Sex Ratio Adjustment in Birds:

Evidence from *Parus* species

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Preface

This thesis has been composed by me, is the result of my own research and contains no work done in collaboration except where stated. No part of this thesis has been submitted to any other university in application for a higher degree.

To John and Nadia

Abstract

Natural selection favours those individuals capable of biasing investment in male and female offspring when the reproductive value of each sex differs. One way in which parental investment can be skewed is through altering the sex ratio, i.e. the numbers of sons and daughters produced. In birds, this can potentially be achieved at the egg stage or through subsequent modification of the numbers of male and female offspring post-hatching. I investigate both sex ratio biasing mechanisms in *Parus major* and *P. caeruleus* breeding on the Swedish island of Gotland.

From deserted *P. major* nests where eggs had been numbered as they were laid, egg sex ratio with laying sequence increased from approximately 0.50 to 0.75. However, sample sizes were small and the effect was not statistically significant when analyses were limited to modal clutch sizes. A cross-species analysis of sex ratio variation with laying sequence suggests that females lay eggs of the larger sex first, whether that is males or females. This relationship is expected to be associated with increased levels of brood reduction. Biasing egg sex ratios in this way might increase the reproductive value of a brood if, by reducing nestling competition through mortality, the quality of remaining offspring is enhanced. There was some evidence that this may only be true for small-brooded species.

Female body condition of both *P.major* and *P.caeruleus* was experimentally manipulated through heating and cooling nest boxes prior to and during egg laying. There was no evidence of alterations to egg sex ratios in relation to female body condition in either species.

After hatching, higher mortality of larger male *P.major* nestlings in poor nesting conditions is expected to result in female biased sex ratios. Cross-fostering nestlings of different sizes and sexes between nests confirmed a male advantage in competitive environments, which can account for observed male biases when female skews were expected. This demonstrates the potential importance of competition amongst nestmates in determining sex ratios, in addition to functional explanations that assume parental control of the sex ratio.

Evidence for primary sex ratio biases in relation to a number of traits and environmental factors was examined for *P.major* pairs over three breeding years. Results suggest that within-individual analyses may be more fruitful than a correlational approach across individuals. A female's brood sex ratio varies with respect to the quality of male she is paired with relative to previous matings, but a male's brood sex ratio does not vary in the same fashion. Amongst blue tits, there was a tendency for sex ratios to increase in relation to paternal tarsus length. These findings support the argument for female control of primary sex ratios.

My results imply that both primary (conception) and secondary (egg, nestling) sex ratio adjustment can occur in *Parus*. Egg sex may be controlled by the female through modifications of the sex ratio with laying order, and in response to the relative condition of the male to which she is mated. Post-hatching modifications are shaped by competitive nestling interactions, regardless of biased parental investment in nestlings of different sexes. However, more work is needed to establish a mechanism of sex ratio control in birds and to demonstrate the adaptive nature of biases. The latter may be achieved through determining fitness payoffs from producing male and female offspring, and data from long-term studies could prove valuable.

Acknowledgements

Most people hate writing up their thesis, but I quite enjoyed these final stages and seeing some structure emerge to my work from the past 3 ½ years. However, when the going got a bit rough in the middle I was lucky in the support I received from a whole bunch of people.

In the department at Edinburgh, Victoria you graciously took me under your wing as a student even though I was nothing to do with your area of expertise. Thank you for your encouragement and especially your help with administrative matters, which were more complicated than usual with me essentially split between two departments. Thank you especially to yourself and Andrew for helping me out at the end of year one. Apart from allowing me facilities to work in the lab, Josephine you always asked how my field season/conference had been, and also how I was getting on generally, and for this interest I thank you. During the writing phase, a special word must go to Loeske. You read through draft manuscripts at short notice and also put up with my mutterings and interruptions in the office as my eyeballs popped out in disbelief before I discovered where those missing cases had disappeared to. I learned so much those last weeks, shame I didn't 'discover you' earlier (shame for me, probably not for you!) Thanks. For comments on manuscripts before I handed in, thank you Ben and Loeske. Thanks also Ben for getting the money for the study, for introducing me to Gotland and Uppsala, and for always reading so very quickly through manuscripts. No sooner had I sent one than it was returned the next day with useful comments which greatly improved the work and guidance to references. Thanks for that.

I thoroughly enjoyed my trips to Uppsala, which were a source of inspiration, lots of interesting and interested people to talk to, and without which I probably would have felt extremely isolated. In the department, thank you my life-history group session peers (crikey, that sounds like some sort of therapy group...) for regular scientific discussions. In particular thanks to Mariusz, Karen, Ineke, Henk, Lasse H, and Robert. We also had some pretty good parties eh, Revolutions and defences...?!

In my first year I spent an incredibly tedious 6 months trying to get the PCR recipe right to sex nestlings. I was a complete molecular virgin and not impressed one bit, but Richard and Kate helped me out with visits to Glasgow or vice versa. The recipe worked fine in Edinburgh using dirty Kelvin water posted through. Thanks for putting me up guys; and the terrible films ('Kiss the Girls', 'Desperado'??). In Uppsala, Simon you lead me through the silver staining procedure and the relief of polyacrylamide after agarose. As for threatening to kill me if anything happened to the micropipette, as if I wouldn't be dead soon anyway after all those chemicals....Good luck in your next place.

Help in the form of moral support, and fun times shared, probably deserves greater thanks in this thesis than academic help. Of course everybody thanks their parents in their theses, but I do feel mine put up with especially a lot. Well, I am supposed to be the happiest person to finish my thesis, but actually this accolade must go to my mother. Ma and Pa, thank you for going through the ups and downs with me and always providing a safe nest for this chick to return to. Dad I'll never forget in the plane from Spain (yes, and the rain...) you telling me 'If it's not fun do something else instead'. You are always full of sound advice. For keeping my feet on the ground, thank you Oma and Opa, you are just brilliant:

Opa: 'So Kate, you look at birds in their nests, all day for a couple of months?'
Me: 'Yes, something like that, measure them and watch them and take blood samples'.
Opa: 'From little garden birds?'
Me: 'Yes'

Opa: 'And this is your job?'
Me: 'Well, for the next two or so years'
Opa: 'And someone pays you to look at birds?'
Me: 'Yes. Well, it's not a huge amount........'
Opa: 'Who pays you?'
Me: 'er, well ,you actually, it's government money. I guess the taxpayer'

Which leads me on to say I should probably thank NERC for the grant and the taxpayers of Britain.........

I should certainly thank the people, landowners, farmers and Folkerids café staff on Gotland for letting us study the birds on their land and in their woods, and pizzas. Gotland is a truly beautiful and peaceful island and I loved fieldwork there. Incredible plant life, geology and birds (not to mention the infamous Stone Museum), I feel privileged for the opportunity to work in such a wonderful place. Keeping track of 250 pairs of tits was exhausting, but little beats mist-netting that male in the tranquil evening calm at the forest's edge. Thank you birds for all your blood and measurements. I actually learned to handle birds from my 'assistant' in the first year, thanks Michele, for that and a million good times. Luke, you turned out to be the most reliable nest-box checker, cheers (and I'm really glad you came). Thanks to Dan, Andy, Constanze and Andy Russell for your help too, and to Robert for catching blue tits. The times spent on Gotland were especially fun because of the good company, good food, good parties and the annual pilgrimage to the clubs of Visby. Cheers guys to Måns 'gadget' Andersson, Michele, Harrie, Luke, Freddie and his ladies, Andy R and Virpi,, Jonas and Steffan from Gotenburg, Mariusz, Beata, Anna and Joanna, to Robert for all the bottles of wine I won, and especially to Blandine.

During the course of this work I have never lived on one place longer than 3 or 4 months. I have a lot of people to thank hugely for their practical help and unfailing friendships, in dealing with a nomadic lifestyle when you are constantly unsettled living out of a bag. For accommodating me in the UK thanks to Katie, Peter, Martyn for coming up trumps at a tight time, Ken and Tracey in Oxford, and to Anna and David. For your encouragement and being second mum and dad, thank you Nadia and John. Thanks also Kotska and Steve, not least for all the times you've provided me with a place to stay and good food. In Uppsala, thank you Karen, Ben, Ineke and especially to Camilla for three months rent-free zed-bedding in the lounge. I miss those cold, dark, snowy evening discussions around the kitchen table.....You are a brilliant friend, even now with your support packages of tea and biscuits across to Edinburgh for writing-up breaks! A hugest thanks also to you Mariusz, for everything, always smiling, and just for being a one-in-a-million person and a most wonderful enduring friend.

Charlie since I have been doing this you have had the hardest time yourself, but always were both an inspiration and a lesson to me, keeping so calm and paddling out the storm. You're the best friend and I am sooooo happy to see how far you've come. The future looks good eh? Thank you also on this score to Jennifer. For coming to pay the rent and supporting me in the final stages, thank you Christophe. Not to mention the tedious reference-checking! I'm sure it wasn't easy for you to figure out this thesis business and the oddities of the academic world. Thank you most for your patience, and showing me some beautiful places. I hope you get where you want, and you can count on my reciprocal help. ☺

Finally, Dan, in the last thing you wrote you said 'send me a copy of the old *Parus major* paper when it comes out'. Well I guess you'll never read that paper, but I know you'd be happy I did this (happier because you'd be out in the savannah!). I'll miss you at graduation this time, I would gladly burn it all for one last Castle with you. Take care mate, you taught me not to waste life.

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1. Sex allocation theories and applications to birds

Evolutionary biologists have long been intrigued by relative numbers of males and females in populations and relative investment in sons and daughters by individuals. Particular interest since the 1930s catalysed the development of sex allocation theories, which have been applied to explain differential allocation to male and female function in almost every biotic system imaginable, from dioecious plants to haplodiploid insects, from sequential hermaphrodites to animals exhibiting environmental sex determination.

Empirical support for these theories has been found amongst animals from a wide variety of taxa, including crustacea (Charnov 1981), arthropods (e.g. Brunson 1937, Krombein 1967, Werren 1980), amphibia (Hertwig 1912), fish (Warner 1982) and mammals (Snyder 1976, Clutton-Brock & Albon 1982). However, despite the considerable theoretical framework, and huge interest in avian biology, investigations of sex allocation biases in birds have only recently received much attention, and consistent patterns of biasing have not yet been identified. There are several reasons for this, but probably the most influential of these is that studies have been inhibited by the inability of researchers to determine offspring sex by eye, making studies of differential investment in males and females impossible. This thesis is an investigation of sex allocation and sex-specific nestling performance in wild populations of two related bird species. The problem of sexing offspring visually has been removed by recent development of molecular genetic techniques for identifying sex.

1.1 Sex Allocation Theories

It is apparent to most people that many populations of animals consist of equal numbers of males and females. Why selection should act to produce this situation is believed by most people to have been first explained verbally by Fisher (1930), although Fisher most probably derived his idea from previous publications (Darwin 1874, Düsing 1884, Gini 1908, Cobb 1914, see review by Edwards 1998). The theory was subsequently modelled mathematically (Shaw & Mohler 1953, Kolman 1960, Bodmer & Edwards 1960). Since then a number of extensions to Fisher's equal allocation theory have been proposed (e.g. Charnov 1981, Hamilton 1967, Trivers and Willard 1973). Some models of sex allocation describe forces acting at the level of the population (e.g. negative frequency dependent selection, Fisher 1930) whereas much recent attention has been paid to forces acting at the individual level or within sub-populations (e.g. local mate competition, Hamilton 1967).

Sex allocation theories are used to describe investment in males and females, which may be reflected in the ratios of males to females in nature. They explain the relative amount of investment in sons and daughters either by a population or by an individual. One way in which investment of resources in each sex can be adjusted is through production of different numbers of males and females. This results in deviations of the **sex ratio** from parity. However, such sex ratio skews are possible without reflecting unequal **allocation** to males and females. To illustrate, if the primary sex ratio does not equal 1:1 (i.e. different numbers of males and females are produced) and the amount of resources subsequently invested per individual of each sex is balanced, sex allocation is unequal. If the sex ratio does not equal 1:1, sex allocation may still be balanced when the lack of primary production of one sex is compensated by subsequent over-investment in that sex. Thus the total investment in male function equals that in female function. Alternatively, numbers of males and females produced may be equal, but parental care to each offspring sex may differ. In this case there is unequal sex allocation, even though the male:female primary sex ratio is equal.

Selection acts on sex allocation, the total investment of parents in male and female offspring. Changes in sex ratios may therefore not represent true biases of resource allocation to sons and daughters, but are frequently assumed to do so and are used in studies because of the ease with which numbers of males and females produced can be observed. In contrast, allocation of resources to males and females is extremely difficult to measure. At the level of the population, all breeding individuals must be taken into account, and the problem becomes even more complex with the need to consider overlapping generations. Sex ratio studies at the level of the individual may be no less challenging, presenting problems such as the quantification of fitness returns of sons versus daughters and in delimiting the period of parental investment over which selection acts. Furthermore, trade-offs between current and future reproductive success or sex allocation over an individual's lifetime can be very difficult to measure, especially in wild, motile organisms.

Sex allocation theories predict sex ratio skews when the reproductive value (RV) of sons and daughters differ. The RV of offspring to parents is measured as the number of grandchildren left by that offspring, relative to the number of grandchildren left by an average offspring of the population. Measuring changes in RV of offspring can suggest how parents should allocate resources to male and female offspring given the fitness returns from producing each sex . Despite the limitations involved in studying *numbers* of males and females produced rather than RV of each sex, sex ratios continue to be a popular measure of sex allocation, and observations can be made at a number of levels (Table 1). Typically, sex ratio is expressed as the proportion of offspring born or hatching that are male, so that a sex ratio <0.5 is female biased, >0.5 male biased. Similar notation will be used throughout this thesis unless explicitly stated.

The following sections give brief summaries of major sex allocation theories (see also Frank 1990, for historical summary). The application of sex allocation ideas particularly pertinent to avian studies is highlighted and the outstanding problems for sex allocation studies in this taxon discussed in chapter 2.

1.1 i) Sex allocation theories: Population Level

Frequency dependent sex allocation – equal male:female

Parents allocate resources between males and females and receive certain genetic gains from their investment (in terms of grand-offspring). As long as fitness returns per unit of investment from producing sons remain equal to those from producing daughters, natural selection favours equal investment in males and females. In most diploid organisms, individuals gain equal amounts of genetic material from their mother and father, i.e. the genetic contribution from males equals the genetic contribution from females. Reproductive profits from producing sons are therefore no greater than reproductive profits from producing daughters, so the evolutionary stable allocation strategy is equal investment in sons and daughters (Fisher 1930).

Sex ratio	Definition	Application to avian studies
Primary	Male:female ratio immediately following fertilisation	In birds oocytes develop hierarchically in the ovary, and one per day is released and fertilised as it passes into the oviduct (see Section 2.1)
Secondary	Male: female ratio at birth	In birds this is the egg sex ratio. In many studies where egg sex ratio is impossible to measure (because of eggs failing to hatch or infertile eggs) hatching sex ratio is recorded. If nestlings hatch but die and are removed by parents before sexing is possible, brood sex ratio is observed.
Tertiary	Male:female ratio at maturity	This is measured in the field as the number of males and females recruiting to the breeding population, also termed 'recruitment sex ratio'
Fledging	Ratio of male:female offspring leaving the nest	In birds, fledging sex ratio is commonly measured. This is neither a secondary sex ratio (being well after 'birth') nor a tertiary sex ratio (being well before individuals are sexually mature). However, its widespread use reflects the ease with which this parameter can be measured in birds
Operational	Reproductively active male:female ratio	
Population	Total proportion of males to females in population	

Table 1: Classification of sex ratios commonly observed and recorded in empirical studies.

The population sex allocation is thus maintained at equality because there is no advantage in producing one sex over another, and natural selection drives the sex ratio towards parity. To illustrate, suppose there were an excess of females in a population. Natural selection would favour a mutant female who produced more sons, as she would gain more profit for investment in sons. Because of her sons' higher genetic contribution to the population, the mutation would spread. This would result in more males being produced to the point at which genetic returns from males no longer exceeded those from females. Thus when reproductive profits from each sex are equal (as expected when offspring result from one male mating with one female), the population is always pulled towards equal allocation to the two sexes.

