Parental Investment and Reproductive Success in the Reed Bunting *(Emberiza schoeniclus)*, Investigated by DNA Fingerprinting

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by

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CONTENTS

5 DETERMINANTS OF MALE REPRODUCTIVE SUCCESS

6 HIGH LEVELS OF EXTRA-PAIR PATERNITY AND PROTECTION OF PATERNITY

Abstract

Parental investment and reproductive success in the reed bunting *(Emberiza schoeniclus)*, investigated by DNA fingerprinting

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1 This study investigated the mating behaviour and parental behaviour of reed buntings *(Emberiza schoeniclus)*, at Rutland Water, Leicestershire. The study integrated behavioural, molecular and anatomical approaches to examine the evolutionary consequences of sperm competition in the species.

2 The frequency of extra-pair paternity (EPP) was extremely high in the study population. 86% (50/58) of broods held at least one extra-pair offspring, whilst 55% (118/216) of young were extra-pair young (EPY). Female participation in extra-pair copulations (EPCs) was virtually ubiquitous at 97% (33/34). Most, i.e., 83% (98/118), EPY were sired by males from an adjacent territory. This pattern of extra-pair paternity was best explained by the indiscriminate female copulatory behaviour associated with the 'genetic diversity' hypothesis.

3 DNA fingerprinting revealed that just over half the males (15/28) breeding in the core study population obtained at least one extra-pair fertilisation (EPFs), and that EPFs accounted for an average of 40% (range 0 - 100%) of a male's reproductive success. There was no relationship between a male's paternity in his own nest and the number of EPFs achieved. Neither was there any consistency in the level of paternity among broods of multiple-brooded pairs. Old males had more EPY in their own nests than young males. There was no relationship between male reproductive success and any of the male phenotypic characters tested in a multivariate analysis.

4 An examination of the paternity protection behaviour of male reed buntings revealed that males did not attempt to expand their territory during the female's fertile period in order to reduce the risk of cuckoldry. There was some evidence of weak mate guarding in the species. It is proposed that the primary paternity guard in the species is through frequent copulations.

5 In the study population, 27% (8/30) of males were polygynous. Males benefit from polygyny through an increased reproductive output on their territories, though not to a significant extent due to a higher level of EPY in secondary nests. Polygynous males fed at only one nest on a territory, thus secondary females incurred a potential cost in terms of increased parental effort.

6 Male and female reed buntings exhibit morphological adaptations associated with a high degree of sperm competition. Males have larger cloacal protuberances, testis and sperm than expected for a bird of comparable size. Females have extremely long sperm storage tubules.

7 Analysis of provisioning behaviour revealed that male feeding rate was significantly related to their level of paternity in the brood. It is proposed that males can assess the confidence of paternity and adjust their feeding rate accordingly. This indicates that there is a potential cost to females of participating in EPCs.

Acknowledgements

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My supervisor Terry Burke allowed me the freedom to develop the project in my own way, but was always willing to provide advice when needed. Over the last year, like me, he has probably become sick-to-death of reed buntings but did eventually manage to battle through a previous draft of this thesis. Loathe as I am to admit it, his comments are usually worth the wait! In addition to his role as supervisor Terry has been a good friend and contributed in no small way to my enjoyment of working at Leicester.

During my three seasons of field work I received help from several people. I inherited the project from Sean O'Malley, who didn't laugh too much when I told him I gave up a good job to study reed buntings. His help in my first year was invaluable. Martin Lester taught me a great deal about bird-ringing and was always willing to help out. My final year of study would not have been so productive without the help of Douglas Ross, who was excellent in the field, despite mistaking a water rail nest for a coot. Good luck with the PhD Doug.

In the Department of Zoology I received invaluable assistance from Douglas Ross with both the video analysis and fingerprinting work. Olivier Hanotte was always willing to advise and give practical help in the alien world of molecular biology lab, and so too were Mike Bruford, Iris van Pijlen, Diogo Tomaz, Tim Robson, Terry Burke, Andy Krupa and Graham Rowe. Thanks also to Elaine and Lesley. Many people have taken the time to discuss ideas and make suggestions throughout my study and I particularly wish to thank the following: Ian Hartley, Dave Curry, Terry Burke, Sean O'Malley, Ian Owens, Mike Double, Douglas Ross, Ian Henderson, and Ben Sheldon. Prof. Tim Birkhead has been particularly helpful, not only for his interest and encouragement throughout my study but also for the loan of an incubator and kindly agreeing to carry out the dissection of reed buntings for me. Thanks.

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Chapter One

INTRODUCTION

1:1 Introduction

Most modern studies in behavioural ecology take an adaptive standpoint, with the assertion that natural selection will favour the genotype which best promotes its genes into future generations, and that individuals are expected to behave in their own selfish interests and not for the good of the species or the group (Dawkins, 1976; Krebs & Davies, 1987).

There are many components which make up overall 'fitness' for individuals. Within each of these components, there are many and varied strategies which individuals can adopt to maximise their fitness. This thesis is an investigation into the behavioural strategies adopted by the reed bunting which contribute to the fitness component of reproduction. This reproductive effort can be further subdivided into mating effort and parental effort (Low, 1978) and individuals should strive to distribute their reproductive effort between these two categories in a way which maximises their reproductive success. Natural selection acts differently on males and females, thus the strategy for maximising reproductive success may not be the same for each sex. The resolution of this sexual conflict is reflected in the evolution of different reproductive strategies exhibited in the various mating systems seen in birds.

Monogamy is by far the most common avian mating system, occurring in 90% of bird species (Lack, 1968). This is the situation where one male pairs with one female in order to reproduce. Lack's hypothesis for the evolution of such a system was that both parents increase their reproductive success by remaining to rear the brood together. However, biparental care is not essential in all species

to rear some offspring (see review in Bart & Tornes, 1989), so any partner which deserts and remates elsewhere will have a higher reproductive success.

It was not until Wittenburger & Tilson (1980) amalgamated several proposed hypotheses on the preconditions necessary for the evolution of monogamy that an explicit 'theory of monogamy' was developed. Thus in species where biparental care is not essential for some reproductive success, additional factors operate, such as: reduced female reproductive success when pairing with a mated male; male-male competition which prevents acquisition of sufficient resources to support more than one female; or that female-female competition prevents a second female from settling.

In passerine birds it is generally the female who has the most to lose by desertion (Trivers, 1972), however, there is only an advantage to a male deserting if he can find another mate (Maynard Smith, 1977). Møller (1986), in a review of the mating systems of European passerines, showed that occasional polygyny was not uncommon in many monogamous species (39% of 122 well studied species). There is, however, another way in which monogamous males can increase their reproductive success. The opportunity for this was first outlined in a seminal paper by Trivers (1972). Trivers presented a model which showed that in species which exhibit some degree of biparental care there will be competition between the sexes to minimise their parental contribution in order to have additional offspring. This gives rise to the conditions where a mixed reproductive strategy will be the optimal course for a male to follow, i.e., in helping a female raise young, while not passing up opportunities to mate with other females whom he will not help. Prior to this it was assumed that the social association between individuals reflected an exclusive mating relationship.

Once the theoretical framework was established, the empirical work followed to investigate the adaptive significance of a mixed reproductive strategy. Most studies have focussed primarily on male mixed reproductive strategies through the pursuit of extra-pair copulations (EPC) (See Birkhead & Møller, 1992a). An EPC is defined as a copulation that occurs when a paired bird copulates with an individual other than its social (pair bonded) mate (Westneat *et al.*, 1990). However, recently more attention has been placed on the females pursuit of EPCs (e.g., Hatch, 1987; Smith, 1988; Birkhead & Møller, 1992a; Kempenaers *et al.*, 1992).

For an EPC to be functionally effective it must result in the fertilisation of an egg. It is virtually impossible to determine by observing a copulation whether

insemination occurs, let alone fertilisation. As a result, the link between behaviour and actual paternity has only been made for a few species using a variety of techniques (reviewed in Birkhead & Møller, 1992a). By far the most accurate measure of parentage can be obtained through genetic fingerprinting (Jeffreys, 1985a, b; Burke & Bruford, 1987; Wetton *et al.*, 1987). Parentage assignments using multilocus DNA fingerprinting have shown the frequency of extra-pair young among bird species to vary between 0% e.g., fulmar *Fulmarus glacialis* (Hunter *et al.*, 1992) to 35%, e.g., indigo bunting *Passerina cyanea* (Westneat, 1990). The drawback of multilocus DNA fingerprinting is that although exclusion of an incorrectly assigned male from paternity is relatively straightforward, paternity inclusion from a large number of possible fathers is very difficult (Burke *et al.*, 1991). Such an analysis is important when answering questions about male reproductive success and female mate choice.

The use of single locus probes allows easier paternity 'inclusion' analysis because each individual has only two bands in its fingerprint, one maternally and the other a paternally derived allele. Although some probes can produce a single locus pattern in some species, e.g., the MHC probe in red-winged blackbirds *Agelaius phoeniceus* (Gibbs *et al.*, 1990) the widespread use of the single locus system has been limited by the unavailability of probes, because they usually need to be cloned from the species under study (Burke *et al.*, 1991a). However, the development of a cloning technique using charomid vectors (Armour et al., 1990) has led to an easier and cheaper way to obtain single locus probes and probes for several avian species have been cloned in this way (see Burke *et al.*, 1991a). The application of such probes to a field study of the blue tit has recently been published by Kempenaers *et al.*, (1992).

The fact that EPCs can result in fertilisation, and so produce extra-pair young, means that any male expending time and energy to help rear a brood containing extra-pair young has been effectively parasitised by the cuckolder. Thus, following the logic of current evolutionary thought, behavioural and physiological mechanisms to prevent such cuckoldry should evolve in tandem with parental care (Trivers, 1972). The two main behavioural mechanisms that have been extensively studied in a range of species are mate guarding and copulation frequency. The former is the term given to the close following of a female by the male during her fertile period to prevent EPCs (e.g., Beecher & Beecher, 1979; Birkhead, 1979; 1982, and see Birkhead & Møller, 1992a). The use of frequent copulation as a paternity guard is most commonly seen in those species

where ecological constraints prevents the possibility of effective mate guarding (Møller & Birkhead, 1991). These two methods of paternity protection are the most widely recognised forms of this behaviour employed by birds, but these are usually only a part of the male's total defensive armoury which will be discussed more fully in the relevant chapters which follow.

Cuckolded males potentially incur a large cost to their fitness if they invest parental care in extra-pair young and so help to propagate an unrelated individual's genes as though they were his own. Though the adage says that 'prevention is better than cure', we may well expect a further suite of behaviours to have evolved by which males can assess their likelihood of paternity in a brood and so adjust their level of parental investment accordingly (Trivers, 1972; Maynard Smith, 1977). To date there have been very few studies which have been able to combine parental investment data with the technology of DNA fingerprinting necessary to identify true parentage, the notable exception being the work of Nick Davies on the dunnock (see Davies, 1992 for review). However, the theoretical basis of how a male should adjust his level of parental care to his level of paternity has not been fully developed and as yet no conclusive empirical evidence of such a response has been found.

1:2 Aim of the thesis

This project follows on from two previous years of field study of the reed bunting by Sean O'Malley at the same study site (O'Malley, 1993). The overall aim was to investigate the reproductive strategies of both male and female reed buntings by examining the mating behaviour and subsequent parental investment of individually marked birds and determining actual parentage of offspring through the use of single locus DNA fingerprinting. The earlier study investigated the mating behaviour of individuals, particularly the pursuit of extra -pair copulations and the behavioural response of males to protect their paternity. Inevitably, there has been some duplication of results between the two studies as they were both brought to a conclusion around the same time. This study, however, provided data in a number of additional areas, and the specific developments and aims were as follows:-

(1) A major aim was the development of single-locus probes which

enabled more efficient identification of extra-pair fathers among a large population of potential cuckolders. It was already known at the start of the study that extra-pair paternity occurred frequently in the reed bunting (S O'Malley, pers. comm.), but it was not known which males were the successful cuckolders. An initial aim was to discover the identity of extra-pair males and discover if some males were more successful than others. This identification of individual parentage was essential for the analysis of phenotypic correlates of male reproductive success.

(2) Reed buntings were known to exhibit polygyny at the study site and the effect of this variable mating system on male and female reproductive success was studied.

(3) The relationship between male parental investment and the level of extra-pair paternity in the brood was investigated using an observational and experimental approach.

1:3 Outline of chapters

The thesis is composed of ten chapters, each presented in the conventional manner of scientific publications. These individual chapters fall into three broad categories, as follows:-

Part One of the thesis (Chapters 1 to 3), provides an introduction to the theoretical and methodological background of the study. Chapter 2 deals with the study species and a description of the field studies, whilst Chapter 3 explains the procedures involved in the genetic analysis of parentage.

Part Two of the thesis comprises Chapters 4 to 8 and deals with aspects relating to sperm competition in the reed bunting. Chapters 4 and 5 investigate why female and male reed buntings engage in extra-pair copulations, respectively. Chapter 6 deals with male adaptations to protect their paternity in their own broods. In Chapter 7, I study the influence of the social mating system on the pattern of extra-pair paternity. Finally, in Chapter 8 I study the physiological adaptations of the species to high levels of extra-pair paternity.

The third and concluding part of the thesis deals with parental investment patterns exhibited in the reed bunting. In Chapter 9, I study the effects of the social mating system on parental investment and in the final Chapter 10, I

investigate the factors which potentially influence the level of parental investment between and within individual birds.

I have decided not to include an overall summary or concluding chapter as the thesis as been organised in such a way that each chapter represents a development of ideas from that investigated in its predecessor. A brief summary of the main conclusions is provided in the abstract.

Chapter Two

THE REED BUNTING, STUDY SITE AND GENERAL METHODS

2:1 The reed bunting

2:1:1 Basic biology

The reed bunting *(Emberiza schoeniclus)* is a small passerine belonging to the subfamily Emberizinae, which is widely distributed throughout the Palearctic region. The Emberizinae originated in the New World and have spread via one or a few colonisations to the Old World, the reed bunting being a member of the only genus that has undergone adaptive radiation (Campbell & Lack, 1985).

Reed buntings exhibit a marked dichromatism and slight size dimorphism between the sexes (Svensson, 1992). Immediately after the post-nuptial moult males superficially resemble females as their characteristic black head and bib feathers are covered by a layer of buff tips which gradually abrade during the breeding season, exposing the black coloration. In a few males a limited pre-

nuptial moult was recorded by Bell (1970). An examination of skins from the national collection at the Natural History Museum, Tring, illustrates this transition clearly (Figure 2.1).

In Great Britain and Ireland the reed bunting has an extremely widespread distribution, reflecting the wide variety of habitats that this species will utilise (Bell, 1969; Sharrock, 1976; Kent, 1964). It is a resident which is mainly sedentary in the winter (Prys-Jones, 1984), often forming flocks with other granivorous species and feeding on arable land in particular. Reed buntings tend to roost communally outside the breeding season and the sex ratio within these roosts tends to be male-biased, possibly due to differential local migration patterns or mortality between the sexes (Bell, 1968; Fennel & Stone, 1976). The equal sex ratio found in this study at Rutland Water at the start of each breeding season suggests that the former rather than the latter reason is more likely.

Male birds return to the breeding areas in late February to early March and establish a territory, the females arriving a little later (Bell, 1968; Ewin, 1977). The pairing period between female arrival on the territory and commencement of laying can be up to two months, as clutches are not initiated until late April or early May. Nests are usually built by the female alone, on or close to the ground and well hidden in rank vegetation. In Great Britain the modal clutch size is five, with a decline as the season progresses, in common with other multi-brooded British passerines (Crick *et al.*, 1993). Incubation lasts around 11 days and is undertaken mainly by the female. The young are usually fed by both adults and remain in the nest for up to 9 days, leaving before they are capable of flight. Genuine second broods after a successful nest are not uncommon, but repeat laying after predation more commonly occurs. Sometimes many repeat clutches are necessary before one is successful.

2:1:2 Previous studies on the species

One of the earliest workers who studied the breeding biology of the reed bunting in detail was Eliot-Howard, who used the species to illustrate his original and seminal theories on territoriality (Eliot-Howard, 1929). This work was particularly innovative in its interpretation of behavioural data and his explanations for such behaviour were highly perceptive and many are still accepted today.

Very little detailed research was published on the species up until the late

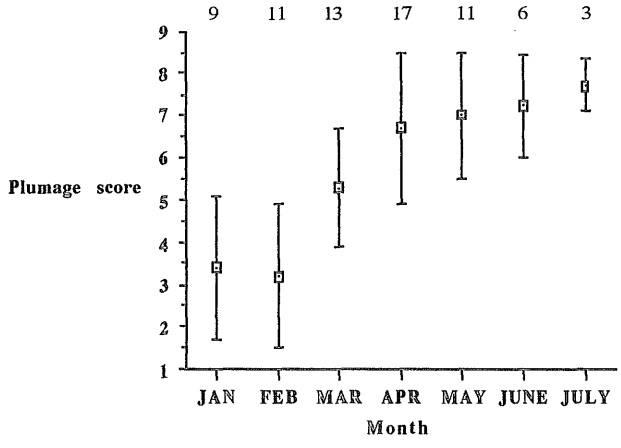

Figure 2.1: Seasonal variation in male plumage score, measured from skins at the Natural History Museum, Tring. Plumage score refers to the amount of visible black on the head and bib feathers, the highest score being the darkest bird. Values shown represent the number of birds scored in each month and bars show standard deviations.

1960's when two research students undertook consecutive studies on the species at Attenborough Nature Reserve in Nottinghamshire. Both investigations were principally descriptive studies of reed bunting demography (Bell, 1968; Hornby, 1971). These studies provide a comprehensive discription of the basic breeding ecology of the species in the East Midlands of Britain (but also see O'Malley, 1993).

In line with the gradual shift of emphasis in behavioural ecology, later studies concentrated on behaviour at the level of the individual rather than the population. Ewin (1977) undertook a detailed study of the song output and repertoire of the species at a study site in Rye Meads, Hertfordshire. This work primarily focussed on the seasonal variation in song and its role in the reproductive cycle.

Finally, the most relevant research to this study was that completed by O'Malley at Rutland Water, Leicestershire. His study was initiated in 1988 and served as a baseline from which the present project was developed. In his thesis (O'Malley, 1993), he investigated the reproductive behaviour of the reed bunting through the combination of intensive behavioural observation and multilocus DNA fingerprinting. The findings and conclusions drawn from his study are discussed in the relevant chapters which follow. In addition, he provided a review of the breeding habits of the species at Rutland Water, which is not therefore repeated here.

2:2 Study site

The field work was carried out from April to July in 1990-92 inclusive, at the Rutland Water Nature Reserve, near Oakham, Leicestershire (SK8908).The reserve is entirely man-made and owned by Anglian Water but managed by the Leicestershire and Rutland Trust for Nature Conservation. The area has been designated as a Ramsar site and Special Protection Area in recognition of its status as a major international wildlife sanctuary.

The core study site comprised an area of approximately 35 ha surrounding Lagoon III (Figure 2.2). The area was relatively undisturbed with limited public access and included a variety of habitat types, which are listed below. Most of these habitats were created artificially when the reserve was set up in 1976 :-

(A) Grazed grassland: short, sheep grazed and mowed grass.

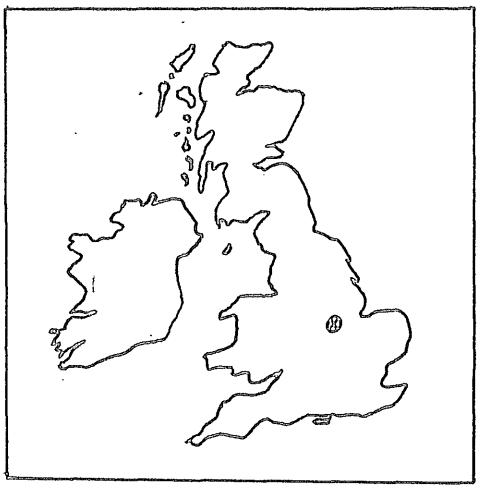

Figure 2.2a: Location of Rutland Water in the British Isles

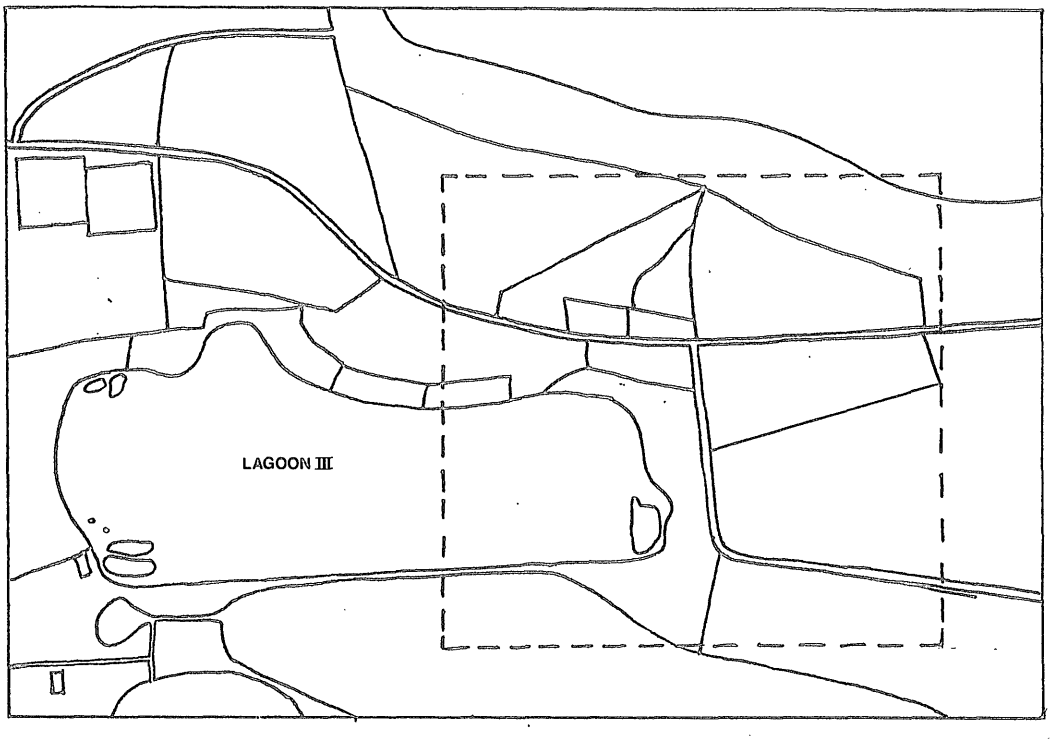

Figure 2.2b: Detail of area surrounding Lagoon III of the Rutland Water Nature Reserve. Core study site is indicated by dashed line.

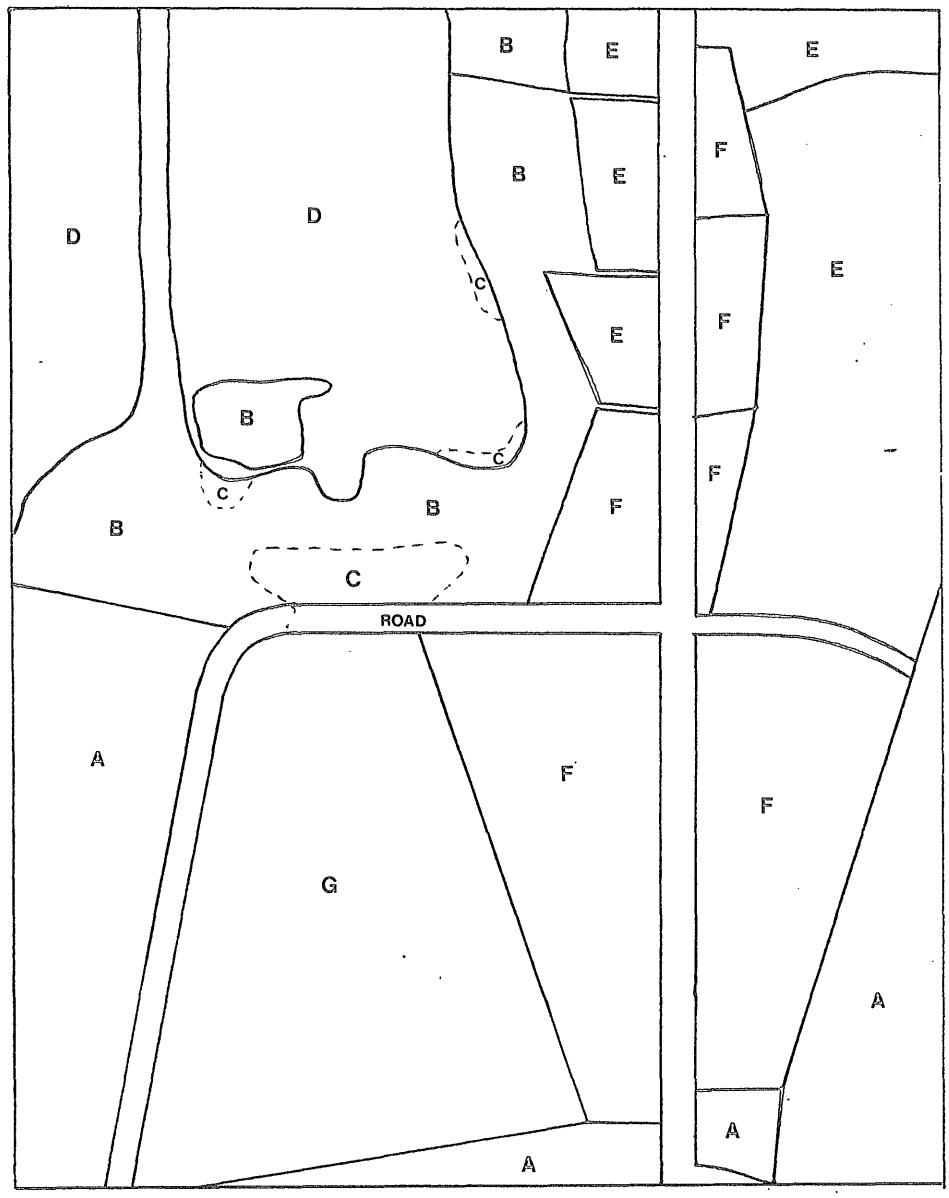

Figure 2.2c: Habitat characteristics in core study area. (A) grazed/mown grassland; (B) marsh meadow; (C) reedbeds; (D) open water; (E) closed canopy plantation; (F) scrub plantation and (G) arable land.

(B) Ungrazed grassland/marsh meadow: overgrown pasture with a vegetation community dominated by hair grasses *Deschampsia* spp., with patches of willowherb *Epilobium* spp., bramble *Rubus fruiticosus* agg., and, in the wetter areas, rushes *Juncus* spp.

(C) Reeds: dense beds and water fringe strips of *Phragmites* spp. and *Typhus* spp.

(D) Fen carr: areas of marsh meadow and reeds with the establishment of willow *Salix* spp.

(E) Wooded plantation (closed canopy): wooded areas predominantly 3 to 5 m tall comprising mainly of oak *Quercus* spp., alder *Alnus* spp., cherry *Prunus* spp., poplar *Populus* spp., pine *Pinus* spp., hawthorn *Crategus* spp. and ash *Fraxinus* spp. Typically, such areas had a sparse field layer with patches of bramble.

(F) Scrub plantation (open canopy): sparse plantation areas with all the tree species listed above present but with a much richer field layer of grasses and bramble patches.

(G) Agricultural crops: these varied from year to year but were mainly wheat *Triticum aestivum* and rape *Brassica napus.*

This rich mosaic of habitats provided excellent feeding and nesting habitat for reed buntings.

2:3 Field Methods

2:3:1 Trapping

Birds were caught entirely by means of mist nets and baited cage traps (Potter traps). Details of these trapping methods can be found in Davis (1981). Birds were caught mainly in the early part of the breeding season i.e., March and early April or during the provisioning period for some females. In order to catch specific males, occasionally a decoy (a freeze dried male) and taped song playback were used in conjunction with mist netting on the target territory.

Once caught, all birds were fitted with a British Trust for Ornithology (BTO) metal leg ring and a unique combination of coloured plastic rings to enable individual identification in the field. The birds were processed as outlined below.

2:3:2 Processing birds

All adult birds, but not juveniles, were sexed using plumage characteristics. It was not possible to age adults with any degree of certainty during the spring (Svensson, 1984) so new birds were coded as 4 i.e., 'hatched before the current calender year'.

Biometrics were recorded in the manner outlined in 'The Ringers Manual' (Spencer, 1984). Weight (to the nearest 0.5 g), was measured using a Pesola 50 g spring balance and wing length (to the nearest 0.5 mm), was measured using a butted metal rule (maximum chord, flattened). A Vernier calliper was used to measure the following to the nearest 0.1 mm: tarsus length (from the posterior notch at the intertarsal joint to the front of the tarsal bone with the toes bent down); head length (from the tip of the bill to the back of the nape); bill depth (at the maximum width at the base of the bill); male cloacal dimensions i.e.,width and height.

Blood samples were collected from all adult birds by brachial venipuncture using a small sterile hypodermic needle. The blood was drawn into 50 µl capillary tubes and evacuated into a 1.8 ml microfuge tube containing 500 µl of blood buffer; 50 - 150 µl of blood was taken from each bird. See chapter 3 for full details.

All male birds were given a head and bib plumage score, from 1 to 9, by comparison with a reference set of nine photographs of skins representing varying degrees of 'blackness'. Figure 2.3, shows the male head coloration for each of categories used. The assignments were made by myself alone and were highly repeatable.

2:3:3 Nests

Attempts were made to find all nests before clutch initiation by observation of the female nest building. However, nests can be built extremely quickly (2 days; pers. obs.) and so to increase the chances of actually seeing the female building, nest material (horse-hair from a mattress lining) was prominently placed in territories known to be at the nest building stage. This material was readily used by female reed buntings for lining their nests. Females also occasionally roosted on or near an incomplete nest (pers. obs.), and could be flushed by 'hot' searching suspected areas in the half light before dawn. Similarly incubating birds

Figure 2.3: Series of photographs to illustrate the nine categories used in scoring the degree of blackness in the head and bib plumage of male reed buntings. The lowest score of 1 represents the least black plumage, i.e., with extensive areas of buff feathering. At the other extreme, a score of 9 represents a male exhibiting pure black head and bib feathers with no buff tips.

could be flushed from close range by searching likely areas.

The data for each nest was recorded on a separate record sheet. Nests were checked on a daily basis during the egg-laying stage and all eggs were numbered in sequence order with a permanent ink pen. Nests were checked at 2 - 3 day intervals during incubation to ensure they were still active. Some nests were visited several times a day during hatching in order to obtain the hatching sequence of the chicks, but this could only be achieved in a very few instances.

All chicks were bled after 2 days using a similar procedure to that used on adults, except that blood was obtained by puncturing a vein in the leg. From small chicks only 10 - 30 µl could be obtained. These small chicks were individually marked by toe clipping. Repeat bleedings were later obtained from those 5 and 6 day old young which were not predated by brachial venipuncture, taking 50 - 100 µl of blood. At this age chicks were given a BTO metal ring and a unique combination of coloured plastic rings.

2:3:4 Observations

Behavioural observations were carried out on individual target males in rotation. Daily timetables were drawn up for each male to ensure that all birds were observed at different times of the day. The observations were carried out from vantage points within the territory; generally the same place was used at the start of each focal watch. Timed observations of behaviour were carried out for 30-minute periods, taken from when the bird was initially sighted. If a male was lost during a timed watch, it was recorded as 'unseen' until relocated or the 30 minutes had elapsed. Behaviours were recorded at 30-second intervals during each watch.

The following behaviours were recorded as occuring or not occuring during each 30-second bout: Song; copulations (within-pair & extra-pair); foraging; male-male fighting/chasing and preening. Each flight over 5 m was recorded. When the female was present during a watch, it was noted which sex initiated a flight and whether the mate followed or not. Also, when the location of both sexes was known, the male-female distance was recorded at each 30-second interval.

Parental investment data was collected in two ways. In 1990 nests were watched for periods of 1 h from a hide or car placed about 10 m away from the nest. In 1991 and 1992 data was collected using video cameras placed on tripods

1 - 2 m away from the nest and powered by car batteries. To enable birds to become used to the placement of video cameras near the nest, dummy cameras were placed 10 m away from nests and gradually moved into position day by day during the incubation period. Video tape data was collected for periods between 1.5 - 3 h. The actual data collected was similar in each year and consisted of the number and timing of visits made by the provisioning adult of each sex.

2:3:5 Male removal experiment

A male removal experiment was carried out under licence from the Nature Conservancy Council. This experiment was designed to influence male confidence of paternity by temporary removal during the females fertile period. This involved catching a male during his mate's presumed fertile period. Birds were trapped as described above, placed in a holding cage and supplied with food (mealworms) and water. The birds were detained for exactly 24 h and then released back onto their territory.

2:4 Statistical methods

All statistical tests can be found in Siegel & Castellan (1988) and Sokal & Rohlf (1981). Analysis was carried out using the Statview 512+^{TM} package produced by Abacus concepts, Inc.

Chapter Three

SINGLE-LOCUS DNA FINGERPRINTING

3:1 Introduction

The theoretical work of Trivers (1972) and subsequent empirical work such as that by Bray *et al.* (1975) led to the recognition that social mating relationships are not necessarily reflected in the genetic matings that follow. This meant that techniques for assigning parentage were required to answer fundamental questions regarding individual reproductive success. However, until recently the techniques available to workers studying avian behaviour were limited to just three options, these being polymorphic genetic plumage markers, sex-specific heritable morphological characteristics and allozyme markers. Unfortunately, none of these techniques was totally satisfactory for the tasks for which they were required and this is reflected in the small number of studies on true genetic parentage prior to the advent of appropriate DNA techniques.

