SEXUAL SELECTION IN THE GREAT TIT, *PARUS MAJOR*

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by

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"There's more to life than DNA"; Harrie in the lab on a wet Wednesday

ABSTRACT

This thesis investigated whether there was evidence for sexual selection in the great tit *Parus major*. The sexually dimorphic badge carried by the great tit was negatively associated with survival of both sexes. This badge, the black chin area, has previously been shown to have a role in male-male competition. The potential benefits to a female great tit of mating with large-badged males were assessed to investigate whether this trait also has a role in sexual selection. Large males produced a greater response to territorial intrusion than their smaller counter parts, a behaviour which may be a form of parental investment. Chicks were transferred between nests in a cross-foster experiment to distinguish between direct and indirect consequences of a male's badge size on offspring development. A potential direct benefit that covaried with badge size was identified, male badge-size was positively related to condition and number of (unrelated) chicks raised. An indirect benefit was also suggested: male's chin area was related to the survival of his offspring raised away from his nest. Large-badged males tended to produce more surviving chicks. When chicks were raised by their own father, male chin area explained variation in chick leukocyte count. Large-badged males produced chicks with low leukocyte counts and low leukocyte count improved chick survival. Female great tits, therefore, may experience both direct and indirect benefits according to the badge size of her mate. Females may also adjust their behaviour according to the phenotype of their mate. For example, there was evidence that the sex ratio of the brood may vary according to male size, with larger males producing more sons. These results are discussed with respect to the current theories of sexual selection.

CONTENTS

ACKNOWLEDGEMENTS

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION TO SEXUAL SELECTION

Many species possess sexually dimorphic traits associated with courtship. These traits are likely to be costly to produce and maintain. The theory of sexual selection was first introduced by Darwin when he described "the advantage which certain individuals have over others of the *same sex* and species in exclusive relation to reproduction." (Darwin, 1871; 1874). Darwin attempted to distinguish between natural and sexual selection of traits, the novelty of sexual traits being that they may be detrimental to survival. In order for a species to maintain such traits there must be some benefit to the individuals carrying them which results in a net fitness gain. Although a display may be costly in some aspect of an individual's life-history, the advantage it conveys may outweigh this cost.

It is generally the male that possesses extravagant traits. Females are usually the choosy sex as, ignoring all other constraints, female reproductive success is limited by the number of gametes she can produce, whereas male reproductive success is limited by the number of females he can fertilize (demonstrated in *Drosophila* by Bateman, 1948). This is a consequence of anisogamy, the fundamental difference between sexes, which results in females investing more than males in each gamete. Female preference for exaggerated male traits in birds has been demonstrated in a number of species (reviewed by Andersson, 1994). Large eye spans in stalk-eyed flies (Wilkinson & Reillo, 1994) and large repertoires in songbirds (Searcy, 1992) are two diverse examples of a preferred trait. In birds, female preference is commonly directed toward a plumage trait. Preference for exaggerated traits has been demonstrated in lekking species (long-tailed widowbird, Andersson, 1982a; peacock, Petrie *et al*, 1991; 1994). Preference has also been demonstrated in species with parental care (house finch, Hill, 1990; zebra finch, Burley, 1988).

Darwin (1871) proposed two mechanisms that may explain the evolution of costly ornaments: male-male competition and mate choice. Males whose ornaments provide them with an advantage in monopolising fertile mates will have a reproductive advantage over males with relatively small traits; this system is common among mammals. Alternatively, if males possess

1

traits attractive to females and thus improve their chance of females identifying or choosing them, this should increase their reproductive success. In both situations the benefits of increased reproductive success may outweigh the survival costs imposed by carrying the trait or ornament.

1.1.1 Models of inter-sexual selection

There have been a number of suggestions to explain the evolution of epigamic traits (sexually selected characters that have evolved in response to mate choice). These fall into three main categories: when the trait is neutral in what it signals (Fisherian process, Fisher, 1930), when the trait signals the genetic quality of the holder ("good genes" model; Wallace, 1891; Zahavi, 1975; Trivers, 1972; Hamilton & Zuk, 1982) and when the trait signals potential direct fitness benefits for the female ("good parent" model; Hoelzer, 1989).

The Fisherian process assumes that female preference is genetically determined, and such a preference is thought to become linked with the male ornament. When development of the ornament is linked with an initial advantage, not due to sexual preference (which may be quite "inconsiderable in magnitude" Fisher, 1930), choosiness may spread through the population. If the female preference trait becomes common then males carrying the trait will experience a mating advantage, as will their sons. The intriguing aspect of the Fisherian model is the consequence of this system. Fisher (1930) suggested this cycle could "runaway", resulting in the trait evolving far beyond the optimum determined by *natural* selection, so long as the disadvantage is more than counterbalanced by the advantage in sexual selection.

The "good genes" models are based on the idea that females benefit by selecting the most viable male on the basis of his "handicap" (Zahavi, 1975). The assumption of these models is that handicaps are costly to produce and maintain, and are more costly for low than high quality males. When there is additive genetic variance for fitness amongst males, therefore, a female can improve her reproductive success by choosing a mate with "good genes" (Andersson, 1982b; Nur & Hansson, 1984; Pomiankowski, 1987; 1988). Hamilton & Zuk (1982) proposed a specific revealing handicap, when the signal reveals an individual's resistance to parasitic infection.

Finally, there are direct benefits models. The leading model is perhaps the "good parent" model, which predicts that secondary sexual characters signal direct fitness benefits (Hoelzer, 1989). When paternal care influences the viability of offspring the male contribution is likely to be very important, as is seen in many socially monogamous species with biparental care. An

alternative direct benefits model is the transmission-avoidance model, developed in the light of the Hamilton-Zuk good genes model (Hamilton & Zuk, 1982; see Clayton, 1991), which predicts that females should select males on the basis of the ornament to avoid mating with parasitized individuals.

This thesis considers sexual selection in the great tit, a bird which possesses a non-extravagant, sexually dimorphic plumage trait. This badge is thought to be preferred by females (Norris, 1990 a and b), and also has a function in contest competition (Järvi & Bakken, 1987; Lemel & Wallin, 1993). The great tit differs from the classical species considered in sexual selection such the peacock, or red deer; species in which the male can increase his reproductive success by attracting, or monopolising access to, more mates. The great tit is a socially monogamous species, in which the male typically pairs with just one female within one breeding season. I will consider how the theories of sexual selection apply to such a species.

1.1.2 Sexual selection in a socially monogamous species

How does the theory of sexual selection fit monogamous species? Sexual selection was also proposed to occur when mates differ in fecundity (and fecund females are ready to breed earliest, see Darwin, 1871; Fisher, 1958), or if the sex ratio is skewed (Fisher, 1958). Without further study of mating behaviour, however, one cannot take variance in the number of potential mates as evidence of sexual selection. Similarly, to make conclusions about sexual selection from the sex ratio we need to estimate the *operational* sex ratio, a main determinant of the opportunity for sexual selection. The operational sex ratio is the ratio of females available for fertilisation to sexually active males at any given time (Emlen & Oring, 1977).

Until recently it was generally considered that social monogamy in birds had reduced the disparity in reproductive success between males and females (due to success being restricted by the amount of parental care received by the young, see: Greig-Smith, 1980; Sasvári, 1986 & Björklund & Westman, 1986 in Bart & Tornes, 1989; Ketterson & Norlan, 1994). Paternity studies in socially monogamous populations, however, suggest an additional route to variance in reproductive success, through extra-pair matings (Westneat *et al*, 1990; Birkhead & Møller, 1992a; 1992b; Kempenaers & Dhondt, 1993). Extra-pair young have so far been found in approximately 65% of socially monogamous passerines (Owens & Hartley, 1998). This suggests a mixed reproductive strategy, in which the birds are socially monogamous whilst engaging in extra-pair copulations.

There is evidence for extra-pair behaviour in the great tit. Allozyme analysis suggested that the frequency of extra-pair paternity in an English population is approximately 14% (Blakey, 1994). DNA fingerprinting revealed a frequency of 15% (Gullberg *et al*, 1992). Estimates do vary, with extra-pair young found in 8.5% of nests in an island population (accounting for 3.5 % of offspring; Verboven & Mateman, 1997) and in 33% of nests in a population in Belgium (Dhondt *et al*, 1996).

The female may mate with more than one male to replenish depleted sperm supplies, or as a hedge against their mate having low fertility (Sheldon & Burke, 1994; reviewed by Kempenaers & Dhondt, 1993). Sperm may be sexually selected within the female's reproductive tract and females might copulate with several males in order to ensure their eggs are fertilized by males with a high fertilization ability, so that their sons may inherit this trait (Keller & Reeve, 1994; review by Birkhead & Møller, 1996). Females might also trade copulations for access to another male's territory, either for immediate benefits or for access later on in the season. Alternatively, a female may take an extra-pair mate for indirect benefits, either to acquire good genes, or to increase the genetic diversity of her offspring. Genetic diversity may increase the chance of some offspring having an increased survival chance (Williams, 1975).

Therefore, extra-pair behaviour may increase reproductive variance within the population, though it may carry associated costs. By not assigning paternity to the chicks in this study, the estimates of genetic contribution to traits may be underestimates. It should still be possible to assess direct benefits to the female according to the badge size of her mate, since we are interested in looking at the general trends of direct benefits available in relation to male badge size, irrespective of the female's extra-pair behaviour.

1.2 AIMS AND CHAPTER OUTLINES

This thesis investigates whether there is evidence for direct or indirect benefits for females mated to males with relatively large badges. Chapter 2 describes the badge of the great tit and considers which morphological variables can be used to assess individual quality. Whether the female gains direct fitness benefits by selecting a highly ornamented male is addressed in Chapters 3, 4 and 5. Chapter 3 examines nest defence by the male and Chapter 4 investigates provisioning behaviour. Chapter 5 looks for both direct and indirect benefits by using experimental manipulations to reveal the heritability of traits in the absence of a common environment.

If females can manipulate sex ratio, do they do so to bias their brood toward what they perceive will be the more successful sex? Chapter 6 considers the sex ratio of each brood in relation to the parent's characteristics. It is possible that decisions are based on the cost of raising each offspring, or on the likelihood of a particular sex surviving to successfully produce many offspring. Chapter 7 investigates the parasite fauna infecting this population of great tits and also considers whether there may be some indirect benefit to females mated to large-badged males, by looking at the heritability of a fitness-related immune trait.

1.3 INTRODUCTION TO THE SPECIES

The great tit (*Parus major*) is a small, cavity-nesting bird. It is a socially monogamous passerine in which the male displays a variety of behaviours during the reproductive period. These include territory defence, mate guarding, mate feeding and provisioning behaviour. Great tits live in small flocks during the winter (Hinde 1952; Drent 1983; Matthysen 1990).

The great tit is the largest of the paridae family (c. 20 g); in many populations the males are 4% larger than the females (Gosler, 1993). Males have bolder and brighter plumage than the female; the female has similar plumage patterns, but they are less contrasting. In the male the ventral stripe is jet black and reaches from the beak to the base of the legs, while it is greatly reduced in the female. Great tits generally exploit a greater range of ecological resources than other tit species (Gosler, 1993).

The great tit has been a favoured research subject for avian ecologists. A thorough review of the various studies is provided by Gosler (1993). Many studies have considered the variables that may influence reproductive success in different populations of great tits. For example, Dhondt *et al* (1996) found that pairs that remained together tended to lay relatively earlier in the following year. Since pairs that lay early tend to recruit more offspring in that population, fidelity may be adaptive to both partners. Hatch date tends to affect nestling growth, but its effect is expected to differ according to the ecological differences between years (Smith *et al*, 1989). Great tits are thought to prefer roost sites and breeding sites with few ectoparasites (Christe *et al*, 1994; Merilä & Allander, 1995). Both ecto- and endoparasites have been shown to influence reproductive success in great tits (Richner *et al*, 1993; Richner, 1995; Hõrak & Ots, 1998).

The great tit is a convenient study species because it is conspicuous in its daytime activities, and relatively easy to trap and identify. The great tit's breeding season is regular but with enough asynchrony to allow one person to study a large set of individuals. Most importantly as a study species, it readily breeds in nest boxes, which significantly aids in catching the birds. With the competitor for nest sites in this population, the collared flycatcher, arriving after initial nest construction, the majority of the population is free from the restriction of finding nesting holes, even if they later loose them to flycatchers. Nest box populations have been suggested not to be representative of the natural situation (Møller, 1989a). This "unnatural" situation, however, has a certain advantage. Individuals that might not otherwise successfully

breed due to competition over nest sites can be included in the data, hopefully increasing the variation observed in the population under study.

1.4 INTRODUCTION TO THE STUDY SITE AND DATA COLLECTION

I studied a breeding population (approximately 200 pairs) of great tits on the southern part of the island of Gotland (57°10'N, 18°20'E), off the eastern Swedish coast in the Baltic. The study area is dominated by coppiced deciduous forest. Tree species found include oak (*Quercus robur*), ash (*Fraxinus excelsior*), and hazel (*Corylus avellana*). For more detail see Pärt and Gustafsson (1989).

Regular nest box inspections were performed from the beginning of May, with each box being checked at least every other day during its expected hatch. For each brood I recorded the clutch size and hatch date of the first chick (day 0). The hatch date of the first chicks is taken as the hatch date of the nest, irrespective of asynchrony. Chicks were ringed at day 14, fledgling success was taken as the number of chicks that survived to day 14 (only a single mortality was recorded after ringing). Chicks were also measured on day 14 when nestling tarsus-length can be considered as fully developed (Gosler, 1993).

Parents were caught between April and July, using mist nets or box traps. Box traps were made by pinning a small rectangle of acetate on the inside of the box to cover the hole. This allowed parents to enter the box but not leave. In 1996 I attempted to catch all parents on day 14; in 1997 parents were caught on or after day 14. They were classified as either yearlings (birds that have have only moulted once, in late summer, after fledging) or adults (birds that have had a full moult, i.e. that have survived two summers) following Svensson (1984). Each year I failed to catch a small proportion of adults, usually the male; hence the data for some broods is incomplete. All birds, parents and young were blood-sampled immediately on handling from the cutaneous ulnar vein for a DNA sample and a thin blood smear. Blood for DNA analyses was stored in 70% ethanol, and the thin smear air-dried in the field, for fixing and staining after the field season.

All birds were measured; for weight (to the nearest 0.5 g) using a pesola balance and plastic weighing cone; tarsus (to the nearest 0.05 mm), tarsus measurements were made with vernier callipers from the posterior notch at the inter-tarsal joint to the front of the tarsal bone, with toes bent down. Adults were measured for wing length (to the nearest 0.5 mm; following Svensson, 1984) and chin area, male's breast area was also measured. I favoured the right tarsus and right wing for measuring, but the left wing for blood sampling. Body condition index (BCI) was calculated as the residuals of the regression of the cube of tarsus on

standardized weight. I used the cube of tarsus to obtain a dimensionality similar to body weight. Weight was standardized by taking the mean weight from each individual measure, weight was standardized for 1996 and 1997 separately to remove year effects, hence each individual was given a score placing its size relative to the other individuals caught that year.

The black stripe was measured as follows: the male was held in the hand with his head held back, the chin area is quadrilateral and the length and breadth were measured with callipers allowing the area to be calculated Figures 1 and 2 (see Lemel, 1993). Breast stripe was measured by gently placing a piece of acetate with a grid of squares traced onto it on top of the breast, (see Figure 3). The breast "in 3" is the total black area in three (vertical) cm of black stripe. The number of squares in three vertical cm more than half-filled with black was counted. The number of squares multiplied by the area of a square gives the breast area. The chin area and the breast area measures were both repeatable (Chapter Two). Chin area plus breast area gave a measure of the black stripe; referred to as CB3. The chin plus breast in three cm describes the majority of the black stripe (excluding the area between the legs).

All analyses were performed in SPSS version 9.0. For analyses non-normal data were log or square root transformed. Models were backwards-selected, then interaction terms were included and the model backward-selected again. The residuals of all general linear models and multiple regressions were examined for homogeneity of variance, for outliers and normality as described by Crawley (1993).

Figure 1. Great tit with head held back to allow chin area to be measured

Figure 2. Great tit male - with black chin area visible.
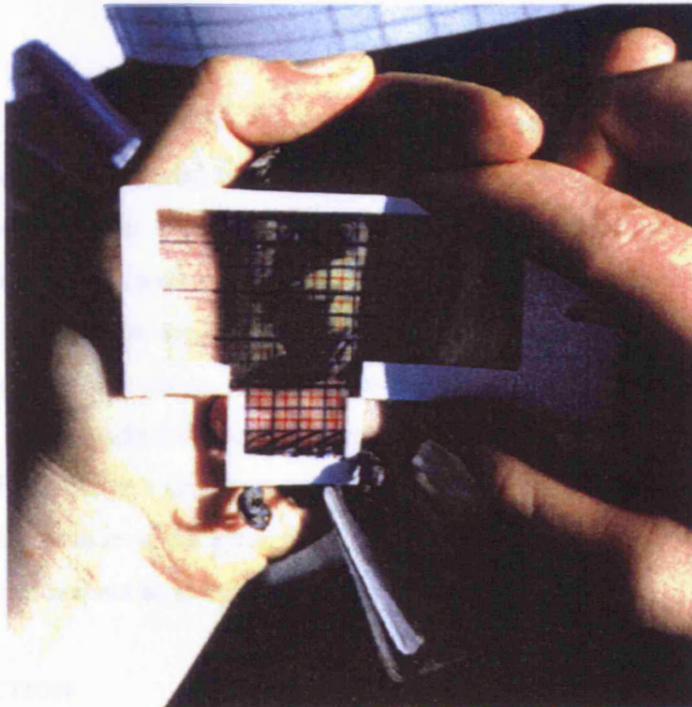
Figure 3.. Great tit male breast-stripe measure. The measure started from where the chin measure stopped, all the squares more than half filled with black feathers were counted.

BADGE SIZE AND SURVIVAL IN THE GREAT TIT

2.1 ABSTRACT

This chapter describes the sexual dimorphism in the great tit and how morphological traits influence survival. Males tended to be larger than females as yearlings and adults. Females also differ from males in that they do not possess a true breast stripe. The largest measured dimorphism was between male and female black "chin" areas, with females having the smaller area. A large chin area was negatively associated with long-term survival. Chin area tended to increase with age in the male. The various measures of badge size in the great tit are considered. It was concluded that for subsequent analyses, where possible, the different components of the badge - chin area and breast stripe - should be used separately in models examining the behaviour of the great tit male. The issues of signal theory and sexual selection are discussed with respect to the great tit badge.

2.2 INTRODUCTION

Plumage characteristics may serve as signals of dominance status in social animals. Individuals with relatively large traits therefore may gain access to resources without paying the costs of agonistic interactions with conspecifics (Rohwer, 1975). These same traits may be also be preferred by females which maintain control over the choice of their mate. In this situation the sets of sexual selection and signal theory become joined. Fisher (1930) began to approach this problem of dual uses of signal traits. He suggested that when the "war paint" used by females in mate choice is also used by competing males to assess an individual's status, it would be an honest indicator of quality. The implication was that only an honest signal would influence competing males. Zahavi (1981), in his discussion of signal selection, suggests that a signal would be absolutely reliable if the cost of using the signal were greater than the potential gain from cheating.

In the great tit, two main signals have been identified in the male: the badge area and vocalizations. Dominance status has been shown to be positively related to badge size in some circumstances (Järvi & Bakken, 1984; Järvi et al, 1987; Lemel & Wallin, 1993, but see Wilson, 1992), and is suggested to be used in mate choice (Norris, 1990a). Survival is related to winter

dominance (Delaet, 1985) and McGregor *et al* (1981) found that males with better singing ability had higher survival rates. Song repertoire size is important in territory defence but not, apparently, in female choice (Krebs *et al*, 1978).

The purpose of this chapter is to describe how morphological traits in the great tit vary with age and sex. I also considered whether the traits, including badge, change in size over time from a longitudinal study of breeding birds. If the badge does signal condition, then we would predict that the badge size should covary with survival. The direction of the relationship is more difficult to predict. Should birds in good condition display a large badge and subsequently trade-off this investment against a life-history trait such as reproductive success or survival (see Stearns, 1992)? Or is the "silver-spoon" effect at work (Cockburn, 1991), when birds in good condition are able to carry a larger badge, and due to their good condition, have better survival and reproductive success? The direction of the relationship between survival and badge size is also informative in addressing models of signal theory: does the badge signal genetic or current condition? I therefore aim to investigate whether there is evidence of the badge covaring with survival and, if this is the case, the direction of the relationship. I will discuss how the behaviour of the great tit's badge fits with signal theory. I will also consider the various measures of badge that have been used in the great tit.

2.3 METHODS

2.3.1 Morphological measures and survival

All measures were made as described in the Introduction (1.4). For repeatability analyses, each measure was made twice within the same incidence of catching an individual; the second measure was the last measurement taken from the individual before release. For survival analyses, all individuals were entered into the data set once only. Birds were classified as having survived one year if they were found breeding in the year after first capture, or within two years after that. They were classified as having survived two years if they were found breeding two years after first found breeding. 1998 data were supplemented by, and 1999 catch data provided by Kate Oddie, University of Edinburgh.

2.3.2 The great tit badge

The badge in the great tit has been measured in as many different ways as there have been people interested in measuring it. I split the badge into chin and breast area to differentiate between the measures made.

Lemel (1993) measured chin area, as described in the Introduction, but referred to this as breast stripe area or badge size. Järvi & Bakken's (1984) breast stripe measure was made by measuring the width of the breast stripe at the base of the sternum. Kölliker et al (1999) photographed the breast stripe and calculated the black area. I assume that in this way they are describing the total area (chin + breast areas), which would be closest to my cb3 (chin plus breast area in 3cm). Norris (1990a) measured the breast area, but not the chin area described by Lemel (1993).

2.3.4 Analyses

Models were backwards selected, and then run with interaction terms included in a second round of backwards selection. All analyses were carried out in SPSS 9.0.

2.4 RESULTS

2.4.1 Repeatability

To ensure that the measurements were repeatable, two measures were taken and an anova performed (repeatability R was then calculated from the between and within group variance; see Harper, 1994). Tarsus R= 0.99, n= 41; chin R= 0.99, n= 57; breast in 2 vertical cm; R= 0.99, n= 75; breast in 3 vertical cm R= 0.99, n= 60. In linear correlations all the measures have an r of greater than 0.7.

2.4.2 Morphological traits, sex and age

I examined how the traits measured differed between the sexes and age classes. In all instances, the mean male measure was greater than the mean female measure. This difference was significant for wing length and chin area for all age groups, and for adult tarsus and wing length (Tables 1 and 2). I also examined how the traits differed between different age classes within the same sex. The general trend was for morphological traits to be larger in adults.

Comparison of traits between age groups does not distinguish between individuals changing over time, or selection acting against specific traits, or sizes of traits; all potentially resulting in a population displaying a different range of sizes after a period of selection. To address this. I considered those individuals caught more than once to investigate how an individual's traits change over time.

Females

Direct comparison of yearling and adult females suggests that tarsus increases with age, or that larger females are more likely to survive (Table 4). From individual analyses, there was no significant difference in tarsus length between years (Tables 5 and 7). There was a significant increase in wing length following the first moult, i.e. as yearling birds become adult, and evidence that wing length tends to increase in each subsequent year (Tables 5 and 7). Comparison of adult and yearling female birds showed no significant difference between chin areas, with the average for both groups being 317 mm^2 (Table 4). By considering individuals recaught when older, however, it is clear that chin area did increase significantly over time (Tables 5 and 7).

Table 1. Comparison of morphological traits of yearling males and females

trait	mean ± sd	comparison of means	corrected p
yearling tarsus (mm)	m: 19.42 ± 0.62	t= 1.05, df= 98, p= 0.29	0.12
	f: 19.25 ± 0.66		
yearling weight (g)	m: 17.52 ± 0.98	t= 1.34, df= 49, p= 0.19	0.76
	f: 17.11 ± 1.11		
yearling wing (mm)	m: 75.9 ± 1.2	t= 2.66, df= 76, p= 0.01	0.04
	f: 74.4 ± 1.9		
yearling chin area (mm²)	m: 369 ± 44	t= 4.43, df= 81, p< 0.001	<0.005
	f: 317 ± 48		

m is male, f is female

Table 2. Comparison of morphological traits of adult males and females

trait	mean ± sd	comparison of means	corrected p
adult tarsus (mm)	m: 19.81 ± 0.79	t= 3.46, df= 87, p= 0.001	0.004
	f: 19.31 ± 0.59		
adult weight (g)	m: 17.91 ± 0.86	t= 1.54, df= 54, p= 0.12	0.58
	f: 17.52 ± 0.96		
adult wing (mm)	m: 77.4 ± 0.8	t= 6.37, df= 64, p< 0.001	<0.004
	f: 75.3 ± 1.4		
adult chin area (mm²)	m: 375 ± 52	t= 5.07, df= 64, p< 0.001	<0.004
	f: 317 ± 46		

m is male, f is female

Table 3. Comparison of morphological traits of male yearlings and adults

Trait	mean ± sd	comparison of means	corrected p
tarsus (mm)	y: 19.42 ± 0.62	t= -1.94, df= 53, p= 0.06	0.36
	a: 19.82 ± 0.79		
weight (g)	y: 17.52 ± 0.98	t= -1.59, df= 55, p= 0.12	0.72
	a: 17.92 ± 0.86		
wing (mm)	y: 75.9 ± 1.3	t= -4.07, df= 31, p< 0.001	<0.006
	a: 77.4 ± 0.86		
chin area (mm²)	y: 369 ± 44	t= - 0.48, df= 51, p= 0.63	>1.0
	a: 376 ± 53		
breast in two (squares)	y: 37.4 ± 7.5	t= 1.06, df= 51, p= 0.29	>1.0
	a: 35.1 ± 7.6		
breast in three (squares)	y: 56.1 ± 10.6	t= 0.40, df= 44, p= 0.69	>1.0
	a: 54.7 ± 10.8		

y is yearling, a is adult

Table 4. Comparison of morphological traits of female yearlings and adults

trait	mean ± sd	comparison of means	corrected p
tarsus (mm)	y: 19.25 ± 0.66	t= -1.94, df= 53, p= 0.06	0.02
	a: 19.30 ± 0.58		
weight (g)	y: 17.11 ± 1.11	t= -1.34, df= 48, p= 0.19	0.76
	a: 17.52 ± 0.96		
wing (mm)	y: 74.4 ± 1.9	t= -2.76, df= 109, p= 0.007	0.03
	a: 75.3 ± 1.4		
chin area (mm²)	y: 317 ± 48	t= 0.06, df= 101, p= 0.95	>1.0
	a: 317 ± 47		

y is yearling, a is adult

Table 5. Paired comparison of morphological traits in retrapped yearling females

trait	mean ± sd	comparison of means, paired t-test	corrected p
tarsus (mm)	y 19.25 ± 0.65	t= -1.48, df= 25, p= 0.15	0.45
	a:19.34 ± 0.53		
wing (mm)	y: 74.4 ±2.2	t= -4.30, df= 19, p< 0.001	<0.003
	a: 76.1 ± 1.5		
chin area (mm²)	y: 274 ± 32	t= -4.39, df= 14, p= 0.001	<0.003
	a: 322 ± 41		

y is yearling, a is adult

Table 6. Paired comparison of morphological traits in retrapped yearling males

trait	mean ± sd	comparison of means, paired t-test	adult larger
tarsus (mm)	y: 19.86 ± 0.51	t= -3.27, df= 16, p= 0.005	0.03
	a: 20.10 ± 0.49		
wing (mm)	y: 76.7 ± 0.98	t= -7.24, df= 11, p< 0.001	<0.005
	a: 78.9 ± 0.87		
chin area (mm²)	y: 402 ± 49	t= -3.22, df= 15, p= 0.006	0.03
	a: 448 ± 35		
breast in two cm (squares)	y: 31.5 ± 10.1	t= 0.73, df= 14, p= 0.48	>1.0
	a: 29.0 ± 6.9		
breast in three cm (squares)	y: 51.0 ±14.2	t= 0.24, df= 13, p= 0.81	>1.0
	a: 49.8 ± 9.3		

y is yearling, a is adult

Table 7. Paired comparison of morphological traits in retrapped adult females

trait	mean ± sd	comparison of means, paired t-test	corrected p
tarsus (mm)	1: 19.16 ± 0.46	t= -2.02, df= 22, p= 0.06	0.18
	2: 19.29 ± 0.49		
wing (mm)	1: 75.2 ± 1.8	t= -0.25, df= 19, p= 0.80	>1.0
	2: 75.3 ± 1.8		
chin area (mm²)	1: 298 ± 45	t= -2.41, df= 15. p= 0.03	0.09
	2: 332 ± 35		

1 is the first incidence of the bird as an adult, and 2 the measure taken from the bird the following year.

Table 8. Paired comparison of morphological traits in retrapped adult males

trait	mean ± sd	comparison of means, paired t-test	trait increased
tarsus (mm)	1: 20.06 ± 0.44	t= 0.31, df= 26, p= 0.76	>1.0
	2: 20.04 ± 0.53		
wing (mm)	1: 78.8 ± 1.47	t= -1.25, df= 25, p= 0.22	>1.0
	2: 79.4 ± 2.34		
chin area (mm²)	1: 435 ± 69	t= -1.49, df= 27, p= 0.15	0.75
	2: 454 ± 35		
breast in two cm	1: 30.8 ± 8.0	t= 1.31, df= 26, p= 0.18	0.9
(squares)	2: 28.6 ± 27.0		
breast in three cm	1: 50.3 ± 11.1	t= 1.39, df= 25, p= 0.18	0.9
(squares)	2: 47.0 ± 6.6		

1 is the first incidence of the bird as an adult, and 2 the measure taken from the bird the following year.