Frequency dependent sex allocation – unequal male:female

Extensions of Fisher's argument, although also based on frequency dependence, demonstrate that equal allocation to males and females may not always be predicted. Although not explicitly stated, Fisher's theory relies on the fact that marginal returns from male investment equal marginal returns from female investment. Where returns on investment are not equal, however, equal allocation to males and females is not expected. This is frequently likely to be the case in higher vertebrates, where investment in, or returns from, one sex do not equal investment in or returns from the other. For example, in red deer, increasing investment in sons gives greater rates of return than increasing investment in daughters for females in good condition (Clutton-Brock *et al.* 1982). Unequal sex ratio has been identified as an ESS by Frank and Swingland (1988), who found support for the argument of overproduction of the cheaper sex. However, the predicted sex allocation ratio may be biased towards either sex.

This argument was formalised by Charnov (1979a, 1979b), who demonstrated that an unequal ESS allocation was possible when doubling investment in one sex did not necessarily lead to doubling the genetic returns to the parents. Accordingly, it is referred to as Charnov's non-linear model. It is easy to envisage how non-linear returns may particularly apply to polygynous species, where life-histories of males and females differ considerably. For example, polygynous mating systems often go hand in hand with sexual size dimorphism, where the larger sex may give overall lower fitness returns because of higher expenditure required for its production. In order to predict the stable equilibrium allocation, knowledge of the functions describing male and female fitness returns is necessary. This often poses problems for empiricists, who must determine the currency of fitness, how to measure this currency effectively, and define the total period over which parental care should be measured.

1.1 ii) Population size and variance in sex allocation

Kolman (1960) provided a mathematical model of Fisher's equal allocation argument. He extended his paper to address the question of variance in sex allocation among pairs within the population. At Fisherian equilibrium, the total population's allocation to sons is equal to its allocation to daughters. In a large population, an individual pair's production of males and females has little effect on the population allocation ratio. The fitness of this pair then depends on its total allocation to male and female offspring, rather than on how this total is divided between the sexes. Pairs may thus vary their individual investment in male and female offspring to any degree (Kolman 1960). For example, it may be that all pairs allocate equally between sons and daughters, or that half the pairs allocate resources entirely to sons, and half entirely to daughters.

Kolman's argument has caused controversy since it suggests that natural selection acts at the population rather than individual or genetic level. (The only evidence for group selection theories of sex allocation, with supporting empirical data, has come from studies of social spiders exhibiting extremely female biased sex ratios (Avilés 1986). These spiders live in common webs, which females share the work of maintaining; egg sacs and young are also cared for by females, mother or not. Avilés claimed the highly skewed sex ratios observed were due to natural selection acting on groups (ie. at colony level) favouring the overproduction of daughters that would disperse to form daughter colonies. Because of the high degree of inbreeding, Avilés suggested genetic variance within colonies to be much lower than between colonies and identified this as 'group selection').

Although controversial, Kolman's model highlights the importance of population size for sex allocation. In small populations, variance in sex allocation will decrease (Verner 1965, Fiala 1981). Here selection will act to drive each pair to produce a sex ratio nearer equality, rather than a sex ratio which would bring the population sex ratio towards 1:1 (for mathematical proof, see Verner 1965).

This argument is devised for populations where allocation to males and females is equal, i.e. it assumes linear returns from investment in each sex. Where there are non-linear returns, the sex ratio variance among pairs can only be predicted from a thorough understanding of individual patterns of investment and resource availability to parents (see Frank 1987). Energy available to parents, and the functions describing male and female fitness returns to parents are difficult to estimate. Because of this complexity, variance in sex ratios between pairs where there are non-linear returns from investment in each sex has not been modelled for any species.

1.1 iii) Sex biases at the individual level

Under certain circumstances, selection may favour individual investment in one sex over the other. If one sex gives higher reproductive returns from increased parental investment than the other, then parents with relatively more resources are expected to favour allocation towards this sex. This idea was proposed by Trivers and Willard (1973) with specific reference to maternal condition. They argued that females in good condition should produce more sons if returns from increased investment in sons exceeded those from daughters, and evidence in support of this was subsequently provided in red deer *Cervus elaphus* (Clutton-Brock *et al.* 1984). Although the paper focused on increasing production of sons with better maternal condition, the idea of sex ratio biasing in favour of that sex which gives higher fitness returns can be extended to suit other biological situations. For instance, amongst primates it has been shown that returns from daughters exceed those of sons when mothers occupy high positions in group social hierarchy, therefore high-ranking females in good condition, in this instance, benefit from increased production of daughters (Gomendio *et al.* 1990).

Trivers and Willard's hypothesis was clearly based on consideration of sexually dimorphic mammals, and makes three assumptions. The condition of young at the end of the period of parental investment should correlate with that of the mother (i.e. healthy mothers produce healthy young). The condition of the young should correlate with their condition as adults. Lastly, and most importantly, the condition of the individual as an adult affects reproductive potential differently in the two sexes. Adjustments to sex ratios by mothers according to their phenotype or environmental quality can thus be considered adaptive if it can be shown that these females enjoy increased fitness returns from such biasing. However, a wealth of breeding parameter estimates might influence the sex specific RV of offspring, and differential fitness returns in relation to these variables should be investigated before it is concluded that a pattern represents an adaptation. It is possible that sex ratio biases resulting from simple physiological responses could be interpreted as adaptive skews (e.g. Clutton-Brock *et al.* 1985).

The Trivers and Willard effect is one of the most investigated ideas in sex allocation theory in terms of empirical studies. However, questions have arisen about whether natural selection would act in this way. The original theory proposed that adaptive sex ratio variation occurs through greater mortality of sons born to mothers in poor condition (thus selection favours production of daughters by mothers in poor condition). Such mortality is likely to be costly, and it was suggested that natural selection was unlikely to favour evolution of this costly mechanism of sex ratio adjustment (Myers 1978). Alternatively, it was proposed that mothers adjust primary sex ratio. Myers also argued that mothers would not invest preferentially in the sex that had the highest reproductive success, but adjust sex ratio in order to maximise the number of offspring produced. Females producing more of the 'cheaper' sex were likely to preserve their own body condition for future reproduction, therefore enhancing their own reproductive success. Williams (1979) questioned outright the ability of animals with chromosomal sex determining mechanisms to adaptively manipulate the sex of their offspring given the genetic constraints of meiosis.

Leimar (1996) showed that the sex ratio may not actually be adjusted by high quality mothers in favour of sons, even when sons would have higher reproductive success than daughters. Instead, through state-dependent life-history models, he showed that females should actually prefer to produce offspring of the sex with higher reproductive *value*, and that this may be daughters. This may occur when, for a high quality mother, the fitness returns from grand-offspring through daughters exceeds the fitness returns from grand-offspring through sons. This is particularly likely to be the case when there is strong maternal transmission of quality. Thus, even though a high quality son may achieve high reproductive success, his offspring might be less fit than those of his sister. Leimar proves this mathematically, but empirical evidence has not yet been presented for vertebrate species, presumably because of the difficulties in gathering data of fitness measures and condition estimates, especially in animals with long generations. Nevertheless, Leimar's model is consistent with data from primates (e.g. Gomendio *et al.* 1990) where there is strong maternal transmission of social status, and high ranking mothers produce more daughters.

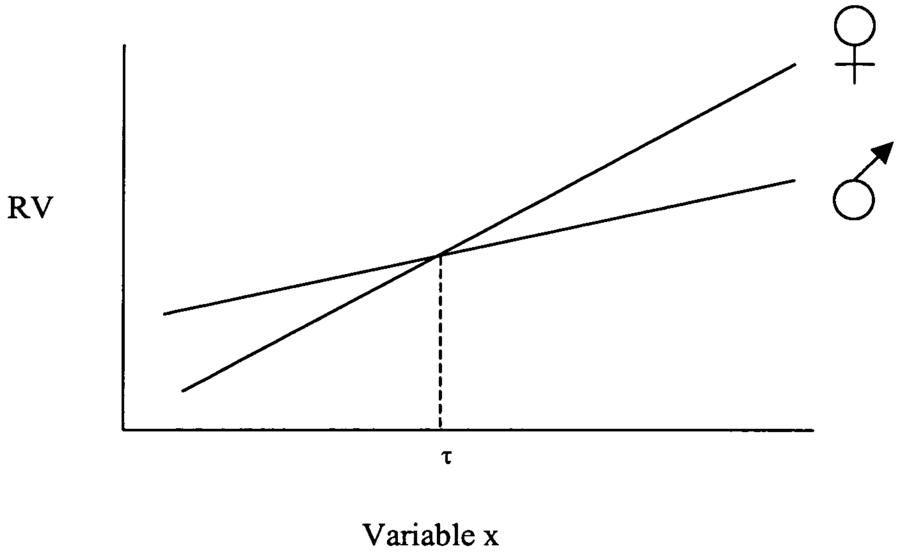
Despite the questioning of Trivers and Willard's theory, it has proved a popular idea for empirical tests. In the original 1973 paper, the theory was illustrated by evidence of sex ratio biases in caribou. Subsequently, many empirical tests of the theory focused on ungulate species. Results from the last 25 years have proved equivocal. A recent review of ungulate literature suggests that evidence does not support the hypothesis that high-ranking mothers bear more sons. However in the most dimorphic and polygynous ungulates, high quality mothers may indeed favour investment in males post-conception (Hewison & Gaillard 1999). Biased investment after birth may be more widespread than primary sex ratio manipulations if mechanisms exist to allow post-natal adjustment (e.g. increased suckling by male offspring) and there are constraints on primary sex ratio manipulation (e.g. random chromosome segregation, Williams 1979).

The Trivers and Willard idea can be adapted to predict sex ratio biases in relation to a number of factors which could potentially effect female condition such as age, stress, habitat quality, brood size, dominance rank and parity. There may also be selection for sex ratio skews when unequal returns from investment in males and females occur if females are mated to particularly high-quality males. Here a female may benefit by skewing offspring sex towards sons if they have higher reproductive success when fathered by a high quality male than daughters will (e.g. Ellegren *et al.* 1996, Sheldon *et al.* 1999, Pen & Weissing 2000).

The underlying idea of Trivers and Willard was that of varying parental investment in sons and daughters because of differences in relative gains from production of each sex offpsring. This idea was generalised to other systems, the most notable examples and models stemming from studies on parasitic wasps *Lariophagus distinguendus* and *Heterospilus propspoidis* (Charnov & Bull 1977, Charnov 1979a, Charnov *et al.* 1981b, Bull 1981a, Bull 1981b). Some female parasitoid wasps lay single eggs on insect hosts of varying sizes. Eggs hatching on large hosts develop into big adults because of the large amount of food available to growing larvae, and those on small hosts develop into small adults. The fitness returns from wasps of various sizes differ for each sex – reproductive values of large females are much greater than that of small females, compared to little difference in reproductive values of large and small males. Thus the slopes of the fitness function relating RV to host body size are likely to differ for the two sexes (see Fig 1). Accordingly, female wasps adjust their sex ratios, laying male eggs on small hosts and females on large. The result is male biased sex ratios from small hosts and female biased ratios from large hosts (Charnov *et al.* 1981b). More than 50 species now support the host size advantage model (e.g. Godfray & Hunter 1994). Predictions of both Charnov and Trivers and Willard assume some maternal control of the sex ratios they produce.

Charnov applied the same basic idea to investment in male and female function by simultaneous hermaphrodites, following work by Ghiselin (1969) and Warner (on hermaphroditic fishes, 1975). In the hermaphroditic pandalid shrimp family, female egg production increases with body size. After reaching a certain size, some individuals mature immediately into females (early maturing females, EMF), but others mature as males which one year later sex reverse to females. Charnov plotted changes in RV at each age for each sex and found that data collected on age of sex change closely matched that predicted by theory. Given the returns from investment in male and female function, he could also predict the proportion of EMF and male shrimp (the ESS is when reproductive gains from each life history pathway are equal; Charnov 1979b; Figure 1.1).

Figure 1.1: Representation of variation in reproductive value (RV) of male and female offspring across a gradient x. In this case, selection should act for overproduction of males when x is low, and females when x is high. At some threshold point, τ, RV of sons and daughters are equal and no bias in sex allocation is expected. In the case of parasitoid wasps, x represents host body size, and in pandalid shrimps represents age (see text). The shape of the fitness functions for each sex need not be linear.



Local mate or resource competition

Models of sex allocation often assume patterns of selection in an outbreeding population, where every individual's offspring competes freely for mates and resources within a population. However, this is not always the case, and Hamilton (1967) proposed that Local Mate Competition (LMC) could select for biased sex ratios, illustrating the idea with the case of extremely female-biased sex ratios in fig wasps. These wasps mate within a constricted area, the fig, and sons of a single female (foundress) compete with each other for matings within the fig. As one son can mate with many females, the number of grand-children produced is limited by the number of females, and genetic returns per unit of investment in males declines with the number of males produced. Where more than one foundress lays in a fruit, selection acts to favour mothers who increase the proportion of males they lay. Thus with increasing foundress number, the proportion of males increases, and competition between brothers in the fig for mates decreases (Herre 1985). Furthermore, in species where there is more variation in the number of foundresses per fruit, greater variation in the sex ratios of broods was observed (Herre 1987). Females of these species exhibit this adaptive plasticity of the sex ratio because of stronger selection to bias sex ratios in those species which commonly encounter multiple- and varying-foundress situations.

Extreme biases in sex ratios have probably evolved in fig wasps because of their life-histories; it seems unlikely that such extreme biases might be found in birds and mammals. Theory is well ahead of empirical evidence and sex ratio predictions require an understanding of relatedness between individuals competing for mates. Sibmating may occur in fig-wasps, but is very infrequent in natural populations of vertebrates. One could consider an analogous situation for birds being where females disperse before mating and males compete with their brothers for mates (modelled by Taylor 1981, Bulmer & Taylor 1980). However, even in this situation there may be some degree of relatedness between breeding individuals which makes predictions about sex ratios difficult.

Offspring may not only compete for mates, both between themselves and their parents, but also for access to resources or territories (LRC – local resource competition). If one sex tends to disperse whereas the other remains near their birthplace, parents may bias sex ratios towards the dispersing sex when local resources are limited (e.g. bush-babies, Clark 1978). One problem in applying both LMC theory and LRC theory to birds and mammals, is that both assume unequal RV of sons and daughters are caused by competition for mates or resources. As previously discussed, this assumption is unlikely to hold true in these taxa.

LRC has been discussed with particular reference to birds by Gowaty (1993). She suggested that LRC could be one source of selection acting on sex allocation in birds, resulting in overproduction of the dispersing sex where philopatry differs with sex. In this way, competition for resources (nest-sites, food, shelter) between parents and offspring would be reduced. As most passerines have predominantly female-biased dispersal, and anseriforms mainly male-biased dispersal, Gowaty predicted female biased sex ratios in passerines and male biases in anseriforms. She presented evidence of significantly lower sex ratios than the equilibrium 50% sex ratio in passerines, and higher sex ratios amongst anseriforms (although the difference was not significantly statistically in the latter case). However, Weatherhead and Montgomerie (1995) pointed out problems with these analyses due to, among other things, lack of control for phylogeny. Because species with the same sex ratio biases are more closely related to each other than to those with other sex ratio biases, it may be that the relationship between sex ratios and sex-specific philopatry is due simply to common ancestry. More importantly, they argue against LRC in birds for biological reasons: the degree of natal philopatry is so low amongst most birds that it is unlikely that kin would be competing for resources. With so few recruits there is little selection for parents to modify sex ratios (Weatherhead & Montgomerie 1995). As a general phenomenon, it is unlikely that LRC will drive sex allocation in birds, although there may be cases where it should be considered, such as in restricted populations with limited dispersal opportunities and scarce resources.

Local resource enhancement

A situation which could be considered the converse of LRC may apply where help from offspring can increase genetic gains of a parent. If offspring offer help to parents with further reproductive attempts, and the sexes do so differentially, this essentially modifies the fitness returns from producing each sex of offspring. Thus, parents may benefit by biasing their sex ratio in favour of the helping sex. Helper theories have been outlined with specific reference to avian systems (Emlen *et al.* 1986, Lessells & Avery 1987), and interesting patterns of sex ratios biases have been observed in three species with helpers at the nest.

Gowaty and Lennartz (1985) noticed a male biased sex ratio (59%) in red-cockaded woodpeckers, and proposed that overproduction of sons was beneficial, as males (the helping sex) increase fitness returns to parents by contributing to their parent's investment in future broods. Older females and those already with helpers produced equal numbers of males and females, whereas young females (which had not yet produced any helping sons) produced sex ratio biases of 69%. It was argued that no male biased sex ratio was observed amongst older females because they had already produced male offspring that would help to raise the current brood. Further production of helping sons would be not only unnecessary, but even detrimental to genetic returns of parents if 'new' sons would then compete for resources with their older, philopatric brothers.

