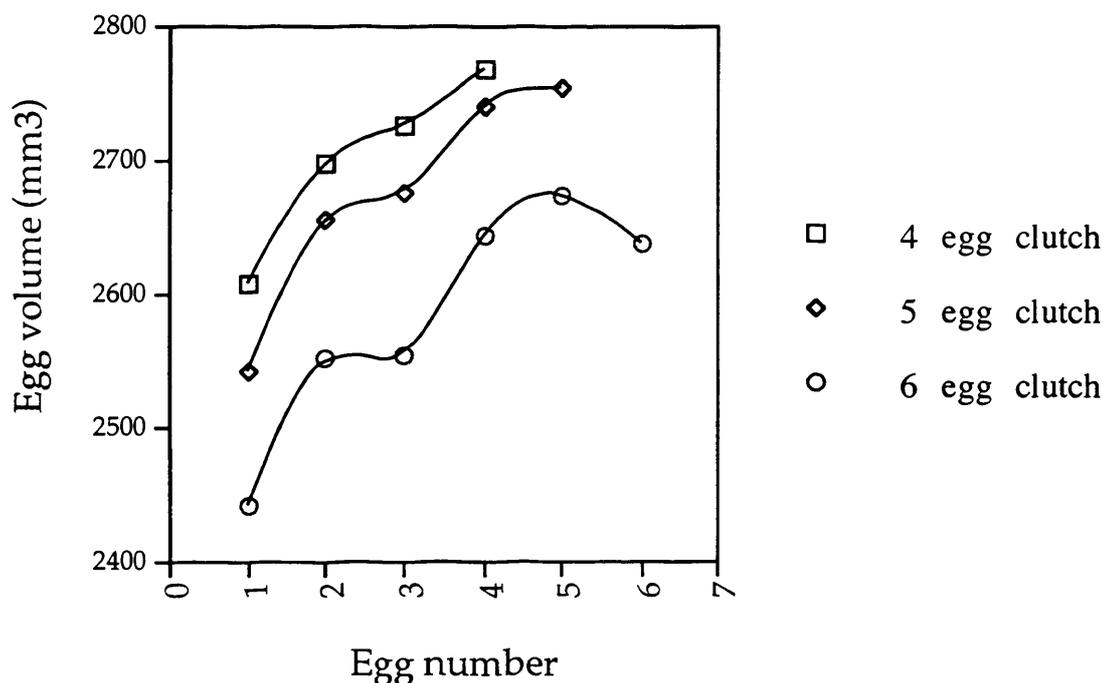
Although Figure 2 suggests that 4-egg clutches were significantly larger in volume at all stages than the 5- and 6-egg clutches, there was no significant difference in mean volume between clutch sizes ($F_{2, 76} = 1.26$ NS).

In all clutches, however, there was a considerable overlap in egg sizes irrespective of their position in the laying sequence (Figures 3, 4 and 5). A

significant difference between the means was only present in the modal clutch size of five eggs (F $_{4,230}$ = 7.62 p = 0.001) (cf. Veiga 1990). Comparing volumes of penultimate and ultimate eggs, 73 % of 4-egg clutches showed an increase, whereas this dropped to 60% in 5-egg clutches and 33% in 6-egg clutches. Although this is the pattern suggested by the curves in Figure 2, there was actually no significant difference between the volume of the penultimate and ultimate eggs in any clutch size (4-egg clutches t = -1.392 df = 21, 5-egg clutches t = 0.79 df = 46, 6-egg clutches t = 1.144 df = 8 all NS).

Figure 3. Volume increase with laying sequence in 4-egg clutches (mean + SE)
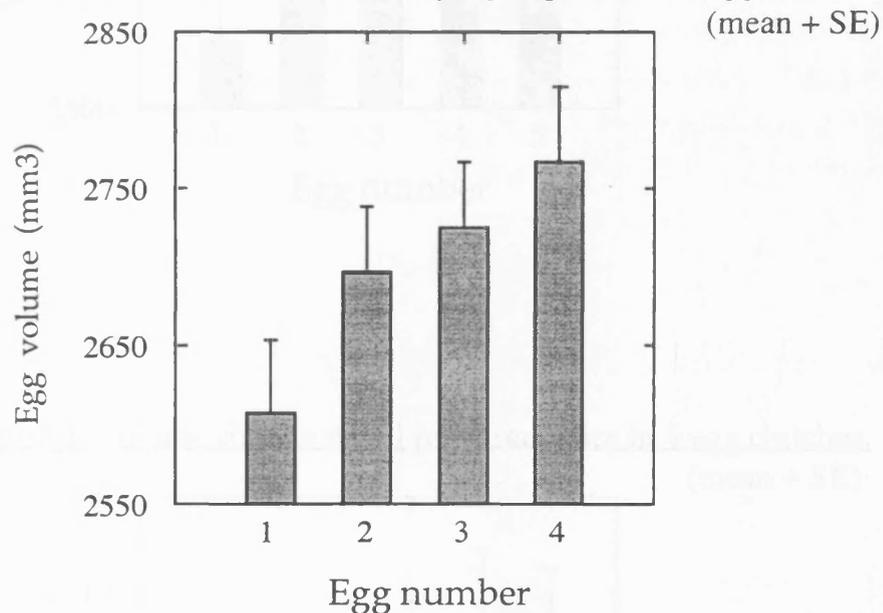
Figure 4. Volume increase with laying sequence in 5-egg clutches.
(mean + SE)
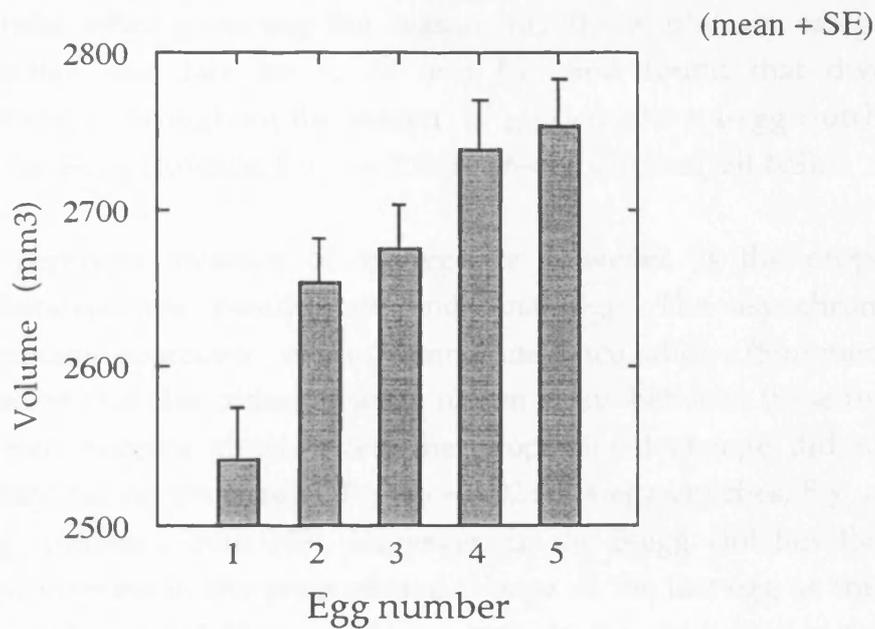
Figure 5. Volume change with laying sequence in 6-egg clutches.
(mean + SE)
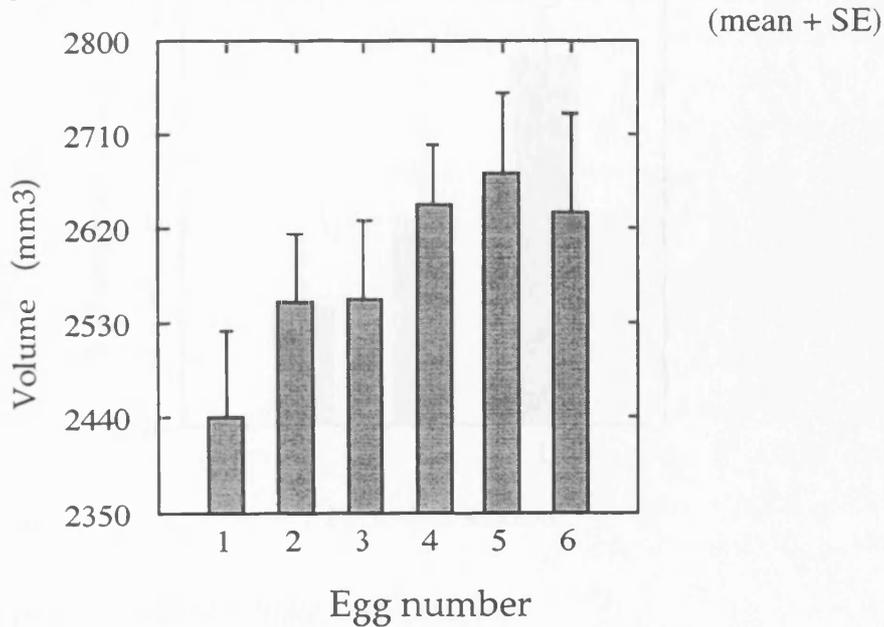
Lowther (1990) found house sparrow clutch sizes showed a clear seasonal trend of progressive divergence of the last-laid egg ('divergence' being the volume increase between the first and last egg of a clutch). He interpreted this as the moderative effect of a large final egg becoming increasingly

important as food supplies become more available. This study did not find such a trend when grouping the season into three arbitrary categories of early, median and late (or E, M and L) and found that divergence remained stable throughout the season (F $_{2, 19}$ = 1.13 for 4-egg clutches, F $_{2, 44}$ = 0.81 for 5-egg clutches, F $_{2, 7}$ = 2.66 for 6-egg clutches, all NS).

A more pertinent measure of 'divergence' however, is the proportional change between the penultimate and final egg. The asynchrony with which female sparrows often commence incubation (Summers-Smith 1963) ensures that the critical discrimination exists between these two eggs. For the two extreme clutch sizes, the proportional change did not vary significantly during the season (F $_{2, 19}$ = 2.02 for 4-egg clutches, F $_{2, 6}$ = 0.415 for 6-egg clutches , both NS). However, in the 5-egg clutches there was significant increase in the proportional change of the last egg as the season progressed (F $_{1, 51}$ = 5.79 p = 0.02), contrary to the prediction of the brood reduction strategy (Slagsvold et al. 1984) (Figure 6).

Figure 6. Proportional change in volume of last-laid egg.

(mean + SE)

Period of season
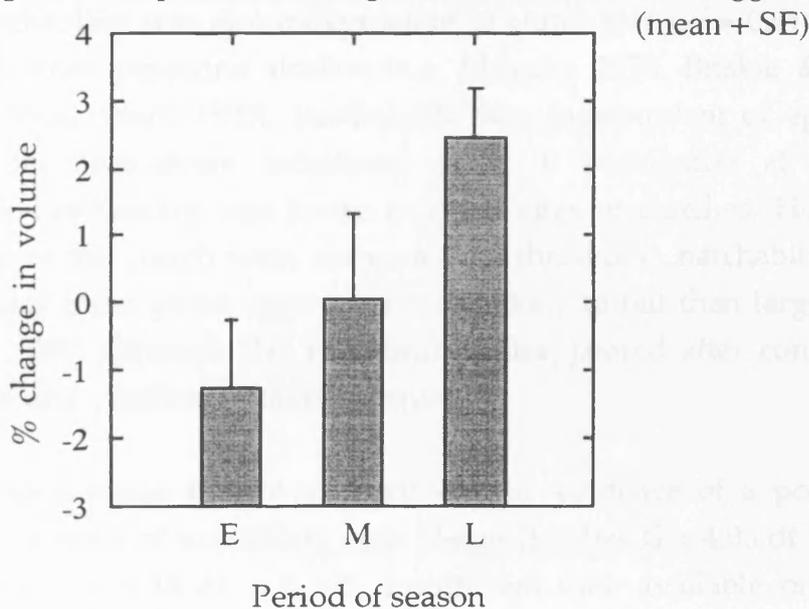
Environmental conditions/time of season

Several studies have related egg size variation to the time of season in which the clutch is produced (Lowther 1990. Birkhead 1991). This is indirectly a measure of environmental conditions (mostly temperature) and therefore food availability. In the present study, the time of season

correlated strongly with average air temperature (1995 Spearman rank $r = 0.946$ $n = 33$ p < 0.01; 1996 Spearman rank $r = 0.731$ $n = 83$ p < 0.01). Hence, days became warmer as the summer progressed.

However, First Egg Date (FED) of clutches during the 1995 season did not explain a significant proportion of the variation when entered into a multiple regression with the female as a variable (FED $F_{1,30} = 10.35$ NS, BIRD $F_{1,30} = 3.182$ NS). Even when considering temperature *per se*, there was no correlation with egg size in either year (1995 Spearman rank $r = -0.017$ $n = 33$ NS; 1996 Spearman rank $r = 0.117$ $n = 77$ NS). Clutches were grouped into three arbitrary 35-day periods corresponding to the early, median, and late phases of the 1996 breeding season. There was no difference in mean egg size between each phase ($F_{2,80} = 0.506$ NS).

10.3.7 Hatchability

Hatchability was independent of egg size, since the volume of unhatched eggs in a clutch was not smaller than the others in their clutch ($t = 0.852$ df = 26 NS). Hatchability was also independent of clutch size ($\chi^2 = 0.814$ df = 2 NS). In most other passerine studies (e.g. Murphy 1978, Briskie & Sealy 1990, Veiga 1990a, Ward 1995), hatchability was independent of egg size, although in the intensively monitored great tit population at Oxford (Perrins 1996), hatchability was lower in small eggs of clutches. However, when all eggs of the clutch were above a 'size threshold', hatchability was random. Smaller snow goose eggs were more likely to fail than larger ones (Cooke *et al.* 1995), although this relationship disappeared after controlling for clutch size and position in laying sequence.

In Maine Chance house sparrows, there was no evidence of a positional bias in the occurrence of unhatched eggs (4-egg clutches G = 4.03 df = 3 NS, 5-egg clutches G = 6.48 df = 4 NS, insufficient data available on 6-egg clutches). In contrast, both Murphy (1978) and Veiga (1990a) found that the first egg in house sparrow clutches was most likely to fail.

10.3.8 Population egg sizes between years

Unexpectedly, the average egg volume was significant larger in 1996 than in 1995 ($t = -3.55$ df = 177 p < 0.001) (Figure 7).

Figure 7. Average egg volume between years.

(mean +SE)
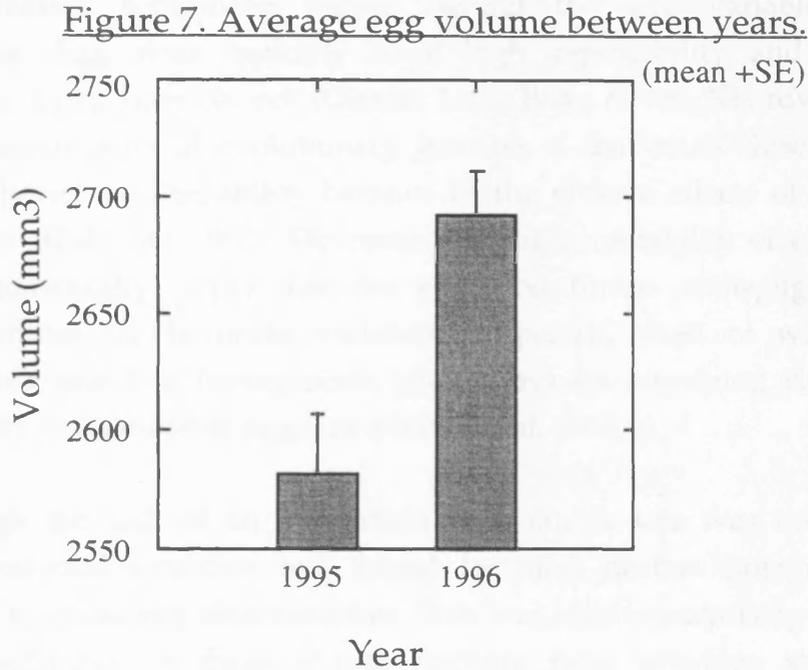
A closer analysis revealed that the mean egg sizes were similar between years at barns C and D (Table 2) but that the mean egg size at barn B was significantly larger than in the previous year (t = 3.09 df = 76 p = 0.003).

Table 2. Mean volume of all clutches laid at each barn in 1996.

Barn	1995 volume	Clutches	1996 volume	Clutches
B	2541	25	2713	53
C	2613	17	2674	25
D	2606	19	2678	40

10.4 Discussion

10.4.1 What affects egg size variation in the house sparrow?

Egg size in house sparrows was independent of clutch size. This is consistent with virtually all previous studies of both passerines and non-passerines (e.g. Briskie & Sealy 1990, Cooke *et al.* 1995, Ward 1995) and suggests that the two reproductive variables are controlled by different mechanisms. For example, large clutches are not composed of small eggs, either as an energetic trade-off, or to improve incubation efficiency.

The ultimate controlling factors behind the two variables are also disparate. Egg sizes typically have high repeatability and heritability, whereas clutch sizes do not (Ojanen 1983, Boag & van Noordwijk 1987). A fundamental tenet of evolutionary genetics is that traits closely related to fitness have low heritability because of the erosive effects of strong prior selection (Falconer 1960). However, the high heritability of egg size does not automatically imply that the effect on fitness is negligible. Several opportunities for heritable variation to persist, most of which involve combative selection (antagonistic pleiotropy) on correlated characters not obviously equated with egg size (Cooke *et al.* 1995).

Although the lack of an interaction with clutch size was not surprising, only equivocal evidence was found for each of the three main factors posited to cause egg size variation. This was also unsurprising however, as the significance of these factors appears from previous studies to be ambiguous itself (Ward 1995).

Parentage

Female size influenced egg size in the second year of the study but not the first. This is difficult to explain, particularly because the existence of an effect varies between species (Kendeigh 1975). Within passerines, the correlation between female size and egg size ranges from being absent (e.g. Otto 1979, Ward 1995), to weak (e.g Ojanen *et al.* 1981) or strong and positive (e.g. Birkhead 1991, Potti & Merino 1996). Murphy (1977) found a correlation between female weight and egg weight in house sparrows, which suggests that the 1995 result was anomalous rather than that in 1996. Although the breeding season was deferred significantly in the second year due to colder spring temperatures, environmental conditions between seasons were basically similar (Chapter 1).

The disparity between the two years could be confounded by an age effect. Birkhead (1991) found that yearling magpies produce smaller eggs than older birds, presumably because female foraging efficiency increases with age and experience. However, eggs laid by yearling sparrows were no different in size than those laid by older sparrows. Thus, a change in the age-structure of the population between years would not account for the difference.

Laying sequence

For all house sparrow clutch sizes, volume increased with laying sequence (as found by Murphy (1978) and Lowther (1990), but not Veiga (1990a)). In common with Murphy (1978) the first-laid egg was generally the smallest, although contrary to Murphy, the last egg was not consistently the largest. Egg volume increases with lay order in many passerines (see Lowther 1990), although in some species egg size is irrespective of position (Ward 1995). The significance of sequential increase, particularly the relative size of the final egg, is discussed below. Egg size declined with laying sequence in a population of house sparrows in southern India (Pinowski & Myrcha 1977), presumably in relation to tropical breeding phenology (an exceedingly long breeding season, low clutch size, constant food supply etc).

There was a striking trend for the last-laid egg to be paler in pigmentation, as reported by Lowther (1988). However, this is probably merely an artefact of physiological change as clutch completion approaches, and is unlikely to have any adaptive significance (Stewart & Walker 1998).

Environmental factors (biotic and abiotic)

The time of season (and hence mean daily temperature) had no effect on egg size (when considering repeat clutches of the same females and mean egg size of the population generally). A similar patterns was found in the house sparrow by both Lowther (1990) and Veiga (1990a).

Several studies have demonstrated significant effects of seasonality on egg volume. Ojanen *et al.* (1981) found that the volume of pied flycatcher eggs was correlated with the mean daily temperature about seven days beforehand, which accords with the period needed for accumulation of reserves. Using the same logic, Ward (1995) related the size variation in barn swallow eggs to the temperatures experienced during their formation (around six days). It may be significant that aerial insects figure heavily in the diet of these two species, and that the insects' activity is strongly dependent on the prevailing weather conditions. In opposition to this, no effect of seasonality on egg size was found in the least flycatcher (Briskie &

Sealy 1990), a species with a similar feeding ecology to the swallow and pied flycatcher.

The more catholic diet of the house sparrow (Summers-Smith 1963) presumably means that egg formation is less restricted by environmental conditions than it is for aerial insectivores. This would explain why egg size is unaffected by seasonality. Although it seems reasonable to assume a link between nutrient availability and egg size, the effect of variable food supply is not an automatic predictor of egg size variation. Fieldfares laid larger eggs when the earthworm density was high (Otto 1979), as did magpies provided with additional food (Birkhead 1991) (although the increase was due to extra water rather than yolk). Great tits did not lay larger eggs when provided with supplementary caterpillars (Perrins 1996).

The direct biotic environment appears to have affected egg size between years. The nesting density at barn B was almost doubled in 1996, when more nest-boxes were erected as part of a study on breeding density, and this apparently led to an increase in average egg size. Whilst this is not a natural comparison, as the population at the barn is not composed of the same females between years, it is a clear result. Older birds did not lay larger eggs than yearlings (see above), and hence the result was probably not due to changes in the age structure of the local breeding population.

In direct contrast to the current study, great tits in the Oxford population laid smaller eggs when the nest-box density was increased (Perrins & McCleery 1994). This result, and the finding by Otto (1979) that fieldfares lay smaller eggs in dense colonies, was interpreted as a local response to increased competition for food. Clutch size is known to decline in many birds for this same reason (Perrins 1979), although there is no direct trade-off between the two life history parameters (as discussed earlier). As it seems obvious that competition for food must have increased at barn B between the years with the doubling of the breeding density (since few non-box pairs had been present in the previous year), the parallel trend of *increased* egg volume is difficult to explain.

10.4.2 The bioenergetics of egg production in house sparrows

The significance of the egg size results, particularly the absence of any seasonal effect, can be placed into perspective by considering the costs involved in egg production (Perrins 1996). In large non-passerines such as snow geese, clutch size is strongly limited by food reserves acquired during spring migration (Cooke *et al.* 1995). Although endogenous reserves do not generally restrict clutch size in the house sparrow and other passerines (Schifferli 1976, Krementz & Ankney 1986) they could conceivably restrict egg size.

The two obvious factors which limit egg production in birds are the amino acids which are imputed into the yolk, and the calcium which is essential for eggshell formation (Perrins 1996). As opposed to large birds such as geese, which draw on 'stored capital' (Cooke *et al.* 1995), house sparrows (like most passerines) are classed as 'reserve users' (Perrins 1996). Protein reserves are not laid down well in advance of breeding (unlike significant amounts of fat (Schifferli 1980)), but are acquired a matter of days before use and stored temporarily (possibly in the oviduct).

Krementz & Ankney (1986) considered that egg production in house sparrows would not be constrained by energy acquisition *per se*, as the maximum cost of ovogenesis per day is unlikely to exceed 50% of basal metabolic rate (BMR). Because house sparrows generally operate at about three times BMR, egg formation probably only requires about an extra 15% of daily energy (Perrins 1996). Krementz & Ankney (1995) also concluded that obtaining sufficient calcium for eggshell formation is unlikely to be difficult for female sparrows. Calcium levels are renewed daily through ingestion of grit and mollusc shells, and stored in the medullary bone, which functions as a labile , ephemeral source of mineral (Krementz & Ankney 1995). Although eggs with noticeably thin and fragile shells were encountered at Maine Chance, this was confined to two particular females, suggesting individual malady rather than any environmental deficiency.

Taken together, the bioenergetic analyses suggest that egg size in the house sparrow is unlikely to be restricted by short-term physiological constraints such as acquisition of energy, protein or minerals.

10.4.3 The significance of egg size on fledging size

Hatchling mass was strongly and positively correlated with egg size, as has been found in most birds (listed in Ward 1995). Although this study did not investigate hatchling size as such (tarsal length, wing length etc), this has only correlated with egg size occasionally. The advantage of hatching from a large egg appears to be manifested in greater nutrient reserves rather than larger size (Williams 1994). Unequivocally, house sparrows which lay larger eggs imbue their hatchlings with greater food reserves. Furthermore, the existence of a within-clutch hierarchy in egg size should, initially at least, result in a hatchling hierarchy (O' Connor 1975).

10.4.4 Does egg size variation have any ultimate significance?

Despite the clear relationship between egg and hatchling size, variation in egg size can only have evolutionary significance if it results in increased reproductive success of the parent. Nestling survival in several passerine species (including the house sparrow (Griffith 1998)) is known to be largely determined by body condition at fledging (commonly designated as the regression of mass on structural size) (Perrins 1979).

This raises two critical issues concerning egg size and fledgling survival. The first issue is whether fledglings which hatched from large eggs are causally in better condition than those which hatched from small eggs. The second issue is whether the presumed initial size hierarchy is commensurate with an eventual fledging hierarchy.

There is a paucity of data which unequivocally relate egg size to eventual fitness (which is perhaps not surprising given the difficulties experienced in assigning individual eggs to chicks in a field situation). In this study, no correlation was found between egg size and fledging condition (estimated from mass at ringing age) when using either individual assignments or whole clutches/broods as data points. However, this is only an indirect analysis. To assess the effects of egg size variation on subsequent nestling size variation, it is essential to assign all hatchlings to eggs to counter any subtle effects of hatching order.

172

Several studies have managed to achieve this level of control, finding that hatchlings from larger eggs grow more rapidly (e.g Schifferli (1973) in great tits and Magrath (1992) in blackbirds). Perrins (1996) monitored the significance of egg size throughout the rearing phase of great tits, and found an effect of egg mass on both fledgling size and success. Insufficient data was available to test whether the hatchling size hierarchy which may have been caused by egg size variation in house sparrows persisted until fledging (= ringing) age. Jarvinen & Ylimaunu (1984) found that the initial weight advantage gained by pied flycatchers hatching from larger eggs persisted for a further ten days, but was not evident at fledging.

10.4.5 Experimental work

The correlations which several authors have reported between egg mass and fledging mass are obviously confounded by the attributes of one or both parents (i.e. large eggs may have been laid by large females, who also provide more food for the nestlings. Hence the large fledgling is not a result of the large egg size *per se*). Several studies have controlled for this effect by exchanging eggs between nests (e.g. Smith *et al.* 1995). In one of the few examples involving passerines, Magrath (1992) found a residual effect of egg size on the growth of nestling blackbirds, although this did not translate into improved survival. The outstanding egg-swapping study was performed by Bolton (1991) using Lesser black-backed gulls. Gulls hatching from larger eggs had intrinsically better growth and survival, even after controlling for parental input and hatching patterns.

These experimental studies demonstrate that, in some species, well provisioned (= larger) eggs have an intrinsic advantage which persists until fledging (Perrins 1996). Whether the same advantage exists in house sparrows is unknown, although several lines of evidence argue to the contrary. As explained above, no effects of egg size permeated through to ringing mass (and presumably fledging mass and the consequences thereof). Furthermore, the size or age of either parent was unrelated to the average fledging mass and structural size of the offspring (Chapter 3).

It does appear that the size variation in fledging house sparrows is far removed from egg size variation. However, this does not nullify the significance of egg size variation on parental reproductive success. Each

173

fledgling emerges from only a single egg, whereas females lay several eggs per clutch, and several clutches per season (Perrins 1996). Since females attempt to maximize their *total* reproductive success, any egg size variation associated with the laying sequence would be favoured if it increased the number of recruits produced from the *brood*, not each individual egg.

10.4.6 Egg size, hatching asynchrony and brood reduction revisited

There was no evidence that house sparrows at Maine Chance pursued the 'brood survival' strategy of synchronous hatching allied with a large final egg (Slagsvold *et al.* 1984). Although volume did generally increase with laying sequence for all clutch sizes, hatching synchrony was rare. Nor was there any conclusive evidence for the 'brood reduction' strategy of asynchronous hatching and a small final egg, as although most clutches did hatch asynchronously, the final egg was relatively large.

The size increase ('divergence') between the first and last egg remained constant throughout the season, as did that between the penultimate and ultimate egg in 4- and 6-egg clutches. 5-egg clutches showed increasing 'divergence' with season, with the final egg becoming progressively larger. This is in direct contrast to the brood reduction strategy (Slagsvold *et al.* 1984), which assumes (reasonably) that the quality and availability of nestling food declines towards the end of the season, and hence predicts that the last laid egg should become progressively smaller.

House sparrows complied with neither of the two strategies proposed by Slagsvold *et al.* (1984). This was also found in the house sparrow by Veiga (1990), although his results were more in accordance with brood survival than brood reduction. Although the theories of Slagsvold *et al.* (1984) are perfectly reasonable, this and other studies reveal that the influence of egg size variation is either negligible or masked when compared to the differences caused by hatching asynchrony. In both the shag (Stokland & Amundsen 1988) and the tree swallow (Zach 1982), hatching asynchrony was much more important in establishing a size hierarchy within the brood than any differences in egg size. A more direct comparison in the yellow warbler (Herbert & Sealy 1993) found no support for the brood

survival strategy. The mass of the last-laid eggs which successfully gave rise to a fledgling was not significantly different to those which did not.

It is difficult to avoid the conclusion that in house sparrows, egg size variation is merely ancillary to hatching asynchrony as the primary force in establishing initial size asymmetries.

Chapter 11

Parasites, host fitness and sexual selection in the house sparrow

11.1 Introduction

11.1.1 The evolution of sexual reproduction

Every year, several international conferences and hundreds of journal articles are devoted to describing who mates with whom, and more importantly, why they mate selectively. The more intriguing and silently omnipresent question (Hamilton 1975) is, why mate at all?

Sexual reproduction (the fusion of separate male and female gametes) represents the most perplexing phenomenon in evolutionary biology (Wilson & Gleeson 1983), particularly because it is overwhelmingly the most common method by which animals (and plants) pass on their genes to following generations. Following the individual selection argument popularised since (but not by) Richard Dawkins (1976), animals behave so as to maximise their fitness ('fitness' being the number of copies of their genes present in subsequent generations). Sexual reproduction, by definition, dilutes the fitness of each parent, as all offspring contain an equal amount of genes from another individual. Hence, sex has the paradoxical consequence of halving individual fitness, an effect described by Williams (1975) as 'the cost of meiosis' and Maynard Smith (1983) as 'the cost of males'. By contrast, asexual reproduction automatically replicates the entire genotype of each individual. In a mixed population, if all else were equal, asexual organisms should rapidly outcompete sexual conspecifics.

That sex is so prevalent indicates that mutant asexually reproducing individuals have either never appeared in sexual populations, or that those which appeared did not flourish (Maynard Smith 1986). It is equally plausible that the current plethora of sexually reproducing species evolved from populations originally comprised of parthenogens, and that mutant sexual individuals progressively dominated until the asexual individuals were excluded. The fact that sexual reproduction persists, despite theoretical predictions to the contrary, suggests that sex confers some benefit (s), or asexual reproduction invokes costs (Williams 1975).

11.1.2 The costs and benefits of the forms of reproduction

Early disputes on the benefits of sex versus parthenogenesis centred around the frequency of genetic mutations. For example, mammals accumulate around 100 mutations per genome per generation (Ridley 1994). A strikingly obvious risk of self-perpetuation is therefore the accumulation of deleterious mutations with successive generations. This effect is known as 'Müller's ratchet', and is perhaps best explained by the analogy of the fuzzy, illegible document which soon results from sequential photocopying of the original (Ridley 1994). In contrast, sexual reproduction provides individuals with an opportunity to 'replace' any mutations (albeit in the genome of their offspring) with intact alleles drawn from a conspecific. Although almost all mutations (99%) are perceived as either neutral or deleterious, several may actually be beneficial (as seen in sickle-cell anaemia for instance) (Alcock 1988). Continual recombination allows sexual individuals to deliberately accumulate these beneficial mutations at a substantially faster rate than parthenogens, which simply have to 'wait' for them to occur. Sexual reproduction therefore appears to confer two genetic benefits; purging deleterious mutations, whilst aggregating those which are beneficial.

Despite the elegance of these genetic suppositions, the mutation theories are now regarded as flawed. Firstly, evolution is not preordained, nor is it inexorable (Dawkins 1976). Secondly, any genetic benefits which individuals accrue by virtue of sexual reproduction would be massively outweighed within a few generations by the two-fold loss in fitness they suffer when compared to parthenogens. Three main hypotheses have since been proposed to account for the origin and maintenance of sexual reproduction. One of these concerns the influence of the abiotic, physical surroundings, while the remaining two focus on the interaction between individuals and their biotic environment.