Males

There was no significant difference between tarsus length of yearling and adult males (Table 3), though measures for recaught males tended to increase with age (Table 6). As with females, wing length tended to increase with age, with a significant increase in wing length following the first moult (Table 6). From direct comparison of yearlings and adults, there was a significant increase in chin area following the first moult (Table 6), and a tendency for chin area to increase through adulthood (Table 8). There was no significant change in breast area (Tables 6 and 8).

2.4.3 Morphological traits and survival

I investigated which variables explained female survival using binary logistic regression. I separated the models by female characteristics, reproductive information and her partner's characteristics. Female (standardized) weight, tarsus, age, wing and first year of capture had no significant effect and were dropped from the model. Female chin area however, did explain some of the variance in survival to the next breeding season (Table 9). It appears that females with smaller chin areas had a better survival chance (mean chin area (± s.d) of survivors was 295 ± 51 mm², and of those not caught again was 316 ± 48 mm²). Similarly, female chin area explained survival for the following two years, and tarsus length also adds to the model (Table 9); smaller females appeared to fare better (mean tarsus length of survivors was 19.15 ± 0.50 mm, and of those not caught in the next two years is 19.34 ± 0.64 mm).

The second model, investigating the effects of reproductive variables, was non-significant; hatch date of clutch, clutch size, day 14 survival and year did not explain the variance in female survival (χ^2= 2.96, n= 131, df= 4, p= 0.56). Of the partner's characteristics, only male survival covaried with female survival (see Table 9); male age, tarsus, wing, chin area, and male breast area were all dropped from a backwards-selected model. The relationship between male survival and female survival was positive; i.e. if the male survived the female tended to have a

higher chance of survival. None of the male characteristics explained female survival for two subsequent breeding seasons. This was despite there being some evidence for assortative mating (correlations between female and male partner's traits: female-male tarsus length r= 0.17, p= 0.02, n= 196 and female chin area-male chin area r= 0.27, p= 0.002, n= 134).

Table 9. Models to investigate which factors explain variance in female survival

dependent variable	model description	variables	n	$\beta \pm$ s.e.	χ^2	p
survived 1 year	female characteristics	chin area	139	- 0.01 ± 0.01	5.39	0.02
survived 2 years	female characteristics	full model	130		7.72	0.02
		chin area		- 0.01 ± 0.01		0.04
		tarsus length		- 0.83 ± 0.41		0.08
survived 1 year	male characteristics	male survived 1 year	245	0.61 ± 0.34	3.11	0.08

Similarly, I investigated which variables covary with male survival. None of the male characteristics explained survival to the next breeding season; age, tarsus, wing, chin area, breast area (in 2 and 3) and year of capture were all dropped. Unlike the females, males with larger tarsi tended to have better survival chances (mean tarsus of those that survived one year, 19.9 ± 0.58 mm, those that were not caught again, 19.8 ± 0.67 mm), though the effect in the model was non-significant. When we consider male survival to two years, chin area becomes significant (Table 10), with surviving males having a smaller chin area (χ^2= 6.23, n=159, p= 0.01). Including the interaction term between chin area and age made this model non-significant. By splitting the data into yearling breeders and adult breeders, it is possible to investigate this relationship in more detail. The relationship remains for adult males only (Table 10).

The pattern of survival for the two following years in relation to chin area was the same for those adult males caught whilst breeding in the next year (not a significant variable in our model). Those males that survived one year had smaller chin areas (mean 426 ± 69 mm^2 compared to 412 ± 57 mm^2 for the survivors; for those males which survived at least two breeding seasons, where the difference was significant in the model, mean 422 ± 62 mm^2 compared to 386 ± 53 mm^2 of the survivors).

Table 10. Models to investigate which factors explain variance in male survival with males separated by age class

dependent variable	model description	variable(s)	n	$\beta \pm$ s.e.	χ^2	p
survived 2 years	yearling characteristics	chin area	70	-0.003 ± 0.006	0.25	0.61
survived 2 years	adult characteristics	chin area	86	-0.01 ± 0.005	3.8	0.05

The model of reproductive variables was non-significant; hatch date, clutch size, day 14 survival, and year did not explain the variance in male survival (χ^2= 2.62, n= 118, df= 4, p= 0.62). None of the female characteristics (age, tarsus, wing, chin area or survival) covaried with male survival (χ^2= 9.64, df= 5, p= 0.68).

2.5 DISCUSSION

2.5.1 Variation in traits and survival

Morphological traits vary with age and sex in the great tit. Males tend to be larger than females as yearlings and adults, as in most passerines. The greatest measured difference was found between adult male and female chin areas. Females have no real breast-stripe, with some females having a small band of scattered, grey-black feathers compared to a black band that reaches from the "chin" down to the legs in males. The black areas on the male also tend to be glossier than in the female, and the yellow richer (Perrins, 1979; Slagsvold & Lifjeld, 1985). There may be an aspect to the badge that we, as humans cannot see, particularly reflection of light in the ultraviolet (Derim-Oglu, 1993; Hunt et al, 1998; Andersson, 1998), which has been shown to be used in mate choice in blue tits (Hunt et al, 1999). Slagsvold (1993) attempted to manipulate female breast stripe to resemble males by dyeing the breast area with ink unsuccessfully. It is possible that to the birds this was not a true manipulation of breast stripe. In males the outer fringes of the primary coverts are blue-grey in males and more brown-green in females (Dhondt, 1970).

The chin area of the great tit may be best defined as a static signal, whilst the breast stripe may be an adjustable signal. The chin area is not concealable, and it is determined by the plumage laid down after moult. Though it may vary in brightness, due to oils in the feathers, its size is set. The breast stripe, however, appears to be a trait over whose size an individual may be able to exert some control. The great tit may be able to move the ventral yellow feathers, which may sometimes obscure the breast stripe in a captured bird (see 1.4, Figure 1). I would also propose that the white eye patch of males and females may differ in appearance. I did not, however, attempt to quantify the difference in this trait. Zahavi specifically discussed the potential use and cost of a trait such as the eye patch (Zahavi 1993). He suggested that a cost might be imposed by carrying such a trait because it is an uncheatable signal of where the individual's attention is focused.

Females

Tarsus length explained some of the variation in survival of females, with smaller females being more likely to survive. It is possible that selection is acting against large females. The same is true of female chin area. Chin area does not differ between yearling and adult females despite there being a significant increase in chin area over time. This suggests there may be selection against females with larger chin areas. There is evidence that the male, or the territory he holds, may affect female survival. Females mated to males which subsequently reproduced in the local population were more likely to survive. It is possible that this is a indirect relationship, because we would predict that males holding a good quality territory would also have a better survival chance.

Males

Male tarsus and wing length, as with female tarsus, tended to increase with age. There was also a trend for chin area to increase. As with females, surviving males tended to have smaller chin areas. It is possible that a large chin area may confer advantages in some situations, but may impose long-term costs, such as a reduction in longevity as suggested by these results. Lemel (1993) reported a tendency for yearling males to suffer from a higher mortality when chin area was enlarged.

The breast area tended to decrease over an individual's lifetime. Norris (1990a), however, found that, as with my measure of chin area, breast stripe tended to increase with age. This suggests that our measures of breast stripe may not be directly comparable. The question therefore arises of why an individual would begin life with a large breast area. Perhaps it has a function before the first moult that is subsequently lost. I would also refer to Appendix 1, which compares the various measures of stripe against a set of behavioural variables (see Chapters 2 and 3 for descriptions of the behavioural data). Whilst the breast stripe measure varied with the length of the vocal response made by the great tit male defending its territory, the chin area described as much of the variation and tended to vary with behavioural variables more often than the measure of breast area. Whilst I do not discount the possibility that breast stripe has a function, when the data allow I will use the components of the badge - chin area and breast stripe - separately in analyses to attempt to differentiate between them. Where such separation will reduce the degrees of freedom within a model, I propose that the total area measured is a more suitable measure to use.

2.5.2 The badge of the great tit and signal theory

The chin area of great tits appears to be a trait which may reduce the survival of individuals who possess exaggerated examples of the trait. Zahavi (1975) suggested that such traits may have evolved because they are a handicap to their bearers, and as such signal the bearer's quality. In some situations, the real cost to carrying the trait has been demonstrated (in guppies, Endler, 1983; in sage grouse, Vehrencamp *et al*, 1989; in the great tit, Lemel, 1993; in the house sparrow, Veiga, 1995). For such a trait to evolve there must be some benefit to balance the cost of developing or carrying it. This benefit may be increased reproductive success if the trait is favoured by the selecting sex, or resources acquired in competition when competitors can reliably base their behaviour on the signal. A male that advertises beyond his abilities may reduce his viability more than he raises his mating success.

The idea that females select mates on the basis of potential benefits signalled by the male's badge is compatible with the theory that the trait carried by the male is important in male-male competition. Rohwer (1975) put forward a hypothesis, originally introduced to account for behaviour outside the breeding season, to suggest that the trait may have a function in agonistic encounters, reflecting social status.

The similarity of the theories, whether the trait is sexually selected or selected because of its use in communication outside the arena of mate choice, is that the signal evolves under opposing forces of selection (Zahavi, 1993). Natural selection will act against exaggeration of the trait, whilst there will be selection for the trait because of the gain from signalling. Alternatively, the trait could also be self-limiting, for example if greater development of a secondary sexual character increases mating success in one way but reduces it in another (Andersson, 1994).

2.5.3 Chin area in the great tit, an example of a handicap?

If the plumage characteristics do influence dominance relationships as previously suggested (Järvi & Bakken, 1984; Lemel & Wallin, 1993), this will translate into different foraging tactics according to the size of the badge. Variation in avian foraging behaviour influences reproductive success and survival (Lemon, 1993), and in the great tit survival is related to winter dominance (Delaet, 1985). Therefore, an enlarged badge may improve success of an individual great tit. How then, can it be a handicap?

The great tit differs from some of the more classical subjects of sexual selection studies such as the long-tailed widow bird (Andersson, 1982a), because the signal thought to be important in mate assessment is not an enlarged plumage structure (such as the tail feathers in the case of the widowbird), but rather an area of pigmentation. How can a plumage pigmentation construe a handicap and how could its development be restrained? There is considerable evidence from a wide range of signallers and signalling systems that animal displays are expressed in a condition-dependent manner, even when they are not apparently physically constrained to do so (see Johnstone, 1995 & Johnstone *et al*, 1996). The cost may be defined as physiological, or social, though social costs must be paid in terms of a bird's physiology (Johnstone, 1995). Models suggest that an honest plumage signal can be maintained if cheats suffer a cost imposed by aggressive individuals (Owens & Hartley, 1991), or if individuals differ in their ability to withstand a contest-independent cost of aggression (Johnstone & Norris, 1993).

The immunocompetence theory (Folstad & Karter, 1992), proposed a mechanism through which carrying the badge may be costly, or more specifically disproportionately costly to a poor quality individual. Folstad & Karter suggested that sexual characters might be honest indicators of the bearer's ability to cope with its parasite burden because the development of many such characters is testosterone dependent. Since testosterone is also known to suppress the immune system (Folstad & Karter, 1992 and references therein), then individuals can only produce a "good" signal by producing high levels of testosterone. In turn, they can only do this when their parasite burden is low, or trade-off increased testosterone levels against increased parasitemias. This mechanism therefore may maintain honest signals. In support of this theory, increased ectoparasite counts have been found in red-winged blackbirds with naturally elevated testosterone (Weatherhead *et al*, 1993) and in swallows, elevated ectoparasite levels were found in testosterone-implanted males (Saino *et al*, 1995). However, contrary to

conventional wisdom the development of showy male plumage has only rarely been shown to be testosterone dependent (Owens & Short, 1995).

Testosterone could still be a mediating factor behind the cost of a plumage trait, without it being responsible for development of that trait. Circulating testosterone levels are associated with aggressive behaviour (Wingfield *et al*, 1987), and evidence from deception experiments suggests that elevated testosterone may influence dominance status. Combining testosterone implants with plumage manipulations can create successful cheaters (Rohwer & Rohwer, 1978; Järvi *et al*, 1987). Without experimentally altered testosterone, cheating may not be so easily achieved. When Møller (1987a) manipulated badge size in house sparrows the experimental birds did not achieve higher dominance rank than controls, but were involved in more aggressive encounters. Rohwer & Rohwer (1978) observed significant differences in the fighting ability of male Harris Sparrows, despite individuals being experimentally altered to resemble subordinates.

Successful deception has been achieved, however, without manipulation of the natural testosterone levels (for example Rohwer, 1985; Fugle *et al*, 1984; Holberton *et al*, 1989). It may be that such deception results in individuals producing unnaturally high levels of testosterone. For example in Hegner & Wingfield's (1987) study on testosterone-implanted house sparrows there was an effect of (experimentally) increased aggression on neighbouring males. Unmanipulated neighbouring males were found to have increased testosterone levels compared to controls. Neighbouring males were involved in more aggressive encounters than males not in contact with the experimentals, and appeared to increase their circulating testosterone as a result.

Behaviour of an individual covaries with badge size. For example, Järvi & Bakken (1987) observed that great tits with experimentally altered dominance status (achieved by altering badge size) are more frequently involved in fights than their subordinates. If testosterone levels are associated with the social situation in which a relative badge size places an individual, then testosterone may still be the mediating factor involved in imposing costs. Holberton *et al* (1989), however, found no correlation between their manipulated level of dominance and hormone levels in dark-eyed juncos. The relationship may not be simple, for example it may be that testosterone is only a mediator in the way described in times of flock formation (see discussion by Holberton *et al*, 1989). Given that manipulating badge size away from that "chosen" by an individual appears to alter its behaviour, or the behaviour of other

individuals toward it, it is conceivable that social costs are translated into physiological costs relative to the size of the badge.

Hence, even when the signal is an apparently cheap-to-produce plumage colouration - such as the "bib" of the house sparrow or "chin area" of the great tit - the cost of taking part in an aggressive encounter may be mediated not through direct physical damage to the individual, but rather through the hormonal and physiological systems of the bird. Hence, testosterone may be the mediating force behind the honesty of the signal, as proposed by Folstad & Karter (1992). In support of this hypothesis, an aviary study in the great tit suggests that increased social interaction may be expressed as a cost on the immune system (Incagli, in press). Also, Gustafsson *et al* (1995) demonstrated an immunological cost to cheating in male collared flycatchers that were given enlarged badges.

2.5.4 Conclusions

There is significant dimorphism between male and female great tits. It appears that both sexes may experience reduced longevity if they have large chin areas. In support of this a previous study found that experimentally increased chin area tends to increase mortality in males (Lemel, 1993).

TERRITORIAL DEFENCE IN THE MALE GREAT TIT

3.1 ABSTRACT

In direct models of inter-sexual selection, the sexual dimorphism in plumage traits is attributed to female preference for males that signal the resources they can offer. This chapter investigated whether territorial defence against an experimental intrusion, and provisioning behaviour (both potential direct benefits to the female) covaried with badge-size in the male great tit. As predicted, large-badged males produced a longer vocal response to the experimental intrusion into their territory. Large-badged males, however, were also more likely to be caught following the experimental trial, suggesting a bias in the sample population. The hormone testosterone, thought to mediate aggressive behaviour, was assayed. Analyses of the testosterone response suggests that small-badged males may raise their testosterone in response to the intrusion more than large-badged males, however more data are required to confirm this. Similarly, the data suggest that there may be a trade-off between producing the testosterone response to a territorial intrusion and provisioning later in the season; again further investigation is required to support this result.

3.2 INTRODUCTION

Sexual dimorphism exists in many species with a bias toward males being more conspicuous or brightly coloured than the female (Andersson, 1994). Several studies have shown that females prefer males with larger or more conspicuous traits (see for example Andersson, 1982a; Petrie *et al*, 1991). Females mating with attractive males may do so to obtain direct or indirect benefits (Andersson, 1994). This chapter considers the two main theories explaining parental behaviour in monogamous birds, and investigates which direct benefits the female great tit gains by choosing a male on the basis of his sexually dimorphic trait.

The relative size of a badge may advertise some "quality" in the holder, which could be of potential benefit to its mate or offspring. The signal may indicate an individual's current condition (and hence potential as a parent) or may advertise some genetic quality, which could be inherited by offspring. There are two main hypotheses predicting the relationship between parental behaviour and the trait used in inter-sexual selection. Firstly, the "good parent

27

hypothesis" (Hoelzer, 1989); assumes that the badge signals current condition and predicts a positive relationship between the size of the male's badge and his provisioning effort. Secondly the "differential allocation hypothesis" theory assumes that badge size reflects genetic quality and suggests that it may benefit the female to invest more in her offspring when mated to a good-quality male (Burley, 1988). This theory predicts a negative relationship between a male's badge size and his parental effort, as the female may increase her investment if mated to an attractive male.

The differential allocation hypothesis is not the only hypothesis to predict a negative relationship between the badge size of the male and his parental care. The female might provide parental care at a level irrespective of her partner's attractiveness. The negative relationship described, however, may still be seen if the male suffers a trade-off between his ability to invest in parental care and maintain his attractive badge. The relationship between parental care and the sexual trait may also be sensitive to the behaviour we choose to record, as parents could be facing a trade-off between different aspects of parental care.

Alternatively, the trait may be used in intra-sexual communication. During the breeding season competition for resources such as nest sites and territories may be severe. A signal of fighting ability is thought to prevent agonistic encounters by reducing the need to interact physically to assess an opponent's abilities (Rohwer, 1975; Maynard Smith, 1982). For such a signalling system to be maintained the signal must be an honest reflection of an individual's status. In fact, models suggest that when the value of a contested resource is high (relative to the cost of fighting) badges of status would not be used to settle the interaction (Maynard Smith & Harper, 1988). Some authors have gone as far as to suggest that the potential benefit of winning a contest over a territory will always outweigh the cost of taking part in an antagonistic encounter, whatever the signalled ability of the opponent (Maynard Smith & Harper, 1988; Wilson, 1992; Johnstone & Norris, 1993).

The cost of taking part in an agonistic encounter could mediate honesty of signalling. The cost of aggressive interactions, such as territorial defence, may not be limited to physical harm. A trade-off may occur at the physiological level since aggressive behaviour is associated with elevated hormone levels, which may stress the individual's immune system (Grossman, 1984; 1985). The suppressive effect of testosterone on the immune system could be the mechanism through which the cost of sexual behaviour is translated into health costs (Folstad & Karter, 1992; Chapter 2).

Testosterone influences the development of certain bird ornaments (reviewed by Owens & Short, 1995), and an individual's level of aggression (reviewed by Wingfield *et al*, 1987). More specifically, testosterone regulates the expression of reproductive behaviours, both sexual and aggressive (Wingfield *et al*, 1990; Alatalo *et al*, 1996). Males may face a trade-off between these behaviours. Experimentally increased testosterone levels have been shown to suppress parental behaviour in favour of territorial aggression and mate guarding (Silverin, 1980; Hegner & Wingfield, 1987).

Testosterone is not alone in influencing antagonistic behaviour, and studies of dominance relationships in birds suggest that testosterone does not influence behaviour outside of the breeding season (Belthoff & Dufty, 1994; see also Gwinner & Schwabl, 1994). Corticosterone also plays a role in aggressive behaviour. Within dyads in the house finch subordinate birds exhibited significantly greater levels of circulating corticosterone than dominant individuals (Belthoff & Dufty, 1994). It must be held in mind that a number of hormones may be involved in aggressive and stressful situations, and indeed that the relationships between these may change according to the season and the social situation presented to the individual.

Norris (1990a) proposed that in the great tit the thick black breast "stripe", a sexually dimorphic plumage trait, acts as a signal to other individuals. Lemel & Wallin (1993) showed that stripe size had no impact on the outcome of conflicts between individuals with prior experience of each other. In pairs of unacquainted birds, however, the size of the black area determined the outcome of a competitive event. It has been demonstrated previously that the great tit will behave toward a stuffed dummy of its own species much as it would respond to a live bird (Järvi & Bakken, 1984) and that the resident great tit will fight to defend its territory from challenge by an intruder (Krebs, 1982). A stranger's song should represent a threat to territory tenure because there are no agreed boundaries (McGregor, 1993), and initially, the resident male does not know its relative status. By experimentally staging inter and conspecific interactions during nest building, and the early stage of egg laying, this study contested ownership of a resource of great value to the resident male. In the population studied great tits and collared flycatcher compete for the same nest sites. Complete clutches may be lost in these disputes and the fights are sometimes to the death (personal observation).

This study investigated how agonistic behaviour, provisioning behaviour and testosterone response of the great tit varied with its plumage trait. Each of these variables may be considered to be a component of "parental investment". Defined by Trivers (1972), "parental investment" is any investment in an offspring that increases the offspring's chance of

surviving at the cost of the parent's ability to invest in other offspring. Other offspring include potential extra-pair young or young of future reproductive attempts. I considered the relationship between the size of the plumage trait in the male great tit and his territorial behaviour to address the question of whether the badge signals direct benefits to the female. The two theories: (1) of direct benefits to the female and (2) of advertising social status lead us to the same prediction: males with relatively large badges should be dominant in aggressive situations. I also considered whether there was a trade-off between territorial behaviour and provisioning behaviour of the male, a potential cost to the female.

3.3 METHODS

3.3.1 Measurements

In the 1997 breeding season dummies of a dead male collared flycatcher (frozen adult), and a stuffed male great tit (yearling) were presented at 23 experimental boxes. The dummies were protected in a "cage" of mist net. The dummy was attached to the nest box and a recording of the respective species' territorial song played back from the base of the tree. The songs played were from birds outside of the local area. A guy rope with 1, 3, 6 and 12 metre markers was stretched out horizontally to aid the observer in estimating the distance of the resident bird from the box. The flycatcher trial was always carried out first. The advantage of presenting an unrelated species at the nest is that it ensures the great tit male's response is not an attempt to protect his fertile mate from intruding males. Observations were carried out for ten minutes, but trials were stopped in the event of heavy rain, effectively removing the male from the data set. The following behavioural responses were scored: whether the male attacked the dummy, and how many times; how close the male got to the dummy; the number of "jumps" made by the male, as a measure of his activity; the time lag to the first observed response (vocal and visual); the length of time the male spent within 1 m of the dummy; the total length of the vocal response and the total length of time for which the male was observed for. As a control, mist nets were placed on a small number of territories to catch males without any territorial challenge.

Provisioning behaviour at each nest was observed for one hour, using either a telescope or video recorder. Watches were conducted on day seven (between 06:00 and 08:00) and day ten (between 09:00 and 11:00), with day 0 being the day the first egg hatched. The frequency of parental visits was taken as an indication of provisioning behaviour. To aid in the identification of the sexes, the female great tit's tail was marked with white correction fluid (Tippex ™) whilst she was incubating the eggs (see Chapter 4, Figure 1).

To catch the resident male mist nets were placed on the territory, with continuous great tit song playback and after the day 10 provisioning watch a box trap was set to re-catch the male. The male, when caught on either occasion, was bled immediately from the cutaneous ulnar vein (collecting 100-150µl of blood). Blood sampling from a bird in this manner is not considered to have detrimental effects (Kovách, 1969; Stangel, 1986). The blood was collected in heparanised capillaries and stored vertically in a cool box in the field. The samples were

31

kept refrigerated and centrifuged for 15 minutes (at 1400 rpm) later the same day. The separated plasma was stored at -20°C until testosterone assays were carried out in Professor Silverin's lab in Gothenburg, Sweden (Röhss & Silverin, 1983).

The black stripe was measured as described in the Chapter 1 (1.4). For analyses the total badge area was used (CB3) for this chapter only. Using this variable did not significantly affect the results (see Appendix 1). Each male's tarsus, weight, and wing length were also measured (following Svensson, 1984) after the blood sample was taken. The Body condition index (BCI) was calculated as the residual of the regression of tarsus³ on standardized weight (standardized data has the mean taken from each measure; data was standardized for 1996 and 1997 separately, taking into account any year affects), see 1.4 for a full description.

3.3.2 Analysis

All analyses except power analyses were carried out in SPSS version 9.0. Power analyses were carried out using the public access web site provided by UCLA:
(http://www.stat.ucla.edu/calculators/powercalc/).

3.4 RESULTS

3.4.1 Territorial Response

The species of dummy presented affected the likelihood of observing a response by the resident male (χ^2= 12, p< 0.05). Of the adult birds 29% percent responded to the flycatcher dummy whereas 79% of the adult birds responded to the great tit dummy. The intensity of the response also differed significantly between the two dummy types, (see Table 1), with the great tit dummy evoking a more intense response than the flycatcher dummy. As a result, for the behavioural analyses, I concentrate on the bird's response to the great tit, i.e. the larger dataset.

To ensure that time of day was not affecting the great tit's behaviour, I compared the timed behavioural variables in the great tit trial taken at the same box in the morning with those taken in the afternoon (Table 1). The relationships between response and time of day are shown in Figures 1 and 2. Time of day did not affect the level of response; therefore data was pooled for subsequent analyses.

The likelihood of the male responding did not differ according to whether the birds were nest building or if the female had laid eggs (Fisher's exact test p= 0.55). Similarly, the intensity of the response did not vary according to the nest stage (Table 1), allowing the data to be pooled.

Table 1. Non-parametric tests analysing effect of the species of the intruder, time of day and nest state on the behavioural response of the resident male great tit.

a.   Wilcoxon's matched-pairs test comparing the response of the resident male great tit to the great-tit versus collared flycatcher dummy (n = 23).
b.   Wilcoxon's matched-pairs test comparing the a.m. and p.m. response of the resident great tit male (n = 6).
c.   Mann-Whitney U test comparing the responses of males with and without eggs to protect at the time of the trial (n= 18 and 6).

Time	Species [a]		Time of day [b]		Nest state [c]	
	Z	P	Z	P	U	P
To first response	-2.1	ns	-0.5	ns	34	ns
Within 1m of box	-2.9	ns	-1.5	ns	41	ns
Vocal Response	-3.4	ns	-0.9	ns	34	ns
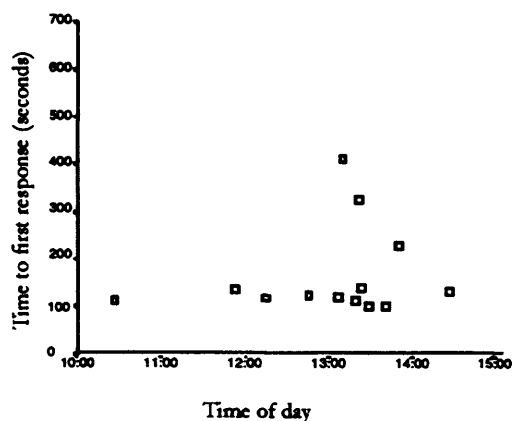
Figure 1. Time to male's first response to great tit dummy
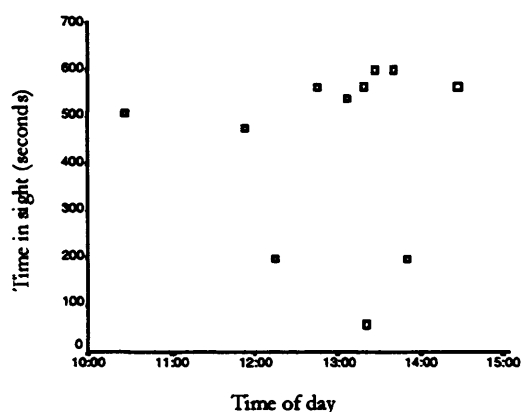
Figure 2. Total time male in sight whilst responding to great tit dummy

3.4.2 Hormonal Response

Four control birds were caught to compare their testosterone measures with those of the experimental birds to investigate if the trial resulted in a raised testosterone level. A direct comparison of the testosterone levels showed no significant difference in the testosterone measures (Mann-Whitney U test, U= 13, n= 4 and 14, p= 0.12). There may be no significant difference between the experimental and control measures because the experimental group included males caught long after the trial, in which the testosterone levels could have been raised but then returned to normal.

In a paired test to compare males caught at both the trial and at provisioning, the drop in circulating levels of testosterone from the territorial experiment to the level during provisioning was close to significance (Wilcoxon's matched pairs test, Z= -1.8, n= 8, p= 0.07). It is possible that these results are explained by testosterone levels reflecting a seasonal change, however the control levels did not differ from those at provisioning (Mann-Whitney U test , U=14.5, n= 4 and 9, p= 0.57). It is not clear from this small sample of control males whether the trial resulted in an elevated level of circulating testosterone.

There was a significant negative association between the circulating testosterone level and the time to capture, shown in Figure 3. A linear regression was carried out to investigate which variables influenced the level of testosterone. Alone, time to capture explains approximately 32% of the variation. If we hold the effect of time constant, total badge area was found to hold a negative relationship with testosterone, (Figure 4). In a linear regression the total badge area adds to the model, however its effect is non-significant, (see Table 2).