In green woodhoopoes the opposite was true: female-biases in the sex ratio were recorded amongst female breeders that had only few helpers. Females with many helpers exhibited no bias. In this species female helpers contribute significantly more care to subsequent broods than their brothers. The female biased sex ratio may also reflect an overproduction of the 'cheaper' sex (Ligon & Ligon 1990).

Another case where parents bias sex allocation towards daughters provides the most extreme case of sex ratio biases amongst birds so far recorded (Komdeur 1996, Komdeur *et. al.* 1997, Komdeur 1998). A bias producing a sex ratio of 13% males at the egg stage was reported for the Seychelles warbler, where again daughters are the helping sex. Seychelles warbler females on high quality territories which had no helpers produced the extreme skew, as daughters can contribute to parents' future reproductive success through helping; pairs on low quality territories or those already with 2 helpers produced predominantly sons, which would disperse and not compete for scarce resources. In a unique experiment Komdeur was able to demonstrate the ability of females to switch from producing predominantly male eggs to female eggs when territory quality improved by moving pairs to a new, previously unoccupied island. Here all territories were 'high quality'. Birds on previously high quality territories which were also moved continued to produce biases toward female eggs (Komdeur *et al.* 1997). Furthermore, Komdeur provided evidence that sex ratio biases were adaptive by measuring the fitness gains to parents from production of sons and daughters by following these offpsring's own reproductive activities (Komdeur 1998). He also tested this experimentally by cross-fostering different sexed offspring between nests of varying territory quality. On high quality territories, fostered daughters had higher reproductive success than fostered sons, whereas on low quality territories the reverse was true.

1.1 iv) Genetic causes of sex ratio biases

A basic assumption of all sex ratio theories discussed so far is that parents have control over the sex ratio. The theories do not specifically apply to primary sex ratio biases but to overall allocation of resources to males and female offspring. However, consider the application of a theory to explain a primary sex ratio bias: the supposition of parental control assumes that parents can overcome constraints to sex determination. However, the genetic mechanisms of sex ratio determination can have important consequences for sex ratios. As well as genetic sex determining mechanisms that might constrain sex allocation, genetic determination of the sex ratio may exist, also influencing offspring sex ratios.

Genetic constraints to sex ratio biases

The genetic mechanisms of sex determination must be understood in order to appreciate possible limitations for sex ratio biasing. Birds have a system of female heterogamety, where males contribute Z gametes to fertilise females' Z or W ova. It has been argued that such systems of genetic sex determination particularly restrict sex ratio variation (Williams 1979): it is unlikely that female birds make adaptive sex ratio biases because of the random process of meiosis dividing chromosomes to produce Z and W ova. However, there is very little known about the physiological regulation of sex determination of developing oocytes by female birds, an area which is ripe for further investigation (see chapters 2 and Oddie 1998, Appendix 3).

Genetic sex ratio distorters

Even less is known about possible genetic sex determining mechanisms which may operate in birds. Obviously the possession of either a Z or W chromosome in an egg determines its sex, although other genetic elements may act to bias production of either sons or daughters. Certain genetic elements have been identified in other organisms that can influence progeny sex ratios. These were first modelled explicitly by Hamilton in 1967, who pointed out that 'selfish' genes can violate Fisher's genetic assumptions of parental sex ratio control. For example, any piece of DNA that is inherited through the cytoplasm will favour sex-biasing towards females, as cytoplasmic DNA is inherited through the egg rather than the sperm (Eberhard 1980). These pieces of DNA spread through production of females, but males are essentially an evolutionary 'dead end'. These genetic elements include mitochondrial DNA, viruses and bacteria found in the cytoplasm.

Meiotic drive is another process through which sex ratio may be modified, in either direction. 'Driving chromosomes' have been found to operate in several insects and in mice (Crow & Dove 1988, Lyon 1991). For example, a Y-linked gene causes the X chromosome to break down in meiosis, resulting in over-production of Y sperm. This drives meiosis away from a random process of 50% chance of producing males and females.

Most investigations into the presence of such sex ratio distorters have concentrated on arthropod species (Hurst 1993) with an overwhelming majority of studies conducted on *Drosophila* species. Although such genetic mechanisms may indeed influence avian sex ratios, a discussion of previous investigations in other taxa and possible existence of similar systems in birds adds little to this introduction. This thesis is primarily concerned with the testing of the sex allocation theories previously described, particularly how selection acts on parents to manipulate sex ratios and whether any observed skews could be interpreted as adaptive. For an excellent review of genetic conflict and the sex ratio see Werren & Beukeboom (1998), and for recent work on the genetic basis of sex determination see Fridolfsson (1999).

1.2 Sex allocation theory applied to birds

Studies of sex allocation in any taxon are faced with the common problems of i) describing the functions relating male and female offspring fitness to parental investment, and ii) understanding the physiological mechanisms which potentially constrain sex allocation decisions. Avian sex ratio studies present no exception, and face some other particular obstacles (below).

Nevertheless, birds are particularly appropriate and interesting subjects for sex allocation studies because they have the potential to modify investment in the sexes through manipulations of both primary and secondary sex ratios. In birds (unlike mammals), females are the heterogametic sex, producing Z and W gametes whilst males produce only Z sperm. Females thus have the potential to manipulate the sex ratio of offspring they produce by releasing an oocyte with either a Z or W chromosome. There are several ways in which offspring sex manipulation inside the female could occur, which are outlined in chapter 2 after a brief discussion of the avian reproductive system. The phenomenon of female heterogamety in birds also makes studies of this taxa particularly interesting as it provides an opportunity to compare avian sex ratio predictions with those from mammals and other taxa exhibiting male heterogamety.

Further modifications of sex ratios are possible in this taxon because birds exhibit parental care, and this period of care provides an opportunity for both parents to bias investment in their sons and daughters. This is especially true for altricial species which may receive food, cleaning and defence for weeks after hatching.

1.2 i) Difficulties of sex allocation studies in birds

Life-histories

Predictions of sex allocation require quantification of resources invested in males and females and the marginal increase in fitness returns to parents from each sex. These data are challenging to collect from organisms of any taxa.

The fact that many birds are long-lived relative to most study periods means that estimates of investment over a life-time are not easy to record. Insects have provided fine examples of sex ratio biasing in line with predictions from theory (see previous sections). In the case of parasitoid wasps, in laboratory studies investigators are able to quantify the entire life-time reproductive effort of a female (wasps are relatively short-lived and die soon after ovipositing). Furthermore, female wasps can judge resources available to developing offspring, because they can see each host when laying eggs. Many birds produce young in environments with varying food availability, and have long incubation and rearing periods. Consequently, an accurate prediction of resource availability by the parent during the future period of care, and consequent manipulation of offspring sex, may be more difficult (Oddie 1998, Sheldon 1998).

One exciting area for sex allocation studies in birds is to identify which selective factors are most important in influencing sex allocation. In any species, several variables may influence the sex ratio (timing of breeding, maternal and paternal characters, environmental factors etc.) and methods should be developed to distinguish which best predict sex ratio variation in a species.

Field studies

Many sex allocation studies of birds use sexual size dimorphism as a measure of differential investment by parents, presuming the larger sex to require more resources during rearing and consequently representing decreased marginal returns to parents (Clutton-Brock *et al.* 1985, Howe 1977, Cronmiller & Thompson 1981, Røskaft & Slagsvold 1985, Ligon & Ligon 1990, Weatherhead & Teather 1991). However, it may not necessarily be the case that the larger sex is more costly. Of importance is the total investment of parents in young of each sex. 'Parental investment' is often measured in terms of 'parental care', because to measure investment one must demonstrate the cost of such investment to future breeding attempts (e.g. Lessells 1991, Roff 1992, Stearns 1992). The amount of care given to each sex is often measured by food delivery rates, but repeated measures should be taken as feeding rates may not be constant over time. The same applies to nestling weights, which may vary considerably between and within days. In cavity nesting species, it can be difficult to tell which individual nestling receives food items, but this can be overcome with the use of infra-red cameras in nest-boxes, providing good data on parental feeding choices. Estimates of post-fledging care and of dispersal prove more of a challenge.

In birds species where life histories involve co-operation, predictions of sex allocation require knowledge of relatedness between individuals exhibiting helping behaviour. To measure benefits and costs of 'help', both must be defined and quantified.

Sex identification of young

Although adults of most bird species exhibit some degree of sexual dimorphism, their nestlings most often do not. It is impossible to investigate either hatching or brood sex ratio skews when young cannot be sexed. This has been a major factor hampering sex ratio studies in birds. Early studies of sex allocation often relied on sexing by some phenotypic character which had developed sufficient sexual dimorphism by the time young were ready to fledge. However, this made it

Box 1: Molecular method of sex determination

In this study, primers of Griffiths *et. al.*, 1998 were used to determine sex of offspring, which hybridise with a region on the highly conserved CHD1 gene. The method involves first extracting nuclear DNA and then amplifying DNA fragments from the Z and W chromosomes using a polymerase chain reaction (PCR). The area of the sex chromosomes which is amplified is the CHD1 gene, which contains introns of different lengths on the Z and W chromosomes.

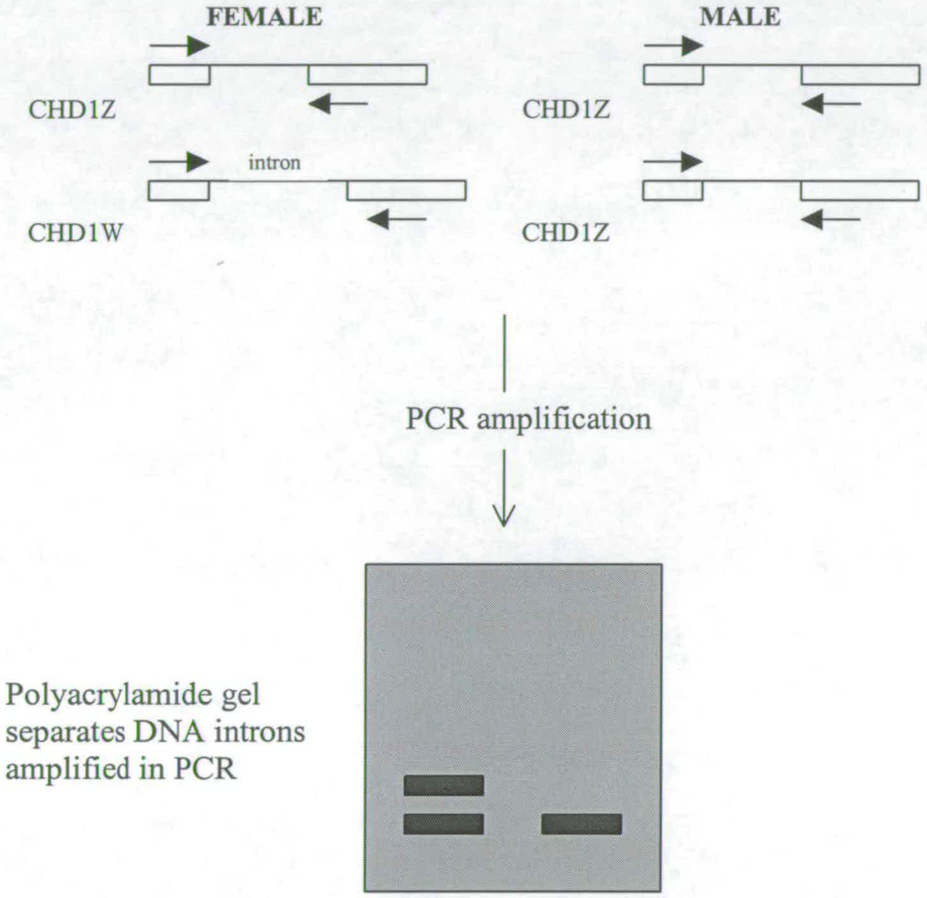


Figure 1: Sexing determination from introns of different lengths on the Z and w chromosomes of birds

Following amplification, the products of the PCR are run on a polycarylamide gel and visualised using a silver staining process (Promega, 1996). Females are recognised by the appearance of two bands on the gel, which represent a copy of the short intron found on the Z chromosome, and a longer intron found on the W chromosome. Homogametic males posess only Z chromosomes and are therefore identified by only a single band on the gel (for exact procedures and PCR conditions see Appendix 1, gel photo Appendix 2).

impossible to determine whether any sex biases were present at the egg stage or due to differential mortality in the nest with respect to sex.

In the 1990s molecular techniques were developed which allowed genetic sex of young to be determined from DNA extracted from tissue, blood or feather samples (Griffiths & Tiwari, 1993, for review see Ellegren & Sheldon, 1997). Procedures were further developed to produce a molecular genetic method of sexing all birds except ratites (Griffiths *et. al.* 1998, see box 1). The method involves amplification of introns in the CHD gene; because the exons are quite conserved, the PCR primers work across species.

The process involves PCR amplification of DNA, and therefore sex can be determined from extremely small (e.g. 1-2µl) blood samples (For protocol used in laboratory sexing in this study see Appendix 1, and example of processed samples Appendix 2). The universal applicability of this sexing method, and its reliance on such small quantities of DNA has removed an essential problem in avian sex ratio studies, and avian sex ratio literature is currently expanding accordingly.

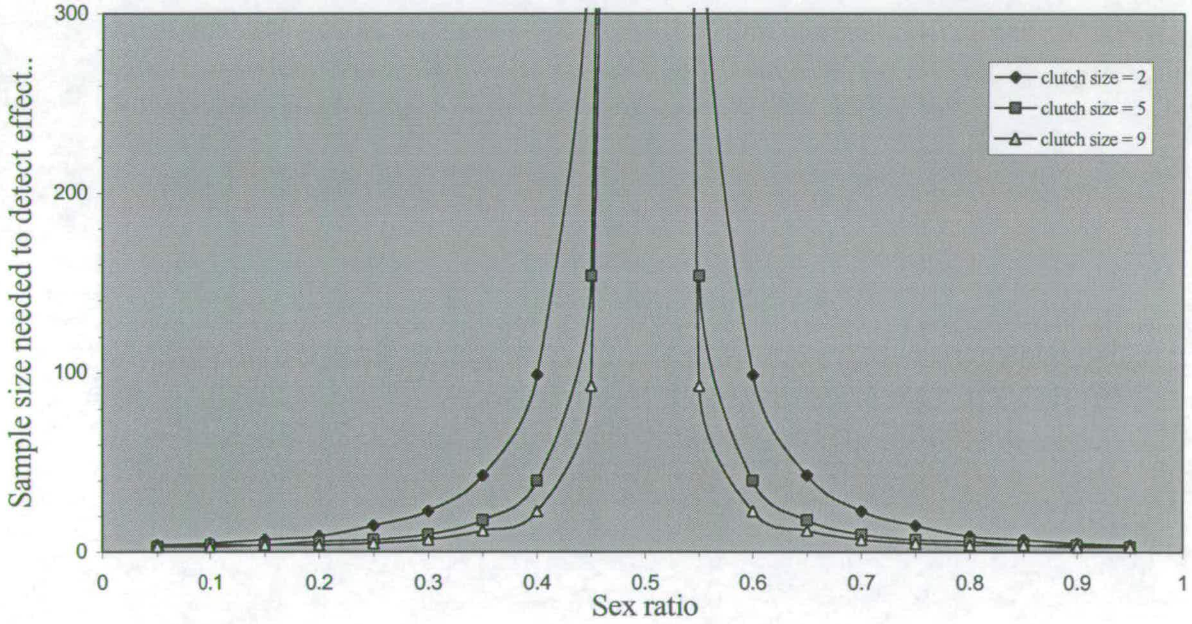
Magnitudes of sex ratio biases

Sex ratio biases are likely to be very small, and therefore only detected with large sample sizes, not always possible in avian studies. Figure 1.2 plots the necessary sample sizes required to detect sex ratio deviations from 50:50 of different magnitudes. It is evident that very small biases (less than 55:45 ratio) require large data sets to detect skews. For example, a bias of 45:55 requires n=387 2-egg clutches to detect, 154 5-egg clutches to detect or 93 9-egg clutches (e.g. great tit clutches) to detect. Sample sizes necessary to detect only slightly larger sex ratio skews are indeed possible to obtain in field studies (Fig. 1.2).