The lottery hypothesis

Asexual reproduction conserves genotypes. Sexual reproduction, by definition, results in the continual formation of novel genotypes. Williams (1975) recognised that all individuals are at the mercy of

changeable and unpredictable environmental conditions, and that survival in such circumstances is akin to a lottery. Rather than purchasing many tickets with exactly the same number (as is the strategy of asexual species), individuals would improve their chances of success (i.e. survival) by purchasing a suite of different numbers. Any deleterious consequences of environmental change would be suffered equally by asexual parents and their clones (such as enzyme inactivity associated with colder temperature). Asexual parents, together with their entire progeny , could die simultaneously because of an inability to adapt to new environmental regime. In contrast, although temperature-induced enzyme inactivity could cause the death of both sexual parents and the vast majority of their offspring, a small proportion may be able to adapt by virtue of their novel array of genes.

Although the lottery hypothesis was appealing, Bell (1982) found no correlation between environmental instability and sexuality. Indeed, species found in stable, predictable environments such as oceans were most likely to reproduce sexually, whereas animals existing in notoriously variable habitats such as mountain sides and freshwater pools were most likely to be asexual (Bell 1982). Any faith in the abiotic lottery was undermined further by Bell (1982), who realised that only a small proportion of animal mortality results from physical factors, and that the most important threat comes from other animals.

The biotic hypotheses

Bell (1982) favoured the 'tangled bank' model, which proposes that sexual reproduction is an adaptation to counter biotic factors, most visibly *intra*specific competition. By producing diverse offspring through recombination, individuals avoid competition with 'themselves', particularly in dense colonies where offspring may settle locally. The tangled bank model was plausible, but was weakened by theoretical criticisms (Ridely 1994). Firstly, individuals possessing a successful genotypic design would be more likely to *lose* from change, rather than gain, and secondly, the tangled bank model fails to recognise that *stasis* is the hallmark of evolution, not change (Gould 1989).

The second biotic model, which is currently the most credible, is a subset of the broader coevolution hypothesis (Alcock 1988). This focuses on an individual's interaction with sympatric species that it seeks to exploit (and vice versa). The changes in one species cause changes in others. Several workers, most prominently W. D. Hamilton, elaborated on the most intricate example of coevolution; that which occurs between a host and its parasite(s). Parasites represent a ubiquitous environmental component of virtually all extant species, and hence all of these are locked in an immediate, inevitable combat with 'the enemy that fights back' (Ladle 1992). This conflict can be illustrated most simply by considering a single host/single parasite system (Hamilton *et al.* 1990).

Hosts vary in their susceptibility to parasites, and parasites vary in their ability to infect hosts. Any susceptible hosts which reproduced asexually would by definition produce susceptible offspring. If, however, a mutant individual arose which reproduced sexually, the continual recombination would eventually produce a novel, resistant genotype. Even a small degree of resistance would be favoured in a population of debilitated individuals, and hence individuals possessing the resistance genes would become more frequent. The original parasitised individuals become progressively outnumbered, and consequently their specific parasite become rare. Selection would thus favour sexually reproducing parasites, which similarly shuffle genes to produce novel, virulent genotypes. The virulent parasites rapidly invade the population of previously resistant hosts, which consequently strive for yet more resistant genotypes through recombination.

The cycle of host/parasite coevolution thus perpetuates, with only temporary respite for successful genotypes of either entity. Frequency dependent selection between host and parasite ensures that rarity remains at a premium, although, as in any arms race (as a host-parasite system *almost* represents), the conflict is ultimately internecine. This endless struggle between parasite virulence and host resistance is an example of a broader range of biotic interactions described by evolutionary biologists as Red Queen scenarios (van Valen 1973). This derives from a passage in Lewis Caroll's (1871) children's book "Through the looking glass", in which the Red Queen explains a curious lack of progress to Alice with 'Now, *here* you see, it takes all the running you can do to keep in the same

place!'. The Red Queen was an ingenious analogy, as once initiated, the struggle is theoretically infinite, and could not be invaded by an asexual mutant. Thus coevolutionary races between host and parasite could be responsible for the maintenance of sexual reproduction (Hamilton *et al.* 1990). Two prime studies lend credence to the host-parasite theory, both of which were performed by Curtis Lively.

Sex and snails

Lively (1987) studied the prevalence of sexual reproduction in two populations of hermaphroditic snails (one in a stable lake, the other in an unpredictable river). Sexual reproduction was more common in lakes than in rivers, where coincidentally, the trematode parasite *Microphallus* was most common (thereby discounting the lottery hypothesis, and supporting the Red Queen). When snails and parasites from two separate lakes were allowed to mix in the laboratory, parasites were much more likely to infect snails from their own lake, which demonstrates the tight genetic interaction necessary for the Red Queen hypothesis. The tangled bank theory was summarily dismissed, as parasites should have been equally virulent to snails, irrespective of any prior association.

Sex and fish

The second study involved the Mexican topminnow fish, which is particularly intriguing as it freely hybridises with other fish to produce asexual triploids. Topminnows themselves can be either sexual or asexual, and are regularly parasitised by helminth cysts ('black-spot'). Where hybrids and asexual topminnows were present, whichever clone was more common was most susceptible to black spot, illustrating the frequency dependence of parasite populations. The most intriguing situation, however, occurred in a pool recently colonised by minnows after a drought. As a result of this founder effect, sexual minnows were actually more parasitised than clones, as the minimal genetic variation meant recombination conferred no advantage. When fresh sexual females were introduced to the pool, the advantage of recombination with novel genotypes lead rapidly to the relative resistance of sexual fish over parthenogens (Lively *et al.* 1990).

11.1.3 Sex, sexual selection, and parasites

Hamilton's genius was realizing that individuals preferring to mate with certain conspecifics could be attempting to obtain resistance genes for their offspring through recombination. This process would be facilitated by the evolution of behavioural or structural characters (as seen in the strut of a grouse or the train of a peacock), with which individuals could (indirectly) advertise their possession of such genes. This simultaneously explained both the maintenance of sexual reproduction, and the presence of Wallacean eugenic characters. In collaboration with Marlene Zuk, Hamilton tested his theory, and the empirical support uncovered was published in *Science* (1982) under the speculative heading 'Heritable true fitness and bright birds : a role for parasites?'.

The Hamilton & Zuk (1982) hypothesis (hereafter HZ) is essentially a 'good genes' (eugenic) model, in which parasite resistance has a heritable, genetic basis. Females benefit indirectly from mating with unparasitised, resistant males, as their offspring inherit the genes which confer resistance. The differential expression of epigamic traits enables females to 'view' the array of such genes possessed by each male. This version of the hypothesis (known as 'sosigonic' selection [Hamilton 1990]) has indisputably attracted the most attention, for two reasons. Firstly, sexual selection theory is obsessed with lekking species such as the peacock, in which females receive only sperm (and hence genes) from males (Andersson 1994). Despite this apparently minimal input, females exhibit definite preferences for certain males as copulation partners (Petrie *et al.* 1992). The genetic basis for resistance proposed by HZ suggested a *precise* advantage which females would accrue through preferential mating, as opposed to the previously used , broader concept of 'viability genes'.

Secondly, in the decade following HZ, an accelerating accumulation of molecular data demonstrated that females of many species seek copulations outwith their pair-bond (Birkhead & Møller 1992). The unfaithful females rarely gain any direct assistance from the extra-pair male, and like the lekking females, receive only sperm (genes). If these sperm imbued the offspring with a greater degree of parasite resistance than those of her partner, then HZ sosigonic selection would provide a definite explanation for the pursuit of extra-pair matings.

PMSS could still operate without heritable resistance. Two variants of the theory suggest that female choice for unparasitised males could still evolve, provided that tangible benefits were accrued (reviewed in Møller 1990a and Clayton 1991). In contrast to the eugenic models, which stress the directedness of female choice *for* particular males, the direct benefits models emphasise the strength of choice *against* certain males.

The 'resource provisioning' model

In species where males make a significant contribution to nestling rearing, females could gain material benefits by preferentially mating with a unparasitised male, or more accurately, avoiding mating with a parasitised male. All else being equal, an infested male would have to divert proportionately more resources into combating the effects of parasitism, and hence less effort would be available for provisioning (Clayton 1991). Alternatively, an infested male may be less efficient at foraging , or in the most extreme scenario, may be more likely to be captured by a hawk (the traditional concept of predators selecting the old and sick). The female in both situations (particularly the latter) would be forced into either increasing her own rate of provisioning to compensate, or risk rearing fewer, undersized nestlings with low probability of survival.

Avoidance of contagious parasites

The 'transmission avoidance' model posits that females select mates according to their level of contagious parasites (i.e. those transmitted by direct contact, Able 1996). This theoretically includes all ectoparasites (particularly the mobile arthropods such as mites and hippoboscid flies), some endoparasitic helminths, and also microparasites such as bacteria and viruses (Hamilton 1990). The latter are usually classed as sexually transmitted diseases (STDs) (Sheldon 1993), although in practice this is a broad category, as ectoparasites such as lice may also be transmitted during copulation (e.g. Hillgarth 1996). The distinction between contagious parasites and STDs is an important one however. By definition, STDs are only transferred between adults, whereas contagious parasites potentially transfer both between adults, and between adults and nestlings (and theoretically , between nestlings). Indeed, most ectoparasite transfer in

altricial birds probably occurs in the nest (Rothschild & Clay 1952), as the nestlings are brooded for long periods by the female (if not the male) and hence the opportunity for transfer is frequent. Any female mating with a mite- ridden male would suffer a added fitness loss, as her nestlings would also receive contagious parasites. (Naturally, this cost does not apply to most precocial birds, where 'nestlings' are not genuinely brooded).

Indirect and direct benefits are not mutually exclusive in most birds of course, as a female pairing with an unparasitised male could simultaneously receive a greater proportion of male assistance in addition to acquiring resistance genes. Equally, a mite-ridden male would probably contribute little to nestling provisioning, and would also present a contagion risk to the female and brood (Møller 1994b).

The original Hamilton & Zuk hypothesis

HZ proposed that secondary sexual characters had evolved because they indicated an individual's resistance to parasite infection. If parasites (and therefore resistance) were an important determinant of fitness in a given species, then female choice would have favoured the evolution of indicator characters. HZ made the interspecific prediction that the species in which visible signals of resistance are most pronounced ('bright birds') should be those which are intrinsically prone to parasitism. 109 species of North American passerines were scored for 'brightness' using illustrations in several field guides. A simple scoring system was used, based on the extent of colouration or striking patterning, and the presence of extra features such as crests, long tails or fleshy eye rings (Zuk 1989). Species were ranked for brightness (dull species such as wrens = low, colourful species such as tanagers = high), and this was compared with haematozoan data taken from several parasitological surveys. A significant, positive correlation existed between several aspects of a species brightness and parasite prevalence. Species in which blood parasites were rare or absent tended to be dull and plain, whereas species in which blood parasites were common tended to be colourful or strikingly patterned. HZ were deliberately tentative in their explanation of this correlation, although they extended their hypothesis to suggest that, if intraspecific brightness correlated with intraspecific variation in blood parasitism, a mechanism for female choice would occur.

The hypothesis was not publicly challenged until 1989. Read (1987) had repeated the HZ analysis on an expanded dataset of haematozoan prevalence from American passerines, supplemented by equivalent data from Europe. The analysis was improved by including phylogenetic associations and several environmental and behavioural variables associated with host breeding ecology. The significant positive correlation between parasitism and brightness persisted. However, when Read & Harvey (1989) repeated Read's (1987) analysis using independent scoring techniques, the correlation between brightness and parasitism disappeared, partly because they excluded species where only few birds were sampled ($n < 10$) and partly because their observers gave several species lower brightness scores than HZ. Zuk (1989) replied by justifying her scoring method as the more reliable, and attributed the discrepancy to Read & Harvey (1989) failing to give species 'credits' for possessing long tails or crests as she had done. Read & Harvey (1989) obdurately (and unjustly) dismissed this, and challenged HZ's assumption, for statistical purposes, that female choice for unparasitised males does not increase a species' resistance. HZ (1989) acknowledged that this increase could occur (as they had done in a footnote to the original paper), and that showy species may actually have lower intensities if they are coevolving more rapidly. HZ also argue that the phylogenetic association between brightness and parasitism emphasised by Read & Harvey (1989) is not inconsistent with HZ, as a high parasite prevalence coupled with intense sexual selection could accelerate speciation.

Although several of the early criticisms were valid, most were either petty (based on minor nuances of the scoring method), or objective rather than constructive (John 1997a, b). A common 'argument' stated that the whole concept was futile, due to the incredibly complex interplay between the parasite and host, and the general dearth of knowledge of how parasites affect their hosts, if at all. Cox (1989) questioned the knowledge of the authors (both behavioural ecologists) stating famously that parasitologists "hoped that the whole matter would die quickly and quietly ".

The validity of the haematozoan data was questioned at the outset, firstly because the use of prevalence data can be misleading (Cox 1989), and more pertinently, there was little evidence that blood parasites actually had any

effect upon their hosts (McClellan & Brookes 1991). Even if haematozoa were pathogenic, determining a host's 'resistance' on the basis of a single blood smear taken at one point in time is a dangerous extrapolation (Cox 1989). Simple assignations of parasite load can, moreover, be entirely ambiguous. An unparasitised host may never have been exposed to pathogens, or may be completely immune as a consequence of earlier infection. Equally, a high parasite load may be the result of a long-standing infection in an unresistant host, or may be the result of a sudden infection in a resistant host, pending activation of an immune response.

Several studies found parasitism oscillated during and between seasons. Boyce (1990) found coccidia to be common in sage grouse during a pilot study, and yet were very rare during his investigation into the HZ . In the same species, *Haemoproteus* prevalence varied between years, and within years, becoming more common as the season progressed (Gibson 1990). Large temporal differences existed in haematozoan infections in birds of paradise (Pruett-Jones *et al.* 1990).

The original HZ study has been heavily criticised for pooling data from different haematozoan surveys (Yezerinac & Weatherhead 1995), whereas it has since been shown in at least two species of bird that prevalence of haematozoa varies between sites (Merila *et al.* 1995, Bennett *et al.* 1995). Ironically, HZ were aware of this potential bias, and averted it by using within-zone surveys (John 1997b).

The attack upon the original interspecific HZ still rages, with several authors stating that the scope of the hypothesis should be narrowed. The doyen of blood parasite studies, Gordon Bennett, argued that several uninfected host species should be excluded from the HZ, as their environment is sufficiently harsh to be free of vectors (e.g. penguins). In all probability, these problems will never be resolved to the satisfaction of all parties. (A thorough rebuttal of the parasitological and theoretical criticisms of the HZ is given in John 1997a, b).

11.1.4 How strong is the evidence to support the HZ theory?

Interspecific tests of HZ

Ironically, although this was the inaugural aspect of the HZ, it has proved the most controversial. A flurry of comparative papers emerged following the 1989 HZ debate, wringing complex analyses from 50 years worth of blood parasite surveys. Read & Weary (1990) re-analysed the HZ paper using the original data on haematozoan prevalence but a different interpretation of aural 'brightness'. When song was scored using sonagram complexity, and the confounding effects of phylogeny were controlled for, the correlation between haematozoan prevalence and song elaboration disappeared.

Pruett-Jones *et al.* (1990) examined haematozoan prevalence in 10 species of birds of paradise, and found a significant, positive correlation with brightness and mating system (promiscuous species being brighter and more heavily parasitised than monogamous species). Johnson (1991) assessed an expanded data set for North American passerines while disentangling ecological and environmental correlates of species brightness. The twin factors of phylogeny and predation risk exerted a more substantial influence upon brightness than parasitism (e.g. ground nesting birds such as American sparrows tend to be more cryptic). Garvin & Remsen (1997) simultaneously investigated HZ and the neglected suggestion of Bennett & Fallis (1960) that blood parasitism is largely determined by nesting height (due to stratification of ornithophilic vectors). Nest height was as good or better a predictor of parasite prevalence as was sexual dichromatism or plumage brightness.

Intraspecific tests of HZ

Curiously, despite the broad significance of the HZ hypothesis, it apparently did not stimulate further tests immediately. Empirical investigations slowly began to appear in the late 1980s, peaking in 1990 with the publication of a suite of papers from an HZ symposium. A plethora of both field and aviary experiments have attempted to test the within-species prediction of the HZ (reviewed in Møller 1990a, Clayton 1991). Many have managed to satisfy one or more of the following criteria

186

necessary for the HZ to be supported, with nine (listed below) satisfying the first three. Five studies (listed in Møller (1990a) have demonstrated the fourth criterion, heritablity of resistance (but see discussion below).

Criteria necessary to demonstrate PMSS.

1. parasite is pathogenic
2. expression of SSC varies with parasite load
3 preferred males have fewer parasites
4. heritable variation in resistance

Table 1. Studies fulfilling the three immediate criteria.

	Host	Parasite	Reference
	guppy	nematode	Kennedy *et al.* 1987
	stickleback	ciliate	Milinski & Bakker 1990
	sage grouse	haematozoan	Boyce 1990
	pheasant	coccidium	Hillgarth 1990
	jungle fowl	nematode	Zuk *et al.* 1990
	rock dove	feather lice	Clayton 1990
	barn swallow	haematophagous mite	Møller 1994
	turkey	coccidium	Buchholz 1995

In contrast, several studies have found no support for any of the HZ criteria.

Table 2. Studies fulfilling none of the HZ criteria.

	Host	Parasite (s)	Reference
	swift	lice , louse flies	Lee & Clayton 1995
	sage grouse	haematozoan	Gibson 1990
	redpoll	haematozoan	Seutin 1994
	red-winged blackbird	several taxa	Weatherhead *et al.* 1993
	stickleback	haemogregarine	Fitzgerald *et al.* 1993

Although all of these studies lend credence to the notion of coevolutionary cycling mediated through sosigonic selection, most could

also be partly explained by the transmission avoidance model. Two studies merit further description, as they illustrate this overlap tidily, as well as demonstrating the synthesis of parasite and host life histories necessary for a full understanding of how coevolutionary cycling could operate.

Sage grouse

Although Gibson (1990) could find no support for the HZ in sage grouse, Boyce's (1990) study of the same species represents one of the most compelling pieces of evidence. This is a particularly important study, as the most extreme asymmetry in avian mating success has been found in sage grouse, with one male obtaining almost half of all copulations seen at one lek. The fact that this does not rapidly erode variation in the genetic component of fitness is known as the lek paradox (Kirkpatrick & Ryan 1991), and host-parasite coevolution is arguably the most promising means of its resolution.

Furthermore, sage grouse (and the Galliformes generally) possess several phenotypic ornaments, most notably fleshy wattles and inflatable air sacs, together with intense behavioural displays. All of these represent immediate indicators of host condition (as opposed to feathers), and hence females can judge the precise condition of all displaying males under the same environmental and ecological circumstances (i.e. accounting for seasonal and diurnal patterns of infection, a rapid local emergence of vectors etc). Furthermore, females can presumably judge changes in condition over a short period of time, as they revisit the leks over a period of days before deciding with whom to mate. Whereas feathers do not change over this interval *per se*, colouration of fleshy wattles or display vigour are plastic, and more amenable as daily indicators of parasitism. Males infected with both lice and malaria had reduced reproductive success as a result of infrequent lek attendance (attendance being virtually a *sine qua non* for copulation). Parasitised males which did manage to obtain copulations did so later in the season, with younger, lower quality females.

The presence of lice and malaria suggest that two of the HZ models could operate simultaneously; transmission avoidance and sosigonic selection. Lice create haematomas on the large frontal air sacs of males, and hence

their presence is immediately revealed. After copulation, and before leaving the lek, female grouse shake vigorously and preen themselves, suggesting that a risk of acquiring parasites *in coitus* is at least perceived. Louse populations do not increase rapidly however, and the successive lek visits suggest females are seeking cues of malarial resistance.

In partial support of this theory, in captive trials females preferred to mate with males given a general antibiotic (which would have reduced malarial infection but would have had a minor effect upon ectoparasitic lice).The malarial parasite involved is not *Plasmodium relictum* , which occurs in many passerines, but *P. gallinarium* , which is specific to sage grouse and may thus represent a prolonged evolutionary association. The life cycle of avian malaria involves an eruption of sporozoites from infected erythrocytes, which releases toxins into the blood stream. The timing of this event varies markedly between parasite species, and occurs in the early morning in *P. gallinarum*. Intriguingly, this coincides with peak lekking activity of the host, and hence female inspection occurs precisely when males are under most physiological duress.

Barn swallows

Perhaps the major criticism of the HZ is that most studies have been purely observational and have relied on correlative evidence, which does not imply cause (Møller 1994). This is mostly because of practical difficulties, as levels of many parasite taxa are difficult to manipulate.

A notable exception was performed by Møller (1994) during his outstandingly detailed long term study of the barn swallow. The haematophagous mite *Ornithonyssus bursa* exerted strong negative effects upon parental reproductive success. Although most mites remain in the nest and obtain blood meals from nestlings, they are highly contagious and a proportion transfer to the parents whilst incubating, brooding or feeding. Swallows of both sex should have evolved mechanisms to detect mite levels in nests or on conspecifics, and thus avoid any contact with mites.

Møller (1994) experimentally manipulated the levels of these mites in swallow nests (i.e. by either adding mite propagules from other nests or by spraying with a pesticide). The numbers of mites present on males was

strongly correlated with the mite levels in their nests. Females could assess the mite load of males at a distance, by virtue of the symmetry and length of their tail streamers (males with longer, more symmetrical tail streamers had fewer mites).

Female swallows preferred to mate with males with fewer mites (advertised by the differential expression of their tail streamers). This supports the transmission avoidance model, as females preferring less parasitised males thereby avoid receiving mites during copulation, and would also indirectly select a less parasitised nest. Moreover, pairs tended to mate assortatively with respect to mite load, suggesting that mutual mate choice exists based on parasite load.

However, sosigonic selection is also supported, as females may prefer males which advertise their genetic resistance by possession of longer, more symmetrical tails. The resource provisioning hypothesis was not supported, as mite load was independent of feeding rate. Møller (1994) could only conclude that female preference for males with longer tails and fewer parasites had evolved to provide a choosy female with an overall combination of direct and indirect fitness benefits.

The 'resource provisioning' model

The resource provisioning model has no unequivocal support to date. Parasitised stickleback males provided no care for eggs and young after spawning (Milinski & Bakker 1990), and as parental care in sticklebacks is only provided by the male, this suggests that parasitism represents at least a potential risk to offspring survival (and hence female reproductive success). Although female sticklebacks do prefer to mate with unparasitised males, it was unclear whether this represented sosigonic selection or resource provisioning.

The 'direct transmission' model

Female rock doves in captive trials mated with males which were uninfested with feather lice in preference to those which had been experimentally infected (Clayton 1990). These lice subsist merely on feathers, and presumably do not invoke an immune response, which led

Clayton (1990) to posit this as evidence for the transmission avoidance model than sosigonic selection.

Borgia (1986) found that female satin bowerbirds preferred to mate with males which had fewer lice, although like Clayton (1990), he could find no effect of lice on physical condition or outward appearance of the host. Ectoparasites may be viewed directly, such as feather lice clustered around the head region of peafowl (*pers. obs.*) and satin bowebirds (Borgia 1989) , or ticks of seabirds (Birkhead 1993). Although he did thus favour the 'transmission' avoidance model, he suggested an alternative scenario of 'correlated infection', in which ectoparasite prevalence correlates with endoparasite prevalence (through their capacity as vectors). This blurs the distinction between the direct transmission model and sosigonic selection.

Multi-parasite   surveys

A common criticism of field studies of HZ was (and still is) the restricted nature of their parasitological assays, which were usually limited (understandably) to the use of non-invasive methods. Two outstanding intraspecific studies have attempted to correlate attractiveness and resistance by performing a broader survey of both ectoparasites and endoparasites.

Weatherhead *et al.* (1993) found no relation between red-winged blackbird secondary sexual characteristics and their parasite load (encompassing four species of blood parasite, plus several intestinal helminths and ectoparasitic arthropods). The survey (unusually) included many individuals which were examined at necropsy. Curiously, the most suggestive relationship was a positive, though insignificant, correlation between epaulet size and mite incidence: attractive birds tended to be more heavily parasitised. This contradictory result was counterbalanced however, as these males were relatively free of the other parasites surveyed, illustrating the value of broad surveys (and simultaneously illustrating how misleading single parasite studies can be).

Buchholz (1995) investigated PMSS in both wild and captive populations of the turkey, which, like several gamebirds, has a bizarre array of ornamental characters (e.g. snood, beard, facial caruncles, large tail, tarsal

spurs). Females preferred to mate with males with longer snoods, which were inversely correlated with levels of the protozoan coccidium *Eimeria*. As Buchholz had thoroughly investigated several taxa of ecto- and endoparasites and found their levels to be minor, they could confidently be discounted as ancillary.

Heritability of resistance

For sosigonic selection to evolve, parasite resistance must have a genetic basis. Møller (1990a) stated that five studies found evidence of heritability or, at least, repeatability of parasite infection between years (Kennedy *et al.* 198, Borgia & Collias 1989, Hillgarth 1990, Møller 1990c, Zuk *et al.* 1990). Repeatability of individual infection is not equivalent to resistance however, and personally, Møller's study is the only one of these five which is reasonably convincing.

Møller (1990c) used a partial cross fostering approach to transfer barn swallow broods between nests with low or high mite levels. Fostered nestlings resembled their genetic parents more closely than their foster parents in terms of mite infestation. Nestlings produced by parents with few or no mites had relatively few mites when reared in nests of heavily infested parents. As Møller later admitted (Møller 1994) this is not a true demonstration of heritability, as nestling infection is being compared against adult infection. To produce stronger (but not unequivocal) evidence for heritability , these nestlings would have to resemble their parents in mite level when they are themselves adult.

Although heritable disease resistance appears to be widespread in domestic animals (Wakelin & Blackwell 1988), an unequivocal demonstration in wild animals is lacking. This should not be surprising, given the large amount of confounding factors which affect parasite load, and the difficulties encountered when simply trying to measure 'resistance'.

11.1.5 Aims of this chapter

This chapter represents a test of the Hamilton & Zuk hypothesis in the house sparrow population at Maine Chance. The male house sparrow possesses a black throat patch or 'badge' which is though to be under

sexual selection (Møller 1987a, Veiga 1993a, but see Kimball 1996). Although females may prefer to mate with males with large badges, the benefits of this choice are unknown. The Hamilton & Zuk hypothesis predicts that large-badged males have a higher genetic resistance to parasitism, and therefore have lower levels of disease, with respect to both physical condition and the infestation present in their nest. I also investigate a necessary precursor of the Hamilton & Zuk hypothesis, that parasites have a detrimental effect upon their host.

To ensure the stringency of the test, several parasite taxa were surveyed in concert. To aid clarity, this chapter is therefore split into sections according to the parasite sampled.

I : Ectoparasites

11.2.2 Methods

Adults were held in the 'ringers grip' and examined for feather lice (Insecta:Phthiraptera) and haematophagous mites (Acari:Acarina) by carefully searching through the major plumage tracts of the body. The head and badge feathers were gently raised with a pencil to ease inspection of their concave undersides, as these are favoured sites for lice in particular (I. R. K. Stewart *unpub.*). Each wing was fanned out and held before a light source (usually the sun). The underside of each primary and secondary was scanned for feather mites (Acari:Proctophyllodidae), and to a lesser extent, lice. The retrices were examined likewise, and finally, the downy feathers around the cloacal region and inner thigh were ruffled and searched. Nestlings were similarly searched for ectoparasites. All examinations were performed in strong light wherever possible, and a hand lens was used for close inspection of suspect entities. Ectoparasites were identified using Séguy (1944) and McDaniel (1988).

11.2.3 Results

A single feather louse was recorded on the underwing of a juvenile in 1995 (of the elongate *Degeeriella* form). None of the characteristic round holes described by Møller (1994) were noted in this bird, or any house sparrow examined during the study. No louse eggs ('nits') were found.

Two juveniles trapped at the end of the 1995 season each carried a single feather mite of the *Proctophyllodes* genus. No other ectoparasites were found.

11.2.4 Discussion

The rarity of ectoparasites was surprising, considering the diversity previously recorded from the house sparrow at several localities. Although a regurgitation of Brown & Wilson's (1975) extensive catalogue is unwarranted, the pertinent records deserve recounting. Five systematic studies of house sparrow ectoparasitism have been undertaken in the USA (Table 1). For convenience, these are referred to in the text by the American state in which they occurred, rather than author.

Table 1. Ectoparasite surveys of the house sparrow in the USA.

Indiana	McGroarty & Dobson 1974
Kentucky	Wilson 1958
Maryland	Wilson 1956
Massachusetts	Brown & Wilson 1975
Wisconsin	Woodman & Dicke 1954

Phthiraptera (feather lice)

Perhaps the most surprising absence (or near-absence) was of feather lice (Insecta : Mallophaga). Thompson (1958) lists four species of lice regularly found on the house sparrow in Britain: *Menacanthus annulatus, Myrsidea quadrifasciata, Bruelia subtilis* and *Philopterus fringillae. Philopterus fringillae* is reasonably common (*c* 30% of adults) on house sparrows in Leicestershire (*pers. obs.*). Lice, especially *Brüelia* sp, were regularly recorded in the each of the five American surveys (Table 2). Indeed, the Wisconsin study specifically investigated the fluctuation in the host louse population in each month of the year. In this study, and in the equally methodical survey (albeit of a broader range of ectoparasites) in Indiana, peak louse populations were found in early spring. This coincides with the presence of the first brood in the nest (allowing the lice maximum

opportunities for transmission) but more importantly, coincides with peak trapping (and thus handling) at Maine Chance.

Table 2.*Bruelia* sp records from the house sparrow in the USA.

State	N	% infested	mean	range
Maryland	55	20	?	?
Kentucky	64	12	4	?
Mass.	34	29	20	1 - 204
Wisconsin	391	45	?	1-68
Indiana	300	55	19	1 - 146

Proctophyllodidae   (feather   mites)

Feather mites are also well recorded from house sparrows in Britain and Europe (Thompson 1958, Behnke *et al.* 1995, I.R.K. Stewart, unpublished data). Proctophyllodid mites were common in three of the five American studies (Table 2). Feather mites were not assessed in the Wisconsin study, and appeared to be absent from the sparrows surveyed in Kentucky. The presence of single mites on two juvenile sparrows is difficult to explain, although as both were unringed birds, they may have originated from a separate breeding population in which feather mites were present. If this is the case, then the variation in parasite presence would have to persist over a relatively small area, as house sparrows show little natal dispersal (Fleischer *et al.* 1984), usually settling within a mile of their birthplace.

Table 2. *Proctophyllodes* sp prevalence in three sparrow populations.

State	N	% infested	mean	range
Mass.	34	65	11	1-437
Maryland	55	55	-	-
Indiana	300	83	62	1 - 1204

The house sparrow has spread dramatically across North America since its original introductions at various localities (Wing 1943). These populations would have overlapped and interbred, and hence a degree of parasite transfer must have occurred (and still be occurring). The two juveniles upon which the feather mites were found may represent transitional birds

originating from local 'pockets' of infection, which could introduce contagious ectoparasites to Maine Chance sparrows. Conversely , the Maine Chance sparrows themselves may represent a 'pocket' of uninfection. Without knowledge of feather mite presence/absence in nearby populations, this explanation remains speculative. However, house sparrow populations of some description must have existed and mingled freely around the Maine Chance area for at least 30 years, and it is curious that feather mites are still absent.

Hippoboscidae   (louse-flies)

These are large (*c*10 mm) crab-shaped, blood-sucking flies which emerge from the plumage when the host is handled. As they usually leave the bird and settle in the beard or hair of the handler (*pers. obs.*), it is extremely unlikely that they were missed. Two main genera of Hippoboscidae occur, *Ornithomyia* of Europe and *Ornithoica* of North America. Both have been recorded on house sparrows. Thompson (1958) records *Ornithomyia fringillina* as a regular ectoparasite of sparrows in Britain, and Hutson (1984) considers sparrows as typical hosts of both *O. fringillina* and *O. avicularia*. Specimens of *Ornithoica* were obtained from each of nine sparrows controlled at a banding station in the US (Main & Anderson 1970). Wilson (1958) found *O. vicina* on five of 70 sparrows examined in Kentucky.

Validation of field technique

The most accurate method of censussing most ectoparasites (dissolution in caustic alkali) unfortunately requires freshly killed birds. The results presented here were obtained by *in situ* examination, since destructive sampling was undesirable at the field site. To avert the criticism that the apparent rarity of ectoparasites was an artefact of methodological limitations, house sparrows were trapped in rural Leicestershire during Autumn, and likewise examined.

Repeating the exact methodology employed in Kentucky, nymphal and adult feather lice (*Philopterus* sp.) were easily located, mostly on the underside of the crown feathers, and occasionally those of the badge. Low numbers of wing lice were found. Feather mites were also eminently

visible as dark spots (or more accurately, rectangles), mostly clustered on the inner primaries. Despite their small size, feather mites were detectable with the naked eye, burrowing quickly towards the feather vane when the wing was opened.