The use of genetic plumage markers in parentage anslysis has the

disadvantage that the genetic basis of the polymorphism needs to be understood before any conclusions can be drawn and as such the technique lends itself more readiliy to captive laboratory studies (see Cheng *et al.* 1983; Birkhead *et al.* 1988, 1989). Little work has been carried out on wild avian populations. Grant and Grant (1989) detected a single case of extrapair paternity in the large cactus finch *Geospiza conirostris* by using bill colour polymorphism. The most notable study using plumage markers on wild birds was on a population of lesser snow geese *Anser caerulescens* (Lank *et al.* 1989) which produced an estimate of 2.4% of goslings resulting from extra-pair copulations and 5.6% from intraspecific brood parasitism.

The basis for the use of heritable morphological traits as a paternity marker relies on the fact that if the offspring tend to resemble their true parents, then a lower heritability for males would suggest that a proportion of the young are sired by extra-pair males. This method has been used in wild populations by taking tarsus length (Alatalo *et al.* 1984; 1989; Lifjeld & Slagsvold 1989; Møller 1989; Dhondt 1991; Parkin & Wetton 1991) or wing length (Payne & Payne 1989; Lessells & Ovenden 1989) as the heritable trait. There are, however, a number of important assumptions which have to be made when using this approach, namely that there is (1) no environmental effect on the trait in question, (2) there is no sexual dimorphism exhibited in the trait and (3) that intraspecific brood parasitism is rare. Criticism has also been levelled at the significance of the sex differences found in the heritabilities in some studies (Lifjeld & Slagsvold 1989; Dhondt 1991; Gebhardt-Henrich & Nager 1991). Despite these criticisms there does seem to be general agreement between heritabity estimates of extrapair paternity level and those subsequently found by DNA fingerprinting (Alatalo *et al.* 1984 and Gelter 1989, *cf* Birkhead & Møller 1992a; Lifjeld & Slagsvold 1989 and Lifjeld *et al.* 1991). This approach can only be used at the population level and the assumptions that need to be made mean that any results from such a study need to be evaluated carefully.

Allozyme polymorphisms can be detected using blood or tissue samples and are more frequently employed by population biologists than behaviourists, mainly due to the fact that the heterozygosity shown by many allozymes is too low for efficient parentage analysis. Indeed, in birds the heterozygosity is generally much lower than that of other taxa (Evans 1987). Some enzymes in various species do show a high degree of polymorphism and have been used in a parentage study (e.g., Burke, 1984; Westneat 1987b; Evarts & Williams 1987;

Wrege & Emlen 1987; Sherman & Morton 1988; Price *et al.* 1989). The main problem with this approach stems from the low variability of allozymes in that only a proportion of extra-pair paternity or intraspecific brood parasitism can be detected because some putative parents and actual parents will have similar genotypes; this has to be corrected for to provide a more accurate population estimate (Burke, 1984; Westneat 1987b; Wrege & Emlen 1987).

The use of recombinant DNA technology allows direct analysis of genomic DNA sequence variation by electrophoretic methods analogous to those used for the enzyme polymorphisms already discussed. Quinn *et al.* (1987) used restriction fragment length polymorphism (RFLP) analysis. This technique utilises bacterial enzymes (restriction endonucleases) which cleave DNA at specific polynucleotide sites. Electrophoresis and hybridisation with a radioactively-labelled DNA probe can then be applied to detect polymorphism in restriction endonuclease recognition sites at a single locus. The utility of RFLPs as genetic markers is limited by their low heterozygosity (Burke, 1989). For a given diallelic marker the maximum frequency of heterozygotes in a population is only 50% as it detects only the presence or absence of a restriction site. A technique to detect DNA polymorphism at multiple loci was discovered by Jeffereys *et al.* (1985a), whilst working on the human myoglobin gene. These authors discovered a family of minisatellite sequences (i.e., a region comprised of repeated units of a short sequence) which shared a common core sequence and that this core, when radioactively labelled and used as a probe, detects hypervariable minisatellites at many separate loci (Jeffreys *et al.* 1985a; 1985b). The band pattern produced by autoradiography is unique to an individual (except in the case of identical twins) and thus the term 'DNA fingerprinting' was coined.

The function of the minisatellite core sequence is unknown but it has been suggested that it might be involved in the process of DNA recombination and has therefore been conserved in evolution throughout vertebrate taxa (Jeffreys 1987). DNA fingerprinting has proved to be very useful in parentage studies of wild birds (Burke & Bruford 1987; Wetton *et al.* 1987 and other subsequent studies reviewed in Birkhead & Møller 1992). However, the limitations of the system are that it cannot be used to estimate the relatedness of two individuals with great precision due to background band-sharing levels (Lynch, 1988). In situations where there are large numbers of offspring to analyse and many potential parents, it can be cumbersome and time-consuming. The development of locus-specific minisatellite probes eliminates this problem.

Single locus probes (SLPs) are probes which hybridise to a single minisatellite locus giving a simple two-band autoradiogram pattern and they usually need to be cloned from the species under study (Burke *et al.* 1991a; but see Gibbs *et al.* 1990 and Hanotte *et al,* 1992). Before an efficient cloning system was developed, minisatellite sequences were very difficult to isolate and this was therefore usually only carried out for humans and in organisms of economic importance, an exception being the work of Gyllensten *et al.* (1989) on *Phylloscopus* warblers. The development of a charomid-based cloning system (Saito & Stark 1986; Armour et al. 1990, Hannotte *et al.*, 1992) has led to easier and more efficient isolation of minisatellite loci and has been used to obtain SLPs from several avian species (see Burke *et al.* 1991a). Such a system has yet to be used extensively in studies of wild animal populations but the applications are many and varied and much work can be expected in the future.

This chapter briefly outlines the methodology for DNA fingerprinting and the isolation of SLPs. Details of the properties of the single locus probes and the methods used to score the fingerprints subsequently obtained are presented.

3:2 Methods

The methods used were as described in Bruford *et al.* (1992). Details specific to this study will be described below.

(A) General

3:2:1 Blood sampling procedure

Blood samples were collected and stored in 500 μl of a buffer solution as described in Chapter 1. Two types of buffer were used in the study: 1 x SSC (0.15 *M* NaCl, 15 m*M* trisodium citrate, pH 7.0), 10 m*M* EDTA, pH 7.4 and a Urea based lysis buffer (8 *M* urea; 0.4 *M* Tris-HCl, pH 8.0; 20 m*M* EDTA; 0.5% SDS) (Galbraith, 1989). The former buffer should be frozen as soon as possible after blood collection, whereas the latter can be stored at room temperature for several months and after which it still yields high molecular weight DNA.

3:2:2 Extraction and restriction of genomic DNA

Most of the DNA used in the study was obtained from blood samples. However, in a few cases DNA was extracted from tissue (mainly from dead embryos or starved chicks). Brain tissue was used wherever possible because it was found that the DNA was less likely to have degraded than in other tissues. Tissue samples were first macerated in liquid nitrogen to a fine powder and the DNA extracted in the same way as for blood. The initial proteasing stage was carried out in small volumes using 1.8 ml screw-top microfuge tubes. An appropriate amount of blood/buffer solution containing approximately 25 μl of blood was suspended in 500 μl of 1 *M* Tris-HCl, pH 8.0, 0.1 *M* NaCl, 1 m*M* EDTA, 0.5% SDS with 5 units of proteinase K (Sigma). This was then incubated for 3 h at 55°C or overnight at 37°C, until the solution was homogeneous.

The technique used for DNA extraction from the proteinased solution was based on that given in Sambrook *et al.* (1990) using a phenol/chloroform treatment, again carried out in small volumes using 1.8 ml screw-top microfuge tubes. Once extracted the DNA was dissolved in 500 μl of H_2O overnight and stored at -20°C. The condition of the DNA was assessed by electrophoresis and the concentration of the solution estimated by use of a DNA flourimeter (Hoefer).

3:2:3 Running gels and blotting filters

Approximately 5μg of DNA was digested overnight at 37°C with *Mbo* I restriction enzyme, according to the manufacturer's instructions. The digested DNA, together with a loading buffer comprised of 0.25% bromophenol blue, 0.25% xylene cyanol containing internal marker DNA (*Xho* I-digested lambda DNA, 1 kb DNA ladder (BRL)), was separated by electrophoresis in a 20 x 20 cm, 0.8% agarose gel (Sigma, Type 1) in 10 x TBE running buffer (0.089 *M* Tris, 0.089 *M* Borate, 2 m*M* EDTA, pH 8.8). The progression of the electrophoresis was gauged by the position of the xylene cyanol and bromophenol dyes. The former migrates at approximately the same speed as 4 kb duplex DNA and the latter's migration is equivalent to that of 0.5 kb DNA. The samples were run at 50 V until the bromophenol blue band had just run off the gel, which took about 18 h. Up to 27 samples could be run on a single gel and produce readily scorable fingerprints.

After electrophoresis the gels were washed in 0.25 *M* HCl then in 0.5 *M* NaOH, 1.0 *M* NaCl and finally 1 *M* Tris, 3 *M* NaCl, pH 7.4. This first wash

depurinates the DNA, whilst the second makes it single-stranded, as required for hybridisation. The DNA was then transferred in 20 x SSC onto Hybond-Nfp (Amersham) nylon membranes by capillary blotting. The DNA was fixed to the membranes by exposing them to UV light using a previously calibrated UV transilluminator. The membranes were then ready for hybridisation.

3:2:4 Hybridisation and autoradiography

Approximately 10 - 20 ng of probe DNA was radiolabelled with [α ^{32}P]dCTP. Multilocus fingerprints were obtained by hybridisation of *Mbo* I-digested DNA with the Jeffreys 33.15 probe (Jeffreys *et al.*, 1985) and 3' alpha-globin hypervariable region (Jarman *et al.*, 1986; Fowler *et al.*, 1988). Single locus probes were hybridised overnight at high stringency (65°C) in the presence of reed bunting or house sparrow *Passer domesticus* competitor DNA and labelled 6.6 kb λ *Hin*d III fragement (Bruford *et al.*, 1992) at a concentration of 5 - 10 μg/ml of hybridisation solution (0.5 *M* NaPO4, 1 m*M* EDTA, 7% SDS and 1% bovine serum albumin, pH 7.2). Once hybridisation was complete, the membranes were washed at 65°C in 40 m*M* sodium phosphate, pH 7.2, 1% SDS for 10 min, followed by 0.1 x SSC, 0.01% SDS for 10 min, after which the radioactivity on the membranes was monitored. The last wash was repeated until the radioactivity in the areas where there was no DNA had subsided to background levels. Probed membranes were exposed to autoradiographs for 8 - 120 h in the presence of one or two intensifying screens at -70°C, using either Fuji RX or Amersham MP film.

Filters were stripped prior to reprobing by incubation in 0.4 M NaOH for up to 45 minutes at 45°C. This did not always remove all the radioactivity so membranes were sometimes stored dry until the remaining radioactivity had decayed or reprobed immediately and the 'new' bands identified by overlaying the autoradiogram from the previous probing experiment.

(B) Isolation of single locus probes

3:2:5 Construction of the genomic library

Equal amounts (50 μg) of genomic DNA were pooled from 19 presumed unrelated individual reed buntings (10 males and 9 females) and digested with

*Mbo*I. This enzyme recognises the 4 bp sequence (5' GATC 3') included within the 6 bp recognition sequence of *Bam*HI (5'GGATCC 3'), which is present in the polycloning site of the charomid vector used in the cloning procedure. The highly variable, minisatellite-rich 4 - 16 kb region (determined from a multilocus fingerprint) was collected by electroelution onto dialysis membrane after agarose gel electrophoresis (Bruford *et al.*, 1992). Of the recovered DNA, 200 ng was ligated to 720 ng *Bam*HI-digested charomid vector 9-36 (supplied by the Japanese Cancer Research Resource Bank) at a ratio of 2:1 charomid:insert DNA. A charomid is a type of cosmid vector which is particularly suitable for cloning small DNA fragments.

The recombinants formed were then packaged in vitro using Gigapack plus (Stratagene) packaging extraction following the manufacturer's instructions, and then *E. coli* NM554 bacteria (Raleigh *et al.*, 1988) were infected and titred by serial dilution. These titres were then cultured and plated on petri dishes containing LUA with ampicillin (50μg/ml). The number of colonies produced by each titre allowed an estimation of the total number of recombinants in the packaging mix.

3:2:6 Screening the library

The bacterial culture was plated out to give approximately 200 colonies per plate, and allowed to grow overnight at 37°C. These colonies were then transferred individually into the wells of eight microtitre plates containing 100 μl of LUB, 15% glycerol + 50μg/ml ampicillin. Although the physical process of picking colonies from petri dishes to microtitre plates is tedious and time consuming, it is a worthwhile stage as positively hybridising colonies are much more easily identified and also the plates can be stored at -20°C for periods of up to a year. These colonies were then replicated on to nylon filters (Hybond Nfp) at double density and grown on sterile LUA overnight. The DNA was fixed on the filters in a microwave oven and hybridised as described for multilocus fingerprinting with the 33.15 probe. The positively hybridising clones were then detected by autoradiography.

3:2:7 Isolation of probes

The positively hybridising clones were cultured and the recombinant

charomid obtained as described in Bruford *et al.* (1992). The isolated charomid was restricted with *Sau*3AI and the inserts isolated by electrophoresis in a low melting point agarose gel (Sea Plaque). The size and concentration of these inserts was determined by comparison with a standard marker (λ *Hin*d III) of known concentration. The inserts were then cut out of the gel, labelled and hybridised to membranes containing the DNA of three unrelated reed bunting individuals digested with *Mbo*I, as described earlier. After autoradiography the banding patterns were interpreted. The probes used in this study were named following the nomenclature recommended by Hanotte *et al.* (1991).

3:3 Results

Characterisation of single locus probes

3:3:1 Screening the library and positive recombinants

After size-selection for DNA fragments rich in ministellites, the genomic library constructed contained approximately 2.5 x 10^4 recombinants. The screening of 744 colonies (i.e., eight plates each with 93 colonies) with the multi-locus probe 33.15 resulted in 32 positively hybridising clones, representing only about 4% of the total number of recombinants. Of these 32 positives, 13 were screened for polymorphism. Of these, three recombinants gave a monomorphic or satellite band pattern; two failed to detect any bands at all; four detected two - four bands in each individual (indicating either the presence of internal restriction sites or that they are double locus probes). The remaining four all detected a maximum of two bands, providing useful single locus probes for this parentage study. One vector contained two inserts, both of which detected different loci giving a maximum of two bands. In addition, a single locus probe obtained from chickens *Gallus gallus* (c*Gga*MS2) (Bruford, 1992), was found to detect a single locus in the reed bunting.

Three single locus probes derived from the reed bunting library (c*Esc*MS1, c*Esc*MS2 and c*Esc*MS7) and the probe obtained from chickens *Gallus gallus* (c*Gga*MS2) were subsequently used in the parentage analysis.

3:3:2 Allele frequencies

The number and sizes of the alleles were determined for all the individual

males which bred in the study area over the three-year period ($n = 25$). The allele sizes were determined by comparing their migration distance with that of DNA markers of known length run in the same lane on the gel. In some gels the DNA had migrated further than in others, thus the degree of resolution for the accurate size assesment of alleles differed between gels. To account for this, the degree of resolution for assessing allele size was determined from the gel with the shortest DNA migration distance i.e., the gel with the least separation between the internal size marker bands. Alleles < 6.0 kb in length were therefore measured to within 0.1 kb. Because the separation for larger alleles was less, those in the range 6.0 kb to 9.0 kb could only be measured to within 0.2 kb and for alleles above 9.0 kb the degree of resolution was within 0.5 kb. A table was compiled and the allele sizes recorded for each of the probes used on the individual males. The allele size distributions of the four loci used in this study are shown in Tables 3.1 a-d.

Allele variability and the heterozygosity of the loci detected by each of the probes was estimated according to Wong *et al.* (1987). However, the estimation of allelic variability was more conservative in this instance (because of the use of size bins described above) and was not restricted to comparisons within the same gel (see Table 3.1). The level of allele sharing, s, for the 25 males examined was determined for each of the probes as follows: c*Esc*MS1, $s = 0.12$; c*Esc*MS2, $s = 0.04$; c*Esc*MS7, $s = 0.10$ and for c*Gga*MS2, $s = 0.09$. Once the level of allele sharing between individuals was assessed the mean allele frequency, q, and the heterozygosity could be calculated as described in Table 3.2.

The mutation rates of the probes were determined by observation of the segregation of alleles in families. The mutation rates for the probes were as follows (the n value in parentheses is the number of offspring tested and they vary because not all offspring were hybridised with each probe): For probes c*Esc*MS1 ($n = 43$), c*Esc*MS7 ($n = 198$) and c*Gga*MS2 ($n = 128$) there were no mutations detected. For probe c*Esc*MS2 ($n = 199$) there were two mutations, both of which involved the maternal allele. This gives a frequency of 0.01 mutations per meiotic event; this rate is not dissimilar to that found in other studies (Jeffreys *et al.*, 1988; Kelly *et al.*, 1989). All excluded offspring mismatched the putative male at more than one loci (unpublished data), thus the chance of false paternal exclusion through mutation was negligible. In addition the vast majority of excluded offspring could be assigned to other males in the population (see Section 4:3:4).

kb	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	1	1	3
5	4	2	1	0	0	1	3	2	3	1
6	0	3	1	0	0	1	0	0	2	1
7	0	0	0	0	0	1	0	0	0	0
8	2	0	0	0	0	1	0	0	0	0
9	0	0	0	1	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0

Table 3.1a: Allele size frequencies detected by probe c*Esc*MS1.
The level of allele sharing (*s*) between 17 presumed unrelated males was calculated as 67/561 = 0.12.

	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	1	0	0	0	1	0	1	0	0
4	2	0	1	2	0	1	1	1	1	0
5	1	1	0	2	0	0	1	0	0	1
6	1	0	1	2	1	0	1	1	1	0
7	1	1	0	0	0	1	0	0	0	0
8	0	0	2	1	0	1	0	0	0	1
9	0	2	0	0	0	0	0	0	1	1
10	0	1	0	0	0	2	0	0	0	0
11	2	0	0	1	0	0	0	0	0	0
12	1	0	0	0	0	3	0	0	0	0
13	3	0	0	0	0	0	0	0	0	0
14	1	0	0	0	0	0	0	0	0	0

Table 3.1b: Allele size frequencies detected by probe c*Esc*MS2.
The level of allele sharing (*s*) between 26 presumed unrelated males was calculated as 59/1326 = 0.04.

kb	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
1	0	0	0	0	0	0	0	0	0	1
2	2	0	0	0	1	0	3	5	5	2
3	2	4	2	1	1	0	2	2	1	3
4	0	0	0	2	1	1	1	0	1	0
5	0	2	0	0	0	0	0	1	0	2
6	0	0	1	2	0	1	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0

Table 3.1c: Allele size frequencies detected by probe c*Esc*MS7.
The level of allele sharing (*s*) between 26 presumed unrelated males was calculated as 129/1326 = 0.10.

	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
1	0	0	0	0	0	0	0	0	0	1
2	0	0	0	0	0	0	0	0	0	1
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	1	0	0	1	2	0	3	3	2	2
6	2	1	0	1	2	0	1	2	0	1
7	2	2	2	1	0	2	2	0	2	0
8	1	0	0	1	0	1	0	0	1	1
9	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0

Table 3.1d: Allele size frequencies detected by probe c*Gga*MS2.
The level of allele sharing (*s*) between 22 presumed unrelated males was calculated as 84/9466 = 0.09.

Probe	Allele size range (kb)	Heterozygosity#	Probability of genotype sharing¶	Probability of false inclusion†
c*Esc*MS1§	4.7 - 9.3	94%	7.0×10^{-3}	0.12
c*Esc*MS2*	3.1 - 14.0	98%	7.9×10^{-4}	0.04
c*Esc*MS7*	1.9 - 6.5	95%	4.9×10^{-3}	0.10
c*Gga*MS2@	1.9 - 9.1	95%	4.9×10^{-3}	0.10
Combined	-	-	1.3×10^{-10}	4.8×10^{-5}

Table 3.2: Characterisation of single locus probes.

Heterozygosity was calculated as (1-q), where q is the mean allele frequency derived from the equation $q = 1 - (1-s)^{1/2}$ (Wong *et al.*, 1987).

¶ For a single locus the probability that two unrelated individuals will share the same genotype is given by q^2 (2-q) (Wong *et al.*, 1987), where q is the mean allele frequency.

† For a single locus the probability of false paternal inclusion is given as $2q - q^2$ (Wong *et al.*, 1987). Correct maternity is assumed because conspecific nest parasitism was not observed and no maternal mismatches were detected.

§ Based on the analysis of the alleles of 17 breeding males from the study area.

* Based on the analysis of the alleles of 26 breeding males from the study area.

@ Based on the analysis of the alleles of 22 breeding males from the study area.

Chapter Four

EXTRA-PAIR PATERNITY AND FEMALE PARTICIPATION IN EXTRA-PAIR COPULATIONS.

Abstract

The functional significance of extra-pair copulations (EPCs) from the female's perspective was analysed by testing a suite of predictions borne from hypotheses of the benefits of EPCs to females. An analysis of paternity using single-locus DNA fingerprinting revealed that 55% of offspring (118/216) were extra-pair young and 86% (50/58) of broods held at least one extra-pair young. Female participation in EPCs was virtually ubiquitous at 97% (33/34). Broods of two or more which held at least one extra-pair young were not sired predominantly by the extra-pair male, and neither was there any relationship between within-pair paternity and extra-pair fertilisation, suggesting that females are not actively seeking good genes through EPC. 83% (98/118) of extra-pair young were sired by males from adjacent territories. This pattern of extra-pair young is best explained by indiscriminate female copulatory behaviour associated with the 'genetic diversity' hypothesis. In reappraising the theoretical validity of this hypothesis it is proposed that females possibly benefit from engaging in EPC through the passive selection of high quality males via intraspecific male competition.

4:1 Introduction

The theoretical work of Trivers (1972) led to the recognition that social mating association between individuals need not necessarily reflect an exclusive mating relationship. Subsequent empirical work indicated that this was indeed the case in many avian species previously presumed to copulate only with the social mate (e.g., Bray *et al.*, 1975; Burns *et al.*, 1980; Burke and Bruford, 1987; Wetton *et al.*, 1987; Lank *et al.*, 1989). Mate infidelity in social pairings can occur in two ways. Either member can engage in extra-pair copulations (EPCs), or either member can form polygamous bonds with additional individuals. However, the potential for increasing reproductive success is different for the two sexes, as a mated male may increase his reproductive success by engaging in EPCs with the mates of other individuals, but a mated female cannot do so (although she may gain fitness benefits if there is an advantage to producing a brood sired by more than one male).

Westneat *et al.* (1990), in their review of the ecology and evolution of EPCs, defined an EPC as one that occurs when a paired bird copulates with an individual other than its social (pair bonded) mate. This definition excludes copulations that occur outside or during the process of forming pair bonds, copulations associated with mate switching, copulations between females and auxiliaries in communal breeding and also polyandrous matings and multiple matings in species that do not form pair bonds.

For an EPC to be genetically effective it must result in the fertilisation of an egg, termed an extra-pair fertilisation (EPF). It is impossible to determine by directly observing copulations whether insemination occurs, let alone whether fertilisation occurs; the link between behaviour and the genetic result has only been made for a relatively small number of species using a variety of techniques. DNA fingerprinting (Jeffreys *et al.*, 1985a; 1985b; Burke and Bruford, 1987; Wetton *et al.*, 1987) has proved to be the most powerful tool in assigning parentage in birds, the information provided being more precise and comprehensive than that prviously available through allozyme studies or other methods (see section 3:1 for further details).

In recent years DNA fingerprinting has been applied to an ever increasing range of bird species from several genera (see review in Birkhead & Møller, 1992a), though studies combining behavioural data with fingerprinting are still few in number (e.g., Burke *et al.*, 1989; Westneat, 1990; Morton *et al.*, 1990; Birkhead *et al.*, 1990; Graves *et al.*, 1992; Smith *et al.*, 1991; Jones *et al.*, 1991;

Lifjeld *et al.*, 1991; Hunter *et al.*, 1992). However, the ability to identify extra-pair fathers using 'traditional' multilocus DNA fingerprinting is limited, especially where the number of potential fathers is high. The development of single-locus probes has made the procedure of paternity inclusion much simpler where there are a large number of potential sires (Burke *et al.*, 1991b). The technique of single-locus DNA fingerprinting has already been applied to paternity studies of wild birds (Gyllensten *et al.*,1990; Gibbs *et al.*, 1990) and studies combining paternity and behavioural data (Kempenaers *et al.*, 1992).

Research into the phenomenon of EPCs has, until recently, been biased towards the more obvious benefits to the male of increased reproductive output. However, there are potential costs and benefits for females which engage in EPCs, which in turn will affect the likelihood of success for males (Halliday & Arnold, 1987; Sherman & Westneat, 1988). The functional purpose of extra-pair copulations by females is likely to vary between species, and proposed hypotheses fall into two broad categories: genetic benefits and non-genetic benefits.

(1) GENETIC (INDIRECT) BENEFITS TO FEMALES ENGAGING IN EPC

(a) *'Good genes'*. Females may benefit from EPCs if the genes they obtain through EPCs are better than those of their mate. Such genes may increase the attractiveness of their sons ('sexy son' hypothesis; Weatherhead & Robertson, 1979) or general viability of their offspring (Zahavi, 1975. 1977; Hamilton & Zuk, 1982). The good genes hypothesis relies on the assumption that females benefit from mating with phenotypically superior males. If this applies then females might benefit from an EPC if the male to whom they are paired is not the best one available. Factors such as site fidelity, female choice for territory quality and a limit to the numbers of females able to settle on a territory can result in an uncoupling of the link between female choice of breeding site and phenotypically preferred males (Wittenburger, 1981; Searcy, 1982). Thus not all females can settle on the territory of the best male and most have to 'make do' with relatively poorer quality males. Such females may possibly increase their fitness by surreptitiously copulating with males which are of better quality than their mate whilst maintaining a pair bond with the latter to help with rearing the resultant brood. The good genes hypothesis has been put forward as a possible explanation for the pursuit of EPCs by female blue tits *Parus caeruleus*

(Kempenaers *et al.*, 1992).

(b) *Genetic variability.* In an uncertain environment increased genetic diversity of offspring may increase the likelihood of some surviving and reproducing (Williams, 1975; Gillespie, 1977; Rubenstein, 1982). However, there are no data to support this in birds and intra-brood competition would in theory work against any fitness gains acquired through genetic variation (Sherman, 1981). Benefits through increased genetic variation might be additional to the benefits of good genes. Hamilton & Zuk (1982) proposed the situation where females preferentially choose to mate with males in good condition, which may be a reflection of their resistance to parasites. If male resistance to pathogens is not reflected by any visual or behavioural signal then females may increase the chance that some of their offspring might be resistant to them by undergoing multiple matings (Sherman *et al.*, 1988).

(c) *Insurance of fertilisation.* Females may ensure that they have some reproductive success through participating in EPCs even if their pair mate is sterile (Walker, 1980; Gibson & Jewel, 1982; McKinney *et al.*, 1984). This hypothesis depends on the occurrence of sterility or temporary infertility among males. There are few data available on sterility rates in wild birds, but it is probably very rare because genes that prevent infertility will be under strong positive selection.

(2) NON-GENETIC (DIRECT) BENEFITS OF EPC TO FEMALES

(a) *Access to foraging areas.* Females may participate in EPCs in return for the right to forage in the extra-pair male's territory (Cronin & Sherman, 1976; Wolf, 1975). Females may forage in an extra-pair male's territory prior to egg laying when their energy requirements are high in forming the clutch. Alternatively, females may trade-off EPCs for foraging rights later in the breeding cycle when demands for food to provision nestlings and fledglings are high.

(b) *Paternal investment.* Females may benefit from increased nest defence or help in rearing the brood if extra-pair males have some chance of paternity in the brood. There is no published evidence from any socially monogamous species to suggest that this occurs, but it may act as an insurance if

the social mate dies or deserts.

(c) *Mate appraisal.* Females of long lived species may use EPCs as a means of appraising the suitability of a male for future pairing (Gibson & Jewel, 1982; Wagner, 1991; Heg *et al.*, in press).

Each of these hypotheses as to why females engage in EPCs has a set of predictions, which when tested can provide an indication as to the relative merits of each hypothesis in the species under examination. There are five predictions as to the pattern of paternity that would be exhibited in the reed bunting for each of the hypotheses of the functional significance of extra-pair copulations. These are presented in Table 4:1.

Table 4:1

	Prediction				
Hypothesis	EPC results in EPF	Most broods should have some EPY	EPY majority in broods with EPY	Relationship between WPP & EPF	EPF achieved mainly by neighbours
Genetic benefits					
Good genes	YES	YES	YES	YES	NO
Genetic diversity	YES	YES	NO	NO	YES
Fertility insurance	NO	NO	YES	YES	YES
Non-genetic benefits					
Foraging rights	NO	NO	NO	NO	YES
Paternal investment	YES	NO	NO	NO	YES
Mate appraisal	NO	NO	NO	NO	NO

Extra-pair copulations must result in fertilisation if the female is to gain any genetic benefit, though the 'fertility insurance' hypothesis only requires the female to be inseminated by the extra-pair male without necessarily being fertilised by him. If there were non-genetic benefits to be accrued from paternal investment by the extra-pair male one might expect males only to invest in circumstances where their chances of paternity are high, resulting in selection for fertilisation.

If there are genetic benefits to be gained from EPCs, the prediction is that the majority of females will engage in EPCs and most broods should have some extra-pair young. In a population of territorial, socially monogamous birds, the majority of females will not be paired to the best male in the area. Thus if the good genes hypothesis is true, one might expect the majority of females to

engage in EPCs with males of higher quality than their own mate. Likewise, the genetic diversity hypothesis also predicts that the majority of broods will hold some extra-pair young as mixed paternity confers a fitness advantage over genetic monogamy.

There are conflicting predictions between the 'good genes' and 'genetic diversity' hypotheses at the level of extra-pair young within broods. The good genes hypothesis proposes that females actively choose mates of better quality than their own, so predicts that in broods where the female was known to engage in EPCs (i.e., broods which contain extra-pair young), the majority of the offspring should be sired by the extra-pair male. This assumes that the female has control over her mating decisions, however, these can be constrained by male paternity protection behaviour (see Chapter 6). Genetic diversity relies on quantitative genetic benefits rather than different quality genes, thus one can predict that broods with extra-pair young should not be sired predominantly by a single extra-pair male.

Similarly, in the good genes scenario, one can predict a relationship between the paternity in a male's own brood and the number of extra-pair fertilisations he achieves because high quality males should be preferred by their own mates as well as by other females, resulting in a positive correlation between within-pair paternity (WPP) and extra-pair fertilisations.

The prediction that extra-pair paternity will be achieved predominantly by neighbours follows from the hypotheses of genetic diversity and fertility insurance in that one might expect a female to engage indiscriminately in EPCs with the nearest available extra-pair male. The good genes hypothesis, on the other hand, predicts that females should choose to engage in EPCs with selected individual males rather than with their immediate neighbour.

4:2 Methods

4:2:1 Parentage analysis

For general fingerprinting methods see Chapter 3.

All offspring and their putative parents were run on the same gels whilst immediate neighbouring males were also included on the same gel if there were enough lanes available in which to run them. All maternal alleles in the offspring

were identified by direct comparison with the mother's profile and the inferred paternally-derived alleles were compared to the genotypes of all breeding males on the study area to identify fathers. In the single case where the female was not caught and sampled the maternal genotype was deduced by checking the offspring alleles against the males in the population. It was concluded that those that did not match with any male must have been maternally derived and that all chicks had the same mother. All other females breeding in the area were sampled and could be excluded as possible mothers in every case. In the one instance where a breeding male was not sampled, all the inferred paternal alleles were compared with all the other males' genotypes in the population (all other breeding males in the area were sampled), and those which could not be assigned to any male were assumed to be derived from the unsampled attendant male. There was one case where an unmated, territorial male was not sampled, but was inferred to be the father of four extra-pair young in two neighbouring territories in 1992. All other breeding males in the population were sampled and could be excluded in the paternity analysis.

4:2:2 Patterns of paternity within broods

In addition to variation between broods, the pattern of extra-pair paternity can also vary within broods. In this analysis the ratio of extra-pair young to within-pair young in broods of two or more, which contained at least one extra-pair young, was examined.

4:2:3 Within-pair paternity versus extra-pair paternity

The relationship between within-pair paternity of a male's own brood and his success at obtaining extra-pair fertilisations was examined in the following ways:

(A) The actual numbers of both within-pair young and extra-pair young per male were compared. This method may be affected by variation in the number of offspring in a male's own brood and the number of neighbouring offspring that were sampled. In order to counter this quantitative effect, a second analysis was also performed as follows.

(B) The proportion of within-pair young in the brood was compared with the proportion of extra-pair fertilisations obtained in relation to the number of

neighbouring offspring sampled. This method is also influenced to some extent by sample size, particularly in the measurement of within-pair young, because in small broods the proportion of within-pair young is greater than that found in larger broods with the same actual number of within-pair young. The two measurements of paternity success were compared using the Spearman rank-order correlation coefficient (r_S).

4:2:4 Spatial distribution of extra-pair paternity

The distribution, size and shape of individual male territories in each of the years of study were plotted from male movements recorded during behavioural observations (see section 5:2:1 for details). For the purpose of clarity, the maps were simplified to exclude any overlap among territories. The number of young sampled and the number of young sired by the territory owner were recorded for each territory, together with the location and number of extra-pair young sired. Males which returned in subsequent years are colour-coded, and the two returning females are labelled A and B.