Figure 1.2: Sample sizes necessary for detection of sex ratio skews of different magnitudes. The functions show sample sizes needed to detect significant differences in clutch sex ratios from 50:50 using a one sample t-test. Significance levels are fixed at $\alpha=0.05$ and power (the probability that a test will yield a significant result) at 0.80. Curves were drawn from calculations of sample sizes necessary when the difference between the mean sex ratio of clutches and a sex ratio of 50:50 was 0.05, 0.10, 0.15...........0.45, 0.50. The expected standard deviation for sex ratios of sampled clutches was calculated from the following formula:

$$\sigma=\sqrt{pq/n}$$

(Sokal & Rohlf 1995, p78). This is the standard deviation of samples with an expected binomial frequency distribution. p and q represent the mean incidence of males and females in a brood in proportional terms (e.g. for a mean clutch sex ratio of 0.60, p=0.6 and q=0.4). n is the clutch size, and thus standard deviations of clutch sex ratios vary with clutch sizes. The curves below represent sample sizes necessary to detect sex ratio biases from 50:50 in species with clutch size of two, five and nine.



2. Avian reproduction and the potential for sex ratio adjustment in tits

The potential for egg sex biasing by female birds has led to many researchers searching for evidence of adaptive sex ratio variation in birds. In order to address the question of adaptive significance of sex ratios, an understanding of the physiological or genetic mechanisms which could potentially cause such sex ratio variation is useful (Krackow 1995, Oddie 1998, Appendix 3, Pen 2000). The nature of any sex ratio adjustment mechanism will affect the cost of manipulations (Maynard-Smith 1980, Frank 1990, Pen *et al.* 1999), suggesting whether observed sex ratio skews represent adaptive modifications or simply reflect physiological constraints. Recent data has suggested that females may indeed exert control over egg sex (Heinsohn *et al.* 1998, Kilner 1998) and also over the chemical constituents of eggs (Schwabl 1993, 1996, Schwabl *et al.* 1997, Gil *et al.* 1999). One way in which offspring sex ratio may be manipulated by females is through control of egg sex with laying sequence, explored in chapter 3 of this thesis.

2.1 The avian reproductive system and opportunities for primary sex ratio adjustment

Female birds have only a single functional oviduct and ovary. In the ovary of a newly hatched chick there are millions of oocytes, most of which die during growth. Of the surviving fraction, a few develop each breeding attempt when layers of yolk are deposited sequentially around them. At maturity the ovary contains a hierarchy of follicles, the largest being next to ovulate. Once released from the ovary, the follicle passes to the end of the oviduct and is fertilised by sperm waiting in the folds of this structure. The fertilised egg then spends a day passing through the oviduct, where first albumen and later shell is secreted around it prior to laying (Romanoff & Romanoff 1949, figure 2.1). Currently available data indicate that sex determination occurs during meiosis in the follicle about 3-4 hours before ovulation (Olsen & Fraps 1950, Sturkie 1986). There is evidence that yolk has already been deposited at this time (Lehrman 1961, Gilbert 1970, 1971).

Figure 2.1: Avian reproductive system
Ventral view of dissection of domestic fowl, from King & McLelland (1984)

In principle there are two ways in which female birds could alter offspring primary sex ratio. They might either (i) determine the sex of the follicle they ovulate through pre-ovulation control of chromosome segregation or (ii) detect the sex of the of the ovulated follicle and reabsorb follicles of the 'wrong' sex. This latter explanation would result in delays in the production of eggs and might delay hatching dates. Emlen (1997) suggested that in species which lay more than one egg, females may be able to bias the sex of the first egg and leave the sex of subsequent eggs to chance, thereby avoiding any cost of hatching asynchrony caused by laying gaps. Alternatively,. females might differentially provision oocytes of different sexes to determine the order in which they are released from the ovary, if the sex determining meiotic phase occurs quite some time (i.e. days) before ovulation. The evidence that this meiotic division occurs three to four hours before ovulation comes from studies of domestic fowl only (Romanoff & Romanoff 1949).

At present both genetic and physiological methods of determining offspring sex in birds are poorly understood (Romanoff & Romanoff 1949, Ellegren 2000). It seems likely to be costly to produce and ovulate an oocyte which may then be reabsorbed, and intuitive that selection favours sex modification at as early a stage as possible in the female tract in order to minimise wasted production of oocytes. The costs and benefits of such processes depend on the energetics of egg production and reabsorption mechanims. Recently, Pen *et al.* (1999) demonstrated that the costs of female control of the sex ratio (which they measured as a time delay in laying) need only be negligible to outweigh the adaptive benefits of modifying the sex ratio. Further studies of sex determining mechanisms in birds should concentrate on a species where offspring sex can be predicted reasonably reliably, and investigate ovary and oviduct physiology at the time of laying. This would unfortunately involve sacrificing individuals.

2.2 Evidence of sex ratio variation in birds

Parents can potentially manipulate investment in the sexes either by altering the primary sex ratio or by adjusting subsequent parental care to male and female nestlings, or both. Most studies investigate sex ratio variation between families. This is largely due to the majority of investigations seeking to provide evidence of individual adaptive sex ratio biases. Trivers & Willard/ Charnov & Bull type arguments provide the theoretical basis for studies, often mistakenly mixed with population level ideas of equal sex allocation. Typically species with males much larger than females are chosen as study subjects, or those exhibiting reverse sexual dimorphism (e.g. raptors), because the costs of rearing one sex are assumed to exceed costs of rearing the other.

Table 2.1 documents a number of studies that have reported statistically significant sex ratio biases, usually correlated to some environmental variable or parental trait. The suggested explanation for each reported observation is presented. The recent interest in primary sex ratio studies follows the advent of molecular sexing techniques. However many investigations have found no significant deviation of sex ratios from equality (lesser snow goose, Harmsen & Cooke, 1983; domestic fowl, Leonard & Weatherhead 1996; northern shovelers, common pochard and tufted duck, Blums & Mednis 1996; great reed warblers Westerdahl *et al.* 1997; corn bunting, Hartley *et. al.*, 1999; barn swallow, Saino *et al.* 1999; yellowhammer, Pagliani *et al.* 1999; great tit, Radford & Blakey 2000a, see also chapter 6), and no doubt there are many more such studies unpublished. So far evidence of sex ratio manipulation has proved inconsistent and no general patterns of sex ratio adjustment in birds have been identified.

2.3 Measuring sex allocation and sex specific performance in two passerine species: thesis aims

The aim of this thesis is to make a comprehensive investigation into factors affecting primary and secondary sex ratios in two small passerine species, the blue tit (*Parus caeruleus*) and great tit (*Parus major*). Work was carried out on populations of both species nesting on the Swedish island of Gotland (57°10'N, 18°20'E) in the Baltic Sea. Great tits breeding in nestboxes erected in woodlands on the southern part of the island have been studied for 15 years. These populations were chosen for the study for a number of reasons. Firstly, sex ratio variation in other populations of great tits (Lessells, *et al.* 1996) and blue tits (Svensson & Nilsson 1996) had already been demonstrated. Secondly, long-term data sets existed for great tits, and a great deal of life-history trade-offs had already been thoroughly investigated in these species (e.g. Hinde 1952, Perrins 1963, Dhondt 1971, Kluyver 1971, Amundsen 1993, Verhulst 1995, Svensson 1997, Verboven 1998, Kunz 1999). From a practical point of view, hole-nesting species represent an ideal opportunity to collect breeding data because of the ease with which nests can be located and data gathered, allowing large sample sizes necessary to detect what might be weak sex ratio skews.

I begin by examining the possibility of female control of offspring sex pre-laying (chapters 3 and 4). In **chapter 3** I investigate a mechanism which may facilitate sex ratio adjustment in birds: changes in sex ratio of eggs with respect to their position in the laying sequence. I ask whether females have control over the distribution of males and females within their clutch, and whether any such control can be described as adaptive. A comparative analysis of reported sex ratio biases with egg sequence is used to determine whether any patterns of sex ratio manipulation with egg sequence existed across a number of species. I also examine sex biases with egg laying sequence from a small sample of deserted great tit clutches on Gotland.

Chapter 4 describes the results of an experiment that again investigates pre-hatching sex ratio biases in great tits, looking at the effect of female condition on egg sex ratio. I experimentally manipulated nestbox temperatures of females before and during laying in order to manipulate female body condition, and examined subsequent effects on reproductive success, including clutch sex ratios. In a previous experiment on great tits, Nager & van Noordwijk (1992) found that by manipulating nest-box temperatures by just 2.9°C, female great tits nesting in cold boxes decreased egg volumes by 14%. In this sexually dimorphic species, it may be that males hatch from larger eggs than females (e.g. Mead *et al.* 1987, Anderson *et al.* 1997, Cordero *et al.* 2000), and thus we may expect to find females in better condition producing larger eggs and male-biased sex ratios (or vice versa for poor condition females). Chapter 4 reports on relationships between egg volumes, weight and sex with respect to this heating and cooling treatment.

Following hatching, both parents may potentially direct care to male and female offspring to bias nestling (brood and/or fledging) sex ratios (Lessells 1998). Chapter 5 investigates post-hatching sex ratio biases. Great tits exhibit sexual size dimorphism, with this dimorphism apparent very early in the nestling stage (Oddie 2000). Assuming that males are therefore more costly produce, for there to be selection to bias the sex ratio, one would expect decreasing fitness returns for investment in males, and to observe an overproduction of female offspring from poor nesting environments. Studies of other dimorphic species predict similar sex biases through post-natal mortality of the larger sex (Howe 1977, Cronmiller & Thompson 1981, Røskaft & Slagsvold 1985, Weatherhead & Teather 1991) although this has not always been demonstrated (Richter 1983, Weatherhead 1983, Newton 1979, Clutton-Brock & Iason 1986). Evidence from three different populations of great tits does not support the expectation that more females will be produced from poor environments. Instead, more males are produced from these environments (Dhondt 1970, Drent 1984, Smith *et al.* 1989, Lessells *et al* 1996). One possible explanation for this is that these studies have overlooked mechanistic processes in the nest which could also lead to biased sex ratios, especially if focused on adaptive explanations for sex ratio variation. In **chapter 5** I investigate the importance of proximate mechanisms of sex ratio adjustment, i.e. scramble competition, amongst nestlings in determining numbers of males and females produced.

Over the three year study period, great tit nests were monitored for routine breeding data and as many blood samples as time would allow were taken to obtain sex ratio data for unmanipulated broods, as well as those included in experiments. Brood sex ratios from the whole data set are analysed to determine factors influencing primary sex ratios of great tits. **Chapter 6** presents correlations between environmental and parental attributes and sex ratios, and discusses whether these results are in line with expectations. Individual variation in sex ratios across years is also reported from data collected from ringed individuals caught breeding in more than one year.

2.4 The study species: Great tits, *Parus major* and Blue tits, *Parus caeruleus*

The two species were studied whilst breeding in nestboxes on Gotland between 1997 and 1999. On the island, 1,100 nestboxes are dispersed between 13 different woodlands. Each year, these are occupied by 150 to 250 pairs of great tits, and 60-120 pairs of blue tits. Nestboxes are also occupied by collared flycatchers (*Ficedula albicollis*), and occasionally by coal tits (*Parus ater*) and wrynecks (*Jynx torquilla*). The woods are mainly composed of deciduous stands of oak (*Quercus robur*), ash

(*Fraxinus excelsior*), hazel (*Corylus avellana*) and hawthorn (*Crataegus spp.*) with some birch (*Betula spp.*) and coniferous species (*Pinus sylvestris, Picea abies*).

Parus major and P.caeruleus

Great tits and blue tits are small sexually dimorphic (~5% body mass dimorphism, with males larger than females) and dichromatic passerines, weighing about 18g and 11g respectively. Neither species is migratory, and over winter individuals can be found roosting in boxes overnight (Dhondt & Eyckerman 1979). Breeding begins in spring with territory acquisition and pairing, and eggs of first clutches are laid in mid to late April. Great tits are socially monogamous with low levels of extra-pair paternity (e.g. Blakey 1994, Verboven & Mateman 1997), and although extra-pair paternity has been recorded for some populations of blue tits (Gullberg *et al.* 1992, Kempenaers *et. al.* 1992), this study population is thought to be monogamous (J. Merilä, unpublished). However, no estimates of extra-pair paternity have been established for either species on Gotland. Great tits in this population typically produce a clutch of nine eggs, but may lay clutches of between six and 13. Modal clutch size in blue tits is 11, but varies between six and 16. Females lay one egg per day, although occasionally there are laying gaps, particularly when weather is cold and wet and females must spend time foraging to maintain their own body reserves. Once the clutch is complete, females incubate for approximately 14 days, with incubation sometimes beginning before the last egg is laid in both species. The majority of the clutches hatch synchronously, with one or two eggs hatching up to four days after the initial hatch date. Late-hatching eggs usually hatch, however, the day after the first hatching. Nestlings are fed by both parents, on a diet of mainly *Lepidoptera* larvae (Przybylo 1995). They fledge approximately 19 days after hatching , and parental care continues after fledging before young form flocks over winter. Second clutches may be laid by great tits nesting on Gotland, but not by blue tits.

2.5 General Study methods

Nestboxes were inspected daily from mid-April to establish box occupancy and laying dates. For nests of great tits and blue tits in some woodlands, eggs were numbered the day that they were laid (see chapters 3 and 4 for more details on egg-labelling methods). For each nest the date of clutch initiation, clutch size, hatch date, brood size and fledgling number were recorded, time permitting. Blood samples were taken from nestlings typically when they were one day old. Individual nestlings were identified first by clipping tufts of down from their heads or bodies, then later with aluminium bird rings. Parents were captured with mist nets or nest-box traps, ringed, sexed and measurements of tarsus, wing length and weight taken. Where data on condition of offspring at fledging were collected, blue tits were generally measured at 12 days old and great tits at 15 days old. Nestling and adult sexes were determined by PCR amplification of DNA as described in Chapter 1 (Box 1) and Appendices 1 and 2. Details of both field and statistical methodologies vary between experiments and are expanded in each chapter.

Species	**Factor affecting Sex Ratio**	**Sex ratio trend and suggested explanation**	**Reference**
Quiscalus quiscula	Seasonal variation	Early nesters produce more female eggs (because they face more severe conditions, and females are smaller therefore less costly than males)	Howe (1977)
Xanthocephalus xanthocephalus	Brood status	Primary broods are provisioned more than secondary or tertiary broods by the male. In primary nests, overrepresentation of male chicks; in tertiary nests, overrepresentation of female chicks	Patterson & Emlen (1980)
Taeniopygia guttata	Parental attractiveness	Birds with attractive leg bands produce more same-sex offspring; those with unattractive bands produce more opposite-sex offspring. Increase fitness by production of offspring with superior mate-getting opportunities	Burley (1981, 1986)
Agelaius phoenicius	Laying sequence (Egg size)	Last laid egg hatches significant more females in 4-egg clutches, no effect in 3-egg clutches. Also increase in egg size with lay sequence, so possibly sex ratio variation with egg size	Fiala (1981)
Gallus gallus domesticus	Laying sequence	Female bias from early hatching eggs, equal males and females from mid-hatching eggs, male bias from late-hatching eggs	Davies and Payne (1982)
Chen caerulescens	Laying sequence	Clutch size = 4; First 2 eggs significant male bias (64% male), 2nd two significant female bias (72% females)	Ankney (1982)
Agelaius phoenicius	Female age	Old females hatch significantly more males, medium-aged females produce even sex ratios, young females produce twice the number of females to males	Blank & Nolan (1983)
Larus delawarensis	Laying sequence	Male bias in first eggs	Ryder (1983)
Agelaius phoenicius	Seasonal variation	Early and late breeding females produce more female offspring; mid-season produce male-biased clutches. Explained because more costly (larger) males are produced when food abundance peaks	Weatherhead (1983)

Agelaius phoenicius	Hatch sequence	In 3 egg clutches sex ratio decreases with hatch sequence; In 4 egg clutches last laid eggs more likely to be female	Weatherhead (1985)
Taeniopygia guttata	Paternal quality	Polygynous males have more male-biased sex ratio than monogamous males	Burley (1986)
Haliaeetus leucocephalus	Hatch sequence	In 2 egg clutches; females most likely to hatch first	Bortolotti (1986)
Zonotrichia leucophrys	Egg size Hatch sequence	Eggs which produce males are significantly larger than those producing females Eggs 1 and 2 male sex ratio bias	Mead, Morton & Fish (1987)
Phoeniculus purpureus	Seasonal variation	Early in season, when it is likely food is more scarce, significantly more females produced. Assumed adaptive as females are 20% smaller than males, therefore require less resources to rear	Ligon & Ligon (1990)
Falco tinnunculus	Seasonal variation	Males bias early in season, as male reproductive success (RS) as a yearling is affected by males laying date, whereas females RS is not	Dijkstra, Daan & Buker (1990)
Parabuteo unicinctus	Hatch sequence	Significant male bias in first hatched eggs	Bednarz & Hayden (1991)
Circus aeruginosus	Seasonal variation	Increase in proportion of males with lay date. Probability of females breeding as yearlings declines with lay date	Zijlstra, Daan & Bruinenberg-Rinsma (1992)
Taeniopygia guttata	Hatch sequence	Decreasing sex ratio with egg hatching sequence	Clotfelter (1996)
Circus pygargus	Hatch sequence	Female bias in first eggs produced, others male-biased	Leroux &Bretagnolle (1996)
Raptors (5 species)	Seasonal variation	Biased production of males or females early in the season, changing with species, but dependent on differential RS of males and females as yearlings	Daan, Dijkstra, Weissing (1996)