After sacrificing each bird, the wings were crudely chopped up with scissors and shaken individually in a sealed jar of weak detergent. Large feather fragments were removed with forceps, and the remaining solution was filtered. Feather mites, most of which survived this process, were counted while still on the filter paper. Both of the common feather mite genera were represented *(Proctophyllodes* and *Analges)*, with a very strong bias towards the former. Mite counts obtained from the filter paper were less than the total estimated *in situ*, presumably because a proportion of the mites were not dislodged by the detergent, although they were significantly correlated (Spearman Rank $r = 0.54$ $n = 34$ $p < 0.05$). The ease with which both feather lice and mites were located upon house sparrows in Leicestershire indicates that these parasites were genuinely rare at Maine Chance.

Comparison with other studies

The exotic nature of the house sparrow in Kentucky does not automatically explain the paucity of ectoparasites. A comparison of the parasite fauna of house sparrows in North America and Britain revealed that 49% (34 of 49) of parasites have accompanied this host since its introduction (Brown & Wilson 1975). Furthermore, the house sparrow is technically an alien in Britain, and hence is an ancestral population itself.

Their exotic status almost certainly explains the absence of louse-flies. These are only found on the bird when feeding, and their pupiparia are deposited away from the nest (in *Ornithomyia* at least [Hutson 1984]). The introduced sparrows would not have physically transported louse-flies into North America. Hippoboscids are notoriously catholic in their range of hosts (Hutson 1984), and the *Ornithoica* records on house sparrows in the US would have been derived from native passerines.

Feather lice and mites are obligate ectoparasites and pass their entire life-cycle on the host. Both parasites live in direct contact with the plumage

and would not have been dislodged in transit, particularly the eggs which are firmly glued to the feathers. As expected from this, *Bruelia* sp was recorded in all five of the published North American surveys, and feather mites were recorded in all but one (ironically, that in Kentucky). The near-absence of lice and feather mites at Maine Chance is most plausibly a legacy of parasite-free ancestors. Louse populations show the classic negative binomial distribution, where most of the lice are aggregated between a few hosts, and most hosts have few or no lice. If, by chance, the founding population of house sparrows around Maine Chance were those with few or no lice, a 'bottleneck' effect would have caused subsequent generations to be louse-free.

Admittedly, this is a speculative explanation. An absence of data is notoriously difficult to interpret (Gould 1989). Whether 'pockets' of lice and feather mites actually exist could only be ascertained by sampling house sparrow populations within a practical radius of Maine Chance.

II : Haematozoa (blood parasites)

11.3.2 Methods

A drop of blood was obtained by brachial venipuncture, and smeared on a clean, dry slide following Bennett (1970). A single smear was taken from nestlings and juveniles, whereas two were usually taken from adults. Smears were air dried immediately, and stored temporarily in slide trays whilst in the field. At approximately weekly intervals, accumulated smears were brought back to the laboratory, fixed in 100% methanol for two or three minutes, and allowed to dry before staining.

In 1995, fixed smears were stained for 30 minutes in Kodak-Eastmann Giemsa (diluted 1:10 with distilled water, pH 7.2). In 1996 a commercially obtained stain was used, which was supplied pre-buffered and diluted, and reduced staining time to 10 minutes. Following staining, slides were rinsed throughly in distilled water, and air dried before long-term storage. Unfortunately, although both stains were sufficiently pervasive for blood parasite identification, the various forms of leucocytes were relatively faint. Consequently, all smears were restained at the Leicester Royal

Infirmary Haematology Unit in 1997 using an automated slide stainer. Slides were examined using a X 40 objective lens to detect leucocytozoids and haemoproteids, and a X 100 oil immersion to detect plasmodiids and microfilariae. The blood smears from the 1995 field season were examined by myself and Dr F. Clark. The much larger sample of smears from the 1996 season were examined exclusively by Dr Clark.

11.3.3 Results

400 blood smears were examined from 122 adults (50 in 1995, 60 in 1996) and 230 nestlings (120 in 1995, 130 in 1996). No blood parasites were found. 12 smears were selected at random and examined by Professor Ellis Greiner, University of Florida. Professor Greiner also failed to detect any blood parasites. Although the blood was not screened for the presence of haematozoan DNA using highly sensitive molecular techniques, as has been used to identify avian malaria (Feldman *et al.* 1995) and *Leucocytozoon* (Solano et al. 1995).

11.3.4 Discussion

The absence of haematozoa was surprising. Although several substantial surveys in Europe have either failed to find blood parasites in house sparrows (e.g. Baker 1975, Stewart *et al.* 1997) or described them as rare (A. P. Møller, *pers. comm.*), many records exist both in Europe and North America. This is fortuitous, as most parasitological surveys are of birds handled for ringing, usually migrants captured at bird observatories.

Blood parasites of the house sparrow at other localities

Peirce's (1981) mammoth review of haematozoan records from the birds of Western Europe cites the house sparrow as host to several classic genera. 1132 house sparrows had been examined (more than any other species cited), and of these, 486 were infected with one or more genus of blood parasite (43%). The 486 infections, listed generically here although several were described to the species level, were represented by *Haemoproteus* (167), *Plasmodium* (83), 'other parasites' (259) and a single case of *Trypanosoma*. The 'other parasites' listed had uncertain taxonomic status

at the time of writing (*Atoxoplasma, Toxoplasma* and *Lankesterella*), which is discussed below.

The infections were recorded in a range of countries;

Haemoproteus	Germany, Greece, Italy, Spain, Switzerland, UK
Plasmodium	Germany, Greece, Italy, Spain, UK
Trypanosoma	Portugal
Atoxoplasma	Holland, UK

The records from Italy should be viewed with caution, as the common Italian sparrow *Passer italie* was treated as a variant of the house sparrow in parasitological surveys. The two are now regarded as separate species (Summers-Smith 1988). Pierce's 1981 review emulated and embellished the equivalent compilation by Greiner *et al.* (1975) of haematozoa from North American birds. Again, the house sparrow was the most frequently sampled species, with 4070 individuals examined. 463 of these were infected (11.4%), mostly with the malarial parasite *Plasmodium* (383 records). 120 records were of 'other parasites', and several other genera being represented, albeit sporadically (*Leucocytozoon, Haemoproteus, Trypanosoma,* microfilariae). 'Other parasites' again refers to *Atoxoplasma, Lankesterella* and lesser haemogregarines).

The prevalence of haematozoa in the states surveyed is shown in Table 1. The total number of birds sampled (6, 520) is obviously much larger than the total used by Greiner *et al.* (1975). Several of the reports listed in Table 1 were not included because of methodological incompatibilities. For example, surveys relying on tissue smears have a higher probability of detecting low infections of *Atoxoplamsa* (Lainson 1959), and malaria could have gone undetected where subinoculation of blood into canaries was the means of diagnosis. Although studies involving these techniques are indicated, the table merely illustrates that house sparrows are infected with haematozoa in many states, although in none are they particularly prevalent.

Table 1. Haematozoan prevalence in North American house sparrows.

(1 = some tissue smears used, 2 = subinoculation used)

State	Number examined	% positive	Author
California	2880	8.4	Herman *et al.* 1954
California	41	7.3	Wood & Herman 1943
Georgia	489	14.5	Jordan 1943
Georgia	250	17.2	Love *et al.* 1953
Georgia	41	7.3	Thompson 1943
Hawaii	70	11.4	van Riper *et al.* 1986
Illinois	125	12.0	Huff 1939
Maryland	516	11.2	Micks 1949
Massachusetts	41	73.0	Herman 1938
New York	703	12.1	Manwell 1957
New York	245	2.9	Manwell & Herman 1935
South Carolina	323	6.5	Hart 1949
South Carolina	225	14.2	Hunninen & Young 1950
Texas	282	42	Box 1966
Utah	209	14.4	Grundman *et al.* 1952

The Atoxoplasma conundrum

The taxonomy, and indeed identity, of *Atoxoplasma* has been a source of dispute among protozoologists. After several years of being described as simply a '*Toxoplasma*-like organism', Garnham (1950) recognised it as being distinct from *Toxoplasma*, and hence renamed it '*Atoxplasma*'. Lainson (1959) attempted to elucidate its life cycle after finding *Atoxoplasma* in all of 99 adult sparrows examined in suburban England. In a methodical study, various stages of the house sparrow ontogeny were examined (eggs, hatchlings, nestlings and fledglings). Several nestlings were infected as early as the sixth nestling day, and heavy infections of *Atoxoplamsa* was found in all fledglings examined. As haematophagous mites were common in house sparrow nests (although not on adults) Lainson (1959) supposed that the mites were actively transmitting the parasite while feeding on nestlings. Ironically, the reverse scenario was eventually invoked, as although squash-smears identified the presence of

sporozoites inside the mites, these had not developed to become infective. Thus, Lainson (1959) presumed (understandably) that nestlings acquired *Atoxoplasma* by ingesting infected mites, as opposed to vector transmission, and renamed the parasite as *Lankesterella*. Unfortunately, this link could not be demonstrated experimentally because all nestlings in the area were naturally infected already.

Box (1970) investigated this link, but could not induce atoxoplasmosis by feeding sparrows with infected mites. Other attempts also proved unsuccessful, including orally inoculating birds with infected blood, and exposing canaries to mites which had fed on infected sparrows. Although this discounted Lainson's (1959) mite theory, the life cycle of *Atoxoplasma* remained an enigma, until it was elucidated serendipitously. While investigating malarial transmission, Box (1970) found that sparrows kept together in unclean conditions developed heavy infections of *Atoxoplasma*, suggesting a coccidial nature to the disease. This was established conclusively when oocysts of *Isospora lacazei*, a common parasite of house sparrows, were inoculated into healthy sparrows and *Atoxoplasma* was subsequently identified in touch smears of internal organs. Hence, *Atoxoplamsa* is not haematozoan, but is a developmental stage of the intestinal coccidian, *Isospora* sp, as later confirmed by Kruszewicz (1995).

Reasons for the absence of haematozoa at Maine Chance

Several publications have appeared within the last five years describing negative records of haematozoa from birds. This reflects the interest in bird parasites stimulated by the Hamilton-Zuk hypothesis: presumably many previous absences went unpublished. Three hypotheses have been proposed to account for these absences, none of which are mutually exclusive.

Hypothesis 1 : Vector deficiency

Arthropod vectors may not be able to tolerate the extremes of temperature found in certain species' breeding range. Little & Earlé (1995) argued that African sandgrouse and sociable weavers were free from haematozoa

because the desert environment was too arid to support vector populations, particularly as most insect vectors require water in which to lay eggs. At the other extreme, unusually cold conditions probably preclude the existence of vectors in Antarctica, where Chinstrap penguins are free from blood parasites (Merino *et al.* 1997). These scenarios of temperature inhibition are unlikely at Maine Chance. Although conditions are cool and wet at the start of the breeding season, and warm and dry thoughout most of the summer, neither environment is dramatically inclement. Avian blood parasites are common at both latitudinal extremes of North America (e.g. Bennett & Fallis 1960 for Canada, Box 1966 for Texas), and the central position of Kentucky ensures environmental conditions are similarly intermediate.

Bennet *et al.* (1995) examined haematozoa from three populations of pied flycatchers in Fenno-Scandinavia, and found that the prevalence and incidence of the various genera differed between sites. This was attributed to local variation in vector populations due to (often subtle) differences in both habitat structure and environment. Although volant insects are common around the Maine Chance area, including flies which bite humans (*pers. obs.*), subtle features of the local habitat may be responsible for a paucity or absence of suitable vectors. The vector deficiency hypothesis could be tested at Maine Chance by operating suction traps at various heights around the barns. The hypothesis would be rejected if known disease-transmitting insects such as mosquitoes or other biting flies were trapped. The hypothesis would also be rejected, indirectly, if haematozoa were found in blood smears taken from other passerines resident at Maine Chance, particularly nestlings of species with a similar breeding ecology (e.g. any species nesting in a cavity at least a metre from the ground, such as the bluebird, tree swallow, house finch, chickadee and wren).

Hypothesis 2 : Evolutionary lag

Arguably the most nebulous theory was proposed by Earlé & Underhill (1993) to explain why several wader species (Charadriiformes) were uninfected on their tundral breeding grounds. They hypothesized that insufficient evolutionary time had elapsed for the host-parasite-vector cycle to become established, as the tundra is a relatively recent

biogeographical zone. As house sparrows have only been present in North America for less than 150 years, the evolutionary lag hypothesis is particularly appealing. However, blood parasites have been identified from house sparrows in over 20 other American states, distributed widely over the country (see Table 4). Hence, the evolutionary lag hypothesis is untenable in Kentucky.

Hypothesis 3 : Innate resistance

The absence of haematozoa in waders breeding on tundra was brought into perspective when Figuerola *et al.* (1996) found Kentish plovers breeding in Spain to be free of blood parasites. The Mediterranean biogeographical zone is not of recent origin, and the climate is warm and conducive to vector reproduction. Hence, the twin hypotheses of evolutionary lag and vector deficiency were not applicable. As haematozoa have only rarely been recorded from waders world wide (Greiner *et al.* 1975 , Peirce 1981), it was suggested that birds such as waders have an innate resistance to blood parasites, irrespective of their breeding distribution, or the local conditions pertaining to vector abundance.

As a striking example of apparent resistance, no blood parasites have ever been recorded from the Procellariformes (Greiner *et al.* 1975, Peirce 1981). Procellarids represent a primitive order of birds which usually nest on offshore islands, weakening the evolutionary lag hypothesis. Despite the remote and windswept nature of these islands, the vector deficiency hypothesis has also been falsified (Peirce & de la Brooke 1993), as several sympatric species of gulls and auks are infected with blood parasites. The presence of some vector (s) is certain (probably hippoboscids and ticks), as several nestlings were also infected. The innate resistance hypothesis was indicted to explain a lack of blood parasites in Cory's shearwaters (González - Solís & Albella 1997), storm petrels (Merino *et al.* 1998) and several procellarids in the Pitcairn Islands (Peirce & de la Brooke 1993).

At least two studies have been published which found no evidence to support either of the three hypotheses. Rytkönen *et al.* (1997) could find no reason why willow tits in a deciduous woodland in Finland were uninfected, and Stewart *et al.* (1997) came to the same conclusion in a large

204

sample of house sparrows in arctic Norway. None of the three hypotheses appear to explain the absence of haematozoa at Maine Chance.

Is there really an 'absence' of haematozoa?

The failure to detect *Atoxplasma* in blood smears was particularly intriguing following the Lainson-Box dispute (discussed above), as several house sparrows at Maine Chance were infected with *Isospora*. The most obvious explanation for this anomaly is the low level of coccidial infections. Peak oocyst counts of Maine Chance sparrows corresponded to less than 1,000 per gram of dried faeces, whereas Box (1970) was experimentally inoculating nestlings with 5, 000 oocysts. Furthermore, *Atoxoplasma* is much more abundant in the macrophages of the internal organs than the peripheral blood (Lainson 1959). If several Maine Chance sparrows had been sacrificed and touch smears taken from their spleen or liver, *Atoxoplasma* proably would have been found. Obviously, this was not desirable during an ongoing study of reproductive success, and until house sparrows are examined according to Box's (1970) methods, the absence of *Atoxoplasma* is only tentative. However, as *Atoxoplamsa* is taxonomically only a stage of an intestinal coccidian (Box 1981), true haematozoa are probably genuinely absent.

III : Intestinal parasites

11.4.2 Methods

Faecal samples were obtained from adults and nestlings, and stored in 1 ml of formal-saline solution (0.05M NaCl, 2M formaldehyde). It was difficult to obtain faecal samples from individual nestlings, as the physical act of extracting the brood tended to induce simultaneous defecation among most or all nestlings. Most faecal samples were therefore taken on a per-brood basis, by pooling samples produced by two or three nestlings. Despite this difficulty, several samples were obtained from particular nestlings by carefully removing them from the nest individually.

Useable adult samples were scarce, as these birds tend to produce watery faeces when held as opposed to the gelatinous lumps produced by nestlings. To counter this, most adult samples were collected

opportunistically when a bird under observation defecated in a convenient point (e.g. a fence post). Several unassigned samples were obtained from around trapping stations where adults had been observed to feed. Also, several samples attributable to parents were taken from the tops of previously clean nest-boxes after mate-guarding observational periods, as parents occasionally defecate during intersexual encounters *pers. obs.*

The samples were transferred to Leicester at the end of each field season for analysis. The 1995 samples were agitated with a thin glass rod while still in the eppendorff, shaken vigorously for 30 seconds, then allowed to sediment for 30 - 60 minutes. Disposable pasteur pipettes were used to independently sample around 100 μl from the surface layer and sediment interface. Each sample was placed on a clean slide and examined under a coverslip until the whole field was scanned. The samples collected in 1996 were treated differently, as approximately 0.2g of the wet faeces was placed in a sieve (100μm), and washed into a tube with 10 ml of formal-saline (performed by Dr F. Clark). The subsequent protocol was identical.

11.4.3 Results

Eighty seven faecal samples were collected in 1995. Ten were obtained from individual adults, but the remaining 77 were 'brood' samples, eleven of which were sampled twice. Six samples (6.9 %) contained oocysts of the protozoal coccidian *Isospora* sp. (most probably *Isospora lacazei*, Kruscewicz 1995). The mean number of oocysts per sample was 10, with a range of 2 - 120. One of the positive samples was taken from an adult male, whereas the remaining five were brood samples.

In 1996, 331 faecal samples were collected. The majority (290) were taken from nestlings, particularly those at barn B (197). The remaining samples were taken from adults (thirteen), traps (seven), nest box lids (ten) and juveniles. Of the 331 faecal samples examined, 32 contained oocysts of *Isospora* sp (9.7%). Several samples contained tapeworm eggs (Annelida: Cestoda), but these were sufficiently large to be of mammalian origin, most probably equine. They were presumably acquired incidentally by the sparrows whilst foraging in the surrounding horse pasture.

The 290 nestlings sampled were drawn from 82 separate broods, provided that the definition of 'a brood' was at least a single nestling sampled, irrespective of its age. When a more rigid definition was applied (a brood sample obtained from two or more nestlings aged at least nine days), then only 55 broods were sampled. Furthermore, for comparative purposes, a further six uninfected broods were discounted, as their age at ringing had only been estimated.

Two of the positive samples were obtained from adult males, and 23 were from broods of mature nestlings. Six of the broods were represented by two or more samples, and hence 17 broods overall were infected with *Isospora*. The other positive samples were taken from traps (four) and nest box lids (three). The mean number of oocysts per sub-sample was 38, and ranged from 2 - 110, although these data have to be interpreted with caution because of the difficulty in performing a standardized analysis.

Age of acquisition

Sufficient samples were collected during 1996 to enable age of acquisition to be estimated. Although nestlings of a variety of ages were sampled, most were between nestling day 7 (ND_7) and ND_{13}. Only four broods were completely sampled (i.e. faecal matter obtained from each nestling on the same day). Twenty broods were sampled at ND_4 and ND_7 , as well as the main sampling point of ND_{10}.

No birds younger than ND_9 were infected, which suggests that either the latent period of infection is at least nine days, or that the nestlings do not acquire oocysts immediately. Kruszewicz (1995) orally inoculated nestling sparrows in Poland with sporulated *Isospora* and retrieved oocysts from the faeces within six days. Assuming that *Isospora* in Kentucky house sparrows has the same life cycle, then this suggests the nestlings are not initially infected until at least ND_4. Of the 20 broods where samples were obtained at several nestling ages, only three were infected. These infections were present at the final sampling (ND_{10}) but not in the earlier samples.

The significance of Isospora infection

For both years, the ND_{10} measures of the infected broods were compared with those of the uninfected broods. In 1995, there was no significant difference in either mean mass (t = -0.339 df = 17 NS) or mean tarsal length (t = -0.338 df = 17 NS) between the groups. In 1996, when the 17 infected broods were compared with the 32 uninfected broods, there was no statistical difference between either median ND_{10} mass (Mann-Whitney U = 269 NS) or median tarsus (Mann-Whitney U = 218 NS).

Several of the apparently uninfected broods were sampled when young ($<$ ND_7). As *Isospora* has been reported to have a latent period of infection of at least five days (Kruszewicz 1995), a failure to record oocysts in faeces from young birds is not commensurate with an absence of infection. Thus, the broods classed as uninfected in the comparison were those which had no coccidia present in faecal samples obtained when ringed at ND_{10}. However, no significant difference in mass was found between infected and uninfected broods, even when deleting those nestlings of unknown age (t = 0.595 df = 11 NS). Tarsal length is immutable beyond about ND_7 (O' Connor 1978), and hence this comparison is genuine.

11.4.4 Discussion

Epidemiology of coccidiosis

Coccidia such as *Eimeria* and *Isospora* have a direct life cycle which commences with the shedding of unsporulated oocysts from an infected bird (Anwar 1966). *Isospora* oocysts are apparently produced year-round (Hopkins & Wheaton 1934) and by virtue of a thick outer layer are sufficiently resistant to cold temperatures that they can overwinter. After a period of environmental stimulation (involving temperature and humidity cues) the sporozoites within the cyst develop. Once a cyst is accidentally ingested by a feeding or preening bird, the wall is dissolved by digestive enzymes and the infective sporozoites are released, to lodge within the wall of the upper duodenum (Anwar 1966).

The most economically important example of a protozoal coccidian is the gamebird parasite *Eimeria*. This can have devastating effects in

commercial broiler houses and pheasant rearing farms, as the infection rapidly spreads between closely confined birds. *Isospora* appears equally contagious. Flocks of house sparrows maintained in aviaries became ubiquitously infected with *Isospora* (S. Norris, *pers. comm.*) and samples taken from house sparrows on Lundy Island, where the entire population fed communally in a large farmyard, were also all infected (S. Griffith, *pers. comm.*). House sparrows often feed in flocks, particularly during the autumn and winter, and hence the risk of infection must be considerable.

House sparrows and coccidia

Several studies have reported a high prevalence of *Isospora* in adult house sparrows, although these are not wholly comparable as sampling methods varied (for instance, infection was occasionally assessed by scraping the intestinal mucosa of sacrificed birds, which is obviously more reliable than faecal sampling of live birds). These studies are listed in Table 1 (drawn from Kruszewicz 1995).

Table 1. Published prevalences of *Isospora* in house sparrows.

Reference	% infected
Boughton (1929)	66
Skidmore (1934)	97
Schwalbach (1960)	100
Pellerdy (1974)	97
Kruszewicz (1991)[a]	11
Kruszewicz (1995)[b]	100
This study	15

(a = not accounting for periodicity, b = accounting for periodicity)

Patterns of infection at the farms

The presence of heavily infected faeces around the traps suggests that the main route of infection is via communal or sequential feeding at favoured sites. Although sparrows tend to defecate in flight or when perched, they occasionally defecate when feeding (*pers. obs.*). Infected faeces would inevitably accumulate around a concentrated food source, such as a grain spill or a productive patch of pasture. Sparrows feeding in these areas

would incidentally ingest sporulated oocysts, or may be infected indirectly when preening feathers soiled with faeces.

The cases of nestling infection recorded at Maine Chance presumably derived from parents obtaining invertebrates from infected areas. When caterpillars and crane flies were fed to the brood, any mature oocysts which were attached would also be transferred. Whether these infected nestlings survived to become infected parents was unknown, as none were present at the farms the following year. It thus remains unknown how many parasitised adults are infected before they leave the nest, and how many acquire coccidiosis as adults.

Limitations of results

There are several flaws with these comparisons however, which reflect both the vagaries of the sampling regime and the natural pattern of *Isospora* infection. Ten of the 17 infected broods were represented by more than one sample taken on the same day. Two of these broods tested positive in both samples examined, whereas four broods were infected in one sample only. The remaining four broods contained a mix of infected and uninfected nestlings.

This suggests that it it is invalid to categorise a brood as uninfected without having sampled all of the nestlings. Equally, the presence of coccidia in a brood sample merely shows that at least one nestling is infected, as opposed to the whole brood. No repeated sampling of the same individual on the same day was performed, which would have established the reliability of identifying infection. A larger sample of intact broods would have been needed to determine whether the infections of individual nestlings are independent of their brood mates.

Peridoicity of expulsion

Kruszewicz (1995) captured infected house sparrows from the wild and maintained them in aviaries to monitor the period of expulsion. There was a pronounced periodicity, with a strong peak in oocyst production between 2pm and midnight. Oocyst production in nestlings did not show such a marked periodicity, although oocysts usually only appeared in the

faeces after 3 pm. Although relatively few nestlings were infected in any circumstance (11 %), Kruszewicz's estimates of *Isospora* prevalence in adults increased dramatically when accounting for the artefact of periodicity (Table 1).

Implications of periodicity on my own results

The standard way to counter this is to maintain infected birds in aviaries and sample their faeces at hourly intervals (Kruszewicz 1995). Once a curve of oocyst production has been constructed, it can be used to standardize an infection recorded at any time of the day. Of course , this method is only applicable when *some* oocysts are found.

Unfortunately, the confounding effects of periodicity render the results of adult infection ambiguous, as the field work was carried out in ignorance of Kruszewicz's results. Most adults were trapped at their boxes during the morning, as this was the peak period of nestling provisioning. Both of the infected adults were sampled between noon and 2pm. In this population at least, noon seems the earliest sampling period in which *Isospora* appears in the faeces. Of the 11 uninfected adults, only two were sampled during this period, and the other nine were sampled several hours earlier. As this apparently coincides with a period when oocyst production is either low or non-existent, the nine birds could concievably have harboured a latent infection. Of the 17 infected broods, 15 were sampled after noon (12 of them later than 2pm). Of the 32 uninfected broods, 25 were sampled after noon (19 of them later than 2pm). As there was no dramatic temporal bias in the sampling of broods in either category, the uninfected broods were probably correctly assigned.

Pathogenicity of Isospora

Although in this study *Isospora* appeared to exert no influence on nestling growth, the data was inconclusive. Indeed, pathogenicity is probably difficult to assess without an experimental approach. Kruszewicz (1995) infected one member of a sibling pair of house sparrows with 1, 000 - 4, 000 oocysts, and found a significant deleterious effect on growth in the late nestling stage. Infected birds averaged 2.3g lighter at ND_{12} than their uninfected siblings, although no difference was found in masses before

this period. The infected nestlings were not lighter than the survival threshold of 19.2g proposed by Dawson (1972), although they would still presumably be less likely to recruit into the breeding population.

Is Isospora an important pathogen of Maine Chance sparrows?

The observation that sparrows defecate around their own feeding areas, whereas they could easily defecate in flight, suggests that they are not aware that faecal matter contains potential pathogens. Furthermore, they do not appear to shun feeding areas where other sparrows have defecated previously.   However , all birds trade off the probability of disease acquisition against the constant threat of starvation (of themselves and on behalf of their young).

Although the data is questionable with regard to the adults sampled, the low number of nestlings infected was probably a genuine result, as was also found by Kruszewicz (1995). This does not imply that coccidiosis is unimportant as a selective factor, as the differential mortality of infected versus uninfected nestlings was unknown.

The levels of infection recorded in the current study were low, although this was an artefact of methodological differences. The highest recorded total in 0.2g of wet faeces was 110 oocysts, which corresponds to about 1, 000 oocysts in 1g of dry faeces (the standard used by several workers). The levels of adult infection found by Kruszewicz (1995) were extremely high, ranging from 20,000 to 300,000 oocysts/g faeces/hr, with an incredible maximum rate of 35 million oocysts shed per gram per hour.   Equally, Boughton (1929) regularly obtained between 200,000 - 2 million oocysts from a gram of dried faeces.

Nestling infection was substantially less however, ranging from 200 - 800/ g/hr, which was comparable with the results presented here. Although several hundred oocysts may or may not have a discernable effect on adult hosts, it is difficult to believe that several hundred thousand shed per hour from the intestinal wall would not have a detrimental effect upon food assimilation. The unavoidable conclusion, at present, is that coccidian infection appears to have had no discernable effect upon nestling growth, nor was it sufficiently common or severe in adults to explain any

proportion of variance in reproductive success. However, the lack of systematic sampling, coupled with knowledge of oocyst productivity patterns, means that these conclusions are at best, suspect, and at worst, completely untenable.

Future directions

A recent technique for obtaining faecal samples involves placing processed adults within a small box lined with greaseproof paper (A. Bickle, *pers. comm.*). After around 15 minutes the adults are freed, (usually) leaving behind a useable faecal sample. The greaseproof paper is then replaced before the next bird is sampled.

Although the main difficulty still remains in catching the birds, the current study could be successfully repeated by trapping birds during the early evening period of peak oocyst production, and placing them in holding boxes as described above. Furthermore, nestlings could be placed into individual boxes likewise, and one or more samples obtained from each member of the brood. This would allow familial patterns of infection to be investigated.

The source(s) of nestling *Isospora* infection could be traced with the use of ligature sampling. Recovered food from various localities (invertebrates, grain spills etc.) could be examined for the presence of infective oocysts. As a corollary of this, a deliberate effort to trap and sample fledglings would provide more detail on the age at which infection is acquired. Any deleterious effects of infection should be more profound if they interfere with nestling development, rather than in infections acquired when body size is fixed.

It is presently unknown whether 'uninfected' birds sampled at ND_{10} are genuinely uninfected or have latent coccidiosis. Several fledglings return to their natal area around two weeks after leaving the nest. If individuals classed as 'uninfected' at ND_{10} were resampled as fledglings and were again classed as 'uninfected', it is reasonable to assume that the original classification was justified. Any of these fledglings later recovered as infected adults obviously did not acquire coccidiosis in the nest.

IV : Carnid flies

11.5.2 Methods

About six weeks after the field season commenced in 1995, several nestlings were found to harbour small black fly-like insects beneath their wings, which were also found in the nest lining. Several specimens were collected and identified as *Carnus hemapterus* (Milichidae: Diptera) (Rothschild & Clay 1952), a parasite of bird nestlings which is apparently widely distributed throughout North America (Capelle & Whitworth 1973). To my knowledge, this is the first record of *C. hemapterus* from Kentucky.

These were identified as counted *in situ* on entire broods of hatchlings, and on individual older nestlings by turning the bird over in the hand and carefully lifting each wing. Nestlings were initially checked for flies as hatchlings, and thereafter during weighing at ND_4, ND_7 and ND_{10} (when they were ringed). Broods were held in white cloth bags before and after processing, and care was taken to ensure no flies remained in the bags after the nestlings had been returned to the nest. Once the brood had fledged, the nest material was sifted (as for nidicoles) and any flies were counted before being returned to the box with the nesting material.

11.5.3 Results

In 1995, 15 of 29 active nestboxes (51%) contained carnids on at least one occasion during the season. Of 68 broods initiated, 19 (28%) were infested with carnids. Carnid flies were equally common in the following season, with 23 of 47 active nestboxes (49%) being infested at least once. 32 broods were infested with carnids in 1996, of which three were outwith the main study area and only visited for ringing. Discounting these, *Carnus* flies were found in 29 (30%) of the 97 study broods which survived to at least one monitoring. Most brood infestations were low (less than five flies per brood) with a maximum record of 25. Nest infestations were equally low, with a maximum of 50 flies recorded from a nest sifted in 1995.

In 1996 the breeding composition of the carnid population at each stage was assessed by counting the number of adult females on each bird (identifiable by their pale, distended abdomens, Guiguen *et al.* 1983). 51

flies were identified from 19 broods, of which 15 (30%) were females. At least one female was present in 12 of these broods.

The effects of carnid parasitism

Carnid infection was classified on a presence/absence basis for analyses, because the average number of flies per brood was generally low. In 1995, nestlings in infested broods were not significantly lighter ($t = -0.122$ df = 58 NS) or had smaller tarsi ($t = -0.774$ df = 58 NS) at ND_{10} than those in parasite-free nests. There was no difference in the interbrood interval (discussed more fully with respect to nidicole infestation in 4.4.2) between infested and uninfested nests (14 infested nests, 30 uninfested nests; Mann-Whitney $U = 193.5$ NS).

In 1996, carnid infestation had no effect on average nestling mass (Mann-Whitney $U = 522$ NS) or average tarsal length (Mann-Whitney $U = 567$ NS). There was no influence of parasitism upon the interbrood interval (24 infested nests, 36 uninfested nests; Mann-Whitney $U = 425$ NS).

The discrepancy between the number of nests parasitised by carnids in each year, and the number used above to analyse interbrood intervals, arises because several parasitised nests were not used again in a particular season.

Seasonal patterns of infection

The seasonal occurrence of carnids differed between years, with flies appearing significantly earlier in 1996 than in the previous year (Figure 1, Mann-Whitney U-test, $z = 15.3$ p< 0.01). In 1995, carnid infestations were not noted until after the first broods had fledged, and showed a strong peak in mid-summer. In contrast, *Carnus* flies were found in several first broods in 1996, and were most common in late May/early June.