4:3 Results

4:3:1 Frequency and distribution of extra-pair young in broods

The results of the parentage analysis showed no evidence of intra-specific brood parasitism by females, but revealed a very high degree of extra-pair paternity. Figure 4:1 shows a representative example of a single-locus DNA profile obtained in the reed bunting. In the paternal inclusion analysis, the identity of the sire was ascertained for 203/216 young. Of the young whose fathers were unidentified, four were probably sired by the unsampled male which reared them in the 1990 season, and four were probably the extra-pair young of an unsampled, unmated male in the 1992 season. The remaining five chicks with unidentified fathers were definitely not sired by territory-holding males in the study area, and all occurred in the 1990 season when the breeding density was highest. In these five young, from two different nests, the number of different alleles ($N = 4$) detected by probe c*Esc*MS2 suggests that at least two males were responsible for siring the unassigned young which were not accounted for.

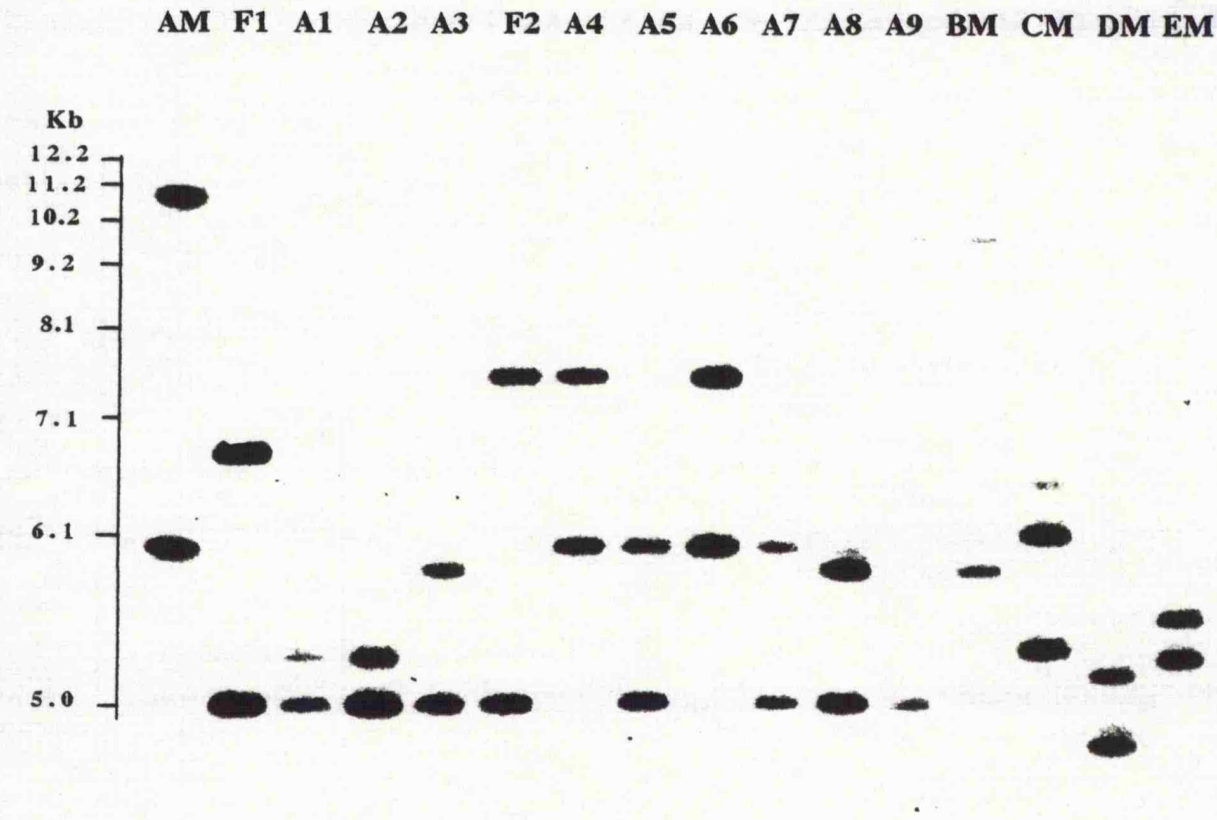

Figure 4:1. Single-locus fingerprint analysis of two broods of a polygynous reed bunting, male (AM) paired with two females (F1 and F2), using probe c*Esc*MS1. Female F1 was the mother of chicks A1 to A3, all of which were sired by extra-pair males. Female F2 was the mother of chicks A4 to A9, of which chicks A8 and A9 were sired by an extra-pair male. BM, CM, DM and EM were neighbouring males. Male BM sired extra-pair chicks A3, A8 and A9, whilst A1 and A2 were sired by male DM.

The results of the paternity analysis are summarized in Table 4:2. Over the three years of study, 55% (118/216) of young were extra-pair. This supports the prediction of the 'good genes', 'genetic diversity' and 'paternal investment' hypotheses, that EPCs should result in fertilisation.

The results of the parentage analysis also show that 86% (50/58) of broods contained at least one extra-pair young and that 97% (33/34) of females laid clutches which contained at least one extra-pair young, indicating that participation in EPCs is virtually ubiquitous amongst females. This supports the predictions of hypotheses proposing genetic benefits to females in engaging in EPCs, but not proposing direct benefits.

4:3:2 Patterns of paternity within broods

The distribution of extra-pair young within broods is given in Table 4:3. Only 8 out of 56 broods had two extra-pair fathers and no broods had three extra-pair fathers. A larger proportion of smaller broods was sired completely by extra-pair males than that seen in larger broods (Table 4.3). Smaller broods are a consequence of several factors, including partial sampling due to degradation of DNA (mainly from dead embryos and chicks), failure of eggs to hatch and, to a lesser extent, predation and starvation of chicks. Partial sampling is a possible explanation for this ambiguous result because extra-pair young in smaller samples will represent a larger proportion of the offspring than they would in a larger brood. For example, in a series of 10 clutches of five eggs with three extra-pair young in each clutch, the proportion of clutches comprised completely of extra -pair young would increase with decreasing sample size if eggs were removed randomly.

To determine whether or not extra-pair young made up the majority of offspring, broods of two or more young which held at least one extra-pair young were compared. There was no significant difference between the number of extra-pair young observed and that expected ($G_3 = 2.55$, $p = 0.49$). This contradicts the prediction of the 'good genes' hypothesis, in that a disproportionate number of extra-pair young would be expected in broods with extra-pair young.

	Frequency of extra-pair young		Proportion of females
	Chicks	Broods	obtaining EPY
1990	58% (57/98)	92% (23/25)	100% (17/17)
1991	45% (18/40)	70% (7/10)	88% (7/8)
1992	55% (43/78)	87% (20/23)	100% (9/9)

Table 4:2. Summary table of results from the parentage analysis.

		Brood size				
		5	4	3	2	1
Extra-pair young	0	0	3	3	3	0
	1	2	2	3	1	1
	2	6	3	4	5	
	3	5	5	3		
	4	2	4			
	5	1				

Table 4.3 Distribution of extra-pair young within broods

4:3:3 Within-pair paternity versus extra-pair fertilisation

(A) Number of within-pair young versus the number of extra-pair fertilisations achieved.

There was no significant relationship between within-pair paternity and the number of extra-pair fertilisations achieved ($r_S = 0.294$, $N = 31$, $P > 0.1$).

(B) Proportion of within-pair young in the brood versus the proportion of extra-pair fertilisations obtained in relation to the number of neighbouring offspring sampled.

There was no significant relationship between the proportion of within-pair paternity and the proportion of extra-pair fertilisations achieved ($r_S = 0.042$, $N = 25$, $P > 0.5$).

These results run counter to the prediction of the good genes hypothesis in that we might expect a positive correlation between within-pair paternity and the number of extra-pair fertilisations achieved by a male.

4:3:4 Spatial distribution of extra-pair paternity

Figures 4:3 a-c show the spatial pattern of extra-pair fertilisations in each year of the study. The fraction in each territory represents the number of offspring sired by the resident male over the number of offspring sampled from all the nests on the territory. Where no fraction is shown, the territorial male was unmated. Arrows indicate the instances of extra-pair fertilisations, the origin of the arrow showing the identity of the cuckolding male and the arrow-head the territory in which he fathered extra-pair offspring. The number in the square represents the number of extra-pair offspring sired by a male. Territories marked with an asterix contained extra-pair young which could not be assigned to any male in the study area.

The vast majority of extra-pair young were sired by males from the immediate neighbouring territory (84%; 98/117), though there were a few instances of males fathering young in territories one removed from their own (10%; 12/117). The remaining seven extra-pair young were not sired by territorial males in the study area. Possibly these fathers were territorial birds from outside the area or non-territorial roving males. Such individuals were occasionally seen and caught in the study area throughout the breeding season. All the unassigned

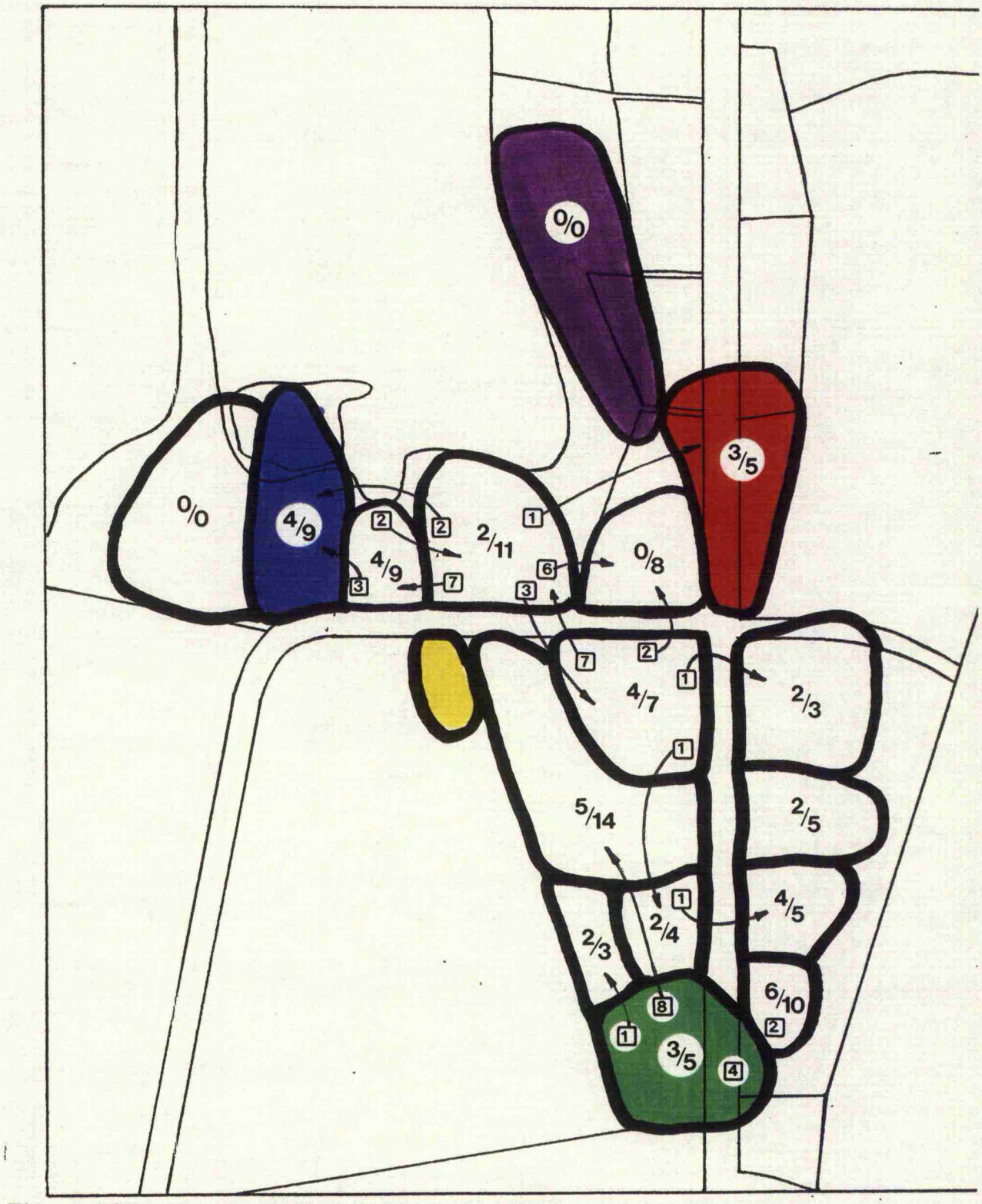

Figure 4.3 a: The distribution of breeding territories and the pattern of paternity during the 1990 breeding season.
2/5 indicates number of young sired over the number of young sampled on a territory. Boxed numbers represent extra-pair fertilisations and arrows indicate the territories in which the extra-pair young were fathered.

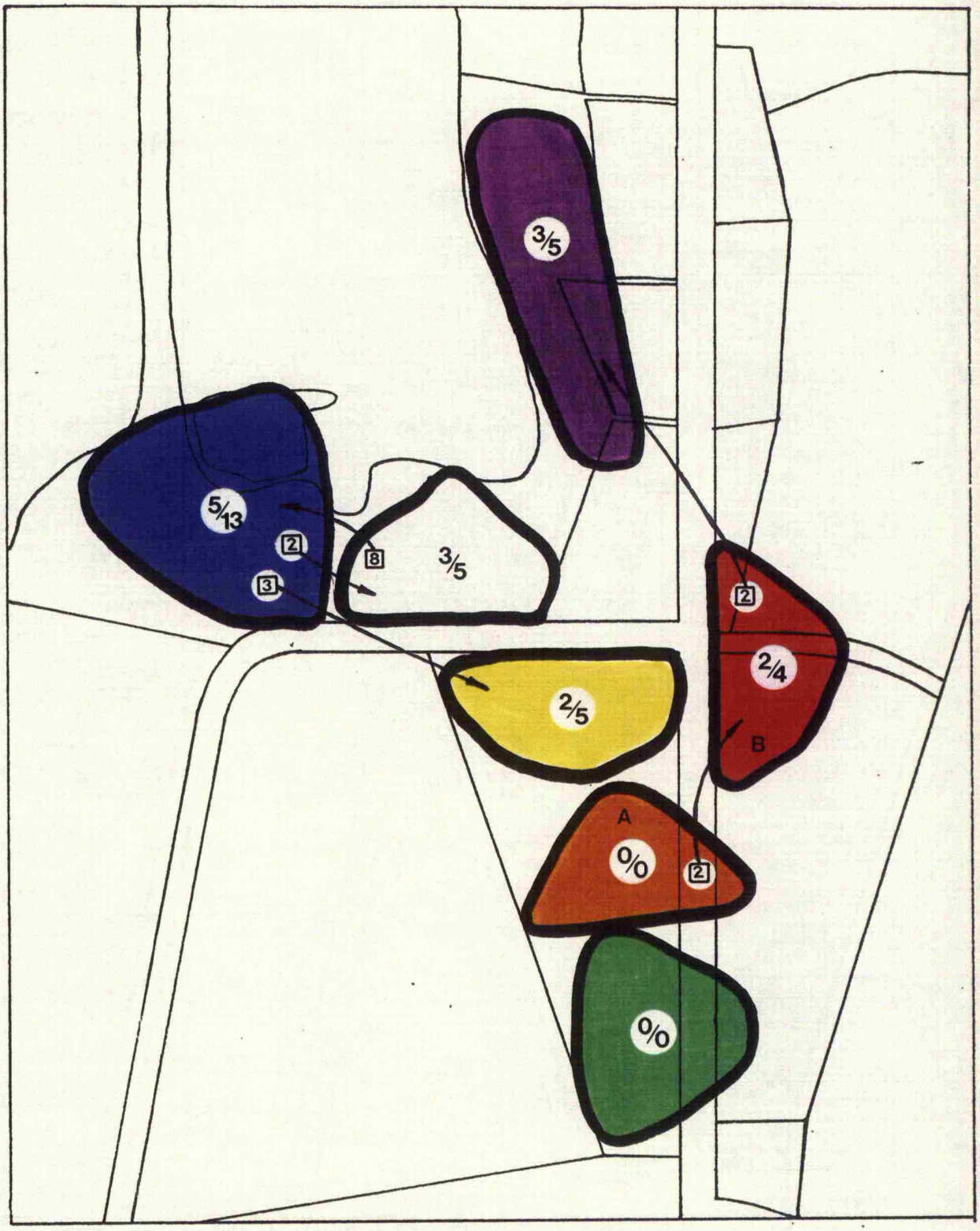

Figure 4.3 b: The distribution of breeding territories and the pattern of paternity during the 1991 breeding season.
2/5 indicates number of young sired over the number of young sampled on a territory. Boxed numbers represent extra-pair fertilisations and arrows indicate the territories in which the extra-pair young were fathered.

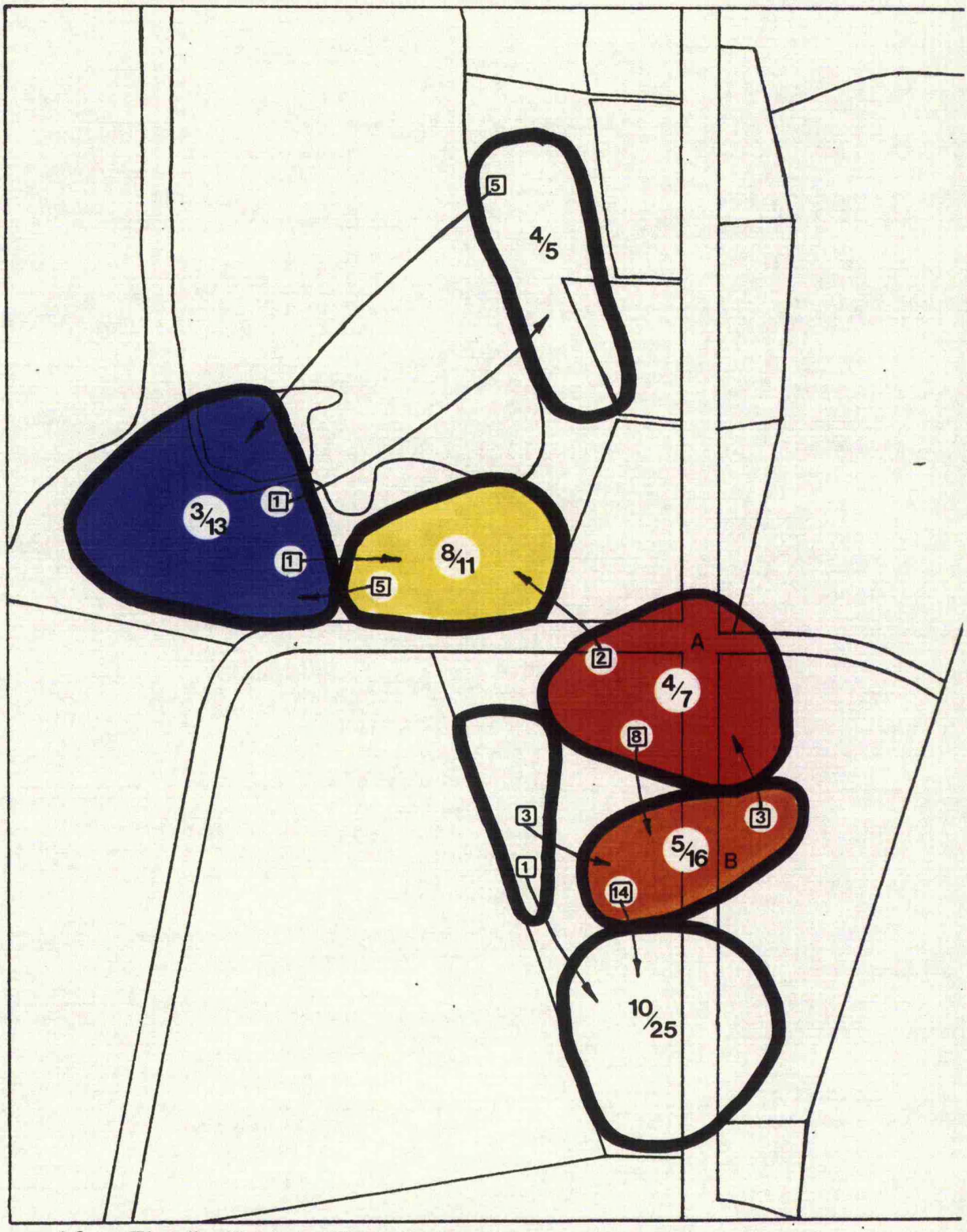

Figure 4.3 c: The distribution of breeding territories and the pattern of paternity during the 1992 breeding season.
2/5 indicates number of young sired over the number of young sampled on a territory. Boxed numbers represent extra-pair fertilisations and arrows indicate the territories in which the extra-pair young were fathered.

young, i.e., those young who were not fathered by local males, occurred in 1990; the year with the highest breeding density. The number of unassigned alleles in these offspring indicated that at least two unidentified males were responsible.

The spatial pattern of extra-pair paternity exhibited in the population is consistent with the predictions of the 'genetic diversity', 'fertility insurance', 'foraging rights' and 'paternal investment' hypotheses, but runs counter to the prediction of the 'good genes' hypothesis.

4:4 Discussion

The benefits to males of engaging in EPCs is plain in that these copulations can result in fertilisation and so increase a male's reproductive success. The extent to which these benefits are affected by potential costs such as loss of within-pair paternity through a reduction in mate guarding intensity or sperm depletion are explored in Chapter 8. Here I investigate the functional significance of EPCs from a female perspective to attempt to answer the question, why do female reed buntings engage in EPCs?

The fact that virtually all females participate in EPCs suggests that either the benefits are very high or the costs are so low as not to negate any small potential benefit. Birkhead and Møller (1992a), in a review of costs and benefits to females, concluded that the most likely cost to females engaging in EPCs is a reduction in male parental care (Trivers, 1972). Recently, theoretical models have been developed to assess under what circumstances cuckoldry will result in reduced paternal care (Whittingham *et al.*, 1992; Westneat and Sherman, 1993).

To date, empirical evidence for such a male response in socially monogamous birds is limited; the only published study to show such a reduction in paternal care (Møller, 1988a; 1991a) has been criticised in its analysis and interpretation (Wright, 1992). However, studies of the polyandrous dunnock provide compelling evidence that the level of paternity plays an important role in determining the degree of paternal care. In this species it has been found that males in polyandrous trios which did not copulate with the female did not provision nestlings, and in situations where both males copulated with the female each male fed the nestlings in relation to their access to the female (Houston and Davies, 1985; Burke *et al.*, 1989; Hatchwell and Davies, 1990; Davies *et al.*, 1992).

In Chapter 10, I provide evidence of a reduction in paternal care in terms of nestling provisioning, in relation to a male's level of paternity. Females do not fully compensate for their mate's reduction in effort, resulting in a reduced overall provisioning rate for broods with extra-pair young. However, this is not translated into a reduced fledging success, as even unassisted females rear a similar number of young to fledging as do male-assisted females (see Chapter 9). It is still possible that some cost is borne to females in terms of the recruitment of offspring into the breeding population, because fledging weights may be less and over-winter survival lowered, or in terms of a reduction in the female's condition and her own future survival and reproduction.

Given that it seems likely that there is some cost associated with EPCs, it is necessary to explore the potential benefits of EPCs to discover their functional significance for females. Table 4.1 is reproduced below as Table 4.4, but with each of the predictions that were supported highlighted in bold type. A problem with this approach is that each hypothesis is not mutually exclusive and that many of the predictions overlap. All hypotheses supported at least one of the predictions, and for three hypotheses there were no positive predictions rejected (i.e., genetic diversity, foraging rights and paternal investment). The relative validity of each hypothesis in the case of the reed bunting is discussed below.

Table 4.4

	Prediction				
Hypothesis	EPC results in EPF	Most broods should have some EPY	EPY majority in broods with EPY	Relationship between WPP & EPF	EPF achieved mainly by neighbours
Genetic benefits					
Good genes	**YES**	**YES**	YES	YES	NO
Genetic diversity	**YES**	**YES**	NO	NO	**YES**
Fertility insurance	NO	NO	YES	YES	**YES**
Non-genetic benefits					
Foraging rights	NO	NO	NO	NO	**YES**
Paternal investment	**YES**	NO	NO	NO	**YES**
Mate appraisal	NO	NO	NO	NO	NO

Direct (non-genetic) benefits

Foraging rights: There was no obvious tendency for females to be seen foraging in the territory of a particular male. Instead, casual observations suggest

that females generally foraged on the territory of their mate or in non-territorial areas such as along the water edge, hedgerows and arable fields (S. O'Malley pers comm.; pers. obs.). This indicates that the exchange of foraging rights for EPCs is not the reason for female participation in EPCs.

Paternal investment: The paternal investment hypothesis also seems an unlikely explanation for female participation in EPCs. The feeding of young by an extra-pair male occurred in only one instance and then was observed on only one day after the female had disappeared (presumed dead). In view of the fact that the number of broods with EPY was so high and that some received no male provisioning at all (i.e., secondary broods in cases of polygyny), one might have expected parental investment from extra-pair males to have been more prevalent if females participated in EPCs to gain male parental help. Additionally, alarm calling by neighbours when nests were checked was not a regular occurrence (pers. obs.), suggesting little attempt at brood defence by extra-pair males.

Mate appraisal: The mate appraisal hypothesis is difficult to test as it produces no positive predictions but is unlikely to be of any importance in a species with such a short average lifespan. In the case where a female (A) and her extra-pair mate (red) returned the following year, the female did switch mates in the direction predicted by the mate appraisal hypothesis, but then engaged in EPCs with the original mate of the previous year.

In conclusion, non-genetic benefits do not appear to offer an explanation as to why female reed buntings participate in EPCs.

Indirect (genetic) benefits

Good genes: The 'good genes' hypothesis requires the female to gain some qualitative genetic benefit from EPCs with males of higher quality than their social partner. The inheritance of fitness through good genes is highly controversial because population genetic theory predicts that natural selection will exhaust the additive genetic variance in fitness (Fisher, 1930; 1941; but see Trivers, 1985; Charlesworth, 1987). In contrast, many behavioural ecologists work on the assumption that behaviour is adaptive and that traits closely related to fitness are heritable in a constantly changing environment.

The suite of predictions generated from the good genes hypothesis were not fully supported, though this could be because female genetic mate choice is constrained by other factors such as mate guarding and direct sperm competition (Birkhead *et al.*, 1990c). However, from the results of this analysis it seems

unlikely that females are actively choosing high quality males for qualitative genetic benefits.

Genetic diversity: The distinction between female choice for good genes and choice for genetic diversity is muddied by the fact that females can get both by copulating with high quality males, either actively though female recognition of male signals of quality or passively via intrasexual male competition. However, as indicated in the introduction the theoretical basis for the genetic diversity hypothesis is unsound (Williams, 1975; Sherman, 1981; Parker, 1984 but see Sherman *et al.*, 1988), yet the indiscriminate female copulatory pattern associated with this hypothesis appears to offer the best explanation for the pattern of extra-pair paternity found in the reed bunting. It may be that the best signal of male quality for a female to recognise is the ability of a male to participate in EPCs. Interspecific competition between males and the allocation of time and energy by males to the pursuit of EPCs would result in males of higher quality winning more male-male contests and having the ability to invest more time in the pursuit of EPCs. Thus, if a male has the ability to be in a position to engage in EPCs with a female, he is of high enough quality for a female to reciprocate.

Chapter Five

DETERMINANTS OF MALE REPRODUCTIVE SUCCESS

Abtstract

Single locus DNA fingerprinting analysis revealed that 15 out of 28 males sired at least one extra-pair offspring, and that extra-pair fertilisations accounted for an average of 40% (range 0 - 100%) of a male's reproductive success. There was no relationship between a male's paternity in his own nest (percentage of within-pair young) and the number of chicks sired outside the territory. The standardised variance in male reproductive success was therefore only 14% higher when based on actual reproductive success (0.77) as compared to apparent reproductive success (0.61). Thus, despite the high incidence of extra-pair paternity there is still a significant relationship between the observed reproductive success and actual reproductive success of males. Stepwise multiple regression analysis of non-phenotypic environmental factors and male phenotypic characters provided some indication that older males had lower levels of paternity in their own nest than younger males, though whether this was due to variation in male behaviour between age classes or due to some interaction with their female partner is not known. Polygynous males reared more young on

their own territories than monogamous males, though this was not reflected in their total reproductive success, probably because of constraints resulting from increased time spent in paternity protection behaviour or some other aspect of maintaining social polygyny. The variation in the distribution of male reproductive success did not differ from that expected by chance and these chance effects may serve to mask the significance of the other variables in natural systems.

5:1 Introduction

The subject of sexual selection is currently much in vogue in behavioural ecology and has witnessed a number of important developments from the theories originally proposed by Darwin (1859, 1871) and later expanded by Fisher (1915, 1930). However, the collection of empirical evidence to support theoretical hypotheses has proved difficult because of the practical problems of separating the forces of natural selection from the forces of sexual selection. In addition, within the field of sexual selection it is necessary to differentiate between selection due to male-male competition and that due to female choice. Recent advances in genetic marker techniques have provided the tools for behavioural ecologists to examine the costs and benefits of behaviour patterns for specific individuals. This technology has proven to be helpful in distinguishing between the various selection forces acting upon individuals.

The ratio of costs to benefits will affect an individual's behaviour, which is itself constrained by the environment which the individual inhabits. Differences in individual behaviour will determine differences in individual success which in turn will drive natural and sexual selection. Sexual selection has been traditionally seen as competition for access to mates but recent studies have shown that sperm competition can result in a discrepancy between social mating and gametic mating. Behavioural differences within and between the sexes in gametic mating behaviour will be expected to contribute to the variance in individual reproductive success, so providing a subtle form of sexual selection.

Sperm competition resulting from extra-pair copulations (EPCs) is well documented in birds, yet techniques which allow parentage to be assigned accurately have only recently been developed and so few studies have been able to test sexual selection hypotheses from the standpoint of EPC behaviour. It appears that the currently most appealing hypothesis to behavioural ecologists is that females choose their gametic mates on the basis of quality, possibly to obtain

'good genes'. There have been many behavioural studies to support this hypothesis and additional evidence from genetic studies comes from studies on the red-winged blackbird *Agelaius phoeniceus* (Gibbs *et al.*, 1990) and the blue tit *Parus caeruleus* (Kempenaers *et al.*, 1992). Alternatively, in purple martins *Progne subis* (Morton *et al.*, 1990), older males increased their reproductive output by forcibly mating with the females of younger males, suggesting a greater degree of male-male competition than female mate choice.

In Chapter 4, I examined the functional significance of EPCs for females. Here I investigate the pattern of paternity exhibited in the reed bunting from the male's perspective in an attempt to answer the following questions. Despite the extremely high incidence of extra-pair paternity, is the observed number of chicks reared related to the actual number sired? Does male reproductive success vary nonrandomly among individuals? If so, why? Three major hypotheses to explain why variance might be found in individual male reproductive success are outlined below.

(A) Female mate choice hypothesis.

Females may seek out EPCs and preferentially mate with specific males which have certain phenotypic characters that convey a superiority over other males (Møller, 1991b). Under such conditions one might expect the evolution of secondary sexual characters that reflect the phenotypic, and possibly genetic, quality of the male. Plumage colouration and badge size are sometimes positively related to dominance status among individuals (Fugle *et al.*, 1984; Jarvi & Bakken, 1984; Møller, 1988b), and as a result can function both as signals of dominance status and as sexual ornaments (Møller, 1990).

(B) Male-male competition hypothesis.

Males may get a disproportionate amount of EPFs because they are in some way superior to other males. For example, a male more efficient at foraging has more time to spend in pursuit of EPCs, or larger males may be able to compete more favourably than smaller males for females. In this scenario the female can be regarded as a passive acceptor of EPCs and though genetic benefits may be gained there is no active female choice of mate. In contrast to the female choice hypothesis, there is no inter-sexual selection for the evolution of plumage indicators of male phenotypic quality.

(C) Environmental variability hypothesis.

Females may mate preferentially with males which have high quality territories, possibly as a trade-off for foraging rights. Alternatively, the

demographic environment may influence individual male reproductive success, possibly through neighbour density.

The female mate choice hypothesis produces a suite of predictions which were examined in Chapter 4, the results of which did not support the theory that females actively seek EPCs with particular males. A corollory of the female choice hypothesis is that male secondary sexual characters, either morphological or behavioural, will most accurately reflect male reproductive success. This is also true of the male-male competition hypothesis, though the signals of male quality will be directed at other males and not at females. In this respect it is difficult to distinguish between the two hypotheses, and any attempt to do so must determine whether or not female mate choice is active or passive. In the reed bunting, behavioural data on EPCs is sadly lacking because of the rarity with which they were seen (see Chapter 8), but there is no evidence to suggest that female mate choice is an active process, i.e., females are not seeking copulations with superior males (see Chapter 4).

Despite this apparent indiscriminate copulatory behaviour of females, intra-specific male competition may still result in the selection of high quality males for EPCs. Thus the male-male competition hypothesis can be examined from the male perspective by investigating the relationship between male phenotypic characters and individual reproductive success. These characters can be physically measured (morphological variation), quantified (behavioural variation) or inferred through female choice (social mating status).

Phenotypic variation in male reproductive success may be influenced to a greater or lesser degree by genetic and environmental factors. It is outside the realms of possibility to determine which is of greater importance in this study, but what can be achieved is a distinction between the importance of male phenotype and other extraneous variables. A strong phenotypic influence on individual reproductive success would lend credence to 'good genes' theories of sexual selection whereas an overriding importance of external environmental and social factors would support theories of neutral, non-additive selection. These non-phenotypic factors can be quantified and related to individual male reproductive success.