Ficedula albicollis	Paternal attractiveness	Females mated to attractive males produce significantly more males in their clutch than females mated to unattractive males	Ellegren, Gustafsson & Sheldon (1996)
Parus caeruleus	Paternal quality (survival)	Females mated to males with high survival prospects bias the hatching sex ratio of broods in favour of sons	Svensson & Nilsson (1996)
Acrocephalus sechellensis	Local resource enhancement	Females are more likely to help parents with future broods than males. Pairs on high quality territories produce females to increase future RS, pairs on low quality territories produce males which disperse, so not competing for resources. When shifting parents from low to high quality territories a corresponding shift from production of males to females is observed	Komdeur *et. al.* (1997)
Falco sparverius	Egg size	Eggs which produce males are significantly larger than those producing females	Andersson, Reeve & Bird (1997)
Strix aluco	Food abundance	Female-biased clutched laid on territories with more abundant prey	Appleby *et. al.* (1997)
Taeniopygia guttata	Hatch sequence	Early laid eggs, tending to hatch first, produce significantly more daughters than sons	Kilner (1998)
Taeniopygia guttata	Diet quality	Experimental food restrictions result in females producing more male-biased sex ratios (RS of females produced when food is scarce is more adversely affected than males)	Kilner (1998)
Eclectus roratus	Unknown	Females produce succession of males followed by females, 'switch' from producing offspring of one sex to another	Heinsohn, Legge & Barry (1998)
Acrocephalus arundinaceus	Female mating status	Male biased sex ratio of primary broods (where males give more care)	Nishiumi (1998)
Larus fuscus	Maternal condition	Females forced to lay large clutches after experimental removal of eggs producing increasingly female biased sex ratios as more eggs produced	Nager *et. al.* (1999)
Sula nebouxii	Food supply	Male biased sex ratios in years of short food supply	Torres & Drummond (1999)
	Hatch date	When food short, proportions of males fledging increases with hatch date	
Parus major	Male trait	Proportion of sons increases with male tarsus length	Kölliker *et al.* (1999)

Parus caeruleus	Male traits	Positive relationship between proportion of sons in a brood and measures of paternal UV crown coloration.	Sheldon *et al.* (1999)
Haematopus ostralegus	Female age and experience	Hatchling sex ratio is positively correlated with female age and female breeding experience	Heg *et al.* (1999)
Acrocephalus arundinaceus	Female mating status	Primary females have a higher proportion of sons in brood	Westerdahl *et al.* (2000)
Falco tinnunculus	Laying date	Years of good food supply: proportion of males decreases with laying date Years of moderate/low food supply: proportion of males increases with laying date	Korpimaki *et al.* (2000)
Falco tinnunculus	Food abundance	Male biased sex ratio during a year of food scarcity	Korpimaki *et al.* (2000)
Falco tinnunculus	Parental body condition	Proportion of males negatively correlated with body condition of both male and female parents	Korpimaki *et al.* (2000)

Table 2.1: Previous studies of sex ratio variation in birds and explanations for biases observed
Studies are listed in chronological order of publication.

I. PRE-HATCHING SEX RATIO BIASING

3. Non-random allocation of egg sex and patterns of sex ratio biases with egg sequence

In birds, the potential for differential investment in male and female function by parents can arise as early in the breeding attempt as egg laying, through manipulation of the numbers of sons and daughters within a clutch. The fact that female birds are both heterogametic and responsible for egg production has led to speculation that in this taxon it is females that determine primary (conception) and secondary (egg) sex ratios. Indeed there is evidence of sex ratio skews at the egg stage from several bird species (e.g. Komdeur *et al.* 1997, Nishiumi 1998, Kölliker *et al.* 1999, Nager *et al.* 1999, Sheldon *et al.* 1999, Westerdahl *et al.* 2000). Females also have the potential to bias investment in male and female offspring at this stage through differential material provisioning of eggs (e.g. Howe 1976, Ankney 1982, Ryder 1983, Weatherhead 1985) with respect to egg sex (Mead *et al.* 1987, Anderson *et al.* 1997, Cordero *et al.* 2000). The questions of how sex ratio biases at the egg stage occur (i.e. the mechanism by which the bias is achieved), and of why females may do this (i.e. what selects for a biased sex ratio), may be illuminated though investigations of sex ratio variation with the order in which eggs were laid. The rationale behind this approach is discussed below.

a) What does sex ratio adjustment with laying sequence tell us about the mechanism of sex ratio manipulation?

The mechanisms through which any primary or egg sex ratio modifications are achieved in birds are unknown and present a great challenge to avian sex ratio studies. Haplodiploidy amongst some insects, particularly hymenoptera, is an example of a system where a clear mechanism of sex determination has been identified. Quantitative predictions about sex ratio biases have been tested in some of these organisms and have been upheld (e.g. Werren 1980, Herre 1987 see also

Charnov 1982 Chapter 5). No such evident mechanism of sex determination has been elucidated for birds.

Statistical analysis which reveals a pattern of egg sex ratio change with egg rank from simple observational data would suggest that the existence of such biases are more than a matter of chance, and that females can have some control over egg sex. The case for sex ratio control with laying order would be particularly convincing if patterns were repeatable across populations or species, or varied with respect to breeding circumstances or life-history characteristics in a predictable manner.

Investigations of egg sex variation with egg rank can also shed light on the nature of egg sex control: does the mechanism allow a one-off change from male to female production - a ‘switch’ from producing one sex to the other - or is control finer so that sex of each egg can be modified independently? Eclectus parrots (*Eclectus roratus*) lay much longer sequences of eggs of the same sex than expected by chance, producing runs of one sex before switching to the other (Heinsohn *et al.* 1998). Why female Eclectus parrots should do this is unclear. However, sex ratio biases with respect to egg sequence in lesser black-backed gulls (*Larus fuscus*) appear to be modified in an adaptive manner. Female gulls which were experimentally induced to continue laying up to 17 eggs (rather than the usual three) biased later eggs in the laying sequence towards females (Nager *et al.* 1999). This was interpreted as an adaptive female modification of egg sex ratio, because male offspring survival declined with laying sequence whereas female survival did not.

b) Are patterns of egg sex ratio with laying sequence adaptive?

Egg sex ratio variation with laying sequence can be considered adaptive if the relative fitness of either sex of offspring changes with respect to egg rank. For example, if fitness returns decline more strongly with egg rank in one sex than the other, then females would benefit from producing eggs of this sex early in the laying sequence. Sex-specific offspring fitness in respect to egg rank may be particularly prevalent in sexually size dimorphic species. In these species, greater fitness returns from the larger sex compared to the smaller sex might be expected amongst later egg ranks. This is because after hatching, offspring of the larger sex may have higher chances of survival when competing with earlier hatched nestmates in the scramble for parental care. Likewise, earlier hatching offspring of the smaller sex from earlier laid eggs would have a 'head start' in growth to increase their chances of survival when competing with larger, later-hatching offspring. There is some evidence that amongst great tits, this sex-specific size advantage in competitive interactions does exist (Oddie 2000). In this way a female could increase the reproductive value of her brood by minimising chances of mortality amongst nestlings (brood reduction).

Conversely, a mother could increase the RV of her brood by laying eggs of the larger sex first. Through enhancing the likelihood of brood reduction, she could be sure that at least some of her remaining offspring have a fair chance of survival, and fledge in good condition. Alternatively, selection may favour females laying the larger sex first because of greater variance in the reproductive success of individuals of the larger sex (e.g. Kempenaers *et al.* 1997). If early ranked eggs fledge offspring in the better condition than later eggs (Kendeigh 1963, Ricklefs 1965, Klomp 1970, Howe 1976, Zach 1982, Forbes 1990), females may benefit from laying offspring of the sex with greater variance in RS first (Trivers & Willard 1973). If the RV of a brood can be increased for either of the above reasons, we might observe sex ratio biases in favour of laying the smaller sex last.

All of the above arguments assume that hatching order of eggs reflects the order in which they were laid. Although there is some evidence that this is the case (Kendeigh 1963, Kilner 1998), it may not be true for all species, especially those where incubation begins after clutch completion.

In this chapter I use a meta-analysis of published data to determine whether common patterns of sex ratio variation exist across bird species and the direction of any such relationships. A meta-analysis transforms statistics presented in individual investigations into 'effect sizes', common measures which can be then compared between studies. Combining effect sizes can give an estimate of the direction and magnitude of a biological phenomenon across species. Comparing effect sizes between species can identify factors that describe significant variation in effects (Hedges & Olkin 1985, Rosenthal 1991, Arnqvist & Wooster 1995). All published relationships between egg rank and sex ratio are included in the present meta-analysis, regardless of whether trends reported were significant or not. In particular I test for differences in trends in relation to sexual size dimorphism. I report whether females facilitate or avoid brood reduction by biasing egg sex ratio in favour of laying the larger sex earlier or later in the laying sequence respectively. I also examine whether any relationships depend on brood sizes, for example if relationships are prevalent only amongst species with large broods where nestling competition might be more intense.

Secondly, I examine patterns of sex ratio variation with egg sequence in a small sample of great tit, *Parus major*, clutches. I discuss whether these results for great tits are in line with those from the cross species comparison. I also test whether female great tits manipulate investment in eggs with the laying sequence through varying egg volumes and masses with egg rank. If females do not assign egg sex randomly with respect to laying order, and there is sexual dimorphism in egg size (Mead *et al.* 1987, Anderson *et al.* 1997, Cordero *et al.* 2000), we might expect to observe an association between egg size and laying order. Finally, I examine directly whether males hatch from larger eggs than females in great tit eggs where both morphometrics and sex are known.

3.1 Cross species comparison of sex ratio patterns with egg sequence in dimorphic species

3.1 i) Methods

Data were collected on 25 bird species where egg sex ratio had been examined with respect to the laying or hatching sequence of eggs. Data for egg sex ratios at both laying and hatching were included in order to produce a data set with reasonable sample sizes for statistical examination. For each species, a standardised measure of effect size was calculated as Pearson's r statistic, using raw data presented in the papers. Specific notes on the derivation of each standardised effect size from the published data are given in appendices 3.1 and 3.2. The correlation coefficient r demonstrated the correlation between the proportion of males and egg rank. An exact relationship between sex ratio and laying/hatching rank is represented by a correlation of 1 or –1 depending on whether the sex ratio increases with laying or hatching sequence (1) or decreases (-1). If there is no pattern of sex ratio alteration with laying or hatching sequence, r equals zero. r is not normally distributed, hence the correlation coefficients were then converted to Z_r values by the following transformation:

$$Z_r = \frac{1}{2} \log_e (1+r/1-r)$$

(Fisher 1928). All analytic procedures were then carried out on Z_r rather than r.

Compiling the data

Some species were represented by more than one study, and some species exhibited different trends in sex ratios within study populations, e.g. according to habitat area (Dzus *et al.* 1996) or time in the season (e.g. Dijkstra *et al.* 1990). Therefore, analyses were carried out on two data sets: one with each species represented by a test statistic, Z_r (*'species specific'*, appendix 3.1), in which all reported statistics for that species were combined to give a single value, and one where each trend was presented (*'trend specific'*, appendix 3.2). Although the latter introduces problems of pseudoreplication, each trend potentially represents a biological phenomenon and this information is lost by combining Z_r values for each species to give a single statistic. Where more than one Z_r had been reported for a species, a single Z_r value was determined by summing the two or more individual Z_r scores and dividing by the number of studies (i.e. finding a mean Z_r of the reported Z_rs, Rosenthal 1991).

Data were combined in this manner to give a single species estimate for the snow goose (*Chen caerulescens*), bald eagle (*Haliaeetus leucocephalus*), great-tailed grackle (*Quiscalus mexicanus*), european shag (*Phalacrocorax aristotelis*), house sparrow (*Passer domesticus*), european kestrel (*Falco tinnunculus*) and laughing kookaburra (*Dacelo novaeguineae*). Data for red-winged blackbirds could not be combined. This was because Weatherhead (1985) reported sex at every egg position, whereas correlations from Fiala (1981) were calculated from knowing the sex of the last egg alone compared to the previous eggs. In this case, the standardised effect size for red-winged blackbirds was taken from data in Weatherhead (1985). For zebra finches (*Taeniopygia guttata*), the standardised effect size was represented by the correlation at laying rather than hatching, as the aim of this study was to investigate maternal control of egg sex ratio with respect to egg rank. However, the correlation between sex ratio and laying order (0.282) is similar to the correlation between sex ratio and hatching order (0.298).

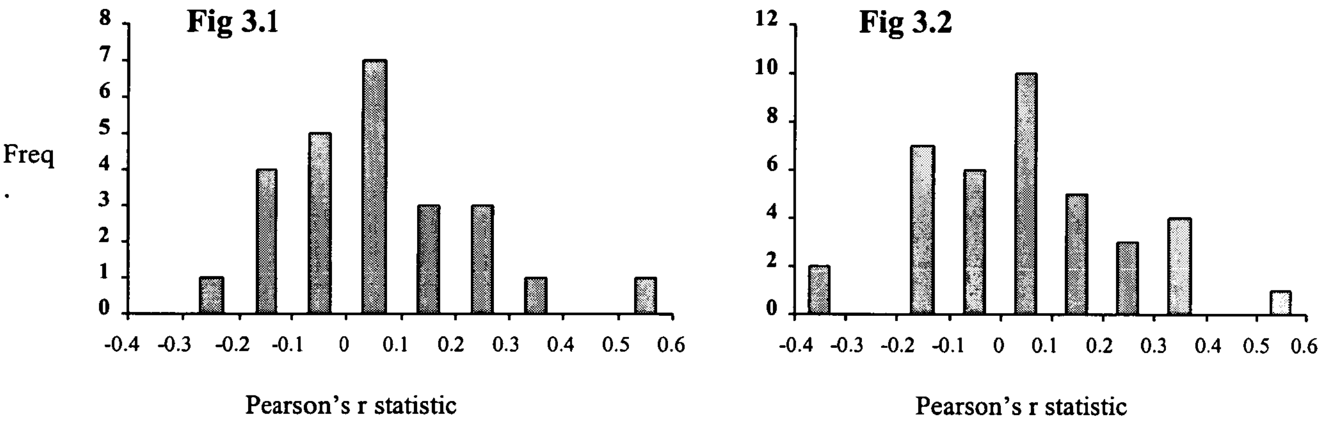
The aim of the analyses was to determine i) whether there were different patterns of egg sex allocation according to egg rank, and ii) whether these patterns differed according to whether males or females were larger. For the first question, comparisons of effect sizes between species (or trends) were made by calculating the statistical heterogeneity of effect sizes. Significance was ascertained from a χ^2 because the sum of the difference in effect sizes from the mean effect size ($\Sigma(N_j - 3)(Z_{rj}$ - mean $Z_r)$) is distributed as χ^2 with K-1 degrees of freedom, where K is the number of studies. In this equation, N is the number of sampling units (i.e.clutches or broods), and mean Z_r is the weighted mean Z_r, (Rosenthal 1991 p80).

For the second question, I gathered data on sex-specific adult body masses of each species (where possible from Dunning 1993) to obtain measures of sexual size dimorphism (SSD). Adult masses were used as a measure of SSD because data on nestling SSD are rarely published, and most species exhibit SSD to some extent as nestlings (e.g. Richner 1991). No published measure of SSD could be found for the roseate tern (*Sterna dougallii*) or western gull (*Larus occidentalis*). Species were categorised as those with larger males or females. To compare whether Z_rs differed between species with males larger and species with females larger, I examined variance in Z_r due to SSD and calculated its significance with a one way ANOVA.