Figure 1. Seasonal distribution of Carnus infestation.
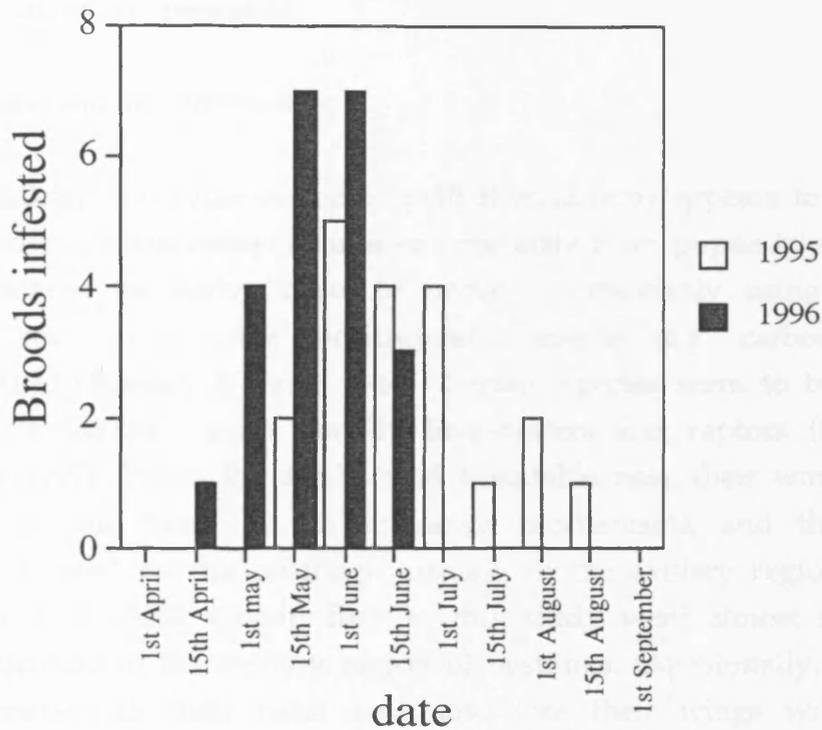
This pattern could not be reconciled with the breeding ecology of the house sparrows. Although environmental conditions were reasonably similar between years, the breeding season started significantly later in 1996. Accordingly, one might have expected carnid appearance to be delayed in 1996.

Nestling age and carnid infection

In 1995, carnids were most commonly found on hatchlings aged less than ND_4 (in 10 of 16 infested broods). Carnids have also been taken from starling hatchlings less than a day old (Walter & Hudde (1987). Three of the six other infested broods were aged ND_7 or less. Of these 16 broods, carnids were only retrieved from four when sifted. Conversely, carnids were found in four sifted nests where the broods had apparently been uninfected. Infection only persisted into the following brood in three nests in 1995.

In 1996 however, carnids were equally common at all nest checking stages (i.e. hatchlings, ND_4, ND_7 and ND_{10}). No carnids were found in any sifts, although infection persisted

The parasite and its life cycle

The specialised life cycle of these small flies($c2$ mm) appears to be unique among avian ectoparasites. Adults emerge alate from pupae in spring, and quickly search for active nests to occupy, presumably using the same locatory stimuli as other ornithophilic insects (e.g. carbon dioxide, temperature) (Bennett & Fallis 1960). Certain species seem to be favoured as hosts for *Carnus* , most usually hole-nesters and raptors (Dawson & Bortolotti 1997). When the adults find a suitable nest, their wings become detached at the base (by an unknown mechanism), and they merely scramble around on the nestlings, usually in the axillary region or groin (Guiguen *et al.* 1983). Carnid flies in this study were almost invariably found clustered in the axillary region of nestlings. Occasionally, the adults merely remain in their natal nest, and lose their wings without ever having used them (Rothschild & Clay 1952).

Mating occurs in the nest or on the bird, with the eggs being deposited within the nest lining. After emergence, the larvae scavenge on nest detritus for several weeks, before forming a pupa in which they then overwinter (Guiguen *et al.* 1983). Emergence in the following spring is thought to be stimulated by an increase in average environmental temperature, or by conditions in the nest microclimate itself. This has evolved to synchronize adult emergence with the presence of nestlings.

Guiguen *et al.* (1983) elucidated the life cycle of *C. hemapterus* in the laboratory and found that the eggs hatched after five days, the larvae pupated 21 days after eclosion, and the resulting puparium remained in diapause for between eight days and many months (corresponding to the overwintering period). Thus, the minimum generation time is around 35 days. Although a cormorant was used as the host, the duration of each stage is probably conserved irrespective of the host species. Evidence in support of this statement is provided by Capelle & Whitworth (1973) who also found a five day hatching period for carnid eggs taken from the nest of a red-shafted flicker

11.4.4 Discussion

The parasitic nature of carnids

The parasitic nature of adult *Carnus* flies has been disputed historically. Although originally viewed as merely saprotrophic upon flakes of sloughed skin and feather debris (Bequeart 1942), more recent authors have associated *Carnus* infestation with axillary bruising and the presence of dried blood within the feather sheaths (e.g. Cannings 1986). H. Smith (*pers. comm.*) found the breast feathers of heavily infested starling nestlings to be matted with what appeared to be dried blood. Most conclusively, Guiguen *et al.* (1983) demonstrated haematophagy by observing carnids feeding on captive cormorants.

Carnids probably only rarely feed on house sparrow feathers and skin debris, if at all. During 1995 in particular, they were most frequently found upon naked hatchlings, and numbers appeared to decline as feather development progressed. Closer examination of these hatchlings with a hand lens revealed areas of rasped skin around the axillary region, and occasionally, faint smears of fresh blood. Spots of dried blood were found on the feather sheaths of older nestlings (aged about ND7) which suggests that flies only consume the feather covering to gain access to the proteinaceous pulp within the developing shaft. Furthermore, when several carnids from non-target nests were squashed, smears of a distinctly sanguine nature were produced.

The evidence strongly suggests that *Carnus* flies are haematophagous in house sparrows, and are thus potentially threatening both directly (as a cause of blood loss) and indirectly (as vectors of disease).

Pathology of Carnus infestation

As far as I am aware, no deleterious effects of *Carnus* have been reported (Walter and Hudde 1987, Kirkptrick & Colvin 1989, Dawson & Bortolotti 1997), even in visibly severe infestations (e.g. in starlings H. Smith *pers. comm.*). Canning (1986) presented circumstantial evidence attributing partial loss of a single brood of saw-whet owl (*Aegolius acadicus*) nestlings

to heavy *Carnus* infestation. Each owlet that died carried over 50 flies, whereas the maximum infection recorded in this study was five flies per nestling.

Host defences against Carnus

Feeding carnids break the skin and sample the blood, and consequently must invoke the same host responses as other haematophagous arthropods such as fleas and mites (Marshall 1981). However, considering the relative manoeuvrability of the host and parasite, house sparrows probably have no direct means of countering carnid flies. Nestlings would be too sluggish and inflexible to preen aggregations of flies from their axillary region, and parental allopreening would probably be ineffective within the dark confines of the nest. Axillary preening is practiced by adults (*pers. obs.*), although this is unlikely to be in response to carnids, as even adults caught while brooding infested nestlings were free from parasites themselves. Carnids do not possess hooked claws (Capelle & Whitworth 1973), and hence any which transferred from nestlings to adults (e.g. during brooding or feeding) would simply be lost during flight. Nestlings presumably have softer, thinner skin than adults, and are probably a more penetrable substrate. Flies may use skin toughness for host selection while in the nest.

The European starling is a common host of carnids (Cannings 1986), and routinely removes all old nest material from boxes or cavities before starting a fresh nest (Cannings 1986, *pers. obs.*). This may represent an evolutionary adaptation to reduce or remove overwintering carnid pupae. Starlings (and coincidentally, raptors) also exhibit the unusual and inventive anti- parasite mechanism known as 'herbalism', in which adults deliberately line their nests with fresh sprigs obtained from certain green plants (Wittenberger 1984). The release of aromatic compounds from these sprigs reduces mite levels, and could conceivably also reduce carnid levels. Although house sparrows could easily remove old material from nests, they did not, nor was there any evidence of 'herbalism'. This suggests that carnids are not yet a selective force to provoke the evolution of avoidance behaviours.

Although both house sparrows and starlings nest readily in boxes, it is probably significant that starlings are hole nesting birds in their native habitat, and may have had an ancestral association with carnids. In contrast, house sparrows are actually weaver birds, and construct loose ball-shaped nests of straw in dense bushes. Cavity nesting is a relatively recent habit.

Life cycle of Carnus on house sparrows

The first carnids of the season would have emerged from puparia overwintering in the nest. The different seasonal distributions of carnids in 1995 and 1996 made it difficult to identify a single stimulus which causes emergence. It seems logical that the overwintering pupae are stimulated by the warmth of the female during the 10-day incubation period. However, this is not consistent with the 1995 data, where all first broods were uninfected, and suggests a less proximate, environmental stimulus, such as a threshold ambient temperature. In 1996, the ambient temperatures were comparable, but the emergence times were much earlier, and actually suggested an incubation stimulus.

Emergence is probably the result of an interaction between both environmental and immediate (i.e. within the nest) stimuli. Whatever this mechanism may be, it is remarkable that in most of the host species studied thus far (house sparrows, starlings, owls, kestrels and cormorants), carnid emergence coincides with nestling emergence, despite the huge variation in incubation periods.

Why the anomalies between nest and nestling infection?

On several occasions, carnids were found when nests were sifted, whereas none had been found on the broods (and vice versa). It was also unusual for successive broods to be infected. The most parsimonious explanation for these 'lost' carnids is that the adults or eggs were destroyed during the nest sifting process. This scenario is possible, although unlikely, as the adults themselves are physically robust, and appeared unharmed when replaced in the nest following sifts. Puparia are thick-walled to maximise overwinter survival, and would presumably be undamaged by agitation. The most vulnerable stages in the life cycle of *Carnus* are evidently the

eggs and larvae, and it is possible that the discrepancies are a result of damage to one or both of these. This hypothesis remains to be tested, although like stages of all nest parasites, the twin threats of predation by other nidicolous arthropods, and compression caused by a brood of nestlings, results in an element of hardiness.

As flies were easily visible due to their colour and mobility, an absence of carnids is probably genuine, and hence, flies present on nestlings either died or moved before the nest was sifted. Carnids are extremely phototactic, and would usually scuttle beneath the bird or between finger flanges when handled. However, as they were reluctant to drop from the bird in hand, it is reasonable to assume that few were lost. Several studies have found broods to be infested when young, but apparently uninfected later (e.g. Cannings 1986). It is unlikely that they were eaten by the nestlings or parents (as occurs with fleas), nor do they leave attached to fledglings (as no infected juveniles were found). Capelle & Whitworth (1973) found several alate males in red-shafted flicker cavities, and presumed that these could readily fly between nests. Three alate adults were found in this study, and hence these carnids at least could have flown to a new nest once the current brood had fledged.

The only plausible general explanation for the anomalies is that the adult life cycle is very short (around a week), and flies die in the nest soon after breeding. Dead flies would have been much more difficult to espy during nest sifts. Assuming that the sifting technique was not critically disruptive, surprisingly few boxes had a second generation of carnids. The interbrood period of house sparrows (i.e. hatchling - hatchling) is around 30 - 40 days. Most house sparrows at the field site rear at least two broods per season, and several pairs rear three or even four. If the generation time of *Carnus* is around 35 days (as elucidated on cormorants by Guiguen *et al.* 1983) , then at least one more generation of carnids could theoretically have been produced.

The data in both seasons suggest that of the original cohort of adults which emerge from the overwintering puparia, only a proportion successfully breed, despite females being commonly distributed. Furthermore, the progeny of these breeders enter a prolonged diapause at the pupal stage, and do not emerge as adults until the following season. This hypothesis

could be tested by thoroughly sifting nests after each fledging to quantify the presence of puparia in particular (as eggs and larvae would be difficult to retrieve in practice).

The studies of *Carnus* published thus far all refer to host species which are usually only single brooded (raptors being a classic example, Brown 1976). For reasons which are currently unclear, carnids may have evolved in association with single brooded host species, and their life history is not finely tuned to continual generations within a season.

V : Nidicoles (nest parasites)

11.6.2 Methods

The level of nidicole infestation was estimated *in situ* by placing the palm of one hand in direct contact with the nest lining for ten seconds, and counting any parasites which had transferred to the skin (after Møller 1990). This was done at three stages of the nesting cycle; mid-incubation, mid-brooding (nestling days 7, ND_7), and post-fledging. For convenience, sampling coincided with nest visits made for other purposes (egg measurement, nestling weighing, and nest retrieval respectively).

To determine the efficacy of the method, the entire nest was removed from the box after the post-fledging sampling. This occurred within three days of the presumed fledging date (using ND_{17} as a conservative estimate). The nest was placed immediately into a purpose-built, box-shaped metal sieve (mesh size 15mm), which was enclosed in a large white plastic bag. The nest was loosely teased apart by hand, and then shaken vigorously within the sieve for approximately 30 seconds. The nest material was then returned to the nest box, and carefully compressed and reshaped into a reasonable approximation of its original state.

The sifted detritus remaining in the white bag was searched for parasites. If none were found, the residue was shaken into the nest cup and gently tapped beneath the surface of the material. If low numbers of parasites were present (e.g. less than 50 mites) they were counted at the field site and returned to the nest within the residue. If the infestation was more severe, the plastic bag containing the residue was tied and taken back to the

laboratory, where it was sieved again through a finer grid (mesh size 3mm). This allowed the passage of dust and nidicoles, but not the nest material. Three subsamples of this secondary filtrate (of known mass) were examined closely, and the numbers of parasites present in each was averaged to provide a figure representing parasites per gram of filtrate. This was multiplied by the weight of the entire secondary filtrate to obtain an accurate estimate of nest infestation.

Although these nidicole counts are not flawless, it is difficult to conceive of a more accurate method which, crucially, is non-destructive. Furthermore, as the parasites discovered were all very mobile, infested nests were unlikely to have been missed, and hence any errors are proportional rather than categorical. This treatment had no discernable effect upon subsequent breeding activity, as parents would merely augment and/or reshape the nest prior to laying.

11.6.3 Results

During the 1995 breeding season, 17 of the 29 active boxes were infested with mites at least once (59%). Mites were present in 22 of the 68 successful breeding attempts (32%), although the constancy of their 'presence' differed between attempts. On five occasions, mites were found on nestlings at the time of ringing (ND_{10}), although none were found later when the nest was sifted (usually within ten days). The opposite situation was found in 11 nests, when mites were found during sifts whereas none had been recorded on the nestlings.

Two extreme examples merit more detail. At one nest, around 10 mites were found on a brood of hatchlings, although none were present when the birds were ringed eight days later. When the nest was sifted however, 350 mites were found. At a second nest, mites were not recorded on nestlings at any visit, whereas when the nest was sifted, eight days after ringing, an estimated 1, 500 mites were found (the highest total recorded from any box during the study). Despite these massive infestations evidenced through sifts, no more than 50 mites were ever found on a live brood, and usually less than 10.

Mite populations in 1996

Mites were less common in the following season. Only 15 of 47 active nestboxes were infected at some point during 1996 (32%), and 18 of 97 broods were infected (19%). Mites were only found in a single sifted nest in 1996 (ironically, from a nest in which the brood was uninfected). Mite incidence averaged around 18 individuals per infected brood (a total of 331 mites found on 18 broods), corresponding with around five individuals per infested nestling (331/61). The maximum brood infestation was 70 mites, distributed evenly between three nestlings.

Although the low mite prevalence in 1996 meant that insufficient data were available for statistical analysis, mites were most frequently found clustered in the axillary region and the under coverts, where feathering is sparse even in old nestlings. Mites were occasionally found on the head and in the scapular depression, particularly in heavy infestations.

2.5.4 The effects of parasitism

Despite the temporal fluctuation in mite populations, nests were classified as 'infected' if mites were found at any stage of the nestling cycle, including the post-fledging period when the nest was sifted. In 1995, there was no significant difference in nestling mass ($t = 0.736$ df = 60 NS) or structural size (tarsal length $t = 0.769$ df = 60 NS) between parasitised broods and unparasitised broods, when measured on nestling day 10. In 1996 however, broods from infested nests were lighter and structurally smaller than those from nests where no mites had been recorded (Table 1).

Table 1. Morphometric difference between uninfected and infected broods.

(standard data taken on nestling day 10)

	Uninfested ($n = 74$)	Infested ($n = 14$)	Mann-Whitney U - statistic	Significance
Mass	24.79	22.09	782	0.002
Tarsus	18.80	18.32	705	0.025
Wing length	50.64	47.35	708	0.023

As an illustrative comparison, the difference in mass between parasitised and unparasitised nests is shown in Figure 1. As expected *a priori*, if one variable differs between the two treatments, the other two also differ, as the morphometric measures are strongly intercorrelated (mass *vs* tarsus Spearman $r = 0.484$, mass *vs* wing length Spearman $r = 0.374$, tarsus *vs* wing length Spearman $r = 0.631$; using each brood as a independent data point , df = 88, all p < 0.001).

Figure 1. Average brood mass versus mite infestation.

(columns refer to mean + SE)

Infested
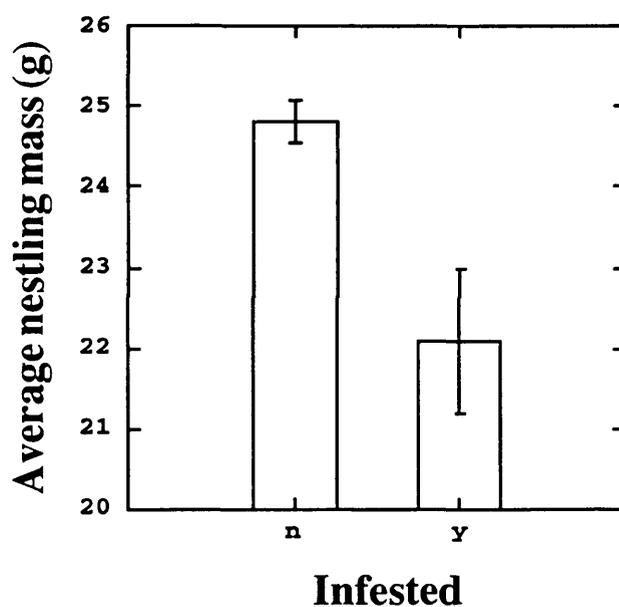
Brood reduction was not associated with parasitism ($\chi^2 = 0.089$ df = 1 NS , using the following contingency table).

	Mites present	No mites present
Nestling(s) lost	7	32
No nestlings lost	8	41

Interbrood interval

Ideally, the interbrood interval is measured as the difference between the fledging date of one brood and the first egg date (FED) of the following clutch. At Maine Chance however, the exact date of fledging was very difficult to obtain, and hence the 'interbrood interval' was measured as the

period between ND_{10} (when the nestlings were ringed) and the FED of the subsequent clutch.

The interbrood interval was only calculated if nestlings had been ringed and presumed to have fledged naturally (i.e. any broods which had been predated, or removed by hand following desertion, were not included in the analysis). Nor were broods included if the observational data on the parents was weak, and an unusually long period (> 30 days) had elapsed between ringing and FED (as the natural cycle in these events may have been interrupted by partner loss or change).

In 1995, the interbrood interval was not independently affected by either mites (15 infested nests, 29 non-infested nests; Mann-Whitney U = 277 NS) or *Carnus* flies (14 infested nests, 30 uninfested nests ; Mann-Whitney U = 193.5 NS). Coincident infestation with both parasite types had no effect on this interval, when comparing nests with both parasites againt those with one parasite or none (six infested nest, 38 uninfested nests; Mann-Whitney U = 107 NS).

Median interbrood intervals were not significantly different between parasitised or unparasitised nests in 1996, when considering either mites (12 infested nests, 48 uninfested nests; Mann-Whitney U = 227.5 NS) or carnids (24 infested nests, 36 uninfested nests; Mann-Whitney U = 425 NS). Coincident parasitism with both mites and carnids tended to lengthen the interbrood interval (uninfested broods = 12.04 days n = 51, infested broods = 14.78 days n = 9), although the difference was not significant (Mann-Whitney U = 140 p = 0.062).

The discrepancy between the number of nests parasitised by carnids and mites in either year (as reported in the general results), and those used to calculate the interbrood interval arises because several parasitised nests were not used again during a particular season.

Age of mite acquisition

In contrast to carnid infestation, nestlings were usually uninfected when young. In 13 of the 18 infected broods, mites were only found on the nestlings when they were ringed at ND_{10}. This accords with the finding of

226

Phillis (1972) that mite populations peak in house sparrow nests when the nestlings are large. Only four cases of hatchling infection were recorded, and in all but one case, the infection had disappeared by the following visit. In the outstanding case, infection increased steadily as the nestlings aged, and the entire brood was found dead in the nest about a week after ringing, covered with mites.

Coincident patterns of nidicole infection

In 1995, either mites or carnids were found in 30 of 68 successful broods (44%). Concurrent carnid and mite infestations were noted in 10 of these. There was a significant association between carnid and mite infestations (Table 1) (χ^2 = 6.26 df = 1 p < 0.05).

Table 1. Contingency table of coincident infection in 1995.

	Carnids present	Carnids absent
Mites present	10	12
Mites absent	8	38

Parasites were equally prevalent in 1996. Either mites or carnids occurred in 40 of 97 broods (41%), although they were only coincident in seven. In contrast , no significant association was found in 1996 (χ^2 = 2.24 df = 1 NS) (Table 2).

Table 2. Contingency table of coincident infection in 1996.

	Carnids present	Carnids absent
Mites present	7	11
Mites absent	22	57

11.6.4 Discussion

The regularity of the nest visitation schedule, in which nestlings were weighed and examined at fairly short intervals (hatching, ND_4, ND_7 and ND_{10}), ensured that few mites would have been missed. Engorged mites in particular were readily visible, as were those on unfeathered nestlings.

Mites from infected nestlings frequently crawled onto the handler during the several minutes of contact associated with ringing, which aided counts.

It may be misleading that the 'first' sighting of mites in many broods was not until ND_{10}. Powlesland (1978) found that starling mites changed both their feeding site and behaviour as the nestlings aged. When the nestlings were young, the mites fed on the soles of their feet, and would rapidly return to the nesting material when disturbed. Hence, the low prevalence of mites found on house sparrow hatchlings could have been an artefact of the disturbance caused by nest visitation. Although scabs and what appeared to be blood clots were found on hatchlings in nests which were definitely parasitised, none were found on hatchlings in nests which were presumed to be unparasitised. Hence, mites were assumed to be genuinely rare on hatchling house sparrows.

As nestling starlings aged and their skin hardened, their mites became more common near the skin of the body (Powlesland 1978). Deliberate efforts were made during this study to separate feather tracts of older nestlings and ensure all mites were found. This revealed a similar age-related pattern in house sparrows, with mites on older nestlings appearing to favour the patches of bare skin around the axillary region.

11.6.2 Why the discrepancy between nestling infection and nest infection?

There were striking differences between the mite numbers found on the birds, and those found later during the sifts. The drops in infestation between the nestling stage and the sifts would have been caused by a mass emigration of mites during the post-fledging period. The reverse situation (where sifts produced mites when the nestlings were uninfected) is more difficult to explain, particularly when several hundred mites were retrieved. The difficulty arises because there was a substantial period between when the nestlings were ringed and the nests sifted, during which the nests were not visited to avoid the risk of premature fledging.

The nestlings in these cases probably were uninfected at ND_{10} (see 'validation of counts' above), and thus the population of mites must have emerged at some point before sifting. The day of this emergence is critical to the survival of the nestlings (and hence parental RS). If the parasites

228

appeared within days of the nestlings being ringed, the resulting burst of intensive haematophagy could cause the nestlings to either fledge in poor condition, or fledge prematurely as a response. Either scenario would lower the survival probabilities of the fledglings, and thus constitute a late-acting selection pressure of parasitism.

In the cases described above however, the massive populations of mites were of a uniform pale grey colour, which suggested that they had not had a blood meal within the last few days. If this were true, then it would be misleading to classify these nests as infected. Although the uncertainty remains, describing these nests as 'infected' for the purposes of the analyses was the more justifiable option .

How do the mites disperse?

Most (75%) starling mites leave the box within days of the brood fledging (Petersen 1979), and move to the perch and lid. This migration behaviour occurs with many ectoparasites of cavity nesting birds, including the house sparrow, where fleas, mites and bugs move to the nest entrance under positive phototaxis (Alves 1997). A considerable proportion remain to breed, which results in greater infestations in second broods. Powlesland (1977) produced strong circumstantial evidence that starling mites disperse on the fledglings.

This is not implausible in house sparrows, as three juveniles were found with heavy (> 50) mite infestations. However, no mites were found on the other 43 juveniles trapped in 1995 and 1996. Common sense suggests that, all else being equal, the infested juveniles would be less likely to survive overwinter. Furthermore, although it is not known whether yearlings (or indeed adults) harbour mites during the winter, all adults caught in early spring were uninfected.

Starling mites also transferred to potentially usurping adults investigating neighbouring boxes (Powlesland 1977). This could occur at Maine Chance as house sparrows did visit the boxes of their rivals (*pers. obs.*), and in both seasons, several birds changed boxes and/or partners. In heavy infestations, mites swarmed over the front and top of the nest box, and readily transferred to any object which touched the box (*pers. obs.*). Any

house sparrow which merely perched on a heavily infested box would doubtless be infested, although whether this would happen if only a few mites were present is debatable. Furthermore, it is difficult to imagine that a sparrow would deliberately perch upon a heavily infested box, as the swarming movement of mites is visible from some distance.

The vast majority of adults handled during the study were uninfected (175 of 177 individuals processed), with two males each carrying a single mite. However, the observation that these mites were wandering aimlessly over their host, whereas nestling mites tend to remain close to the skin, suggests that the adult infestations were aberrant and caused by 'stragglers'. The parsimonious explanation for their presence is that the cloth bag used to hold birds before processing contained several mites left over from previously processed nestlings.

The bags were regularly cleaned of contaminants by turning them inside out and brushing off any mites, faeces, feathers and nesting material, as well as being laundered monthly, which would definitely have destroyed any mites or adhered eggs. Despite this rigour, it is conceivable that a single mite from a previous brood had burrowed into the seam of a cloth bag, and emerged a day or two later when one of these two adults was placed in the bag.

Juvenile starlings occasionally prospected at active house sparrow boxes at the farms, and were thus a potential source of mites (Powlesland 1977). However, as these did not appear until late June (*pers. obs.*), they could not have been the source of mites found in the earlier months of the season at least.

No mites were found on the vaseline trails daubed around the boxes, which suggests that they do not merely crawl between nests. Considering the size of the mites (< 2mm), crawling may only be a successful strategy when the distance between nests is small. In the colony studied by Phillis (1972), where nests were closely juxtaposed in a clump of ivy , crawling would probably be an effective dispersal method (as it would also be in their natural tree colonies).

The day on which the trails were applied may have rendered them ineffective. The vaseline dried within a few days in the summer sunshine, and hence any mites which left the box several days after fledging could have crawled over the hardened surface. Also, as the vaseline was applied using a conservative estimate of fledging date, mites which had left the nests within hours of fledging would have been missed.

Despite these limitations, the use of trails could theoretically determine whether mites move between boxes, and if so, at which stage in the breeding cycle the flux(es) occur. If a substance were found which remained tacky for longer periods than vaseline, it could be applied at the beginning of the breeding season, and simply monitored at the important periods.

Mites may normally overwinter in the nest material, but during this study may have been damaged by the sifting procedure and died. This is countered by the fact that dermanyssid mites overwinter as adults, and should thus be sufficiently robust to survive sifting. The possibility that sifting causes damage to the mites is, admittedly, a concern. This risk was not addressed during the study (due to the general paucity of infested nests), although it could easily could be established using 'control nests' (i.e. nests which were not sifted), and examining nests for mites during the winter (as done by Powlesland 1978, Petersen 1979).

Why do the mites disperse?

Individual mites have two options once the nestlings have fledged: to stay in the nest or to leave. These options are mutually exclusive, and individual mites probably 'decide' which to use in the prevailing conditions, rather than having a fixed behavioural strategy (i.e. 'stayers and leavers'). The most important factor influencing this decision must be density of conspecifics. Mites which remain in the nest are only guaranteed further blood meals (and hence for females, the opportunity to produce more eggs), if the nest is used again. However, further broods are less likely to be initiated if the mite density is high (as occurred on two occasions at the farms).

Emigration would be preferred in these circumstances, which by definition is the more risky option. The sensory system of mites is crude, and could probably only detect other birds or nests if they were very close. Hence, these mites are not assured of locating another nest before dying through starvation and/or desiccation. This is not mere speculation. At two of the heavily infested nests, many of the mites which had been observed swarming over the box were later found as shrivelled specks, drying in the sun.

Powlesland (1977) found thousands of dead mites in starling nests examined during winter, suggesting that these had failed to emigrate with the final brood and had starved. Sit-and-wait is presumably only a realistic strategy in nests with low infestation, during the early to middle stages of the season, when more broods are due. Presumably mites have no way of knowing whether a current brood is the final event of the season, and those found dead in the starling nests examined by Powlesland (1977) during winter decided, fatefully, to stay and wait for a further brood rather than attempt to move onto the adult.

Do mites overwinter in boxes?

D. gallinae can overwinter in nests of the colonial purple martin (R. Dawson, *pers. comm.*). If house sparrow mites overwintered in the nestboxes, one would intuitively have expected the nests which were infested towards the end of the 1995 season to be most likely to be infested at the beginning of the following season. This was not so.

Starling nests examined outwith the breeding season contained only dead mites (Powlesland 1977, 1978). No overwintering mites were found in the crevices of nestboxes, nor did any emerge when a live bird was trapped in the box overnight. Mites which overwintered in the nest would, moreover, probably be lost because starlings routinely remove all nest material before each breeding attempt.

Instead, mites overwintered on the adults themselves, nestled within gular folds of skin. 32 % of starlings trapped during the winter carried residual populations of non-breeding adult mites in these folds, with usually less than 10 mites per bird. The starlings probably cannot remove

these mites because of their location, and they remain within the folds until the onset of breeding in spring. At this point they presumably leave the bird in anticipation of nesting activity, which would explain why adults are only rarely infected during the breeding season, whereas mites become common in the nests.

Theoretically, house sparrow mites are much more likely to overwinter within the nest than on the bird, as sparrows have no similar skin folds within which mites could seek refuge (*pers. obs.*). However, the data obtained does not concur with this theory. If dermanyssid mites overwintered in the nest, then the boxes parasitised at the start of one breeding season would most parsimoniously be those which were parasitised at the end of the previous season. This was not true of Maine Chance house sparrows. Of the 15 boxes which were parasitised in 1996, only two had been infested at the end of 1995. Furthermore, five of these parasitised boxes had only been erected in late-winter to increase the breeding density at barn B, and were devoid of any nesting material. Considering the reverse situation, the final broods of 10 boxes were parasitised in 1995. Mites were only found in one of these during the following season.

Furthermore, the seasonal pattern of infection in both years suggested that mites did not originate from overwintering populations in the nest material, or at least, this was not their exclusive source. Although 17 nestboxes were parasitised at some stage during 1995, only three infections were associated with first broods. In 1996, 15 nestboxes were parasitised, of which six were first broods. One of these occurred in a newly erected box, which had not held any nesting material until breeding was initiated in April.

House sparrows and mites : a comparable study

Phillis (1972) extracted ectoparasites (using a Tullgren apparatus) from 71 house sparrow nests collected at various times of the year. 75% of nests were infected with at least one of two *Dermanyssus* species, *D. americanus* and *D. hirundinis*. Mites were present, albeit in in low numbers, outwith the breeding season, and increased sharply during the late brood stage

(ND$_8$ and older). There was a drastic drop in mite numbers between late broods and post-fledging, due to emigration of mites (Table 1).

The nests formed a loose colony constructed in a thick growth of English ivy, which is probably a better approximation of their native state than a nestbox colony. Thus, mite transfer between nests would be facilitated, as it probably is in colonies built in African trees. The fact that Phillis finds mites in nests in winter suggests that mites overwinter in nests at Maine Chance. Furthermore, although his numbers are higher at each stage, the pattern of infestation parallels house sparrow mites at Maine Chance, where the mites appear to increase in late brood stages, and then decline sharply when they fledge, so I am at least partially vindicated.

Table 1. Seasonal abundance of mites in house sparrow nests.