34
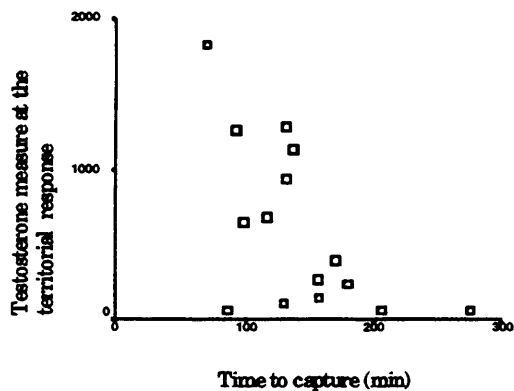
Figure 3. Testosterone against time taken to capture the male.
Spearman's r= -0.58, n= 15, p= 0.01
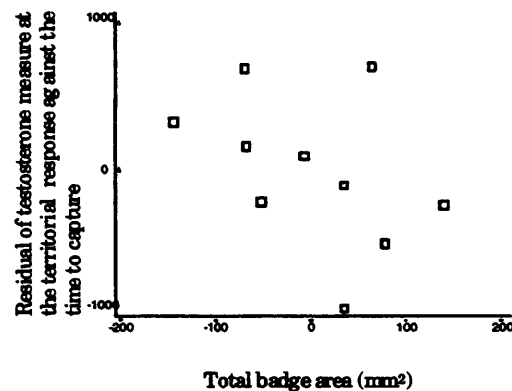
Figure 4. Total badge area against territorial testosterone (with the effect of time to capture removed). See Table 2.

Table 2. Multiple regression examining the variables that explain variance in the territorial testosterone measure.

Y= territorial testosterone levels	r^2= 0.43, df= 10, p= 0.10	
independents	$\beta \pm$ s.e.	p
time to capture	-10.58 , 4.44	0.04
total badge area	-2.57, 1.92	0.25

Table 3. Comparison of medians of those birds that did and did not attack the great tit dummy.

Variable	Mann-Whitney U	df	p
testosterone level	-1.49	6 and 4	ns
BCI	-0.31	12 and 5	ns
total badge area	-0.52	12 and 5	ns

There was no difference in circulating testosterone level, stripe measure or BCI between those birds that did versus those that did not physically attack the great tit dummy; an extreme aggressive behaviour (Table 3).

3.4.3 Defence behaviour

I examined how the behavioural responses varied in relation to morphological traits. Firstly the vocal response by the male is considered. A multiple regression was used to investigate

which factors explained variation in the length of the vocal response (in the form of bout singing). Males with larger tarsi and badges showed a longer vocal response (Table 4 and Figures 5a and b). Nest attentiveness was measured as the time lag to the first response recorded by the observer. Only the male's tarsus measure improved the model (Table 5). The following variables were dropped from the model; standardized male weight, male wing length, breast area, BCI, final clutch size and hatch date.

Table 4. Multiple regression examining the variables explaining variation in the length of vocal response by resident great tit males.

Y= length of vocal response		r^2=0.62 p=0.002, df=15	
independent	β	s.e.	p
tarsus	0.8	0.72	0.001
stripe measure	0.4	0.52	0.045

Table 5. Regression describing the relationship between the time lag to the first response by the defending male and his tarsus length.

Y= ln (time to first response)		r^2=0.22, p=0.06, df=16	
independent	β	s.e.	p
tarsus	-1.5	0.74	0.06
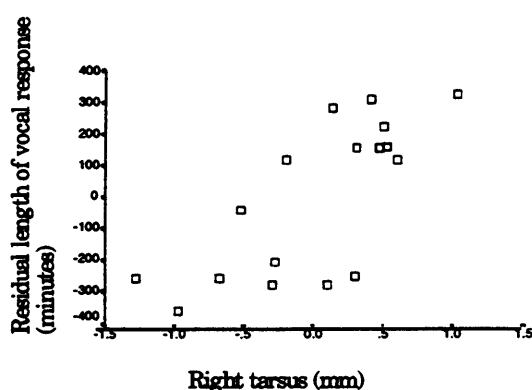
Figure 5a. Partial regression plot explaining variation in the length of the vocal response in the absence of the effect of total breast area, see Table 4.
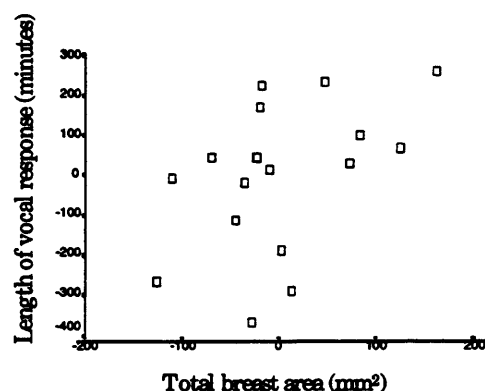
Figure 5b. Partial regression plot explaining total breast area against variation in the length of the vocal response in the absence of the effect of tarsus, see Table 4.

The circulating testosterone levels did not add to any of the models of behaviour measured, even after correcting for time to capture.

None of the other behavioural responses were significantly explained by the bird's morphology (Table 6). This may be due to the small sample size involved. The majority of trials produced a vocal response (58% of nests), whereas only 42% of birds stayed within one metre of the dummy. To be able to exclude the possibility of a type II error - not rejecting the

null hypothesis when it should be rejected - larger samples are required. For example, to give the test an 80% power (at 0.05 significance), when investigating the relationship between the time spent within one metre of the dummy and male body condition, we would need a sample of 39 individuals compared to the 10 used. In all cases the sample size is below that required to eliminate the probability of committing a type II error, and more data would be required to investigate this further.

Table 6. Spearman's Rank correlations between morphological variables and various measures of the behavioural response.

morphological measure behavioural variable	BCI	stripe	wing length	standardized weight
time within 1m (n=10)	r=0.44, ns	r=-0.14, ns	r=-0.17, ns	r=0.45, ns
number of jumps (n=10)	r=0.62, ns	r=0.54, ns	r=0.08, ns	r=0.46, ns
time in observers' sight (n=17)	r=-0.05, ns	r=0.09, ns	r=-0.20, ns	r=0.24, ns

When the behavioural response was converted to binomial data there was no significant relationship between the territorial behaviour of the male and the morphological measures (Figure 6a and b). To investigate whether the sample of males caught during the territorial trial was biased, I compared the means of the stripe area of those birds caught at the territorial response and those caught later, whilst provisioning their chicks (Figure 6c). Males caught at the territorial trial had significantly larger stripes than those caught at provisioning only. This suggests that the males that responded to playback and the dummy might not have been a random sample of the population. This was not reflected in the male's behaviour: there was no apparent relationship between the behaviour at the territorial trial and the likelihood of capture (Table 7)
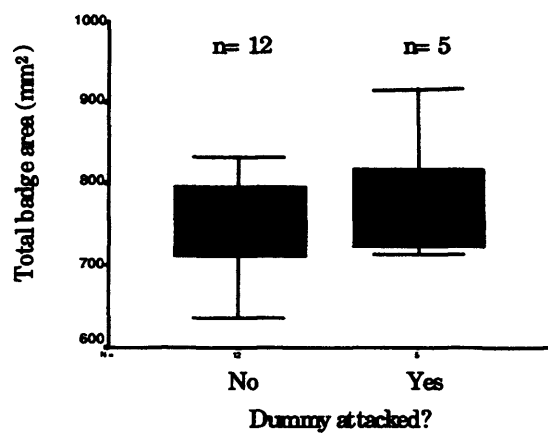
Figure 6a
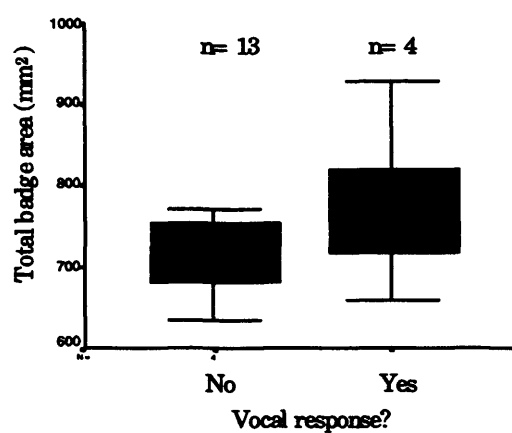
Figure 6b
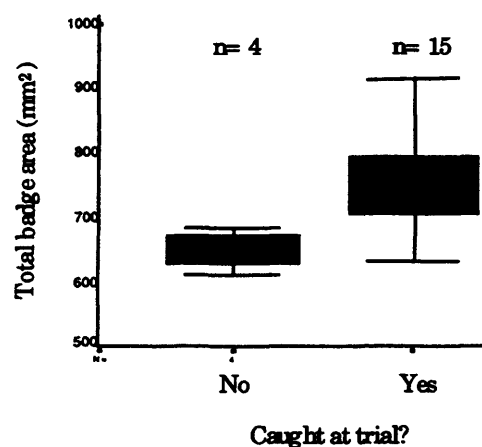
Figure 6c

Figure 6. Comparison of male total badge area of birds that:
a. did or did not physically attack the dummy (Mann Whitney U test, U= -0.52, df= 17, p=0.64)
b. did or did not respond vocally to the dummy (Mann whitney U test, U= -0.79, df= 17, p= 0.47)
c. were caught at the territorial trial (Mann Whitney U test, U= -2.4, df= 19, p= 0.02)
a-c "n" refers to the sample size

Table 7. Comparison of medians of the behavioural responses - with males separated on the basis of whether or not they were caught at territorial trial (n=35).

Behavioural trial variable	Mann-Whitney U test	significance
time to first response	U=-0.06	p=0.96
time spent in 1m	U=-0.02	p=0.98
length of vocal response	U=-0.02	p=0.98
date of trial	U=-0.34	p=0.73

3.4.4 Provisioning behaviour

The variance in the measure of testosterone levels in provisioning males is too low for analysis, with four out of the six individuals having the same testosterone levels. There was no significant relationship between territorial testosterone and male's provisioning at day 7 (Figure 7a). There was a negative relationship, however, between the testosterone measure (taking into account time to capture) and the provisioning behaviour at day 10 (Spearman's r= -0.86, df= 6, p= 0.02, see Figure 7b).
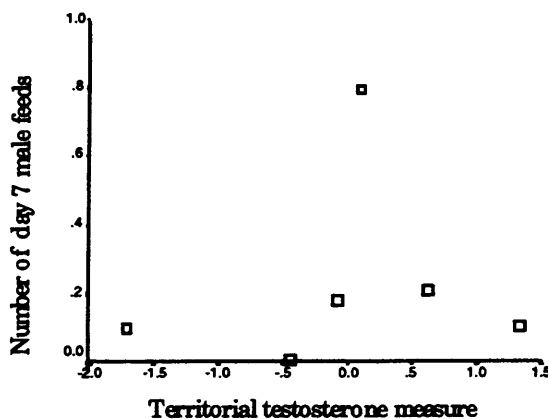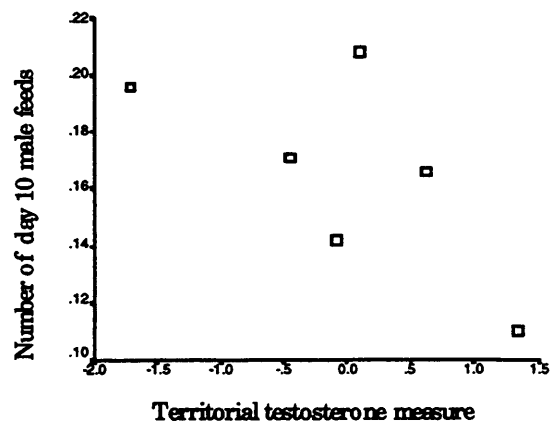
Figure 7a   **Figure 7b**

Figure 7. Territorial testosterone level (using the residuals of testosterone measure against time to capture) against:
a. The number of male feeds on day 7 (Spearman's r= 0.15, df= 6, p= 0.78)
b. The number of male feeds on day 10 (Spearman's r= -0.86, df= 6, p= 0.02)

3.5 DISCUSSION

This study shows that in one measure - the length of the vocal response - males with a larger stripe behaved as if they were more dominant, or in better condition, than small-striped males. Thus, female great tits may be able to use the badge size of their potential partner to assess his ability to defend a nest-site. In great tits, males that successfully defend a territory against intruding breeders have a higher reproductive success (Dhondt & Schillemans, 1983), which suggests successful defence behaviour should increase reproductive success. Whether the ability to defend a nest-site means that bigger badged birds acquire a better territory deserves further investigation. As great tits suffer from woodpecker predation (Smith *et al*, 1989, personal observation, Figure 8), it would be interesting to know whether great tits able to defend a good-quality territory are also able to repel predators. The data suggest a trend for males which elevate their testosterone during the antagonistic behaviour experience a trade-off against their provisioning behaviour. I found no evidence, however, to suggest that the circulating testosterone as measured directly explained any of the aggressive behaviours observed.

3.5.1 Hormonal Response

The most significant contribution to the variation in testosterone levels identified was the time taken to capture each bird following the trial. As expressed by Wingfield & Hahn (1994), the focal male was captured "usually within 1-2 min for highly aggressive birds, but sometimes taking much longer for less responsive individuals". It may be that those birds which raised their testosterone levels the most were caught quickly. In this circumstance the testosterone levels may not be a reflection of how long it took to catch the bird, but rather vice versa: birds that raised their testosterone levels most were caught the quickest because of their aggressive behaviour. The data do not show any immediate effect of testosterone on the male's behavioural response. In his study on the pied flycatcher, Silverin (1993) found that paired males and males that did not attack the decoy had similar testosterone measures; both lower than that of the attacking, unpaired males. It is possible that at the nesting stage that I chose to study (when all males were paired), testosterone was not the main mediating factor behind the aggressive response.

The only male trait that covaried with the elevated testosterone, after controlling for time to capture, was the total badge area, although the effect was not significant. Males with smaller badges tended to elevate their testosterone levels more than large-striped males in response to

the challenge (Figure 4). Such elevation of testosterone may be necessary for small-badged males to be in a position to protect their territory or mate against the perceived threat. Raising testosterone levels, however, may have an associated cost, if not through physical and immunological effects on the individual (Grossman, 1984; 1985; Folstad & Karter, 1992), then perhaps through indirect costs transferred to the current breeding attempt.

One of the limitations of a study such as this is that the sampled group may have behaved differently to those males that were not captured following the trial. It is possible that, by the nature of the experiment, I captured a particular subset of males, with larger striped males being caught more readily. It may be especially important to sample from those birds with small badges- those individuals not so easily caught in the field. Considering the possibility that I only observed a particular subset of males, it is difficult to draw conclusions. The problem with the hormone assay is that the measure of testosterone may not be sensitive enough, and at this stage of nesting (egg laying) the territorial behaviour of the bird may not be mediated by testosterone levels alone. It may be necessary to catch non-responsive males to observe the relationship between the hormone and behaviour.

3.5.2 Defence Behaviour

Paternal defence behaviour in the great tit has been shown to vary with the number of nestlings fathered (in one year of the two studied, Lubjuhn et al, 1993). This experiment was performed so early in the season that levels of cuckoldry should not be a consideration. The benefit of using an unrelated species is that the response observed is not one of mate guarding. Such mate-guarding behaviour has been demonstrated to be negatively related to the male bluethroat's experimentally altered sexual attractiveness (Johnsen & Lifjeld, 1995). In this study the response to the conspecific dummy, the collared flycatcher, appeared to be less vigorous than the response to the great tit dummy. In Kral & Bicik's (1992) study, 63% of flycatchers approached the great tit dummy, however, only in 22% (compared to my 29%) of cases did the great tit approach the flycatcher dummy. It is possible that this may be a problem common to such an experiment. The low response to the flycatcher dummy made the data unsuitable for analysis.

Great tits are bout singers; this means they match each other's song type (Dabelsteen et al, 1996). The birds responded by imitating the playback song. It is a potential problem that such a non-interactive challenge may be so unnatural as not to imitate a territorial challenge. However 79% of the birds did respond to the great tit dummy, suggesting that the great tit trial produced a familiar stimulus. As the response to the great tit, which was displayed after

the flycatcher, was greater we can be assured that the experimental subject had not become conditioned to the stimulus of a territorial challenge. It is possible that the bird would become bolder as a result of the lack of response by the dummy (Knight & Temple, 1986). Aggressive behaviour toward an experimental intruder correlates with nesting success in other species (Grieg-Smith, 1980; Andersson *et al*, 1980 Blancher & Robertson, 1982; but Melivang *et al*, 1997). This suggests that the behaviours measured may be a good indication of an individual's willingness and effectiveness of nest defence.

We would predict that the males with the largest black area would be of better phenotypic condition than those with a small stripe; whether explaining the function of the male's stripe in terms of the male advertising his relative social status to other males, or advertising some quality to potential mates. The data show that males with larger stripes give a longer vocal response. Norris (1990b) found that the male's breast stripe size differed between males that responded to his presence at the nest within three minutes and those that showed no response (a significant relationship in only one year of the two studied). My measure of nest attentiveness, the time lag to the first response observed, however, is explained only by tarsal length. Adding the breast measure improves the model, though its effect is non-significant. Male tarsus is an indication of size, thus larger males apparently are more attentive. This may be because they are also dominant, and therefore may spend less time searching for food and more time in territorial defence.

There was a tendency for males with larger breast stripes to provide a greater response by attacking the dummy and responding vocally to the dummy. However there is no significant difference in these analyses. The analyses are likely to be sensitive to the "uncaught males", for if there were a relationship between likelihood of attacking and stripe size then it would be more difficult to catch unresponsive, small-badged males. The data suggest that I was more likely to catch large-badged males, which indicates using playback is not a reliable way to catch a random population of males.

3.5.3 Provisioning Behaviour

There was no association between the male's circulating testosterone level at provisioning and his provisioning behaviour. The variance of provisioning testosterone levels was very low; this could be due to too little blood being collected for reliable assay at provisioning, or due to the testosterone having fallen to basal levels. There was a negative relationship between the male's provisioning behaviour at day 10 and the circulating testosterone at the territorial trial. This

result may reflect a trade-off facing the male (and his partner if she is choosing her mate). However more data are required before any firm conclusions can be drawn.

If this relationship does reflect a natural trade-off then the female may face a choice between a male who will take part in nest defence and a male who will provision her chicks well. Qvarnström (1997) observed increased male competitive behaviour and reduced parental care due to experimentally increased badge size in the collared flycatcher. Also a number of studies have demonstrated reduced parental care in males with artificially increased testosterone (Silverin, 1980; Ketterson *et al*, 1992; Hegner & Wingfield, 1987; and - through indirect observation - Raouf *et al*, 1997). This study does not establish whether the reduced male provisioning affects female or offspring survival (a true test of direct benefits). Furthermore, there does not appear to be any direct relationship between the parental effort at the territorial trial and that at provisioning, hence this study can give no support to a trade-off theory through analysis of the behavioural data alone.

The male's provisioning behaviour could not be explained by his morphology in this sample. Norris (1990b) also found no evidence to suggest that the feeding rate of males varied in relation to his stripe. In using an alternative measure of parental care, nestling mass, he found a significant, positive relationship between nestling mass at days 7 and 15 and the male's stripe size. The problem with such a measure is you can not remove the effect of the female response to the situation she is presented with. If the female follows a rule such as "invest more in the chicks if mated to an above average male" (as proposed by the differential allocation hypothesis, Burley, 1988), then we might expect such a result. Chapter 4 considers provisioning behaviour in the great tit in more detail.

One of the problems with the data is the small sample sizes used, as in some cases it is not possible to exclude a type II error (not rejecting the null hypothesis when it should be rejected). Also, such sample sizes do not allow investigation into which measure of the black stripe area may be important. (For a summary of separate analyses of the badge measures see Table 1, Appendix 1). Previous studies of the great tit have used very different measures of the stripe. Lemel & Wallin (1993) used a measure of the chin area, whereas Norris (1990a) measured the black stripe below the chin area. The measure used in these analyses was a combination of the two areas, hence it describes the majority of the black area on the great tit's ventral side. Chapter 4 considers the measures of chin and breast and male provisioning behaviour, a measure of potential direct benefit to the female, in more detail.

3.5.4 Conclusions

To conclude, large-badged males produced a longer vocal response to the great tit dummy, but may, in doing so, raise their testosterone levels less than their small-striped counterparts. Whether the elevation of testosterone by the small striped males reflects an option taken by the males is impossible to determine without sampling from the small-striped males (that were not caught during this study). It is possible that some males were not "choosing" the route of raising their testosterone, perhaps because of the potential associated costs. We would expect testosterone levels in the breeding season to represent an optimal compromise between allocation of effort to male-male competition and to parental care (Hegner & Wingfield, 1987). This study suggests there may be some benefit to being mated to a male with a large stripe if such aggressive, defensive behaviour influences reproductive success. There may, however, be a trade-off between aggressive and provisioning behaviours, suggesting that to test the direction of the relationship between male badge size and direct benefits to the monogamous female we need to consider more than one aspect of parental care.

Figure 8. A greater spotted woodpecker predating a great tit nest. The entire brood was lost over a number of days.

CHAPTER 4

THE SEXUAL TRAIT, REPRODUCTIVE SUCCESS AND PROVISIONING

BEHAVIOUR IN THE GREAT TIT

4.1 ABSTRACT

One of the explanations for sexual dimorphism in plumage traits is that males signal the resources they can offer. This chapter considers whether variation in the badge carried by the male great tit signals direct benefits available to the female. The study uses both direct and indirect measures of parental care. There was a negative relationship between the male's provisioning behaviour and his badge area. There was no evidence, however, that this reflects an overall reduction in investment by large-badged males. The reason why apparently reduced provisioning behaviour does not result in a lower reproductive success is discussed. In direct measures of reproductive success there was a positive relationship between the male's badge area and the condition of fostered chicks, and total number of chicks that survived to fledge, as predicted by theories of direct benefits. The possibility can not be excluded, however, that this was due to the female's response to being mated to a large-badged male, as predicted by the differential allocation hypothesis.

4.2 INTRODUCTION

Female mate choice may be based on a number of features: on the basis of direct benefits, such as the resources the male is offering, the male's ability to raise offspring, or on the basis of genetic quality (indirect benefits). This chapter addresses whether direct benefits available to the female are correlated with male badge size, a signal upon which the female may base her choice of mate.

The more a parent provides food and guards against predators (direct benefits), the better its family should fare, and this includes its mate (Andersson, 1994). It has been demonstrated in a number of studies that the male's contribution to the offspring is important in the great tit. For example Sasvári (1986) and Björklund & Westman (1986) both found that removal of the male negatively affected the mass of the young and reduced the number surviving to fledging. Therefore, paternal care may influence female fitness both directly and through her offspring's success.

Experimental manipulations of clutch size or parental care have demonstrated that the level of avian parental care is flexible. For example, experimentally enlarged broods are fed more (Nur, 1984; Gustafsson & Sutherland, 1988; Gustafsson & Pärt, 1990). Similarly, male removal studies have shown that the widowed female increases her effort (Gowaty, 1983; Bart & Tornes, 1989, reviewed by Gowaty, 1996). In some instances, however, reduced assistance by the male has little or no effect on the level of parental care provided by its mate (Slagsvold & Lifjeld, 1988; Whittingham, 1989; Westneat & Sargent, 1996; Lozano & Lemon, 1996; see review by Clutton-Brock, 1991).

Biparental care may offer fitness advantages to each parent, although conflict may arise over the relative contribution each makes to the offspring (Williams, 1966; Trivers, 1972; Parker, 1979). Experimental manipulations suggest that parental care may be costly; brood-size manipulations have been found to affect subsequent survival, condition, or reproductive success of the parents (Askenmo, 1979; Ekman & Askenmo, 1986; Reid, 1987; Nur, 1998; Gustafsson & Sutherland, 1988; Gustafsson & Pärt, 1990; Dijkstra *et al*, 1990; Gustafsson *et al*, 1995; Daan, 1996; Griffith, 1998 (chapter 5); see also Clutton-Brock, 1991). Life-history theory assumes that an organism cannot simultaneously maximise all its life-history traits, since available energy is limited, hence individuals have to trade-off their investment in one life-history trait against that in other traits (Stearns 1976). In the zebra finch partners given "unattractive" coloured leg rings showed a higher level of parental expenditure (Burley, 1988). Attractive birds might benefit from reducing expenditure because this raises their ability to acquire extra matings. Conversely, unattractive birds may benefit from increasing their level of expenditure because it permits them to acquire attractive mates and to retain them (Burley,1988).

This chapter considers whether parental care by the male great tit is correlated with his badge size. The "good parent model" (Hoelzer, 1989; Heywood, 1989), predicts that the male badge size should be positively correlated with male provisioning rate. Hill found that in the house finch, the male's colouration signals his nutritional condition, and that brighter males were better parents (Hill, 1991; 1994). In an alternative model, the differential allocation hypothesis (Burley, 1988), the female is hypothesized to alter her level of investment according to the quality of her mate, which may result in a negative relationship between the male's badge size and his level of parental care.

It is possible that a negative relationship between paternal care and male's badge size may be found depending on the measure of parental care used. I propose that a negative relationship may be observed between provisioning rates and badge size where other behaviours (such as territorial defence) are traded off against other aspects of parental care. Previous studies suggest that males may have to trade off aggressive parental behaviours against provisioning behaviour (Silverin, 1980; Hegner & Wingfield, 1987). My study of territorial defence showed a significant negative relationship between the male's circulating testosterone during a territorial challenge and his provisioning behaviour whilst feeding the chicks at day 10 (Chapter 3, 3.4.4).

The aim of this study was to identify whether females paired to males with large badges received any direct benefits. Norris (1990a) proposed that the black stripe of the male great tit is a sexual signal. I investigated whether female great tits benefit directly in terms of help in provisioning nestlings by selecting males with a large stripe. I use two distinct measures of the black badge of the great tit male. Direct benefits of mating with large-striped males may be missed if only considering one aspect of parental behaviour - such as feeding rates - so I also examine which factors explain variance in reproductive success.

4.3 METHODS

4.3.1 Measurements

In 1996 and 1997 I investigated which variables explained parental feeding behaviour at 40 randomly selected great tit nests. These represented a sub-set of approximately 200 breeding pairs from a nest box population of great tits in southern Gotland, Sweden (see 1.4, Introduction to the field site, for more detail). For each nest, clutch size, hatch date of the first chick (day 0) and the number of chicks successfully fledged was recorded. These give a crude estimate of reproductive success. The parents were caught on their territory using mist nets or box traps on, or after, day 14. All birds were measured for tarsus, weight, and wing length, and BCI calculated (as described in section 1.4). The black stripe was measured as described in the introduction (see 1.4). The chicks were measured at day 14.

For the purposes of this study each visit to the nest was assumed to be a visit to provision young. Each nest was observed for one hour, by telescope or video, on day seven (between 06:00 and 08:00) and day ten (between 09:00 and 11:00), GMT+1, day 0 being the day the first egg hatched. To aid in the identification of the sexes the female great tit's tail was marked, whilst she was incubating her eggs, along the vein at the end of the tail with correction fluid (Tippex™; see Figure 1).

4.3.2 Analyses

For analyses of nestling condition the mean tarsus and weight measures of cross-fostered young were used, hence removing any heritable effects from the analyses. Nests were matched according to hatch date and on day 2 approximately half the clutch exchanged for the same number of chicks from a matched box. Thus chicks were exchanged without altering the clutch size of the receiving nests. The procedure is described in more detail in section 5.2.1.

Figure 1. Female great tit making a provisioning visit to the nest. Note the white mark on the tail making identification of the sexes easy.

4.4 RESULTS

4.4.1 Provisioning behaviour

Relative parental contribution

Male parental behaviour was negatively related to his chin area; whether expressed as the male's contribution per chick or his proportion of the total feeds. (Table 1, Figures 2 a, c and e; the following variables were dropped from the model: male age, tarsus, wing, breast area, standardized weight, clutch size, hatch date and year). No model could be found to explain the variance in the number of female feeds per chick at day seven or day ten (the following variables were dropped from the final model: female age, tarsus, wing, chin area, standardized weight, BCI, clutch size, hatch date and year). The female proportion of the total feeds in relation to male badge size is shown in Figures 2 b, d and f. As the data is presented here in terms of the total proportion feeds, female feeds are a mirror of the male feeds.