Alternatively, the variation in male reproductive success may not be found to be different from that expected by chance. It will be shown here that, despite a relatively powerful data set, there was no association between any of the

variables examined to test the phenotypic and non-phenotypic hypotheses and male reproductive success, and that the differences in male success might be attributable to chance events.

5:2 Methods

5:2:1 Observed reproductive success versus actual reproductive success

The measurement of observed reproductive success was simply the number of offspring sampled on a particular male's territory, whilst the measurement of actual reproductive success was the number of young sired by a male within his own brood and in other broods as EPFs.

5:2:2 Alternative measures of male reproductive success

The following measures of male reproductive success were used in the analyses. Each of the measures of male reproductive success had an inherent confounding factor which needed to be controlled for in the subsequent analyses.

(1) The total number of young fathered (i.e., the number of offspring sired in own brood + extra-pair offspring obtained in other broods). This measure is obviously affected by sampling effort. There was a high degree of variance in the number of offspring sampled between territories within years due mainly to nest predation and also between years due to variable predator control efforts. In addition, each fertilisation is not an independent event, because one copulation can result in the fertilisation of a whole brood (see Chapter 7).

(2) The number of extra-pair fertilisations males achieved. Again, this measure is affected by variation in sampling effort but is particularly influenced by the non-independence of fertilisations.

(3) The number of extra-pair mates. This measure removes the confounding effects of non-independence among fertilisations, but may reduce the resolution of the data set.

(4) The proportion of potential extra-pair fertilisations achieved by the male (i.e., the proportion of all the offspring sampled in neighbouring territories which were sired by the male). This measure reduces the bias due variation in sampling

effort, but is still confounded by non-independence of fertilisations.

(5) The proportion of extra-pair young found within a male's own brood. As for (4), this measure reduces the bias due to variation in sampling effort, but is still confounded by the non-independence of fertilisations.

(6) The number of within-pair young sired by a male. This measure is affected by the same factors which influence the total number of young sired by a male.

(7) The number of extra-pair young fathered by a male. This measure is also affected by the same factors which influence the total number of young sired by a male.

5:2:3 Non-phenotypic factors

Environmental variables which might be expected to influence variance in male reproductive success include climatic conditions during the breeding season, thus year needs to be as a variable to detect any potential influence on male reproductive success. There is a relationship between territory size and population density (Chapter 10) which remains fairly constant within a year. However, within each year, there may be an effect of territory quality which influences male reproductive success. Therefore, using territory size as an independent variable may distinguish within season influences on reproductive success.

The number of neighbouring territories adjacent to a male's own territory is also related to population density, but as in the case of territory size, analysis of the number of adjacent neighbours allows within season influences on male reproductive success to be revealed.

5:2:4 Phenotypic characters

The phenotypic characters investigated fell into three broad groups: morphological, behavioural and inferential. The morphological characters could be measured directly (see chapter 2 for details) and the following were used in the analysis: wing length (mm); weight (g); tarsus length (mm) and full head length (mm). In isolation these characters may not be good indicators of relative male quality because males were caught at different times of the year, which influences the extent of primary feather abrasion and hence wing length, and variation in male weight could be extreme (unpublished data). To counter this, a

formula was used to produce an index of body condition. The formula used was [weight/(wing length)^3] X 100 (after Møller, 1988a). Plumage score was a measure of the relative blackness of the head and bib feathers on a scale of 1 to 9 (see chapter 2). Age could not be measured accurately, but males were classified as either 'young' (= unringed, therefore age unknown) or 'old' (= ringed, thus known to be at least in their second year) (see chapter 2).

Behavioural characters were also analysed because they may reflect male phenotypic quality. Male song was taken as the residual mean 'number of minutes in which song bouts occurred' during 30 minutes' observation Using the residual song data from the second stage of the multiple regression analysis described in section 6:2:1 removed much of the variation in song rate due to non-phenotypic factors. Male feeding rate was taken as the residual mean number of male feeds/chick at its own nest (see Chapter 10). In this case, using the residual data from the first stage of the stepwise multiple regression analysis described in section 10:2 removed the variance in feeding rate due to the age of chicks.

The inferential factors analysed which may reflect male phenotypic quality were the proportion of extra-pair young in a male's own nest and the male's social mating status (i.e., polygamous or monogamous).

5:2:5 Stepwise multiple regression analysis

The separate factors influencing male reproductive success do not act in isolation in the natural world, but act in concert to exert their varying influences on individual males. To account for this combined effect, a stepwise multiple regression analysis was carried out in which male reproductive success could be regressed against all the factors simultaneously. However, in order to separate the non-phenotypic variables from the phenotypic variables, the regression procedure had to be separated along those lines. Thus the first stage of the analysis examined the relationship between male reproductive success against the non-phenotypic factors. In this way, residual values for reproductive success were produced for each male, which were then used in a second stage regression against phenotypic factors. The robustness of the regression results was tested by 'jack-knifing' the variables, i.e., omitting each variable from the analysis in turn to see if significant variables remained in the regression model.

This analysis required the residual data to be normally distributed. The following variables were therefore log $(1+x)$ transformed: number of extra-pair

mates; total number of offspring sired; number of extra-pair fertilisations; number of within-pair young sired by a male; and the number of extra-pair young reared by a male. The following data was arcsin transformed: proportion of extra-pair fertilisations achieved and the proportion of extra-pair young in a male's own nest.

5:2:6 Distribution of male reproductive success

In this analysis I tested whether the variation in male reproductive succes differed significantly from that expected by chance. The best available measure of male reproductive success was the number of females with which males sired extra-pair young as this was the most unbiased of all the alternatives. To undertake this analysis I plotted a simple frequency distribution for the observed male reproductive success and calculated the mean number of extra-pair partners per male. From this mean, the expected frequency distribution was derived (to the nearest whole number) and the observed versus expected frequencies compared by a chi-squared test.

5:3 Results

5:3:1 Observed reproductive success versus actual reproductive success

The DNA fingerprinting analysis showed that 15 out of 28 males sired at least one extra-pair offspring, and that extra-pair fertilisations made up an average of 40% (range 0-100%) of a male's reproductive success. There was no relationship between a male's paternity in his own nest (percentage of within-pair fertilisations) and the number of chicks sired outside the territory (Kendall rank correlation, tau = 0.045 (corrected for ties), $P > 0.1$). There was therefore no evidence that the gains and losses to be made balanced each other, nor that males which obtained high levels of extra-pair paternity tended to lose paternity in their own nests. The standardised variance in male reproductive success [variance/(mean)^2] was therefore only 14% higher when based on actual reproductive success (0.77) as compared to apparent reproductive success (0.61). So, despite the high incidence of extra-pair paternity there was nonetheless a significant positive relationship between the observed reproductive success and

actual reproductive success of males ($r_S = 0.691, N = 31, P < 0.001$).

5:3:2 Stepwise multiple regression of non-phenotypic variables on male reproductive success

The results of the first stage of the regression analysis are presented in table 5:1. The results for each dependent variable (Y_1 toY_7) will be discussed below.

(Y_1) The total number of young fathered. In 1992 the total number of young fathered by each male was significantly higher than in the previous two years ($F = 6.09$, $df = 30$, $P < 0.03$). This was due to the virtual elimination of nest predation, the collection of clutches for artificial incubation and the high degree of polygyny which resulted in more clutches laid per male. The combination of these factors explained 15% of the variance between males for this measure of reproductive success.

(Y_2) The number of extra-pair fertilisations that males achieved. In 1992 the number of EPFs achieved by each male was not significantly higher than in the previous two years ($F = 4.14$, $df = 30$, $P < 0.10$), but showed a strong trend for the same reasons outlined above.

(Y_3) The number of extra-pair mates. In 1992 there was a non-significant trend towards males having more extra-pair partners than in the other years ($F = 3.92$, $df = 29$, $P < 0.10$). This may be explained in terms of the male:female sex ratio which was highly biased towards females in 1992, resulting in a high degree of polygyny. The effect of an increase in polygyny was that more males had two potential extra-pair partners on some of their neighbouring territories as opposed to just one on territories of monogamous birds.

(Y_4) The proportion of potential extra-pair fertilisations achieved. In 1990 there was a non-significant trend towards males achieving proportionately less EPFs than in other years ($F = 3.29$, $df = 28$, $P < 0.10$). In 1990 the breeding density was much higher than in the following two years, resulting in males having more neighbours on average and thus achieving proportionately less extra-pair fertilisations in relation to the number of offspring sampled on adjacent territories.

(Y_5) The proportion of extra-pair young found in a male's own brood. There

$F(V_2;V_1)$	Y_1 F(30;1)	Y_2 F(30;1)	Y_3 F(30;1)	Y_4 F(28;1)	Y_5 F(29;1)	Y_6 F(29;1)	Y_7 F(29;1)
Non-phenotypic variables (X_1 to X_4)							
1990	0.00	-0.12	0.00	3.29	-0.22	0.15	-0.44
1991	0.00	0.12	0.00	0.18	0.01	-0.15	-3.19
1992	6.09	4.14	3.90	-0.18	0.21	2.30	0.44
No. of neighbours	0.13	0.00	0.14	0.00	2.68	0.19	0.07
Adjusted r^2	0.15	0.10	0.10	0.08	0.06	0.04	0.07

Ket to Y variables:

Y_1 = Total number of young fathered by a male.
Y_2 = Number of extra-pair fertilisations achieved by a male.
Y_3 = Number of females with which males sired extra-pair young.
Y_4 = Proportion of potential extra-pair fertilisations achieved by a male.
Y_5 = Proportion of extra-pair in a male's own brood.
Y_6 = Number of within-pair young sired by a male.
Y_7 = Number of extra-pair young reared by a male.

Table 5:1. First stage stepwise multiple regression analysis of non-phenotypic variables with each of the measures of male reproductive success (i.e., Y_1 to Y_7). Values exceeding the F-to-enter value of 1.96 (i.e., equivalent to $P < 0.10$ in simple regression) are highlighted in bold type. Variation in the V_2 values was due to missing values in some of the Y variables.

were proportionately more extra-pair young on territories which had more adjacent neighbours than others. However, this relationship was not significant ($F = 2.68$, $df = 25$, $P < 0.10$). This suggests that there might be a relationship between the number of adjacent neighbours and frequency of extra-pair young independent of overall year by year variations in density.

(Y6) The number of within-pair young sired by a male. There was a non-significant trend for more within-pair young to be sired in 1992 than in any other year ($F = 2.3$, $df = 29$, $P < 0.25$), presumably for the same reasons as outlined above in (Y1).

(Y7) The number of extra-pair young reared by a male. There was a non-significant trend for five extra-pair young to be sired in 1991 than in any other year ($F = 3.19$, $df = 29$, $P < 0.10$). This was possibly related to the small sample sizes obtained in that year.

The mean residual values for the Y variables were obtained for each male, and regressed against a series of phenotypic variables in the second stage analysis

5:3:3 Stepwise multiple regression of phenotypic variables on male reproductive success

The results of the second stage of the regression analysis are presented in table 5:2. The results for each dependent variable (Y1 toY7) will be discussed below.

(Y1) The total number of young fathered. There was a non-significant trend towards the total number of young sired being related to male song rate ($F = 3.46$, $df = 14$, $P < 0.10$). However, the jack-knife analysis showed that this regression was not particularly robust as the relationship only entered the model when the sample size was reduced by 10 on the addition of the feeding rate variable. This reduction was due to not having both song and feeding data for a number of males - any missing value results' in the entire case being deleted from the analysis.

Furthermore, inspection of the data showed that this non-significant trend was dependent on one male in 1990, which sang for much longer periods than other males and sired a large number of chicks. The high song rate of this male (A) can be attributed to the outcome of a removal experiment (see Chapter 2) on

$F(V_2;V_1)$	Y_1 F(0;0)	Y_2 F(0;0)	Y_3 F(0;0)	Y_4 F(0;0)	Y_5 F(14;1)	Y_6 F(20;1)	Y_7 F(20;2)
Phenotypic variables (X_1 to X_8)							
Male age	-0.16	0.43	0.62	0.02	3.02	-1.28	0.76
Social status	1.02	-0.68	-0.01	-0.35	0.00	11.40	7.98
Plumage score	-0.03	0.00	0.44	-0.09	-0.05	0.54	0.41
Body condition index	1.21	0.32	0.33	0.07	-0.25	1.37	0.79
Male feeding rate	-----	1.34	0.28	1.89	-0.05	-----	-----
Proportion of EPY	-----	0.00	0.01	0.04	-----	-----	-----
Song rate	0.43	1.75	1.35	061	1.38	0.20	2.38
Female age	0.79	0.93	0.49	1.32	-0.36	0.14	-0.03
Adjusted r^2	0.00	0.00	0.00	0.00	0.09	0.29	0.33

Table 5:2. Second stage stepwise multiple regression analysis of phenotypic variables with each of the measures of male reproductive success (i.e., Y_1 to Y_7; see table 5:1). Values exceeding the F-to-enter value of 1.96 (i.e., equivalent to $P < 0.10$ in simple regression) are highlighted in bold type. Jack-knife analysis was carried out to test the robustness of the regression model. The variation in V_2 values is due to the removal of the male feeding rate variable from the analysis, for which data was only available for 14 males and so increased the sample size from 14 to 20.

a neighbouring male (B). The female of (B) was predated during incubation, and he remated with another female soon after. Male (B) was caught and removed for 24 hours five days before the new female laid her first egg. During the removal male (A) took over the territory and (B) subsequently failed to regain it. Male (B) was displaced to a territory at the edge of a cereal field but continually intruded upon his old territory throughout the breeding season, resulting in an increased song rate of male (A).

(Y_2) The number of extra-pair fertilisations achieved by males. There was no relationship between any of the phenotypic characters tested and the number of extra-pair fertilisations achieved by males.

(Y_3) The number of extra-pair mates. There was no relationship between any of the phenotypic characters tested and the number of extra-pair partners fertilised by males.

(Y_4) The proportion of potential extra-pair fertilisations achieved. There was no relationship between any of the phenotypic characters tested and the proportion of extra-pair fetilisations achieved.

(Y_5) The proportion of extra-pair young found in a male's own brood. The proportion of extra-pair young in a male's own brood was positively related to male age ($F = 3.02$, $df = 14$, $P < 0.10$). This relationship is not statistically significant but does show a strong trend.

Figure 5.1 is a graphical representation of a bivariate analysis of age versus the proportion of extra-pair young (Mann-Whitney, $z = 1.657$, $P < 0.05$). However, this relationship did not translate into any overall effect on reproductive success (i.e., actual number of young sired), because the non-significant increase in actual number of extra-pair young found in old males' broods (Mann-Whitney, $z = 1.46$, $P < 0.08$) was offset by the non-significant trend for the number of within-pair young to be higher in old males' broods (Mann-Whitney, $z = -0.62$, $P = 0.27$).

(Y_6) The number of within-pair young sired by a male. There was a significant relationship between social mating status and the number of within pair young sired ($F = 9.18$, $df = 14$, $P < 0.03$), which became even more significant after the jack-knife analysis ($F = 11.4$, $df = 20$, $P < 0.001$). The trend towards a negative relationship between age and the number of within-pair young was lost after the jack-knife analysis. The number of within-pair young sired by polygynous males was higher than that of monogamous males simply because the number of samples obtained was higher (two females laying clutches).

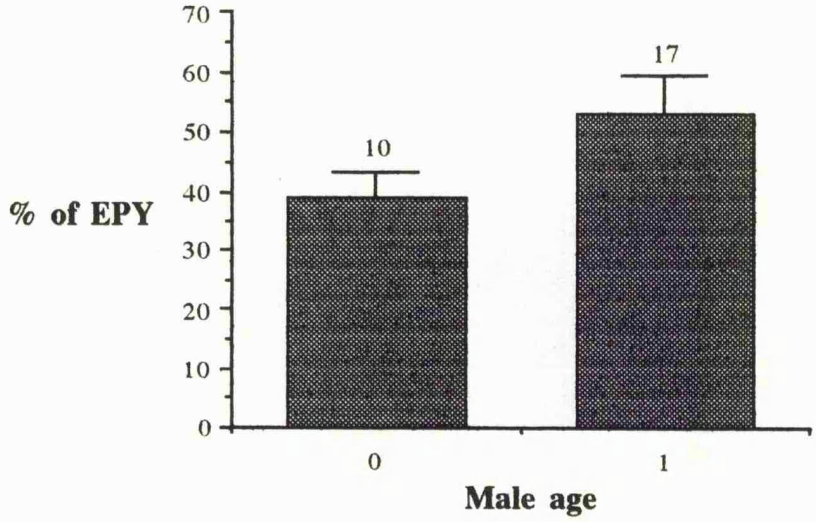

Figure 5.1 Male age and the percentage of extra-pair young in the brood. Male age was defined as young i.e., first year of breeding in study area (0) or old i.e., previously observed breeding in study area (1). Older birds had significantly more extra-pair young in their broods than young birds.

Mann-Whitney U-test, $z = 1.657$, $P < 0.05$.

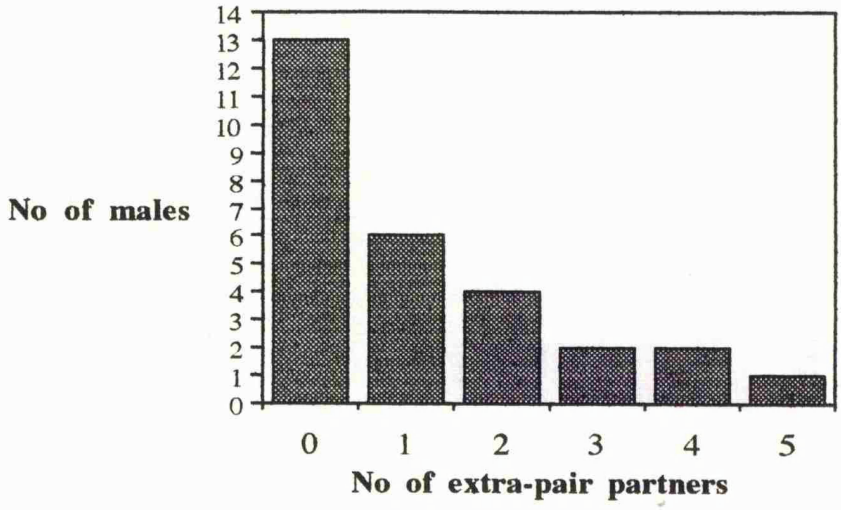

Figure 5.2: Variation in male success at obtaining extra-pair partners. The columns represent the distribution in male success in terms of the number of females with which they sired extra-pair young.

(Y7) The number of extra-pair young reared by a male. There was a significant relationship between a male's song rate and the number of extra-pair young raised ($F = 8.16$, $df = 14$, $P < 0.005$); however, the significance was lost following the jack-knife procedure ($F = 2.38$, $df = 20$, $P < 0.25$), suggesting that the regression was not particularly robust. Social mating status was significant before and after jack-knifing ($F = 7.98$, $df = 20$, $P < 0.005$) and the fact that polygynous males have more extra-pair young in their own broods can be explained in both numerical terms (more young sampled), and that secondary females have more extra-pair young in their broods than either monogamous or primary females (see chapter 7).

5:3:4 Distribution of male reproductive success

The frequency distribution of the number of extra-pair partners fertilised by each male is shown in figure 5:2. The distribution is highly skewed, with most males having few or no extra-pair partners and a small number having many. This distribution could be caused by variation in male quality, with a few high quality males achieving the most extra-pair partners. Alternatively, the distribution could be derived totally by chance. Comparison between the observed and expected values showed that there was no significant difference between the two ($Chi^2 = 5.05$, $df = 5$, $P > 0.05$), thus the variation in male success at obtaining extra-pair partners may be explained purely in terms of chance.

5:4 Discussion

This analysis is essentially a progression of that undertaken in chapter 4 to discover why female reed buntings engaged in EPCs. The conclusion of that analysis was that females apparently participate in EPCs indiscriminately, possibly obtaining genetic benefits through intraspecific male competition. Here, I investigate the system from a male perspective, first to elucidate why males participate in EPCs and secondly to try and identify those characteristics which best explain the variance in male reproductive success and hence provide an indication about the functional significance of EPCs for females.

The most obvious benefit of EPCs to males is the numerical increase in reproductive success. On average, extra-pair fertilisations made up 40% of male

actual reproductive success. Another benefit of extra-pair fertilisations is that a male's genes are spread out over a number of nests, thus increasing the likelihood of some passing on to the next generation despite high predation rates on nests, providing a natural example of the old advice, 'don't put all your eggs in one basket'. Males which obtained extra-pair fertilisations did not suffer a consequential increase in the level of extra-pair young in their own broods, so these birds had a higher reproductive success than those who did not achieve any extra-pair fertilisations. The purpose of the stepwise multiple regression analysis was to identify the causes of this variation among males.

The final conclusion of the multiple regression analysis was that the only phenotypic variables which were related to male reproductive success were male age and male mating status. The non-significant relationship of the former is contrary to what would be expected intuitively in that older males have a greater proportion of extra-pair young in their own broods than do younger males. A similar relationship was found in a North Carolina population of the indigo bunting (Westneat, 1990) but the opposite was found in a Michigan population of the same species (Westneat, 1987a). It is possible that this result is due to some behavioural difference between young and old males during their mate's fertile period, perhaps because older males protect their paternity less. However, the relationship between male age and the proportion of extra-pair young in the brood is weak.

The relationship between social pairing status and the number of both within-pair young and extra-pair young in a male's broods is more straightforward to explain simply through the numerical difference in the number of young sampled for polygynous males as opposed to monogamous males. The fact that polygynous males do not sire more total young than monogamous males suggests that there is some trade-off by polygynous birds between the pursuit of EPCs and maintaining social polygyny, even though the number of extra-pair fertilisations achieved is not related to social mating status. Polygynous males have twice as much to do as monogamous birds in terms of paternity protection, thus this may act as a constraint on EPC behaviour.

The absence of any strong significant association between any phenotypic or non-phenotypic variable and male reproductive success suggests that chance may be the major factor which explains the variance in male reproductive success. The frequency distributions for the number of extra-pair partners did not differ significantly from that expected by chance.

In conclusion, there appears to be some indication that male age plays a role in a male's reproductive success at his own nest, though whether this is due to variation in male behaviour between age classes or due to some interaction with the female partner is not known. Polygynous males rear more young on their own territories than do monogamous males, though this is not reflected in their total reproductive success, probably because of constraints resulting from increased time spent in paternity protection behaviour or some other aspect of maintaining social polygyny. To investigate these possibilities further, a larger data set, possibly obtained through a long-term study, is required to tease out the significant factors affecting male reproductive success which are undoubtedly clouded to a greater or lesser extent by chance events in this natural system.

Chapter Six

HIGH LEVELS OF EXTRA-PAIR PATERNITY AND PROTECTION OF PATERNITY

Abstract

This chapter investigates the paternity protection behaviour of male reed buntings in light of the high levels of extra-pair paternity exhibited by the species. An examination of the relationship between territorial behaviour and paternity revealed that territory size was not related to levels of paternity, indicating that larger territories are not advantageous in terms of protection of paternity. Nor was it found that reed buntings enlarge their territories during the fertile period of their mate as a means of reducing neighbouring males' opportunities for EPCs. Male song rate was lower during the periods when their mates were fertilisable than during other periods, suggesting that males allocate their time to some other behaviour during this period. There was some evidence of weak mate guarding in the species, with males generally spending more time closer to the female in the fertile period than during the stages of the breeding cycle when the female was not fertilisable. There was no tendency for second broods (when mate guarding is constrained by fledgling provisioning) to have a higher proportion of extra-pair young than first broods, suggesting that close guarding and following of the female is not the primary behaviour adopted by males to prevent cuckoldry. It is suggested that the primary paternity guard adopted by male reed buntings is a high copulation rate.

6:1 Introduction

In species where there is a significant amount of paternal care, males should be under strong selective pressure to ensure their paternity of any young they help to rear (Trivers, 1972). A corollary of the pursuit of EPCs by males as part of a mixed reproductive strategy has been the evolution of behavioural and physiological mechanisms of paternity protection (see review in Birkhead & Møller, 1992).

Mate guarding is the term applied to a behavioural paternity guard in which the male closely follows its social mate during her fertile period (Beecher & Beecher, 1979; Birkhead, 1979). Mate guarding can be viewed as a trade-off between the benefits of protecting paternity versus the benefits of competing alternative actions (Westneat *et al.*, 1990). This method of guarding paternity has received by far the most attention of researchers to date, and has been shown to occur in a wide variety of avian species (see Birkhead & Møller, 1992a). Mate guarding as a paternity guard necessarily involves not only the association of a male and a female during the fertile period but also the direction of the association, which needs to be ascertained to distinguish if it is the male that is following the female rather than vice versa.

Recently, sperm competition has been used to explain the evolution of territorial behvaiour in birds (Møller, MS, *cf* Birkhead & Møller, 1992). This hypothesis stemmed from the fact that territory sizes do not remain constant throughout the breeding season, and that the size of the area defended is likely to vary depending on the relative costs and benefits of defence (Brown, 1969). The likelihood of a female engaging in an EPC will decrease with increasing distance between the female and the potential cuckolder. A study on the yellowhammer (Møller, 1990b) showed that the size of the territory increased during the fertile period of the female, as predicted by the sperm competition hypothesis.

A consequence of territoriality as a paternity guard is that males will invest most effort in territory defence during their partner's fertile period. The means by which the males in most passerine species defend their territory is by song. This produces two mutuallly exclusive predictions. Mate guarding should result in a reduction of song during the fertile period because the male spends more time in close following of the female, whereas territory expansion should result in an increase in song activity during the fertile period.

In many species, ecological factors prevent mate guarding by close

following, and so yet another mechanism of paternity protection has evolved, that of frequent copulation. This method is regarded as a secondary strategy to that of mate guarding and it is predicted that the level of extra-pair young should be higher in frequent copulators than in guarding species (Møller & Birkhead, 1992).

This chapter investigates what paternity guards, if any, are employed by male reed buntings through testing the following hypotheses:

Territoriality

(A) If territory size is important as a paternity guard than one might expect those males occupying larger territories to have fewer extra-pair young in their own nest in comparison to those with smaller territories.

(B) Territory size is expected to increase during the females fertile period if territory size plays an important role in paternity protection.

(C) Another aspect of territorial behaviour is song output. It is expected that song output will increase with territory expansion during the female's fertile period. Conversely, a decrease in song output is expected during the female's fertile period if males protected their paternity through mate guarding.

Mate guarding

(A) If mate guarding is an important strategy employed by reed buntings to protect their paternity, then we would predict that males would spend more time close to their mate during the fertile period and that this association is caused by the male following the female.

(B) Reed buntings occasionally rear two broods within a season, the second brood being initiated prior to the independence of the first brood. During this period males continue to provision fledglings, which presumably constrains a male's mate guarding behaviour. If mate guarding is the primary paternity guard in reed buntings then we can predict that the level of extra-pair young in the second brood should be higher than in the first brood.

Copulation behaviour

(A) If the frequency of within-pair copulations is expected to higher during the female's fertile period

(B) If the pursuit of EPCs increases the likelihood of being cuckolded through the separation of the male from the female during her fertile period, then we can predict that males would be less likely to pursue EPCs during their mate's fertile period than outside this period.

6:2 Methods

6:2:1 Territorial behaviour

For the purposes of this analysis, a period of 6 days, encompassing 3 days before the first egg and the first three days of egg laying was chosen as the time during which the female is most effectively fertilisable. The period after the third day of egg laying and throughout the incubation period was taken as the time period when the female was not fertile. These periods are somewhat arbitrary and were chosen to provide an adequate sample size for the comparative analysis.

Reed buntings frequently used only one song post making it impossible to plot territories from the distribution of song posts. The territory area was therefore calculated by plotting the position of males onto a scale map during the behavioural observations (as described in Chapter 2). Observation periods were combined for each male into two groups, fertile and non-fertile. Convex polygons were drawn up for the territory of each male by joining the peripheral male co-ordinates on the scale maps. Obvious intrusions into neighbouring territories were ignored. This analysis was only carried out for the years 1991 and 1992 as male positions were not marked directly onto maps in 1990.

In all, eight territories provided enough data points in both the fertile and non-fertile periods to draw up meaningful polygons (i.e., a minimum of three days and ten different co-ordinates for each period). The internal area of each polygon was calculated by overlaying graph paper onto the scale maps and calculating the territory area. The territorial ranges of individual males were then compared between the two periods.

In order to test whether territory size had any influence on the number of extra-pair young found within a male's nest, the size of each male's territory was regressed against the proportion of extra-pair young found on the territory. In this analysis, the internal area of each polygon was calculated as above for all stages of the breeding cycle in order to obtain a larger sample size and a greater number of co-ordinates per territory.

For the analysis of song output in relation to the stage of the breeding cycle, a different set of criteria were used to delineate the fertile period. The breeding cycle was divided into five distinct stages, these being: early season (i.e., a period at least seven days before the first egg); pre-fertile (i.e., the period of 6, 5

and 4 days before the first egg); fertile (i.e., the period of 3, 2 and 1 days before the first egg); laying (i.e., the period of days 1, 2 and 3, where the first egg is laid on day 1); incubation (i.e., the period starting at least 4 days after the first egg and while the female was incubating). *N.B. Pre-fertile does not mean that the female is not fertile, but simply that this category preceeds the defined fertile category.*

Again, the naming of the categories is somewhat arbitrary, but they do reflect distinct times in the breeding cycle, such as egg laying and incubation. The distinction between pre-fertile and fertile, unlike the latter periods, does not follow any obvious behavioural cue, but there are subtle behavioural changes between each period (O'Malley, 1993). Early season observations could only be made before the first clutch was laid. After the first clutch, any replacement clutches were always laid within five days of the loss of the first brood.

Song output was recorded as the proportion of time in each of the 30-minute observation periods in which the bird was singing. These data were first regressed against time of year, as this was found to have the greatest influence on song output. The mean residual variance remaining from this regression was then calculated for each stage of the breeding cycle.

6:2:2 Mate guarding

Mate guarding involves two components, the first is the close association of the male and female during the fertile period and the second is that this association is brought about by the male following the female. The behaviours associated with mate guarding were compared between stages of the breeding cycle, as described above.

Data were only collected from timed observations of the male bird, thus they were heavily biased towards periods during which the female was close to the male. Focal female watches were not undertaken as females were very difficult to keep under observation for any length of time. The number of 30-second bouts in which the position of both the male and female were known was recorded for each of the 30-minute behavioural observation periods. From these, the proportions of bouts in which the distance between the male and female was less than 5 m and greater than 10 m was calculated.

Additionally, the total number of flights made by the male and the female was calculated. From this, the proportion of the total flights initiated by the male

or the female were calculated respectively. Data were also collected on the following behaviour of either sex after the other initiated a movement. These were recorded as male flight followed by female or male flight not followed by female and vice versa for female initiated flights. From these data, the proportion of female initiated flights in which the male followed and the proportion of male initiated flights which the female followed was also calculated.

In the analysis of paternity in first and second broods, only those nests which followed the successful fledging of at least one chick of the first brood were considered as a second brood. Replacement nests of failed attempts were not included in the comparison.

6:2:3 Copulation behaviour

In this section behavioural evidence is examined to explore the possiblity that male reed buntings use direct sperm competition through copulatory behaviour as a means of protecting their paternity.

Copulations in reed buntings occur singly or in bouts, with two or more mountings in quick succession. It was impossible to determine whether cloacal contact occurred so copulations were separated into two classes, successful (i.e., were there may have been cloacal contact) and unsuccessful (i.e., where there was definitely no cloacal contact). Copulations were recorded during the 30 minute behavioural watches and also casually. Because there were instances of multiple copulations in short time periods, each bout was treated as a separate data point, rather than each individual copulation. Each copulation bout was plotted against the stage of the female's breeding cycle.

In the analysis of the timing of EPCs by males relative to the stage in their own mate's breeding cycle, the breeding cycle of the pair female was separated into five arbitrary stages: pre-lay was a five day period from day -10 to -6; the fertile period was -5 to -1; the laying period was day 1 to 5, the incubation period was the time from clutch completion to hatching (12 days) and the provisioning period was the time from hatching to fledging (10 days). The fertile period of females with which the males sired some extra-pair young was taken as the five-day period from day -3 to day 2 (where day 1 is the first egg date). The time of this fertile period was related to the stage of the male's own mate's breeding cycle, for each of the males which obtained extra-pair fertilisations.

6:3 Results

6:3:1 Territoriality and paternity protection

The results of the territory area analysis are given in table 6.1. It can be seen that five males occupied larger territory ranges during their mate's fertile period, whereas three males occupied smaller ranges. Only one comparison resulted in a change of more than 10%, and that was in the direction opposite to that predicted by the hypothesis (i.e., the territory size was smaller during the female's fertile period).

There was no significant relationship between territory size and the proportion of extra-pair young found on a territory (Spearman rank-order correlation coefficient, $r_S = 0.009$, *NS*). Again, this does not support the hypothesis that a larger territory confers a greater protection of paternity than a small territory.

The analysis of the song data showed that there was a strong correlation between the amount of time spent singing and the time of year, with males spending more time in song later in the breeding season (Spearman rank-order correlation coefficient, $r_S = 0.707$, $P < 0.001$). The residual variance from this regression was compared for each stage of the breeding cycle (figure 6.1).

There were significant differences in male song rates among the nesting stages (Kruskal-Wallis ANOVA, $df = 4$, $H = 8.82$, $P < 0.05$). Males sang significantly more in the early season than in the pre-fertile and fertile periods, and there was a significantly lower song output in the fertile period compared to the incubation stage. Although all the comparisons did not differ significantly, a pattern was revealed where song output was lower in the periods when the female was fertile. This result is contrary to the prediction of the territory theory whereby territorial behaviour is expected to increase during the females' fertile period. The pattern of song output exhibited does, however, support the mate guarding hypothesis, where territorial behaviour is constrained by close following of the female.