Period in annual cycle	Prevalence (%)	Mean incidence
prebreeding	50	1.5
early clutch	71	2.3
late clutch	100	27
early brood	100	35
late brood	100	3,000
post-fledging	100	84
dispersal	80	4.5
winter roosting	66	6.3

The significance of mite infestation upon house sparrow breeding success

Although most detrimental effects of parasitism were relative (i.e. a reduction in mass or condition of nestlings), the significance of the five heavy infestations (> 500 mites) was drastic. None of these nestboxes were used again during the season, and in two boxes, the entire brood of nestlings was dead. It is reasonable to assume that mites were the cause of nestling mortality, either directly or indirectly. Although several hundred feeding mites would cause substantial blood loss, the mortality was probably the direct result of parental desertion because of the mites. The nestlings were fatally trampled by two of the four horsemen of the apocalypse, famine and pestilence (namely 'starvation and parasitism').

Parents would be reluctant to visit heavily infested broods, even those sufficiently developed to make it unnecessary to enter the box to feed. There would be a visible risk of acquiring mites from merely touching the entrance of the box, and any mites which transferred to adults could act as propagules of infection in future breeding attempts (even at a fresh nest). Moreover, the benefits of further feeding become proportionately small in heavily infested nests, as much of the food provided is (indirectly) taken by the mites. House sparrow nestlings probably cannot rid themselves of mites, and it is doubtful whether this task could be performed by parents. Hence, blood loss would be continual and unavoidable.

Even if the nestlings did manage to fledge, they would have a very low probability of recruitment into the breeding population. Hence, selection would favour desertion in favour of a new box, especially at Maine Chance where there was a surplus of nesting sites. The fact that two broods were deserted within days of fledging, when relatively little further investment would have been required, is testament to the strength of this selection. The mites present in these boxes were uniformly grey. This implies that the population expansion was largely a consequence of blood feeding by parental mites, and that the birds may well have been dead before nymphal emergence. Avian blood congeals rapidly after death (*pers. obs.*), and mites could not have fed upon dead nestlings.

A negative relationship between ectoparasite levels and clutch size has been reported in other nestbox passerines (see Richner & Heeb 1995). This was not assessed in the current study, because in virtually all breeding attempts, nidicoles were only ever present on nestlings. Parasites were never recorded on any adult females trapped. Nidicoles could only influence clutch size in Maine Chance sparrows (and for that matter, egg size) if females were exposed to parasitism during the period of clutch formation (around a week before egg laying commences). The interbrood breeding behaviour of female house sparrows minimizes exposure during this critical period.

The main period of exposure to females is during brooding of infested nestlings (or, more accurately, nestlings in infested nests). After about ND_7, females do not brood the nestlings or habitually enter the box to

deliver food, and hence contact with infested nestlings is restricted. Once the brood fledges, females stay away from the nest, simultaneously shepherding nestlings and feeding in readiness for the subsequent clutch. Even when eggs are laid, the nest is only visited briefly at around 6am. Females only risk exposure to nidicoles once they commence incubation, by which time the clutch size is 'decided' (if not completed).

For all of these arguments, it was assumed that the detrimental effect of mite parasitism is blood loss. However, mite infestation may have more indirect consequences. Although no blood parasites were found in this study, it is possible that mites acted as vectors for several parasite which were not assessed, such as viruses or tissue protozoa.

The significance of mites in mate choice

The observation that adults were not infected by mites is crucially important when testing the Hamilton-Zuk hypothesis. Unlike barn swallows, females could not visually judge the direct and indirect costs or benefits accrued by mating with a particular male (either within-pair or extra-pair). Powlesland (1977, 1978) did not find mites on sparrows he examined during his starling work.

As there is no variation in mite load between males, females mating randomly have an equal chance of obtaining (latent) resistance genes for their offspring than those mating preferentially. Furthermore, the risk of acquiring mites through the physical act of copulation is equally negligible from all potential partners.

Females could not estimate the number of mites in each nest from the number present on the resident male. Furthermore, the absence of mites during the early stage of the season suggests that females could not visit several nests in turn to select the least infested, nor could they even obtain any cues of future mite levels.

The evidence suggests that ectoparasitic mites would not affect the choice of either social or sexual mates, nor would they influence which nest a female initially chose to breed in. However, because mite infections become apparent as the nestlings age, ectoparasites could influence the

decision of the female (if not the male) to initiate a new breeding attempt in the same nest, or move to a different nest (and presumably, a different mate). Thus, mites probably do not affect initial mate choice in house sparrows at Maine Chance, although they could affect mate retention and hence reproductive success.

SPECIAL NOTE

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Chapter 12. Immune defence and spleen size in the house sparrow

12.1 Introduction

12.1.1 The avian spleen

A recent and timely review of splenic function in birds (John 1994) contained the apt sub-title 'A neglected organ'. Whereas in mammals, the activities and significance of the spleen are well documented, the avian spleen has received little attention, particularly in wild populations. Despite this inequality, the basic histological structure and function of the spleen are known to be similar in these taxa (Fänge & Silverin 1985), and hence the same two broad functions have been ascribed. These concern somatic oxygenation via the circulatory system, and immune defence.

The main roles of the spleen with regard to the circulatory system include the formation of red blood cells (erythropoiesis) and their subsequent removal and destruction (Silverin 1981). Together with the liver, the spleen is the principal blood filtering organ (Powell 1987), and removes a range of toxins and metabolic by-products, including foreign bodies.

Immunologically, the spleen is a lymphoid organ responsible for both humoral and cell-mediated responses. John (1994) recognises eight forms of disease resistance performed by the spleen, most of which are concerned with lymphocyte production and recirculation. In addition to simple phagocytosis and destruction of antigens, the complex differentiation of lymphocytes into their various types occurs in the spleen, including the production of 'memory cells' important in the residual, secondary immune response (John 1994).

Although the spleen performs these functions in both birds and mammals, their relative allocation differs. Avian spleens are relatively small compared with their mammalian counterparts (as was known by Aristotle), and have a much higher proportion of tissue devoted to immune function (John 1994). The more vacuous tissue concerned with somatic oxygenation is less common in birds (Fänge & Silverin 1985), which may be compensated by anatomical improvements of their circulatory system such as air sacs.

237

12.1.2 Sexual variation in splenic activity

According to epidemiological theory, the two sexes often differ in their susceptibility to parasitism and disease (Zuk 1990). The males are assumed to be the most susceptible, for three reasons which are not mutually exclusive;

1. the intensity of sexual competition is greatest in males (and this energetic demand detracts from immune defence)

2. males suffer from the immunosuppressive effects of testosterone

3. males are more heavily exposed to parasitism

If these assumptions are correct, the male-bias in disease susceptibility should be associated with a male-bias in spleen size.

12.1.3 Spleens, sexual selection, and the immunocompetence handicap

Folstad & Karter (1992) posited that the level of testosterone is regulated in *response* to parasite burden, and that the immunosuppressive effects of testosterone consequently affect the expression of male secondary sexual traits. Genetically resistant males have low parasite burdens, and thus suffer from a greater degree of immunosuppression because their testosterone level is high.

Only the best quality, resistant males can tolerate this inevitable 'immunocompetence handicap'(Folstad & Karter 1992). Males with little or no resistance could not bear the immunosuppression associated with the production and/or maintenance of large ornaments, and hence seek to minimize this by producing only a small ornament.

An androgen/immunity trade-off appears to exist with regard to spleen size variation. Spleens are smallest during the period of gonadal recrudescence prior to the breeding season (Silverin 1981, Fänge & Silverin 1985), and Oakeson (1953) recorded an interplay between gonad development and spleen size.

238

If the immunocompetence handicap prevails in house sparrows, only high quality, resistant males could afford the production and maintenance costs of bearing a large badge (Owens 1994). If, as seems reasonable, spleen size paralleled any manifestation of infection (John 1994), a negative correlation should exist between badge size and spleen size.

The predictions of the immunocompetence handicap, and the hypothesized sexual difference in splenic activity, were tested in a free-living population of house sparrows. This work, undertaken with Mr S. Griffith, attempted to minimise confounding variables by obtaining sparrows which had recently undergone their single annual moult.

12. 2 Methods

30 house sparrows (20 males, 10 females) were mist-netted from two sites in rural Leicestershire between late-September and early-October 1997. Vernier callipers were used to measure the length of the right tarsus, and the 'hidden length' of the male badge. This corresponds approximately with 'white badge length' used at Maine Chance (Chapter 2), and is the perpendicular distance between the bill base and the point of insertion of the lowest melanised feather. Following brachial venipuncture, a 50μl capillary tube was filled with blood and blocked at both ends using a plasticene sealant. A blood smear was then taken, and the bird sacrificed by suffocation in a sealed box of frozen carbon dioxide ('dry ice') for 60 seconds.

Upon returning to the laboratory (usually within six hours), the capillary tubes were centrifuged at 12,000 rpm for five minutes, and the haematocrit was measured (the percentage height comprised of packed red blood cells) (Dawson & Bortolotti 1997). The corpse was weighed on an electronic balance, and examined for ectoparasites by sifting through the plumage under a strong lamp, concentrating on the head and breast region in particular. The wing was fanned open and held before the lamp to reveal the presence of feather mites (Bhenkhe *et al.* 1997), which were then counted, and several specimens taken for identification.

The corpses were scalped, and aged as either yearlings or adults depending on their degree of cranial ossification (Nero 1951). The bird was opened

ventrally by dissection, and the spleen exposed by rotating the gizzard and proventriculus (John 1994). After extraction with a scalpel, surplus mesenteries or tissue were trimmed away, and the spleen was rinsed briefly in water. As each spleen had a cylindrical appearance (shaped like a bowling pin to be precise), Vernier callipers were used to measure their length and breadth, and the splenic volume calculated as $\pi r^2 l$. Finally, each spleen was patted dry on tissue paper and weighed to the nearest tenth of a milligram using a microbalance.

12.3 Results

Spleens were of a consistent shape, as evidenced by a high correlation between their length and width ($r = 0.709$ $n = 28$ p < 0.001). The calculated splenic volume was very highly correlated with mass ($r = 0.847$ $n = 28$ p < 0.001), and although the latter was used as the measure of spleen size throughout all calculations, volume is an equally applicable measure. Relative spleen size was calculated as the percentage of spleen mass on body mass (mean = 0.12 % , range = 0.029 - 0.204 %).

27 birds were aged unequivocally, of which 11 were adults and 16 were yearlings. Although there were no intersexual differences in morphological variables, all subsequent calculations controlled for an effect of age. When sexes were pooled, mass did not differ between categories ($t = 1.266$ df = 25 NS), although adults had significantly longer tarsi than yearlings ($t = 2.957$ df = 25 p = 0.007). Consequently, an index of body condition (BCI, the residuals from a regression of mass on tarsus) was higher in adults ($t = 2.594$ df = 25 p = 0.016). Adult males had significantly larger badges than yearlings (mean of six adults = 35.22 mm, mean of 11 yearlings = 33.26 $t = 2.42$ df = 15 p = 0.029).

Splenic mass was independent of physical characteristics (tarsal length $r = -0.275$, mass $r = -0.057$, BCI $r = -0.273$; $n = 30$, all NS). No correlation was found within the two categories (adults $r = -0.013$ $n = 11$ NS ; yearlings $r = 0.058$ $n = 16$ NS).

There was no intersexual difference in splenic mass when age-classes were pooled ($t = 0.542$, df = 28 NS). However, yearlings had significantly larger spleens than adults (adult spleen = 22 mg, yearling spleen = 38 mg, $t = -$

4.20, df = 25 p = 0.001). A two-way ANOVA did not detect any interactions between sex and age (below).

Source	SumSq	df	F - ratio	p
Age	1849.9	1	17.73	0.001
Sex	2.0	1	0.02	0.891
Age * Sex	90.7	1	0.87	0.361

Splenic size was negatively correlated with badge size when pooling males of both age classes ($r = -0.541$ $n = 20$ p < 0.01) (Figure 1). However, this correlation does not persist when considering males within age classes (adults $r = -0.313$ $n = 6$ NS ; yearlings $r = -0.426$ $n = 11$ NS).

Figure 1. Spleen size correlates inversely with badge size.
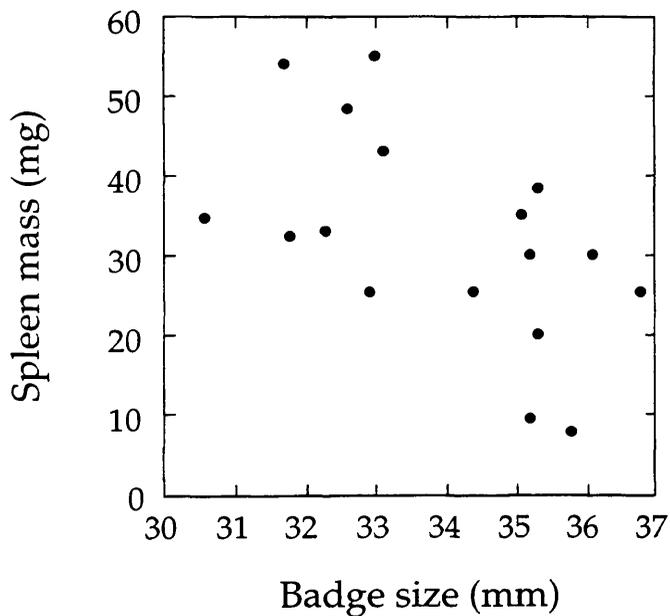
Spleen size was independent of the parasitological variables (haematocrit r = 0.008, feather mite load $r = 0.274$, louse number $r = -0.024$ all $n = 23$, NS). None of these varied with sex (Mann-Whitney U-tests, $z = 1.67$ for haematocrit, $z = 2.35$ for mites, $z = 1.77$ for lice, all from 20 males and 10 females), or between age classes (Mann-Whitney U-tests $z = 0.154$ for haematocrit, $z = 0.55$ for feather mites, $z = 1.12$ for lice).

Substituting relative spleen mass into the calculations (in lieu of spleen mass) did not affect the significance of any correlations. The two important

conclusions persist, namely that spleen size is greater in yearlings (t = - 2.43 df = 15 p = 0.028), and badge size and spleen size are negatively correlated (r = - 0.516 n = 20 p < 0.05).

12.4 Discussion

Admittedly, this survey represents a single, brief phase in the annual cycle of the house sparrow, and any extrapolations to events during the breeding season have to be made tentatively.

Although Silverin (1981) and Silverin *et al.* (MS) have described seasonal patterns of change in spleen sizes, little comparable data exists on the significance of spleen size variation in wild birds. It is physically impossible to follow temporal changes in an individual's spleen size, simply because the bird has to be sacrificed before the spleen can be exposed and measured. Changes can only be assessed indirectly, by sacrificing several individuals at intervals during the annual cycle and using average measures.

Silverin (1981) performed a notable study of this ilk with pied flycatchers. Flycatcher spleens were relatively small upon the initial arrival, but increased in size dramatically following early reproductive activity (corresponding to larger and more numerous lymphoid follicles). Spleens were small during the winter, and relatively large during the moult. This suggests that the size variation recorded in house sparrow spleens during the moult may correspond with that during the breeding season. In two species of tits however, spleens were larger during the moult than in the summer (Silverin *et al.* MS)

12.4.1 Spleen size, immunity and condition

The physical characteristics of house sparrows examined were not related to spleen size, although this was not surprising. Several extraneous variables and internal factors influence body condition (e.g. weather, food supply), which may be only indirectly related to any form of disease or concomitant immune status.

242

Spleen size appeared to be independent of ectoparasite load, which in retrospect was not surprising given the ecology of the two taxa assessed. Both subsist on feather detritus and by rasping at the feathers themselves (Marshall 1981), which are non-living structures composed of keratin (Ginn & Melville 1994). Theoretically, this mode of ectoparasitism would not have challenged the host's immune system. However, as several of the sparrows were still in the final stages of moult, the region of bloody pulp at the base of their shafts could conceivably have been affected.

This illustrates the general importance of monitoring immunity and host condition during the moult, as feathers, once produced, are sealed at their base and are merely dead pieces of protein (Ginn & Melville 1994). The full expression of the house sparrow badge coincides with the onset of male display and (presumably) mate choice in the following spring (Veiga 1993a). As this occurs around five months after the moult, the feathers can only provide limited information concerning the immediate condition of their bearer.

The absence of a correlation between haematocrit and spleen size was also found in pied flycatchers by Silverin (1981), although these results have to be interpreted with caution, as the reliability of the haematocrit as an indicator of immune activity is currently a moot point (Dawson & Bortolotti 1997). Several other variable may affect the haematocrit independently of any splenically derived response (such as general 'stress' [Ots & Horak 1996]).

12.4.2 Age differences in spleen size

The finding that juveniles had significantly larger spleens than adults was contrary to expectations. The immune system of yearling birds is initially represented by the Bursa of Fabricius, a small knob which projects from the cloacal wall (Glick 1989, Møller *et al.* 1996). Prior to bursal involution, B-lymphocytes cells emerge and relocate either in the bloodstream, or become lodged in the spleen (John 1994). Although the spleen thereby assists with the establishment of the immune system in juveniles, and should increase in size as B-lymphocytes accumulate, bursal involution does not occur until several months after the moult (Glick 1989). The *a priori* expectation was that yearlings would have smaller spleens than

adults, as much of their immune activity was being undertaken by the bursa.

12.4.3 Sexual differences in spleen size

The parity of spleen size between males and females, which persisted after controlling for age effects, suggests that the sexes have equal exposure to pathogens and/or stresses which invoke a splenic response.

It is possible that this sexual equality is an artefact of the autumnal sampling, and sexual variation may only become apparent during the breeding season as a consequence of differential reproductive effort and exposure to pathogens. Silverin (1981) found no intersexual difference in spleen size of pied flycatchers upon their arrival on the breeding grounds.

12.4.4 Spleen colouration and activity

Spleens varied in colour from pale pink to deep red, which presumably reflects differing levels of haemoglobin deposition following erythrocyte destruction. Pied flycatcher spleens were small and white during the winter (Silverin 1981), but large and dark red/blackish during the breeding season. Silverin (1981) concluded that this represented increased erythrocyte storage, although whether the spleen actually performs this function (particularly on a long-term basis) is uncertain (John 1994). John (1994) suggests that the colouration change merely results from an increase in general splenic activity during the breeding season.

12.4.5 Spleen size and badge size

The immunocompetence handicap hypothesis was supported in house sparrows. Males with small spleens (and hence assumed to possess greater genetic resistance) had large badges, and conversely , males with large spleens (presumably suffering from an undefined infection) had small badges.

As the badge size during the breeding season equals the post-moult badge size using this measure (Griffith 1998), females preferentially pairing with large-badged males would obtain a mate which had little infection after

the moult. Unfortunately, it cannot be known whether these males would have also had little infection during the breeding season.

Although this relationship disappeared when considering each age class in isolation, this may not be important. By choosing males with larger badges, the females mate with males that had smaller spleens, and consequently are presumably less likely to become infected in the future. Whether differential mortality during the winter would have removed the males with the heaviest infection, or a disproportionate number of yearlings, again cannot be known. If each bird had an equal chance of surviving to breed, the negative correlation between badge size, spleen size, and infection would persist.

The lack of a correlation between badge size and spleen size when considering the age classes separately is, however, inconsistent with the hypothesis. In both classes, the males are expected to differ in their degree of disease resistance, which should be manifested by differences in spleen size. The relationship among yearlings at least could be confounded by small differences in fledging time, since Veiga (1993a) reported that yearling males which fledged earlier in the season had significantly larger badges than those which fledged later, presumably because the latter had less time to acquire essential resources. Unfortunately, fledging dates of the yearlings involved in this study could not be known. The relationship among adults may be an artefact of small sample sizes, since only six individuals were available.

Although these results support the immunocompetence handicap, they are also consistent with the classical Hamilton & Zuk (1982) theory of parasite mediated sexual selection. Under the Hamilton & Zuk (1982) hypothesis, females prefer males with larger badges as these signal the greater parasite resistance of their bearer, or condition with respect to parasitism, irrespective of any interaction between androgen and trait. In this respect, males with large badges would be expected to be in better condition, and thus have smaller spleens, since they have a greater genetic resistance to parasitism.

Indeed, to what extent testosterone affects the expression of the badge (if at all) is currently a moot point (Owens & Short 1995, Møller et al. 1996), and

hence this support for the immunocompetence handicap hypothesis remains tentative pending conclusive evidence of a direct link. Keck (1934) demonstrated that castrated male house sparrows still produce an apparently normal badge in successive moults, despite the assured absence of testosterone. However, as Evans *et al.* (MS) maintain, the persistence of a secondary sexual character in the absence of testosterone does not automatically mean the immunocompetence handicap hypothesis should be rejected. The hypothesis could still be supported, provided that variation in testosterone levels is positively related to variation in the size of the character. Nonetheless, the study reported here is one of the few to detect a negative correlation between ornament size and a measure of disease resistance.

Preface

In advance of my first field season, 60 nest-boxes were constructed and erected at four separate breeding colonies of house sparrows (three on the outskirts of Leicester, one at Whipsnade Park Zoo, Bedfordshire). 35 boxes were erected in the spring of 1993, and a further 25 were added in the spring of 1994. Unfortunately, none of these boxes were occupied, despite repeated efforts to encourage this (e.g. removing existing nests, blocking all crevices). Moreover, the situation was exacerbated by the dramatic national decline in the British breeding populations of the house sparrow (Marchant *et al.*, 1990).

An alternative population had to be found for my first field season, preferably retaining the house sparrow as the study species. An ideal population of house sparrows was spread over several coastal islands in Northern Norway. Dr B-E. Sæther and his graduate students from the Nordic Institute for Nature Conservation (NINA) in Trondheim had recently (1993) initiated a long-term study on these islands, concentrating on differential reproductive success of house sparrows with particular regard to inbreeding, migration, and the extent of extra-pair paternity. Dr. T. Burke, my joint supervisor, had been consulted during the initial stages of Dr. Sæther's project, and had agreed to provide laboratory facilities for the DNA fingerprinting required. A collaboration was thus proposed, enabling me to conduct work in parallel with Dr Sæther's students.

Chapter 13   Breeding ecology of Helgeland house sparrows

13.1 Introduction

13.1.1 The field site and logistics

The study was carried out between mid-May and August 1994, over several islands of an archipelago ('Helgeland', 66.3°N, 1.4°E) off the coast of Norway. Hestmannøy, a large, central island, was used as a resident base from which other islands were visited using local ferries or fishing boats (see Figure 1).

Figure 1. Map of the Helgeland archipelago, with study islands in black.
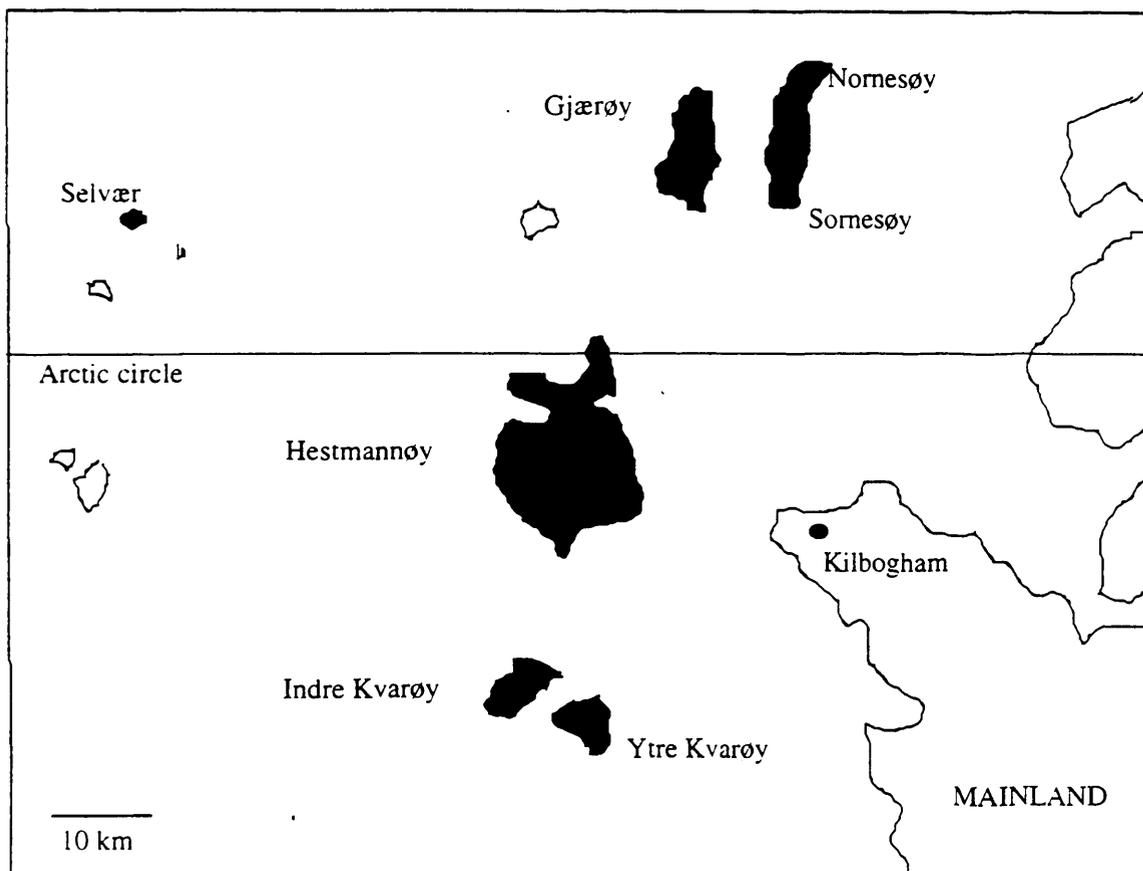
The house sparrows on most of the islands were nesting in storage barns and cowsheds, where the nests were reasonably accessible. The number of study farms on each of the six main islands, and an estimate of the number of nests which each barn held, is given in Table 1.

Table 1. Barns and nests present in the six study islands.

Island	Farm (Family name)	Nests
Hestmannøy	Heen	24
	Klausen	12
	Mathiesen	10
Hestmona	Wilhems	14
Gjærøy	Aakre	12
	Monsen	5
Indre Kvarøy	Young	5
	Dutta	7
	Hill	3
Ytre Kvarøy	Janssen	14
Selvær	Harbour/fish factory	6
Nesøy	Karlisle	4

Hestmona was actually a peninsula of Hestmannøy, and was sufficiently distant that it was considered to be isolated from the remainder of the three farms on Hestmannøy, which were all reasonably close to one another. Similarly, Aakre and Monsen were two family farms located on the same island (Gjærøy), but since they were several miles apart they were referred to separately as Gjærøy Aakre and Gjærøy Monsen.

13.1.2 Aims and predictions

The hypotheses which were tested in Helgeland were very similar to those later tested in Kentucky. Since the theoretical basis for these hypotheses is discussed fully in earlier chapters of this thesis, only a brief rationale is presented before each of the predictions made below.

Sexual selection

Evidence from both captive trials and field studies suggests that the throat patch or 'badge' of the male house sparrow is under directional sexual selection (Møller 1987a, Veiga 1993a). If this is correct, then large-badged males are predicted to have access to the highest quality females, commence breeding earliest, and have higher seasonal reproductive success than small-badged males.

Extra-pair paternity

If female house sparrows seek extra-pair copulations outside of their pair bond in an attempt to gain direct or indirect benefits (particularly the latter), then one would expect that these copulations would be sought from males of a higher quality than her social mate. If badge size is an indicator of male quality, then large-badged males should be more likely to achieve extra-pair matings than small-badged males. Moreover, small-badged males are more likely to be cuckolded than large-badged males.

Parasites

Although parasites were traditionally thought to exert little or no detrimental effect upon their avian hosts, more recent work has overthrown this view (for examples, see Møller *et al.* 1990). If house sparrows at Helgeland are subject to constant attack from debilitating parasites, then females should preferentially pair with a male who provides either a parasite-free nest environment, or possesses disease resistance genes which would consequently be inherited by her offspring. If male badge size is an indicator of quality, then large-badged males should be more likely to occupy a parasite-free nest, and be subject to lower levels of parasitic infection.

13.2   Methods

13.2.1 Field protocol

Nest searches and monitoring

Barns were searched for active nests from early May onwards, generally by 'cold' searching in likely places, or by checking sites used in previous years. During the chick rearing period, new nests were occasionally found by tracking the flight of feeding adults. Nests were usually scruffy masses of hay jammed between the supporting beams and the roof or side of a barn. Other common sites were the apexes of barns, and inside small cracks and gaps in the barn walls, both inside and outside. All nests in each barn were routinely searched, whether or not they had been used during the season.

As the study involved continuous travel between islands, nest visitation

schedule was not standardized. Thus, almost all active nests were found when clutches were complete or partially complete, or occasionally, when well developed nestlings were present. To collect data on hatching success, and thereby predict the day on which nestlings would be old enough for processing, visits to particular islands were arranged to coincide with a period when several nests were expected either to hatch or to have hatched very recently. Average clutch size was treated as five, and thus a nest found with two eggs on first inspection would be expected to produce a further three eggs at a rate of one per day. Assuming an incubation period of ten days (defining incubation period as the time between laying and hatching of the last egg), the nest would therefore be expected to hatch 13 days (3 + 10) following the original check. Any clutch containing five or more warm eggs was assumed to be complete. The stage of incubation was crudely assessed by placing eggs in small containers of water. Fresh eggs, being relatively heavy, sink to the bottom, whereas incubated eggs lose water and thus weight, and so float at a correspondingly higher position in the water (T. H. Ringsby, *pers. comm.*). Eggs which sank were predicted to hatch 9/10 days later, those which were suspended in the water were predicted to hatch 4/5 days later, and those which floated completely were predicted to hatch within two days. Nests were classified as deserted if eggs were found cold on visits more than a week apart. Laying sequence was not known.

Despite these careful predictions of hatching date, and the loose breeding synchrony often noted at individual barns, it was only realistically possible to gauge visits to embrace a spread of hatchings. Exact hatching dates and thus nestling ages were rarely known, although fresh hatchlings could be identified as their initial deep red colour fades to pink after this period. Hatching sequence was not known, and as nest visits were not sufficiently detailed to determine hatching asynchrony, broods were given an average age. Any unhatched eggs still present in the nest were examined, and those obviously infertile or dried out were removed. Eggs which appeared otherwise healthy were replaced. In nests where developed nestlings were found on first inspection, average age of the brood was estimated after Weaver (1942).

Nestling processing

Following hatching, nests visits were usually only made when the nestlings

were around 10 days old, when they were fitted with an individual combination of three or four plastic colour rings (supplied by A. C. Hughes Ltd., London) and a single numbered metal ring (Norwegian Natural History Museum, Stavanger). The colour rings were fitted on one leg according to the code for the particular island (e.g. red/metal on the left leg for Selvær, metal/blue on the right leg for Nesøy), with two or three colour rings fitted on the remaining leg. If it was known in advance that a nest visit on the appropriate day would not be possible, then the nestlings were ringed earlier if their tarsi were sufficiently developed (usually at around 3/4 days old). Thus, even if circumstances were such that the nest could not subsequently be visited to fit colour bands before the nestlings fledged, the ring number would at least ensure the origin and therefore age of the bird could be determined if recaptured. On several occasions where the nestlings had been ringed before the 10 day old ideal, nests were revisited in an attempt to colour ring the nestlings before they fledged. Thus, the morphometric data taken during processing represents nestlings ranging between 7 and 14 days of age.

Nestlings were weighed to the nearest 0.1g using a Pesola spring balance. The length of each tarsus was measured to the nearest 0.1mm with Vernier callipers following the method of Svensson (1985). The length of each wing (flattened chord method [Svensson 1985]) was usually, but not always, measured.

Approximately 100μl of whole blood was obtained following brachial venipuncture and stored in 1ml of Queens lysis buffer (0.2M NaCl, 0.2 M SDS, 0.5M EDTA, 100mM Tris/HCl pH 8.0). Any dead nestlings present were decapitated, and a blood sample obtained by tightly compressing the thorax and neck. Blood samples were stored at room temperature.

Adult capture and processing

Adults were caught invariably by mist-netting. Mist nets were placed across barn door openings and inside the barns themselves across regular flight paths. On some island sites, the birds were nesting inside houses, and in these cases adults were mist-netted in private gardens and around outhouses.

Adults and juveniles were processed in a similar fashion to nestlings with regard to blood sampling and measurement of tarsi and wings, although

individuals were not bled if a sample had already been obtained during the 1993 season. A Body Condition Index (BCI) was calculated for all adults as the residual of body mass on tarsal length.

Additional morphometric data was collected on bill depth (at base) and bill length (from tip to feathering), both measured to the nearest 0.1mm with Vernier callipers. The dimensions of the throat patch or 'badge' of males were measured with the bird held in a natural, 'sitting' position. The length and width was recorded of the 'black badge' (i.e. the region of pure black feathers) and the 'white badge' (i.e. the region of feathers which still retained their buff tips). Badge size was calculated following the equation of Møller (1987a);

$$\text{Badge size (mm}^2) = 166.7 + 0.45 \times (\text{badge length} \times \text{badge width})$$

The size of the black and white badges are strongly correlated ($r = 0.537$ $n = 81$ p <0.01), and thus the area enclosed by the white badge was designated as 'badge size', as practised in other house sparrow studies (Møller 1987a, Veiga 1993a, Kimball 1995, Griffith 1998).