Table 1. Models to investigate which factors explain variance in male provisioning behaviour.

dependent variable	Model description	df	β ± s.e.	r²	p
Male contribution per chick at day seven	Full model	38		0.30	< 0.01
	Male chin area		-0.007 ± 0.98		< 0.01
	Male BCI		0.33 ± 0.13		< 0.05
Male contribution per chick at day 10.	Male chin area	32	-0.007 ± 0.003	0.28	< 0.01
Male proportion of total feeds	Male chin area	23	-0.002 ± 0.006	0.32	< 0.05

In Appendix 1 (Figure 1), I consider the alternative measures of male stripe. This is the first dataset large enough to allow separate (meaningful) analyses of male chin and breast areas. Male chin area is the only measure to explain male provisioning behaviour. The measure of male breast stripe does not explain the variation in visits to the nest.
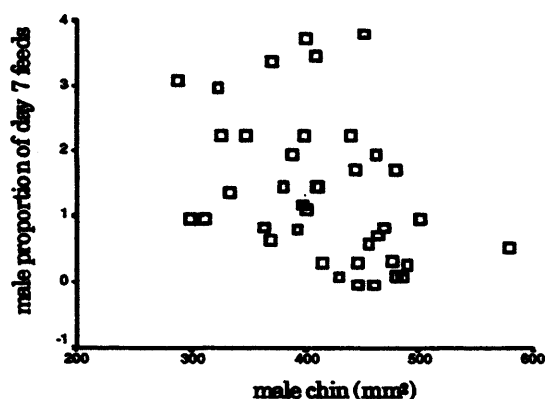
Figure 2a. Relationship between the proportion of male feeds at day 7 against his chin measure.
r= -0.45, df= 38, p=0.005
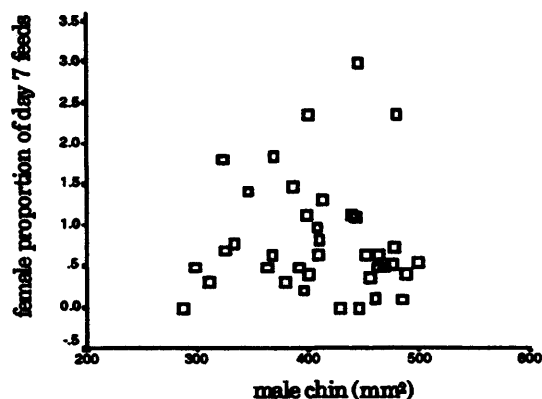
Figure 2b. Relationship between the female proportion of feeds at d7 against male chin measure.
r= -0.08, df= 38, p=0.61
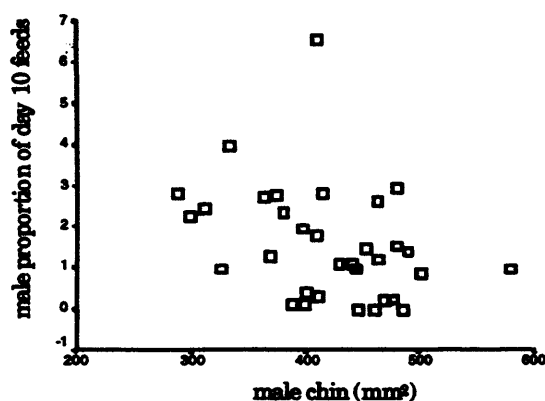
Figure 2c. Relationship between the proportion of male feeds at day 10 against his chin measure.
r= -0.39, df= 33, p=0.02
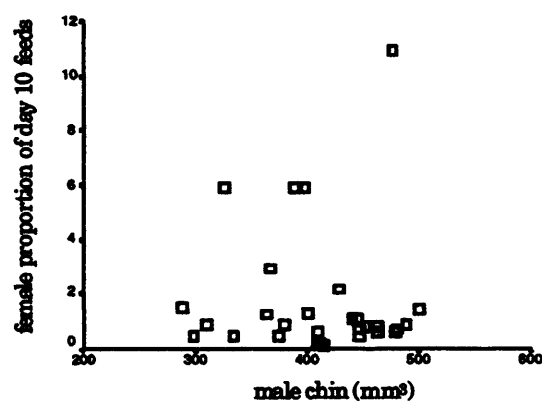
Figure 2d. Relationship between the female proportion of feeds at d10 against male chin measure.
r= -0.09, df=29, p= 0.63
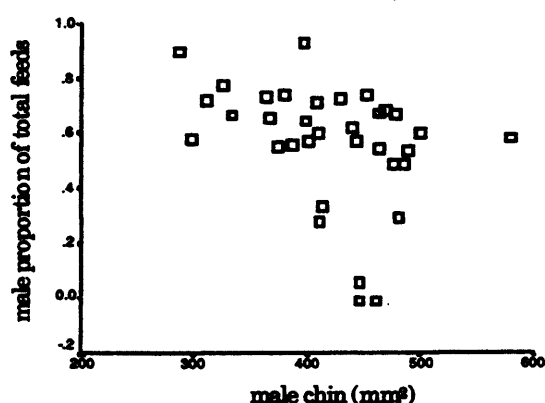
Figure 2e. Relationship between the male proportion of total feeds against his chin measure.
r= -0.47, df= 33, p<0.001
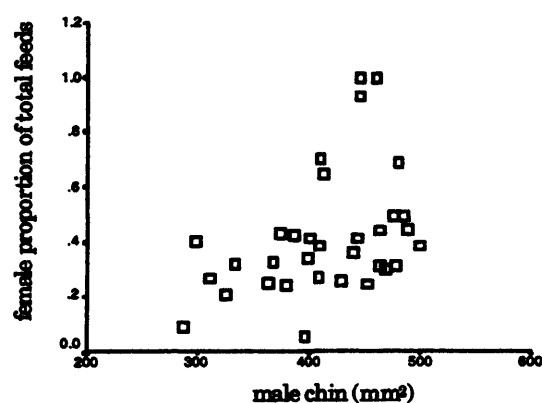
Figure 2f. Relationship between the female proportion of total feeds against male chin measure.
r= 0.47, df= 33, p<0.001

52

Total feeds

The important variable in terms of chick survival, whatever the relative proportions of feeds from each parent, is the amount of food actually being delivered to the nest. There was no relationship between the total feeds at the nest and the male badge size (r^2= 0.08, df= 34, p= 0.08). When total feeds per chick was used (total feeds/number of chicks alive at day 10), there is a negative relationship between the number of feeds per chick and male chin area (r^2= 0.17, df= 33, p= 0.01, Figure 3; day seven mean visit rate 9 ± 7, day ten mean visit 10 ± 9). Hence the total rate of feeds received by each chick was negatively related to the badge size of the father.

There was no relationship between the total female feeds or total female feeds per chick and male chin area (r^2<0.01, df= 33, p> 0.80 in both cases). The data suggest therefore that the female does not compensate for a reduced number of visits by the male.
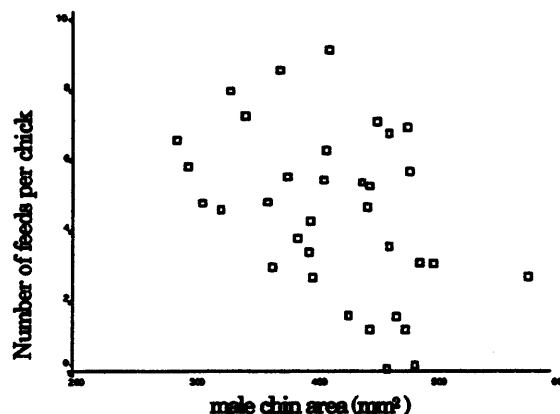
Figure 3. Total number of feeds per chick (total feeds/ number of chicks alive at day 10) against male chin area against the r^2= 0.17, df= 33, p=0.014

4.4.2 Condition of the nestlings

There was a positive effect of hatch date on nestling weight (Figure 4 and Table 2- the effect of hatch date on nestling weight remains to explain variation in BCI). This effect was removed by taking the residuals of nestlings' standardized weight against hatch date and plotting these against the tarsus³ to produce a new estimate of the fostered young's BCI — in the absence of an effect of hatch date. Male chin was the only variable to explain some of the variation in the new estimate of fostered-young's BCI, suggesting a positive effect of foster male's chin area on chick's BCI (see Table 2 and Figure 5). All measures of provisioning were dropped from the model. The provisioning measures did not co-vary with the final fledgling weight.

Table 2. Models to investigate which factors explain variance in measures of fostered-nestlings' body condition.

Dependent variable	Model description	df	β ± s.e.	r²	p
Mean tarsus	Full model	20	27.29 ± 4.88	0.34	< 0.05
	Female tarsus		-0.63 ± 0.29		< 0.05
	Clutch size		7.81 ± 0.01		< 0.05
Mean BCI	Hatch date	71	-4.06 ± 0.03	0.14	< 0.01
Mean standardized weight	Hatch date	71	-0.002 ± 0.07	0.28	< 0.05
Mean BCI (adjusted to remove the effect of hatch date on weight)	Male chin area	74	0.006 ± 0.002	0.10	< 0.01
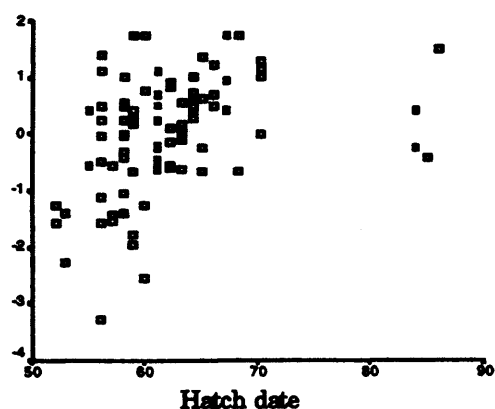
Figure 4. Scatter plot of the fostered chicks (standardized) weight against hatch date. This effect is removed statistically for analyses in Table 2.
r= 0.43, df= 75, p<0.001
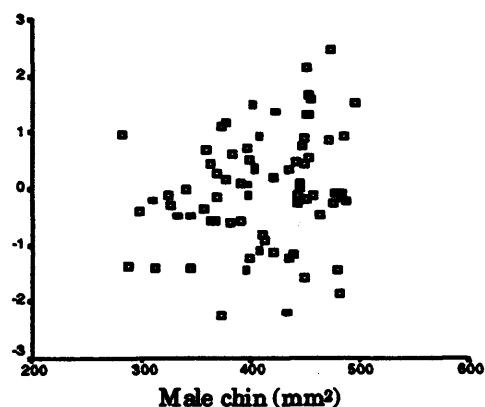
Figure 5. Scatter plot of the fostered young's BCI against male chin measure. The BCI has been recalculated removing the effect of hatch date on chick weight, seen in Figure 4.
r= 0.32, df= 74, p= 0.005

4.4.3 Reproductive success

As a direct estimate of the effect of parental care, I considered the factors affecting the success of each clutch. Table 3 shows the results of a model to examine which factors - measured in the same breeding season - contributed to a female's clutch size. The only significant variable remaining in the model was the male's body condition index. This indicates that either females lay clutches according to the condition of her mate, or females pair non-randomly, with fecund females mating with males of large BCI. Female age had no effect on clutch size (t= -5.45, df= 106, p= 0.58) as has been found in other populations (Perrins & McCleery, 1985).

I investigated, by using the proportion of the clutch that survived, which variables may predict direct benefits to the female and the clutch. Male chin area and male age both contributed significantly to clutch success, though hatch date and the female BCI also add to the model (see Table 3). The actual number of chicks produced should be more important in fitness terms. As expected, the more fledglings produced at a nest, the higher the chance the nest would produce at least one local recruit (mean number of chicks at day 14 in successful nests is 7.1 ± 1.5 compared to 6.0 ± 2.0, t-test t= 2.05, df= 88, p= 0.04; this is not true of clutch size as there is no significant difference between clutch sizes of nests which did and did not produce a recruit Mann-Whitney U= 439, n= 90, p= 0.18). I therefore investigated a model to explain the variation in survival of chicks to day 14. Male chin area, tarsus length and age all contribute to the model, the relationship between these measures and chick survival was positively correlated. Male standardized weight, however, has a negative correlation with chick survival; heavy males tended to produce less chicks (Table 3).

Table 3. General linear models to investigate variation in measures of reproductive success

Dependent variable	Independent variables	df	β ± s.e.	r²	p
Clutch size	Male BCI*	61	-0.62 ± 0.19	0.24	<0.001
Proportion of clutch survived (only including those clutches that produced at least one chick)	Full model	33		0.75	<0.001
	Male chin area		-0.001 ± 0.00		<0.01
	Hatch date		0.004 ± 6.81		<0.001
	Female BCI		0.02 ± - 3.38		<0.01
	Male age		-0.008 ± 0.04		<0.05
	Clutch size		-0.42 ± 0.15		<0.01
Number of young surviving to day 14	Full model	56		0.50	<0.001
	Male age		1.68 ± 0.56		<0.01
	Male chin		0.0023 ± 0.007		<0.01
	Male tarsus		1.59 ± 0.43		<0.001
	Male standardized weight		- 0.85 ± 0.28		<0.01
	Hatch date		0.006 ± 0.026		<0.05
	Clutch size		0.22 ± 0.09		<0.01

*see Appendix 2 for an alternative model

4.5 DISCUSSION

4.5.1 Provisioning behaviour

Both the proportion of male feeds at the nest and the total number of feeds were negatively correlated with male chin area. Females could be compensating for reduced parental care, or may provision more when mated to a male with a large chin area (Burley, 1988). If the female was attempting to compensate for reduced parental care she appears to be doing so incompletely, as the total number of feeds per chick decreased with male chin area. Alternatively our measure of provisioning is not a good reflection of the value of the food brought to the nest.

The provisioning measure did not explain variation in nestling body condition in this study. We would expect that the amount of food brought to the nest should influence chick development. It is possible, however, that the provisioning rate may not reflect the food-consumption by nestlings. The relationship between the provisioning rate and the total weight of food may not be simple. In Royama's (1966) study, for example, the total weight of food brought to the nest decreased as the number of visits increased by males visiting late broods of great tits. There may also be differences in provisioning behaviour between the sexes; in the wheatear males fed larger prey to the nestlings than females (Currie, 1995; Chapter 6).

Even if the negative relationship between provisioning behaviour and male chin area truly reflects the male's reduced food input, it does not necessarily imply differential parental investment. If males trade-off their effort spent in acquiring and defending a territory against their parental care we might expect to see such differences in parental feeding rates. Whether the size of the male's chin area also indicates the quality of his territory is not yet clear in the great tit.

The results presented in Chapter 3 suggest that a male's chin area is positively related to his defence behaviour and Lemel & Wallin (1993) showed that, in pairs of unacquainted great tits, the size of the badge determined the outcome of a competitive event. Hence it is possible that females mated to large-badge males are acquiring a mate who holds a good-quality territory. There is experimental evidence to suggest that a more conspicuous plumage display positively influences territory size and provisioning behaviour in opposite directions (Marchertti, 1993; Qvarnström, 1997). In an observational study blackcap male's song frequency is negatively

related to the male share of parental care, but positively correlated with territory quality (Hoi-Leitner *et al*, 1993; 1995). In collared flycatchers experimentally increased badge size led to an increase in territorial behaviour but decreased provisioning behaviour (Qvarnström, 1997). Similarly, experimentally increased testosterone levels positively increase aggressive behaviour but negatively affect provisioning behaviour (Silverin, 1980; Hegner & Wingfield, 1987). If a trade-off between parental care and territory quality occurs then we might expect females mated to large-badged males to be able to provision their young more, or with a smaller associated cost than females mated to males holding poor quality territories.

Aside from the assumed variation in paternal care due to differences in male quality, what might affect the amount of care provided by the social mate? Westneat & Sherman (1993) predict from their model of paternal care that age of, and relatedness to, young should influence paternal behaviour (see also Møller & Thornhill, 1998). It is an assumption of this study that extra-pair behaviour is not affecting provisioning behaviour. It is also assumed that the cross-fostering experiment had no effect on parental behaviour. Chapter 5 suggests there is no detrimental effect of cross-fostering as control clutches actually performed worse than experimental clutches (5.3.1). It is also important to bear in mind that the paternal behaviour performed may be influenced by the "perceived" potential for future, as well as immediate, reproductive success (Freeman-Gallant, 1996). Nur (1984) proposes that parental feeding frequency reflects a trade-off between fledgling and parental survival.

4.5.2 Condition of the nestlings

After taking into account the effect of hatch date on chick weight, there was a positive relationship between the average BCI of the fostered chicks and male chin area. By using fostered nestlings, we remove the possibility that this relationship is due to heritable effects. Female tarsus length was the only parental measure that influenced the tarsus length of fostered offspring. These data suggest that there may be some direct advantage of being mated to a male with a large chin area. Norris (1990b) found no evidence to suggest that the feeding rates of males varied in relation to his breast stripe measure. In using an alternative measure of parental care (nestling mass) he found a significant, positive relationship between the male's stripe size and nestling mass at days 7 and 15. Similarly, this study found a positive relationship between male chin area and fostered chicks' body condition. The problem with such a measure it that it is not possible to remove the effect of the female response to the situation she is in. If the female follows a rule such as: "invest more in the chicks if mated to an above-average male" then we might expect such a result. The differential allocation hypothesis proposes that females mated to males with large badges invest more in their young

(Burley, 1988). It seems unlikely that the female could do this without incurring serious costs (Qvarnström, 1998).

4.5.3 Reproductive success

The central question is; was there a direct benefit of being paired to a large-badged male? The most direct measure of this is the number and quality of offspring produced, as the likelihood of a nest producing at least one recruit was dependent upon the number of chicks that survived to day 14. When considering the proportion of the clutch that survived to day 14, there was a negative relationship with the male's chin area in the two years studied. However, the proportion of the brood that survived was highly influenced by the original clutch size, which did not influence whether a nest produced at least one recruit or not. When considering the total number of chicks that survived, male chin area remained in the model, with a positive relationship between the number of offspring and the male's chin area.

In his analyses of reproductive success, Norris (1990a) found a significant relationship between clutch size and his measure of male badge: large-striped males tended to mate with females which produced large clutches. In this study, male BCI was the only variable to explain variance in clutch size. It is not possible to distinguish between the two most likely explanations for the relationship: assortative mating or the female responding to being mated to a male with a relatively high BCI by laying more eggs. Norris (1990a) found evidence for assortative mating.; in his study females mating with large-badged males had previously produced a large clutch.

Nestling condition may influence the subsequent success of the brood. Smith *et al* (1989), for example, found that local recruitment of great tit chicks correlated with nestling weight and tarsus. By taking the effect of hatch date on the offspring's weight into account, a significant association between the fostered young's BCI and male chin area was observed.

The relationship found here agrees with that found in the majority of studies: a positive relationship between the reproductive success of a male and the size of his sexual ornament (Andersson, 1994 and Johnstone, 1995 for reviews; but see Griffith *et al*, 1999a).

4.5.4 Testing the theories

The "good parent" hypothesis predicts a positive effect of female choice on the viability of sons and daughters as a result of increased parental investment (Hoelzer, 1989). Thus the "good parent" model should ideally be tested by assessing all aspects of parental investment.

The model can not be rejected on the basis of provisioning rates alone. Despite the reduced provisioning by males with large chin areas, the reproductive success of a brood was positively correlated with male chin area. This suggests that the female may gain some direct benefit from being mated to a male with a large badge.

If the increased success of broods from males with large chin areas were due to differential allocation by the female, and not due to direct benefits, then we would predict a detrimental effect on female survival and subsequent breeding success. Such analysis requires large-scale longitudinal studies, as have been carried out in the collared flycatcher. Qvarnström (1998, paper IV) found a significant negative effect of a male's badge size on his provisioning behaviour (though only in late breeders), as did this study. She also demonstrated that females mated to large-badged males, although they did not benefit from help in provisioning chicks, benefited from slightly better reproductive success the following year. Hence even if the male does not provide direct benefits to the female through provisioning it is still possible that the female benefits directly by mating with a large-badged male. This study can not therefore reject the "good parent" hypothesis. In fact, the data suggest that there may be some direct benefit to being mated to a male with a large chin area. Chapter 3 suggests that the reduced provisioning behaviour may be due to a trade-off with behaviours such as territorial defence. This warrants more study in the great tit.

Burley (1988) proposed that the relative contribution to parental care may be dependent on the relative attractiveness of the mates. She described two possible situations. Firstly "incomplete investment compensation", i.e. withdrawing less investment per offspring than is added by mates willing to increase their relative investment. If this had occurred then we would have expected to observe a positive relationship between male chin area total feeding rate; the relationship with provisioning behaviour is actually negative.

In "complete investment compensation" the total investment per offspring is fixed, but the relative contribution by the parents varies with mate quality (Burley, 1988). Attractive individuals, therefore, would benefit by a lowered per-offspring investment, which should result in making resources available for investment in other life-history traits (Stearns, 1976). There was no evidence for this from the data as the total per-offspring care actually decreased with male chin area. However, as discussed, the provisioning behaviour recorded may not represent a true estimate of the value of the parental care. This study did not allow investigation into whether this apparent asymmetry exists in all aspects of parental care, though Chapter 3 suggests that it does not. In her experimental test of the differential

allocation hypothesis, Burley (1988) determined the incidence and duration of nest-defence activities as well as parental care. I would suggest that the great tit would be a suitable species in which both areas of parental care may be studied, however the sample sizes should be increased over those used in Chapter 3, for considering defensive behaviour.

4.5.5 Conclusions

In conclusion there was a significant negative relationship between the male's provisioning rate and his chin area. There was no indication, however, that this reduced visit rate to the nest was detrimental to the success of the clutch. In direct measures of reproductive success there is a positive relationship between the condition of the (fostered) chicks and the number of chicks that survived to fledge, and male chin area. Given the similarity in results obtained here and in a flycatcher study (Qvarnström, 1998 paper IV), I would suggest that there might be some direct benefit to being mated to males with large chin areas.

ENVIRONMENTAL AND GENETIC INFLUENCE ON CHICK MORPHOLOGY
AND SURVIVAL- DIRECT OR INDIRECT BENEFITS IN THE GREAT TIT?

5.1 ABSTRACT

Theoretically there are two types of benefits a female may receive by choosing to mate with a large-badged male: direct and indirect benefits. These categories need not be mutually exclusive (Andersson, 1994). To separate genetic (indirect benefits) from environmental effects (potential direct benefits), a cross-foster experiment was performed in great tit broods. This study provides evidence of both direct and indirect benefits to the female, dependent upon the phenotype of her mate. Fostered chicks were more likely to survive if raised by large foster-males, potentially a direct benefit to the female. Though we can not discount the possibility that females invest differentially according to the phenotype of her mate, which may account for this result, this is not a problem when considering indirect benefits. Fostered chicks whose true father had a large chin area were more likely to survive than those with small-badged fathers, suggesting that females may gain indirect benefits through mate choice.

5.2 INTRODUCTION

Female mate choice may be based on the potential benefits offered by her mate, which are defined as direct or indirect. She may choose the male on the basis of resources he is offering, or on the male's ability to raise offspring (direct benefits). Alternatively choice may be made on the basis of genetic quality (indirect benefits). Even when such mechanisms are not exclusive to one mating system, we can draw a clear distinction between the two using cross-foster techniques.

By separating the environmental and genetic components that influence the development of chicks in the nest, we can investigate if there is a covariance between the morphological condition of the nestling and parental phenotype. Several studies have investigated this by swapping complete clutches between nests (Smith & Dhondt, 1980; Dhondt, 1982; Gustafsson, 1986; Wiggins, 1989; Norris, 1993; Griffith et al, 1999b). This experimental design allows separation of genetic and environmental effects. Alternatively, a partial cross-foster

design allows us to look at the effect of shared genome and environment, of shared genome, and of shared environment. Such a technique has been used to tease apart the influence of genes and environment in a number of studies (Lemel, 1993, paper IV; Merilä, 1997; Sheldon *et al*, 1997; Qvarnström, 1998 paper V).

The phenotype expressed by any individual is determined by the genotype, environment and any interaction between the two (Falconer, 1989). The genotype is a selection of parental genes, whilst the environmental effects are the non-genetic influences on the phenotype. In the case of parent-offspring comparisons certain assumptions are made. Primarily, the assumption is that the developmental conditions experienced by the nestlings are similar to those experienced by the parents during their early development. If this assumption is broken, then the heritability of a character will be underestimated, as the similarity between parents and offspring may be low due to the dissimilar nature of the environment experienced during development. We cannot quantify the effect of year on the parents' development and hence can not estimate the likelihood of similarity between parent and offspring characteristics.

A second assumption is that estimates of heritability are not affected by the presence of extra-pair young. Extra-pair young have so far been found in approximately 65% of socially monogamous passerines (Owens & Hartley, 1998). Inclusion of half-sibs in full-sib analyses would result in conservative estimates of the genetic contribution to characters. Thirdly, it is generally assumed that estimates of heritability are not affected by prehatching or early posthatching maternal effects. Recent study shows that maternal effects may take effect at the egg stage, and may do so according to the phenotype of the male (Gil *et al*, 1999).

This study aimed to quantify the relative importance of genetic and environmental factors in determining the size of morphological characters, and in determining the recruitment of chicks into the local population. Any environmental contribution that varies with the male phenotype is potentially a direct benefit, or cost to the female. This chapter focuses on the question of whether there is evidence of direct or indirect benefits to the female according to the characteristics of her mate.

5.3 METHODS

5.3.1 The cross-foster

In 1996 and 1997 110 clutches were matched according to their hatch date and for each pair of nests the number of chicks moved was half of the smaller clutch size. In this way, no attempt was made to match nests for clutch size and the final size of both clutches was unaltered. When the number of chicks in a brood was uneven, I transferred less than half the clutch. The mean number of chicks transferred was 3.4 ± 0.6. Chicks were exchanged on day 2. In 1996 the total weight of the chicks for transfer and the total weight of chicks left was measured. In 1997 the weight of each chick at day 2 was taken before fostering. Chicks were carried in an insulated bag, under the field worker's jacket to use body heat to keep them warm. Control nests were those at which no fostering took place.

As a result of the manipulations there are three categories of chicks:

1) those born and raised in the same nest, referred to as home chicks,

2) those chicks moved out of their home nest, referred to as crossed chicks

3) those chicks moved into a nest with the home chicks, referred to as fostered chicks

Home and crossed chicks therefore are related siblings reared apart, and home and fostered chicks are unrelated chicks raised in the same nest.

Chicks that were moved out of their nest are associated with two sets of parents:

1) those unrelated parents that raised them as a result of the manipulation, referred to as foster parents

2) their genetic parents, referred to as true parents.

5.3.2 Measures

The parents were caught at their box whilst provisioning young on, or after, day 14. All birds were measured as described in Chapter 1 (1.4). Each chick's tarsus and weight were measured at day 14, when a blood sample was taken from the cutaneous ulnar vein, and stored in 70% ethanol for subsequent sexing (described in Chapter 6).

5.3.3 Analyses

Statistical analyses were performed on parent-offspring similarity using mean values from each category of chicks, giving mid-offspring values. Averages of the morphological traits, were also calculated separately for each sex within each category. Narrow sense heritability is calculated by regressing mid-sex and mid-offspring measurements against mid-parent or single-parent values. To calculate heritability estimates in the case of resemblance between offspring and one parent, the regression coefficient was multiplied by 2 (as the genetic resemblance with one parent equals 0.5; Falconer, 1989). We cannot quantify the magnitude of the environmental effect on resemblance, hence the slopes of the regression of parent-offspring resemblance are presented for easy comparison.

To analyse which variables influenced chick survival I used binary-logistic regression, entering each chick into the model. To take into account over-dispersion due to each parent being entered more than once a backwards-selected model was run to identify contributing factors with a significance of less than 0.1. This reduced model, only containing variables of interest, was then run forcing the box identification (and therefore parent identification) into the model as a categorical term. From this σ^2, an estimate of the over-dispersion can be estimated from:

$$\text{from: } \frac{-2\log(likelihood)}{(n-(df+1))}$$

The model was then run again without box identification, using our estimate of σ^2 as a user-defined estimate of the variance (see McGullagh & Nelder, 1988), to investigate which factors remain in the model.

Power analyses were carried out using the public access web site provided by UCLA: (http://www.stat.ucla.edu/calculators/powercalc/).

5.4 RESULTS

5.4.1 Experimental versus control boxes

It is essential that cross-fostering the chicks had no deleterious effect on their survival. To check this, I compared the hatch date (using April 1st as day 1), clutch size, condition and survival of experimental and control broods (Tables 1 and 2). Whilst there was no difference in final offspring condition and clutch size, fewer chicks were produced in control broods (Table 2). This may be attributable to a difference in hatch date between the two categories (Table 1), however it appears that control clutches in the years studied were more likely to suffer from desertion or complete clutch failure (there was no significant difference in brood success when clutches which produced at least one chick were compared, Table 2). There was no evidence from these analyses that experimental clutches suffered from the manipulation.

Table 1. Hatch date of experimental and control great tit broods for 1996 and 1997.

Variable	Mean ± s.e.	Median Date	Range
Hatch date of control nests	60.7 ± 1.12, n=44	29th May	22nd May – 25th June
Hatch date of crossed nests	67 ± 1.9, n=48	2nd June	17th May – 6th July
Comparison of median hatch dates	Mann Whitney-U test: U= -2.77, N= 92, p= 0.006		

Table 2. Comparison of clutch size, condition and survival of chicks from experimental and control great tit broods.

Variable	Category	Mean ± s.e.	Range	Comparison of medians, crossed nests v control nests (Mann Whitney-U test).
Clutch Size	crossed	8.7 ± 0.34	5 – 12	Z= -0.25, N= 92, p= 0.80
	control	8.9 ± 0.26	6 – 11	
Day 14 survival	crossed	5.98 ± 0.39	0 – 10	Z= -2.75, N= 84, p= 0.006
	control	4.08 ± 0.51	0 – 9	
Day 14 survival when at least one chick survived				Z= -1.92, N= 78, p= 0.054
Day 14 BCI index				Comparison of means (t-test) t= -1.18, df= 130, p>0.05

Table 3. Comparison of day 2 weights of experimental clutches.

clutch weights (total weight of chicks originating from the same nest at day2).	Mean ± s.e.	Comparison of total weight of chicks crossed and left (paired t-test).
home young	11.52 ± 3.39	t= -0.84, df= 106, p=0.40
crossed young	11.83 ± 4.32	

Table 4. Correlation between day 2 and day 14 weights (1997 chicks).

home female			home male			crossed female			crossed male		
r	df	p	r	df	p	r	df	p	r	df	p
0.06	17	0.80	0.18	18	0.45	0.24	15	0.38	0.32	16	0.21
power: 0.06			0.11			0.14			0.22		

To ensure that the manipulation was not biased I compared the total weight of chicks crossed out of the nest with those that remained. There was no significant difference in weights (Table 3), indicating that the experimenter did not favour large or small chicks for transfer. Similarly, I checked that the weight at the day 2 manipulation did not correlate with the day 14 weight. None of the correlations were significant (Table 4), suggesting that even if there were some maternal effect on chick condition at day 2 it did not influence fledgling condition.