Territory size during the fertile period	Territory size during the incubation period	% change
4662 (3)	4482 (6)	+ 3.8 %
3141 (4)	3486 (4)	- 9.9 %
5328 (2)	4887 (5)	+ 8.3 %
10005 (5)	9899 (6)	+ 1.0 %
9012 (4)	8910 (6)	+1.1 %
2589 (6)	2612 (7)	- 0.8 %
12728 (6)	12430 (5)	+ 2.3 %
8328 (4)	9593 (7)	-13.2 %

Table 6.1: Territory area occupied by eight males during the fertile period and the incubation period of their mate. The figures in brackets represent the number of observation days from which the territory area was calculated.

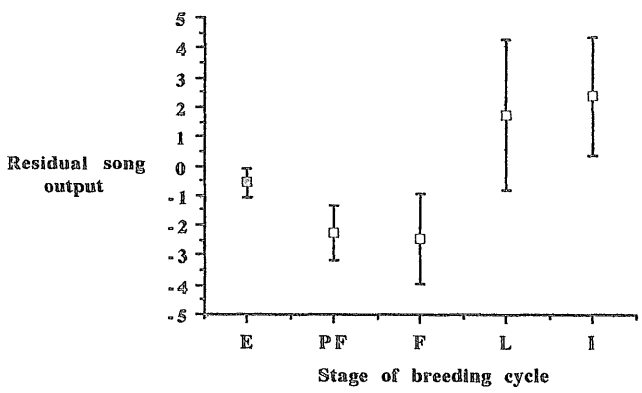

Figure 6.1: Mean (+/- SE) residual song output for each stage of the breeding cycle after controlling for the time of year.
Kruskal-Wallis ANOVA, $df = 4$, $H = 8.82$, $P < 0.05$.
Mann-Whitney U-tests:

Early V Pre-Fertile	$z = -2.69$, $P = 0.003$
Early V Fertile	$z = -2.83$, $P = 0.002$
Early V Lay	$z = -0.29$, $P = 0.38$
Early V Incubation	$z = -0.29$, $P = 0.38$
Pre-Fertile V Fertile	$z = -1.33$, $P = 0.09$
Pre-Fertile V Lay	$z = -0.99$, $P = 0.16$
Pre-Fertile V Inc'n.	$z = -1.13$, $P = 0.13$
Fertile V Lay	$z = -1.50$, $P = 0.07$
Fertile V Incubation	$z = -1.65$, $P = 0.05$
Lay V Incubation	$z = -0.23$, $P = 0.41$

6:3:2 Mate guarding

The results of the analyses of mate guarding behaviour are presented below:

(A) Proportion of time when male and female were less than 5 m apart

There was no significant difference between the proportion of time when the male and female were less than 5 m apart for the pre-fertile, fertile, laying and incubation periods. However, males and females spent proportionately more time closer together during the early season than at any of the other periods (figure 6.2). This does not support the hypothesis that males closely guard their mates during the fertile period.

(B) Proportion of time when male and female were more than 10 m apart

Pairs spent proportionately less time more than 10 m apart during the early season than the fertile, laying and incubation periods, but not significantly so in the laying period (Mann-Whitney U-test, $z = -1.31$, $p = 0.07$). There was also a significant difference in the proportion of time pairs spent more than 10 m apart between the pre-fertile stage and the incubation stage, with birds spending more time further apart during the latter period (figure 6.3). There was a tendency for males to spend a greater proportion of time more than 10 m away from their mates outside the fertile period, providing some evidence of guarding behaviour by males as a means of protecting their paternity.

(C) Proportion of all flights initiated by the female

There was no significant difference in the proportion of female-initiated flights in each of the stages of the breeding cycle (figure 6.4), indicating that there was no change in female behaviour with stage of the nesting cycle.

(D) Proportion of female flights followed by the male

There was no significant difference in the proportion of flights made by the female which the male followed in each stage of the breeding cycle (figure 6.5). There is however a trend towards more following flights by males during the fertile period of the female, suggesting that some degree of mate guarding may occur.

(E) Proportion of male flights followed by the female

Females followed significantly more male-initiated flights in the early season than at any other stage of the breeding cycle (figure 6.6). This indicates a change in female behaviour between the early season and other periods of the

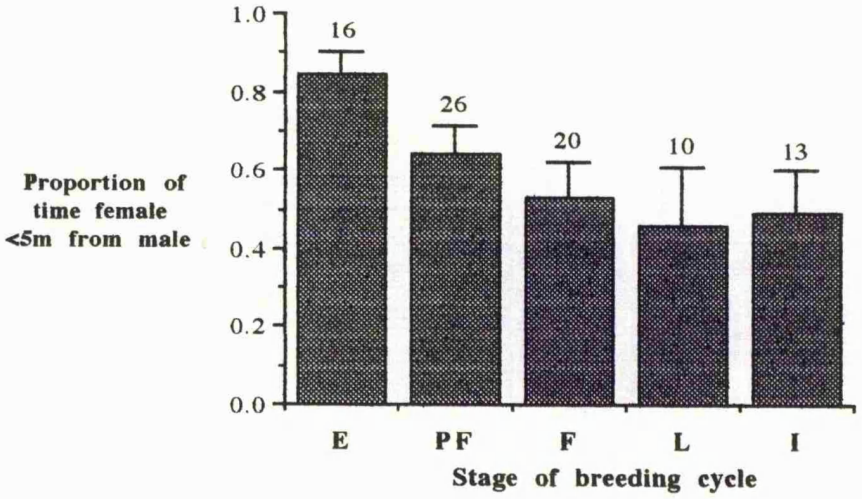

Figure 6.2. Proportion of visible observation time that the male and female were less than 5 m apart for each stage of the breeding cycle. The columns show the mean (and SE), and the values above each column indicate the number of separate observations used each stage.
(Kruskal-Wallis ANOVA, df = 4, H = 8.09, P < 0.05).
Mann-Whitney U-tests:

Early V Pre-Fertile: Z = -1.39, P = 0.08
Early V Fertile: Z = -2.08, P = 0.02
Early V Lay: Z = -1.93, P = 0.03
Early V Incubation: Z = -2.34, P = 0.001

Pre-Fertile V Fertile: Z = -1.05, P = 0.15
Pre-Fertile V Lay Z = -1.23, P = 0.11
Pre-Fertile V Inc'n. Z = -1.39, P = 0.08

Fertile V Lay Z = -1.41, P = 0.34
Fertile V Incubation Z = -0.34, P = 0.37

Lay V Incubation Z = -0.13, P = 0.45

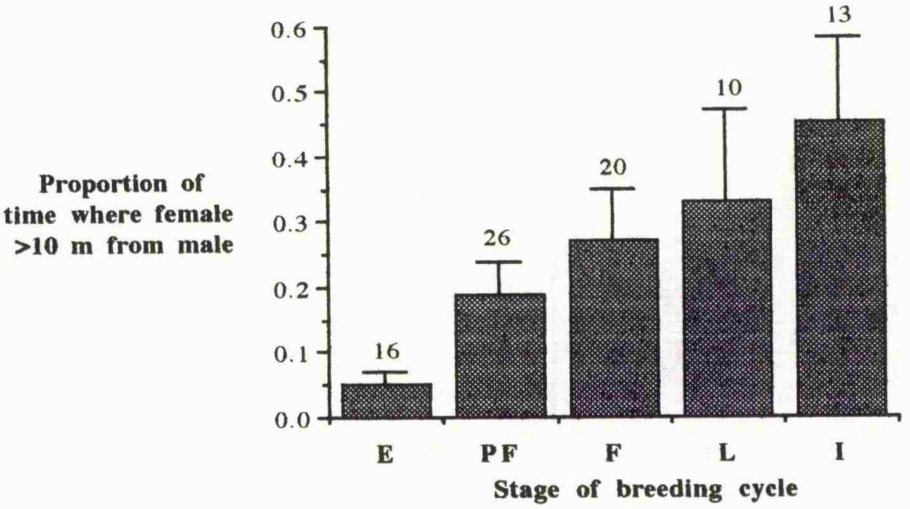

Figure 6.3. Proportion of visible observation time that the male and female were more than 10 m apart, for each stage of the breeding cycle. The bars show the mean (SE), and the values above each bar indicate the number of separate observations used for each stage.
(Kruskal-Wallis ANOVA, $df = 4$, $H = 6.64$, $p < 0.05$)
Mann-Whitney U-tests:

Early V Pre-Fertile	$z = -1.31$, $p = 0.1$
Early V Fertile	$z = -1.83$, $p = 0.03$
Early V Lay	$z = -1.47$, $p = 0.07$
Early V Incubation	$z = -2.17$, $p = 0.02$
Pre-Fertile V Fertile	$z = -0.74$, $p = 0.23$
Pre-Fertile V Lay	$z = -0.67$, $p = 0.25$
Pre-Fertile V Inc'n.	$z = -1.68$, $p = 0.05$
Fertile V Lay	$z = -0.12$, $p = 0.45$
Fertile V Incubation	$z = -0.81$, $p = 0.21$
Lay V Incubation	$z = -0.62$, $p = 0.27$

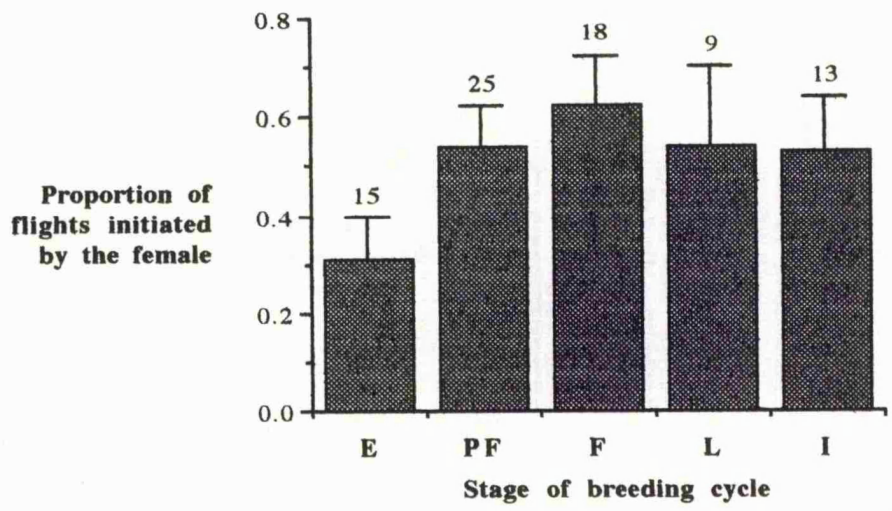

Figure 6.4. Proportion of the flights made whilst both the male and female were visible that were initiated by the female during each stage of the breeding cycle. The bars show the mean (SE), and the values above each bar indicate the number of observations used for each stage.
(Kruskal-Wallis ANOVA, $df = 4$, $H = 4.94$, *ns*)

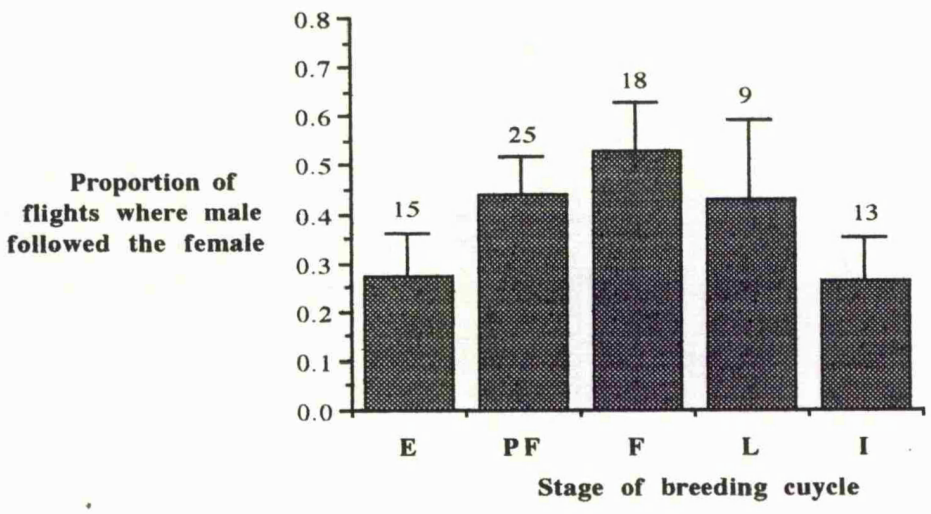

Figure 6.5. Proportion of the flights initiated by the female which were followed by the male, for each stage of the breeding cycle (see Figure 6.4). The bars show the mean (SE), and the values above each bar indicate the number of observations used for each stage.
(Kruskal-Wallis ANOVA, $df = 4$, $H = 4.44$, $p > 0.05$)

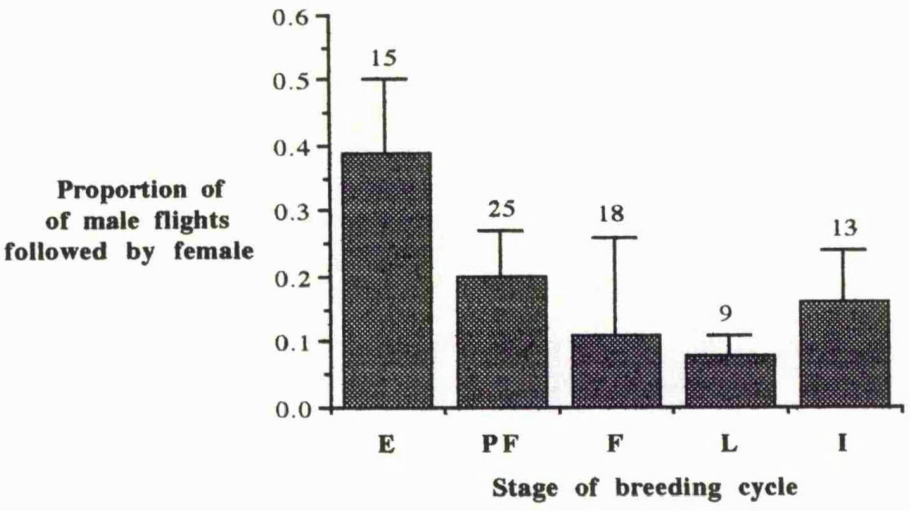

Figure 6.6. Proportion of the flights initiated by the male which were followed by the female, for each stage of the breeding cycle (see Figure 6.4). The bars show the mean (SE), and the values above each bar indicate the number of observations used for each stage.
(Kruskal-Wallis ANOVA, $df = 4$, $H = 7.66$, $p < 0.05$)
Mann-Whitney U-tests:

Early V Pre-Fertile	$z = -1.34$, $p = 0.09$
Early V Fertile	$z = -2.21$, $p = 0.01$
Early V Lay	$z = -1.81$, $p = 0.04$
Early V Incubation	$z = -1.84$, $p = 0.03$
Pre-Fertile V Fertile	$z = -1.40$, $p = 0.08$
Pre-Fertile V Lay	$z = -1.01$, $p = 0.16$
Pre-Fertile V Inc'n.	$z = -1.04$, $p = 0.15$
Fertile V Lay	$z = -0.19$, $p = 0.42$
Fertile V Incubation	$z = -0.32$, $p = 0.37$
Lay V Incubation	$z = -0.05$, $p = 0.48$

breeding cycle.

In the comparision of levels of paternity in first and second broods it was found that the level of extra-pair paternity did differ, though not in any particular direction (table 6.2). Observations showed that in all the cases analysed males were seen feeding fledglings at the time of laying of the first egg of the second brood. The mean period for initiating a second brood was 9.8 days after the first brood had left the nest, thus the chicks from the first brood were still dependent on adults for food at this time. This does not support the hypothesis that male mate guarding behaviour is constrained by parental duties at the second nest.

6:3:3 Within-pair copulations

In total, 42 copulatory bouts were witnessed during the three years of study, of which 12 were classed as unsuccessful copulations. The observed copulation frequency does not appear to be particularly high in relation to the number of hours spent in the field. This does not support the hypothesis that males copulate frequently as a means of protecting their paternity.

The majority of copulations occurred in the period four days before the first egg up to the day of the second egg (figure 6.7). There was, however, a degree of spread in the distribution of copulation bouts, indicating that copulations can occur in the early stages of the breeding cycle. There are not enough data available to determine any peaks in copulatory activity or to attempt to define the fertile period of the female. However, it does not appear that males target their copulatory activity to a specific time in the female's breeding cycle in order to maximise their chances of paternity.

6:3:4 Extra-pair copulations

The observed rate of EPC was very low. In fact, no successful EPCs were seen. There were, however, nine 'EPC events' - these were not actual copulations but apparent extra-pair copulation attempts. In all cases these took place during the fertilisable period of the female.

Inferential evidence of male extra-pair mating behaviour in relation to their own mate's breeding cycle can be drawn from the pattern of extra-pair fertilisations revealed by DNA profiling. This analysis assumes that the EPCs which resulted in the extra-pair fertilisations must have occured during the five-

PAIR	% EXTRA-PAIR PATERNITY 1st BROOD	2nd BROOD
A	75 (4)	0 (3)
B	80 (4)	60 (5)
C	100 (4)	75 (5)
D	100 (4)	0 (3)
E	100 (2)	75 (4)
F	50 (4)	60 (5)
G	66 (3)	100 (4)
H	0 (3)	60 (5)
I	80 (5)	50 (4)
J	40 (5)	20 (5)
K	100 (4)	66 (3)
L	0 (4)	100 (2)
M	100 (3)	100 (5)
N	0 (3)	100 (4)

Table 6.2. The level of extra-pair paternity in first and second broods of 14 double-brooded pairs of reed bunting. The figures in brackets indicate brood size. The level of extra-pair paternity differed between the first and second brood in 13 cases, of which 8 first broods had a higher proportion of extra-pair young than second broods.

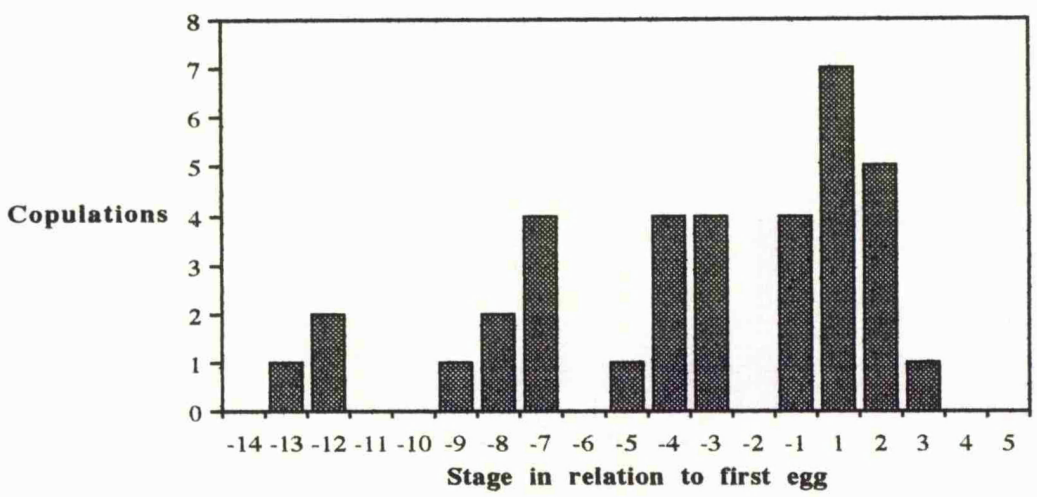

Figure 6.7. Number of copulation bouts seen in relation to the day of the breeding cycle, where day 1 represents the laying of the first egg. Three bouts at days 17, 19, 20 and -27 have been omitted.

Stage of pair-female's breeding cycle

	Pre-lay (5)	Fertile (5)	Lay (5)	Incubation (12)	Feeding (10)
Observed	6	7	7	15	6
Expected	5.5	5.5	5.5	13.3	11.1

Figure 6.8. The frequency of extra-pair fertilisations in relation to the stage of the pair-female's breeding cycle. The figures in brackets indicate the length of each breeding cycle stage in days. The observed values represent the number of males which obtained extra-pair fertilisations at each stage of their own mates' breeding cycle. There was no significant difference in the timing of a male's extra-pair fertilisations in relation to the stage of the breeding cycle.
$X^2 = 2.7$, $df = 4$, $p > 0.05$.

day fertile period of the extra-pair female (see methods). The results indicate that males obtained EPCs at all stages of their own mate's breeding cycle, and that the distribution of EPCs did not differ from that expected by chance (figure 6.8).

6:2:3 Discussion

The data showed that larger territories did not confer any advantage for males, in terms of the number of within-pair young in their own nests, and there was no evidence to suggest that males defended larger territorries during the fertile period of the female as a means of protecting paternity. An examination of 38 different bird species found that nearly all had their largest territory during the fertile period of the female, the few exceptions being during the pairing period or in the nestling period (see Møller, 1990b for review), though it was not stated whether the species under review were predominantly guarding species or frequent copulators. If the primary paternity guard in the reed bunting is not mate guarding then the need to defend a larger territory would not arise.

The song rate of males is lower during the fertile period, is possibly due to males altering their time budgets and so devoting less time to song and more to some other activity. There was little evidence from this study to suggest that males mate guard to any great extent (but see O'Malley, 1993), so it is unlikely that males are spending their time closely pursuing their mate. In a closely related species, the yellowhammer, a similar absence of mate guarding behaviour has been reported (Sundberg, 1992). The fertile period of the female is the time of the breeding cycle where territorial intrusions are greatest (O'Malley, 1993; pers obs.), so males may well be spending more time chasing and fighting intruders.

Alternatively, there may be more intruders because males spend less time singing. My own observations and the work of O'Malley (1993) suggest that males spend a greater proportion of time perched on some prominent point, apparently watching for intruders during the fertile period. O'Malley termed this behaviour as 'vigilance'. This certainly does not constitute mate guarding in the strict sense of closely following the female, but does serve a similar purpose in attempting to prevent males gaining access to the female.

Close association of males and females was, prior to the interest in EPCs, regarded as being related to the formation of the pair bond or to guard against predation and allow more efficient foraging. The close association by males and

females during the early season is unlikely to be related to sperm competition and the guarding of paternity by the male. In fact this association is apparently the result of the female following the male. It is possible that during this period, when the female has a high energy requirement prior to breeding, the most efficient way to forage is under the watchful eye of the male. In this way the female can devote more time to feeding than watching out for predators.

Female reed buntings are capable of initiating the second clutch within a week of the first brood leaving the nest, i.e., before the first brood becomes independent of parental care. This has important implications for the reproductive behaviour of the pair, particularly on mate guarding by the male to protect paternity in the second brood (Weatherhead & McRae, 1990). If males mate guard to ensure their paternity, then male care for widely dispersed fledglings will result in a physical separation of the male and female, so making close following and guarding of the female an impossibility, thus males face a higher risk of their mate engaging in EPCs.

Weatherhead and McRae (1990), reported that male American robins apparently guard the female for the first brood (Gowaty & Plissner, 1987) but not the second brood. There was no difference in the paternal investment between first and second broods, from which it was inferred that there was no difference in paternity (based on the results of Møller, 1988a and Westneat, 1988). The hypothesis Weatherhead and McRae proposed to explain this apparent discrepancy was that females initiating second nests refrain from participating in EPCs if their mates have proven themselves by the success of the first brood, because confidence of paternity ensures male parental care at the second nest.

The above hypothesis could not explain the situation in the reed bunting, where females obviously do not refrain from extra-pair copulatory behaviour for the second nest. A more plausible explanation in this species is that male reed buntings do not mate guard the female to any great extent at either nest. The level of extra-pair fertilisations underlies the fact that whatever paternity guard males employ it is not very efficient, but neither is it less efficient for second broods. The provisioning of widely dispersed fledgelings constrains male mate guarding, but would not necessarily prevent males from copulating frequently to protect their paternity.

Møller & Birkhead (1992) predict that non-guarding species are more likely to have high rates of extra-pair young than guarding species. Given the degree of sperm competition present in the reed bunting it does seem surprising

that intensive mate guarding behaviour has not evolved. This may be due to the ecological constraints of the habitat type in which reed buntings breed. Traditionally reed buntings were known to breed only in marshy habitats, and the ancestral home of the species is likely to have been in dense reed beds. Evidence that suggests that reed beds are indeed the ancestral habitat of the species comes from the fact that several sub-species, with different bill sizes, have evolved in different regions of the world to adapt to the different characteristics of the reed species found in the region. Close following and guarding of the female in dense reed bed habitats would be futile and so some other paternity guard would need to evolve.

Mate guarding alone is not an effective paternity guard unless the female can be watched at all times, as a missing female may well have engaged in an EPC. Such circumstances must be very rare in nature, so unless mate guarding is backed up by copulatory behaviour it's value as a paternity guard is minimal. To make a footballing analogy, with the score at nil : nil it is OK to defend and 'guard' against the opposition scoring, but once they score, continued guarding is useless unless you score one back. There is evidence to suggest that in some guarding species, if the male loses sight of the female for any period, he will copulate when she returns (Davies, 1992). In species where the female is frequently 'lost', males must copulate on many more occasions and this is possibly the route of the evolution of frequent copulation as a paternity guard.

Frequent copulation is regarded as a less efficient and secondary strategy to mate guarding, and is prevalent in species where environmental and ecological constraints prevent the adoption of the primary paternity protection strategy (Birkhead & Møller, 1992a). Considering I was in the field for approximately 10 hours per day virtually every day from the beginning of April to the end of July in each of the three years and yet only saw 42 copulation bouts and no extra-pair copulations, it would seem fair to say that copulations must be relatively infrequent. However, as shown previously in chapter 4, 85% (49/58) of broods held at least one extra-pair young. Thus there must have been at the very least 49 EPCs, and probably many more. Similarly, the 42 within-pair copulation bouts witnessed is likely to represent only a very small proportion of the true number of copulation bouts that actually take place.

In Chapter 8 I provide physiological evidence to support the hypothesis that direct sperm competition through copulatory behaviour is the primary means of protecting paternity in the reed bunting.

Chapter Seven

EXTRA-PAIR PATERNITY AND THE SOCIAL MATING SYSTEM

Abstract

The level of polygyny found in this study of the reed bunting was 27% (8/30 males), a much higher frequency than has been reported in previous studies of this species. This is probably due to a combination of more detailed field study and a dramatic increase in predator numbers (sparrowhawk, *Accipiter nisus*) than in the previous studies. Polygyny was opportunistic in nature and occurred primarily through mate replacement after disappearance of the territorial male. Male reed buntings benefit from polygyny through increased reproductive output, though not to a significant extent. Females, on the other hand appear to gain no benefits from mating polygynously and potentially incur increased costs through the absence of male parental care. It is proposed that the most likely explanation for the evolution of monogamy in the reed bunting is through the lack of opportunity for males to monopolise additional mates. DNA fingerprinting revealed that the true reproductive success of polygynous males is not significantly greater than that of monogamous males. It is proposed that the

species is poorly adapted to protecting the paternity of additional females and that the advantage of polygyny to normally monogamous species may have been over-estimated through the simple use of observed fledging success.

7:1 Introduction

Natural selection acts differently on males and females, with the result that the strategy adopted by an individual to maximise fitness may not be the same for each sex. The resolution of this sexual conflict is reflected in the evolution of different reproductive strategies of the two sexes. Males have the potential to fertilise more eggs than a female can produce thus, theoretically, males can increase their fitness by deserting the female and seeking others to inseminate (Trivers, 1972). A corollary of this is that females would be selected to be choosy in their choice of mate. Having said this, it would appear paradoxical that over 90% of bird species are socially monogamous (Lack, 1968).

There is some degree of ambiguity in the definition of monogamy and polygamy between different authors. Typically, a bird is considered monogamous if it forms a pair bond with only one member of the opposite sex and polygamous if it forms pair bonds either simultaneously or successively with more than one individual of the opposite sex (Lack, 1968; Wittenburger, 1979). However, some authors label mate-switching between breeding attempts as successive polygamy (Radbaugh, 1972; Fraga, 1972; Burns, 1982), while others label such events as successive monogamy (Carey & Nolan, 1975). Mate switching has the same genetic consequence as polygamy but as a behavioural phenomenon it is quite distinct.

For many years the only explanation proposed for the prevalence of monogamy in birds was Lack's (1968) interpretation that shared parental care resulted in the most offspring. This hypothesis explains the obligate monogamy found in many seabirds and shorebirds (Oring, 1982) and also that found in the colonial jackdaw *Corvus monedula* (Henderson & Hart, 1993). Alternative theories for the evolution of monogamy were not put forward for some time, until Emlen & Oring (1977) outlined hypotheses on the preconditions necessary for the evolution of monogamy. This was then further expanded by Wittenburger & Tilson (1980) into an explicit 'theory of monogamy'. The alternative hypotheses proposed by these authors were derived from the boundary conditions of theories put forward by others to explain the various forms of polygamy.

Wittenburger and Tilson's conditions for the evolution of monogamy can be summarised as follows:

(A) Monogamy should always evolve when male parental care is both non-shareable and indispensable to female reproductive success. This is an extension of Lack's (1968) original hypothesis on the evolution of monogamy.

(B) Monogamy should evolve when males are less successful with two mates than with one (Trivers, 1972). In non-territorial species, monogamy should evolve when the majority of males can reproduce most successfully by defending exclusive access to a single female.

(C) In territorial species, monogamy would be expected if pairing with an available unmated male results in higher female fitness than pairing with an already mated male. This has been derived from the competitive female choice model for the evolution of polygyny (Verner 1964; Orians 1969). A review of the literature by Wittenburger & Tilson (1980) concluded that monogamy has evolved this way in most of the passerine species studied.

(D) Monogamy should be expected even though the polygyny threshold is exceeded if aggression by mated females prevents males from acquiring additional mates (Wittenburger & Tilson, 1980).

In many species, removal experiments have shown that biparental care is not essential to rear at least some offspring (see reviews in Bart & Tornes, 1989; Davies, 1991). Thus, if the opportunity to remate is available it would benefit males to mate polgynously. Møller (1986) reported that occasional polygyny has been recorded in 39% of 122 well-studied European passerines. This suggests that monogamy has arisen in many species because of the limited opportunites for polygyny (Davies, 1991). Two obvious constraints on the opportunity of polgyny, as outlined above, are male-male competition for mates and female-female aggression preventing extra pairings. The situation is not always so biased towards male benefits; in the penduline tit *Remiz pendulinus*, either sex may desert leaving the other to care for the brood, and which sex deserts depends on which sex has the greater opportunity to remate (Persson & Öhrstöm, 1989).

There are very few published records of polygyny in the reed bunting, though it was noted by Bell (1967). After realising that there was a possibility of polygyny in the species, Bell (1968) re-examined his past data from his study site at Attenborough in Nottinghamshire, and regarded polygyny as "not being rare in the species", though he did not quantify this statement. Hornby (1971) stated that between 2 - 9% of the reed buntings at Attenborough were polygynous

between 1964 and 1969. He also recorded instances of successive polygyny and polyandry. This low level of polygyny occurred in a slightly male-biased population. Both Bell and Hornby regarded polygynous males to be both older and to occupy superior territories than their neighbours, though evidence for the latter assertion was lacking from both studies.

A previous study on reed buntings at Rutland Water indicated that females become polygynous primarily through loss of a mate (O'Malley, 1993). Widowed females have essentially three possibilities in their social mating circumstance:

(1) Do not remate.

(2) Leave the territory and seek an unmated male elsewhere.

(3) Stay on territory and remate polygynously.

Assuming that the option of remaining unmated and not breeding increases the chances of a female surviving overwinter, it still does not appear to be a viable option. Most females only bred for one season during this study (pers. obs.), so refraining from breeding for one year would appear to be a very risky decision on a lifetime reproductive success basis.

A female choosing the second option may not find an unmated, territorial male or if she does it is likely that this male will be of low quality or occupy a sub-standard territory. However, the relative costs or benefits of choosing this option depend on the time of year when a female's mate dies. A bird widowed early in the season would stand a better chance of finding an unmated male than one widowed later in the season.

A female choosing the third option can still exercise a degree of social mate choice once she has been widowed, in that out of the neighbouring males the female would be expected to pair polygynously with the best male or, alternatively, the best male would be able to monpolise the female over other neighbours. Either way, the female would still gain any possible direct or indirect benefits. Furthermore, the costs to mating polygynously can be ameliorated by a high nest predation rate. Predation of nests means that those females which start to nest first are not necessarily those which hatch their clutch first (and so receive male care); thus polygynous females are in a kind of raffle where chance plays a major part in the hatching order of their nests (Temrin & Jakobsson, 1988; Bensch & Hasselquist, 1992).

In this chapter I describe and propose an explanation for the social mating pattern found in the reed bunting at Rutland Water during the study period from 1990 to 1992. The following predictions from hypotheses on the evolution of

monogamy in birds are tested:

(A) Monogamy should evolve if male parental investment is essential for female reproductive success (Wittenburger & Tilson, 1980 after Lack, 1968).
Prediction - females should be unable to successfully rear a brood without male assistance in parental care.

(B) Monogamy should evolve if the reproductive success of monogamous males is greater than that of polygynous males (Trivers, 1972).
Prediction - Monogamous males should fledge more young than polygynous males.

(C) Monogamy should evolve if the reproductive success of females is higher in monogamy than polygyny (Wittenburger & Tilson, 1980 after Verner, 1964; Orians, 1969).
Prediction - Monogamous females should fledge more young than polygynous females.

The influence of extra-pair paternity has not been included in past models and hypotheses on the evolution of polygyny. Through the use of DNA fingerprinting, I examine how this phenomenon affects traditional measures of male reproductive success and hence the models of the costs and benefits of monogamy. Finally, I examine the options open to females in social pairing to see if there is any evidence of female preference for male or territory characteristics in the social mating decision of polygynous females.