Measurements and repeatability

All morphometric data was obtained by T. H. Ringsby and E. J. Solberg, who had worked on the project since 1993. As they had previously established consistency between each others measurements, they were (understandably) reluctant to introduce error from a third party.

All calculations involving adult size used the average length of their tarsi, since the two did not differ significantly (Paired *t*-test $t = 0.763$ df $= 154$ NS). Tarsal lengths of retrapped birds were significantly repeatable ($F_{18,21} = 77.7$ p < 0.01), as was body mass ($F_{18,21} = 2.79$ p $= 0.013$) (calculated using a one-way ANOVA after Harper [1994]). The area enclosed by the white badge, used as 'badge size' throughout this chapter, was highly repeatable ($F_{14,21} = 6.19$ p $= 0.01$).

Both of the beak dimensions had to be discarded, since they could not be measured consistently (bill depth $F_{14,21} = 0.49$ NS, bill length $F_{14,21} = 0.93$ NS). Wing length was merely an allometric correlate of tarsal length ($r = 0.265$ $n = 76$ p <0.05), and was not used in any further analysis.

Parasite assays

Nestlings and adults were briefly scanned for ectoparasites during processing. During handling, louse-flies (Insecta:Hippoboscidae) (Hutson 1984) often flew from the bird, usually into the clothes of the handler. If possible, these were trapped and placed inside a cloth bag along with their original host for a few seconds, whereupon they usually crawled back on to the bird. Occasionally this did not happen, or the flies were discovered after the bird had been released. In these instances the flies were placed in a small tube of formal-saline for future identification.

Faecal samples were taken opportunistically from both adults and nestlings, and stored in 1 ml of formal-saline preservative.

Blood smears were taken from all birds processed, usually a single smear from nestlings and two smears from adults. Smears were air dried immediately, and fixed, usually within a few days, in 100% ethanol for two minutes. Fixed smears were air dried thoroughly, before storage in clean, dry slide trays. Several non-target passerine species were trapped whilst mist-netting, and blood smears were similarly obtained.

Nests were searched *in situ* for parasites using the method Møller (1994b) successfully used to assay haematophagous mites (Acarina:Acari) in the nests of the barn swallow. I placed the flat of my hand upon the nest cup for a standard period of 10 seconds, and then recorded the number of mites or other ectoparasites which crawled onto it. At the end of the breeding season, nine nests from which nestlings had recently fledged were sealed inside two large sandwich bags and brought back to Leicester for closer inspection.

Observational work

Observations were made using binoculars and a 20 x magnification telescope. The primary objective of this work was to allocate colour ringed parents to their nests. This was most productive during the chick rearing periods, as adults could be followed making feeding trips to particular nests. We assumed (reasonably) that the parents observed feeding were the resident, social parents.

Observational work was difficult, as the nests were typically located in the dark recesses of barns. Also, as they were often beneath the roof, birds often had to be tracked whilst balancing precariously among the rafters. The lack of light rendered colour rings difficult to read, and as one leg of each bird was devoted to the island code (e.g. green/metal) a relatively large number of colours had to be used to generate sufficient combinations on the largest island. Hence, several 'intermediate' colours proved problematic (e.g. burgundy resembled both red and violet unless seen in good light).

Despite these difficulties, it was possible to observe the colour rings of adults seen gathering food outside the barns before flying inside. Although it was not known which nest was being visited, it was reasonable to assume that the individual identified was a parent of nestlings within. However, this assumption may not be true of individuals recorded at particular barns outwith the rearing period, where both males and females may visit other barns in search of extra-pair copulations. A few nests had only a single access route, and in these cases adults could be identified with reasonable certainty, as was true of nests with external entrances. On several occasions, colour ringed females were observed feeding ringed fledglings, and maternal identity was thus established.

A considerable amount of time was spent reading the colour rings of any sparrows seen (whether affiliated to a nest or not) in an attempt to identify their island of capture or birth and thus assess dispersal.

13.2.2 Laboratory protocol : parasite analysis

Faecal samples were shaken vigorously to break up any compacted pellets and free any parasitic stages. Drops of solution were taken from the bottom, middle, and surface of the solution using clean Pasteur pipettes, and placed on a microscope slide. A glass coverslip was carefully placed over the droplet to reduce bubble formation, and the area beneath the coverslip was scanned completely using a standard laboratory microscope.

Blood smears were stained for at least 30 minutes in Giemsa (strength 1:10, pH 7.2), rinsed of excess stain in clean, distilled water, then allowed to air dry. A small drop of Xam mountant was placed in the centre of the slide, and a rectangular coverslip fitted (thickness 0) which covered most of the smear.

Mountant was allowed to spread by capillarity (occasionally with the addition of a small weight) and set overnight, before being examined for parasites microscopically. A x 40 objective lens was used to search for haemoproteids and leucoctozoids, and a x 100 objective lens with oil immersion was used for plasmodiids.

Nests were carefully emptied out over a white plastic tray, and any fleas which emerged were recovered using an aspirator and placed in a vial of 70 % ethanol. The remaining material from each nest was split into several sections, loosely teased apart, and placed into one or two funnels of a multi-chambered Tullgren apparatus. 30ml plastic tubes of 70% ethanol were tightly fitted to the bottom spout of the funnel, and a wide glass Petri dish lid was placed over the top of the funnel to prevent any arthropods escaping. The lamps above each funnel were switched on, and the apparatus was left for three days, excepting daily checks to ensure no light bulbs had exploded.

Arthropods recovered from nests were identified using Smit (1957), and Hutson (1984) was used to identify louse flies taken during the field season.

13.2.3 Laboratory protocol : DNA minisatellites

DNA extraction and digestion

Crude nuclear proteins were isolated by incubating 75μl of the blood/buffer solution with 25 μl proteinase enzyme K in 500μl of stock proteinasing buffer (1M Tris-HCl pH 8.0, 0.1M NaCl, 1mM EDTA). Incubation was performed in a rotisserie oven, and was either at 55°C for three hours, or overnight at 37°C. If the blood was not fully proteinased, a further 20μl of enzyme was added, and re-incubated at 55°C for one hour.

Proteins were denatured by vortexing the proteinased solution with 500 μl of liquified phenolic acid (phenol) and centrifuging for 10 minutes. The supernatant was transferred to a fresh eppendorff, and any residual phenol removed following a similar treatment with 500μl of chloroform/isoamyl alcohol mix. The resultant supernatant was vortexed with 900μl of ice cold 100% ethanol, and centrifuged for 10 minutes, precipitating the DNA into a compact pellet. The alcohol was carefully poured away to isolate the pellet, and 200μl of 70% ethanol was added to absorb residual traces of concentrated alcohol. After further vortexing and centrifuging, the alcohol was siphoned

off, and the DNA dehydrated in a vacuum dryer for 45 minutes. 500µl of ultrapure water was added to the desiccated pellet, and then the DNA was left to dissolve for at least 12 hours at 4°C.

200µl of this solution was then incubated at 37°C in a horizontal shaker for at least three hours with 22.5 µl REact 2 buffer plus 1µl of the restriction enzyme *MbO*1. 10 µl of solution was ran at 100mV for an hour on a 2% agarose gel to ensure evenness and completeness of digestion. If digestion was incomplete or inapparent, a further 0.5µl of *Mbo*1 was added to the solution, which was then re-incubated for three hours. Fully digested samples were purified using the phenol/chloroform/iso-amyl alcohol treatment described above, using 200µl volumes of chemicals instead of 500 µl. 20 µl of ultrapure H_2O was added to the dried DNA pellet, which was then left to dissolve for an hour at 4°C.

Running and blotting of fingerprints

Digest concentrations were determined using a Hoeff DNA fluorometer (calibrated using a standard of 375ng/µl DNA). A volume of solution in loading buffer corresponding to approximately 5 µg of DNA was loaded into preformed wells of a 0.8% high quality agarose gel, and ran at 65 mV for 18 hours. In the earlier stages of the work, a size reference ladder of lambda DNA was loaded in each of the side lanes. This was superceded by using a loading buffer fitted with an internal reference ladder which thus appears in each lane (Taggart & Ferguson 1994).

Wherever possible, putative parents were run in adjacent lanes to broods. In many cases however, putative parents were not known. Thus, several gels were comprised entirely of either broods or adults. The remaining gels were comprised of the surplus adults and intact broods, plus the juveniles and those birds caught late in the season which could have been either parents or offspring, as their age could not be determined.

In addition to the samples collected during 1994, samples from the previous season were also profiled at Leicester by a research technician (Ms P. Spink). The 1993 material included 80 nestlings and adults which survived to become potential parents of the 1994 season nestlings. To avoid the expense and inconvenience of running the same DNA firstly on a gel of 1993 nestlings and

then again on a gel of 1994 parents, all 'double' samples were only run once, and the profile compared between situations. In total, 487 samples from the 1994 season were extracted, digested, and ran out as fingerprints.

The gels were removed from their plastic frames by inversion, and prepared for subsequent DNA transfer to a solid support matrix by immersion in a series of reagents held in a plastic tray on a mechanical rocker. The sequence of chemical treatments is as follows, the gel being thoroughly rinsed with distilled water between each stage.

1) acid hydrolysis in 0.25M HCl (2 x 8 minutes)
2) alkaline strand separation in 0.5M NaOH/1.0M NaCl (2 x 15 m)
3) reduction/neutralization in 1M Tris/3M NaCl pH 7.4 (2 x 15 m)

Treated DNA from the gel was eluted into a Nylon Hi-Bond membrane using Southern blotting. Blots were performed overnight wherever possible. The blot was disassembled carefully, and the membrane washed in 2 x SSC for 60 seconds to remove salt traces, then carefully patted dry between two sheets of Whatman absorbent paper. Spots of concentrated DNA (225ng/μl of lambda DNA) were placed in three corners of the membrane to allow repeatable alignment of probe autoradiographs on the same membrane (Taggart and Ferguson 1994). The DNA was permanently fixed to the membrane by a 60 second exposure to a previously calibrated UV transilluminator. The 1994 sample amounted to 25 membranes, and several parents were spread over about half of the 25 filters which P. Spink used for the 1993 samples.

Oligolabelling and autoradiography

Membranes were wetted briefly in 2 x SSC, and prepared for oligolabelling by immersion in prehybridization solution (0.5M NaPO$_4$, 1mM EDTA, 7% SDS) and 1% bovine serum albumin (BSA) for three hours at 65°C. House sparrow competitor DNA (10μg/ml) was denatured by boiled for 10 minutes before use, and added at the start of the prehybridization stage. Approximately 20μg of probe was radioactively labelled following incubation for 3 hours with 1μl ^{32}P in association with 3μl oligolabelling buffer, Klenow fragments and BSA. To detect the spots of concentrated DNA on the membranes, a weak solution of lambda DNA was incorporated into the probe (0.11ng DNA/μl). Following incubation, the strands of the probe were separated by boiling with 500μl

ultrapure H_2O for five minutes, then added to the prehybridization solution containing the membrane. Hybridization was carried out for at least eight hours, preferably overnight, using either a cylinder or chamber.

Non-bound probe was removed from the membrane in a succession of washing solutions of increasing stringency. Although the number of washes varied according to the strength of the signal detected by a Geiger counter after the first wash, a typical sequence was five minutes in 1 x SSC 0.1% SDS, then a series of two minute washes in 0.1 x SSC 0.01% SDS, until counts dropped to 10-15 per second. The filters were then rinsed of SDS in 2 x SSC, and placed in an autoradiography cassette with a sheet of X-ray sensitive film sandwiched between two fluorescent intensifying screens. The cassette was then left in a -70°C freezer for between 1-3 days depending on the radioactivity level of the membrane. The cassette was removed from the freezer and allowed to cool, and the autoradiograph developed. If the resulting pattern was weak, a fresh sheet of film was added to the cassette, which was replaced in the freezer for seven days before developing. If no bands were detected, or if the result was satisfactory, the membranes were stripped of radioactive probe by placing them in boiling 0.1% SDS, which was left to cool to room temperature. The membranes were rinsed of SDS in 2 x SSC before being pattted dry in Whatman paper, wrapped in cling film, and stored in a cool dark place until further use.

Probing regimes

Initially, membranes were probed using c*Pdo*MS14, a robust probe cloned from the house sparrow and known to work well in this and all other passerine species thus far tested (Hanotte *et al.* 1992). A further 13 probes similarly cloned from the house sparrow were also tested (c*Pdo* MS1, c*Pdo* MS13, c*Pdo* MS22, c*Pdo* MS23, c*Pdo* MS24, c*Pdo* MS27, c*Pdo* MS29, c*Pdo* MS31, c*Pdo* MS39, c*Pdo* MS42, c*Pdo* MS46, c*Pdo* MS64, c*Pdo* MS70).

Scorable single-locus profiles were produced by c*Pdo*MS13, c*Pdo*MS27 and c*Pdo*70, although the latter two were not reliable.

Thus, probes from other species were also tested : three from the European starling (c*Svu* MS1, c*Svu* MS13, c*Svu* MS15) and three from the pied flycatcher (c*Fhy* MS6.1, c*Fhy* MS 6.51, c*Fhy* MS 15.19). These probes produced single-

locus profiles in their parent species (M. C. Double *pers. comm.*; D. Ross *pers. comm.*) and as the house sparrow probes successfully cross-hybridized to DNA from these species it seemed reasonable to expect reciprocity. Unfortunately, none of them produced single-locus profiles in the house sparrow.

I therefore attempted to obtain single-locus profiles from all membranes using the four house sparrow probes MS13, MS14, MS27 and MS70. The band profiles of the successful probes showed a significant amount of overlap and thus only one probe could be used in a single radiolabelling episode (i.e. two or more probes could not be mixed as a 'cocktail' to simultaneously radiolabel two loci).

Scoring of profiles

DNA bands were placed into size 'bins' after Wetton & Parkin (1991) and Wetton *et al.* (1992). 'Binning' was relatively crude for the earlier profiles as the fragments produced by the lambda DNA reference ladder are relatively widely separated. Consequently, relatively few 'bins' were designated. The later gels were a considerable improvement, as the internal size reference marker (Taggart & Ferguson 1994) is considerably more detailed.

Scoring and probing difficulties, and a change of method

Most assignment scoring had to be performed across several autorads because of the dispersed profiles of potential sires. As broods on large islands often had to be compared with potential parents present on more than five autorads, there was a tendency towards conservatism when including possible parents according to their band 'bins'. Hence, nestlings were matched to many adults. It also became apparent when visualizing autorads taken from membranes of 'families' that several of the putative parents were in fact wrongly assigned, and thus these parents had to be treated as potential sires for the other broods, and the presumed 'family' broods had to be searched for sires in return.

By early 1997, many of the membranes had been probed, stripped and reprobed several times, a process which is detrimental to the fixed DNA because of the caustic SDS. In addition, most membranes were over two years

old, and the combined effect of the age and caustic detergent was producing uneven probings. Rather than run fresh fingerprint gels using the original digested DNA samples, I decided to avoid committing the Concorde fallacy (Dawkins & Carlisle 1976) and attempt to obtain families using microsatellite markers (Ellegren 1992). This was an appropriate option, as polymorphic loci had been recently described in the house sparrow (Neumann & Wetton 1996), and another variable locus had been identified by a colleague concurrently working on house sparrows at Leicester.

13.2.4 Laboratory protocol : DNA microsatellites

DNA extraction

DNA was available for all of the individuals involved in the paternity analysis, since it had been previously extracted from proteinased blood samples for use in the single-locus profiling.

Preparation and running of the PCRs

All work was undertaken within a continuous flow hood unless stated otherwise, and equipment was UV sterilized for two minutes before use. The gross reaction mix was prepared in a 1.5ml eppendorff, according to the following standard recipe;

> For 10 reactions :   69µl ultrapure H_2O
> 15µl 'badger buffer' (see below)
> 1µl dNTPs (20µM)
> 8µl Forward primer (10µM)
> 8µl Reverse primer (10µM)
> 1µl *Taq* enzyme

(The badger buffer was comprised of 20mM $(NH_4)_2SO_4$, 75mM Tris-HCl pH 8.8, 0.15 mg/ml DNAase-free BSA, 10 mM ß-mercaptoethanol, 2.5mM $MgCl_2$ [Jeffrey's *et al.* 1988]).

10µl of this reaction mix was aliquotted into strips of 0.1ml PCR tubes containing 4µl of ultrapure H_2O, and 20µl of mineral oil was gently added to cover the diluted mix. The strip tubes were capped and labelled, and taken to a laboratory bench where 1µl of DNA solution (concentration 10-100µM) was

added directly into the mix, ensuring that it was placed beneath the oil layer. The strips tubes were tightly capped, and placed firmly into a Hybaid thermal cycler.

For the paternity analysis, house sparrow primers were used to amplify DNA at four loci. Two of the primers had been previously published ($Pdo\mu3$ and $Pdo\mu4$ [Neumann & Wetton 1995]), whereas the remaining two ($Pdo\mu5$ and TE) had been isolated and optimized by S. Griffith and D. Dawson at Leicester (Griffith *et al.* MS). A fifth primer was used to determine the sex of nestling sparrows (Griffiths *et al.* 1998).

The PCR conditions appropriate to each primer are listed in Table 2. All reaction cycles were preceded by an initial 'hot start' of 94°C for 90 seconds. Note that the conditions used to amplify two of the loci, $Pdo\mu4$ and $Pdo\mu5$, were identical. When the reaction was completed, the tubes were removed from the thermal cycler and stored at 4°C until required.

Table 2. Reaction conditions of the primers used in this study.

(° = degrees celsius, s = duration in seconds)

Primer	Reaction conditons	Cycles
$Pdo\mu3$ / $Pdo\mu4$	94° / 15s , 54° / 30s , 72° / 60s	35
$Pdo\mu$ 5	94° / 60s , 56 - 50° / 30s , 72° / 30s	1 *
	94° / 60s , 48° / 30s , 72° / 30s	25
TE	94° / 20s , 59° / 30s , 72° / 60s	35
P2/8 (sexing)	94° / 15s , 50° / 20s , 72° / 25s	40

(* $Pdo\mu5$ employs a 'touchdown' program, where the temperature of the central, annealing step drops by a single degree celsius with each cycle).

Preparing polyacrylamide gels for electrophoresis

Integrated Plastic Chambers (IPCs) formed by two glass plates were used for electrophoresis of the PCR products (BioRad Ltd.). One of the plates was coated with a binding chemical (3µl of methoxysilane in 1ml of 95% ethanol/0.5% acetic acid), while the other was coated with a chemical repellent ('Repelcote'). 667µl of 0.1% ammonium persulphate solution and 67µl of TEMED were added to 100ml of refrigerated 4% polyacrylamide gel

mix (10ml 10 x TBE, 2M Tris, 10ml 19:1 acrylamide : bisacrylamide). The fluid gel was introduced into the IPC using a syringe, and allowed to set for at least three hours.

Electrophoresis of PCR products

The IPC was filled with running buffer (0.5 x TBE) and prewarmed to 50°C. 8μl of 2 x loading buffer was added to the tubes containing completed PCR reactions, and then the PCR products were denatured into single-stranded DNA by heating at 94°C for three minutes. 3μl of the denatured, buffered PCR product was loaded into wells formed using a 0.4mm sharkstooth comb. Buffered solutions of 50 base pair DNA fragments (Advanced Biosystems Ltd.) were loaded in the outer wells of each gel for use as a reference ladder. Products were electrophoresed at 100 Watts for between 1hr 15 minutes and 3 hours, depending on their size (since smaller fragments move more rapidly through the gel matrix). PCR products amplified from the sex-specific locus show rapid differentiation into the male or female conformation, and were thus run in 'piggy-back' style for the final 30 minutes of paternity gel electrophoresis.

Table 3. Characteristics of the four microsatellite loci used in this thesis.

Locus	Primer sequence 5' - 3'	Product size (bp)
Pdoμ3	F : CTGTTCATTAACTCACAGGT	100 - 200
	R: AGTGAAACTTTAATCAGTTG	
Pdoμ4	F: CGATAAGCTTGGATCAGGACTAC	250 - 450
	R: CTTGGGAAGAGAATGAGTCAGGA	
Pdoμ5	F: GATGTTGCAGTGACCTCTCTTG	150 - 200
	R: GCTGTGTTAATGCTATGAAAATGG	
TE	F: CTGATCATGTGTAGATGTAAGACTGC	300 - 500
	R: CAGATCCTTAAGCAGGAAGTTAGG	

Loading sequence

At least one parent was known at 21 nests (totalling 36 broods). In these cases, the samples were run in families, with the presumed adults alongside the nestling samples. Broods in which the parents were unknown, and adults which had not been assigned to any brood(s), were simply ran together in

blocks. Hence, several gels were entirely comprised of adult samples, whereas other gels were filled by broods.

DNA was amplified at the three most robust loci (*Pdo*μ3, *Pdo*μ4 and TE) from 567 individuals, of which 262 were adults, 287 were nestlings, and 18 were juveniles. Samples were electrophoresed island-by-island, and wherever possible, all nestlings and adults were ran on the same gel to minimize problems of scoring between gels. The proportion of adults and broods sampled at each island is given in Table 4.

Only juveniles which had been observed in close association with a known, colour-ringed adult were included in the paternity determination. Individuals which could not be distinguished in the field as either early-fledged juveniles or early-moulting adults were not included because of a simple genetic objection. While nestlings share 50% of their genes with each parent, siblings also share on average, 50% of their genes (Hamilton 1964). Theoretically, the cross-referencing technique employed here could have erroneously ascribed a female bird as being either a mother or daughter of its sibling.

Table 4. Adults and nestlings sampled from each island.

Island	Males	Females	Nestlings	Broods
Nesøy	5	10	5	2
Selvær	21	15	9	4
Gjærøy Monsen	13	12	10	3
Gjærøy Aakre	17	13	37	13
Ytre Kvarøy	17	12	56	16
Indre Kvarøy	26	20	48	16
Hestmona	9	10	28	8
Hestmannøy	32	30	94	37

Silver staining of gels

The IPC was disassembled at the end the run, and the glass plates were separated. The gel adhered to the glass plate treated with the binding agent, so that the plate functioned as a solid support for the gel during subsequent processing in a series of trays. Gels were fixed in 10% glacial acetic acid for 20

minutes, rinsed thrice for two minutes in clean ultrapure water, and then stained for 30 minutes in a 0.1% w/v solution of silver nitrate and 0.15M formaldehyde. The gel was briefly rinsed, then developed in a chilled solution of 0.3M alkaline sodium carbonate, 0.054M sodium thiosulphate and 0.15M formaldehyde. Developing was halted with the addition of 10% glacial acetic acid, then the gel rinsed and allowed to dry (example shown in Plate 1).

Gel analysis and PCR profiles

The paternity and/or maternity of broods loaded as 'families' was assessed directly by comparing their alleles against those of their presumed mother and/or father. Several broods were mismatched with one or both of their parents, which was presumed to have resulted from misidentification of the parents. These broods and the mismatched adults were pooled with the remainder of unassigned broods and adults on each island, and included in a cross-referencing protocol designed to match nestlings with their adults.

A permanent record was made of each gel (Plate 1) by overlaying it with an acetate sheet and tracing the position of all allelic bands, including the 50 base pair reference ladder, with a fine fibre-tip marker. The acetate tracing was then scanned as a PICT file into a Power Macintosh computer using Adobe Photoshop Version 4. Acetates were analyzed on the computer using NIH Image in conjunction with a macro program designed within the zoology department (courtesy of Mr M. Walker).

Parallel horizontal lines were drawn through matched fragments of the 50 base pair markers, selected according to the size range of the primer-specific PCR products (see Table 2). For example, the products of *Pdoμ4* range between 250 and 450 base pairs. Consequently, *Pdoμ4* gels were analysed by drawing an upper horizontal line between the 450 base pair fragments of two DNA reference ladders, and a lower horizontal line between the 250 base pair bands of the same two ladders. The distance between the 450 and 250 base pair fragments of each ladder was calibrated as 100 (in arbitrary units) using Image. Since the two reference lines are parallel, the distance between them remains fixed at 100 units.

Most individuals are heterozygous at *Pdoμ4*, and consequently produce two bands which fall between the 450 and 250 base pair fragments. Using the

macro program it was possible to measure the relative position of each band in relation to the two horizontal lines (Figure 2). Since the distance between the upper and lower lines had been calibrated as 100, each individual could be represented by a simple two-number PCR profile (e.g. 18, 92). Homozygotes were represented by equal numbers (e.g. 45, 45).

Plate 1. Microsatellite markers visualized through silver staining.
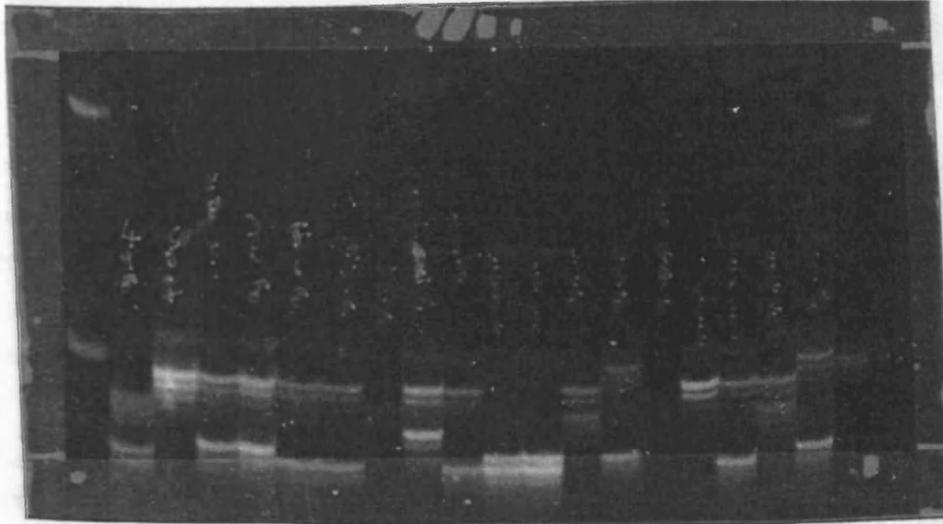

Figure 2. Tracing of a silver stained gel, showing the measurements used to obtain allele profiles for each individual.
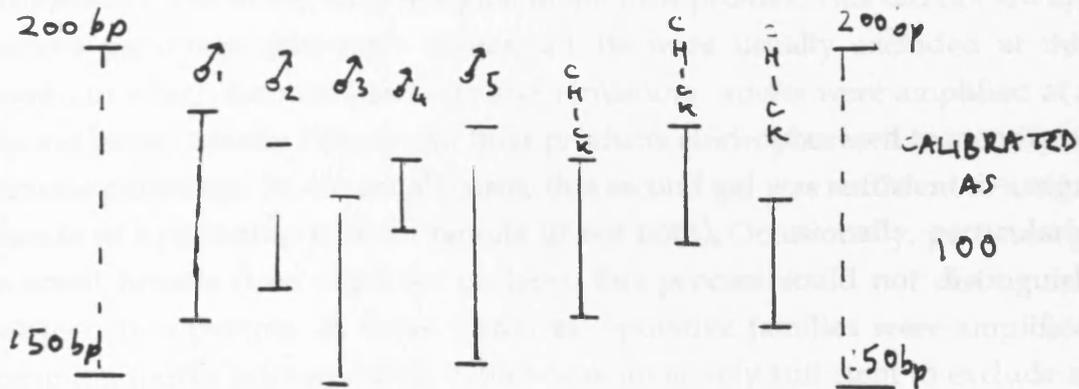
Identifying the parents

Parentage was assessed on each island in turn. The PCR profile of each nestling was compared against the PCR profiles of all the adults which had been trapped and bled on their island. An conservative error limit of +/- 3

266

was assumed in all comparisons of nestling and adult profiles, to account for slight differences in PCR profiles of individuals ran on separate gels.

The range of possible parents was then assessed for each bands of a nestling's PCR profile. For example, if a nestling had a $Pdo\mu4$ PCR profile of (30, 50), any adult (male or female) would be considered as a potential parent if it possessed a band between 27-33 or 47-53 (to correspond with the first and second nestling band respectively). A list of possible mothers and fathers is compiled for this nestling at the locus $Pdo\mu4$. The process is then repeated using the PCR profiles measured at the two other robust loci ($Pdo\mu3$ and TE).

In this way, three lists of possible mothers and fathers were obtained for each nestling. The lists were compared, and any parents which were included at all three loci were identified (known as 'trios'). The nestlings were then grouped into broods to detect any recurrent trios. This narrowed down the list of likely parents.

If the number of possible parents was small (e.g. six possible parents for a brood of four nestlings), then the PCR products from the most variable locus (usually TE) were rerun with the parents alongside the nestlings. This identifies any exact matches, and excludes any adults which were included erroneously due to the error margins in the PCR profiles. This did not always resolve parentage (although several adults were usually excluded at this point), in which case the nestlings and remaining adults were amplified at a second locus (usually $Pdo\mu4$) and their products electrophoresed to identify or exclude parentage. In almost all cases, this second gel was sufficient to assign broods to a particular male or female (if not both). Occasionally, particularly in small broods (two nestlings or less), this process could not distinguish between two parents. In these instances, putative families were amplified using the fourth primer, $Pdo\mu5$, which was invariably sufficient to exclude at least one of the parents.

If the number of potential parents per brood was large (e g. more than six possibilities for a brood of four nestlings), then the nestlings were rerun initially alongside their possible mothers. If one assumes no intraspecific brood parasitism (ISBP), a maternal band should be present in all nestlings produced by the attendant female. This tracking of female bands is much more reliable than relying upon a paternal band appearing in all nestlings,

since ISBP is generally much rarer than cuckoldry (MacWhirter 1989). If the mother of the brood could be identified, the identity of the male could therefore be usually deduced using the PCR profiles (Example 1).

Example 1.   Paternal deduction using PCR profiles.

Using the primer *Pdoμ4*, Female A has been matched exactly to the four nestlings of Brood 1. The PCR profiles are as follows;

Female A = 24, 39
Brood   **1 i)   24, 83**
                 1 ii)   24, 56
                 1 iii)   39, 56
                 1 iv)   39, 83

By deduction, the male PCR profile at locus *Pdoμ4* is 56, 83. The data set of male profiles is scanned for a match, and male B is identified as 55, 84. The PCR profiles of male A and female B at the two other loci correspond with those of Brood 1. To confirm, the two adults are run alongside the four nestlings on a second primer (TE), and if they match exactly, parentage is confirmed.

Assigning extra-pair paternity

The same deductive process was used to identify sires of extra-pair young, by comparing the rogue male of the illegitimate nestling with the PCR profiles of the other males on the island. To assign paternity, the extra-pair male had to match the rogue band exactly when run on all three primers.

13.3   Results

13.3.1 Parentage assessments

Parentage of the families deduced from observational work

Samples from 36 broods were run alongside their presumed parent(s). The mother and/or father was correctly assigned in 16 of these broods, whereas the remaining 20 broods were mismatched.

Paternity determination of nestlings

Of the 104 broods which were assessed for paternity and maternity, the identity of both parents was elucidated for 57. For a further 14 broods, only the male could be determined, and for a further 21 broods, only the female could be determined.

The broods in which both parents were known were combined with those where only the male was known. This generated extra-pair paternity (EPP) data from 71 broods. Although, theoretically, the presence of extra-pair young could have been deduced in the female-only broods by eliminating the maternal alleles from the nestling profiles (M. C. Double *pers. comm.*), this approach is statistically weak considering that the females themselves were only identified in retrospect.

The 71 broods in which the male (at least) was known totalled 208 nestlings. Eight of these (3.9%) were mismatched with the father of their nest-mates at all three loci, and were thus considered to be extra-pair young (EPY). All eight EPY occured in separate broods (11.6% of broods thus contained EPY).

To improve statistical confidence, the analysis was restricted to the 57 broods in which *both* parents were known. Using only these broods, seven of 173 nestlings were EPY (4.0%). Since this was not significantly different to the original level (χ^2=0.143, 1df with Yates' correction, NS), the Helgeland EPP level is assumed to be 3.9%, calculated by including male-only broods.

The percentage of broods which could be included in the paternity analysis varied between islands (Table 5), reflecting the differences in catching success. For instance, mist-netting was much more productive at Gjærøy Aakre than Hestmannøy, since all nests were built inside a single barn, and the adults utilised a small number of easily coverable entrances.

Table 5. Summary of the parental determination achieved at each island.