Fostered chicks were moved into three categories of clutch size: those larger than they started in, those smaller than they started in and those of equal size. To ensure this manipulation did not significantly affect final body size I compared the two extreme classes (smaller and larger, only using nests in which the category applied both to clutch size and survival to day 14). There was no significant difference between the average tarsus measure of those moved into larger or smaller broods (t-test; t= 1.83, n= 27 and 48 nests, p= 0.07), though the trend was for those moved into larger broods to have smaller tarsi (fostered young mean tarsus: in larger broods = 19.23 ± 0.60; in smaller broods 19.63 ± 1.01). Similarly, there was no significant difference in weights between those chicks moved into larger or smaller broods (t-test; t= 0.70, n= 27 and 48, p= 0.49).

5.4.2 Resemblance and heritability estimates

Parent-offspring comparisons

Regressions of fledgling morphological measures against parent measures were made. There were three categories of manipulation: those chicks born and raised in the same nest (shared environment and genome); those unrelated chicks that were fostered into the nest (shared environment); and those chicks crossed out of the nest (shared genome). Using the latter comparison to test for genetic influence, I used both sets of fostered chicks: those that were crossed out of the home nest against their true parents and those fostered into the home nest against their true parents. Thus the results of these two regressions should be similar.

There was near-significant correlation between parent and chick tarsus for those chicks that shared both environment and genome with the parents at the nest (Table 5). There was a significant correlation between crossed chicks and true parent body weight, i.e. there was a significant genetic component to final body weight for one set of the shared genome comparisons (Table 5). Resemblance estimates for mid-chick measures against each parent can be seen in Table 1, Appendix 3, and for chicks separated by sex in Table 2, Appendix 3.

Table 5. Resemblance and heritability estimates for mid-offspring on mid-parent measures from day 14 chicks in the nest: (1) shared environment and genome, (2) shared environment, (3) shared genome (3a) compares chicks fostered into the "home" nest against their true parents, (3b) compares chicks crossed out of the "home" nest against their true parents.

relationship		mid parent - mid offspring				
character		n	slope *	s.e.	p	power
tarsus	1	66	0.37	0.19	0.06	0.87
	2	64	0.02	0.21	0.94	0.38
	3a	56	0.16	0.23	0.49	0.22
	3b	54	0.17	0.24	0.47	0.23
body weight	1	62	0.09	0.11	0.43	0.11
	2	60	0.15	0.12	0.25	0.21
	3a	53	0.28	0.13	0.04	
	3b	51	0.27	0.15	0.07	0.48

*the slope given here is = h^2 (narrow sense heritability) for category 3- the genetic contribution

Table 6. Resemblance and heritability estimates for recruited young against their parents. Three categories are investigated: (1) shared environment and genome, (2) shared environment, (3) shared genome (compares all moved chicks against their true parents i.e. those defined as crossed and fostered).

relationship		recruit measure* – mid parent measure			
character		n	slope	s.e.	P
tarsus	1	15	0.77	0.13	<0.001
	2	12	1.28	0.55	0.04
	3	14	0.42	0.70	0.56
wing	1	12	0.45	0.32	0.20
	2	11	-0.12	0.47	0.80
	3	13	0.30	0.55	0.58
chin	1	10	0.24	0.35	0.51
	2	7	0.06	0.59	0.91
	3	8	0.07	0.57	0.89

* The measures use averages from all incidences of catching an individual.

An alternative comparison can be made using recruited young and their parents. The environmental contribution to tarsus length becomes significant when we consider the correlation between foster parents and their unrelated offspring (Table 6). (Analyses separated by parental and chick sex can be found in Tables 3 and 4 respectively in Appendix 3). Body-weight was not considered as these analyses are based on all averages from all incidences of catching recruits, and body weight varies over season and time (Garnett, 1981).

If there were some indirect benefit to the female from mating with a male of a certain phenotype then there should be evidence of the male contributing to the development of relevant traits. Here I present a male-based index of the relative genetic contribution of both parents. Using this index, male contribution appears to be greater for body weight, whilst the female appears to contribute more to tarsus (Table 7). Consideration of male-indexed genetic influence on the phenotypic expression in offspring separated by sex is given in Table 5, Appendix 3; the trends remain the same whatever category was considered.

Table 7. Comparison of male and female genetic influence on phenotype expression compared to female genetic influence. Indices are calculated as R^2_{male}/R^2_{female} from the resemblance regressions of each sex or mid-offspring values. A value of 1 estimates an equal impact; values larger than 1 suggests a male biased influence.

Character	Influence on mid-offspring values	Influence on mid-recruit values
tarsus	<0.01	0.63
body weight	2.50	1.00
chin area	-	0.50

Sibling comparisons

We controlled for the problem of year-effects on the development of morphological traits by directly comparing related siblings raised in different environments. The relative contributions of environment and genome to tarsal development appears to be equal (Table 8), whilst there seems to be a larger environmental component to body-weight (Table 8). This is not unexpected as tarsus is a skeletal measure, probably less sensitive to environmental variation than a trait such as body weight. The results of resemblance estimates for chicks separated by sex are given in Table 6, Appendix 3.

Table 8. Resemblance estimates for mid-home young on mid-fostered measures from day 14 chicks in the nest: (1) shared environment (chicks fostered into the home young's nest), (2) shared genome (related chicks crossed out of the home young's nest).

relationship		Mid home young- mid fostered young			
character		n	slope	s.e.	P
tarsus	1	84	0.59	0.09	<0.001
	2	68	0.56	0.07	<0.001
body weight	1	84	0.67	0.08	<0.001
	2	68	0.28	0.10	<0.01

The genetic resemblance between full siblings is 0.5 * the additive genetic variance (the correlation coefficient of a character $h^2 = 2$ * the slope). The resemblance due to shared environment should be approximately equal. For easy comparison of the two the slope of the line is presented in place of h^2.

5.4.3 Chick survival

I considered three separate models, using binary logistic regression - to examine chick, clutch and parental characteristics. I analysed which morphological variables explained recruitment of chicks into the next year's breeding population. Unmanipulated chicks (i.e. those raised in their own nest) were used for this analysis.

For the chick's characteristics I considered tarsus length, standardized weight and BCI separately to avoid any problems of colinearity. Tarsus length was the only measure of chick size to explain chick survival (see Table 9). As tarsus is a dimorphic trait, I fitted the model on male and female offspring separately. Both models were non-significant ($\chi^2 = 0.35$, n= 62, p= 0.55, $\chi^2 = 1.89$, n= 57, p= 0.17; respectively). This may be due to the sample size becoming reduced as a result of using these sub-sets of data, the analyses included only ten surviving males and seven surviving females.

The number of chicks that survived to day 14 was dropped from the model investigating the effect of clutch characteristics on chick survival, but hatch date and clutch size remained (Table 9). Chicks from smaller and earlier clutches had a better chance of survival.

To examine the influence of parental characteristics, the data were separated into two groups according to their experimental manipulation. I used crossed chicks, those chicks raised away from their home nest, to consider the influence of foster parents. Secondly I considered the effect of true parents on their away-raised offspring. Of the foster parents' characteristics only male tarsus length covaried with chick survival (Table 9; the following male and female characteristics were dropped from a backwards-selected model: female tarsus, standardized weight, BCI, age, wing, chin area, and for the male breast area). The same model was fitted for fostered chick's true parents; all variables were dropped except for true male's chin area (Table 9).

Table 9. Models to investigate which factors explain variance in chick survival

dependent variable	model description	variables	df	β ± s.e.	χ²	p
survived 1 year	chick characteristics	tarsus length	192	0.46 ± 0.24	4.01	0.05
survived 1 year	clutch characteristics	full model	233		13.58	0.001
		hatch date		-0.07 ± 0.03		0.03
		clutch size		-0.42 ± 0.15		0.01
survived 1 year	foster parents	male tarsus	254	0.98 ± 0.47	4.42	0.04
survived 1 year	true parents	male chin area	224	0.01 ± 0.00	3.94	0.05

The mean tarsus length of those carer-parent males which produced at least one recruit was 19.96 ± 0.43 mm compared to 19.76 ± 0.68 (t=5.87, df= 77, p< 0.001) of males that did not produce recruits in the local population. The mean chin area of those true males which produced at least one recruit was 427 ± 78.5 compared to 405 ± 53.1 for those males that did not produce recruits (non-significant in a t-test, probably due to the high standard deviations).

5.5 DISCUSSION

5.5.1 The assumptions

Certain assumptions were made in this study: that there was no pre or post-hatching maternal effect; that parent-offspring correlations were not affected by the environmental conditions experienced; and that there were no extra-pair young. Two of these are addressed in the analysis. I investigated whether there was any relationship between weight at day 2 and weight or condition at day 14, to investigate whether any such maternal effect influenced final nestling condition. There was no significant relationship between the weights, which suggests any maternal effects on the chicks at day 2 did not influence fledgling weight. Merilä (1997), in a similar cross-foster experiment in the collared flycatcher also found no evidence for a maternal effect on fledgling condition.

The results of our parent-offspring correlations were lower heritability estimates than those obtained from correlations between related siblings raised in different nests. This was also true of other partial cross-foster studies (Alatalo & Lundberg, 1986; Larsson & Forslund, 1992; Lemel, 1993). Merilä (1997) and Qvarnström (1998, paper V) demonstrated that the parent-offspring regressions in the collared flycatcher revealed lower estimates of heritability of body size under poor environmental conditions. Similarly, in *Drosophila melanogaster* ecological factors significantly influenced heritability estimates (Mukai & Nagano, 1983). This confirms the assumption made in such analyses, (that the conditions experienced by the chicks during development are the same as those that were experienced by the parents when they were nestlings), is in danger of being broken. It is therefore probably more useful to compare related chicks raised in different environments than to use parent-offspring correlations to examine the relative contribution of environment and genes. Extra-pair young have been found in the great tit, and may account for up to 15% of chicks. Including such chicks in the analyses will also reduce our estimates of heritability. It is the magnitude of this reduction that is important; for example the variation due to environmental effects may reduce our heritability estimates in a similar magnitude as inclusion of extra-pair young.

5.5.2 Resemblance and heritability estimates

Parent-offspring comparisons

There was a near-significant correlation between mid-parent and mid-fledgling tarsus for those chicks that shared both environment and genome with the parents at the nest. When recruits were compared with mid-parent measures, the environmental component determining development of the tarsus was significant. It is possible that due to the years of study being relatively poor both in terms of caterpillar abundance and mean temperature (Robert Przybylo, personal communication) that the day 14 measure of tarsus was not that of the fully developed chick. This would reduce my estimate of genetic resemblance between parent and offspring. The lack of correlation between true parents' and recruits' tarsi may be due to the low numbers involved. Lemel (1993), in his study on the great tit described a significant heritable component to tarsus. Dhondt (1982), in his clutch-swap in blue tits found no relationship between foster parents and the chicks they raised, whilst he did find a significant association between chicks and mid-true-parent tarsus. Gebhardt-Henrich & van Noordwijk (1991) reported a varying genetic impact on tarsus in the great tit, depending on year. It may be that such an environmental effect masked the heritable component to tarsus length in my study.

There was also a small, significant, genetic component to fledgling body weight. The results differ from those described previously, in which approximately half the deviation in body weight of surviving offspring was described by the body weight of the parents. However, in his study van Noordwijk et al (1980) used weight measures taken outside of breeding and moulting season, i.e. periods when weight fluctuates less. I did not analyse the body weight of recruits because the majority of measures were taken during the breeding season, when weight is expected to fluctuate most widely. (Van Noordwijk et al, 1980). Lemel (1993) also described a significant heritable component to body mass in the great tit.

Sibling comparisons

I compared this study with another that analysed related great tit siblings raised in different environments, and unrelated siblings raised in the same environment (this study and Lemel, 1993). In both, there is a greater effect of environment rather than genotype on body weight, and therefore on body condition of the chick (Table 10).

Table 10. A comparison of studies on resemblance estimates for mid-home young on mid-fostered measures from day 14 chicks in the nest: (1) shared environment (chicks fostered into the home young's nest, or shared environment in shared hatch date), (2) shared genome (related chicks crossed out of the home young's nest).

Study		this study	Lemel (1993)	Lemel (1993)
relationship		mid home young- mid fostered young		unrelated young- with only hatch date in common
character		slope	slope	slope
tarsus	1	0.59	0.45	0.22
	2	0.56	0.46	
body weight	1	0.67	0.60	0.20
	2	0.28	0.44	

Lemel's (1993) study considered that there is probably a small effect of season on the resemblance of chicks. This effect however, is not large enough to account for the resemblance between unrelated chicks raised in the same nest. If the influence of season is additive, sibling heritability estimates may be reduced by approximately 0.20, which would reduce our genetic effect of body weight to almost nothing, but leave the environmental component at approximately 0.40 (see Table 10; there is no significant difference between Lemel's estimate of environmental contribution to body weight and my own, $Z < 1.96$, though there is a significant difference between our genetic estimates, $Z > 1.96$).

5.5.3 Direct or indirect benefits

The purpose of this study was to examine evidence for there being direct or indirect benefits gained by females from being mated to large-badged males. A number of studies have demonstrated that fledgling survival in the great tit increases with body-weight (Perrins, 1965; Garnett, 1981; Smith *et al*, 1989). It seems, therefore, prudent to investigate whether the male makes any substantial genetic contribution to these traits. In our index of genetic influence it appears that the female contributed most to the development of offspring tarsus length, while the male contributes most to the body weight (and therefore the BCI) of the young. This index is however based on non-significant regressions. From sibling comparisons, it was apparent (as also found by Lemel, 1993) that there was a larger environmental component than genetic component to body weight. Therefore, if the male contributes to the chick's body weight it should be largely through direct effects, rather than through passing on his genes.

In this study chick tarsus covaried with survival (see also Smith *et al* 1989). In this population, therefore, or in the years studied, contribution to tarsus growth may be more important than the contribution to body weight. As discussed above, rather unusually there is a large environmental component to tarsus length. I found evidence that the foster male's tarsus influences the likelihood of chicks surviving to recruit into the local population, with chicks raised by larger males being more likely to survive.

Due to the sample size of recruits being small, effectively removing the possibility of looking for genetic influence on survivors' development, I considered which variables influenced chick survival. Offspring survival is a major component of the lifetime reproductive success of parents (Clutton-Brock, 1988). By using chicks fostered out of their home nest it is possible to separate the usually confounding effect of the male's input and the female's response to being mated with a particular male (see Burley, 1988). In this study, the chin area of the true male covaried with chick survival, with chicks born to males with large chin areas being more likely to recruit into the local population. As there was no correlation between day 2 and day 14 weights I propose there was no evidence of direct maternal effects influencing chick development whilst in their foster nest. This suggests, therefore, that females may gain indirect benefits by being mated to males with a large chin area.

5.5.4 Conclusions

In conclusion, it is possible that males make a genetic contribution to a common predictor of offspring survival, body weight. There also appears, however, to be a large environmental component to body weight. In this population, chick tarsus length covaried with recruitment into the local population, and it was the female who contributed most to tarsus development. There was evidence of both direct and indirect benefits to the female, depending on the phenotype of her mate. The survival of fostered chicks which recruited into the local population was related to their foster father's tarsus length, which suggests there may be some direct benefit to mating with larger males. It is not possible to discount the possibility of differential allocation by the female, depending on the size of her mate, accounting for this result. This is not a problem, however, when we consider indirect benefits. Despite finding no correlation between the chin area of crossed recruits and their true fathers, there was a significant effect of true male chin area on the survival of these fostered chicks, which suggests that male chin area does reflect some aspect of genetic quality in the male.

CHAPTER 6

OFFSPRING SEX RATIO IN THE GREAT TIT

6.1 ABSTRACT

It has been suggested that female birds may manipulate the sex ratio of their offspring. This study examined – using molecular sexing techniques - whether the sex of a chick could be explained by its parents' or foster parents' characteristics. There is a significant dimorphism between the sexes, apparent by day 10, which suggests that the cost of raising each chick may be influenced by sex. Neither mortality within the nest, nor foster parent characteristics covaried with chick sex, suggesting that the environment in which a chick is raised does not differentially affect survival according to sex. In one of our experimental categories, home-raised young, and in a constructed "original" ratio, chick sex ratio varied with the parental male tarsus length. More sons were raised by males with larger tarsi. This relationship, if it is a reflection of a decision made by the female prior to laying, should also exist between young crossed out of their nest and their true fathers, but it does not. This result highlights the need to assess primary as well a secondary sex ratios in studies addressing the problem of sex ratio manipulation.

6.2 INTRODUCTION

Fisher (1958) proposed that, within a population as a whole, natural selection will tend to equalize parental expenditure devoted to the production of the two sexes, but that due to sex-based differences in reproductive opportunities, preference towards one sex may give individuals an effective fitness advantage. For example, if the potential reproductive success of large sons were be predicted to be high, then it would be advantageous to produce large sons. Bateman (1948), demonstrated that the variance in reproductive success was greater in male than female *Drosophila melanogaster*. Willson & Pianka (1963) also suggested that parents might be expected to invest more heavily in the offspring of one sex. Parents might be expected to behave in this way when the resources allocated to offspring of one sex provide a greater return in terms of parental fitness. Sex ratio may be manipulated at conception if the mechanism is under the parent's control (and if the relative profitability of sons and daughters can be assessed in advance). Alternatively an equal sex ratio may be produced and

subsequently modified through sex-biased brood reduction (for a review see Krackow, 1995; Dijkstra *et al*, 1998).

When the offspring of one sex are more costly to rear, parents might be expected to vary the sex ratio of their offspring in relation to their ability to expend resources. For example, in red-winged blackbirds, males are around 30% heavier than females, and require approximately 27% more energy (Fiala & Congdon, 1983). The sex ratio might vary with parental quality; superior parents may opt to produce the more "expensive" sex and inferior parents the "cheaper" sex. We would also predict that parents might benefit, in fitness terms, from terminating investment in offspring that have little chance of surviving to breed successfully (Trivers, 1972; Trivers and Willard, 1973).

Sex ratio trends need not be confined to the case when the offspring of one sex are more costly to rear. When the relative attractiveness (and hence the reproductive value) of sons varies according to the quality of her mate, for example, females mated to attractive males might bias the sex ratio of their offspring toward males, whilst females mated to unattractive males might produce daughters (Burley, 1977; 1981; 1986). It is important to note that it does not follow that differing attractiveness results in variation in *lifetime* reproductive success (Newton, 1989), primarily because there may be some cost to being attractive. If such a difference does arise, however, then it follows that each sex may differ in their "value" to their parents according to their potential attractiveness as a mate.

There is probably no clear line to be drawn between the theories of sex ratio manipulation due to the relative cost of raising each sex, and the potential of raising attractive chicks, because the two may be intertwined. When male success depends on fighting ability and body size, the breeding success of sons may often be more strongly influenced by early growth and parental expenditure than that of daughters. Sex ratio has previously been shown to correlate with the parental male tarsus length in the great tit (Kölliker *et al* 1999), with males of longer tarsi producing a greater proportion of sons. In a similar species, the blue tit, Svensson & Nilsson (1996) found that the sex ratio of the brood was more likely to be male-biased if the father was of a high quality, in this instance assessed by subsequent survival.

Parents generally set clutch size at an "optimistic" level (in excess of what normally can be maintained - Kozlowski & Stearns, 1989; Forbes, 1991; Mock & Forbes, 1994). Hence, avian nestlings in many species face a highly competitive environment - sometimes one requiring

brood reduction. In energetic terms, manipulating the survival of male and female offspring after birth is a wasteful method (though may be effective if fitness is increased as a result). Sheldon *et al* (1998, also Clutton-Brock *et al*, 1985), propose that the general pattern found throughout the animal kingdom is that mortality is higher among males than among females, particularly when environmental conditions are poor. In birds, there is a tendency for the extent to which the sex ratio declines to be greatest in dimorphic species (Clutton-Brock *et al*, 1985). Those studies that address this in the great tit have, thus far, indicated that males may out-compete females in poor conditions (Dhondt, 1970; Smith *et al*, 1989). In most birds studied, however, mortality is biased toward the larger sex (i.e. males) (see Dijkstra, 1998; Sheldon *et al*, 1998).

Sex ratio has also been found to vary with season (Dijkstra *et al*, 1990) and maternal age (Blank & Nolan, 1983). In the great tit, Lessel (1996) provides evidence that sex ratio may vary with the timing of breeding, with more males surviving in early broods (see also Dhondt, 1970). Sheldon & Ellegren (1996) found no relationships between the timing of breeding and sex ratio, or between clutch size and sex ratio among natural broods of the collared flycatcher. In their review of the data, Dijkstra *et al* (1998) found the largest bias toward the "cheaper" sex in the largest and smallest brood sizes.

This study examined whether secondary sex ratios covary with parental phenotype, hatch date of the nest, or brood size. The secondary sex ratio is that of the surviving fledglings, not that of chicks hatched. We would predict that there should be more males born to large or good quality male parents, and vice versa - more females born to small or poor quality males. Any size dimorphism found in nestlings must affect the nestlings' demands of the parents. I therefore also investigated whether there is evidence from the data that male and female offspring might require different levels of parental care.

6.3 METHODS

6.3.1 Manipulations and measurements

In 1996 and 1997 nests were matched according to hatch date and approximately half the clutch exchanged for the same number of chicks from the matched box, (see Chapter 5, 5.3.1) for more detail. Chicks were therefore raised in a clutch of the same size, but potentially of a different sex ratio make-up than the original. Clutch size, hatch date of the first chick and the number of chicks successfully fledged was noted.

Nestlings were identified using a unique claw clip made on day 2 and were ringed, blood sampled and measured at day 14 when the claw clip was identified. The breeding pair were caught on their territory with mist nets or box traps on, or after, day 14. All birds were measured as described in Chapter 1 (1.4). For the purposes of this chapter, the different categories of chicks will be referred to as follows. Home chicks are those born and raised in the same nest; crossed chicks are those born to the same parents as home chicks but raised in a different nest from day 2 onwards; and fostered chicks are those moved into the "home" nest, hence crossed and fostered chicks were raised away from their true parents.

6.3.2 Molecular sexing

For molecular sexing, blood was stored immediately in alcohol (70%) in the field. DNA was extracted using 5% chelex (Walsh, 1991). Sexing was carried out using microsatellite primers designed to amplify sex-specific DNA (Griffiths et al, 1998). The polymerase chain reaction conditions were as follows: each 10 µl reaction contained 1 µl of the DNA-extraction solution, 1 µM of each primer and 0.25 units of DNA taq-polymerase (Thermoprime plus, Advanced Biotechnologies, Epsom, UK) in the manufacturer's buffer (Final concentrations: 20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCL pH 9.0, 0.01% Tween, containing 1.0 mM $MgCl_2$ and 0.2 mM of each dNTP). The reaction profile was an initial denaturing period of 94°C for 2 minutes, then 40 cycles of 94°C for 15 seconds, 50°C for 20 seconds and 72°C for 25 seconds. The products of the polymerase chain reaction were separated on a 2.5% agarose gel run for two hours at 90V; two bands indicate a female and one band a male. To confirm the accuracy of the sexing technique, 25 adults of known sex were analysed blindly - all were correctly identified.

6.3.3 Analyses

For binary logistic regression, investigating which variables covaried with sex, each chick was entered into the data set once, meaning each parent was entered into the dataset according to the number of chicks that survived to day 14. To take into account this over-dispersion, a backwards-selected model was run to identify variables that may be of interest. This reduced model, only containing variables of interest, was then run forcing the brood identification (and therefore parent identification) into the model as a categorical term. From this σ^2 (variance), an estimate of the over-dispersion can be estimated from: $\dfrac{-2\log(likelihood)}{(n-(df+1))}$ The model was then run again without the brood identification, using our estimate of σ^2 as a user-defined estimate of the variance (see McGullagh & Nelder, 1988).

6.4 RESULTS

6.4.1 Experimental versus control boxes

There was no difference in final offspring condition and clutch size, though fewer chicks were produced in control broods. This may be attributable to the difference in hatch date between the two categories. The data, however, suggested that control clutches in the years studied suffered more from desertion or complete clutch loss because when those experimental and control nests that produced at least one fledgling were compared there was no significant difference in clutch success. There was no evidence from this analysis that experimental clutches suffered from the manipulation (see Tables 1 and 2, in Chapter 5, 5.4.1).

Table 1. Comparison of weights and secondary sex ratios of experimental clutches.

Experimental clutch day 14 sex ratios (home young and fostered young)	Mean ± s.e.	Wilcoxon's' matched pairs test
home young	0.51 ± 0.31	Z= -0.60, n= 60, p= 0.55
fostered young	0.54 ± 0.35	

To ensure that the manipulation was not biased, I compared the total weight of chicks crossed out of the nest with those that remained. There was no significant difference in weights (Table 3, Chapter 5, 5.4.1) indicating the experimenter did not favour large or small chicks for transfer. Similarly I compared the secondary sex ratios, from those chicks surviving to day 14. There was no significant difference between the ratios of chicks surviving in the fostered or home raised categories of chicks (Table 1).

6.4.2 Clutch characteristics and sex ratio

I used two ratios for analyses here - the home young ratio and a reconstructed ratio. Home young were those born and raised in the same nest, and the "original" ratio was the home young added to their crossed siblings (those moved into another nest). The assumption is that there was no sex-biased mortality affecting either ratio at day 14 (when the samples used for analyses were taken).

Data were analysed using binary logistic regression with each chick entered into the data set once. To take into account the over-dispersion (i.e. chicks shared nests and parents), the model included an estimate of σ^2 (see Methods). For both home and "original" sex ratio;

clutch size, hatch date (of the chick not the nest), the interaction between the two, year, and brood raised in had no significant effect on the model (χ^2= 0.61, df= 6, p= 0.99 (n=267); and χ^2= 1.53, df= 6, p= 0.96 (n= 515) respectively).

6.4.3 Parental phenotype and fledgling sex ratio

Graphical analyses suggest that there is a relationship between the genetic father and the fledgling sex ratio of his chicks. The correlation between male tarsus length and the home ratio is significant (see Figure 1), the correlation between foster male tarsus length and foster young, however is non-significant (r= 0.17, n= 46, p= 0.26).
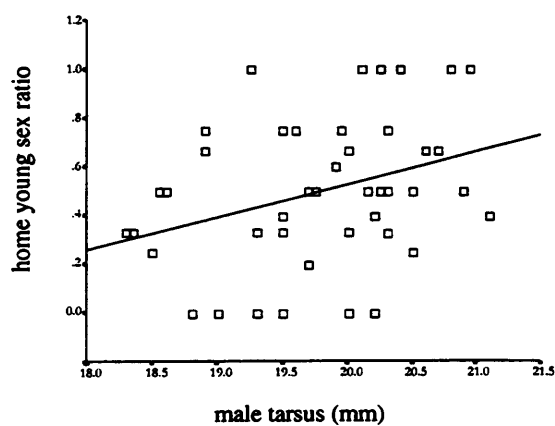
Figure 1. Scatter plot of parent-male tarsus length against the sex ratio (males/total ratio) of home young (those young born and raised in the same nest).
r= 0.33, n=46, p= 0.03

Table 2. Binary Logistic models of male tarsus and sex of chicks.

Dependent variable	Model description	df	β ± s.e.	χ^2	P
home chicks sex n= 256	male tarsus length	1	0.55 ± 0.24	7.22	0.01
crossed chicks sex* n= 200	male tarsus length	1	- 0.02 ± 0.35	0.48	0.49
home plus crossed chicks ** n= 456	male tarsus length	1	0.33 ± 0.20	4.57	0.03
fostered chicks sex *** n= 223	foster male tarsus length	1	0.12 ± 0.25	0.01	0.91

* crossed chicks are those chicks raised in a different nest to that hatched in, male used is the true father.

** home plus crossed chicks gives an estimate of the "original" secondary sex ratio, assuming no sex-biased mortality.

*** fostered chicks are those raised in a different nest to that hatched in, male used is the foster father.

I investigated through binary logistic regression, as described above, which paternal characteristics may explain the chicks' sex in the nest. The following variables were dropped from the models: female tarsus, male and female wing, male and female chin, male and female BCI, male and female survival, clutch size and hatch date. The home-raised chicks' sex, and an estimate of the original sex ratio covaried with male tarsus (see Table 2). Male tarsus length, therefore, influences the likelihood of a chick being male, with more males being produced at nests with a large parental male. This relationship should exist with the crossed chicks if there is no effect of box raised in on the survival of chicks, however the model is non-significant (Table 2).

I investigated therefore, if the ratios produced are a result of differential mortality between the sexes. If they were we would predict an increase in sex ratios with an increase in mortality (as the opportunity for an increased skew would increase with mortality). Such a trend does not arise (see Figures 2 & 3), thus the data suggest the relationship between chick sex and carer-male size may exist at laying and does not arise subsequently.