7:2 Methods

7:2:1 Definitions of social polygyny and monogamy

I have defined social monogamy as the situation where one male forms a pair bond with one female, and social polygyny as the situation where one male forms a pair bond with more than one female simultaneously. There were no instances of polyandry or mate switching during the study. Within polygynous systems, the females are defined as primary or secondary according to whether or not they receive male assistance in parental care. The primary female is defined as the one whose brood receives provisioning by the male, usually representing the first nest to hatch on the polygynous male's territory (only one exception to this occurred). The brood of a secondary female is fed only by the female, and is

usually in the second nest to hatch on a territory.

7:2:2 Paternal investment and female reproductive success

To test whether or not paternal investment in the form of brood provisioning was essential to, or increased, female reproductive success, the fledging success of nests with and without male care was compared. Fledging success was measured as the number of young which left the nest naturally and not as the number surviving to independence because reed bunting chicks always leave the nest before they are able to fly, and are then extremely difficult to observe and count.

7:2:3 Reproductive success of monogamous and polygynous males

Current hypotheses on the evolution of polygyny naturally assume that males benefit from polygyny, yet the incidence of extra-pair paternity can greatly influence a male's reproductive success. The effect of extra-pair paternity on true male reproductive success, particularly in relation to the benefits of opportunistic polygyny in normally monogamous species, has not been examined.

Two methods of measuring the reproductive success of monogamous and polygynous males were used. Firstly, the observed number of young fledged from a territory was compared between the two social mating classes. This is the 'traditional' measure of reproductive success upon which many of the theories for the evolution of avian mating systems are based. To examine the influence of extra-pair paternity on this estimation of male reproductive success DNA fingerprinting was used (chapter 3) to obtain a second measure, i.e., the number of young which fledged which were actually sired by the male.

7:2:4 Reproductive success of monogamous, primary and secondary females

There were no cases of intra-specific brood parasitism recorded in the study (chapter 4), so the traditional measure of the number of young fledged from the nest of a female was a true reflection of female reproductive success. In this analysis I compare the fledging success from the nests of monogamous, primary and secondary females.

7:2:5 Female choice in social polygyny

In this section I examine the phenotypic characters of the polygynous males to see if there was any evidence to support the hypothesis that widowed females preferentially paired with high quality males.

Comparisons were made between the polygynous male and the other males immediately adjacent to the widow's territory, using the Wilcoxon signed-rank test for matched pairs. This is essentially the comparison a widowed female has to make in choosing among potential partners, as there were no cases of polyterritorial polygyny. The methods used to define male body condition index, plumage score, song rate, feeding rate and age have been described earlier (Chapter 5) and the methods used to measure tarsus, wing length, weight and head length have been described in Chapter 2.

7:3 Results

7:3:1 Frequency of social monogamy and polygyny

In the three years of study from 1990 to 1992, overall 27% (8/30) of territory-holding males were polygynous (Table 7:1). This is a higher level of social polygyny than has been described in any previous study on the reed bunting.

Polygyny normally arose through the disappearance of a territorial male, resulting in the widowed female pairing with a neighbouring mated male (5/8 cases of polygyny occurred in this manner). There was one case of polygyny caused by the 24-hour experimental removal of a recently paired male. This male had lost his territory and mate permanently to a neighbouring mated male when he was returned. In the remaining two cases of polygyny, males were seen consorting with two females when observations were first made early in the season. It is possible that these females were already widowed before I started fieldwork.

Of the five males which disappeared, two were known to have definitely died. One was a road casualty and the other was found impaled on a grass stem. There was strong evidence to suggest at least one and probably all of the other

	UNMATED	MONOGAMOUS	POLYGYNOUS
1990	1 (6%)	13 (77%)	3 (17%)
1991	0	5 (83%)	1 (17%)
1992	1 (14%)	2 (29%)	4 (57%)

Table 7:1. Social mating status of territory-holding males in the study area over three breeding seasons.

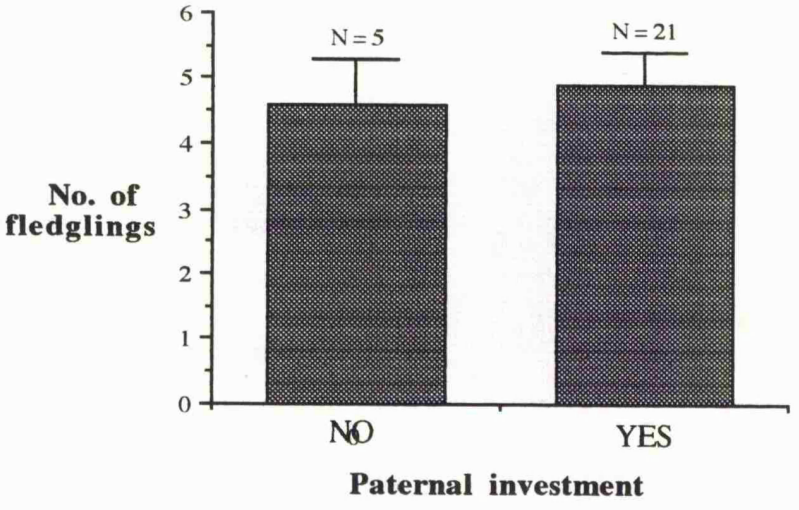

Figure 7:1. Fledging success of nests with and without male assistance in brood provisioning. Values given are means (SE).
Mann-Whitney U-test, $z = -0.06$, $p = 0.5$.

three males which disappeared had been taken by a sparrowhawk. Sparrowhawks were seen attacking males on two occasions.

7:3:2 Paternal investment and female reproductive success

To examine the necessity of paternal investment for female reproductive success, the fledging success of broods which received male assistance in feeding was compared with those which did not. There was no significant difference in the fledging success of these two nest categories (Mann-Whitney U-test, $z = -0.06$, $P = 0.5$, Figure 7:1). It is possible that the survival rate of offspring from unaided nests was lower than that from nests which received male help, but natal recruitment was so low in this population that this possibility could not be tested (no fledglings returned to breed in the study area in 1991 and 1992). It is evident however, that paternal investment is not essential for female reproductive success.

7:3:3 Reproductive success of monogamous and polygynous males

There was a significant difference in the observed number of young fledged on the territories of monogamous and polygynous males (Mann-Whitney U-test, $z = -2.50$, $P < 0.005$, Figure 7:2), with polygynous males fledging nearly twice as many young. However, when the numbers of fledglings actually sired on the territory by polygynous and monogamous males were compared, there was no significant difference between the two (Mann-Whitney U-test, $z = -0.99$, $P = 0.16$, Figure 7:3). There was however a tendency for monogamous birds to fledge fewer young than polygynous males. Further evidence of a difference in reproductive output between monogamous and polygynous males was revealed in the multiple regression analysis of all offspring (not just those surviving to fledge), which showed that polygynous males did in fact sire more young on their own territories than did monogamous males (see Chapter 5). That this is not revealed in the above bi-variate, non-parametric test may well be a reflection of the lower power of this test due to the smaller sample size, because only nests which fledged young were used. It is clear, though, that whatever genetic benefits males may accrue through social polygyny they are not as great as they would appear to be simply from the observed number of young fledged on their territories (the mean apparent number of young fledged by polygynous males was 8.8 chicks, whereas the mean actual number sired was 3.5 chicks).

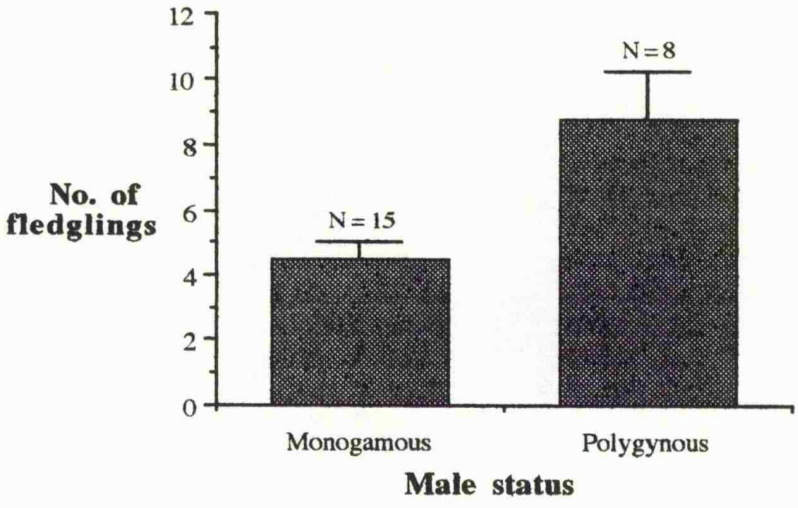

Figure 7:2. Apparent fledging success of monogamous and polygynous males (i.e., total fledging success for two nests on their territories).
Values are means (SE).
Mann-Whitney U-test, $z = -2.50$, $p < 0.005$.

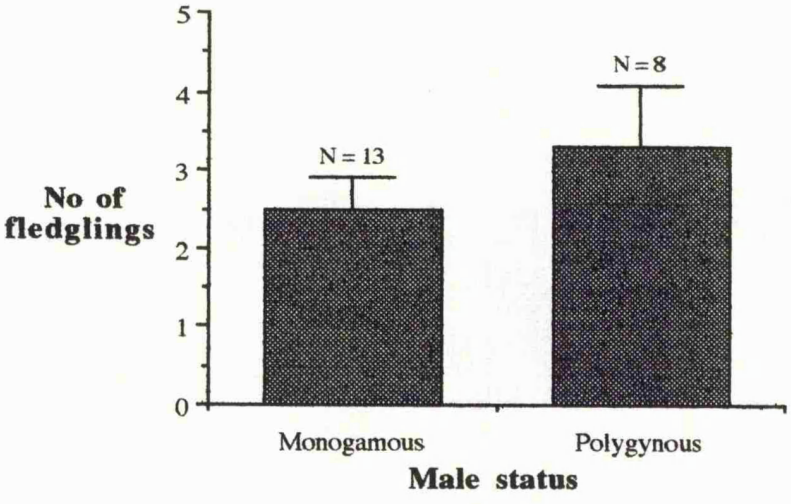

Figure 7:3. Actual fledging success of monogamous and polygynous males determined through DNA fingerprinting.
Values are means (SE).
Mann-Whitney U-test, $z = -0.99$, $p = 0.16$.

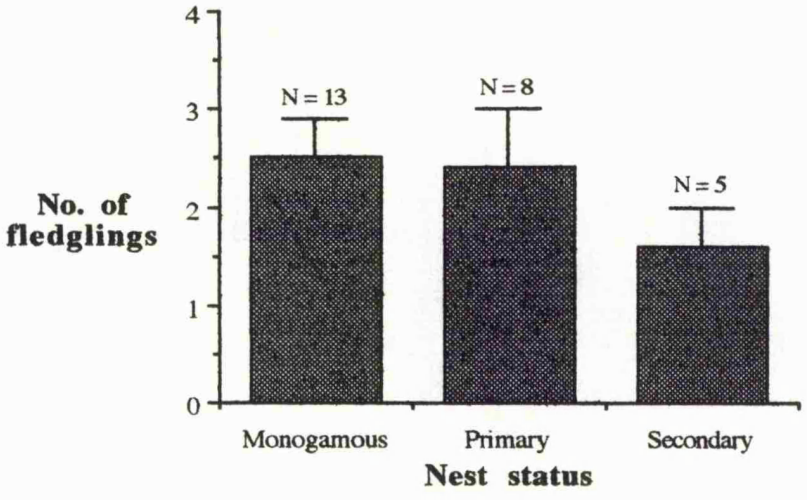

Figure 7:4. Actual male fledging success of monogamous, primary and secondary nests, determined through DNA fingerprinting. Values are means (SE).
Kruskal-Wallis one-way ANOVA, H (corrected for ties) = 1.16, $df = 2$, $p > 0.1$.

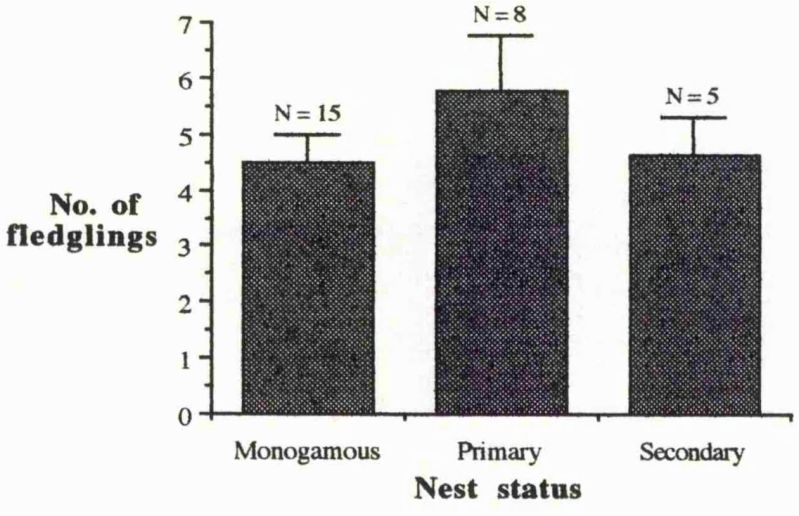

Figure 7:5. Female fledging success in monogamous, primary and secondary nests. Values are means (SE).
Kruskal-Wallis one-way ANOVA, H (corrected for ties) = 1.61, $df = 2$, $p > 0.1$.

	z value	*p*
Plumage Score	-0.308	0.37
Body Condition Index	-0.493	0.31
Tarsus Length	-1.272	0.10
Wing Length	-0.834	0.20
Weight	-0.560	0.29
Head Length	-1.512	0.07
Song Rate	-1.362	0.09
Feeding Rate	-0.297	0.38
Age	-0.000	0.50

Table 7:2. Results from Wilcoxon signed-rank test for matched pairs of phenotypic characters between polygynous males and their monogamous neighbours. *z* values are corrected for ties.

When the number of young sired by monogamous and polygynous males was examined more closely, it was found that the main loss of paternity for polygynous males was in the nests of secondary females, though this difference was not statistically significant (Kruskal-Wallis one-way ANOVA, H (corrected for ties) = 1.613, $df = 2$, $P > 0.1$, Figure 7.4).

7:3:4 Reproductive success of monogamous, primary and secondary females

There was no significant difference between the number of young fledged from monogamous, primary and secondary females (Kruskal-Wallis one-way ANOVA, H (corrected for ties) = 1.162, $df = 2$, $P > 0.1$; Figure 7.5). The importance of paternal investment in relation to female reproductive success is discussed more fully in chapter 9.

7:3:5 Female choice in social polygyny

All widowed females remained on the territory of their former mate. Thus females were not choosing to pair polygynously with neighbouring males simply on the basis of gaining access to superior territories, suggesting that direct environmental benefits to females do not play a major role in female social mating decisions.

There was no significant difference for any of the phenotypic measures tested between polygynous males and their monogamous neighbours (Table 7:2). Thus, it appears that widowed females were not actively choosing to pair with phenotypically superior males from among the available monogamous neighbours.

7:4 Discussion

This study reports a much higher level of polygyny than previous studies on the reed bunting. In the case of the former studies by Bell (1968) and Hornby (1971), the breeding bird surveys were carried out over a much larger area than in this study, the result being that not all nests were found. In these circumstances it is easy to envisage that some females in polygynous trios may have been

overlooked. This study involved very intensive observation of a smaller area and I believe that no nests or females were missed throughout the three breeding seasons.

The presence of sparrowhawks hunting over the study area probably also had a dramatic effect on the frequency of polygyny. Analysis of prey remains at sparrowhawk plucking posts has shown that they contain a disproportionate number, in relation to their abundance, of species which sing from prominent song posts (Newton, 1986). Male reed buntings are thus particularly vulnerable, especially during the early part of the breeding season when most males are in song (O'Malley, 1993). This situation would not have occurred during the studies by Bell and Hornby, because sparrowhawks were very much diminished in numbers in the late 1960s, particularly in central and eastern districts of Britain (Newton, 1986).

Given that polygyny is essentially an opportunistic phenomenon in the reed bunting, the more interesting question is how monogamy has evolved in the species. In the preceding analyses I tested predictions borne from three hypotheses for the evolution of monogamy in birds, the results of which I shall discuss in turn.

(A) Monogamy should evolve when male parental care is both non-shareable and indispensable to female reproductive success.

This study has shown that male parental care is not essential for female reproductive success. Thus the above hypothesis cannot explain the prevalence of monogamy in this species.

(B) Monogamy should evolve when males are less successful with two mates than with one.

Monogamous males do not fledge more young than polygynous males, suggesting that monogamy has probably not evolved in this species as a result of increased male reproductive success. In fact, polygynous males tend to fledge more young (though not significantly so) than monogamous birds. However, fledging success is only one measure of male reproductive success and may not be closely related to the number of young recruited into the breeding population. Thus, monogamy may have arisen because paternal investment increases male reproductive success in terms of offspring recruitment.

The importance of paternal investment in relation to male reproductive success is discussed more fully in chapter 9. Nestlings which received male care were fed more than those which did not, and this may have increased their

survival rate. That this difference in feeding rate was not reflected in fledging success may mean that paternal care is only essential in years of poor food supply and that such a 'bad' year did not occur during this study.

(C) Monogamy would be expected if pairing with an available unmated male results in higher female fitness than pairing with an already mated male.

The results showed that the reproductive success, in terms of number of young fledged, was not significantly different for monogamous, primary or secondary females. Secondary females received no help in rearing their offspring and fed at a higher rate than monogamous and primary females (see chapter 9). This higher feeding rate may well adversely affect female survival or future fecundity but unfortunately I obtained no data which would allow this hypothesis to be tested. It is likely that females benefit in some way, primarily through guaranteed shared parental care, when mated to a monogamous male rather than a polygynous male.

Conversely, there appear to be no benefits for females in polygynous pairings. Females suffer a potential cost in polygynous matings through a possible absence of male parental care, and also do not appear to gain any direct or indirect genetic benefits from such matings. It is worth noting that all females formed socially monogamous pair bonds (only males had two mates), yet virtually all were polyandrous through EPC behaviour (see Chapter 4). Thus the social mate choice was not reflected in their genetic mate choice.

Though the costs and benefits of polygynous pairing appear not to be particularly drastic, there is a clear trend towards polygyny being beneficial for males and costly for females. Thus, males should sieze any opportunity to become polygynous, whereas females may be forced into the situation where they have to make the 'best of a bad job' and mate with an already mated male to ensure that they at least have some reproductive success. In fact, the high predation rate on nests means that a remated widow is just as likely to hatch the first brood on the territory (and so receive male help in rearing the brood) as the original female.

The most plausible explanation for the evolution of monogamy in the reed bunting is that the opportunity for polygyny by is limited (Davies, 1991). Male-male competition for mates, and female-female aggression to ensure male assistance are two of the more obvious constraints which act to decrease the chance of a male obtaining additional mates.

The data from DNA fingerprinting on true reproductive success in male reed buntings suggest that the benefits of polygynous behaviour are not as great

as would appear from direct observation. This is probably due to the inability of males to effectively protect their paternity with two females, either through reduced mate guarding, preferential allocation of copulations to primary females or sperm depletion. Alternatively, secondary females are more active in the pursuit of EPCs than primary females. Unfortunately I have no data on female behaviour to test this hypothesis.

Predominantly mongamous species, such as the reed bunting are possibly not well adapted to polygynous behaviour, and thus less able to protect their paternity in secondary nests as effectively as those species which are regularly polygynous, such as the corn bunting *Miliaria calandra* (Hartley, 1991). Therefore, as well as the more obvious constraint of limited opportunity for polygyny, there may be an additional, more subtle constraint on the protection of paternity. This constraint needs to be incorporated into future models on the costs and benefits of monogamous and polygynous behaviour in birds.

Chapter Eight

SPERM COMPETITION AND THE REPRODUCTIVE ORGANS OF THE REED BUNTING

Abstract

The morphology of the reproductive organs of a male and female reed bunting are described in relation to the high levels of sperm competition exhibited in the species. Males exhibit several morphological adaptations associated with high levels of sperm competition as a result of extra-pair copulation behaviour. The volume of the cloacal protuberance in relation to body weight was 26.5, the higest recorded for a socially monogamous paserine. The weight of testes (0.552g) was 25% greater than that expected for a bird of comparable size. The spermatozoa were amongst the longest recorded for any avian species (mean length = 242 μm). The morphology of the female tract also showed adaptation associated with a high level of sperm competition. The female examined had a low number of sperm storage tubules (469 in 15 mucosal folds of the utero-vaginal junction), but these tubules were extremely long (mean length = 865 μm). An analysis of the sequence of fertilisation in a series of broods revealed that 100% last male sperm precedence is unlikely and that fertilisations are not independent of each other.

8:1 Introduction

If frequent pair copulation works as a paternity guard, then selection should favour males that produce and deliver relatively more, and better quality, sperm than competing males (Møller, 1988c). In addition, females of many bird species are known to store sperm following copulations (Howarth, 1974; Birkhead, 1988). This storage of sperm further increases the possibility of sperm competition through direct competition between sperm within the female reproductive tract (Parker, 1970, Birkhead and Møller, 1992a). Recent comparative analyses have indicated that physiological adaptations of the reproductive organs have evolved in tandem with the degree of sperm competition found in a variety of avian species (Møller, 1991c; Briskie and Montgomerie, 1992; Birkhead *et al.*, 1993).

Males with large testes have been found to deliver more sperm per ejaculate than males with smaller testes (Burrows and Titus, 1939; Møller, 1988c), and an analysis, controlling for phylogenetic effects, on a wide range of avian species revealed that species with a high degree of sperm competition have larger testes than those with little or no sperm competition (Møller, 1991). In addition to quantitative differences in the production of sperm, direct sperm competition within the female reproductive tract is also expected to lead to qualitative differences in sperm (Gomendio and Roldan, 1991; Briskie and Montgomerie, 1992).

Males are known to store sperm in the seminal glomeri, which are situated close to the vent of the cloaca (Wolfson, 1954). During the breeding season the seminal glomera become engorged and this results in a swelling of the tissue of the cloaca; this is known as the cloacal protuberance. The size of the cloacal protuberance varies in relation to the stage of the nesting cycle, reaching maximum size during the fertile period of the female (Hegner and Wingfield, 1986; pers. obs.). A recent comparative analysis of passerine birds has provided evidence that the size of cloacal protuberance is correlated with the degree of sperm competition found in a species (Birkhead *et al.*, 1993).

The females of several bird species are known to store sperm following copulation, prior to fertilisation of their eggs (Howarth, 1974; Birkhead, 1988). The primary storage site is at the utero-vaginal junction (UVJ), where sperm is held in blind ending tubes called sperm storage tubules (Bakst, 1987). The variation in the number of sperm storage tubules between individuals of the same

species is small but across a range of species the number of sperm storage tubules varies by nearly two orders of magnitude (Birkhead & Hunter, 1990a; Birkhead, 1988; Birkhead & Møller, 1992a). There are several hypotheses proposed as an explanation of sperm storage in birds:

(1) It may be a consequence of their common reptilian ancestry.

(2) Storage may be necessary to ensure fertilisation because of the lack of synchrony between copulation and fertilisation.

(3) Sperm storage avoids the potential costs of repeated copulation to fertilise each egg because of sequential ovulation and fertilisation in birds.

(4) Sperm storage may facilitate female manipulation of paternity through increased sperm competition.

At the moment it is not known which of these reasons offers the best explanation and further data are required on sperm viability and fertile periods to explore fully the adaptive significance of sperm storage in birds (Birkhead & Møller, 1992b).

It is evident that sperm competition has not resulted in the evolution of behavioural traits alone, but also in the concomitant evolution of morphological characters. In chapter 6, I investigated the behavioural traits associated with sperm competition in the reed bunting. Here, I provide data on the physiological adaptions associated with sperm competition, which are subsequently discussed in conjunction with the results of the behavioural study. This combined behavioural and physiological approach to provides a more coherent picture of the mechanism of sperm competition operating in the reed bunting.

The morphology of the female reproductive tract provides important clues as to the degree and mechanism of sperm competition exhibited by a species, but to discover the actual process by which sperm competition takes place requires thorough experimental work. Captive experiments have been undertaken on a small number of species to explore explore the process of copulation and fertilisation in birds. The majority of these studies have been carried out on commercially important species (chickens *Gallus domesticus*, e.g. Warren & Gish, 1943); turkey *Meleagris gallopavo*, Payne & Kahrs, 1961; Mallard *Anas platyrhynchos*, Cheng *et al.*, 1983), but there have also been a series of detailed experiments on captive zebra finches *Taeniopygia guttata* (Birkhead *et al.*, 1988a; 1989).

When the sperm of two or more males compete to fertilize an egg, only one sperm will be successful. Experimental studies with temporally separated

copulations have shown that the last male mating tends to fertilise most of the eggs (e.g., Birkhead *et al.*, 1988a; 1989). This is referred to as last male sperm precedence, and the proportion of offspring fathered by the last male to mate is referred to as the P_2 value. A P_2 value of 0.5 means that two males have an equal chance of fertilisation success; a value higher than 0.5 indicates last male advantage to a maximum P_2 value of 1.0, which is 100% last male sperm precedence.

The presence of stored sperm obviously has important implications for sperm competition and last male sperm precedence. There are not enough data available to determine the average period sperm remain viable in the sperm storage tubules of birds, but work on captive zebra finches and bengalese finches *Lonchura striata* (the only passerines for which data exists) suggest a period of sperm viability of 8 - 10 days (see Birkhead & Møller, 1992a).

The exact mechanism of sperm release from sperm storage tubules is also unknown, but it is known that released sperm can only reach the infundibullum (the site of fertilisation) at certain times during the laying period because the developing egg blocks the passage of sperm up the oviduct (Birkhead and Møller, 1992a). The only time that the oviduct is empty and sperm can travel unimpeded from either the vagina or the sperm storage tubules, is during a period of about an hour after the laying of one egg and the ovulation of the next ovum. This period has been referred to as the fertilisation window (Cheng *et al.*, 1983).

It is well documented in birds that extra-pair copulations can result in fertilisation and the presence of extra-pair young in broods (see Birkhead and Møller, 1992a for review), but much less is known of the pattern or sequence of paternity in broods. The sequence of fertilisation is determined by a number of factors: behavioural (i.e., copulation frequency, order and timing), physiological (i.e., sperm numbers, size and storage) and mechanistic (i.e., method of sperm storage and release). In this chapter I present data on the sequence of within-pair and extra-pair fertilisations in the reed bunting. Such data provide an insight into the behavioural, physiological and mechanistic processes of sperm competition in a wild bird species.

7:2 Methods

8:2:1 Examination and dissection of reproductive organs

Males were caught during the breeding season as described in Chapter 2. The dimensions of the cloacal protuberance of each male were measured to the nearest 0.1 mm using a graduated caliper. The dimensions recorded were height and width. The volume of the protuberance was calculated by the equation πr^2 x height, the protuberance being approximately cylindrical in shape. If a bird was caught on more than one occasion during the breeding season the cloacal dimensions were recorded each time. However, there were insufficient data collected to relate protuberance size to the stage of the breeding cycle.

The volume of the protuberance increased to a maximum size as the breeding season progressed (unpublished data). To control for this development in volume, only the maximum protuberance volume recorded for each male was used, and only measurements taken after 1st May (this date approximates to the time of maximal development of the cloacal protuberance; pers. obs.) were used in the analysis of mean protuberance size.

One male was found freshly killed by the side of the road which ran through the centre of the study area and was subsequently stored at -70°C for several months. The bird was taken to the University of Sheffield, where Prof. T. R. Birkhead carried out the dissection. The testes were removed, measurements taken with calipers to the nearest 0.1 mm and individually weighed to 0.001 g. The seminal glomera were removed by dissection, measured and weighed in a similar manner and the sperm they contained were counted. This was done by macerating the tissue in a known quantity of phosphate buffered saline (PBS) and counting the sperm in an improved Neubauer counting chamber.

A female reed bunting was found freshly killed by the roadside on the morning before the third and final egg of her clutch was laid (as evidenced from the dissection). This bird was subsequently stored at -70°C, and gradually thawed at room temerature for the dissection at a later date. The dissection was undertaken by Prof. T. R. Birkhead at the University of Sheffield. The utero-vaginal junction region of the oviduct was removed and examined as described by Birkhead and Hunter (1990a).

8:2:2 Sequence of fertilisation

Nests were found prior to laying and the eggs were numbered sequentially on the day they were laid using a waterproof marker pen. Two methods were employed to determine from which egg offspring were derived. Firstly, in 1991 nests were visited hourly during hatching and the claws of each chick were clipped with fine scissors as they hatched. A different toe was marked in this way for each egg. The clipped toe was distinguishable for about 5 days, after which metal BTO rings were applied. Using this method, data were obtained on the hatching sequence of only two nests because most chicks hatched out overnight.

In the 1992 breeding season, eggs were removed (under English Nature License) and substituted with plaster replica eggs as they were laid. The real eggs were labelled and placed in an incubator. The objective was to return the chicks back to their respective nests on hatching. The egg removal served two functions, firstly to save DNA samples from predators and secondly to provide data on the sequence of extra-pair fertilisations in the brood.

8:3 Results

8:3:1 Male reproductive tract

(A) Measurement of live birds

The cloacal protuberance: Dimensions of the cloacal protuberances of 11 males were obtained. The mean volume of the protuberances was 0.41 cm^3 (range = 0.32 to 0.53 cm^3), whilst the mean height was 6.98 mm. The mean volume index in proportion to the body weight was 26.5.

(B) Dissection

The dissection of the male revealed no major anatomical differences in the reproductive organs to that of any other passerine (Figure 8.1).

Testes: The weight of both testes combined was 0.552 g, which represented 2.6% of the male's body weight (i.e., 21.6 g when weighed alive three days before death). Thus the testes size is approximately 25% greater than that expected for a bird of this size (Møller, 1988). The dimensions of the testes were 10.6 x 8.7 mm (left) and 9.3 x 7.6 mm (right). The mean weight of both seminal

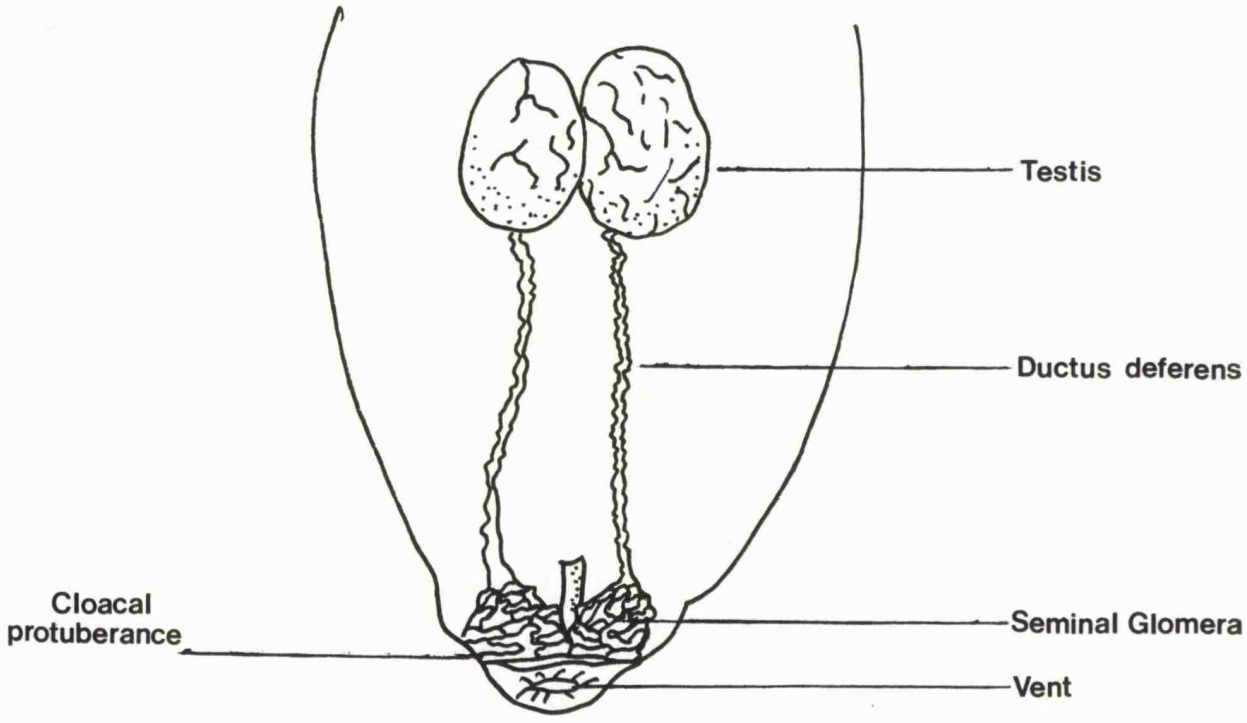

Figure 8.1 The male reproductive system (adapted from Sturkie, 1954)

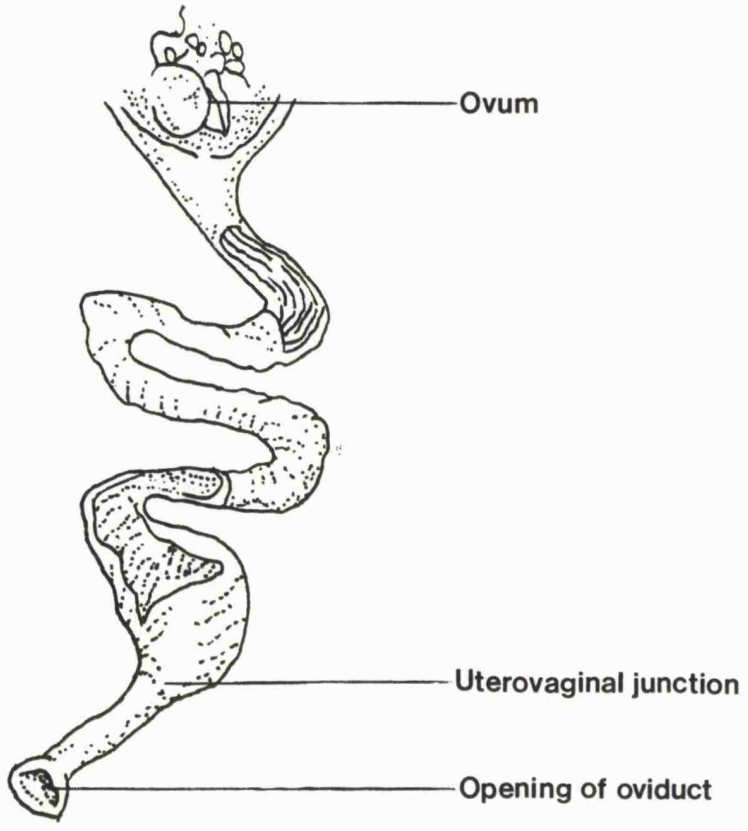

Figure 8.3 Female reproductive system (adapted from Sturkie, 1954)

glomera combined was 0.098 g which represented 0.45% of the male body weight.