(a dash represents 'zero broods')

Island	Broods sampled	Both parents	Male only	Female only	% broods useable	EPP
Nesøy	2	2	-	-	100	0/5
Selvær	4	-	1	2	25	0/2
Gjærøy Monsen	4	3	-	-	75	0/7
Gjærøy Aakre	13	13	-	-	100	3/37
Ytre Kvarøy	17	16	-	-	94	1/54
Indre Kvarøy	16	5	5	5	63	2/32
Hestmona	9	5	-	2	55	0/15
Hestmannøy	39	13	8	12	54	2/56

Identification of extra-pair sires

Only one of the eight extra-pair young could be assigned to its sire at all three loci. The extra-pair father was a nest-owner in the same barn as the cuckolded male.

Paternity determination of juveniles

Two juveniles were assigned to the adults with which they were observed to associate in the field. On one of the smaller islands (Nesøy), all four juveniles which had been trapped inside a storage barn within the same day were attributed to a single pair. Since this pair were also the parents of a brood discovered later in the season inside the same barn, these four juveniles presumably fledged from this nest. Another three juveniles trapped one morning inside the same barn were all attributed to one male. Presumably, they had recently fledged together from an undiscovered nest. The remaining nine juveniles could not be assigned to any parents.

The efficiency of parental determination

Overall, about two thirds of the broods which had been bled could be used in the paternity analysis (71/104 broods, 208/287 nestlings). Several broods on each island usually remained unassigned however, which was surprising, since there were many adults on each which could not be attributed to any

broods (Table 3). Of all the adults which were run out on gels, less than 40% were identified as being mothers or fathers (104/262). Again, this percentage varied considerably between islands (Table 6), since on the well-populated islands such as Selvær and Indre Kvarøy, many pairs nested in inaccessible sites such as housing eaves or brickwork cracks. Most of the unassigned adults were presumably parents at these nests, where the broods had not been bled.

Table 6. The success rates of assigning parentage at each island.

Statistical concerns with the Helgeland analysis

Deducing maternity and paternity by cross-referencing nestlings against a pool of adults is obviously an inferior method to running samples in

Island	Parents compared	Parents assigned
Nesøy	15	5
Selvær	36	5
Gjærøy Monsen	25	4
Gjærøy Aakre	30	17
Ytre Kvarøy	29	16
Indre Kvarøy	46	16
Hestmona	19	11
Hestmannøy	62	30

recognized families. This is particularly true when attempting to detect extra-pair young, and it is possible that the low percentage of EPP reported from Helgeland is an artefact of the unusual method used to determine parentage.

In a typical paternity study, such as that undertaken in the Kentucky house sparrow colony described in Chapter 3, it is possible to quantify the power of the analysis by calculating the probability of false paternal inclusion, or P(*fpati*) (Gundel & Reetz 1981). This is the probability of attributing a nestling to a within-pair fertilization, when it has actually been sired by an extra-pair male (hence 'false inclusion'). The P(*fpati*) is derived from the frequency distribution of alleles at a particular locus, in such a way that extra-pair

young have a higher probability of being detected if the locus is more variable. Unfortunately, the P(*fpati*) is generally used with the assumption that the mother has been correctly identified (Gundel & Reetz 1981). This could never be assumed at Helgeland, since the identity of the mother was usually deduced by the same cross-referencing procedure used to identify the fathers. Hence, the statistical confidence in the EPP result was calculated to account for the probability of the mother being correctly identified.

This calculation was further confounded at Helgeland however, since the population size varied between each of the eight islands, and consequently the number of alleles differed between islands (Appendix 3). At each locus, the number of alleles detected was strongly correlated with the number of adults sampled (*Pdo*μ3 $r = 0.866$ $n = 8$ $p < 0.01$, *Pdo*μ4 $r = 0.765$ $n = 8$, TE $r = 0.822$ $n = 8$ NS). Calculating P(*fpati*) using the allele frequencies from a large island such as Hestmannøy or Indre Kvarøy would be misleading, since the number of alleles would be large, and hence the probability of birds sharing alleles by chance would be low. By contrast, if P(*fpati*) was calculated from a small population, such as Nesøy, stochastic effects associated with low sample sizes may mean the number of alleles is artificially small, which would inflate the P(*fpati*).

To achieve a balance between the two extremes, all inclusion probabilities were calculated using the allele frequencies from Gjærøy Aakre (Table 7 and Figure 3). This island was used as the standard since its population size represented the crude median of all eight islands, Moreover, both parents had been identified for all broods.

Table 7. Alleles detected and P(*fpati*) of the 29 Gjærøy Aakre adults.

Locus	Alleles detected	P (*fpati*)
*Pdo*μ3	8	0.352
*Pdo*μ4	19	0.137
TE	23	0.166
Combined	-	0.008

The probability of false paternal inclusion at all three loci was thus 0.008. This is the figure which would have been used if the mother was assumed to

be correctly identified. However, since this assumption could not be made at Helgeland, the probability of false paternal inclusion at each locus had to be *doubled*, since *both* of the nestling bands could have been paternal. The true probability of false paternal inclusion is therefore given by;

P (*fpati*)   = [2 x P(*fpati*) Pdoµ3] * [2 x P(*fpati*) Pdoµ4] * [2 x P(*fpati*) TE]

   = [2 x 0.352] * [2 x 0.137] * [2 x 0.166]

P (*fpati*)   = <u>0.064</u>

This implies than six of every hundred nestlings assumed to result from a within-pair mating are in fact extra-pair young. Since 208 nestlings were sampled from Helgeland, the real percentage EPP may be 10 % (12 + 8/208).

<u>Figure 3. Allele frequencies of the three microsatellite loci at Gjærøy Aakre.</u>
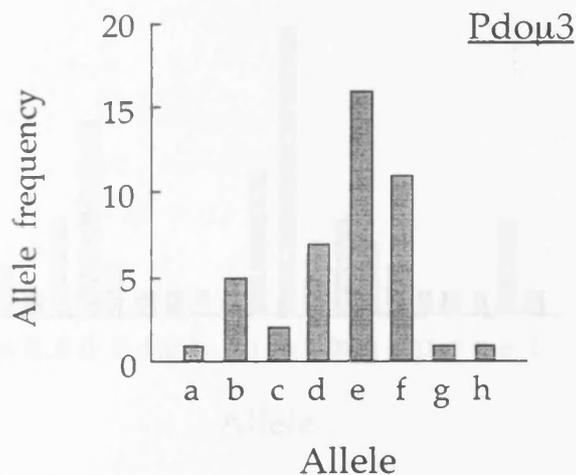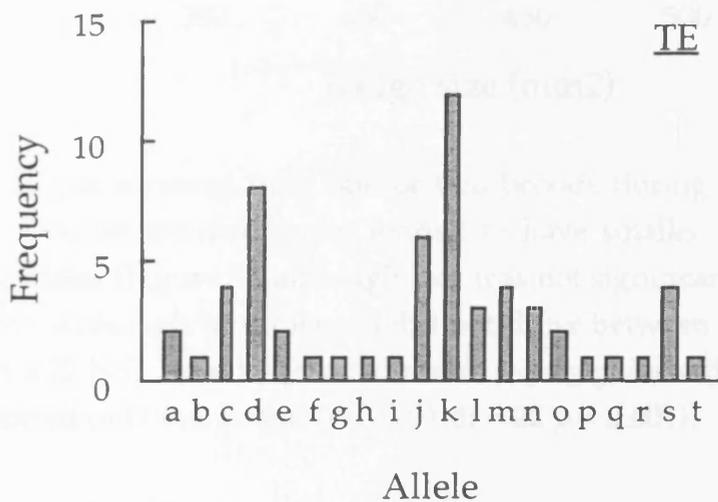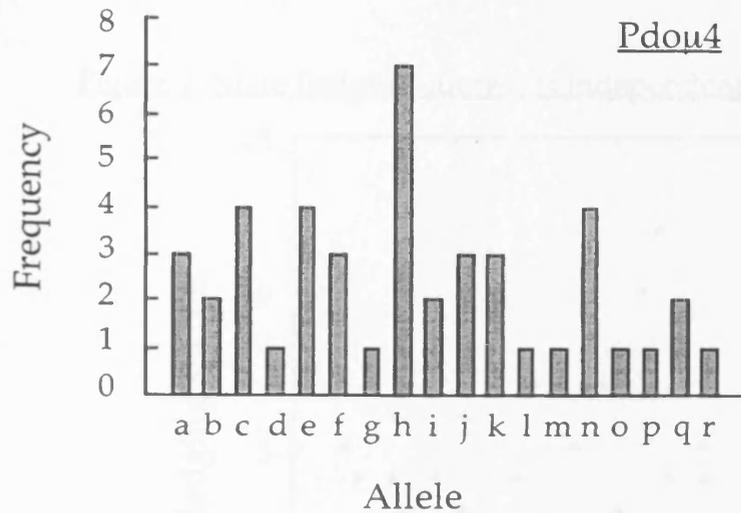
13.3.2 Phenotypic correlates of male fledging success

Badge size was independent of structural size ($r = -0.081$ $n = 77$ NS), and body condition ($r = -0.123$ $n = 76$ NS), although positively correlated with mass ($r = 0.280$ $n = 76$ p < 0.05). Hence, heavier males have larger badges.

The number of fledglings produced by each male was independent of his badge size ($r = -0.115$ $n = 26$ NS, Figure 4). Fledging success was not related to male size (tarsal length $r = -0.011$, mass $r = 0.057$, both $n = 26$ NS) or body condition ($r = -0.015$ $n = 26$ NS).

274

Large-badged males were not paired with females in good physical condition
(r = -0.395 *n* = 13 NS).

Figure 4. Male fledging success is independent of badge size.
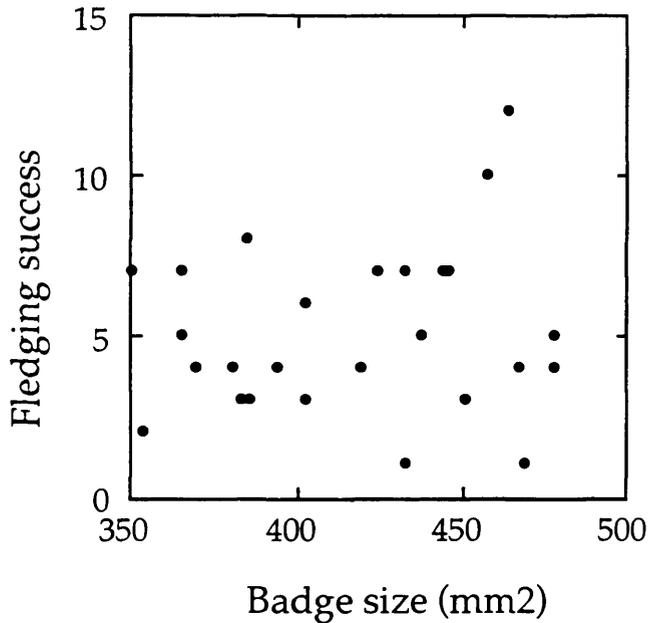
Almost all pairs reared only one or two broods during the entire breeding
season. Double-brooded males tended to have smaller badges than single-
brooded males (Figure 5), although this was not significant (*t* = - 1.80 df = 22
p = 0.086). Although tarsal length did not differ between the two groups (t =
-0.323 df = 22 NS), double-brooded males were significantly lighter than those
which reared only one brood (*t* = -3.91 df = 22 p = 0.001).

Figure 5. Badge sizes of single- and double-brooded males.
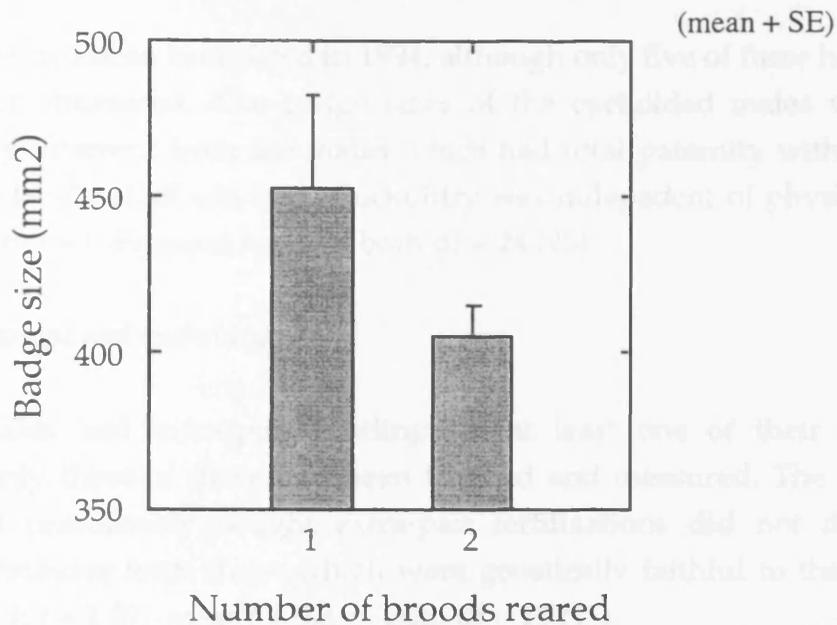
Phenotypic correlates of female fledgling success

The number of fledglings which females produced was independent of their body size (tarsal length r = -0.091, mass r = -0.391, both n = 20 NS) and physical condition (r = - 0.058, all n = 20 NS).

Double-brooded females tended to have smaller tarsi than those which reared only a single brood (t = -1.80 df = 19 p = 0.087), although mass did not differ between the two groups (t = -1.59 df = 19 NS).

13.3.10 Age-related differences in fledgling production

Few definite yearlings of either sex were present in the families grouped by microsatellite DNA analysis. Nestling processing had been less successful in the previous breeding season, and most trapping effort was timed to coincide with the post-breeding flocks, when unfortunately, juveniles and yearlings could not be distinguished with certainty.

The average number of fledglings produced by yearling males (n = 4) did not differ from that of males in at least their second season (t = 0.99 df = 31 NS). This test could not be repeated for females, since only two definite yearlings were present.

Male phenotype and cuckoldry

Seven males had been cuckolded in 1994, although only five of these had been trapped and measured. The badge sizes of the cuckolded males was not significantly different from the males which had total paternity within their own nests (t= -0.141 df =24 NS). Cuckoldry was indepedent of physical size (tarsal length t = 0.89, mass t = 0.89, both df = 24 NS).

Female phenotype and cuckoldry

Seven females had extra-pair nestlings in at least one of their broods, although only three of these had been trapped and measured. The females which had presumably sought extra-pair fertilizations did not differ in physical attributes from those which were genetically faithful to their mate (tarsal length t = 1.07, mass t = 0.366, both df = 18 NS).

Sex ratio variation in relation to parental phenotype

Only broods comprised of at least two nestlings were used to calculate sex ratios (i.e. the proportion of male nestlings in each brood). This was restricted to the first brood which each pair produced during the season, to avoid confounding effects associated with pooling.

The sex-ratio within the first broods of each nest was independent of the father's badge size (r = -0.198 n = 23 NS) and the mother's physical condition (r = -0.194 n = 19 NS).

13.3.3 Results from parasite surveys

Louse-flies

38 of the 409 house sparrows handled (9%) harboured at least one louse-fly. These were equally common on both adults and nestlings (8% vs 10%). Most birds carried single flies, although two or three flies per host was not uncommon, up to a maximum of five. The preserved hippoboscids were identified as *Ornithomyia fringillina*, a reasonably common louse-fly of passerine birds (Hutson 1984). All of the hippoboscids which had been recorded at the field site were assumed to have been *O. fringillina*.

The body condition of adults infested with at least one louse-fly was not significantly different to that of adults from which flies were never recorded (males $t = 1.57$ df $= 66$ NS; females $t = -0.91$ df $= 56$ NS). This calculation could not be repeated for nestlings, since they were not measured at a standard age.

Endoparasites

43 faecal samples were examined, three of which (7%) contained a mixture of sporulated and unsporulated oocysts of parasitic coccidia (7 - 489 detected in a five minute search). These belonged to the genus *Isospora* , which is characterised by the presence of two sporocysts, each containing four sporozoites. Records of house sparrow parasites from other populations suggest this is probably *Isospora lacazei* (Kruszewicz 1995).

Blood parasites

At least one blood smear was examined from 452 house sparrows (122 adults, 36 juveniles and 294 nestlings). No haematozoa were detected.

A single smear was examined from each of the following birds trapped incidentally while mist-netting : one blackcap, 39 bramblings, two crossbills, three fieldfares, five meadow pipits, seven pied wagtails, two redwings, 12 redpolls, eight starlings, five wheatears and five willow warblers. No haematozoa were detected.

Nest parasite counts

No nest parasites were detected by placing a hand on the nest (*sensu* Møller 1994). However, both fleas and mites were found in the nests which were returned to Leicester for closer examination. Fleas (mean 38, range 2 - 131) were found in all nine nests. All specimens were identified as the hen flea, *Ceratophyllus gallinae*. A single nest contained 44 haematophagous mites identified as *Dermanyssus gallinae*.

13.4 Discussion

13.4.1 The extent of cuckoldry at Helgeland

Extra-pair paternity was low at Helgeland ($c4\%$), even when the analysis was restricted to broods in which both parents had been deduced. Admittedly, the indirect means in which both paternity and maternity were determined renders it less likely that extra-pair nestlings would be detected. Despite this statistical concern, the level was lower than that in all other house sparrow populations studied to date, with the single exception of that on Lundy Island, where EPP was rare (Table 8).

Table 8. Levels of EPP in eight populations of the house sparrow.

Population	n	% EPY	Reference
Barcelona	106	10.4	Cordero *et al.* 1998
Helgeland	208	3.9	this thesis
Kentucky	185	10.3	this thesis
Lundy Island	305	0.9	Griffith 1998
New Mexico	55	12.7	Kimball 1995
Nottingham	536	13.6	Wetton & Parkin 1991
Oklahoma	56	19.0	R. Whitekiller *pers. comm.*
Villalba	171	7.0	J. Veiga & L. Boto *pers. comm.*

Although the underlying causes of intraspecific variation in EPP are unclear, current theories attribute this to differences in genetic variation, population demography or local flexibility in the focus of sexual selection. Each of these theories was discussed in detail in Chapter 4, and thus they are not repeated at length here. Superficially however, the first of the three theories appears most relevant to the Helgeland archipelago, since the population of house sparrows expanded to each island in sequence and consequently passed through a series of 'bottlenecks' (see Figure 1, and T. H. Ringsby, *pers. comm.*). If the genetic variation on the islands is low, then the net benefit of pursuing extra-pair matings would diminish (provided that females sought to gain genetic benefits through these matings). However, the allele frequencies at four microsatellite loci were recently assessed in subsets of adults drawn from

three of the populations listed in Table 7 (Lundy Island, Nottingham, Kentucky) (Griffith *et al.* MS). Although the levels of EPP differed significantly between Lundy Island and the sites in Nottingham and Kentucky, all four loci amplified a similar number of alleles in each populations (cf. Marin *et al.* 1994). This suggests that the Helgeland house sparrows are not necessarily genetically impoverished.

Moreover, there were no clear differences in allele frequencies between the study islands, despite the pattern expected from sequential bottlenecks (Appendix 3). House sparrows typically colonize areas of continuous human habitation, relying upon human settlements to provide both nest sites and food (Summers-Smith 1988). The initial step in the colonization of Helgeland would almost certainly have been Hestmannøy, because of its direct proximity with Kilbogham, the isolated port connecting Helgeland with the mainland (see Figure 1). The house sparrows resident on the Kvarøy islands would have mostly been derived from the Hestmannøy birds, possibly supplemented by birds from Kilbogham. The populations on Gjærøy and Nesøy are also most likely to have stemmed from Hestmannøy, and the colonization of the distant Selvær presumably occurred via Hestmannøy, perhaps with ship-assistance. However, since detailed records are not available, these routes of colonization are merely presumptive based on local geography.

13.4.2 Parental phenotype and reproductive success

Males with large badges were not in better physical condition than those with small badges, which refuted Veiga's (1993a) proposal that badges are honest indicators of their bearer's physical condition. Large-badged males did not produce more fledglings during the season than small-badged males, and so the hypothesis that badge size is under directional sexual selection in house sparrows was not supported. However, since nestlings were measured at variable ages, the possibility that large-badged males produce higher quality offspring cannot be discounted. No phenotypic correlates of fledging success could be detected in either males or females, and the occurrence of cuckoldry was independent of male phenotypic traits, including badge size.

13.4.3 Parental phenotype and brood sex ratio

Neither prediction of standard sex ratio theory was upheld at Helgeland.

Females paired to large-badged males did not produce a higher proportion of sons, nor did females in relatively poor physical condition produce a higher proportion of daughters.

13.4.4 Parasite assays

The significance of each taxon will be discussed only briefly here, since a detailed review of parasites of the house sparrow was given in Chapter 11.

Louse-flies

Louse-flies were found on about 10% of the house sparrows handled at Helgeland. These are potentially dangerous parasites, since they are fairly large (*c*10mm in length) and can consequently extract significant volumes of blood (estimated as over 8% of the total blood volume of an average 12 day old nestling swift [Lee & Clayton 1995]). Moreover, they have been documented as vectors of many avian haematozoa (Baker 1967).

There was no evidence that louse-flies had a detrimental effect upon their adult hosts at Helgeland. Saino *et al.* (1998) demonstrated experimentally that louse-flies depress nestling condition, although Lee & Clayton (1995) concluded that they had no detrimental effects upon swift nestlings. Patterns of louse-fly presence are difficult to interpret however, since the extreme mobility and agitative nature of species such as *Ornithomyia* suggests that they frequently move between hosts (Hutson 1984). Hence, a fly found upon a bird could have arrived recently, or may have been taking blood meals from the host for the last week. Furthermore, some species of louse-fly, particularly those associated with hirundines, deposit their pupiparia directly into the nest where they can be counted (Lee & Clayton 1995). However, *Ornithomyia fringillina* deposits its pupiparia away from the nest in crevices, where they cannot be found and counted. It would have been difficult to demonstrate that *O. fringillina* was pathogenic at Helgeland.

Endoparasites

Low levels of the protozoal coccidian *Isospora* sp. were recorded. As discussed in Chapter 11, however, oocysts are expelled in house sparrow faeces with a distinct periodicity (Kruszewicz 1995), and hence the results of my

281

opportunistic faecal sampling are difficult to interpret.

Blood parasites

The lack of blood parasites in Helgeland house sparrows was surprising, since several haematozoan records exist for this host, particularly in Europe (listed in Chapter 11). The absence of haematozoa in the other passerines sampled also contradicted surveys undertaken elswhere (Peirce 1981). None of the three hypotheses which are usually invoked to explain an absence of blood parasites (i.e. evolutionary lag, species-specific resistance, vector deficiency; all discussed in Chapter 2) explained the apparent freedom from haematozoa reported in Helgeland. (See Stewart *et al.* 1997 [Appendix 1] for a further exposition).

Nest parasites

No nest parasites were recorded when the flat of one's hand was placed directly on the nest lining, and yet fleas and occasionally mites were found when the nests were disassembled and carefully searched. It was clear that nidicoles were indeed present, but the field technique was inadequate. House sparrow nests are much more bulky and dense than the dry cup of barn swallows, and any mites present may neither detect a hand placed on the nest lining, nor may be able to crawl with any urgency through the nest. There are no published methods for estimating fleas numbers *in situ*, and it was difficult to envisage how nest parasites could have been estimated non-invasively in the Helgeland house sparrows.

In summary, there was no support for the Hamilton & Zuk (1982) theory of parasite mediated sexual selection in the Helgeland population of house sparrows. However, several classic parasite taxa were either absent (haematozoa) or could not be sampled (e.g. fleas, mites). These results are inadequate to reject the hypothesis.

Chapter 14. Overall discussion and summary of results.

The original aim of this thesis was to study house sparrow breeding ecology, with particular emphasis on sexual selection. Although male and female house sparrows are similar in body size, they are conspicuously sexually dimorphic, with the males possessing a throat patch of black feathers. This patch, known as the 'badge', is thought to have evolved through directional sexual selection, since simple mate choice trials had revealed a female preference for large-badged males (Møller 1988a) and previous field studies had recorded large-badged males having a reproductive advantage (Møller 1989, Veiga 1993a).

However, a more recent mate choice trial was unable to demonstrate a consistent female preference for any male morphological trait, including badge size (Kimball 1995, 1996). Furthermore, although the two main house sparrow researchers concur that badge size is a sexually selected character, they disagree on whether the mode of selection is female choice or male-male competition (Møller 1989, Veiga 1996). Consequently, the current study attempted to aid the resolution of this argument by primarily examining male reproductive success in relation to badge size, but also taking a broader perspective and testing for other attributes of both male and female which may influence reproductive success.

The majority of the data discussed in this thesis was collected at Maine Chance, an agricultural farm on the outskirts of Lexington, Kentucky, USA. Although, chronologically, this was the second site used, the data from this site was more comprehensive with respect to the hypotheses tested, and hence these results are considered first.

The evidence was consistent with the badge being a sexually selected trait, although the data gathered could not resolve whether large-badged males gain their reproductive advantage through female choice or male-male competition. Large-badged males did indeed produce more fledglings at Maine Chance, although this result was weakened after controlling for timing of breeding. Female fledging success was influenced by physical size, with larger females producing more fledglings, independent of the badge size of their partner. There was no evidence that pairing was assortative with respect to quality, since larger, more fecund females were

283

not paired to males which were either physically large or were endowed with a large sexual character.

As is usual for dichromatic passerines, however, although there was considerable variation in male appearance, there were few features which distinguished between females. Consequently, female quality was difficult to assess, and since female house sparrows not only lay the eggs, but undertake the major roles in incubation and brooding, their significance in determining differences in fledging success may have been underestimated. Furthermore, since very few fledglings returned to breed at the study colony, true estimates of reproductive success were impossible to obtain, and hence all the conclusions reported were drawn from the numbers of fledglings produced.

Other manifestations of sexual selection could also have been present in the Maine Chance house sparrows, although these were beyond the scope of this work (e.g. whether females have a pre-existing sensory bias, Basolo 1990, attempt to mate assortatively to produce heterozygous offspring, Brown 1997, or whether they use mate choice cues which are invisible to humans, such as signals which only reflect in the ultraviolet, Bennett *et al.* 1994, Andersson *et al.* 1998).

A more recent theory posits that secondary sexual characters such as the badge of male house sparrows reflect the quality of their bearer in their degree of fluctuating asymmetry (Møller and Pomiankowski 1993). Asymmetries arise in both sexual and non-sexual characters, such as the tarsi, as a result of developmental stresses such as a restricted nestling diet or genetic defects. This is particularly pertinent when considering the expression of the male badge, since this is renewed annually during the autumnal moult. An examination of badge asymmetries in male house sparrows revealed considerable variance in asymmetry between males, although this was not related to fitness parameters such as pairing date or reproductive success. Moreover, since badge asymmetry was difficult to measure objectively, any conclusions are premature, since the key measure is whether female sparrows perceive badges as being variably symmetric. Males with asymmetric tarsi had lower fledging success, which is consistent with the prediction of fluctuating asymmetry theory. However, the level of asymmetry was at the lowest possible limit of detection (in most cases, only one tenth of a millimetre), which could

have easily arisen through observer error. Consequently, the evidence that asymmetry affects fitness was merely suggestive.

The theory of sexual selection has been revitalized within the last decade by the genetical revelation that female birds of many species achieve fertilizations with males other than their social partner (Birkhead and Møller 1992). This was a particularly important advance for those studying reputedly monogamous birds, in which the variance in reproductive success between individuals was supposedly low, and accordingly, the opportunity for sexual selection would be weak (Mayr 1972). This is clearly an underestimate, since females appear to gain nothing from the cuckolding male other than sperm, and hence the latter fathers additional offspring without incurring any rearing costs. Despite the plethora of genetic parentage studies which have appeared in the wake of the original publications, the benefits to infidelitous females are largely speculative, and the factors which influence the extent of extra-pair paternity (EPP) both within and between studies are much debated (e.g. Westneat and Sherman 1997, Owens and Hartley 1998, Petrie and Kempenaers 1998).

This study used DNA microsatellites to firstly determine whether female house sparrows pursue these extra-pair matings, and secondly, to examine the factors which apparently influence the distribution of EPP in other bird species (Ross 1997, Owens and Hartley 1998). Since male house sparrows have a conspicuous secondary sexual trait, which apparently functions in mate choice, this leads to a clear prediction. If, as seems reasonable, females seek extra-pair fertilizations in an attempt to gain genetic benefits (since they rarely receive direct benefits), large-badged males should be cuckolded less frequently than small-badged males, since they are presumed to be of higher quality (Veiga 1996).

The level of EPP at Maine Chance was 10.3% of young, distributed in 28.6% of broods. This is slightly lower than the average figure collated from previous parentage studies of passerines, but was similar to the levels recorded in other house sparrow populations. No phenotypic differences were detected between cuckolded and non-cuckolded males, although the sample sizes for many tests were small. Males with either small or large badges were equally likely to be cuckolded, as was found by R. Whitekiller and D. F. Westneat (*pers. comm.*) and Cordero *et al.* (MS). Cuckoldry was independent of the host male's age, as reported in a British house sparrow

population (Wetton *et al.* 1995), although older males gained a reproductive advantage since they were more likely to achieve extra-pair fertilizations.

There was no association between infertility and cuckoldry in both years of the study, and hence no support for the fertility insurance hypothesis (Wetton and Parkin 1991). Hence, females do not appear to seek copulations from other males to guard against the risk of their partner's infertility. At Maine Chance, the frequency of EPP was unrelated to local breeding synchrony. This was unexpected, since synchrony (i.e. the overlap of the females' fertile periods) is theoretically an important determinant of EPP since it regulates the operational sex ratio (Emlen and Oring 1977). The most interesting finding from the genetic data, albeit not significant, was an unexpected inverse relationship between breeding density and EPP. Although density had no effect upon EPP in tree swallows (Dunn *et al.* 1994) or in a Spanish house sparrow population (Cordero *et al.* MS), a positive relationship between density and EPP has been found in red-winged blackbirds (Gibbs *et al.* 1990), eastern bluebirds (Gowaty and Bridges 1991), European starlings (Double 1995), bearded tits (Hoi and Hoi-Leitner 1997) and northern orioles (Richardson 1997). However, this was confounded by a between-year effect, since the families sampled were taken from a different barn in each of the two years of the study.

To test whether the patterns of EPP were not related to physical factors such as density, but rather to differences in individual behaviours, the study examined the prevalence of two behaviours described from other birds, which are presumed to act as paternity protection devices (Birkhead and Møller 1992). In the first of these, frequent copulation, males are predicted to maintain a high rate of copulation to either limit the amount of time available for their mate to seek other matings, or to physically dilute any sperm introduced from a rival male. In the second of these, mate guarding, males follow their mate to again, limit the amount of time which she has available to seek copulations, and also, to deter rival males intent on seeking copulations.

The strength of both of these activities is expected to vary with regard to the likelihood of a copulation being successful (Ross 1997). Hence, males are expected to exhibit these behaviours most intensively during their mate's fertile period, and within this period, around the early morning

'fertilization window' (Cheng *et al.* 1983), when the previous egg has recently been laid and the unfertilized egg appears briefly in the infundibulum. Moreover, the behaviours are expected to vary between individual males according to how they perceive their confidence of paternity. Consequently, if large-badged males are indeed generally attractive and of higher quality, their paternity protection measures should be more relaxed.

Copulations were easily observed at the study site, since most pairs copulated on or around their box. All copulations witnessed were within-pair. The generally high copulation rates recorded at Maine Chance were consistent with Møller (1990b), who credited house sparrows with about 47 bouts per breeding cycle, each 'bout' containing around four copulations (each bout at Maine Chance contained around six copulations). Large-badged males tended to copulate at higher rates than small-badged males, in contrast to the above prediction. Although this result was not significant, the sample size involved was restrictively small. Despite the notion of the 'fertilization window', morning copulation rates were not higher than those observed in the late afternoon. Large-badged males did not continue to copulate later in the day than small-badged males. The variation in evening copulation frequency may, however, be meaningless, as evidence taken from house sparrows suggests that copulations become less valuable as the day progresses (Birkhead *et al.* 1994). Although temporal fluctuations in copulation frequency were undoubtedly confounded by variation between males, the obvious peak in copulation rate coincided with the day on which the first egg was laid (the First Egg Date or FED). Since it has been demonstrated experimentally that male house sparrows can distinguish between fertile and non-fertile females (Møller 1987c), males presumably increase their rate of copulation in anticipation of this day.

Observations of mate guarding were more difficult to obtain, since both sexes spent a substantial amount of time away from their nestbox where they could not be seen. As the breeding cycle progressed, males spent proportionately less time near the nest, whereas female presence increased. Females presumably spend most of their pre-fertile period away from the barns, to obtain the nutrients required for egg production (Summers-Smith 1963, Murphy 1978a). Naturally, males have no such constraints. The most informative results, and the closest approximation

of true 'mate guarding', concerned the proportion of female presence in which the males were also present. Males were only present for about 50% of the time that females were present, a figure which was used by Møller (1994b) to indicate the termination of mate guarding in barn swallows. Males were not more likely to be associated with the female during her fertile period, despite evidence that they can distinguish when their mate is fertile (Møller 1987c).