It is possible that this relationship may be further influenced by brood size. For example, Zr may be negative in species with females larger, but positive in species with males larger. In this way mothers may avoid brood reduction by hatching the larger (better able to compete) sex last. However, such a relationship may only be evident amongst species where competition for food is likely to be intense, perhaps particularly in large-brooded species. This can be investigated by statistical examination of the interaction effect of brood size*SSD. I analysed the variance in Z_r with a model containing SSD (males or females larger), 'clutch size' to represent brood size, and the interaction term clutch size*SSD (e.g. Rosenthal 1991 p115). Significance of terms was tested with an F test. A more accurate estimate of nestling competition intensity may be obtained from the number of nestlings fledging rather than clutch size (number of eggs) if most mortality occurs early in the nestling period. Hence, analyses were repeated substituting 'number of fledglings' as a factor, though sample sizes were fewer for this variable. Data on clutch sizes and numbers of fledglings were collected where possible, from del Hoyo *et al.* (1992). Clutch sizes used were means unless a modal clutch size was clearly stated. Modal clutch sizes were preferred to means because outliers included in calculations of clutch size influence them less. Certain data were tranformed prior to analyses because of lack of normality. For both trends and species data sets, clutch size was $\log_{10}$ transformed before analyses; for the trends data, fledgling number was also $\log_{10}$ transformed.

3.1 ii) Results

Pearson's correlation coefficient, *r*, varied between –0.21 and 0.57 for species-specific data (Figure3.1) and between –0.37 and 0.57 for trend-specific data (Figure 3.2). For species data, there was a suggestion of heterogeneity in the relationships between egg sex ratio and rank (χ^2=35.89, d.f.=24, p<0.10). For trends data, the effect sizes were significantly heterogeneous (χ^2=56.46, d.f.=37, p<0.01).



Figures 3.1 and 3.2: Distribution of standardised effect sizes describing the relationship between offspring sex ratio and laying or hatching rank. Figure 3.1 shows the distribution for data with each species represented as a case, figure 3.2 with each reported trend in the literature as a case (see also appendices 3.1 and 3.2)

Mean Z_r values for cases with males larger than females were compared to those with females larger than males. For species-specific data, there was no difference in the correlation between offspring sex ratio and laying/hatching sequence according to the pattern of sexual dimorphism of the species ($F_{1,21}$=2.523, p=0.1271). For the trends data, there was a significant difference in effect sizes between species with larger males and those with larger females ($F_{1,34}$=4.988, p=0.0322, figure 3.3).

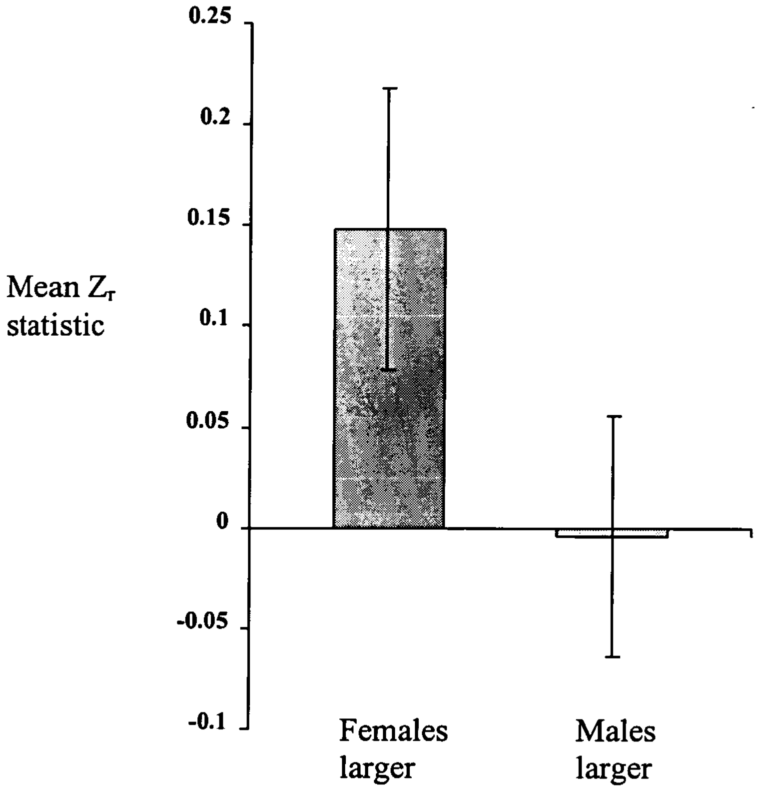


Figure 3.3 Mean standardised effect sizes for sexually dimorphic and reverse sexually dimorphic species.

The mean Z_r value for cases where females are larger than males was positive, indicating increasing sex ratios with laying/hatching sequence. This suggests that female nestlings are laid or hatch first. Conversely, mean Z_r was close to zero when males were the larger sex.

To test whether this relationship was conditional on competitive nesting conditions likely to be faced by hatchlings, the term 'clutch size' was included in a general linear model along with the term SSD, and the significance of the interaction term tested with an F test. There was no effect of clutch size for either species- or trend-specific data (Table 3.1). However, for species-specific data there was an almost significant interaction between brood size when estimated as fledgling number (p=0.057, table 3.1) which was significant when data were analysed for each trend (p=0.021, table 3.1).

The significant interaction effect 'number fledging*SSD' was investigated for the trend-specific data. Sample sizes were too small to allow examination of species specific data. Trends were categorised as having either 'many' or 'few' fledglings according to whether their fledgling number was greater or less than the mean fledging number for all species combined. Even for the trends data, this created only 14 cases fledging 'few' young and seven cases fledging 'many'.

For those cases where few young fledged, there was a similar pattern of variation in standardised effect size with SSD as in figure 3.3. The larger sex tended to be laid or hatched first, here for both species with larger males and those with larger females, i.e. positive mean standardised effect size for cases with larger females and negative mean standardised effect size for cases with larger males. This difference was not significant ($F_{1,12}$=3.389, p=0.0905, figure 3.4). The seven cases where 'many' nestlings fledged could not reasonably be analysed because comparisons would involve two mean values derived from only three and four data points each.

Factor	Species specific data				Trend specific data			
	n	d.f	*F*	*p*	n	d.f.	*F*	*p*
a) Analysis with brood size represented by 'clutch size'								
	23				36			
SSD		1	1.713	0.206		1	2.751	0.107
Clutch size		1	0.124	0.728		1	0.500	0.485
Clutch size*SSD		1	0.953	0.341		1	1.197	0.282
b) Analysis with brood size represented by 'number fledging'								
	12				21			
SSD		1	6.112	0.039*		1	8.324	0.011*
Number fledging		1	0.001	0.975		1	0.119	0.735
Number fledging*SSD		1	4.934	$0.057^{(*)}$		1	6.507	0.021*

Table 3.1: Variation in Z_r (the relationship between offspring sex ratio and laying/hatching order) with pattern of sexual size dimorphism (SSD) and a)clutch size, or b) fledgling number.

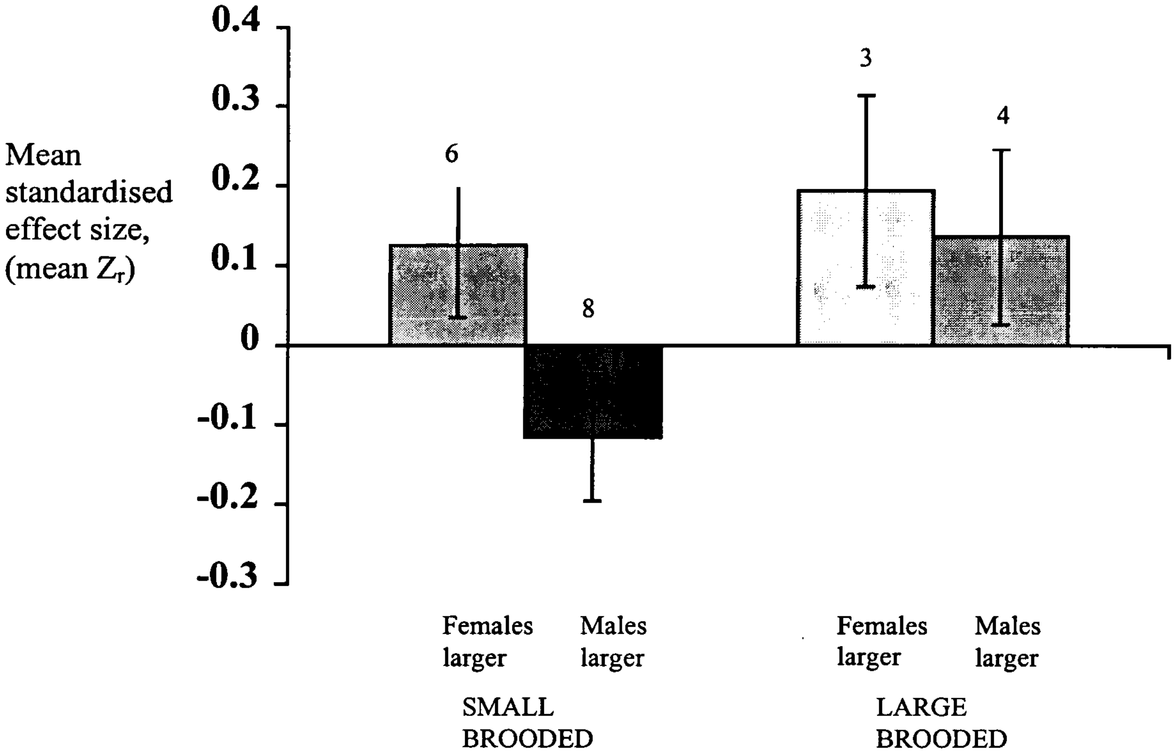


Figure 3.4: Differences in the relationship between offspring sex ratio and laying/hatching order according to size dimorphism, for small and large brooded species. Cases were categorised as small or large brooded according to whether they fledged few or many nestlings. Data were taken from the trends data set. Sample sizes are given above bars. Note the very large standard error bars.

3.2 Great tit egg sex ratio and laying sequence

3.2 i) Methods

The study was conducted on great tits breeding in nestboxes on the Swedish island of Gotland during spring 1999. From mid-April, nest boxes were checked daily for signs of occupation and laying dates and clutch sizes were recorded for each nest. Nestboxes were visited every day following laying of the first egg, to number and weight each egg the day it was laid. Egg mass was recorded on the day of laying as masses may vary over time) Ar & Rahn 1980; Carey 1986). Following the completion of a clutch, egg length and breadth were measured to the nearest 0.5mm using dial callipers, and egg volume calculated according to the formula: volume = length x breadth^2 x $\pi/6$ (Hoyt 1979). Clutch completion was recognised by the initiation of incubation, when eggs were uncovered from nest material, arranged neatly in the nest cup, and were warm to touch. If a female abandoned a clutch, the eggs were removed and developing embryos immediately dissected out and stored in 98% ethanol. Nests were designated deserted if eggs had been left cold and uncovered for seven days and if the nest material became messy and infested with fleas. Sometimes unmeasured clutches were found abandoned, and in this case, eggs were measured even though they could not be assigned rank. Parents of active nests were caught 10 to 14 days after egg-hatching and their mass, tarsus length and ages recorded. Female body condition index was calculated as residual mass from a regression of mass on tarsus length.

The sexes of embryos from eggs collected from 14 abandoned nests were determined using PCR based molecular techniques. Following DNA extraction, PCR was used to amplify sex-specific introns of the CHD1 gene, and the products run out on a polyacrylamide gel and visualised using silver staining. An egg was designated as carrying a female embryo if two bands showed on the gel, representing copies of the CHD1 intron from the Z and the W chromosomes. Male embryos carried two copies of the Z linked intron, and hence only one band showed on the gel. A further five deserted clutches collected in 1998 were added to the sample, so that a total of 154 measured eggs were assigned sex in this way. The laying sequence was not known for these 1998 clutches.

i) Variation in egg sexes with laying sequence

The proportion of male eggs laid at each egg rank was analysed using logisitc regression, fitting data to a model with binomial errors and using a logit link. The model was constructed with the number of males at each rank as the response variable and the total number of eggs sexed for each rank as the binomial denominator. An association between egg rank and sex was tested for by including egg rank as an explanatory variable in the model, and observing the change in deviance after its removal. The significance of the change in deviance was assessed using an F test with appropriate degrees of freedom. Egg measures were initially included as covariates in the model, but as they explained no significant change in deviance when removed, results report effect of egg rank on sex ratio when included in the model alone (i.e. minimal model). The analysis was scaled by the heterogeneity factor (0.23) because the data were underdispersed. Scaling by the heterogeneity factor approximates the data to the binomial distribution, making it suitable for testing in a model with binomial error structure. Data from 17 nests (appendix 3.3) were analysed. Egg ranks 11 and 12 were excluded from analyses as each were represented by only one egg, and the combined sex ratio across clutches for these egg ranks could only be either 0 or 1.

Incubation of great tit clutches usually commences after clutch completion, but occasionally begins before the last egg is laid (*pers. obs.*). If the last laid egg consequently hatches later, the nestling hatching from last eggs may be under particularly harsh competitive conditions in the nest. Therefore, there might be particularly strong selection for the last laid egg to contain an offspring of the larger sex (males in great tits), better able to compete in this disadvantaged position. The sex ratio of last laid eggs was compared to an expected 50:50 random allocation of sex with a G test for goodness of fit.

ii) Variation in egg sizes with laying sequence

The possibility of differential investment in eggs with respect to egg rank was analysed by examining variation in mean egg masses and volumes according to the position of eggs in the laying sequence. Analyses were carried out on 35 nests from 1998 and 36 nests from 1999 (including deserted nests) where eggs were both numbered and measured. Eggs included in the analysis were not independent because within clutches all shared a common mother (and probably father). Hence, tests were carried out on mean egg mass and volume with respect to egg rank. Analyses were split into eggs from 'early' and 'late' nests because a multiple regression of laying date and egg rank on egg measures revealed significant egg rank*laying date interaction terms (egg mass: $F_{1,336}$=44.549, p<0.0001; egg volume: $F_{1,333}$=17.196, p<0.0001).

iii) Variation in egg sizes with sex

For analysis of variation in egg masses and volumes with respect to embryo sex, individual eggs could not be taken as independent data points because of common nest effects. Thus sex differences in egg mass were tested through comparisons of mean male and female egg masses for each nest using a *t*-test. Nine nests were analysed, where there were sufficient egg mass data for eggs of each sex. Similar analyses were carried out for egg volumes on six nests. Whether there was an association over all nests for male or female eggs to be heavier was determined with a Wilcoxon mean sign test. In analyses in part (*ii*) I found a significant effect of egg rank on egg mass. Therefore I also grouped eggs according to rank (rather than nest) and compared male and female egg masses and volumes for each rank grouping.

3.2 ii) Results

i) Variation in egg sexes with laying sequence

For 17 nests where egg sex and laying sequence were known, there was a trend for increasing egg sex ratio with egg rank (figure 3.5a). However, egg rank did not explain significant variation in the proportion of male eggs at each laying position ($\Delta D=2.798$, d.f.=1, $p>0.05$). This was also true when analyses were limited to clutches of only 8 or 9 eggs ($n=12$), the most common clutch sizes ($\Delta D=1.720$, d.f.=1, $p>0.05$ figure 3.5b). The last egg showed no deviation from a 1:1 sex ratio ($G=2.657$, d.f.=1, $p<0.10$).

ii) Variation in egg sizes with laying sequence

Egg rank explained a significant amount of the variation in egg masses (figures 3.6 and 3.7). A polynomial function best relates egg mass to egg rank for early nests (figure 3.6a, $F_{2,9}=31.707$, $p<0.0001$) whilst there is a negative linear relationship for late nests (figure 3.6b, $F_{1,10}=5.784$, $p=0.0370$). Similarly, a quadratic function explains the variation in egg volume with laying sequence in early nests (figure 3.7a, $F_{2,9}=6.405$, $p=0.0186$), but for later nests there is a non-significant decrease in egg volume with egg rank (figure 3.7b, $F_{1,10}=1.1438$, $p=0.3100$).

iii) Variation in egg sizes with sex

There were no differences between male and female egg volumes within six nests with adequate data for testing the effect of egg sex on egg volume (table 3.2). One of nine nests showed variation in egg masses due to egg sex, however given the number of comparisons carried out here, the fact that one is significant would be expected by chance. Hence, this is interpreted as a Type I error. A Wilcoxon ranked sign test demonstrates no overall pattern of male or female eggs to be heavier (T-=21, n=9, T becomes significant at <6). Egg size was not associated with egg sex for egg of any rank (tables 3.3a and 3.3b).

Figure 3.5a and 3.5b: Sex ratio variation with egg rank great tit nests – all clutches included in figure 3.5a, those of eight or nine eggs only in figure 3.5b. See appendix 3.3 for sample sizes.