Seminal glomera: The dimensions of the seminal glomera were 7.7 x 4.6 mm (left) and 8.0 x 4.4 mm (right). Both seminal glomera combined contained a total of 1.78×10^6 sperm.

Sperm: Unfortunately, the sperm within the seminal glomera showed signs of degradation as none of those examined had any visible heads. This was not due to any infertility on the part of the male because the DNA analysis proved that he sired several offspring. Intact sperm were found in the sperm storage tubules of the dissected female; these were extremely long, with a mean length of 242 μm (Figure 8.2).

8:3:2 Female reproductive tract

The dissection showed that there were no major anatomical differences between the reproductive organs of the female reed bunting and other passerine species (figure 8.3).

Utero-vaginal junction: The number of primary mucosal folds found in the utero-vaginal junction was 15. Detailed examination of three of these folds revealed 29, 33 and 32 sperm storage tubules in each (mean 31.5 sperm storage tubules/mucosal fold). Extrapolating from this result, there was a minimum number of 469 sperm storage tubules in the utero-vaginal junction of this particular female. Unfortunately, some tissue degredation had taken place and the mucosal folds were extremely delicate, resulting in the disintegration of many sperm storage tubules. Thus, it is possible that 469 is an underestimate of the actual number of sperm storage tubules possessed by this female.

No branched tubules were noted, all were straight blind ending tubes (figure 8.4). The most striking characteristic of the tubules was their large size; of 26 complete tubules measured, the mean length was 865 μm (SE 200 μm). The largest tubule measured was 1,270 μm in length. The width of the tubule was quite uniform, being between 40 and 60 μm in diameter.

The proportion of sperm storage tubules containing sperm was very low at 4.8% (7/146 tubules examined). The orientation of sperm in the tubules was smilar for all those examined. In every case the sperm heads were found to face the blind end of the sac (figure 8.5).

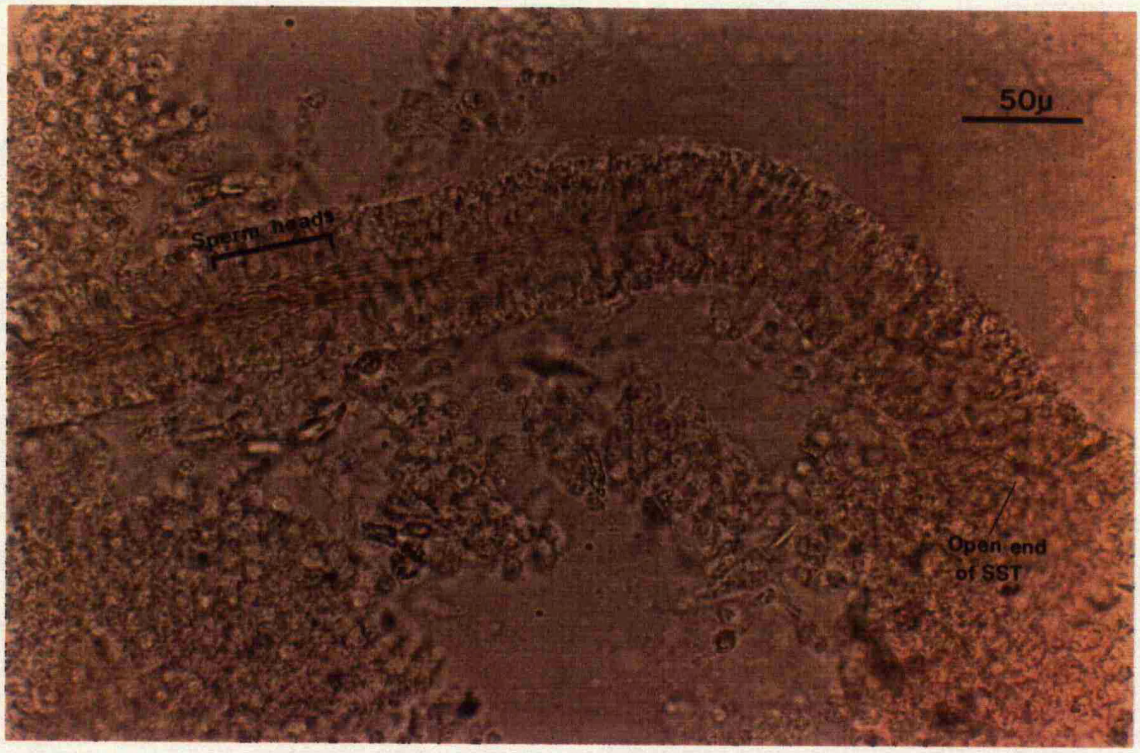

Figure 8.2: Photograph showing detail of spermatozoa. The spermatozoa are stored in a sperm storage tubule of a female reed bunting.

Figure 8.4: Photograph illustrating the simple structure and length of sperm storage tubules in a female reed bunting.

Figure 8.5: Photograph illustrating the orientation of sperm in a sperm storage tubule of a female reed bunting. The sperm heads point towards the blind end of the tubule. *N.B* a coiled loop in the sperm tails can be seen in this photograph.

	Laying sequence				
FAMILY	1	2	3	4	5
A	√	√	*	*	*
B	*	*	?	?	*
C	*	?	√	√	
D	?	?	*	*	?
E	*	√			
F	?	√	?	?	√
G	?	?	*	√	*
H	*	?	?	√	√

Table 8.1. Sequence of paternity for eight broods where chicks could be related to their position in the egg laying order, and hence their order in the fertilisation sequence.

Key: √ = Within-pair fertilisation

* = Extra-pair fertilisation

? = Sire unknown because of DNA degradation

8:3:3 Sequence of fertilisation

The experimental removal of clutches was not particularly successful and was abandoned after manipulation of all the first broods. Of 11 clutches substituted, 6 were deserted, 4 were predated and only one brood could eventually be returned to the nest.

The artificial incubation of eggs also proved difficult and most embryos died during development (usually after about 7 days). The death of the embryos was not discovered until the eggs had passed the duration of the incubation period and, consequently, the DNA was degraded in many instances. Degradation of the DNA samples meant that many offspring could not be included in the single-locus fingerprint analysis.

In total, sequence data was obtained for eight broods. However, not all broods are complete because of DNA degradation (Table 8.1). In those broods where the paternity of more than one offspring was identified, five were of mixed paternity (i.e., broods A, C, E, G and H). In four cases the extra-pair young occurred earlier in the sequence and in a single instance a within-pair fertilisation occurred between two extra-pair fertilisations (i.e., brood G). Brood A provided a revealing result in that the pair male died the day before the first egg was laid (this was the male used in the dissection), and yet still sired the last three eggs of the clutch.

8:4 Discussion

Male reproductive tract and sperm competition

The size of the cloacal protuberance was large in relation to the body size of the male reed bunting, and in comparison to a range of species for which data are available (from Wolfson, 1954; Nakamura, 1990; Birkhead *et al.*, 1991):

	cm^3/g
Rufous-sided towhee *Pipilo erythrophthalamus*	2.6
Field sparrow *Spizella pusilla*	9.6
Song sparrow *Melospiza melodia*	10.5
White-throated sparrow *Zonotrichia albicollis*	10.7
Slate-coloured junco *Junco hyemalis*	13.4
Swamp sparrow *Melospiza georgiana*	17.1
Dunnock *Prunella modularis*	21.7
Reed bunting	**26.5**
Alpine accentor *Prunella collaris*	55.7

Dissection showed that this protuberance consisted mainly of the seminal glomera, which are extensively coiled tubules of the ductus deferens which transport the sperm from the testes. From the size alone, it appears that the reed bunting is morphologically adapted for storage of large sperm reserves in the seminal glomera.

In the specimen dissected, the tissues and the sperm found inside the tubules showed signs of degradation which may well have affected the results of the sperm storage analysis. The number of sperm found in the seminal glomera was very low when compared to other passerines which have been analysed; 1,060 x 10^6 in the dunnock (Davies, 1992) and 7.69 x 10^6 in the bengalese finch (Birkhead, 1991). In species which copulate frequently, the number of sperm per ejaculate is lower than in species which copulate less frequently (Møller, 1988d). Thus, though the low sperm storage number may possibly be an artifact caused by tissue degradation it may also be a function of the copulation frequency in the species.

Møller (1988c) in a cross-species analysis, showed that the amount of sperm produced by a male is related to testes weight. The testes of the reed bunting were 25% larger than predicted by the body size of the species. This result indicates that the species is morphologically adapted for high levels of sperm production.

The length of the sperm in the reed bunting is among the highest recorded for any bird species, with most previous recorded lengths ranging from 50 - 300 μm (Briskie & Montgomerie, 1992). Large sperm size is associated with a high degree of sperm competition in mammals (Gomendio & Roldan, 1991) and in Coleoptera (Dybas & Dybas, 1981), though Briskie and Montgomerie (1992) failed to find a relationship between sperm size and sperm competion in a diverse range of avian species. However, there was a positive correlation between sperm length and the length of the female sperm storage tubules and a negative correlation between sperm length and number of sperm storage tubules (Briskie and Montgomerie, 1992). From these correlations, it was concluded that sperm competition does influence sperm size in birds, because larger sperm swim faster and selection would favour long sperm when sperm storage tubules are in short supply; sperm long enough to fill a storage tubule might also prevent the storage of sperm from other males. The size and number of both sperm and sperm storage tubules found in the reed bunting concur with this conclusion.

Female reproductive tract and sperm competition

The number of sperm storage tubules found in the female was very low in comparison to that found in other species (e.g., 1499 sperm storage tubules in the zebra finch, 1511 sperm storage tubules in the bengalese finch (Birkhead & Møller, 1992b). This was possibly due to the condition of the tissue of the mucosal folds rather than a real lack of sperm storage tubules. Alternatively, the number of sperm storage tubules is low in the species and acts to increase direct sperm competition within the female reproductive tract. The negative correlation between sperm size and number of sperm storage tubules (Briskie and Montgomerie, 1992) suggests that such a mechanism might well operate in the reed bunting.

An interesting feature of the female reproductive tract which was revealed by the dissection was the extreme size of the sperm storage tubules. The mean length was longer than that recorded for any other avain species (Birkhead & Møller, 1992; Briskie and Montgomerie, 1992). Comparative studies suggest that on average sperm are about one-third the length of their sperm storage tubules (Birkhead & Møller, 1992b), and this is broadly similar in the reed bunting (mean sperm length 242 μm with mean sperm storage tubule length 865 μm). If, as seems likely, spermatozoan length is a reflection of the degree of sperm competition in a species (Briskie & Montgomerie, 1992), then the evolution of larger sperm storage tubules in the female is a corollary of this male morphological adaptation.

The low proportion of sperm storage tubules containing sperm was expected as the female had recently laid her second egg and ovulated her third and final egg. Sperm are known to be squeezed from the storage tubules during oviposition (T. R. Birkhead, Pers comm.). The exact mechanism of uptake and release of sperm from sperm storage tubules is still unclear, but only viable sperm enter them (Bakst, 1987) and in some species it is known that sperm continue to enter the sperm storage tubules 48 hours after insemination (Brillard & Bakst, 1990). Once egg laying commences sperm are continuously leaked from the sperm storage tubules. The trigger which initiates the release of the spermatozoa is not known but it is possiblly a result of chemical factors in the female follicular fluid (Birkhead & Møller, 1992b).

Examination of the reproductive organs of both male and female reed

buntings indicates that the species is morphologically adapted for a high degree of sperm competition. The reed bunting is unusual in this respect as most species exhibiting such physiological adaptions have distinct social mating systems associated with sperm competition (e.g., dunnock, Smith's longspur, alpine accentor). Direct observation alone would lead to the assumption that the reed bunting is a typical monogamous species, whereas DNA fingerprinting has shown that females are highly polyandrous. Presently, the reed bunting is in the unique position of being the only species known to exhibit morphological adaptations of the reproductive organs associated with extra-pair copulation behaviour.

Sequence of fertilisation

Despite a very incomplete data set the results of the sequence of fertilisation analysis have provided some insight into the mechanism of fertilisation in the reed bunting.

Last male sperm precedence in the reed bunting is not 100% as indicated from the results of brood A. The P_2 values found in the limited number of bird species so far investigated, suggest that the extent of sperm precedence varies rather little between species at about 0.8 (i.e., 80% last male sperm precedence; Birkhead & Møller, 1992a). In the case of brood A, the extra-pair male was seen associating with the female on the day the pair male died and this association continued until the female began incubation i.e., the morning of the fourth egg. No copulations were observed between the birds, thus it is not known which of the two males was the last to copulate. Circumstantial evidence suggests that reed buntings are frequent copulators and it is known that copulations occur after clutch initiation. In these circumstances it seems very unlikely that the dead pair-male was the last male to copulate with the female.

Another important fact discovered from the case of brood A is that each fertilisation is not the result of a separate copulation, and as such the numbers of extra-pair young in a brood are not independent of each other, as has been assumed in some recent studies (e.g., Kempenaers *et al.*, 1992). Sperm storage must play some role in the fertilisation of eggs in wild birds otherwise eggs number 3, 4 and 5 would not have been fertilised in brood A. A recent paper by Oring *et al.* (1992) has illustrated this point dramatically in the double brooded spotted sandpiper *Actitis macularia*, where sperm can remain stored and viable for lengthy periods. It would seem that fertilisations can result from both stored sperm and from recent inseminations, so this complex pattern of copulation and

fertilisation make empirical measurement extremely difficult. To combat this, theoretical models have been developed as a way of understanding sperm competition in birds (Lessells & Birkhead, 1990).

Lessells and Birkhead (1990) devised three sperm competition models, using data from chickens, to distinguish between several different mechanistic hypotheses that could account for last male sperm precedence. They concluded that a model incorporating both limited sperm storage and sperm displacement seemed to offer the most likely mechanism for the observed patterns of sperm precedence in birds. There is no evidence to suggest that females can regulate the release of sperm from their sperm storage tubules and so control the paternity of the offspring, but they can eject sperm from the cloaca in some species (e.g., dunnock, Davies, 1983; Japanese quail *Coturnix japonica*, Birkhead & Møller, 1992b) and most species are probably able to devalue a copulation through defaecation (T. R. Birkhead, pers. comm.).

It is possible that in the future a more complete data set could be obtained from the sequences by using microsatellite DNA polymorphic marker loci which have been recently identified in the reed bunting (Hanotte *et al.*, submitted), but in the absence of complete copulation data the results would add little else to what as been discovered already, i.e., that last male sperm precedence is less than 100%, stored sperm does result in the fertilisation of some eggs and that extra-pair young in a brood should not be regarded as independent of each other.

Chapter Nine

PARENTAL INVESTMENT AND THE SOCIAL MATING SYSTEM

Abstract

In this chapter I examine the nestling provisioning behaviour of polygynous males and their mates at 21 nests in 8 different territories. All polygynous males had two females nesting on their territory, and all nesting attempts by polygynous females overlapped with each other (11/11 pairs of nesting attempts). Males provided an average of 40% of the nestling feeds to only one nest on the territory (the primary nest), whilst the nestlings in the other were fed solely by the female (the secondary nest). There was a non-significant tendency for secondary (unassisted) females to feed at a higher rate than monogamous and primary (male-assisted) females. This apparent compensatory increase in feeding rate by secondary females resulted in the total level of feeds at secondary nests being not significantly different from monogamous or primary nests. There was, however, a tendency for secondary nests to receive less feeds than primary and monogamous nests. Males based their decision on which nest to feed primarily on the basis of which nest hatched first, i.e., the age of the brood. Males preferentially fed older broods. There was some evidence to suggest that brood size may modify male feeding decisions, where males preferentially feed younger but larger broods.

9:1 Introduction

In monogamous systems, both parents normally have a vested interest in rearing their brood, but because parental investment in offspring may be costly, each sex should try to minimise its investment at the expense of its partner (Maynard Smith, 1977; Trivers, 1972). When the costs and benefits of parental investment differ between either member of a pair, their relative share of the total effort would be expected to change accordingly. So provisioning should be considered as an evolutionary game (Chase, 1980; Houston & Davies, 1985; Winkler, 1987).

The game theory approach to parental investment suggests that biparental care can be stable in situations where the response by either parent is insufficient to compensate for reduced investment by the other (Chase, 1980; Houston & Davies, 1985). Several studies of biparental care in birds have shown that both males and females are capable of increasing their provisioning rate if the other partner deserts, is removed or manipulated experimentally (e.g., Weatherhead, 1979; Lyon *et al.*, 1987; Alatalo *et al.*, 1982; Wright & Cuthill, 1989). Empirical data from such studies show that single females can increase their provisioning rate but not fully compensate for the loss of help provided by the absent mate (e.g., Leffelaar & Robertson, 1986; Alatalo *et al.*, 1988; Hatchwell & Davies, 1990 but see Smith *et al.*, 1982; Wolf *et al.*, 1990).

In polygynous mating systems, the conflict of reproductive interest between the sexes is often expressed in patterns of parental care (Clutton-Brock, 1991). In such systems males will often have offspring in more than one brood simultaneously. The maximisation of their overall reproductive success may be achieved through the partitioning of paternal care between broods (reviewed by Verner & Willson, 1966; Wittenburger, 1981b). The decision on how best to allocate their paternal investment may be based on the relative reproductive value of the broods (Patterson *et al.*, 1980; Whittingham, 1989). The outcome of this decision process often results in asynchronous paternal investment, whereby one brood is favoured over another (e.g. Martin, 1974; Patterson *et al.*, 1980; Davies & Hatchwell, 1992).

The absence of paternal care to secondary nests in polygynous systems has been shown to reduce fledging success in many species (e.g., Alatalo *et al.*, 1981; Catchpole *et al.*, 1985; Davies & Hatchwell, 1992). Additionally, the increased work load associated with the absence of male parental investment may

have a deleterious influence on female fitness, possibly through a decrease in female survival and hence future reproduction (see Clutton-Brock, 1991 for theoretical review) or indirectly through lower survival rates of offspring (e.g., Wolf *et al.*, 1988, 1991; Sasvari, 1986).

The reed bunting is a predominantly socially monogamous species. However, males have the potential to increase their reproductive success by becoming polygynous, whereas females do not (chapter 7). In this study, polygyny was opportunistic and occurred primarily through mate replacement (chapter 7). In this chapter I describe the influence of social mating behaviour on the pattern of parental investment seen in the reed bunting.

Firstly, I examine the potential cost of polygyny to females in terms of parental investment. I have shown previously that there was no measurable cost in terms of fledging success of females in polygynous systems (Chapter 7). However, females may well incur additional costs through increased levels of provisioning. To test this hypothesis, I compare the level of female nestling provisioning provided by monogamous and polygynous females. Polygynous females may also suffer through reduced offspring survivorship due to their incomplete compensation for having no male assistance. To examine this possibility, I compared the total provisioning level at monogamous and polygynous nests, and use this as an indicator of offspring fitness.

Secondly, I examine male parental investment in monogamous and polygynous mating circumstances. Males can respond to the increased demand for paternal investment as a result of polygyny in two ways. They can (A) increase their total level of parental effort, or (B) allocate their investment between broods in such a way as to maximise their reproductive success.

The first of these hypotheses can be tested by simply comparing the level of paternal investment between monogamous and polygynous males. If males are capable of increasing their level of parental care, then it would be expected that polygynous males would work harder to feed two broods. To examine the second hypothesis I analyse what possible factors influence male parental investment decisions. If the feeding behaviour of polygynous males is adaptive, they would be expected to maximise their reproductive success by investing in the brood which is of highest value to them. Such decisions may be based on a number of factors, which I have outlined below.

(I) Polygynous males may be unable to protect their paternity effectively in the nests of both the primary and secondary females (see Chapter 6). Levels of extra-

pair paternity have been shown to be higher (though not significantly so) in the nests of secondary females than in those of primary or monogamous females (Chapter 7). Males may thus preferentially feed the nest where the ability to protect paternity was least constrained, i.e., the first nest initiated. Following the simple rule of always feeding the first brood initiated would then be a non-facultative, but nonetheless adaptive, response to a potential variance in the level of extra-pair paternity in broods. *Prediction* - Males feed the first brood initiated on a territory.

(II) Males may assess their level of paternity in each of the broods of polygynous females and make a facultative decision about which brood to feed in relation to their perceived confidence of paternity. *Prediction* - Males feed the nests in which they have more paternity.

(III) The reproductive value of a brood increases as the young approach independence (Andersson *et al.*, 1980), thus males should preferentially feed older broods. *Prediction* - Males feed the first brood to hatch on the territory.

(IV) The reproductive value of a brood increases with brood size (Winkler, 1987), thus males benefit more through investment in larger broods. *Prediction* - In circumstances where the brood sizes of primary and secondary females differ, males will preferentially feed the larger broods.

9:2 Methods

9:2:1 Measuring parental investment

Parental investment is defined as "any investment by the parent in an individual offspring that increases the offspring's chances of survival at the cost of the parent's ability to invest in other offspring" (Trivers, 1972).

Parental investment may take many forms, and often the distinction between what is parental effort and mating effort is blurred (Low, 1978). For instance, should male territorial behaviour be regarded as parental behaviour (defence of food resources, nest sites etc.) or mating behaviour (attraction of females, competing with other males etc.). To counter this ambiguity, I concentrate on direct care of offspring and in particular the provisioning of nestlings. This aspect of parental care is potentially the most costly in terms of expenditure of time and energy, and is easy to observe and quantify.

The methods used to record provisioning behaviour have been described previously (Chapter 2). In the following analyses, I compare both the actual and relative levels of parental investment between birds in different social mating circumstances. The actual provisioning rates refer to the number of feeds/chick per hour made at a nest i.e, the number of feeding visits per hour divided by the number of chicks in the brood. The relative provisioning rates refer to the proportion of the total feeds/chick per hour made by either sex.

9:2:2 Female provisioning and social mating status

In examining female provisioning rate, I investigate the possibility that secondary females in polygynous systems may incur increased costs through (A) increased parental investment and (B) reduced level of total brood care. Social mating status follows the same definition as that used previously (section 7:2:1), in that secondary females are females which receive no male assistance in nestling provisioning.

9:2:3 Male provisioning and social mating status

In this analysis of male nest provisioning behaviour, I compare both the actual and relative level of paternal investment between monogamous and polygynous males.

9:2:4 Male chick feeding rules

In this section I investigate what possible chick feeding rules polygynous males employ in their investment allocation decisions. The initiation order of nests' was classed as 'first' or 'second' from the first egg date of each female on a territory. Nest predation resulted in repeated nesting attempts, so females which initiated nesting attempts first were not always the females which hatched eggs first. The hatching order of nests was classed as 'first' or 'second' from the date of hatching.

The level of extra-pair paternity (as a proportion of the total number of young in a nest) and the brood size were compared between pairs of nests in each territory.

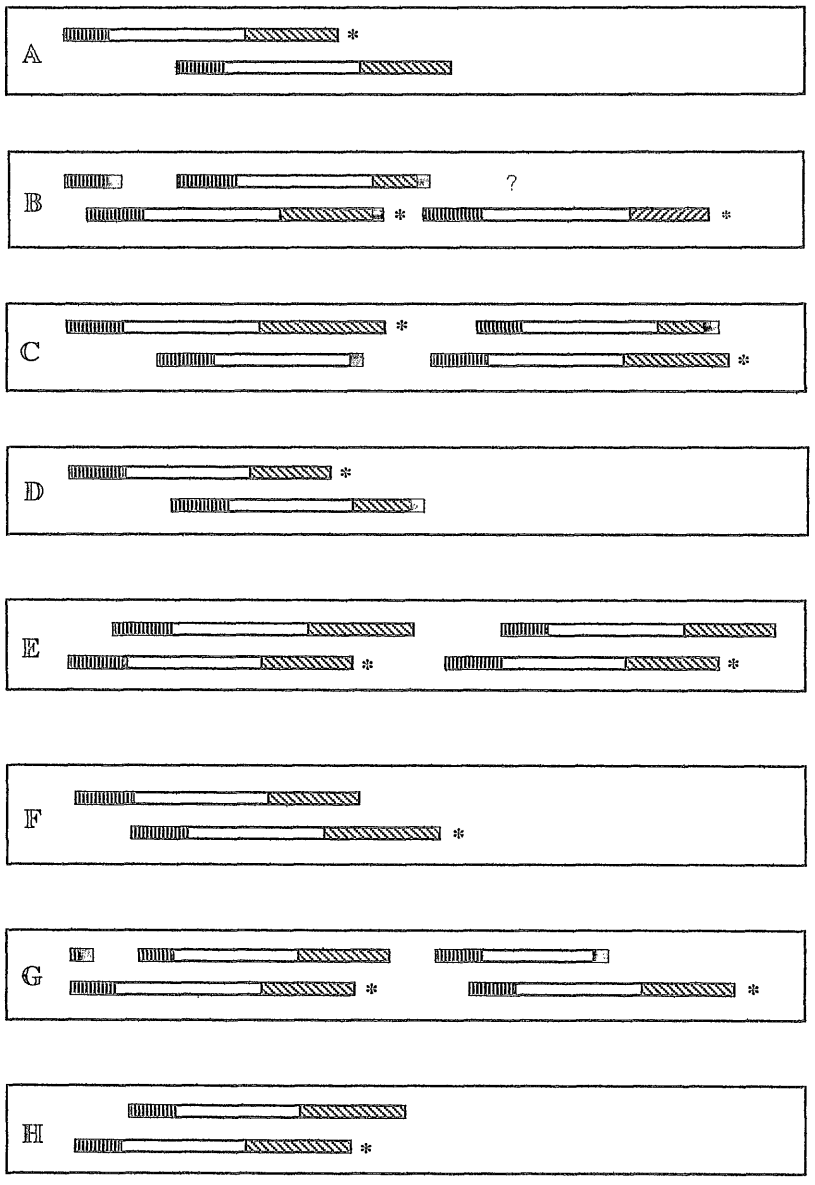

Figure 9:1. Schematic representation of synchrony in nests of primary and secondary females on the territories of 8 polygynous males. Each row refers to a single identifiable female. Vertical shading represents egg laying; clear areas represent the incubation period and diagonal shading denotes the nestling period. Black areas indicate nest predation, whilst an asterix indicates at which nest the male fed. One female unsuccesfully renested in a wheat field in territory B. Access could not be gained to this nest so precise laying dates are not known, however it was known that the nest did not reach the nestling stage.

9:3 Results

9:3:1 Synchrony in polygynous broods

There were eight polygynous males during the study, all of which had two females. A schematic diagram of the nesting period of the polygynous birds is given in figure 9:1. The degree of synchrony between the nests was controlled primarily by nest predation.

The nests of polygynous females overlapped each other at some stage of the nesting period in every case (11/11 pairs of nesting attempts). In two cases (territories A and D, figure 9:1), the young had already left the nest of the primary female before the chicks had hatched in the nest of the secondary female. In neither case did the male feed nestlings at the secondary nest. In the majority of cases (7/11 pairs of nesting attempts) there was some overlap in the nestling period between primary and secondary nests. There were no instances where the nestling period of one nest overlapped with the peak fertile period of the other female (regarded as day -3 to day +1; see Chapter 6).

In the five cases where second broods were produced by polygynous females after the successful fledging of the first brood, three were produced by male-assisted females and two by unassisted secondary females. The mean interbrood interval was apparently not different between the two classes (assisted females, mean interval = 8.5 days; unassisted females, mean interval = 9.0 days).

9:3:2 Female provisioning and social mating status

There was no significant difference in the absolute feeding rate of monogamous and primary females, though there was a tendency for monogamous females to feed at a slightly higher rate. However, there was a significant difference between primary and secondary females, with secondary females feeding nestlings at a higher rate (Figure 9.2).

There was no significant difference between the proportion of total feeds made by the female for both monogamous and primary females (Mann-Whitney U-test, $z = -0.325$, $P = 0.37$, figure 9:3). Secondary females are defined as females which receive no male help in brood provisioning, so obviously they provided proportionately more feeds than males than in the other two classes.

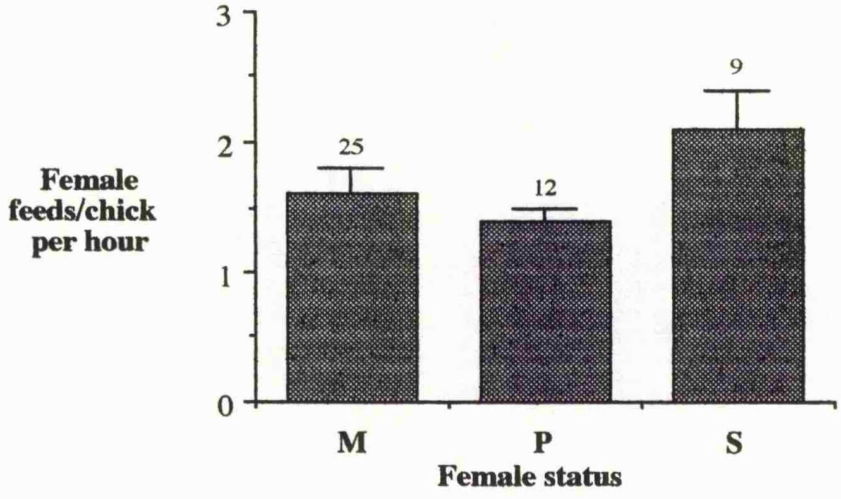

Figure 9:2. Mean (+SE) female feeding rate in relation to mating status. M = monogamous; P = primary; S = secondary. Mann-Whitney tests:
M v P; $z = 0.58$, $p = 0.28$
M v S; $z = 1.47$, $p = 0.07$
S v P; $z = 1.94$, $p = 0.03$

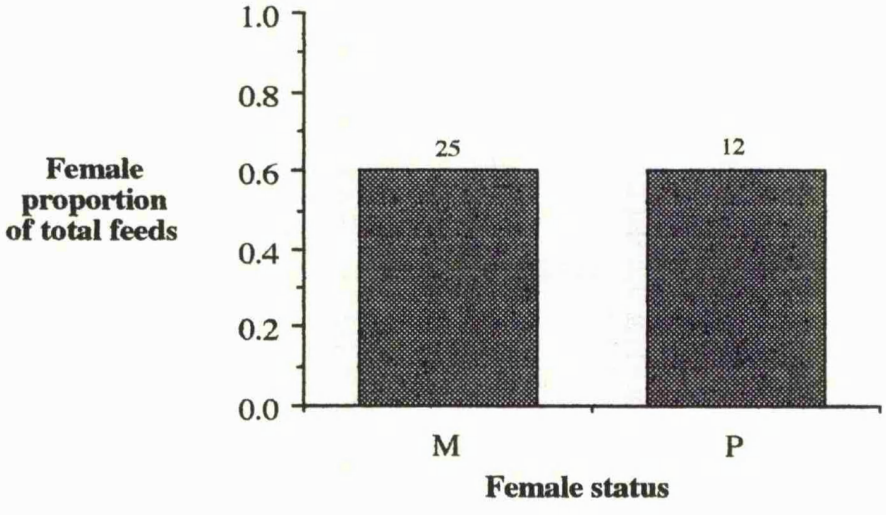

Figure 9:3. Mean proportion of total feeds made by the female in relation to mating status (standard errors = 0.03). M = monogamous; P = primary. Mann-Whitney , $z = 0.133$, $p = 0.43$

There was a trend towards lower levels of total parental investment at secondary nests than at primary nests and higher levels of provisioning at monogamous nests than at primary nests, though these differences were not significant (Kruskal-Wallis one-way ANOVA, H (corrected for ties) = 1.97, $df = 2$, $P < 0.1$, figure 9.4).

There were insufficient data to analyse the annual survival rates of females in different mating systems, and also the very low level of observed natal philopatry in the population did not allow comparison of chick survival rates from monogamous, primary and secondary nests.

9:3:3 Male provisioning and social mating status

Polygynous males did not increase their total level of parental effort and feed the broods of both females nesting on their territory. Instead, males preferentially allocated their parental effort to one of the broods, leaving the other to be reared solely by the female.

There was no significant difference in the actual feeding rate of monogamous and polygynous males (Mann-Whitney U-test, $z = -0.373$, $P = 0.36$; figure 9.5). Males contributed an average of 40% of nest feeds to both monogamous and primary nests.

9:3:4 Polygynous male chick feeding rules

The results of the analyses of polygynous male chick feeding rules are presented below:-

(A) There was no significant relationship between male parental investment and the order of nest initiation on a territory; sign test, $p = 0.25$ (one-tailed; 9 pair-wise comparisons were made from figure 9.1). This result conflicts with the hypothesis that male feeding decisions are based on a non-facultative response to their level of paternity.