Theoretically, males which suffer from the greatest threat of cuckoldry should mate guard most intensively (Møller 1994b).This prediction was not upheld, since the intensity of mate guarding was independent of badge size. Elements of mate guarding were apparent in the proportion of partner flights which were followed, since significantly more males followed female flights than vice versa. These results suggest that mate guarding by physical proximity occurs only weakly, if at all, in house sparrows at Maine Chance. Nevertheless, without knowing the activities of both sexes while they are out of range, it would be unwise to extrapolate too far from focal nestbox observations. Either sparrows do not perceive a threat of cuckoldry (Wright and Cotton 1994), or that an unknown factor(s) precludes efficient guarding. However, the evidence that mate guarding reduces the risk of cuckoldry is itself equivocal (reviewed in Birkhead and Møller 1992). Empirical evidence (mostly from removal experiments) suggests that it can function as an effective paternity guard (Dickinson 1997), although several counter-intuitive results have been reported.

The thesis also investigated individual variation in the size of the cloacal protuberance, the swollen, distal bulb of the male reproductive tract which houses the seminal glomera. The development of the protuberance appears to be related to the intensity of sperm competition at the interspecific level (Birkhead *et al* . 1993a). This did not extend to the intraspecific level, since badge size was actually negatively correlated with the height of the protuberance, contrary to the prediction of the sperm competition hypothesis. Although the data set was small, the height of the protuberance was negatively correlated with copulation frequency, which also contradicts the sperm competition hypothesis.

In addition to the parentage analysis, the work also considered another recent finding which has stimulated sexual selection theory. Although adult birds were traditionally assumed to have no control over the sex of

288

the offspring they produce, this view has been overthrown within the last three years (see Ellegren and Sheldon 1997). In birds, the female is the heterogametic sex, and thus has the opportunity to facultatively adjust the sex of the eggs or embryos before they are laid. Facultative sex ratio adjustment could be adaptive in response to the quality of either the female herself, or that of her mate. Females mated to an attractive, high quality male should theoretically bias their brood towards sons, since this would increase maternal fitness gain should the sons inherit their father's traits. Females in poor physical condition should produce a greater proportion of daughters, since even low quality daughters will probably produce at least some surviving young, whereas low quality males could conceivably remain unmated.

Although male nestlings had significantly longer tarsi, and tended to be slightly larger in all dimensions, sons and daughters were of similar sizes. This suggests that the costs of rearing sons and daughters are similar, and would not confound the analysis. The sex ratio did not differ from unity in either year of the study, although this is largely attributable to the small sample sizes involved (about two hundred). To detect a significant departure from a 1 : 1 ratio requires either a considerable skew, or large sample size (Schifferli 1980). Females did not appear to produce more sons when paired to more attractive, large-badged males, although again, the data were few. There was no convincing evidence of a condition-dependent sex-ratio. In 1996, heavier females produced significantly more males, although their body condition *per se* did not affect offspring sex ratio. The sex ratio did not become biased towards daughters as the season progressed in either year. This was strong evidence against the maternal condition hypothesis, as it seems reasonable to assume that all females suffer a decline in physical condition towards the end of the season, irrespective of their initial condition. The results suggest that either female house sparrows do not possess the subtle control over offspring sex reported from other species, or that the advantages of sex ratio adjustment are minimal.

A substantial amount of fieldwork involved following the fate of each individual clutch, assessing the number of fledged young which resulted from it, and testing adaptive hypotheses which account for any losses. Clutches of birds' eggs rarely hatch simultaneously, which Lack (1947) proposed was an adaptive parental mechanism to optimize reproductive

output. Following on at the next ontogeneic stage, Ricklefs (1965) coined the phrase 'brood reduction' to describe the adaptive loss of one or more nestlings from an otherwise successful brood. Brood reduction refers classically to the starvation of the smallest and weakest individual, and can arise following either active neglect by the parent(s), or competition with nest-mates (Clark and Wilson 1981, Stenning 1997). It is immediately paradoxical since the maximum fledging potential from any nest is reduced at a relatively early stage. However, the mortality would have a net adaptive effect upon adult fitness if effort (usually food provisioning) is diverted away from the smallest individual and apportioned to the remainder of the brood, whose growth and condition improve at the expense of their dead sibling (Stouffer and Power 1991).

Most house sparrow clutches hatched asynchronously at Maine Chance farm, as has been found in other populations (Seel 1968b, Anderson 1986, Veiga 1990a). The final egg to hatch was invariably the last-laid egg, which implies that the asynchrony is caused by incubation being initiated before the clutch is complete (Stoleson and Beissinger 1995). Although larger clutches were more likely to hatch asynchronously than small clutches, this was considered an artefact of the high proportion of synchronous 3-egg clutches. When these relatively uncommon small clutches were deleted from the analysis, hatching asynchrony was independent of clutch size. Hatching asynchrony was independent of clutch size, which caused the 'egg viability' hypothesis to be rejected (Arnold et al. 1987). Under this hypothesis, eggs slowly lose viability if they remain unincubated for long periods. Hence, females commence incubation before the clutch is complete so that the first-laid eggs survive. This should be more important in large clutches, where the time difference between the laying of the first and last eggs is more pronounced.

Brood reduction was also common in both breeding seasons, and was consistent with the sequence of events described in other studies (Zach 1982, Skagen 1988, Magrath 1989). The nestling which starved was almost always the last to hatch, and had therefore usually emerged from the last-laid egg (Magrath 1989, Veiga 1990). Despite this obvious pattern, brood reduction was not facilitated by hatching asynchrony, since it was not more likely to occur in clutches which had hatched asynchronously. One prediction of the classical brood reduction hypothesis (Ricklefs 1965, Lack 1968) was supported however, since the proportion of broods in which one

or more nestlings were lost increased with advancing hatching date (Amundsen and Stokland 1988, Mock 1994). This was presumably in response to a seasonal decline in the quality and quantity of the food supply (Perrins 1979), although admittedly, no data on this were obtained at Maine Chance. The patterns of hatching sequence and selective mortality which were observed at Maine Chance were consistent with several tenets of hatching asynchrony and brood reduction theory. Generally however, the results were equivocal, since neither strategy appeared to improve the mass (and therefore survival probability) of the remaining nestlings.

To broaden the assessment of hatching asynchrony and nestling losses, attention was paid to the significance of egg size variation between females, within females, and within clutches. Previous studies have recorded an influence of egg size variation on hatching patterns and also successes of individual eggs, which illustrates the occasionally subtle influence female birds exert over the survival prospects of their young. For all house sparrow clutch sizes, volume increased with laying sequence (as found by Murphy (1978) and Lowther (1990), but not Veiga (1990a)). In common with Murphy (1978) the first-laid egg was generally the smallest, although contrary to Murphy, the last egg was not consistently the largest. The time of season (and hence mean daily temperature) had no effect on egg size (when considering repeat clutches of the same females and mean egg size of the population generally).

There were few clear patterns in egg size variation between females however. Egg size was independent of clutch size, which suggests that there is no energetic trade-off. Female size influenced egg size in the second year of the study but not the first. However, eggs laid by yearling sparrows were no different in size than those laid by older sparrows. Hatchling mass was strongly and positively correlated with egg size, as has been found in most birds (listed in Ward 1995). Despite the clear relationship between egg and hatchling size, variation in egg size can only have evolutionary significance if it results in increased reproductive success of the parent. Although it proved difficult to match individual eggs to hatchlings and, more importantly, fledglings, the few data which were available failed to show a correlation between egg size and fledging size.

There is a paucity of data in any bird species which unequivocally relate egg size to eventual fitness (which is perhaps not surprising, given the difficulties experienced in assigning individual eggs to chicks in a field situation). It does appear, however, that the size variation in fledging house sparrows is far removed from size variation in eggs. It is difficult to avoid the conclusion that in house sparrows, egg size variation is merely ancillary to hatching asynchrony as the primary force in establishing initial size asymmetries.

The final aspect of the work considered the influence of parasites on host fitness, and ultimately, on sexual selection. The Hamilton & Zuk (1982) hypothesis posits that secondary sexual characters, such as the badge of the male house sparrow, have evolved to indicate either the innate, genetic resistance of their bearer or reflect the current health and disease status of the individual concerned. This was originally to have been one of the main themes of the research, on a par at least with the work on sexual selection which introduces the thesis. Unfortunately, very few parasites were present at the field site. The rarity of ectoparasites was surprising, considering the diversity previously recorded from the house sparrow at several localities in both Europe and North America. Equally surprising was the absence of haematozoa (blood parasites), since these have been recovered from the house sparrow in other American states, despite the exotic nature of this host. This was unfortunate, since a substantial proportion of current research into bird-parasite interactions concerns these protozoa, which can be sampled with only minimal effect upon the bird. None of the three leading hypotheses to account for an absence of parasites could be supported (vector deficiency, evolutionary lag and innate resistance), although at least two recent studies have been unable to explain haematozoan absences (Rytkönen et al. 1997, Stewart et al. 1997).

About 8% of house sparrows (adults and nestlings) did harbour variable numbers of the protozoal coccidian Isospora spp., which were detected as unsporulated oocysts in faecal samples. No pathogenic effects were detected, although a genuine assessment of coccidia level is notoriously difficult to achieve, since there is a definite periodicity to the expulsion of oocysts, and regular faecal sampling of adults in particular was impossible to achieve because of general catching difficulties. However, future work could house sparrows in aviaries, elucidate the cycle of expulsion, and

produce a simple calculation for standardizing oocyst counts taken from faecal samples obtained at different times of the day.

Around 30% of house sparrows broods were infested with *Carnus hemapterus*, a small black dipteran which typically completes its entire life cycle in the nest. The evidence strongly suggested that *Carnus* flies are haematophagous in house sparrows, and are thus potentially threatening both directly (as a cause of blood loss) and indirectly (as vectors of disease). Again however, the presence of these flies did not appear to influence the physical development of the sparrows, or indeed their level of white blood cells. No deleterious effects of *Carnus* have been classified (Walter and Hudde 1987, Dawson and Bortolotti 1997), although Canning (1986) presented circumstantial evidence attributing partial loss of a single brood of saw-whet owl nestlings to heavy *Carnus* infestation. However, each owlet that died carried over 50 flies, whereas the maximum infection recorded in this study was five flies per nestling. It seems reasonable to conclude that, although *Carnus* flies are potentially pathogenic if they occurred in large infestations, the levels at which they occurred at Maine Chance farms was too low to cause detectable pathology.

The only other common and noticeable parasites were blood sucking mites (*Dermanyssus* sp.), which occurring in around 25% of broods. Like *Carnus*, these are primarily nest parasites, and take blood from nestlings and possibly incubating or brooding females. The vast majority of adults handled during the study were uninfected (175/177 individuals processed). The seasonal pattern of infection in both years suggested that mites did not originate from overwintering populations in the nest material, or at least, this was not their exclusive source. The rarity of mites upon adults suggests that they are not transported this way, although they were more often found on fledged birds, which would return to nest boxes at the end of the season to quest for nest sites. Nestlings reared in nest with mites were lighter when ringed on nestling day 10 than nestlings reared in mite-free nests. The significance of heavy infestations (> 500 mites) was drastic. None of these nestboxes were used again during the season, and in two boxes, the entire brood of nestlings was dead.

The observation that adults were not infected by mites is crucially important when testing the Hamilton-Zuk hypothesis. Unlike barn swallows (Møller 1994b), females could not visually judge the direct and

293

indirect costs or benefits accrued by mating with a particular male (either within-pair or extra-pair). This suggests that ectoparasitic mites would not affect the choice of either social or sexual mates, nor would they influence which nest a female initially chose to breed in. However, because mite infections become apparent as the nestlings age, ectoparasites could influence the decision of the female (if not the male) to initiate a new breeding attempt in the same nest, or move to a different nest (and presumably, a different mate). Thus, mites probably do not affect initial mate choice in house sparrows at Maine Chance, although they could affect mate retention and thus ultimately impact on fitness.

Since the thesis had addressed the role of parasitism in affecting house sparrow fitness, it was then pertinent to also consider factors associated with the immune response of the host. Consequently, a sample of house sparrows was obtained from rural Leicestershire, which had recently completed their annual moult, a particularly stressful period for small passerines. The current level of immune activity was estimated by recording the size of the spleen, the major disease resistance organ in birds (John 1994). Larger spleens were presumed to be more active.

The size of the house sparrow spleen was not related to any physical characteristics of the host, such as body size. However, spleen size was strongly affected by age, with juvenile birds having significantly larger spleens than adults. This was attributed to a heightened immune response to novel antigens encountered soon after leaving the relatively constant environment of the nest. There was no intersexual difference in spleen size, even after controlling for age effects, which suggests that, at this time of year at least, male and female house sparrows have equal exposure to pathogens and/or stresses which invoke a splenic response.

Males with small spleens (and hence assumed to possess greater genetic resistance) had large badges, and conversely , males with large spleens (presumably suffering from an undefined infection) had small badges. This supports the hypothesis that badge size is a condition dependent trait (Veiga 1993a, 1996), since poor quality individuals can only produce a small badge since they are having to divert resources into mounting an immune response. Although this relationship disappeared when considering each age class in isolation, this may not be important. By choosing males with larger badges, the females mate with males that had

294

smaller spleens, and consequently are presumably less likely to become infected in the future.

The first study site chronologically was an island archipelago ('Helgeland'), located on the coast of arctic Norway. The aims of the work in Helgeland were virtually identical to those which were later followed in Kentucky, and hence are not discussed in any detail here. One of the major themes of the work was to consider the effects of parasites on host fitness and sexual selection. However, as in Kentucky, these efforts were curtailed by a general dearth of parasites. Blood parasites were absent, although none of the three hypotheses proposed to account for their absence could be supported. Permanent ectoparasites were apparently absent, and although about 5% of the sparrows handled were infested with blood sucking dipterans (Hippoboscidae), no deleterious effects of these could be detected. However, these flies are extremely mobile, were difficult to monitor, and hence it could not be known how long they had been feeding on the host concerned. Several nests examined at the end of the field season contained low levels of haematophagous parasites (fleas and mites), but these proved impossible to monitor during the breeding season, mainly because all of the nests were located in deep crevices in brickwork and were difficult to extricate without permanent damage.

The study also considered the role of sexual selection and extra-pair paternity, although these efforts were restricted because it proved difficult to match adults to individual nests. Also, the logistics of the fieldwork involved regular travel between the islands, such that nests were not monitored at regular intervals. Hence, the basic data obtained in Kentucky concerning clutch sizes, hatching success and brood losses were often not available. Furthermore, paternity and maternity had to be elucidated in retrospect by comparing the DNA profiles, at three microsatellite loci, of all nestlings in the population against all adults trapped. Using this method gave an low EPP level of 4%, even when the analysis was restricted to broods in which both parents had been deduced. However, the indirect means in which parentage was determined renders it less likely that extra-pair nestlings would be detected. Also, this level could be a severe underestimate, since an entire brood assigned to a particular male could actually have resulted from complete cuckoldry. Large-badged males did not appear to have a reproductive advantage as regards number and

quality of offspring produced, although the data for this was obviously limited and assumed parentage was correctly assigned.

To summarize, two of the three main aims of the thesis were investigated satisfactorily. There was suggestive evidence that badge size is under directional sexual selection, since large-badged males reared more nestlings within a season than small-badged males. However, this was not significant when time of breeding was controlled for, and since the survival of the fledglings was not known, it would be premature to conclude large-badged males have greater fitness. No other parental feature clearly influenced reproductive success. It does appear that badge size variation has little significance during the breeding season, although since the field site was not visited during the winter, it is possible that badge size functions more in dominance interactions and territorial disputes outside of the breeding season.

House sparrows at both field sites appeared to exhibit extra-pair behaviour as deduced by the presence of cuckoldry. Although no extra-pair copulations were witnessed, the fact that paternity protection behaviours were present suggests that male sparrows perceive at least an occasional risk of cuckoldry. No phenotypic factors appeared to influence the extent of cuckoldry, and large-badged males were equally as likely to be cuckolded as small-badged males. No parameters associated with local breeding ecology, such as density and synchrony, influenced the incidence of extra-pair paternity.

Attempts to address the third and final aim, the effects of parasites upon host fitness and sexual selection, were curtailed by a general dearth of parasites. Two types of parasites were present at a reasonable frequency (haematophagous mites and flies), although the levels of infection were probably too small to result in serious effects, and consequently, no pathology was detected.

Appendix 1. Alleles detected at each locus on a per-island basis.

Microsatellite locus _Pdoμ3._

Island	Adults	Alleles	Homo-zygotes
Nesøy	15	7	2
Hestmona	17	10	0
Gjerøy Aakre	29	8	6
Gjerøy Monsen	25	10	2
Selvær	35	11	7
Storselsøy	60	16	7
Ytre Kvarøy	29	12	2
Indre Kvarøy	45	12	7

Microsatellite locus _Pdoμ4_

Island	Adults	Alleles	Homo-zygotes
Nesøy	15	15	0
Hestmona	17	20	1
Gjerøy Aakre	29	19	3
Gjerøy Monsen	25	24	0
Selvær	33	23	0
Storselsøy	62	34	2
Ytre Kvarøy	25	22	0
Indre Kvarøy	45	19	1

Microsatellite locus TE

Island	Adults	Alleles	Homo-zygotes
Nesøy	15	16	1
Hestmona	17	17	0
Gjerøy Aakre	30	23	1
Gjerøy Monsen	25	26	1
Selvær	36	29	0
Storselsøy	60	29	3
Ytre Kvarøy	25	24	1
Indre Kvarøy	45	29	1

Appendix 2. Scientific names of species mentioned in the text

Acorn woodpecker	*Melanerpes formicivorus*
Adelie penguin	*Pygoscelis adeliae*
Alpine accentor	*Prunella collaris*
American kestrel	*Falco sparverius*
Aquatic warbler	*Acrocephalus palaudicola*
Arctic skua	*Stercoraria parasiticus*
Australian brush-turkey	*Alectura lathami*
Barnacle goose	*Branta leucopsis*
Barn owl	*Tyto alba*
Barn swallow	*Hirundo rustica*
Bearded tit	*Panurus biarmicus*
Bicolored wren	*Campylorhyncus nuchalis*
Black-billed magpie	*Pica pica*
Black-capped chickadee	*Parus atricapillus*
Black-eared bush tit	*Psaltriparus minimus*
Blackbird	*Turdus merula*
Blackcap	*Sylvia atricapilla*
Black grouse	*Tetrao tetrix*
Black vulture	*Coragyps atratus*
Blue duck	*Hymenolaimus malacorhyncus*
Blue peafowl (peacock)	*Pavo cristatus*
Bluethroat	*Luscinia svecica*
Blue tit	*Parus caeruleus*
Bobolink	*Dolichonyx oryzivorus*
Brambling	*Fringilla montifringilla*
Brown skua	*Catharacta longbergi*
Bull-headed shrike	*Lanius bucephalus*
Chaffinch	*Fringilla coelebs*
Chicken	*Gallus domesticus*
Cliff swallow	*Hirundo pyrrohonota*
Collared flycatcher	*Ficedula albicollis*
Common guillemot	*Uria aalge*
Common loon	*Gavia immer*
Corn bunting	*Miliaria calandra*
Cory's shearwater	*Puffinus diomedea*
Crested tit	*Parus cristatus*
Crossbill	*Loxia curvirostra*
Domestic hen	*Gallus domestica*
Dunnock	*Prunella modularis*
Eastern bluebird	*Sialia sialis*
Eleonora's falcon	*Falco eleonorae*
Erect-crested penguin	*Eudyptula sclateri*
Eurasian dotterel	*Charadrius morinellus*
Eurasian kestrel	*Falco tinnunculus*
Eurasian moorhen	*Gallinula chloropus*
European bee-eater	*Merops apiaster*
European robin	*Erithaculus rubecula*

European starling	*Sturnus vulgaris*
European swift	*Apus apus*
Feral pigeon	*Columba livia*
Fieldfare	*Turdus pilaris*
Field sparrow	*Spizella pusilla*
Galapagos hawk	*Buteo galapagoensis*
Goshawk	*Accipiter gentilis*
Great reed warbler	*Acrocephalus arundinaceus*
Great tit	*Parus major*
Henderson reed warbler	*Acrocephalus vaughani*
Herring gull	*Larus argentatus*
Hooded warbler	*Wilsonia citrinella*
House finch	*Carpodacus mexicanus*
House martin	*Delichon urbica*
House sparrow	*Passer domesticus*
House wren	*Troglodytes aedon*
Indigo bunting	*Passerina cyanea*
Irish elk	*Megalocerus giganteus*
Jackdaw	*Corvus monedula*
Kentish plover	*Charadrius alexandrius*
Kittiwake	*Rissa tridactyla*
Leach's storm petrel	*Oceanodroma leucorrhoa*
Least auklet	*Aethia pusilla*
Lesser black-backed gull	*Larus fuscus*
Lesser kestrel	*Falco naumanni*
Lesser snow goose	*Anser (Chen) caeruluscens*
Loggerhead shrike	*Lanius ludovicius*
Meadow pipit	*Anthus pratensis*
Merlin	*Falco subbuteo*
Northern cardinal	*Cardinalis cardinalis*
Northern fulmar	*Fulmarus glacialis*
Northern oriole	*Icterus galbulla*
Osprey	*Pandion haliaetus*
Oystercatcher	*Haemotopus ostralegus*
Pheasant	*Phasianus colchicus*
Pied flycatcher	*Ficedula hypoleuca*
Pied wagtails	*Motacilla alba*
Pukeko	*Porphyrio porphyrio*
Purple martin	*Progne subis*
Redpoll	*Carduelis flammea*
Redwing	*Turdus iliacus*
Red-backed shrike	*Lanius collurio*
Red-cockaded woodpecker	*Picoides borealis*
Red-winged blackbird	*Agelaius phoeniceus*
Reed bunting	*Emberiza schoeniclus*
Resplendent quetzal	*Quetzalus quetzalus*
Ruff	*Philomachus pugnax*
Sand martin	*Riparia riparia*
Savanna sparrow	*Passerculus sandwichensis*

Screech owl	*Otus asio*
Scrub jay	*Aphelocoma coerulescens*
Sedge warbler	*Acrocephalus schoenobaenus*
Shag	*Phalacrocorax aristotlensis*
Sharp-shinned hawk	*Accipiter striatus*
Short-tailed shearwater	*Puffinus tenuirostris*
Silvereye	*Zosterops lateralis*
Smith's longspur	*Calcarius pictus*
Snow bunting	*Plectrophenax nivalis*
Song sparrow	*Melospiza melodia*
Sparrowhawk	*Accipiter nisus*
Splendid fairy-wren	*Malurus splendens*
Spotted sandpiper	*Actitis macularia*
Stripe-backed wren	*Campylorhyncus nuchalis*
Superb fairy wren	*Malurus cyaneus*
Tasmanian native hen	*Tribonyx mortierii*
Tree sparrow	*Passer montanus*
Tree swallow	*Tachycineta bicolor*
Water/Rock pipit	*Anthus spinoletta*
Western bluebird	*Sialia mexicana*
Wheatear	*Oenanthe oenanthe*
Whiskered auklet	*Aethia pygmaea*
White-throated sparrow	*Zonotrichia albicollis*
White-crowned sparrow	*Zonotrichia leucophrys*
Willow ptarmigan	*Lagopus lagopus*
Willow tit	*Parus montanus*
Willow warbler	*Phylloscopus trochilus*
Wilson's warbler	*Wilsonia pusilla*
Wood warbler	*Phylloscopus sibilatrix*
Yellowhammer	*Emberiza citrinella*
Yellow warbler	*Dendroica petechia*
Zebra finch	*Taeniopygia guttata*

Appendix 3. Acronyms used in the text.

ANOVA	Analysis of variance
BBS	Black badge size
BCI	Body condition index
BR	Brood reduction
BSI	Breeding synchrony index
CP	Cloacal protuberance
DNA	Deoxyribonucleic acid
DPM	Delayed plumage maturation
EPC	Extra-pair copulation
EPF	Extra-pair fertilization
EPO	Extra-pair offspring
EPP	Extra-pair paternity
EPY	Extra-pair young
ESS	Evolutionarily stable strategy
FA	Fluctuating asymmetry
FED	First egg date
HA	Hatching asynchrony
HZ	Hamilton & Zuk
ID_x	Incubation day$_x$
IPC	Integrated plastic chamber
ISBP	Intraspecific brood parasitism
LMC	Local mate competition
MAT	Male awareness time
ND_x	Nestling day$_x$
NND	Nearest neighbour distance
NS	Not significant
OSR	Operational sex ratio
PCR	Polymerase chain reaction
PMSS	Parasite-mediated sexual selection
RAPD	Randomly amplified polymorphic DNA
RS	Reproductive success
SSC	Secondary sexual character
SST	Sperm storage tubule
STD	Sexually transmitted disease
WBS	White badge size
WPC	Within-pair copulation

301

SPECIAL NOTE

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Brief report

Absence of haematozoa in passerines from a Norwegian archipelago

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In 1982. Hamilton and Zuk proposed a novel theory of sexual selection. based on female choice for male secondary sexual characters which reveal the health or infection status of their bearer. Their hypothesis was supported by an interspecifc positive correlation between plumage brightness and haematozoan prevalence. but has since been criticised as relying upon a desultory haematozoan sampling regime with respect to host sample sizes and locations (reviewed in Møller 1990).

We attempted to test the Hamilton and Zuk hypothesis intraspecifically during an investigation into the breeding ecology and differential reproductive success of the House Sparrow (*Passer domesticus*). The study was undertaken during the spring and summer of 1994 on a coastal archipelago ('Helgeland') in northern Norway (66.3°N, 5.4°E).

Blood smears were taken from adult and juvenile House Sparrows caught in mist nets. and from nestlings known from our nest visitation schedule to be around 10 days of age. Blood was obtained following brachial venipuncture, smeared, and air dried in the field.

All House Sparrows were marked with an individual combination of a single coded metal ring (issued by the Norwegian Museum of Natural History. Stavanger) and two or three plastic colour rings. During the study. blood smears were also taken from any non-target species of bird accidentally caught while mist netting. To prevent pseudo-replication. a small square was drawn upon the rear of both tarsi of these birds using a permanent marker pen (effective for the duration of the field season. *pers. obs.*). All birds smeared were sexed and aged wherever possible (Svensson 1984).

The blood smears were fixed in 100% ethanol for two minutes, and thoroughly air dried. Smears were stained (after completion of the fieldwork) in Giemsa's solution for 30 minutes (strength 1: 10; pH 7.2). The long delay (up to three months) between fixation and staining did not appear to affect either the morphology of the blood cells or the penetration of the stain.

Smears were screened microscopically (by IRKS) using a × 40 objective lens for leucocytozoids and haemoproteids. and a × 100 lens under oil immersion for plasmodiids (Weatherhead & Bennett 1991). Nestling smears were scanned for one minute using × 40 power and then examined for an arbitrary period of five minutes using × 100 power (modified from van Riper et al. 1986). Smears from adults were examined for five min-

utes under each magnification. The number of fields examined during five-minute periods averaged 30 using × 40 power. and 80 using × 100 power. Davidar and Morton (1976) considered these levels to be sufficient to classify smears as negative.

At least one blood smear was examined from each of the following birds: 452 House Sparrows (of which 294 were nestlings), eight Starlings (*Sturnus vulgaris*), three Fieldfares (*Turdus pilaris*), two Redwings (*Turdus iliacus*). 12 Redpolls (*Acanthis flammea*), seven Pied Wagtails (*Motacilla alba yarelli*). five Willow Warblers (*Phylloscopus trochilus*). five Wheatears (*Oenanthe oenanthe*). two Red Crossbills (*Loxia curvirostra*). five Meadow Pipits (*Anthus pratensis*). two Rock Pipits (*Anthus spinoletta*). 39 Bramblings (*Fringilla montifringilla*) and one Blackcap (*Sylvia atricapilla*).

No haematozoa could be detected.

As the study was carried out during the host's breeding season. blood parasite levels. for several reasons. should theoretically have been maximal. Adults may suffer a relapse of chronic infections due to the energetic costs of reproduction (Atkinson & van Riper 1991) and/or the immunosuppressive effects of sexual hormones (Folstad & Karter 1992). Vector populations and thus host exposure also increase during the summer (Møller 1994). Hence. the inability to detect haematozoa in breeding birds suggests that Helgeland birds are genuinely uninfected by these parasites.

Published studies reporting an absence of haematozoa are relatively rare (e.g. Figuerola et al. 1996. Rytkönen et al. 1996. Gonzalez-Solis & Abella 1997). although this almost certainly underestimates the number of studies which produced negative results. Three possible scenarios have been proposed to explain negative records.

Earlé and Underhill (1993) attributed the absence of blood parasites in waders (Charadriiformes) breeding on the Arctic tundra to insufficient evolutionary time for the host–vector–parasite cycle to become established.

This would not explain the absence of haematozoa in Helgeland. It may be significant that the House Sparrow has followed an unnatural process of colonisation. spreading northwards into this region during the 19th century following several mid-European introductions (Summers-Smith

1988). However. the other passerine species sampled here are not recent additions to the local avifauna. and there is no reason to presume only a brief evolutionary association with this habitat. Furthermore, Eide et al. (1969) recorded considerable prevalences of haematozoa from several of these bird species on mainland Norway.

A subsequent failure to detect haematozoa in the Kentish Plover *Charadrius alexandrius* in southern Europe. led Figuerola et al. (1995) to suggest waders in general may possess an inherently high resistance to haematozoa. irrespective of breeding latitude.

With the exception of the Starling. however, haematozoa have been recorded from each of the species sampled here (Peirce 1981). including the House Sparrow. which would imply that none possess any particular species-specific freedom from blood parasitism.

Bennett et al. (1995) examined the haematozoan communities of three populations of a single host species. the pied flycatcher. Parasite arrays varied considerably between populations. with a notable absence of *Leucocytozoon* and *Trypanosoma* at one site. This was attributed to a lack of local breeding sites for the appropriate vector.

A deficiency of vectors is unlikely to account for the absence reported here. Regarding House Sparrows on Helgeland specifically. haematophagous mites (Acari: Acarina). fleas (Insecta: Siphonaptera) and flat-flies (Diptera: Hippoboscidae) were regularly found both on the birds and in their nests. Strong evidence (from other host species) indicts both fleas (Allander & Bennett 1994) and hippoboscids (Baker 1975) as vectors of avian haematozoa. and mites could equally plausibly act in such a capacity (Greiner et al. 1975).

Furthermore. despite the northern latitude. the archipelago is reasonably lush in vegetation. with diverse habitats such as clear running streams, wooded marshes and grassland ponds. These would be ideal development sites for the larvae of blackflies (Diptera: Simuliidae). mosquitoes (Diptera: Culicidae) and midges (Diptera: Ceratopogonidae) (Greiner et al. 1975). The summer climate is mild. and the abundance of human-biting midges suggests environmental variables *per se* do not preclude the presence of volant insect vectors.

Therefore. none of the three main hypotheses seems to explain the apparent freedom from

haematozoa reported in Helgeland passerines. This report adds to the burgeoning literature on avian haematozoa, and emphasizes the warning of Bennett et al.(1995) that haematozoan infections are not a property of the species alone.

Selostus: **Veriloisien esiintyminen Norjan saariston varpuslinnuilla**

Kirjoittajat tutkivat veriloisten esiintymistä varpuslinnuilla pesimäaikaan pohjoisnorjalaisessa saaristossa. Verinäyte otettiin yhteensä 452 varpuselta, joista 294 oli pesäpoikasta, ja 89 muulta linnulta 12 lajista. Yhtään veriloista ei tässä tutkimuksessa löydetty. Kirjoittajat käyvät läpi mahdolliset syyt loisien puuttumiseen. ja toteavat. ettei yksikään aikaisemmin kirjallisuudessa esitetty hypoteesi päde tähän tutkimukseen. Veriloisia on löydetty lähes kaikilta tässä tutkimuksessa mukana olleiden lajien muista populaatioista. Tämä sulkee pois sen mahdollisuuden, ettei näille lajeille olisi jostain syystä kehittynyt veriloisia ja että kyseiset lajit olisivat vastustuskykyisiä veriloisille. Saarilla esiintyy todistettavasti loisten kantajia, punkkeja, kirppuja. lintukärpäsiä sekä muita verta imeviä kaksisiipisiä. Tutkimuksen tulos tukee käsitystä. että veriloisten infektiot eivät ole lajikohtaisia ominaisuuksia vaan populaatioiden välillä voi olla suuriakin eroja loisten esiintymisessä.

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314

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322

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