Fig. 3.5a

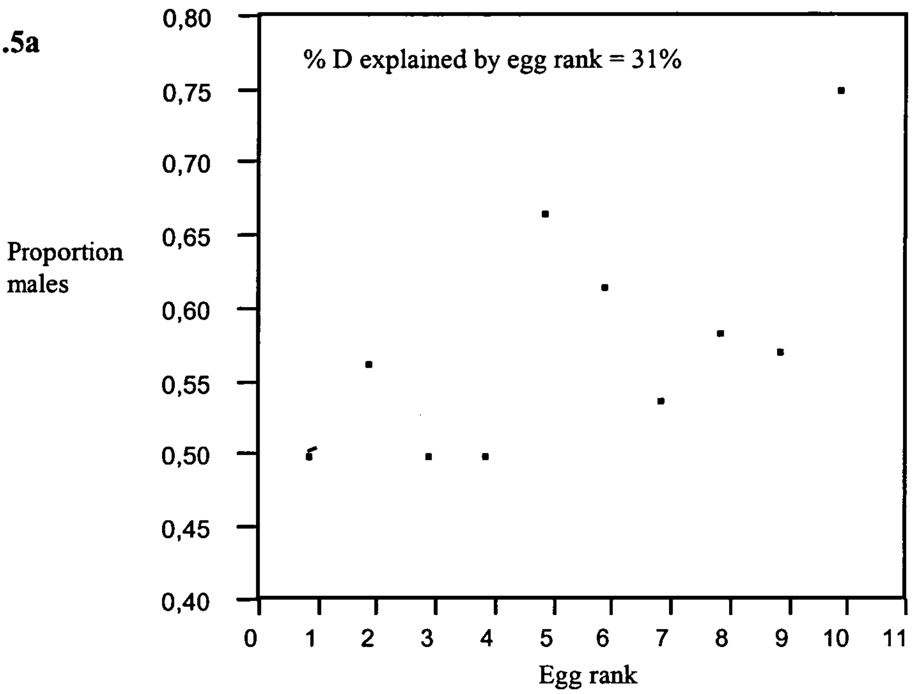
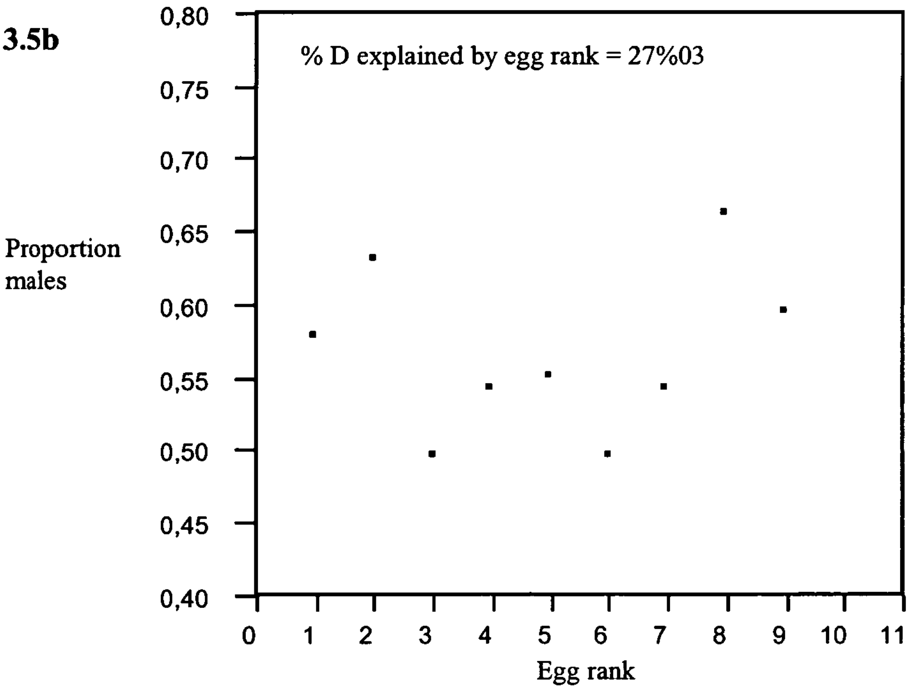


Fig. 3.5b



Figures 3.6 and 3.7: Variation in mean egg masses (figures 3.6a and 3.6b) and egg volumes (figures 3.7a and 3.7b) with respect to laying order of eggs.

Fig 3.6

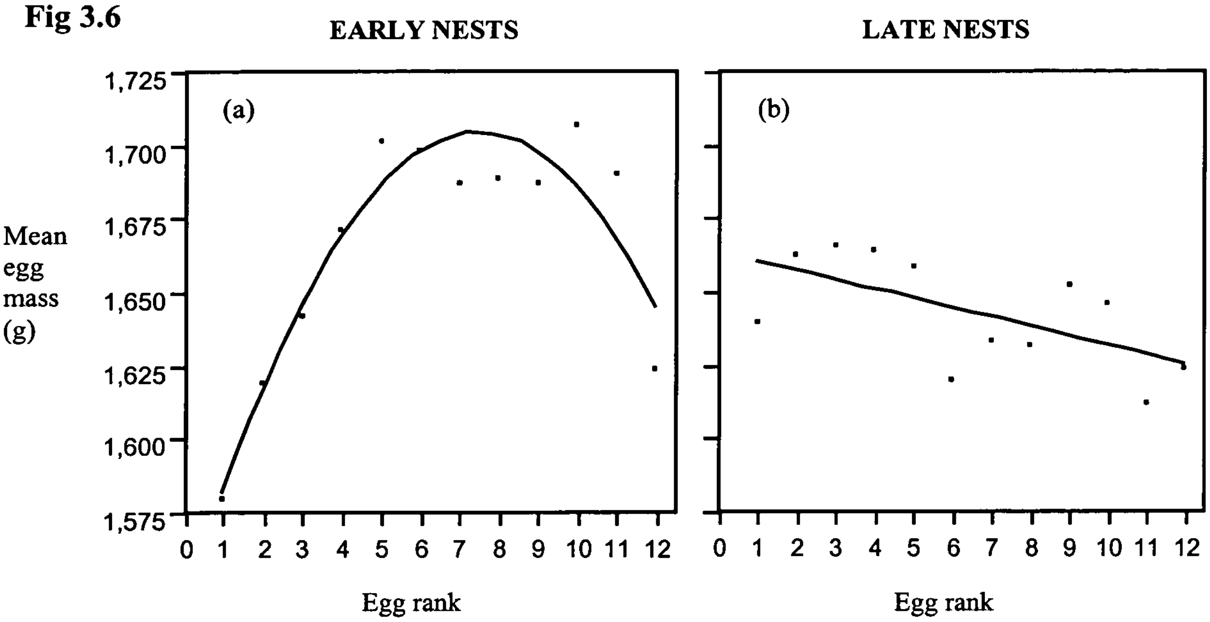


Fig 3.7

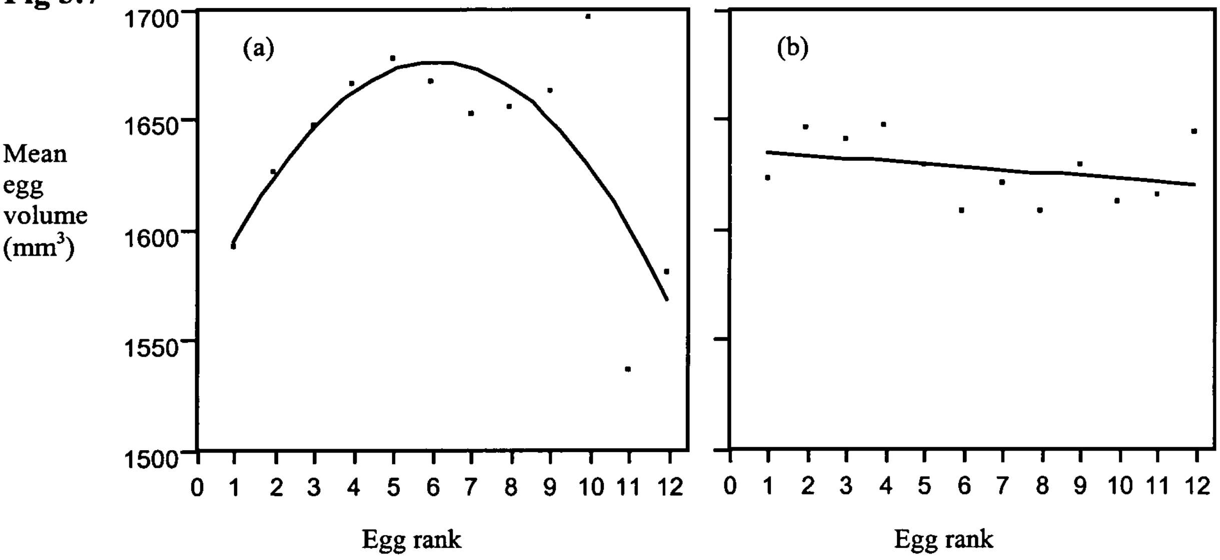


Table 3.2: Effect of egg sex on egg measures for each nest. Results of statistical comparison of egg masses and volumes according to offspring sex. Positive *t* values indicate female eggs larger than males.

Nest number	Egg mass			Egg volume		
	N	*T*	*p*	*n*	*t*	*p*
1	11	0.172	0.868	11	1.107	0.297
2	11	-0.117	0.910	11	-0.045	0.965
3	11	-0.926	0.379	11	-1.962	0.081
4	9	0.516	0.622	9	1.460	0.188
5	9	-1.369	0.213	9	-1.648	0.143
6	9	0.284	0.784	9	0.231	0.824
7	9	0.222	0.831			
8	10	-0.491	0.636			
9	9	2.479	*0.042			

Table 3.3a: Effect of egg sex on egg measures for each rank position of egg. Mean mass (± s.e.) of male and female eggs and results of statistical comparison of egg sizes according to offspring sex. Positive *t* values indicate female eggs larger than males.

Egg rank	Egg mass					
	Mean male egg mass (g)	*n* male	Mean female egg mass (g)	*n* female	Difference	
					t	*P*
1	1.61 ± 0.05	11	1.50 ± 0.05	7	-1.554	0.110
2	1.63 ± 0.04	9	1.64 ± 0.05	8	0.205	0.841
3	1.65 ± 0.06	8	1.66 ± 0.04	10	0.196	0.847
4	1.59 ± 0.05	10	1.70 ± 0.03	6	1.548	0.414
5	1.67 ± 0.03	11	1.66 ± 0.07	6	-1.228	0.240
6	1.63 ± 0.03	11	1.65 ± 0.07	6	0.174	0.864
7	1.65 ± 0.04	10	1.58 ± 0.04	5	-1.161	0.267
8	1.60 ± 0.04	9	1.70 ± 0.06	5	1.319	0.212
9	1.63 ± 0.03	6	1.73 ± 0.09	4	1.231	0.253

Table 3.3b: Effect of egg sex on egg measures for each rank position of egg. Mean volume (± s.e.) of male and female eggs and results of statistical comparison of egg sizes according to offspring sex. Positive *t* values indicate female eggs larger than males.

Egg rank	Egg mass					
	Mean male egg volume (mm^3)	*n* male	Mean female egg volume (mm^3)	*n* female	Difference	
					t	*P*
1	1577 ± 43	7	1530 ± 59	2	-0.532	0.611
2	1620 ± 45	5	1635 ± 63	4	0.201	0.847
3	1613 ± 48	6	1628 ± 55	5	0.209	0.839
4	1563 ± 34	7	1653 ± 15	3	1.679	0.132
5	1653 ± 23	6	1618 ± 65	3	-0.066	0.530
6	1592 ± 41	8	1726 ± 12	2	1.548	0.160

Discussion

Egg sex ratio biasing in respect to the laying or hatching sequence of eggs was investigated using a cross species analysis and field data from great tit clutches. Biasing egg sex ratio with respect to laying order might represent a method of female control of the sex ratio that minimises costs in terms of hatching delays (see later discussion). The aims of the study were twofold: firstly, to probe for evidence that female birds might indeed control the egg sex ratios of their clutches, and secondly, to identify and suggest reasons for any patterns of sex biases with egg rank.

Cross species comparison

The meta-analysis combined effects of egg rank on egg sex ratio for 25 species. In the majority of cases, any relationships between egg rank and egg sex ratio were not very strong (figs 3.1 and 3.2). There was almost significant heterogeneity in effect sizes when effect sizes were analysed as a mean value for each species. This hints that perhaps different patterns of egg sex ratio variation with laying sequence may occur, and this analysis would benefit from including a larger number of studies to further examine this possibility. Within studies, even if a common true effect of egg rank on egg sex exists, a significant result may not have obtained if sample sizes were small (see Arnqvist & Wooster 1995). Hence, increasing sample sizes within studies (i..e. number of broods involved) would also be beneficial to these analyses.

Further analyses partitioned the variance in effect sizes according to species dimorphism patterns – whether males or females were larger. When each species was analysed as a data point, there was no difference in the egg sex ratio-rank relationship between species with larger males, and those with larger females. Analysing each trend reported in the literature suggests that for species with females larger than males, mothers tend to lay the larger sex first (fig. 3.3). However, the fact that this relationship was only significant when each trend was analysed suggests that this result should be treated with caution as it may be due to over-representation of effect sizes for species with larger females.

Supposing that this relationship is real, there are two potentially functional explanations for this pattern. Firstly, a female might increase the reproductive value (RV) of a brood through increasing the quality or chances of fledging of remaining offspring, given that smaller young which hatch later would be competitively disadvantaged and more likely to die (e.g. Howe 1976, Clark & Wilson 1981, Mead & Morton 1985, Zijlstra *et al.* 1992, Wiebe & Bortolotti 1992, Bortolotti 1986). Secondly, females may bias early eggs towards the larger sex because first hatching young fledge in better condition (Kendeigh 1963, Ricklefs 1965, Klomp 1970, Howe 1976, Zach 1982, Forbes 1990) and variance in RS may be greater amongst the larger sex (e.g. Clutton-Brock 1991, Kempenaers *et al.* 1997). This explanation would avoid the obvious costs involved in brood reduction of nestling mortality.

However, it is also possible that any relationship may be confounded by problems of non-independence of traits within categories 'females larger' and 'males larger'. Certain groups of birds might share a tendency to fall into the category 'females larger', and also the tendency to bias sex ratios with rank in a certain direction. For example, in species with larger females, nine of ten exhibit siblicide, compared to two from eleven of those with males larger (see appendices 3.1 an 3.2). This difference is statistically significant (χ^2=14.18, p=0.0002, n=23). Although I have no data to prove a relationship, brood reduction in siblicidal species may be more common. This may lead females of siblicidal species to be more likely to bias last eggs toward the smaller sex, if they stand to lose less from following a strategy enhancing brood reduction because nestling mortality is likely anyway. Teasing out the important determining factors of the egg sex ratio-rank relationship would benefit from analysis taking into account these group differences, i.e. independent constrasts, controlling for common group effects (phylogeny).

This highlights the problem of the current analyses in treating individual species as independent data points. Species exhibiting the same sex ratio biasing patterns may be more closely related to each other than those with other biasing patterns. Relationships between traits may be present for reasons of common ancestry (Harvey 1996, Harvey & Nee 1997). Though here I am limited as to my conclusions concerning the evolution of such relationships (i.e. whether one evolved in response to another, identifying causality), a meta-analyses can adequately demonstrate the existence of an association between traits.

Investigations of variation in an egg sex-rank relationship for the two sexual dimorphism groups with brood size suggested there was no difference in biasing patterns according to brood size. There was a suggestion that the possibility of female eggs being laid later when females are the larger sex may only be the case for small-brooded species (table 3.1). However, this result again is at best speculative, as statistical significance was found only when trends data were analysed (hence possible pseudoreplaiction) and when brood size was estimated as number of fledglings (small sample size). Again any potential relationship cannot be confirmed before enlarging the data set

Great tit egg data

Analysis of field data from great tits similarly suggested no sex ratio biasing in relation to egg laying order. Experimental evidence from great tits found that males (larger) grew better than their sisters in situations of heightened competition (Oddie 2000, Chapter 5). This suggests that mothers might avoid brood reduction by biasing later eggs towards males. Despite these potential benefits, females did not manipulate egg sex ratio in relation to laying order. Neither did they appear to bias the sex of the last laid egg, which is likely to produce the offspring under the most competitive stress. This result is consistent with another published study of sex ratios with laying order in the large-brooded zebra finch (*Taeniopygia guttata,* Kilner 1998). It is also consistent with the findings from the cross species analysis.