Figure 2. Scatter plot of the home-raised chicks' sex ratio(males/ home raised chicks) against the number of chicks dead.
r= -0.08, df= 56, ns (Spearman's rank)
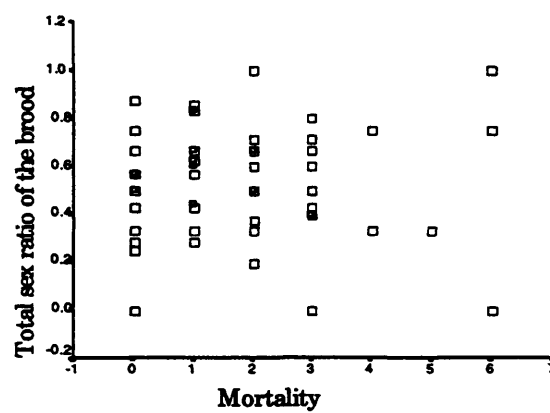
Figure 3: Scatter plot of the total sex ratio (males/clutch size)of the experimental broods against the number of chicks dead.
r= 0.09, df= 56, p< 0.05 (Spearman's rank)

6.4.4 Sexual dimorphism

To investigate whether there was any evidence that male and female chicks could place different demands on the parents I compared the body size of each sex (see Table 3). There is a significant dimorphism between the sexes that is apparent from this data at day 10. I examined which variables explained whether a nest experienced mortality; clutch size and box remained in the model whilst hatch date, home ratio, fostered ratio and total (created ratio) were removed, (see Table 4). There is no evidence from this data, therefore that the sex ratio in the nest covaries with mortality, despite the number of chicks produced being significantly reduced by mortality (t= 2.59, df= 89, p=0.01).

Table 3. Comparison of the means of male and female sibs raised in the same environment.

morphological measure and age of chicks	fostered male v fostered female	home male v home female
	paired t-test	paired t-test
day 14 weight	t= -5.91, df=47, p< 0.001	t= -4.27, df= 56, p< 0.001
day 14 tarsus	t= -7.7, df= 47, p< 0.001	t= -7.45, df= 56, p< 0.001
day 10 weight	t= -0.27, df= 10, ns	t= -1.46, df= 9, ns
day 10 tarsus	t= -9.32, df= 10, p< 0.001	t= -6.34, df= 9, p< 0.001
day 7 weight	t= 0.29, df= 10, ns	t= -1.3, df= 9, ns
day 2 weight	t= -1.7, df= 12, ns	t= 0.45, df= 15, ns
day 0 weight	t= -0.37, df= 19, ns	t= -1.1, df= 19, ns

Table 4. Binary logistic regression to examine which variables explain presence or absence of mortality in the nest.

Dependent variable	Model description	df	$\beta \pm$ s.e.	χ^2	p
mortality	Full model	62		10.25	0.01
	clutch size		0.33 ± 0.008		0.04
	brood id		- 0.013		0.09

6.5 DISCUSSION

What are the benefits of producing more sons? If sons of high quality males inherit characteristics of their father, this may subsequently increase their survival or reproductive success. If this is the case it may benefit females to bias the sex ratio toward sons, when mated with a high quality male. This assumes that daughters either do not inherit these traits or that they do not determine their reproductive success (Weatherhead & Robertson, 1979).

6.5.1 Parental phenotype, clutch characteristics and fledgling sex ratio

The significant, positive relationship between the home-raised secondary sex ratio and male tarsus agrees with Kölliker et al (1999). This study differs from Kölliker et al (1999), however, in that the raising environment is separated from parental phenotype for approximately half of the brood. If the relationship described by this and Kölliker et al (1999) exists at laying, and sex ratio is not subsequently modified due to mortality within the nest, then we have three tests of our prediction from this experimental design; that fostered chicks' sex should not covary with carer-male's phenotype and that home and crossed chicks' sex should. As predicted, home sex covaries with male tarsus and fostered young's sex does not, however, nor does crossed young's sex. This may be a problem with this particular sub-set of the data. When the same analysis is carried out on our estimate of the original sex ratio of the brood, those surviving home young plus the related-crossed young, the relationship between male tarsus and chick sex is significant. It is possible though that the home young are carrying this relationship within the model.

If the relationship between home raised-sex ratio and large males is a reflection of a female's response she may be responding to something other than the male's tarsus length. Territory quality is potentially a confounding variable that may explain these results. There could be a casual relationship between male tarsus and territory quality and females may be using territory quality as a cue to determine the sex ratio of her brood. Komdeur (1996), for example, found evidence of sex ratio adjustment (at laying) in response to the quality of territory inhabited. I would predict, though, that territory quality should influence female survival. That is, females paired to good-quality territory holders should have a higher survival rate. Therefore females laying male biased broods on good-quality territories should have higher survival, however female survival does not remain in models of sex ratio. If females are responding to territory quality rather than male tarsus then I would suggest that any benefits

are transferred into raising larger sons, rather than into female survival as the data show a significant dimorphism between the sexes as chicks.

Burley (1981) found sex ratio is skewed, in favour of males, among females mated to (experimentally) attractive males, thus demonstrating that attractiveness can influence the female's decision. In the great tit it has been suggested that the size of the breast stripe is a heritable characteristic (Norris, 1993), however data within this thesis suggest that the chin area may be an important signal of quality (see also Lemel, 1993). Other studies have found a pre-hatching bias in sex ratio that covaries with attractiveness of the male – for example Ellegren et al (1996) found that females biased their sex ratio in favour of sons when mated to a large-badged male. Kölliker et al (1999) included a non-significant ($0.1 < p > 0.5$) effect of breast stripe size in their model of sex ratio. There was no evidence from this study of male chin area, or male breast stripe influencing sex ratio. Lessells et al (1996) found an effect of hatch date and clutch size on the proportion of males in a brood. This study can not confirm these results. Svensson & Nilsson (1996) also did not find an effect of date on the sex ratio in experimentally delayed broods of blue tits.

6.5.2 Dimorphism and control of sex ratio

There are two possible mechanisms by which sex ratio could be biased, differential mortality or as females are the heterogametic sex, through female control prior to laying (Clutton-Brock, 1986). Differential mortality is more likely to exist in dimorphic species. An inherent difference in nestling condition in the great tit is suggested to exist between sexes (Lessells, 1996, Smith et al, 1989; Dhondt, 1970). This study concurs with the difference between the sexes becoming apparent between day 7 and day 10.

Such size dimorphism as we found must influence the nestlings' demands on the parents. Fiala & Congdon (1983) demonstrated that, in the red-winged blackbird, though the difference is proportionally less than the mass difference, total assimilated energy required by a nestling male is much greater than required by females. Lessells et al (1998) found no effect of experimentally altered sex ratio on their measures of parental effort in the great tit however, Whittingham et al (1994) suggest that measures of fledging success and offspring quality are probably better indicators of parental care than nest-visit rates.

In the great tit two studies have suggested that male chicks out-compete females, predicting sex-related mortality. Dhondt (1970) proposed that more females than males died in the nest;

however the method of sexing the nestlings had a reliability of 87-93%). Dhondt presented evidence that more females than males died in the nest, however if we take a conservative estimate and assume only 87% of the females were correctly identified then the ratio of male to female deaths becomes equal. In enlarged broods proportionately more great tit males recruited into the local population (Smith *et al*, 1989). Recruitment, however follows a intense period of selection and therefore this bias in survival may not reflect mortality within the nest.

The fact that the relationship between male tarsus and fledgling ratio is found in home young, but not between fostered young and male tarsus, suggests that this relationship exists at laying; not through subsequent modification due to differential sex-related mortality. If differential mortality did occur according to the size of the male tarsus we would expect to see the same relationship in the fostered young. However the relationship was not seen in those related young crossed out of the nest. In support of the ratio being determined at laying, there is no trend in sex ratio against the level of mortality in the brood. We would predict an increase in the skew in sex ratio as the level of mortality increases if sex-related mortality occurs. Kölliker *et al* (1999) compared the ratios of great tit nests that did and did not suffer egg failure or mortality and found no difference. They too conclude that the sex ratio is a reflection of the ratio present at egg laying. Similarly in the blue tit, analyses of clutches that did and did not sufferer mortality indicate the bias in sex ratio is present at hatching (Svensson & Nilsson, 1996).

6.5.3 Conclusions

These results suggest that a female may adjust the sex ratio of her clutch according to the size of her pair-male. However, we did not find the expected relationship between the sex of those chicks crossed out of the nest and their father's tarsus. There is no evidence of sex-biased mortality in the nest, so the difference in the relationships between home young and paternal tarsus and crossed young and paternal tarsus is difficult to account for.

If the relationship between home young sex and male's tarsus is a reflection of the female's ability to manipulate primary sex ratio, the question of what the female responds to is raised. It is not possible to determine from these data whether females adjust sex ratio according to the quality of the territory, or to male tarsus itself. The results of this study highlight the importance of determining primary sex ratio, which is relatively easy now that sex can be identified from a very small sample of blood. It is also vital to cross-foster chicks to separate the effects of the environment and parental phenotype on chick survival.

The results show a relationship between fledgling sex ratios and parental phenotype in the direction predicted. The data suggest that males with large tarsi produced more sons, tarsus length has also been shown to be heritable (Gebhardt-Henrich & van Noordwijk, 1991). This relationship, however, exists only between the home-raised young, and a constructed "original" clutch and male tarsus. Male tarsus does not explain the variation in crossed or fostered secondary sex ratio. The data show that male and female chicks differed in size, a dimorphism that becomes apparent by day 10. However, this dimorphism apparently did not result in differential mortality within the nest.

PARASITIC INFECTION IN THE GREAT TIT

7.1 ABSTRACT

Three blood parasites were identified in a population of great tits on the island of Gotland, Sweden. The most prevalent haematozoa was *Haemoproteus majoris*, as recorded previously in this population (Allander & Bennet, 1994). Reproductive effort covaried negatively with the intensity of *Haemoproteus majoris* infection, and prevalence of infection affected adult survival. An estimate of immune status, total leukocyte count, varied with parental badge size, and influenced chick survival. Nestlings were cross-fostered between nests to distinguish between the environmental and genetic influences on development of this immune measure. There was a correlation between paternal badge size and total leukocyte count, but only when chicks were raised by their genetic parents, not when raised by foster parents.

7.2 INTRODUCTION

According to life-history theory, life-history traits should trade-off against one another (Stearns, 1992). This has been confirmed by demonstrating that current reproductive effort trades-off against future effort (Lindén, 1988; reviewed by Lindén & Møller, 1989; Gustafsson *et al*, 1994). Møller *et al* (1990) reviewed the evidence that parasites affect fitness by reducing reproductive success. The trade-off between parasite status and reproductive success must have a mediator; it is possible that reproductive effort may impose life-long costs through immune suppression. In support of this, relapse of blood parasite infections have been suggested to be triggered by hormones associated with reproduction (Chernin, 1952; Atkinson & van Riper, 1991; see also Deerenberg *et al*, 1997). It has long been recognized that stressful conditions may lead to a suppression of immune function (Gross & Siegel 1973; Grossman 1985; Gershwin *et al*, 1985; Cooke, 1993). It is possible, therefore, that a trade-off occurs; infection results in a significant reduction in resources available for other life-history processes, or investment in life-history processes is made at the expense of defence against infection.

The trade-off between reproductive effort and parasite status in the great tit has been demonstrated in several studies. For example, both Norris (1994) and Richner (1995b) found

a higher prevalence of blood parasites in great tit males with experimentally enlarged broods, although Norris' manipulations were not independent of the original clutch size. Richner observed that the enlargement of clutch size increased male feeding rate, which may have imposed costs on the male. Allander (1997a) found an increased prevalence of haematozoa in parents with enlarged broods (and vice versa for reduced broods), but found no difference between the sexes. Hõrak *et al* (1998) found that brood size manipulation affected immune status, but not the intensity of parasite infection. Brood size manipulations in the collared flycatcher suggests a negative relationship between clutch size and measures of immune function (Gustafsson *et al*, 1994; 1995).

Parasites take resources from the bodies of their hosts. Parasite defence can also be costly; some species of haematozoa have been shown to cause extensive host mortality, especially in domestic birds (Bennett *et al*, 1993). Ectoparasites are also known to affect host fitness (reviewed by Lehman, 1993). Evidence suggests that parasites have fitness effects on passerine birds (reviewed by Møller *et al*, 1990; Loye & Zuk, 1991; Brown *et al*, 1995). This fitness effect, however, has been recently questioned (Sheldon & Verhulst, 1996). Sheldon & Verhulst argued the extreme view that the effects of brood size manipulation on immune function, as described above, may be readily detected *because* these changes are not important in terms of fitness.

This argument highlights a consistent problem in ecological, field-based studies of immune function: what is the utility and function of the immune responses measured? Following Hamilton & Zuk's (1982) hypothesis that parasites mediate the evolution of sexually selected characters, many field studies attempted to measure parasite or immunological status. Correlations between badge size and measures of immune function have been taken to support relative badge size or signal intensity reflecting immune condition. "Good" immune status has been cited as high white blood cell counts or low white blood cell counts, according to the direction of the relationship found. For example, the total concentration of heterophils increased with colour intensity in the great tit (Dufva & Allander, 1995), and was taken to be indicative of parasite resistance. Alternatively heterophil counts were negatively associated with reproductive success in the collared flycatcher. This was interpreted as being due to birds with low success having more infections (Gustafsson *et al* 1994). Interpretation of measures of immune function is meaningless without some knowledge of their effects on an individual's fitness.

Does a high leukocyte count suggest the individual is immune-competent, potentially ready to resist infection, or does it reflect infective status (Sheldon & Verhulst, 1996; see also Siva-Jothy, 1995)? Leukocytes are produced to protect against various pathogenic antigens. Elevated leukocyte numbers are generally accepted to be indicative of stress and the inflammatory process (Dein, 1986). Leukocyte elevation is most commonly caused by an increase in the number of heterophils in the blood. Ideally, these relationships would be teased apart by manipulating immune function and investigating its effect on fitness. Observational data, though, can still be informative in suggesting which immune measures or parasite infections have important effects on fitness.

The aim of this two-year field study was to investigate the species composition and prevalence of blood parasites in great tits between years, sexes and age classes for comparison with a previous study on this population (Allander & Bennett, 1994). Experimentally altered reproductive effort has been shown to influence parasite status in this population, as discussed above (Allander, 1995). I therefore considered how infection by parasites and measures of immune function related to natural reproductive effort and components of fitness. The heritability of fitness-related immune measures was also considered. It is possible by cross-fostering nestlings to tease apart the genetic and environmental component of any potentially important trait.

7.3 METHODS

7.3.1 The study population and morphological measures

This study was carried out from 1996 to 1997 in a nest box population of great tits in the southern part of Gotland, an island in south-east Sweden (57°10'N, 18°20'E; for a full description of the site see Pärt and Gustafsson, 1989).

Breeding pairs were caught at their box whilst provisioning young on, or after, day 14 and a blood sample taken immediately for a thin smear. All birds were measured as described in Chapter 1 (1.4). Parents were classified as either yearlings or adults, chin and badge were measured as described in 1.4. The body condition index (BCI) was calculated as the residual of the regression of standardized weight on tarsus^3 (weight was standardized for 1996 and 1997 separately to remove year effects). The chicks' tarsus and weights were measured on day 14, when a blood sample was also taken.

7.3.2 Blood smears

Approximately 100 μl of blood was taken from the cutaneous ulnar vein, using heparinized capillaries. A small drop was used to make a thin blood smear, air-dried in the field for later fixing and staining (with Giemsa) and the rest stored in 70% Ethanol for subsequent DNA analyses. Each slide was given a reference number in the field to allow blind scoring. The slides were stained for 1 hour with Giemsa (Giemsa stock solution: 0.5g Giemsa powder, 33 ml glycerol, 33 ml absolute methanol).

The blood smears were by scored Dr Frank Clark, of Leicester University. The total leukocyte count was made per 1,000 red blood cells, or per 10,000 red blood cells using x100 oil immersion objective lens mounted on a Nikon Labophot compound microscope. White blood cells and haematozoa were identified with the aid of reference slides supplied by Dr Reija Dufva, Uppsala University and demonstrated by Professor Ali Anwar, Oxford University (Campbell, 1995 and Hawkey & Dennett, 1989 were also used as reference texts).

I specify in the results which data set was used. "Group one" was scored per 1,000 red blood cells, as recommended early in the project (Mike Hart, personal communication), and accounts for the majority of smears scored for breeding birds. In " group two", the larger set,

the slides were scored per 10,000 red blood cells which should allow more reliable cross-study comparison. All chicks' smears were scored in this way.

7.3.3 The cross-fostering experiment

To separate the effects of environmental and genetic influences on nestling development I performed a partial cross-foster experiment. Broods were matched according to their hatch date and approximately half of the smallest brood was exchanged on day 2 (see Chapter 5, 5.3.1). No attempt was made to match nests for clutch size, and the final size of both clutches was unaltered. Where the number of chicks in a brood was uneven I transferred less than half the clutch (the mean number of chicks transferred was 3.4 ± 0.6).

7.3.4 Analyses

In all analyses of parasite prevalence or intensity, each individual was entered into the dataset once only. All models, GLM or binary logistic were backward-selected. To analyse which variables influenced chick survival I used binary-logistic regression, entering each chick into the model once. To account for over-dispersion due to each chick being raised with others, a backwards-selected model was run to identify variables that may be of interest. This reduced model, only containing variables of interest, was then run forcing the box identification into the model as a categorical term. From this, σ^2, an estimate of the over-dispersion, can be estimated from: $\dfrac{-2\log(likelihood)}{(n-(df+1))}$

The model is then run again without box identification entered, using our estimate of σ^2 as a user-defined estimate of the variance (see McGullagh & Nelder, 1988). Non normal data was log or square root transformed for analyses.

In the heritability analyses experimental chicks were designated as home crossed or fostered (see Chapter 5, 5.3.1); if there was more than one chick in each category then the averages were used.

7.4 RESULTS

7.4.1 Parasite species

Several parasite species have been described previously in this population including: *Haemoproteus majoris*, *Hepatozoon parus*, *Plasmodium vaughani*, *Plasmodium circumflexum* and *Plasmodium polare* (Allander & Bennett, 1994). We identified *Haemoproteus* parasites as *Haemoproteus majoris*; *Hepatozoon* and *Plasmodium* parasites were identified to the genus level. Figure 1 shows a red blood cell infected with gametocytes of *Haemoproteus majoris*.

Table 1 shows the prevalence of these blood parasites and compares our data with Allander & Bennett's (1994) 1990-1992 study. Bennett scored slides per 100 fields (using a 100x oil immersion objective). Four chicks (three in one nest and one single chick) were found to be infected with *Haemoproteus*; they are not included in Table 1, all other infections found were from breeding birds.
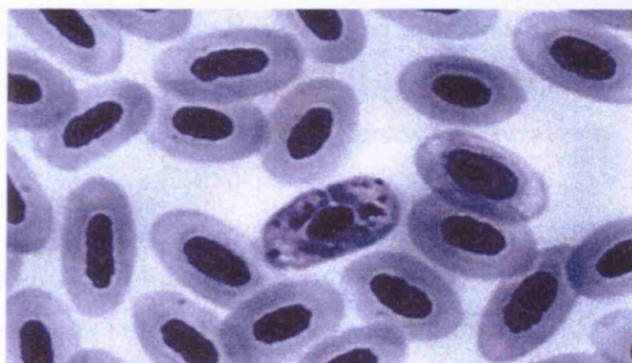
Figure 1. Great tit red blood cell infected with gametocytes of *Haemoproteus majoris*.

Table 1. Prevalence of different species of blood parasites in great tits breeders for 1996 and 1997 (31 yearlings and 39 adults scored).

Parasite species	number of infected yearlings	prevalence in yearlings %	number of adult birds infected	prevalence in adult birds %	prevalence in adults (Allander & Bennett 1994) %
Haemoproteus majoris	13	41.9	20	51.3	75.7
Hepatozoon sp.	5	16.1	0	0	16.5
Plasmodium sp.	2	6.5	1	2.6	4.8

7.4.2 Repeatability

In 1996 two smears were made from each blood sample to assess between-smear repeatability. There was no significant difference between total leukocyte counts on separate smears from the same blood sample (paired t-test, t= -1.3, df= 13, p=0.22, correlation, r= 0.94). Five of the fourteen repeat smears had *Haemoproteus* infection. There was no significant difference in the intensity of infection on matched smears (Wilcoxon's Z= -0.67, n= 14, p= 0.50).

Only two individuals in the repeat smears sample suffered *Hepatozoon* infection. Of these, one was scored as positive for *Hepatozoon* infection from the second smear, the other was scored as negative for *Hepatozoon* infection. Due to the low parasitemias one must assume that prevalence data for *Hepatozoon* infection are less reliable than for more intense infections. This is also suggested by comparing the two main groups within the data set; group one is those smears scored per 1,000 red blood cells and group two those smears scored per 10,000 red blood cells. There was no difference in the prevalence of *Haemoproteus* between groups one and two (47.1 and 50.0% respectively χ^2= 0.05, df= 1, p= 0.82). There was however a (non-significant) difference between group one and two in prevalence of *Hepatozoon* infection (3.9 and 13.6% respectively; χ^2= 2.06, df= 1, p= 0.15), which may be due to the accuracy of the scoring technique rather than due to any real difference in prevalence.

7.4.3 Patterns of prevalence

Prevalence is the percentage of infected individuals in a sample, irrespective of the intensity of infection. For analyses of breeders I combined both groups of data (those scored per 1,000 and per 10,000 red blood cells), as there was no significant difference in prevalence of the infections, as described above. From this sample, 52.8% of individuals were scored as infected with one of the three common parasite genera considered. I investigated the patterns of prevalence of host characteristics and year on *Haemoproteus* and *Hepatozoon* infection. There was no effect of year, sex, age class or their interactions on *Haemoproteus* ($\chi 2$= 2.53, df= 6, p= 0.86). Year, and the interaction between year and age, explain some of the variation in prevalence of *Hepatozoon* infections ($\chi 2$= 16.67, df= 2, p< 0.01), because all infections were found in smears taken in 1996. It appears that the apparent relationship between age and prevalence of *Hepatozooa* in Table 1 is merely a sampling effect and not an age effect *per se*, as more yearlings were caught by chance in 1996, and also all smears scored per 10,000 red blood cells were from 1996.

Five chicks were found to be infected with *Haemoproteus*, of these, three were raised in the same nest, though one was a fostered chick. All these infections were from 1997.

7.4.4 Intensity of infection

Due to the small number of individuals infected with *Hepatozoon* and *Plasmodium* consideration of the intensity of infection is restricted to those birds only infected with *Haemoproteus* (leaving 82 individuals).

Allander & Bennet (1994) found an effect of both year and age class upon the intensity of blood parasites. I investigated whether year, age, sex or the interactions between these variables explained the intensity of *Haemoproteus* infection. None of these variables alone, or their interactions, improved a general linear model of log-transformed *Haemoproteus* counts (F= 0.24, df=30, p= 0.94). By separating the two groups of data there was a suggestion of a year effect, this is found only in group one (those smears scored against 1000 red blood cells), the year effect remains significant only if the age-year interaction is included in the model (F= 3.90, df= 3, p= 0.02). It was not possible to look for a year effect in group two, because scoring against 10,000 red blood cells was only carried out on slides made in 1996.

In a general linear model I examined whether parental characteristics determined their intensity of infection. None of the following variables remained in a backwards-selected model: sex, age, BCI, tarsus, wing, chin or breast area (of males), (F= 0.89, df= 13, p= 0.57). Considering both groups separately did not improve the model (group one, F= 0.64, df= 19, p= 0.67; group two, F= 0.43, df= 10, p= 0.81).

I also considered whether reproductive effort affected individual intensities of *Haemoproteus* infection. Hatch date and clutch size covaried with intensity of infection (Table 2) whilst sex, number of chicks surviving to day 14 (taking into account clutch size and hatch date) and the breeding stage (age of the nestlings when parents were caught) did not remain in the model. When the data were split into the two groups the model did not fit with group two. This may be a sample size problem as there were only 10 degrees of freedom (see Table 2). In group one, sex became an important predictor of the intensity of infection, with males having greater numbers of parasites in their peripheral blood (male average 21.8 ± 55, n= 32, compared to the females' average of 5.8 ± 12 gametocytes, n= 37). Infected males in group one had their clutch hatch, on average, two days earlier than uninfected individuals; the same was true of females from this group.

Table 2. Results of GLM explaining variation in intensity of *Haemoproteus* infection.

Dependent variable	Model description	df	β ± s.e.	F-value	p
ln (Haemoproteus count)	Full model	27		6.94	0.004
	ln (hatch date)	1	-4.46 ± 1.56	7.90	0.009
	clutch size	1	0.22 ± 0.09	5.41	0.028
ln (Haemoproteus count) selected for group one (those slides scored per 1000 rbc)	Full model	17		11.80	<0.001
	ln (hatch date)	1	-4.58 ± 1.01	18.05	0.001
	clutch size	1	0.21 ± 0.08	7.63	0.015
	sex	1	-0.60 ± 0.25	4.08	0.06
ln (Haemoproteus count) selected for group 2 (those slides scored per 10,000 rbc)	As above	10		6.49	0.60

7.4.5 The leukocyte count in adults

All smears were scored for a basic measure of immune function, the total leukocyte count, which is a count of all white blood cells in the peripheral blood. Leukocyte counts from group one (counted per 1,000 red blood cells) and group two (counted per 10,000 red blood cells) differed significantly (t = -3.00, df= 89, p= 0.003). Taking each group separately, therefore, I investigated whether the leukocyte counts were higher in the infected group. Infection with *any* of the common blood parasites did not explain the (log-transformed) leukocyte count for group one (t= -0.04, df=67, p= 0.96) or group two (t= -0.16, df= 20, p= 0.87). Nor did leukocyte counts differ for individuals infected and free from *Haemoproteus* (group one t= -0.51, df= 67, p= 0.61; group two t= 0.85, df= 20, p= 0.40), or *Hepatozoon* (group one Mann-Whitney U= 57, n= 69, p= 0.24; group two U= 18, n= 22, p= 0.24). The presence of these two main pathogens did not explain the variation in total leukocyte count. For the rest of these analyses, I concentrated on the larger group, group one, because models built for group two would have very low degrees of freedom.

It is possible that infection by parasites, bacteria or viruses other than the two parasites scored are affecting the total leukocyte count. I investigated, therefore, whether there was any indication of a year effect, as with *Haemoproteus*. There was a significant year effect (t= 2.36, df= 67, p= 0.02), with leukocyte counts being higher in 1996. This suggests individuals may have experienced different conditions in each year.

Stressful conditions may lead to a suppression of immune function (Gross & Siegel 1973; Grossman 1985; Cooke, 1993). I therefore investigated (in group one) whether any of the measures of reproductive effort covaried with leukocyte count, as with *Haemoproteus* above. None of the reproductive variables, sex or age stayed in the model; the year effect was the only variable which explained a significant amount of variation in the leukocyte count (without year F= 1.25, df= 67, p= 0.31).

I also used a general linear model to examine whether parental characteristics covaried with leukocyte count. Theory suggests there may be some intrinsic difference between individuals' immune response (Hamilton & Zuk, 1982). None of the following variables remained in a backward-selected model: sex, age, BCI, tarsus, wing or breast area (for males). Chin area did explain some of the variation in leukocyte count, with large-badged individuals having lower leukocyte counts (see Table 3 and Figures 2 and 3). I included the non-significant interaction term between sex and chin area because there was a known difference in chin area between the sexes (Chapter 2, 2.4.2); its inclusion did not affect the estimates of the effect of chin area.

Table 3. Results of GLM to explain variation in leukocyte counts.

Dependent variable	Model description	df	β ± s.e.	F-value	p
Total leukocyte count	full model	66		3.91	0.03
	chin area	1	-0.18 ± 0.07	6.75	0.01
	sex*chin area	1	0.03 ± 0.03	1.97	0.17
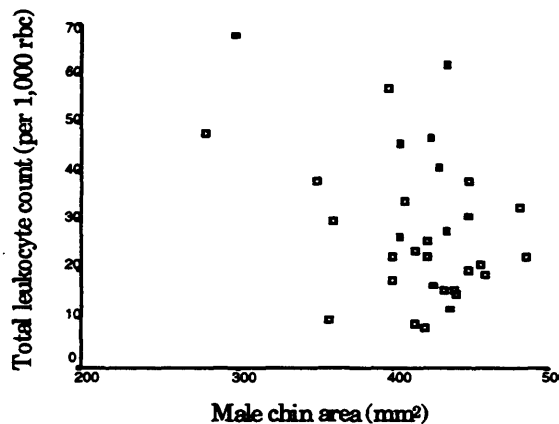
Figure 2 Total leukocyte count against male chin area
r= -0.40, df= 31, p= 0.03
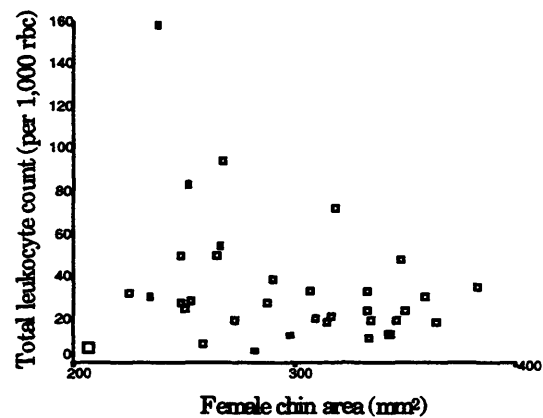
Figure 3 Total leukocyte count against female chin area
r= -0.28, df= 34, p= 0.10

Figures 2 and 3 show the relationship between total leukocyte count and chin area for each sex separately. Though the general trend is similar, the relationship is only significant in males, with large-badged males having lower total leukocyte counts than their small-badged counterparts.