(B) There was no significant relationship between male parental investment and the relative level of extra-pair paternity in nests on a particular territory (figure 9.6). Thus, the hypothesis that males make a facultative decision based on their perceived confidence of paternity is not supported.

(C) There was a significant relationship between male parental investment and the order of hatching, where males preferred the first brood to hatch; sign

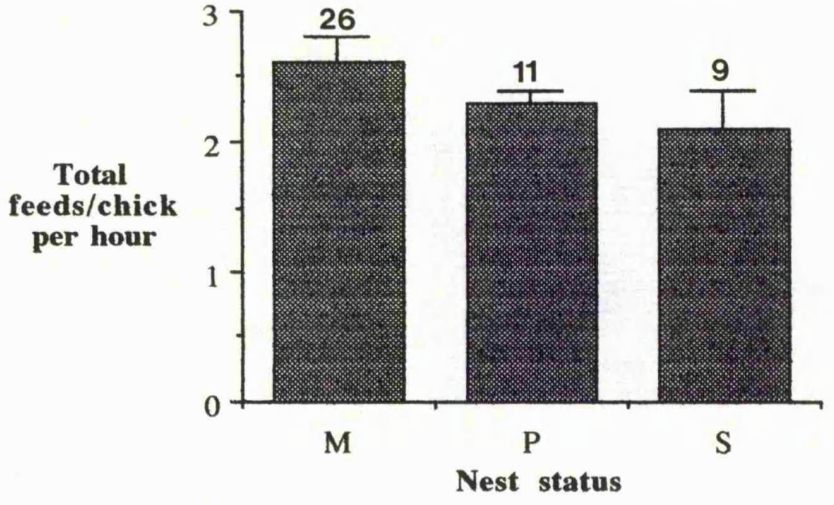

Figure 9:4. Mean (+SE) total nestling feeds (male plus female) in relation to nest status. M = monogamous; P = primary; S = secondary.
Kruskal-Wallis one-way ANOVA, H (corrected for ties) = 2.09, $df = 2$, $p < 0.1$.

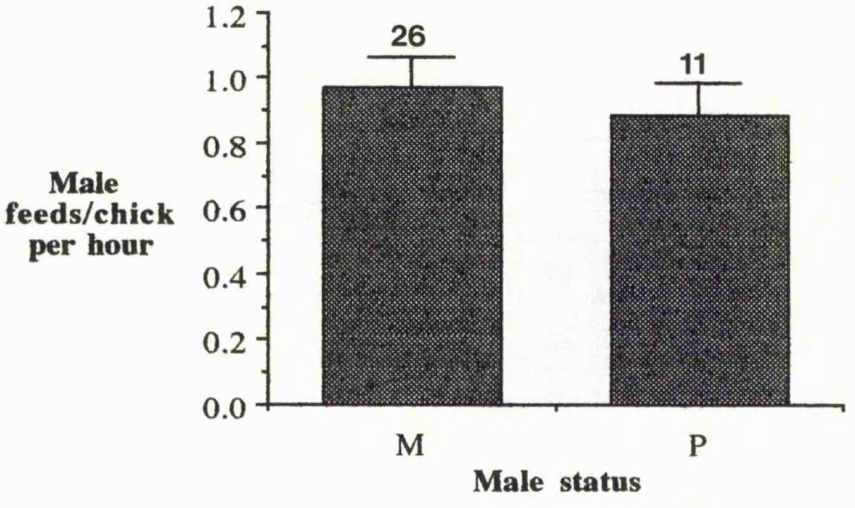

Figure 9:5. Mean (+SE) male feeding rate in relation to mating status.
M = monogamous; P = polygynous.
Mann-Whitney , $z = -3.73$, $P = 0.36$.

PAIR	BROOD SIZE		SIGN
	Male assisted	Unassisted	
A	4	4	=
B	4	3	+
C	5	4	+
D	3	5	-
E1	4	4	=
E2	5	3	+
F	5	3	+
G	2	3	-
H	3	4	-

Figure 9.6. Brood sizes in male assisted and unassisted nests. The hypothesis that males assist the female in nestling provisioning at nests with larger broods is not supported; sign test, $p = 0.50$ (one-tailed).

PAIR	%extra-pair young		SIGN
	Male assisted	Unassisted	
A	100	75	+
B	0	100	-
C	80	50	+
D	100	60	+
E1	100	50	+
E2	60	66	-
F	80	33	+
G	100	66	+
H	?	25	/

Figure 9.7. The percentage of extra-pair young in male assisted and unassisted nests. The hypothesis that males assist the female in nestling provisioning at nests with a lower proportion of extra-pair young is not supported; sign test, $p = 0.15$ (one-tailed).

test, $p = 0.02$; 9 pair-wise comparisons were made from figure 9.1). This supports the hypothesis that older broods are of higher reproductive value and thus preferred by males.

(D) There was no significant relationship between male parental investment and the size difference between broods (figure 9.6). This does not support the hypothesis that males preferentially provision larger broods. However, there was only one instance where the male fed the second brood to hatch and in that case the brood size was larger in the male assisted nest (nest F in figure 9.1), .

9:4 Discussion

There was a tendency for secondary females to provision nestlings at a higher rate than monogamous females, and significantly more than secondary females. There was also a tendency for secondary nests to receive less feeds than monogamous and primary nests. However, these differences were not statistically significant, which probably explains why there was no detectable difference in fledging success between nest classes (see chapter 7). Despite this, the results indicate that there is potentially some direct (female survival and future reproductive capacity) and indirect (offspring quality and survival) cost to secondary females as a result of unassisted parental care.

If females can successfully raise nestlings to fledging without male assistance, why then do males feed nestlings at all? The answer probably lies in the relative survival rates of fledglings from male-assisted and unassisted nests. In some species, male care has little effect on fledging success but a greater effect on subsequent survival or reproductive success of offspring (Smith *et al.*, 1982; Greenlaw & Post, 1985; Wolf *et al.*, 1988). It is possible that fledglings from secondary nests are lighter and less likely to survive than those from monogamous and primary nests. Also, the importance of male provisioning may be more noticeable in years of poor food supply (Bart & Tornes, 1989; Lyon *et al.*, 1987) and such a 'bad' year might not have occurred during this study.

It seems likely that the provisioning demands on secondary females are greatest when the young leave the nest, because the young are larger and spatially dispersed. I did not obtain any quantitative data on post-fledging provisioning but I did see males feeding fledglings from secondary nests ($N = 2$).

In the indigo bunting, *Passerina cyanea*, where there was no post-fledging paternal care of secondary broods, the inter-brood interval for unaided females was greater than that for male-assisted females (Westneat, 1988). Such a difference in inter-brood interval was not evident in this study (though the sample size is small), suggesting that post-fledging paternal care by polygynous males may be similar for fledglings from both primary and secondary nests. Male care at this stage has benefits for both males and females in that it emancipates the female from feeding duties, enabling her to start another breeding attempt.

Assuming male parental investment does increase male reproductive success, I now turn to the decision-making process of polygynous males, in deciding at which nest to allocate care. The best predictor of male provisioning is the hatching sequence of nests, where males prefer the first brood to hatch on a territory. Older broods are of greater value to males because they have already survived a period of potential predation and are closer to independence (Andersson *et al.*, 1980).

The results therefore indicate that males primarily base their investment decision on the age of the brood and not on other factors such as paternity and brood size. However, brood size possibly has some influence (note the difference in brood size in the exceptional case of paternal investment at the second brood to hatch) and the male decision-making process is probably based on a combination of the two factors. An experimental approach is required to investigate in more detail the flexibility of polygynous male nestling provisioning in the reed bunting (e.g., Lifjeld & Slagsvold, 1991; Yasukawa *et al.*, 1993).

Chapter Ten

INDIVIDUAL VARIATION IN PARENTAL INVESTMENT

Abstract

Variation in nestling provisioning behaviour within and among individual reed buntings was examined. Chick age was found to have a major influence on the feeding rate of both males and females, with older chicks being fed more than younger chicks. This relationship was not linear; feeding increased to a maximum level at seven days and then remained stable until fledging. Comparative analysis between individuals did not reveal any significant factors that influenced individual provisioning rate. However, analysis of individual feeding rates of birds which reared two broods in a single season with the same mate revealed that the proportion of extra-pair young in a nest was significantly related to male feeding rate, but not female feeding rate. Males fed less at nests with the greater proportion of extra-pair young. It is proposed that males assess their confidence of paternity in each brood and adjust their feeding rate accordingly. Females did not fully compensate for the reduction in male care, resulting in broods with a higher proportion of extra-pair young recieving less feeds per nestling, indicating a potential cost to females in accepting extra-pair copulations.

10:1 Introduction

Parental care is an energetically costly part of reproduction and individuals investing in such care are expected to incur a survival cost (Williams, 1966; Ricklefs, 1974; Hails & Bryant, 1979). Individual expenditure on parental care might be expected to vary in relation to the payoffs to both the parents and the offspring so as to maximise the individuals' fitness (Winkler, 1987). The relative costs and benefits of parental care are not necessarily the same for each sex or even different individuals of the same sex (Clutton-Brock, 1991).

In species adopting a mixed reproductive strategy through the pursuit of EPCs, one might expect an evolutionary arms race to develop between males achieving EPCs and those who are cuckolded (Smith, 1984). The adaptations evolved by males to counter EPCs can be either preventative i.e., paternity protection behaviour; remedial i.e., adjusting their parental investment to their level of paternity; or both. Many studies have been undertaken which illustrate the presence of preventative counter-adaptations in birds (see Birkhead & Møller, 1992) but relatively few have revealed remedial adaptations in response to EPCs (e.g., Møller, 1988a; 1991a, but see Wright, 1992). In fact, most studies which have investigated the possibility of such a remedial male response to cuckoldry have failed to find any relationship between EPCs and parental investment (Gavin & Bollinger, 1985; Frederick, 1987b; Morton, 1987; Westneat, 1988). Morton *et al.* (1990) reported a relationship between paternity and paternal care but this was confounded by age-related differences between individuals in their susceptibility to cuckoldry.

In this chapter, I examine the variation in a major component of parental investment (nestling provisioning) for male and female reed buntings, both within and among individuals. In table 10:1, I put forward four hypotheses to explain the variation in the level of nestling provisioning between individuals. Many of the factors which potentially influence parental care are interrelated. Thus, to analyse variation between individuals I used a stepwise multiple linear regression model. The predictions arising from these hypotheses are not necessarily mutually exclusive (e.g., age is related to the level of extra-pair paternity; see Chapter 5), though the relative importance of each hypothesis can still be evaluated through the use of multiple regression analysis.

Table 10:1

	Hypothesis	Prediction
	Individual variation in parental nestling provisioning is determined by:-	Variation in parental care is best predicted by:-
𝔸	Previous breeding experience	Age
𝔹	Variation in phenotype	Phenotypic characters/measures
ℂ	Variation in paternity	Level of extra-pair paternity
𝔻	Variation in mating opportunity	Mating potential/success

It can be seen from table 10:1, that some of the hypotheses are only applicable to males, i.e., hypotheses ℂ (variation in paternity) and 𝔻 (mating opportunity). Females had very little opportunity to desert the brood and remate elsewhere with another unmated male, yet males frequently had the opportunity to pursue EPCs instead of provisioning nestlings. These two hypotheses (ℂ and 𝔻), which potentially influence the level of paternal care, may also indirectly affect maternal care. Females are expected to adjust their provisioning rate in response to the level of paternal care (Houston & Davies, 1985; Winkler, 1987; Kacelnik & Cuthill, 1990).

If paternal investment is found to be directly related to the level of extra-pair paternity, then it might be the result of a facultative assessment by a male of the level of paternity in a brood. Alternatively, the level of paternity achieved may partly be determined by paternal phenotype, which may also determine the level of paternal care. In this case a relationship between parental care and paternity might be a non-facultative consequence of phenotype. To discriminate between these two hypotheses, I compared the paternity and provisioning data from each brood of double-brooded pairs, thus allowing for any effect of consistent differences among males to be excluded from the analysis.

10:2 Methods

10:2:1 Measuring parental investment

See section 9:2:1 for details. In the multiple regression analysis where comparisons were made between individuals, only data from male-assisted nests were used, i.e., monogamous and primary nests ($N = 24$). Fourteen pairs raised

two broods in a single season. To avoid problems associated with pseudoreplication this analysis included only the first brood reared in each case.

10:2:2 Definition of variables tested

Five variables which might potentially influence parental investment varied on a daily basis were analysed in the first stage of the stepwise multiple regression analysis (see section 10:2:3). These variables are outlined below:-

Time of day - This was the time at the start of each observation period, recorded to the nearest half-hour. Observations were primarily undertaken between 06:00 and 12:00 hours.

Chick age - Age in days of the young. Observations were carried out from day one to fledging (up to day 10).

Mate visit rate - This is the number of nest visits/chick per hour made by the mate of the sex under consideration.

Minimum temperature - This is the minimum temperature recorded in the 24-hour period in which the observation was made.

Fertile neighbour - This relates to the presence or absence (entered as 1 or 0) of a fertile female in a territory next to that under observation. A fertile female neighbour was recorded as present if the day of provisioning observation fell within a six-day period (from day -3 to day 3, where day 1 = first egg date) of the neighbour's nesting cycle.

In order to combine provisioning data for each of the years of study, I examined the annual variation in feeding rates in the second stage of the stepwise multiple regression analysis (see section 10:2:3). Data were entered as 1 or 0 for each year (1990 to 1992).

To examine the factors which influenced feeding rates among individual nests the following variables were analysed in the third stage of the stepwise multiple regression analysis (see section 10:2:3):

Proportion of EPY - This was the proportion of the brood that were extra-pair young. Data were arcsin transformed for stepwise multiple regression analysis.

Social mating status - Males were recorded as being either socially polygynous or monogamous (data entered as 1 or 0). Only nests where there was some male nestling provisioning were included in this analysis.

Male age - Age could not be measured accurately, but males were classified as either 'young' - previously unringed, therefore of unknown age, and 'old' -

already ringed, thus known to be at least in their second year (see Chapter 2). Data were entered as either 1 or 0.

Temporary male removal - In an attempt to reduce a male's confidence of paternity a removal experiment was attempted in 1990. Unfortunately, the birds were difficult to trap and consequently only a small number of males ($N = 4$) were removed for 24 hours during their mate's fertile period. Any influence of removal on the subsequent feeding behaviour was checked in the multiple regression analysis. Data were entered as either 1 or 0.

Male plumage score - Plumage score is a measure of the relative blackness of the head and bib feathers (see Chapter 2).

Female age - This was recorded and analysed in a similar fashion to male age (see above).

Number of young in nest - This was the number of young in each brood. These data were normalised by log (1+x) transformation for analysis in the multiple regression model.

Scrubland nest site - The study site comprised two main habitat types scrub plantation and marsh - which influence territory quality in terms of food production. The territories were always in one or other of these two habitats and as field boundary hedgerows often delimited reed bunting territories, a territory could always be classified as either marsh or scrub. Data were entered as either 1 or 0.

Additionally, male song rate was initially included in the analysis but it did not explain a significant amount of the variation in feeding rate, nor did it alter the significance of the variables which entered the model when it was excluded. It has not been included because both song rate and feeding rate were recorded for only 14 of the total of 38 males. Thus the exclusion of song from the analysis does not alter the conclusions but does increase the sample size.

10:2:3 Stepwise multiple regression analysis

The interrelationships among each of the separate variables that potentially influence parental investment were analysed in a stepwise multiple regression model. The procedure was undertaken in three stages. The robustness of each stage of the regression was tested by 'jack-knifing' the variables, i.e., leaving out each variable in turn to see if the result obtained from the other variables remained.

First Stage Analysis

The first stage analysed the factors which potentially influence parental investment on a daily basis. Data from each daily observation were used in this stage. The mean residual variation was calculated for each nest after fitting the regression model for those variables found to be significant. However, it was found that chick age had an extremely strong influence on feeding rate, but that this relationship was not linear. To counter this, a bivariate polynomial regression of chick age and feeding rate was carried out and the mean residual variance calculated for each nest.

Second Stage Analysis

The mean residual variance from the first stage analysis was used to examine the influence of annual variation in feeding rate. Data were entered as either 1 or 0 for each year of the study.

Third Stage Analysis

The third and final stage of the analysis was performed to detect and identify the causes of variation in feeding rates among individuals. Again, the mean residual variance for each nest obtained from the first stage analysis was used.

10:2:4 Extra-pair paternity and parental investment

In total (1990 - 1992), 14 pairs of reed buntings reared two broods in a single season. This phenomenon provided an ideal opportunity to examine the male's response to extra-pair paternity. All males were paired with the same partners, nested on the same territories and had the same neighbours between each nest. Thus any differences in the level of paternity between broods might be expected to produce a difference in paternal care if males responded to their level of paternity (Whittingham *et al.*, 1992; Westneat & Sherman, 1993).

The mean residual variance in feeding rate for each nest was calculated after accounting for the influence of chick age in a polynomial regression. The difference in the means between first and second broods was regressed against the difference in the proportion of paternity achieved between broods. A sign test was used to determine whether feeding rate and paternity differed between first and second broods *per se*.

10:3 Results

10:3:1 Daily variation in parental investment

The results of the first stage multiple regression analysis are presented in Table 10:2. It can be seen that of the variables tested the one having the most important influence on the feeding rate was chick age. The older the young, the greater the feeding rate of both males and females. The influence of chick age was much greater than that of the other variables tested, and so was examined in a polynomial regression to obtain daily residual values. The polynomial regression provided a closer fit, and so better residual values than the multiple regression, because the relationship between chick age and parental investment was not linear (Figure 10.1).

Time of day also influenced feeding rate, with more food being provided to nestlings earlier in the day. This time effect was not accounted for in the residual values from the polynomial regression of chick age against feeding rate. However, as nest watches were undertaken systematically in cycles (see Chapter 1), observations were not carried out at different times at different nests. Thus, the mean residual values for each individual nest were unbiased in that respect.

The analysis of the proportion of feeds provided by each sex revealed only one significant association: males fed proportionately less when the daily minimum temperature was higher. This is possibly due to the females brooding the nestlings for longer periods on cold mornings. However, this was not reflected in the feeding rate of either males or females, as neither increased or decreased their actual feeding rate significantly. The amount of variation in the proportion of feeds explained by temperature differences was minimal, accounting for only 2% of the variance.

10:3:2 Annual variation in parental investment

The results of the second stage of the analysis are presented in table 10:3. There was no relationship between feeding rates and the proportion of feeds provided by each sex for any of the years of study.

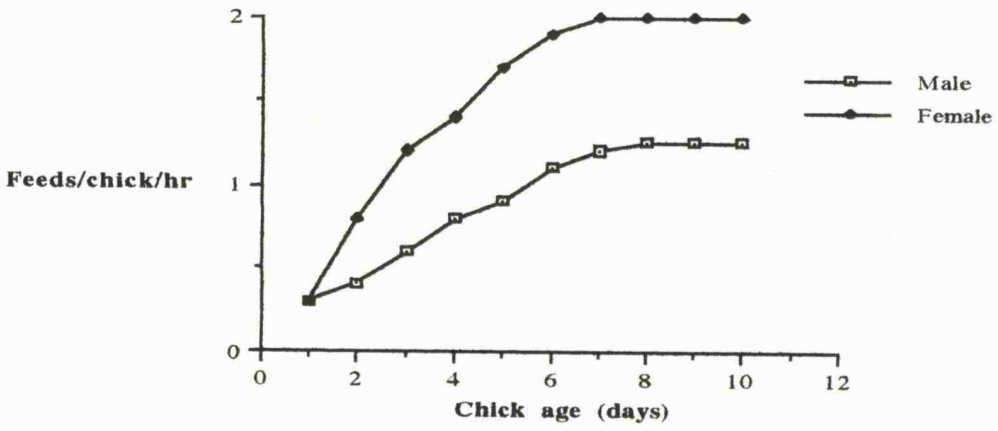

Figure 10:1. Polynomial regression of chick feeding rate against chick age for male ($DF = 208$; *Adjusted* $r^2 = 0.161$, $P = 0.0001$) and female ($DF = 207$; *Adjusted* $r^2 = 0.197$, $P = 0.0001$) reed buntings.

	Y_1 Visits/chick/hour F(207;2)			Y_2 Proportion of total nest visits F(207;2)	
Variables (X_1 to X_5)	*Female*	*Male*	*Total*	*Female*	*Male*
Time of day	-4.22*	-1.61	-4.29*	0.00	0.70
Chick age	65.70†	35.30†	125.80†	2.84	0.16
Mate visit rate	1.71	-1.23	-----	-----	-----
Min. temperature	1.97	0.25	0.88	2.64	-4.60*
Fertile neighbour	0.01	1.95	0.15	0.03	0.38
Adjusted r^2	0.25	0.14	0.39	0.02	0.02

Table 10.2: Columns show F values resulting from stepwise multiple regression of each Y variable on the X variables. The sign attached to the F variable indicates the direction of the relationship between the variables. F values represented in bold type indicate that the X variable explained a significant amount of variation in the Y variable (* = $p < 0.05$; † = $p < 0.001$). It can be seen that chick age accounted for an overwhelming amount of the variation in the number of nest visits/chick/hour for both sexes. However, this relationship was not linear and so the residual values used in further analyses were calculated from a bivariate polynomial regression of Y_1 against chick age.

	Y_1 Visits/chick/hour F(0;0)			Y_2 Proportion of total nest visits F(0;0)	
Variables (X_1 to X_3)	*Female*	*Male*	*Total*	*Female*	*Male*
1990	0.14	0.35	-0.07	0.38	1.41
1991	1.08	0.03	1.36	0.00	-0.33
1992	0.17	-0.62	-0.39	0.50	-0.68
Adjusted r^2	0.00	0.00	0.00	0.00	0.00

Table 10.3: Table showing the influence of year on the feeding behaviour of males and females at each nest. Columns show F values resulting from stepwise multiple regression of each Y variable on the X variables. The sign attached to the F variable indicates the direction of the relationship between the variables; none of the values were significant. The adjusted r^2 values indicate the amount of variance explained by the model, thus there was no annual influence on the feeding behaviour of the pairs.

10:3:3 Overall parental investment

The results of the final stage of the multiple regression analysis are provided in table 10:4. There was no significant relationship between parental investment and any of the variables tested. There was, however, a trend towards older females feeding at a higher rate than younger females. This non-significant trend was apparent in both the measures for female investment ($p = 0.25$) and overall investment ($p = 0.1$), suggesting that broods reared by older females may receive more feeds than those reared by younger females.

10:3:4 Extra-pair paternity and parental investment

Of the 14 double-brooded pairs, the level of extra-pair paternity was different between the first and second broods in 12 cases (in one case both nests contained 100% EPY and in another the level of paternity in one of the broods was not known). In first and second broods with a difference in the percentage of extra-pair paternity, the differences ranged from 10% to 100%. There was no tendency for second broods to have more EPY than first broods; five second broods held less EPY and seven more. Neither was there a tendency for males to feed at a lower rate in second broods; males fed at a lower rate in seven second broods and at a higher rate in five.

A comparison between the difference in male feeding rate and the difference in EPY between first and second broods revealed a highly significant relationship between a male's care and his proportion of paternity (Figure 10.2). There was no relationship between female provisioning rate and paternity (Figure 10.3). There was a non-significant trend towards broods with a higher proportion of EPY receiving less feeds (Figure 10.4). This provides compelling evidence that males adjust their level of parental care in relation to their gametic contribution to the brood.

	Y_1 Visits/chick/hour			Y_2 Proportion of total nest visits	
Variables (X_1 to X_9)	*Female* F(23;1)	*Male* F(0;0)	*Total* F(23;1)	*Female* F(0;0)	*Male* F(0;0)
Social mating status	0.25	-1.19	0.15	0.45	-0.81
Male age	-0.15	-0.08	-0.11	0.39	-0.52
Date	-0.77	0.00	-0.92	0.03	0.03
Male removal	0.18	-0.07	0.22	0.24	-0.47
Male plumage score	0.52	0.11	1.76	-0.17	-0.03
Female age	2.38	-0.04	3.64	0.81	-1.84
No. of young in nest	-0.83	0.00	-0.93	-0.84	0.24
Scrubland nest	0.98	0.24	0.51	0.01	0.00
Proportion of EPY	0.36	-1.27	0.12	0.13	-0.95
Adjusted r^2	0.06	0.00	0.10	0.00	0.00

Table 10:4. Results from the stepwise multiple regression of nine variables which potentially influenced male and female nestling provisioning. Columns show F values between each Y variable and the X variables. The adjusted r^2 values indicate the amount of variance explained by the model. No values exceeded the F-to-enter value of 3.99 (equivalent to $P < 0.05$ in simple regression), thus none of the variables tested could explain a significant amount of the variation in feeding offspring among individual birds.

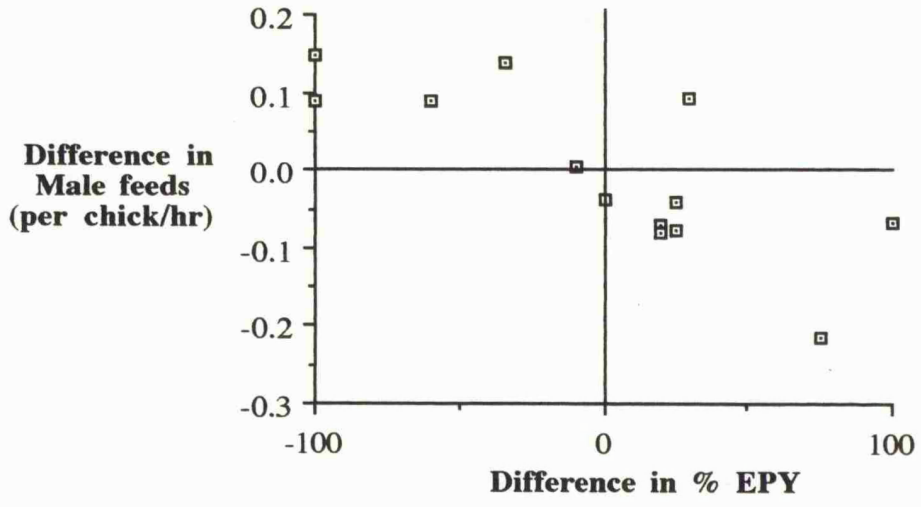

Figure 10.2. Relationship between male nestling provisioning rate and paternity ($df = 11$, *adjusted* $r^2 = 0.57$, $p = 0.002$). The Y axis represents the difference in mean residual feeding rate (after controlling for chick age) between the first nest and the second nest for double-brooded males, whilst the X axis represents the difference in the % of extra-pair young between first and second broods.

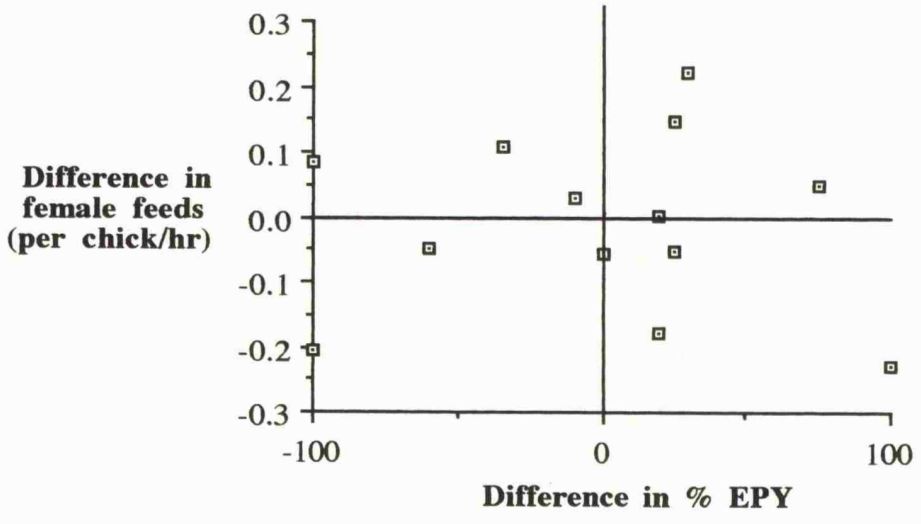

Figure 10.3. Relationship between female nestling provisioning rate and paternity ($df = 11$, *adjusted* $r^2 = 0.00$, $p = 0.9$). The Y axis represents the difference in mean residual feeding rate (after controlling for chick age) between the first nest and the second nest for double-brooded females, whilst the X axis represents the difference in the % of extra-pair young between first and second broods.

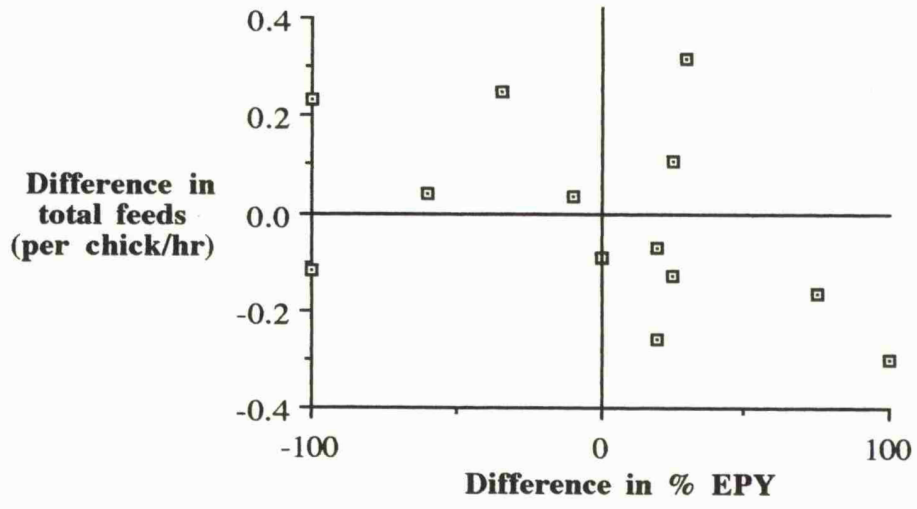

Figure 10.4. Relationship between total nestling provisioning rate and paternity ($df = 11$, *adjusted* $r^2 = 0.13$, $p = 0.125$). The Y axis represents the difference in mean residual feeding rate (after controlling for chick age) between the first nest and the second nest for double-brooded pairs, whilst the X axis represents the difference in the % of extra-pair young between first and second broods.

10:4 Discussion

The multiple regression analysis revealed that the major variable which significantly influenced feeding rate was chick age. A polynomial regression revealed that it was a non-linear relationship, with food being provided at a maximal rate after the young were seven days old. This relationship can be explained by the higher energy requirements of older nestlings.

The fact that the high degree of variation among individual feeding rates remains unexplained suggests that there are many subtle factors influencing provisioning rate which are not easily recorded. In this analysis, I have measured the frequency with which food was brought to the nest, but this may also be influenced by the amount of food carried on each visit and the calorific value of the food load. Differences in the distribution of food items, foraging areas around the nest and differences in foraging ability of individuals all combine to introduce 'noise' into the analysis. What can be said from the multiple regression analysis is that none of the variables tested appears to have a consistent major influence on the variation of feeding ability among individuals.

However, the analysis of parental investment and paternity in the multiple regression model is possibly confounded by differences in feeding ability among individuals. This, together with any unexplained variance in the remaining residual data from the earlier stage of the analysis, may mask any relationship that possibly exists between the variables tested and parental care. For instance, even though the daily variation in male feeding rate was corrected for the age of the chicks, 84% of the variance remained unexplained (see results). This statistical 'noise' is incorporated into the later stages of the analysis (through the use of mean residual values) in which comparisons among individuals are made. Thus, if males vary in their feeding ability then, for example, any reduction in paternal care in relation to paternity may be masked because each male has intrinsically different feeding rates. It is possible to envisage a situation where a male providing 50% of his maximum feeding rate still provides the same actual number of feeds as another male providing 100%.

The analysis of parental investment and extra-pair paternity in first and second broods does not suffer from the possible confounding effects of differing male or female quality because the same individuals are compared within a single breeding season. The results show that males are not responding to any change in female feeding behaviour with paternity. It is male behaviour which is the

determining factor in the relationship between paternal investment and level of paternity. This male response is not dictated by the simple presence or absence of EPY in a brood but is related to the proportion of EPY, the larger the proportion of EPY the greater the reduction in paternal investment.

How do males assess their level of paternity? The fact that males feed broods which are composed entirely of EPY suggests that males cannot recognise their own offspring. The most plausible explanation is that males can somehow assess their level of paternity during the female's fertile period. Evidence for such an hypothesis has been found in the dunnock *Prunella modularis* (Davies, 1992) and swallow, *Hirundo rustica* (Møller, 1988a; 1991a). In the dunnock, males in polyandrous mating trios have been shown to use their share of matings with the female to determine their relative feeding rate. In the monogamous swallow, males were found to allocate their investment in relation to both their absolute number of copulations and to their share of the total copulations engaged in by the female (i.e., within-pair and extra-pair copulations).

The large variation in the level of paternity between successive broods of the same males does not concur with the predictions of 'good genes' hypotheses for the phenomenon of EPCs, where one might expect some consistency between nests. The extreme variation in the level of extra-pair paternity found in this population of reed buntings also suggests that male behaviours apparently aimed at protecting paternity are not particularly effective. Thus, it seems that the best way for males to behave under such circumstances is to adapt their behaviour in relation to their perceived degree of cuckoldry. Recent theoretical models have predicted that such behaviour is adaptive when the likelihood of parentage varies randomly with successive nesting attempts, as appears to be the case in the reed bunting (Westneat & Sherman, 1993).

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