7.4.6 The leukocyte count in chicks

All blood slides taken from nestlings were scored by counting leukocytes per 10,000 red blood cells. There was a significant difference between these counts and those adults scored in the same way (Mann-Whitney U= 1052, n= 22 and 169, p= 0.001), with chicks showing the higher leukocyte counts. There was no significant difference between the total leukocyte counts of male and female chicks (using square root of leukocyte count t= - 0.61, df= 162, p= 0.54, see Chapter 6, 6.3.2 for a description of the molecular sexing technique).

There is a year effect on the leukocyte count; in a GLM clutch size, hatch date and box were dropped from the model, whilst the number of chicks that survived to day 14 (entered after

101

clutch size was dropped to avoid colinearity problems) and year remained (Table 4). Counts were higher in 1996 (82.9 ± 45.5 in 1996 compared to 65.7 ± 37.5 in 1997) suggesting that, as with the parents, the chicks may have experienced different conditions in different years. I also investigated if chick characteristics varied with total leukocyte count day 14; standardized weight, tarsus, box number and hatch date were all dropped from a backwards selected GLM.

Table 4. Results of a GLM explaining variation in chick total leukocyte count.

Dependent variable	Model description	df	β ± s.e.	F-value	p
sqrt (total leukocyte count)	full model	182		6.43	0.002
	year	1	1.17 ± 0.37	10.36	0.002
	brood size	1	-0.20 ± 0.11	3.05	0.082

7.4.7 Fitness effects

Adults

A binary logistic regression was used to investigate which variables explained local survival of parents to the next breeding season. Year, reproductive variables and parental characteristics were all dropped from the model. The intensity of infection, leukocyte counts and prevalence of *Hepatozoa* infection were also dropped. The only variables remaining in the model were prevalence of *Haemoproteus* and age (χ^2= 10.5, df= 2, n= 73, p= 0.02). When the data was separated by age, prevalence of *Haemoproteus* was a significant contributor to survival in adults only (χ^2= 9.7, df=1, n= 39, p= 0.002, compared to first years $\chi2$= 0.05, df=1, n= 32, p=0.82). Uninfected adults, therefore, tended to have a better chance of local survival.

Chicks

I also investigated which variables influenced chick survival to the following breeding season. This analysis was based on 30 experimental clutches. Hatch date, clutch size raised in, day 14 survival, day 14 tarsus and sex were all dropped from a backwards-selected model. Standardized day 14 weight and leukocyte count explained which chicks recruited into the breeding population the following year (Table 5). This relationship holds if standardized day 14 weight taking into account hatch date is used (a relationship described in Table 2, Chapter

4; χ^2= 12.51, df= 2, p= 0.002). Therefore this does not reflect the difference in weights due to hatch date.

Chicks with above average weight at fledging had a better chance of recruiting into the local population (mean of recruits = 17.6 ± 1.5g, compared to those not recaught = 16.9 ± 1.9), whilst chicks with a low leukocyte count had a better chance of local recruitment (mean of recruits = 50 ± 26, compared to those not recaught = 73 ± 46). There was no correlation between the two measures (Spearman's r= 0.05, n= 165, p= 0.49). This suggests that the total leukocyte count can be informative, and may be a fitness-related trait.

Table 5: Binary logistic regression examining recruitment into the local breeding population.

Dependent variable	Model description	n	β ± s.e.	Chi-square	p
chick survival	full model	165		12.77	0.002
	standardized day 14 weight		0.58 ± 0.28		0.002
	square root (leukocyte count)		-0.23 ± 0.10		0.006

7.4.8 Heritability of fitness-related immune traits

As leukocyte counts at fledging may influence chick recruitment, as suggested above, I examined whether this trait was heritable by comparing the immune status of related and unrelated siblings raised in the same environment and related siblings raised in different environments. None of the correlations were significant, though there does appear to be a larger environmental than genetic component (Table 6).

Table 6: Resemblance estimates between mid-home young on mid-fostered measures from day 14 chicks in the nest: (1) shared environment (chicks fostered into the home young's nest compared with home chicks), (2) shared genome (related chicks crossed out of the home young's nest compared with home chicks).

relationship		Mid home young- mid fostered young			
character		n	slope	s.e.	p
leukocyte count	1	23	0.27	0.24	0.26
	2	20	-0.02	0.30	0.96

for category 2 h^2 (heritability) is 2* the slope

The sample of parents and chicks with scored smears was small, so a direct parent-offspring comparison was not possible. There was no correlation between leukocyte counts of birds first scored as nestlings and then as recruits (Spearman's rank, r= -0.50, n= 8, p= 0.20). As

there may be some intrinsic difference between different individual's immune response, which covaries with badge size (Hamilton & Zuk, 1982), I examined if the parental characteristics explained fledgling leukocyte count.

The chicks were separated according to their manipulation category: "home" chicks are those born and raised in the same nest. In a backwards-selected general linear model male and female tarsus were dropped, as were age and male breast measures. The only variable remaining was male chin area (r^2= 0.25, df= 23, p= 0.01; Figure 4). This relationship was not significant for those fostered chicks raised by the same male (r^2= 0.04, df= 25, p= 0.32; Figure 5). This might suggest that the leukocyte count has a genetic component. However the same model considering leukocyte count of fostered chicks against their true father's chin area finds no relationship (r^2= 0.00, df= 25, p= 0.92, Figure 6). This is not due to any differences in the data as there is no difference between home and fostered leukocyte counts (paired t-test t= 1.04, df= 23, p= 0.31), nor is there a difference between the number of home and fostered chicks surviving (Wilcoxon's matched pairs Z= -1.27, n= 24, p= 0.21).
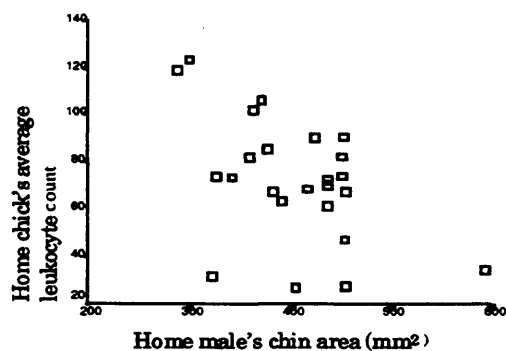
Figure 4. Scatter plot of the average luekocyte count of home-raised (related) chicks against their true father's chin area.

Figure 5. Scatter plot of the average luekocyte count of fostered (unrelated) chicks against foster father's chin area.
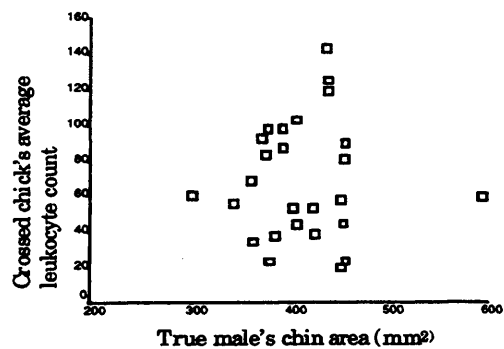
Figure 6. Scatter plot of the average luekocyte count of crossed chicks against their true father's chin area.

7.5 DISCUSSION

7.5.1 Haematozoa infection

The three most frequently occurring genera of haematozoa in this population of great tits were *Haemoproteus*, *Hepatozoon* and *Plasmodium* (in order of prevalence). This agrees with a previous three-year study on the same population (Allander & Bennett, 1994), despite methodological differences (Gordon Bennett looked at the number of parasites per 100 fields, whereas Frank Clark scored the majority of slides as the number of parasites and WBC per 10,000 red blood cells). More than half of the breeders were infected with at least one of the three common haematozoa, and *Haemoproteus* were identified in five chicks.

The intensity of *Haemoproteus* infection covaried with measures of reproductive effort. Infection intensity tended to increase with the natural brood size raised by the bird, and to decrease with hatch date. These results can not confirm Allander & Bennett's (1995) observation that parasite-free females initiated laying significantly earlier than parasitized females (which may have been explained by older birds laying earlier than young individuals). In fact, parasitized birds hatched their clutch, on average, 2 days earlier than infection-free individuals. This study found the relationship between success of the clutch and time of breeding goes against that found previously in the great tit (Perrins, 1965; Verhulst & Tinbergen, 1991; Allander, 1995). In this population, in the years studied, later clutches produced more chicks, and proportionately more of the brood survived to fledging than early clutches (4.4.3 Table 3).

A number of studies have also found an increase in intensity of infection with increased reproductive effort. Previously, Allander (1995) demonstrated that enlargement of clutch size increased parasite prevalence in this population of great tits. It appears from these data that the intensities of infection may vary with the natural reproductive effort, for those infected individuals, confirming Allander's findings (Allander, 1995). Allander described an age effect, with the prevalence of *Haemoproteus* being higher in adults, which this study can not confirm. Ots *et al* (1998) found that *Haemoproteus* intensities were higher in females, whilst the data from this study suggest that it is breeding males which suffer higher intensities of infection. It is difficult to compare two studies of blood parasites, even within the same population between years, because there are many factors which influence parasite prevalence and intensity. For

example, environmental conditions may influence the vector population, (which is responsible for transmitting the parasites) as well as influencing the condition of the host population.

Reproductive effort may increase the stress on the immune system, allowing chronic infections to relapse, or the relationship between reproductive effort and parasitemia may reflect an increased exposure to disease. From Allander's results (Allander, 1995), the most likely explanation is that chronic infections relapse as a response to the current reproductive effort. Hence, observational and experimental data suggest that the cost of reproduction may be mediated through immune suppression, often resulting in increased parasitemias (in the great tit see: Norris *et al*, 1993; Richner *et al*, 1995; Oppliger *et al*, 1997; in the collared flycatcher, Nordling *et al*, 1998; reviewed by Lindén & Møller, 1989).

It has been suggested that the reason we are able to observe this increase in parasitemias, or prevalence of parasites with increased reproductive effort, is that this increase is not important in fitness terms (Sheldon & Verhulst, 1996). The data from this population of great tits, however, demonstrated that both prevalence and intensity of *Haemoproteus* infection increased with reproductive effort (Allander, 1995; this study 7.3.4) and that infection with *Haemoproteus* affected local survival. Hõrak & Ots (in press) described an effect of *Haemoproteus* on survival of yearling great tits, whereas here, infection by *Haemoproteus* influenced adult survival. To date, only a few studies have demonstrated an effect of survival in association with haematozoa infection (in great tits infected with *Plasmodium*, Richner *et al*, 1995; in great tits infected with *Haemoproteus*, Ots & Hõrak, 1998; in collared flycatchers infected with *Haemoproteus*, Nordling *et al*, 1998).

7.5.2 Total leukocyte count

This study found no difference between the total leukocyte counts of individuals with and without scored infection. In a larger sample, Ots & Hõrak (1998) found that individuals infected with *Haemoprotues* had significantly higher concentrations of lymphocytes in their peripheral blood. It may be necessary to investigate the relative abundance of each class of white blood cell to directly investigate the immunological consequences of *Haemoprotues* infection.

In this study, males with larger chin areas had lower leukocyte counts. Data from this study suggest that lower leukocyte count affects the fitness of chicks. This relationship may not continue into adulthood, but it is possible that the lower leukocyte count is a fitness-related

variable. If this is the case then the size of the chin area may signal the general immune condition of the individual. In Allander & Dufva (1995) the total concentration of heterophils increased with colour intensity - a proposed signal. They interpreted this as colour intensity signalling immunity to parasites. There is also evidence from the house sparrow that immune function (measured as the size of the bursa of Fabricius, a trait thought to have a genetic component) covaries with badge size (Møller *et al*, 1996).

7.5.3 Heritability of fitness-related traits

In established host-parasite relationships, where natural resistance is low, the host can only control the infection through the activities of its immune system by developing an acquired resistance to infection. Resistance, or the ability to mount an effective response against parasitic infection may have a heritable component (Møller, 1990; Boulinier *et al*, 1997). Genetic heterogeneity among hosts in their immune response to parasitic infection has been found in all host-parasite systems (reviewed in Wakelin, 1988; Quinnell & Keymer, 1990). If a male's badge signals his resistance (relative to the local population of males) or his ability to mount a response against parasites, then, by choosing large-badged males, females may acquire for their offspring a currently advantageous, genetic-based immunity to the same parasites (see the classification of the various arguments regarding this theory in Westneat & Birkhead, 1998). In this population, male chin area covaried with a measure of immune status - total leukocyte count - a measure which explains some of the variation in local survival of chicks. I make the assumption that recruitment into the local population reflects survival by chicks, since Gotland is too large an island to survey the true survival of individual birds.

It was not possible to distinguish between genetic and environmental effects from these data. In nestlings raised by their true father, there was a significant negative relationship between the father's chin area and their total leukocyte count. This effect is not simply environmental, as this relationship was not found in chicks raised by a foster father (the same male as used in the previous analyses). The effect of the environment provided by genetic parents can be removed by considering those chicks raised away from the nest they hatched in, against their true father. There was no relationship between the total leukocyte count of crossed chicks and their true father's chin area. The leukocyte count of chicks only covaried with father's badge size when the environmental *and* genetic influences of males contribute to chick development.

7.5.4 Conclusions

The results from this and other studies suggest that life-history trade-offs may be linked to physiological trade-offs between reproductive effort and immune defence. The strongest demonstration of this relationship would be through direct manipulation of the immune status of an individual. The relationships found, however, suggest that immune suppression induced by increased reproductive effort imposes a fitness cost by increasing susceptibility to disease. The data also suggest that there may be some heritable genetic component to the nature of an individual's immune status, although this could not confirmed by this study.

DISCUSSION: IS THERE EVIDENCE FOR SEXUAL SELECTION IN THE GREAT TIT?

8.1 SEXUAL SELECTION

It was Darwin who first introduced the concept of sexual selection, when he described "the advantage which certain individuals have over others of the same sex and species in exclusive relation to reproduction" (Darwin, 1874; see reviews by Bradbury & Andersson, 1987; Andersson, 1994).

Sexual selection may occur through intra or inter-sexual selection. *Intra*-sexual selection is classically described in terms of male-male competition. This may be precopulatory, taking the form of fighting for territories, or post-copulatory, for example through sperm competition. Females may also compete for access to males. Good quality females may choose good quality males and exclude poor quality females from acquiring such males as mates (Slagsvold, 1993). *Inter*-sexual selection occurs through "choice" by the opposite sex. In practice it may be difficult to separate the two forms of sexual selection, when more subtle forms of choice may not be distinguishable from male-male competition. It is also possible that both mechanisms of sexual selection are often present in a single species (Bradbury & Davies, 1987). In socially monogamous species where there is biparental care, both sexes may discriminate among and compete over mates (Darwin, 1871; Andersson, 1994; Clutton-Brock, 1991).

The focus here is on inter-sexual selection. When considering inter-sexual selection it is useful to focus on female discrimination between males, i.e. on choice being displayed by one sex only. However, it may also benefit the male to make some form of choice, because the mate he takes may influence his reproductive success in a number of ways:

- male variation in reproductive success may be affected by variation in female behaviour, such as the likelihood that she will engage in extra-pair copulations. (In poultry, for example, copulation frequency is a heritable trait, see Birkhead & Møller, 1992).

- male variation in reproductive success may be influenced by variation in female quality, such as her age, fecundity or parental ability (Darwin, 1874).

- variation in male reproductive success may vary with how attractive his female rates him; for example in Burley's (1988) study in the zebra finch "attractive males" (made attractive by experimental manipulation) acquired better quality mates, while in peacocks the females laid more eggs when mated to attractive males (Petrie & Williams, 1993).

Taking the female point of view clarifies the discussion, but it must be held in mind that this is a biased outlook, with a number of implicit assumptions.

8.2 A SEXUALLY SELECTED TRAIT?

The novelty of sexual traits is that they may be detrimental to survival. The traits may be maintained, however, because they enhance reproductive success. A problem in studies of sexual selection is determining which traits may be sexually selected (i.e. have evolved through mate preference). In passerines, the focus of this discussion, the trait thought to be used in mate choice is often non-extravagant, potentially energetically cheap to produce and often has other functions. The badge of the great tit (Järvi & Bakken, 1984, Lemel & Wallin, 1993), the bib of house sparrows (Møller, 1987b; 1988a; however Kimball, 1996), the forehead patch of the collared flycatcher (Qvarnström, 1997) and plumage brightness in yellow warblers (Studd & Robertson, 1985) are all badges which have been proposed to function as signals of resource-holding potential as well a influence a male's attractiveness as a mate.

Fisher (1930) began to approach the problem of dual uses of signal traits when he suggested that when the "war paint" used by females in mate choice is also used by competing males to assess an individual's status, it would be an honest indicator of quality. The implication being that a dishonest signal would not be acted on by competing males. Fisher (1930) proposed that in such a situation the trait could rarely become extravagant. Zahavi (1975) also proposed a situation in which development of the trait may be restrained. He recognised the constraints of parental care on the development of a handicap, and suggested that mate selection may be

achieved through an easier test, with a smaller handicap (allowing investment in parental care rather than in passing the test of quality, Trivers, 1972).

Both of these theories account for why the trait may be non-extravagant in socially monogamous passerines but, because of its function outside that of mate choice, we cannot assume that these traits are sexually selected. A mate may be selected, but "sexual selection", (i.e. selection upon particular traits due to preference by the opposite sex) may not occur. Darwin (1874) pointed out the difficulty in judging how much of a character developed as a consequence of natural selection, and how much by sexual selection. The effects of both selective pressures may be mixed from the beginning since, he proposed, sexual selection preferably acts on characters which have already been correlated with quality.

Sexual selection through mate choice simply assumes that individuals vary in attractiveness and proposes that there is some benefit to being choosy. Mate choice could be passive with respect to phenotypic traits, for example simply based on opportunity to mate. Darwin (1871) originally suggested that males, by mating with females that are able to breed early, would acquire mates of greater than average quality. In this way "choice" could be passive.

It may be difficult to demonstrate unambiguously that female choice is important in the evolution of the male trait under study. For example, choice could be based on a casually linked factor, such as males with attractive sexual ornaments holding better quality territories, as has been demonstrated in the pied flycatcher (Alatalo *et al*, 1986). Female preference for *exaggerated* male traits has been demonstrated, by both observation (Petrie *et al*, 1991; Petrie & Halliday, 1994) and experimental manipulation (Andersson, 1982a). As discussed, however, the great tit does not fall into this classic category, as it does not carry an exaggerated male trait, but rather an area of pigmentation of the plumage. There is a suggestion that comes directly from paternity studies in passerines, that birds vary in attractiveness. This will briefly be reviewed.

Kempenaers *et al*, (1992) found that extra-pair young are not randomly distributed over nests. This result, however, does not distinguish between extra-pair paternity rates reflecting female behaviour, rather than her mate's quality. There is evidence to suggest that certain males are more attractive than others. For example, female intrusion rates in the blue tit differed markedly between territories; there was a significant inverse relationship between the number of female intrusions into a male's territory and the number of extrusions of his own female

(Kempenaers *et al*, 1992). Also, the males suffering from lost paternity received significantly fewer visits by females than males with no extra-pair young. Blakey (1994) reported that cuckolded male great tits were significantly smaller in tarsus-length than non-cuckolded males; the same was found to be true of the blue tit (Kempenaers *et al*, 1992).

These results may be explained if the female is responding to a factor casually linked to male size, for example territory quality. The female may trade copulations for access to feed on a male's territory, or may be more willing to solicit males who possess good-quality territories. There is a suggestion, for example, that large male great tits may better defend their territories (Chapter 3). However it is not known if this results in them acquiring better-quality territories. Thus, though it is important to consider how the data from this great tit population fit with models of sexual selection, it is equally important to bear in mind that casual relationships may mean that mate choice is passive with respect to the male badge.

8.3 TESTING MODELS OF SEXUAL SELECTION

Models of sexual selection may be applied to account for the evolution of traits used in mate choice. The same models may account for the maintenance of the badge and the preference themselves. Each model will be addressed in turn, with the focus on maintenance of traits in socially monogamous passerines, with evidence presented from this and other studies.

8.3.1 The Fisher Process

The Fisherian process assumes that female preference is genetically determined. Such preference is thought to become linked with male ornamentation because the most choosy female selects the most ornamented male. When this is linked with an initial advantage, not due to sexual preference (which may be quite inconsiderable in magnitude), choosiness may spread through the population (Fisher, 1930). If the female preference trait becomes common in the population then males carrying the trait will experience a mating advantage, as will their sons. The intriguing aspect of the Fisherian model is the consequence of this system. Fisher (1930) suggested this cycle could "runaway", resulting in the trait evolving far beyond the optimum determined by *natural* selection, so long as the disadvantage is more than counterbalanced by the advantage in sexual selection.

The fact that a preferred trait is now indicative of mate quality does not rule out that the preference initially evolved by the runaway process (Johnstone, 1995). I am not taking an

historical approach, rather, asking which model best fits with what we know about the behaviour of the birds and the function of the trait? The badge of the great tit appears to have a function outside that of mate choice; it is used as an signal out side of the breeding season (Lemel & Wallin, 1993) and may have originated as a signal of resource holding potential, rather than evolving through mate choice. It is likely that selection would act upon other individuals to use the information conveyed through this trait, whatever its origins. I therefore suggest Fisher's runaway model would not apply to the badge of the great tit, as the trait is an indicator of resource holding potential, as discussed above. The trait is not free to be selected as described by Fisher's (1930) theory. Similarly, I would suggest that models of sensory exploitation do not fit the evidence from the great tit (Johnstone, 1995 and references therein). Female preference may explain why a particular characteristic initially has the chance to evolve (Searcy, 1992). It does not fit, from an adaptationst point of view, that selection has not acted on this trait, as individuals continue to use as it as a signal in competitive interactions, suggesting that the information conveyed is useful, and the size of the trait appears to affect survival (Chapter 2).

8.3.2 Age dependent selection

Age may also influence choice. Trivers (1972) suggested that females may benefit from selecting older males because they have demonstrated their quality through their ability to survive. A female choosing an older male, expressing attractive secondary sexual characters, may therefore gain "good genes" for her offspring.

The predictions of the age-dependent model are:
- females preferentially mate with older males
- the male trait in question is an accurate indicator of age
- females use the proposed trait during mate choice

Whilst badge size did increase with age in the great tit, the between-individual variance was so high that it would not be a reliable indicator of age (Chapter 2). There is some evidence to suggest that older males may be preferred as mates, Greenwood et al (1979) showed that the average age of a mate was higher for female than for male great tits (for review see Manning, 1985). Wetton et al (1995) showed that female sparrows prefer older males as extra-pair partners.

8.3.3 Good genes models

Advertising your quality is different to demonstrating it through physical prowess. For example, manipulation of condition has been shown to affect the signal intensity in the wolf spider (Mappes, 1996). This, however, may be a direct display of prowess. If a plumage signal of quality does exist, rather than some direct assessment, then how is honesty of the signal enforced? Individuals may benefit by making an accurate assessment of a potential mate's quality, whilst being able to mislead partners as to their own quality so as to gain either more matings or a partner of good quality. To function as an honest advertisement of quality there must be some direct link between the signal and some aspect of the signaller's condition. The signal must therefore be costly to either produce or maintain. This cost may be arbitrarily defined as physiological (for example traits may increase the chances of predation), or social, though social costs must be paid in terms of the bird's physiology (reviewed by Johnstone, 1995).

The idea that the trait signals quality was formally presented by Zahavi (1975), who suggested that sexual selection is effective only by selecting a character which lowers the survival of the individual. Zahavi (1975; 1977) proposed that the degree of ornamentation signals to the female the male's ability to survive despite his handicap, and hence signals his overall viability. In contrast to Darwin's observation (Darwin, 1874) that sexually selected characters may lower survival but increase reproductive success, Zahavi considers the effect of the character on survival a necessary component of the system. Honesty is imposed because the cost of carrying such a signal would be greater for a cheater than for an honest signaller.

Zahavi was describing a *genetic* indicator, a number of people subsequently addressed the handicap model, and as a consequence it has been redefined (for example, Pomiankowski, 1987). The "strategic handicap" interpretation suggests that cost is necessary for the maintenance of honesty. A "quality dependent cost that ensures it is only for superior individuals that the benefits of extreme display outweigh the costs". The "revealing handicap" by contrast, allows that preferred traits need not signal genetic quality, because they are somehow uncheatable, making cost unnecessary for honest advertisement (see review by Johnstone, 1995).

Early models assumed that the handicap size is fixed and that it acted like a filter, with only those that can afford the costs associated with carrying the trait surviving, or displaying the trait (for example, Kirkpatrick, 1982). In practice, it is more plausible that the cost an individual can afford to pay, and therefore the size of the trait, will vary. In the condition-dependent model, investment in the handicap varies with male condition, and reflects the balance of a trade-off between mate attraction and longevity to maximize fitness. That is, the signal may be an advertisement of quality, but quality is determined, at least in part, by the conditions imposed by the environment. Development of the trait has been shown to be affected by environmental conditions in a number of species, for example the antlers of red deer (Clutton-Brock et al, 1982), and the tail feathers in male swallows (Møller, 1989b).

Predictions of Zahavi's handicap model:

* individuals with large characters, or a more intense signal should have lower survival than their smaller counterparts
* individuals with large characters, or a more intense signal have a higher mating success
* the character is positively correlated with male vigour
* females should use the character in mate choice

The primary prediction of Zahavi's handicap model is that the character should negatively affect survival. This is seen in the great tit (Chapter 2), with large-badged females and large-badged adult males having a lower chance of surviving two breeding seasons following the measure. More generally, male relative to female total mortality rate increases with sexual size dimorphism among mammals and birds (Promislow 1992, Promislow et al, 1992). The second condition is also met, with large-badged males producing more chicks in better condition than their small-badged counterparts (Chapter 4). In Nur & Hassons (1984) modelling of the handicap principle they suggest that when the benefit of a character is expressed in terms of fecundity and the cost in terms of survival this would fit their multiplicative model.

The best known example of Zahavi's "revealing handicap" model is the Hamilton-Zuk hypothesis (Hamilton and Zuk, 1982). Hamilton and Zuk proposed that the male ornament reflects his resistance to parasitic infection. A female could assess a potential mate's past or present condition from his secondary sexual trait. In this specific model, therefore, the sexual signal would be a genetic indicator.

Predictions of the Hamilton-Zuk hypothesis (after Andersson, 1994):

•   host fitness decreases with increased parasitic infection

•   ornament condition decreases with increased parasite burden

•   there is heritable variation in resistance to parasites

     female choice favours the most ornamented males (and circularly, hence the least parasitized males)

This thesis identified a parasite that decreased host fitness in the great tit (Chapter 7), however the intensity of infection did not covary with the badge measure. An indirect method of assessing whether the trait is a revealing handicap in this way is to look directly at the immune response. Male badge size covaried with total leukocyte count, a variable which explains survival of chicks. However, there were mixed results on the heritability of leukocyte count, with the negative relationship between parental badge size and total leukocyte count only being apparent in chicks that were raised by their true father. There are also no data on whether females prefer males with low leukocyte count.

All of the above conditions were met in a study in barn swallows (Møller, 1988b and 1990a). Cross-fostering of young suggested that the level of infection by a bloodsucking mite commonly found on swallow chicks, was a heritable trait. The host fitness was negatively affected by the mite; ornament size is inversely related to parasite burden, and the development of tail length, the ornament in the swallow, reflects parasite load.

A number of studies have found results which provide evidence for *some* of these predictions. For example, in sticklebacks females have been shown to prefer males according to their colour, and male colouration reveals parasite load (Milinski & Bakker, 1990). Calling characteristics differ between parasitized and unparasitized field crickets (Zuk *et al*, 1993) Female rock doves prefer males with feathers undamaged by mites (Clayton, 1990). Similarly, experiment in the ring-necked pheasant suggest that females may prefer to solicit matings from males with low intensities of infection (Hillgarth, 1990). In general, the results of such studies are varied, perhaps because the most important parasites, in terms of variance in host fitness, are not usually identified first (Clayton, 1991; Clayton *et al* 1992).

8.3.4 Evidence for good genes from the literature

There is evidence for a genetic correlation between the trait and preference, for example, in sticklebacks (Milinski & Bakker, 1990; Bakker, 1993) and stalk-eyed flies (Wilkinson & Reillo, 1994; see review by Bakker & Pominakowski, 1995; however Ritchie, 1992). The correlation between the trait and preference could be accounted for by the Fisher process and, alone, is not evidence of good genes. There is, though, accumulating evidence that males with large secondary sexual characters may carry good genes. In Milinski & Bakker's (1990) study of sticklebacks, the preferred trait varied with a measure of condition. Similarly, Nicoletto (1993; 1995) found that male ornamentation reflected condition in the guppy.

An experiment by Møller (1988b; 1989b) in the barn swallow suggests that naturally short-tailed males were less able to survive with elongated tails than were males with naturally long tails. However, adding a set length of feather to a short tail results in a proportionately greater increase in size than adding the same length to a large tail. This may lead to a greater impact on flight performance in short-tailed males, even if there were no relationship between natural tail length and male quality (Johnstone, 1995). Moore (1994) showed that offspring of attractive male cockroaches reached developmental maturity faster, which is assumed (but requires evidence) to increase the female's lifetime reproductive success, because the time between clutches is decreased by producing offspring that mature more quickly. Day et al (1996) suggested that male size in the seaweed fly is a reliable indicator of offspring fitness, and that female choice for large males produces fitter offspring (Crocker & Day, 1987; Gilburn et al, 1996). Hasselquist et al (1996) found that male repertoire size, attractive to females, is positively correlated with offspring survival in the great reed warbler.

The great tit badge may reflect a heritable aspect of male quality, with cross-fostered chicks being more likely to survive if their true father had a large chin area (Chapter 5). Norris (1993), by using cross-fostered chicks, found that stripe-size was heritable and that there was a significant relationship between the stripe size of the true father and the proportion of male offspring surviving from a brood. The problem with such cross-fostering experiments is there may be a maternal response to her mate's badge which takes effect at the egg stage (Gil et al, 1999), or that high quality females mate assortatively with attractive males.

Studies have attempted to control for this maternal effect, for example Sheldon *et al* (1997), by using maternal half siblings were able to show that the genetic contribution to chick condition depended on the badge size of the true father. Similarly, in tree frogs, the condition of maternal half siblings depended on the size of the father's signal (Welch *et al*, 1998). In peacocks, assortative mating was controlled for by randomly assigning females to mates, the size and survival chance of offspring were found to increase with male attractiveness (Petrie, 1994).

Not all studies find evidence for good genes. For example, Hughes (1995) found no correlation between mating success and offspring quality in *Drosophila melanogaster*. However, mating success is just one measure of attractiveness. It is possible that sexual selection occurs even if variation in reproductive success is not due to the number of mates an individual acquired, but rather due to the relative number of offspring they leave. This would still allow preferred males to pass on good genes, even if there were restrictions on the number of mates an attractive male acquires.

There is growing evidence that female birds choose to perform extra-pair copulations with a male of better quality than their partner. For example, in several species females paired to young males, or males of low social status, were involved in EPCs more frequently than females paired to older, or more dominant males (Birkhead & Møller, 1992a and references therein). Females also participated more readily with males that were more attractive than their partners (Birkhead & Møller, 1992a and references therein; however see Bjorklund et al, 1992). Whether mating with males of a better "quality" has its associated costs still needs to be established in monogamous breeders. Møller (1994) demonstrated that offspring longevity is positively related to the ornament size of the male parent, and that longevity of the sons is a trait with a significant resemblance to their father's. However, relative paternal provisioning of offspring was negatively related to the tail length of males; thus it is also important to consider the costs to the female of mating with "attractive" males.

8.3.5 Maintenance of variation

Models of genetic indicator mechanisms assume that viability is heritable, and that the secondary sexual trait is expressed in relation to the condition of the individual. In this way, a female choosing a male of good condition makes it likely that her offspring will inherit this high viability from its father. The problem inherent in theories of viability-dependent traits is that if a signal is heritable, then why has it not reached fixation in the population due to

continuous directional selection (Falconer, 1989)? That is to say, following Fisher's fundamental theorem (Fisher, 1930), in a population at equilibrium, characters exerting a large effect on fitness have a much lower heritability than those that do not greatly influence reproductive success. This conclusion is based on the assumption that if a trait important for fitness has a high heritability it will evolve rapidly. This will tend to fix the alleles and hence reduce genetic variability (the heritability) of the trait. However, if the signal is a phenotypic indicator, not influenced by the individual's genes, the model is free from this problem because fitness need not be heritable.

Jones (1987) proposed a number of forces that could account for maintenance of genetic variance in fitness:

- genetic variation maintained by host parasite co-evolution (Morand *et al*, 1996) (any common strain of host will quickly be effectively parasitized by an adapted strain of parasite - so the genetic make up of the fittest individuals is under constant flux: otherwise described as temporal variation).
- changing environment.
- mutation (mutation pressure may create enough heritable variance in fitness to give a selective advantage to choosy females see Charlesworth, 1987).
- immigration (otherwise described as spatial variation).
- outbreeding (again an example of spatial variation)

The relative importance of these forces is very difficult to quantify (although some studies attempt to address the question for example, Houle *et al*, 1992; Fernandez & Lopezfanjul, 1996; Coltman *et al*, 1999). Such propositions allow us to accept, theoretically, the existence of a heritable aspect to both a sexually selected trait and to "good genes". Sexually selected traits seem to have substantial heritabilities (e.g. Norris, 1993; Wilcockson *et al*, 1995; Qvarnström, 1998): there is also evidence of genetic variation in mating preference as well as in the preferred traits (Bakker, 1990; 1993; Wilkinson & Reillo, 1994).

One of the first estimates of heritability of a direct measure of fitness in a natural population produced a result which supports Fisher. Gustafsson (1986) demonstrated that the lifetime reproductive success of male collared flycatchers was virtually zero (as also found in song sparrows, Smith, 1998). We may be forced to accept Fisher's view that "sexy" males pay a price in reduced life expectancy, development time, or another component of fitness to such an extent that females do not improve the prospects of their offspring by choosing them.

Being "sexy" could be considered to be just one of a set of equally rewarding strategies available. Just as dominance and subordination may represent alternative, but equally successful lifetime strategies (Owens & Hartley, 1991), so may exaggeration of the attractive trait result in such costly trade-offs that carrying a small example of the trait may be equally successful in the long term. It is possible, however, that Gustafsson's measure of lifetime reproductive success is not necessarily a good estimate of lifetime fitness. For example, extra-pair success may need to be considered, as also might information concerning the population stability and relatedness (Jones, 1987).

Defining fitness becomes especially difficult when there are direct parental effects on the offspring phenotype, other than the genes that are passed on. Parental care is the most obvious, and perhaps the most biologically important example (Bradbury & Andersson, 1987). Whether fitness-related traits are heritable is currently a moot point because studies in the lab suggest that under some circumstances a population may retain considerable genetic diversity for fitness characters (Jones, 1987 and references therein).

Pomiankowski & Møller (1995) propose that the evidence points toward there being more phenotypic variation in sexual than non-sexual traits and suggest that this is due to genetic modifiers which influence condition-dependent expression of the trait. Wilkinson & Taper (1999) demonstrate that in sexually dimorphic sister species, genetic variation for the attractive male trait is significantly larger than the variation available in monomorphic species. They propose that this variation is due to changes in condition dependence of the trait, rather than due to the action of genetic modifiers.

The behaviour of heritable fitness traits is not intuitively obvious. Although several components of fitness show considerable heritability there may be negative genetic correlations between such components (Rose & Charlesworth, 198; Reznik, 1985; reviewed in Andersson, 1994). So total fitness need not show heritability even if some fitness components do.

Variation in the heritability of an attractive trait may not only arise through variation of the frequency of genes determining possession or the size of the trait. Ecological conditions will determine the heritability of a trait. Different genes differ in their response to changes in the environment (Wilkinson, 1987; Aspi & Hoikkala, 1993; Santos *et al*, 1994; Jia & Greenfield, 1997) and hence may differ in the extent to which they affect fitness according to the

121

environmental conditions experienced. A number of studies have found evidence for the occurrence of genotype-by-environment interactions in natural bird populations (Gebhardt-Henrich & van Noordwijk, 1991; Price, 1991; Merilä, 1997; Qvarnström, 1998).

If varying environmental conditions reduce the genetic contribution parents can make to their offspring's phenotype, then the strength of sexual selection through female choice will be lowered in such years. By themselves gene-environment interactions and lowered selection may explain maintenance in variation of the heritability of a trait (typically) under direct selection. Recent modelling (Kirkpatrick & Barton, 1997) suggests that though preference for good genes may be theoretically sound, its relative importance as an evolutionary force compared to direct selection might be low.

8.4 DIRECT BENEFITS

There are a number of ways that male behaviour may not only affect female reproductive success in the current attempt, but also in future attempts (Gustafsson & Sutherland, 1988). Direct selection can be tested unequivocally since it provides clear correlations between mating patterns and subsequent female survivorship or fecundity. In some circumstances, the "good genes" models need only a small amount of reworking to become models of direct benefits. Nur & Hasson (1984), in their model of the handicap principle proposed that, when males provide parental care, differences in male "quality", signalled by the trait need not be genetic. Similarly, with the age-dependent model, a male may express the badge according to his age, and may survive to express that badge only if he has superior hunting skills, which may influence the quality of his parental care.

What are the potential direct benefits of mate choice? Westneat et al (1990, in Birkhead & Møller, 1992a) proposed that females may pair with a male on the basis of his territory quality, his potential for providing parental care, the familiarity of the territory, or because there may be an advantage to mating with a former mate. Kirkpatrick & Ryan (1991) propose that most evidence points overwhelmingly to the importance of selection acting directly on female survivorship or fecundity (Thornhill, 1983; Price, 1984; Lightbody & Weatherhead, 1988 in Kirkpatrick & Ryan, 1991).

In a socially monogamous species such as the great tit, in which the adults share parental responsibilities, it is thought the resources gained through pairing will be of great importance.

By selecting a good-quality parent a female may improve the condition of her offspring. If the badge expressed is a condition-dependent trait she may also improve her offspring's badge size. By improving an offspring's condition a female may increase its chances throughout its life, through the "silver spoon" effect (Cockburn, 1991). Alternatively, parasite transmission avoidance may be important (Clayton, 1990; Clayton *et al*, 1992), if male badge reveals parasite load.

Models of direct benefits require that the secondary sexual character convey information to the female about the potential direct benefits offered. Benefits must either be apparent in the female or her offspring.

Predictions of models of direct benefits:
* female choice must favour the most ornamented males.
* females mated to males with the most exaggerated characters must subsequently be of better condition, or produce offspring of better condition than those mated to unattractive males.

There is some evidence for direct benefits in great tits. Females mated with large-badged males produced (fostered) offspring of better condition than those mated to small-badged males (Chapter 4). Also, chicks raised by large (unrelated males) had a better chance of survival (Chapter 5). There is also evidence from the bird literature to suggest a direct link between a male's plumage pigmentation and his phenotypic quality (Hill, 1990; 1991; Hill & Montgomerie, 1994).

Zahavi (1975), however, proposed that males which do not invest in parental care can spend more time and energy on "passing the test" of quality. Hence he predicted an inverse relationship between the effort allocated to the male signal and parental care. This has been found in a number of studies. For example, Griffith (1998) found a negative relationship between a males' provisioning behaviour and his badge size in the house sparrow. Burley (1988) experimentally manipulated attractiveness in the zebra finch. Partners given "unattractive" colour rings showed a higher level of parental expenditure (and also have shorter lifespans). The relationship found may depend on the species studied, for example, as discussed above in the house finch, attractive males provide better parental care (Hill, 1990).

Zahavi's prediction is perhaps oversimplified, when the size of the badge truly reflects male quality such males may also be able to provide a better territory or provision young with a smaller associated immediate cost than small-badged males. The bird data suggests that the relationship between badge size and parental care varies between species. One of the major problems is assessing all aspects of parental care.

Territory quality will directly affect the feeding effort of the parents. Evidence that a poor territory can influence reproductive success comes from Perrins & McCleery's study of great tits (Perrins & McCleery, 1994). At high breeding densities egg weight was reduced, and egg weight has been previously shown to influence the chances of hatching, chick fledging and chick weight at fledging (Perrins, 1965). In the pied flycatcher it was found that females are basing their choice of male on the quality of territory he controls (Alatalo *et al*, 1986). Qvarnström (1998, paper IV) suggests that female collared flycatchers mated to males with large badges have a better chance of survival. In this study on the great tit, females mated to males which survived had a better survival chance. This may be due to differences in the territory held, and there is circumstantial evidence that large-badged, or large male great tits may acquire better territories (see Chapters 2 (discussion) and Chapter 3). Survival has also been shown to be affected by mate-quality in insects, for example in the comma butterfly (Wedell, 1996).

Both sexes may suffer from trade-offs, Owens & Bennett (1994) propose that the most costly forms of parental care are feeding and defence of chicks. A direct trade-off between these behaviours has been described, Qvarnström (1998, paper IV) found that experimentally altered attractiveness increased territorial activity, but decreased parental care by the collared flycatcher male. There is a suggestion that raised testosterone levels early in the season may subsequently lower provisioning behaviour in the great tit (Chapter 3). To investigate whether there is an overall cost or benefit to mating with or being a large-badged male, data must be collected on all aspects of parental care.

8.5 THE FUTURE OF TESTING MODELS OF SEXUAL SELECTION

Balmford & Read (1991) propose that even though the predictions for the various models of sexual selection are not conclusive, that there are ways we could find support for a specific hypothesis. By assessing the consistency with which secondary sexual characters reveal heritable variation in a component of male viability we may find evidence for the good genes

model. The parasite mediated sexual selection hypothesis would be supported if most ornaments specifically and consistently reveal relative components of parasite burdens. Owens (1994) more specifically proposes estimating the genetic heritability of survival traits and attractive traits through a cross-fostering regime. The good genes hypothesis will be supported if both lifetime reproductive success and attractive traits are heritable and positively correlated. A direct selection/"honest-signal" hypothesis based on environmental components of condition predicts that neither survival nor attractive traits will be heritable. This approach would not, however, identify what is being gained by the female's choice. The result could either fit the transmission avoidance model, or suggest that parental care is important. Finally, the arbitrary trait model predicts that only attractive traits will be heritable.

8.6 SUMMARY

There is evidence from the great tit that the female may gain both direct and indirect benefits by mating with large-badged males.

1) Males and females with large chin-areas appear to have reduced survival.

2) Large males appear to behave more aggressively during the early nesting stage, with large-badged males tending to produce a longer vocal response to experimental intrusion.

3) Large-badged males produced more chicks, in better condition than their small-badged counterparts. This may reflect the males parental investment, the quality of the male's territory, maternal quality, or the female's response to the quality of mate she is paired to.

4) Large-badged males appear to produce more recruits even when they are raised away from their true parents. This suggests there may be some genetic-contribution to offspring condition which covaries with paternal badge size.

5) This contribution to chick survival may include a genetic component to immune defence. Large-badged males produced chicks with a lower leukocyte count than small-badged males, and low leukocyte count improved the chick's chance of survival.

8.7 CONCLUSION

This thesis investigated whether there was evidence for sexual selection in the great tit. In this, as with other species, the ambiguity lies with the lack of evidence that the female uses the badge in active mate choice (for example the house sparrow, Møller, 1988a; Veiga, 1996, however Kimball, 1996). The data suggest that male chin area potentially signals both environmental and genetic benefits available to the female great tit. I propose that the single most important variable determining the extent of these benefits may be the current ecological conditions. In a bad year it may be the size of the male's territory or his provisioning ability which determines nestling survival (and hence female fitness). Alternatively, in a good year, when most nests successfully fledge their brood, the genetic contribution to an offspring's condition, or dominance status (through badge size), may be a significant factor in influencing offspring's chances of surviving the winter in a large flock of yearlings.

APPENDIX 1

COMPARISON OF DIFFERENT MEASURES OF MALE STRIPE SIZE

Table 1: Consideration of the different measures of male stripe size.

Variables included in the model	r^2	df	significance of badge measure
Dependent: time to capture			
CB3 and tarsus	0.73	8	ns
Chin and tarsus	0.58	8	ns
B in 3 and tarsus	0.74	8	ns
Dependent: variation in length of vocal response			
CB3 and tarsus	0.79	15	**
Chin and tarsus	0.73	15	**
B in 3 and tarsus	0.78	15	**
Dependent: time lag to first response			
CB3 and tarsus	0.47	15	ns
Chin and tarsus	0.29	15	ns
B in 3 and tarsus	0.48	15	ns
Dependent: total proportion of male feeds			
CB3	-0.31	33	ns
Chin	-0.47	38	***
B3	0.09	33	ns
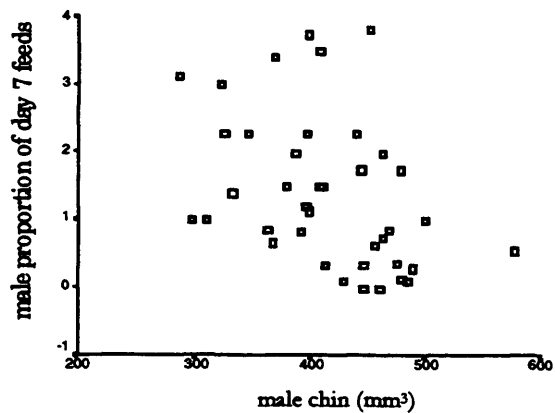
Figure 1a. Scatter plot of the proportion of male feeds at day 7 against his chin measure.
r= -0.45, p=0.005, df=38
if male breast measure is used: r= 0.09, p= 0.61, df= 33
if male cb3 measure is used: r=-0.13, p= 0.47, df= 33
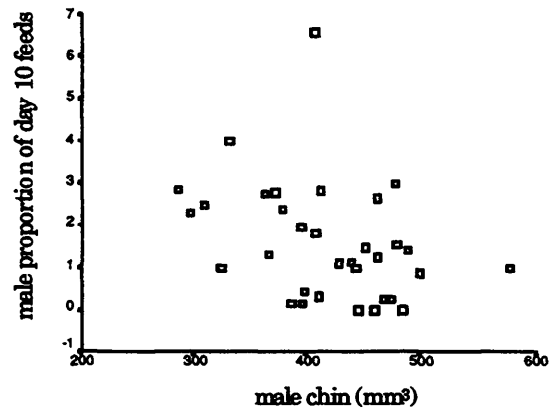
Figure 1b. Scatter plot of the proportion of male feeds
at day 10 against his chin measure.
r= -0.39, p=0.02, df= 33
if male breast measure is used r= 0.18, p= 0.34, df= 30
if male cb3 measure is used r= -0.02, p= 0.92, df= 30
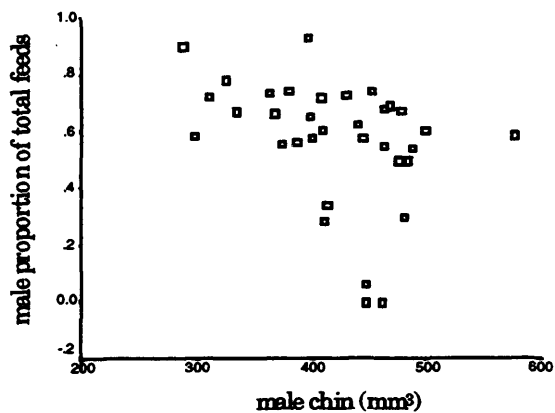
Figure 1c. Scatter plot of the male proportion of total feeds against his chin measure.
r= -0.47, p=0.00, df= 33
if male breast measure is used r= -0.05, p= 0.78, df= 30
if male cb3 measure is used r= -0.31, p=0.09, df= 30
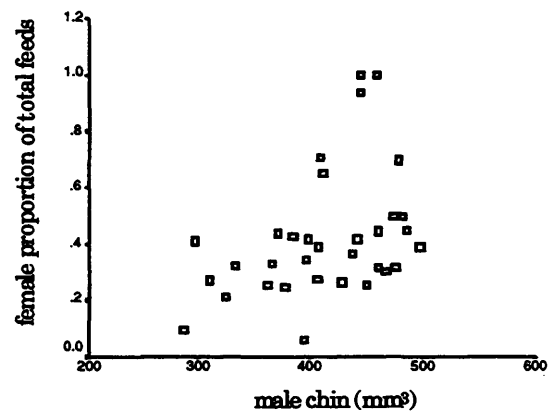
Figure 1d. Scatter plot of the female proportion of total feeds against male chin measure.
r= 0.47, p= 0.00, df= 33
if male breast measure is used r= 0.05, p=0.77, df= 30
if male cb3 measure is used r= 0.31, p= 0.09, df= 30

APPENDIX 2

ALTERNATIVE VIABLE MODELS EXPLAINING VARIANCE IN CLUTCH SIZE

Table 1. Alternative models to investigate variation in clutch size.

Model	independent	df	statistics
model A	male BCI	42	F= 12.88 ***
model B	male weight	42	F= 6.91 *

APPENDIX 3

RESEMBLANCE OF CHICKS AND PARENTS

Table 1: Resemblance and heritability estimates for mid-offspring measures from day 14 chicks on each parent: (1) shared environment and genome, (2) shared environment, (3) shared genome. (3a) compares chicks crossed into the "home" nest against their original parents, (3b) compares chicks crossed out of the "home" nest against the home parents.

relationship		male - mid offspring				female - mid offspring			
character		n	slope	s.e.	P	n	slope	s.e.	P
tarsus	1	73	0.22	0.14	0.10	73	0.23	0.16	0.16
	2	71	-0.03	0.15	0.80	74	-0.10	0.19	0.59
	3a	59	-0.04	0.17	0.98	63	0.28	0.19	0.14
	3b	62	-0.06	0.17	0.98	66	0.28	0.18	0.13
body weight	1	70	0.18	0.11	0.11	70	0.12	0.12	0.31
	2	68	0.12	0.12	0.31	63	0.19	0.12	0.11
	3a	59	0.22	0.13	0.08 †	63	0.17	0.13	0.17
	3b	57	0.22	0.14	0.11	60	0.14	0.13	0.28

† signifies a p of < 0.1
the h (narrow sense heritability) for category 3 is 2* the slope.

Table 2: Resemblance and heritability estimates for day 14 chicks in the nest: (1) shared environment and genome, (2) shared environment, (3) shared genome. (3a) compares chicks crossed into the "home" nest against their original parents, (3b) compares chicks crossed out of the "home" nest against the home parents. (A) considers the male parent's influence and (B) the female parent's influence.

A

relationship		male - male offspring				male - female offspring			
character		n	slope	s.e.	p	n	slope	s.e.	p
tarsus	1	61	0.02	0.13	0.86	58	0.07	0.17	0.67
	2	59	0.02	0.16	0.89	54	0.17	0.16	0.25
	3a	48	-0.09	0.18	0.60	46	0.19	0.17	0.26
	3b	45	-0.09	0.17	0.57	46	0.16	0.17	0.35
body weight	1	60	0.18	0.12	0.14	55	0.16	0.12	0.18
	2	57	-0.05	0.15	0.73	52	0.18	0.14	0.18
	3a	45	0.18	0.13	0.17	45	0.34	0.15	0.03 *
	3b	43	0.24	0.16	0.14	44	0.38	0.15	0.01 **

B

relationship		female - male offspring				female - female offspring			
character		n	slope	s.e.	p	n	slope	s.e.	p
tarsus	1	61	0.37	0.13	0.08	59	0.26	0.18	0.15
	2	61	-0.09	0.20	0.65	54	0.20	0.18	0.26
	3a	52	0.33	0.20	0.11	48	0.27	0.19	0.17
	3b	49	0.36	0.21	0.08	46	0.21	0.19	0.30
body weight	1	59	0.14	0.12	0.27	57	0.13	0.13	0.29
	2	59	0.09	0.12	0.44	52	0.09	0.16	0.55
	3a	49	0.14	0.14	0.30	45	0.14	0.14	0.37
	3b	46	-0.09	0.15	0.55	43	0.12	0.14	0.37

† signifies a p of < 0.1.
the h2 (narrow sense heritability) for category 3 is 2* the slope.

Table 3: Resemblance and heritability estimates for recruits: (1) shared environment and genome, (2) shared environment, (3) shared genome, considering the male parent's influence and the female parent's influence.

relationship		male parent - recruit				female - recruit			
character		n	slope	s.e.	p	n	slope	s.e.	p
tarsus	1	15	0.31	0.13	0.02 *	15	0.42	0.15	0.02 *
	2	13	0.42	0.42	0.34	15	0.96	0.34	0.01 **
	3	15	-0.18	0.35	0.61	16	-0.21	0.46	0.66
wing	1	13	0.68	0.25	0.02 *	13	0.04	0.28	0.87
	2	12	-0.23	0.34	0.51	14	0.16	0.35	0.64
	3	14	0.25	0.24	0.31	15	-0.28	0.33	0.41
chin	1	15	-0.11	0.23	0.64	10	0.29	0.36	0.43
	2	12	-0.30	0.28	0.61	10	0.67	0.44	0.16
	3	15	-0.20	0.25	0.61	9	0.79	0.56	0.19

Table 4: Resemblance and heritability estimates for recruits separated by sex: (1) shared environment and genome, (2) shared environment, (3) shared genome (A) considers the male parent's influence and (B) the female parent's influence. The sample sizes are very low and therefore give unreliable estimates of the slope.

A

relationship		male parent – male recruit				male parent – female recruit			
character		n	slope	s.e.	p	n	slope	s.e.	p
tarsus	1	8	0.07	0.16	0.66	6	0.35	0.26	0.24
	2	6	0.75	0.47	0.17	6	0.48	0.74	0.54
	3	7	-0.06	0.97	0.95	7	-0.19	0.32	0.57
wing	1	6	0.15	0.21	0.49	6	1.01	0.45	0.06
	2	5	-0.74	0.41	0.15	6	-0.09	0.26	0.74
	3	6	0.61	0.32	0.11	7	-0.07	0.16	0.64
chin	1	8	0.21	0.17	0.24	6	-0.21	0.45	0.67
	2	5	0.09	0.19	0.63	6	-0.25	0.14	0.14
	3	7	-0.02	0.14	0.84	7	0.15	0.29	0.62
breast (in 2 cm)	1	7	0.39	1.02	0.71	na			
	2	6	0.04	0.21	0.84	na			
	3	7	0.02	0.30	0.95	na			
breast (in 3 cm)	1	7	-0.06	1.77	0.97	na			
	2	6	0.04	0.28	0.97	na			
	3	7	-0.05	0.34	0.88				

B

relationship		male parent – male recruit				male parent – female recruit			
character		n	slope	s.e.	p	n	slope	s.e.	p
tarsus	1	8	0.23	0.12	0.10	6	0.51	0.22	0.08
	2	7	1.15	0.62	0.11	7	0.74	0.40	0.11
	3	8	-0.24	0.73	0.76	7	-0.36	0.64	0.59
wing	1	7	0.19	0.16	0.43	5	-0.59	0.48	0.29
	2	6	0.39	0.69	0.59	7	-0.07	0.25	0.76
	3	7	-0.27	0.43	0.55	7	-0.21	0.31	0.52
chin	1	7	0.59	0.36	0.15	2	na		
	2	4	na			5	0.15	0.38	0.71
	3	4	na			4	na		

Table 5: Male indexed genetic influence on phenotype expression compared to female genetic influence. Indices are calculated as R^2 male/ R^2 female from the resemblance regressions of each sex or mid-offspring values for chicks (using average R^2 from 3a and 3b, see Table 2). A value of 1 estimates an equal impact; values larger than 1 suggests a male biased influence.

Character	Influence on male chick character	Influence on female chick character	Influence on mid-offspring values
tarsus	0.80	0.86	<0.01
body weight	3.21	6.00	2.50

Table 6: Resemblance estimates for related siblings, using mid-home young on mid-fostered measures from day 14 chicks in the nest (1) shared environment (2) shared genome.

relationship		home male – fostered male				home female – home female			
character		n	slope	s.e.	p	n	slope	s.e.	p
tarsus	1	61	0.39	0.11	<0.001 ***	47	0.67	0.12	<0.001 ***
	2	47	0.60	0.10	<0.001 ***	38	0.58	0.13	<0.001 ***
body weight	1	61	0.36	0.13	0.006 **	47	0.57	0.12	<0.001 ***
	2	47	0.44	0.13	0.002 **	38	0.13	0.13	0.32

The genetic resemblance between full siblings is 0.5 * the additive genetic variance. Therefore the narrow sense heritability h = 2*slope (Falconer, 1989). The resemblance due to shared environment should be approximately equal. For easy comparison of the two the slope of the line is presented in place of h .

APPENDIX 4

SCIENTIFIC NAMES OF SPECIES REFERRED TO IN THE TEXT

barn swallow	*Hirundo rustica*
blackcaps	*Sylvia atricapilla*
bluethroat	*Luscinia svecica*
blue tit	*Parus caeruleus*
cockroach	*Periplaneta Americana*
collared flycatcher	*Ficedula albicollis*
comma butterfly	*Polygonia c-album*
dark-eyed juncos	*Junco hyemalis*
field cricket	*Teleogryllus oceanicus*
gray tree frog	*Hyla versicolor*
great tit	*Parus major*
great reed warbler	*Acrocephalus arundinaceus*
guppy	*Poecilia reticulata*
harris sparrow	*Zonotrichia querula*
house finch	*Carpodacus mexicanus*
house sparrow	*Passer domesticus*
peacock	*Pavo cristatus*
pied flycatcher	*Ficedula hypoleuca*
red deer	*Cervus elaphus*
red-winged blackbird	*Agelaius phoeniceus*
ring-necked pheasant	*Phasianus colchicus*
rock dove	*Columba livia*
sage grouse	*Centrocercus urophasianus*
seaweed fly	*Coelopa frigida*
song sparrow	*Melospiza melodia*
stalk-eyed fly	*Cyrtodiopsis dalmanni*
three-spined stickleback	*Gasterosteus aculeatus*
wheatear	*Oenanthe oenanthe*
wolf spider	*Hygrolycosa rubrofasciata*
yellow hammer	*Emberiza cirtinella*
zebra finch	*Taeniopygia guttata*

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