Although egg sex ratio variation did not vary over the laying sequence, egg masses and volumes did. Previous studies suggest that female birds may be able to manipulate qualities of individual eggs, such as hormone levels, with respect to the order of egg laying (Schwabl 1993, Schwabl *et al.* 1997). The adaptive value of such manipulations of egg constituents has been discussed, if they increase the reproductive value of a breeding attempt (Scwabl 1993, Winkler 1993, Schwabl *et al.* 1997, Gil *et al.* 1999). Most recently, female mallard have been found to lay eggs of different sizes according to whether they mate with a preferred male or not (Cunningham & Russell 2000).
However, variation within the egg size-rank relationship according to timing of breeding suggests that this may reflect resource availability at the time of laying, at least for early nests, rather than an active female strategy to assign certain eggs greater resources.

In early clutches, the relationships between egg mass and volume and egg rank was best described by a quadratic function. The earliest laid eggs amongst these clutches were amongst the first laid in the nesting season, when food resources were scarce following the winter. This might account for their low masses and volumes. Egg masses and volumes increased amongst 'middle' eggs, possibly as a food resources increase, then declined amongst eggs 10, 11 and 12 early in the season, probably attributable to exhaustion of female resources invested in earlier eggs. In late nests, early ranked eggs were of intermediate mass and volume. Declining food resources available for egg production could explain the declining masses of eggs in late nests.

However, 'late' nests were those where laying began after 30 April (mean laying date) and in this population the peak of caterpillar density is in early June (R. Przybylo, *pers. comm.*). Therefore it seems unlikely that the decrease in egg mass with rank in late nests reflects decreasing resource availability. An alternative explanation could involve different age classes of birds adopting different patterns of investment in eggs, as on Gotland late breeders are mainly young birds (Cichon & Lindén 1995).

I found no evidence that females provision male and female eggs differently. Male and female nestlings hatched from eggs of the same size. This is also supported indirectly by the fact that egg size is a function of the laying order (and timing of laying), but egg sex is not.

Concluding, the combined results of both the cross species analysis and great tit data suggest that female birds do not manipulate egg sex ratios with respect to laying order. However, the meta-analysis provided suggestive results of a relationship between egg sex and rank in relation to the sexual dimorphism pattern of a species. This merits further investigation with larger data sets as more studies are published. Factors potentially influencing the sex-specific fitness returns from offspring hatching from each egg, and hence patterns of egg sex ratio with laying order, should be included in such analyses. These include predicted resource availability, likelihood of brood reduction, brood size, incidence and intensity of siblicidal behaviour, parental quality, presence or absence of helpers, or any interactions between these variables.

Sex ratio alteration can only be considered adaptive if the existence of a low-cost mechanism through which offspring sex can be manipulated can be demonstrated (e.g. Maynard-Smith 1980, Pen & Weissing 2000). The cost of modifying sex ratios by reabsorbing or discarding eggs of the unwanted sex (Emlen 1997), may be too prohibitively high for such a sex ratio adjustment mechanism to evolve. Recently, Pen *et al.* (1999) demonstrated that such a time delay cost to sex ratio control through egg reabsorption need only be extremely small for it to outweigh adaptive advantages to sex ratio biasing.

Analysis of the relationships between egg sex ratio and egg rank will benefit from repetition with larger data sets. An increasing number of studies concerned with egg sex ratio manipulation are published, and this meta-analysis is intended to encourage data collection of sex ratios in respect to egg rank. These data are not always easy to collect, particularly in large-brooded and synchronously hatching species, because of the difficulties in matching hatched nestlings (which can be sexed) with their ranked eggs.

Data on egg sex and rank can be collected by numbering eggs when they are laid, and visiting clutches at very frequent intervals around and after hatching to mark or bleed hatched nestlings. . Otherwise, eggs can be removed to an incubator and monitored for hatching. However, this risks decreased hatching success (e.g. Schifferli 1972) and desertion of parents from the nest whilst eggs are removed. Whole clutches may be sacrificed, and embryos dissected from each egg for sexing, although obviously this is ethically questionable. Alternatively, a huge effort numbering eggs must be spent in order to collect reasonable data sets from those nests deserted during the incubation period. Depending on the frequency of nest desertion, the latter is likely to be not only extremely time consuming, but only suitable for species with high breeding densities where a small fraction of deserted nests could contribute reasonable sample sizes.

Appendix 3.1: Species-specific data used in comparative analysis of sex ratio variation with respect to laying/hatching sequence

Common name	n	r stat.	Clutch size[1]	Number fledged[1]	Number fledged category	Female mass[11]	Male mass[11]	Males> Females	Siblicide[10]	When measured	Ref
Canada goose	44	-0.0069	5.5			3043	3690	yes	no	laying	1
Snow goose	91	-0.0745	4.5			2517	2744	yes	no	laying	2,3
Marsh harrier	38	0.0342	4.5	2.8	few	763	492	no	yes	hatching	5
Montagu's harrier	86	0.2484	4.0			370	261	no	yes	hatching	4
Bald eagle	160	0.221	2.0			5350	4130	no	yes	hatching	6,7
Harris's Hawk	74	-0.1975	3.0			998	690	no	yes	hatching	8
Cattle egret	45	-0.1667	4.5[2]	1.53[2]	few	304[2]	311[2]	yes	yes	hatching	9
European kestrel	89	0.0489	4.5	3.1	few	217	186	no	yes	Laying	10
Peregrine falcon	23	0.574	2.8	1.63	few	952	611	no	yes	Hatching	11
Herring gull	66	0.1237	2.3			1044	1226	yes	no	Laying	13
Audouin's Gull	46	-0.0466	3.0			519.5[12]	629[12]	yes	no	Hatching	12
Western gull	73	0.0783	3.0						no	Laying	15
Lesser-black backed Gull	46	0.0178	2.5			755	880	yes	no	Hatching	14
Ring-billed gull	67	-0.1713	2.8			471	566	yes	no	Laying	16
Roseate tern	131	0.0806	2.0	1.25	few				no	Laying	17
European shag	43	-0.2093	3.0	2.43	few	1598	1940	yes	yes	Laying	18
Laughing Kookaburra	90	-0.1385	3.0[3]	2.45[3]	few	369[3]	319[3]	no	yes	Hatching	19
Red-winged Blackbird	164	0.0847	4.0[4]	2.95[4]	few	41.5	63.6	yes	no	Laying	20
Great-tailed grackle	96	-0.0289	3.4[5]			107	191	yes	no	Laying	22
Great Tit	16	0.3671	9.0[6]	6.72[6]	many	17.72[6]	18.63[6]	yes	no	Laying	23
House sparrow	30	0.0474	5.0[7]	3.05[7]	many	27.4	28	yes	no	Laying	24
Zebra finch	16	0.2816	5.0[8]	3.87[8]	many	12.45[8]	12.15[8]	no	no	Laying	25
White-crowned sparrow	47	0.1186	3.0[9]			26.65[9]	28.75[9]	yes	no	Laying	26
Tengmalm's Owl	90	-0.0145	5.6	4.65	many	167	101	no	yes	Hatching	27
Tawny Owl	85	0.1479	4.0	3.4	many	524	426	no	yes	Hatching	28

[1] del Hoyo *et al.*, eds. (1992) Handbook of Birds of The World, Vols. 1-5, unless otherwise stated; [2] Cramp & Simmons, eds. (1977) Handbook of the Birds of Europe, The Middle East and North Africa, Volume 1: Ostrich to Ducks; [3] Higgins, ed. (1999) Handbook of Australian, New Zealand and Antarctic Birds, Volume 4: Parrots to Dollarbird; [4] Beletsky, (1996); [5] Jaramillo & Burke (1999); [6] Own data used for great tit; [7] Cramp & Perrins, eds. (1994) Handbook of the Birds of Europe, The Middle East and North Africa, Volume 8: Crows to finches; [8] Zann (1996); [9] Blanchard (1941); [10] del Hoyo *et al.*, eds. (1992) Handbook of Birds of The World, Vols. 1-5, and references to siblicide within publications used in analyses; [11] Dunning Jr., ed. (1993) CRC Handbook of Avian Body Masses, unless otherwise stated; [12] Cramp & Simmons, eds. (1998) Handbook of the Birds of Europe, The Middle East and North Africa, Volume 2 (1) update, p47-61.

Appendix 3.2: Data for each trend in sex ratio variation with respect to laying/hatching sequence used in comparative analysis

Common name	n	r stat.	Clutch size	Number fledged	Number fledged category	Female mass	Male mass	Males> Females	Siblicide	When measured	Ref
Canada goose	44	-0.0069	5.5			3043.00	3690.00	yes	no	laying	1
Snow goose	29	-0.3189	4.5			2517.00	2744.00	yes	no	laying	2
Snow goose	62	0.026	4.5			2517.00	2744.00	yes	no	laying	3
Montagu's harrier	86	0.2484	4.0			370.00	261.00	no	yes	hatching	4
Marsh harrier	38	0.0342[a]	4.5	2.80	few	763.00	492.00	no	yes	hatching	5
Bald eagle	37	0.3534	2.0			5350.00	4130.00	no	yes	hatching	6
Bald eagle	70	0.3144[b]	2.0			5350.00	4130.00	no	yes	hatching	7
Bald eagle	41	0.0251[c]	2.0			5350.00	4130.00	no	yes	hatching	7
Bald eagle	12	-0.1026[d]	2.0			5350.00	4130.00	no	yes	hatching	7
Harris's Hawk	74	-0.1975	3.0			998.00	690.00	no	yes	hatching	8
Cattle egret	45	-0.1667	4.5	1.53	few	304.00	311.00	yes	yes	hatching	9
European kestrel	39	-0.1914[e]	4.5	3.10	few	217.00	186.00	no	yes	laying	10
European kestrel	50	0.2409[f]	4.5	3.10	few	217.00	186.00	no	yes	laying	10
Peregrine falcon	23	0.574[g]	2.8	1.63	few	952.00	611.00	no	yes	hatching	11
Audouin's Gull	46	-0.0466	3.0			519.50	629.00	yes	no	hatching	12
Herring gull	66	0.1237	2.3			1044.00	1226.00	yes	no	laying	13
Lesser-black backed Gull	46	0.0178	2.5			755.00	880.00	yes	no	hatching	14
Western gull	73	0.0783	3.0						no	laying	15
Ring-billed gull	67	-0.1713	2.8			471.00	566.00	yes	no	laying	16
Roseate tern	131	0.0806	2.0	1.25	few				no	laying	17
European shag	25	-0.3708[h]	3.0	2.43	few	1598.00	1940.00	yes	yes	laying	18
European shag	18	0[i]	3.0	2.43	few	1598.00	1940.00	yes	yes	laying	18
Laughing Kookaburra	90	-0.1385[j]	3.0	2.45	few	369.00	319.00	no	yes	hatching	19
Red-winged Blackbird	41	-0.1166[k]	4.0	2.95	few	41.50	63.60	yes	no	laying	20
Red-winged Blackbird	123	0.1171[l]	4.0	2.95	few	41.50	63.60	yes	no	laying	20
Red-winged Blackbird	42	0.0731[k]	4.0	2.95	few	41.50	63.60	yes	no	laying	21
Red-winged Blackbird	117	-0.1094[l]	4.0	2.95	few	41.50	63.60	yes	no	laying	21
Great-tailed grackle	16	-0.0979[l]	3.4			107.00	191.00	yes	no	laying	22
Great-tailed	80	0[k]	3.4			107.00	191.00	yes	no	laying	22

grackle												
Great Tit	16	0.3671[m]	9.0	6.72	many	17.72	18.63	yes	no	laying	23
House sparrow	8	0.1209[n]	5.0	3.05	many	27.40	28.00	yes	no	laying	24
House sparrow	17	- 0.0173[p]	5.0	3.05	many	27.40	28.00	yes	no	laying	24
House sparrow	5	0.0556[l]	5.0	3.05	many	27.40	28.00	yes	no	laying	24
Zebra finch	16	0.2816	5.0	3.87	many	12.45	12.15	no	no	laying	25
Zebra finch	42	0.2981[q]	5.0	3.87	many	12.45	12.15	no	no	hatching	25
White-crowned sparrow	47	0.1186[r]	3.0			26.65	28.75	yes	no	laying	26
Tengmalm's Owl	90	- 0.0145[s]	5.6	4.65	many	167.00	101.00	no	yes	hatching	27
Tawny Owl	85	0.1479[t]	4.0	3.40	few	524.00	426.00	no	yes	hatching	28

[a] Early versus late ranked nestlings; [b] Besnard Lake, good year; [c] Besnard Lake, poor year; [d] Nemeiben lake; [e] Early nests; [f] Late nests; [g] 2-egg clutches only, insufficient data for others; [h] First 2 eggs of early nests; [i] First 2 eggs of late nests; [j] Data combined for nests with different sexed of helpers or no helpers; [k] 3-egg clutches only; [l] 4-egg clutches only; [m] All sized clutches combined; [n] 6-egg clutches only; [p] 5-egg clutches only; [q] all hatching sequence data combined for food restricted and food abundant broods; [r] Eggs 1&2 and 3&4 combined in calculation of r; [s] Nestlings 1-3 ('early') and 4-8 ('late') combined in calculation of r; [t] Data from all years combined.

References: 1 Leblanc (1987); 2 Ankney (1982); 3 Cooke & Harmsen (1983); 4 Leroux & Bretagnolle (1996); 5 Zijlstra *et al.* (1992); 6 Bortolotti (1986); 7 Dzus *et al.* (1996); 8 Bednarz & Hayden (1991); 9 Voltura (unpublished ms); 10 Dijkstra *et al.* (1990); 11 Olsen & Cockburn (1991); 12 Genovart *et al.* (unpublished ms); 13 Ryder & Termaat (1987); 14 Bradbury & Griffiths (1999); 15 Sayce & Hunt (1987); 16 Ryder (1983); 17 Szczys (unpublished ms); 18 Velando *et al.* (2000); 19 Legge *et al.* (in press); 20 Weatherhead (1985); 21 Fiala (1981); 22 Teather & Weatherhead (1989); 23 Oddie (own data, present study); 24 Cordero *et al.* (2000); 25 Kilner (1998); 26 Mead *et al.* (1987); 27 Hörnfeldt *et al.* (2000); 28 Appleby *et al.* (unpublished ms).

Species names: Zebra finch *Taeniopygia guttata*, white-crowned sparrow *Zonotrichia leucophrys*, red-winged blackbird *Agelius phoeniceus*, snow goose *Chen caerulescens*, ring-billed gull *Larus delawarensis*, herring gull *Larus argentatus*, Audouin's gull *Larus audounii*, western gull *Larus occidentalis*, canada goose *Branta canadensis*, great-tailed grackle *Quiscalus mexicanus*, european shag *Phalacrocorax aristotelis*, house sparrow *Passer deomesticus*, great tit *Parus major*, Harris's hawk *Uparabuteo unicinctus*, bald eagle *Haliaeetus leucocephalus*, European kestrel *Falco tinnunculus*, Peregrine falcon *Falco peregrinus*, Marsh harrier *Circus aeruginosus*, Cattle egret *Bubulcus ibis*, Tawny owl *Strix aluco*, Montagu's harrier *Circus pygargus*, Roseate tern *Sterna dougalii*, Laughing kookaburra *Dacelo novaeguineae*, Tengmalm's owl *Aegolius funereus*, Lesser black-backed gull *Larus fuscus*.

Egg rank	Egg sexes																	n males	Total sample	Prop. males
Egg 1	F		F	F	M	F	F	M	M	M	F	F	M	M	F	M	M	8	16	0.50
Egg 2	F	F	M	F	M	F	F	M	F		M	F	M	M	M	M	M	9	16	0.56
Egg 3	F	M	F		M	F	M	F	F	M	F	F	M	M	F	M	M	8	16	0.50
Egg 4	M		F		M	M	F	F	F	M		M	M	F	F	M	F	7	14	0.50
Egg 5			M	M		M		M	F		F	M	F	M	F	M	M	8	12	0.66
Egg 6	M	M			M	F	F	F	M		F	F	M	M		M	M	8	13	0.62
Egg 7		M	F		M	F		M	F	M	F	F	M	M	F	M		7	13	0.54
Egg 8	M	F	F		M	M	M	F	M		M		M	F	F			7	12	0.58
Egg 9	F		M		M	M	F	M	F									4	7	0.57
Egg 10	F	M	M	M														3	4	0.75
Egg 11	F																	0	0	0
Egg 12	M																	1	1	1
Clutch size	*12*	*10*	*10*	*10*	*9*	*9*	*9*	*9*	*9*	*8*	*8*	*8*	*8*	*8*	*8*	*8*	*6*			

Appendix 3.3: Sex of eggs at each position in the laying sequence for 17 great tit nests.

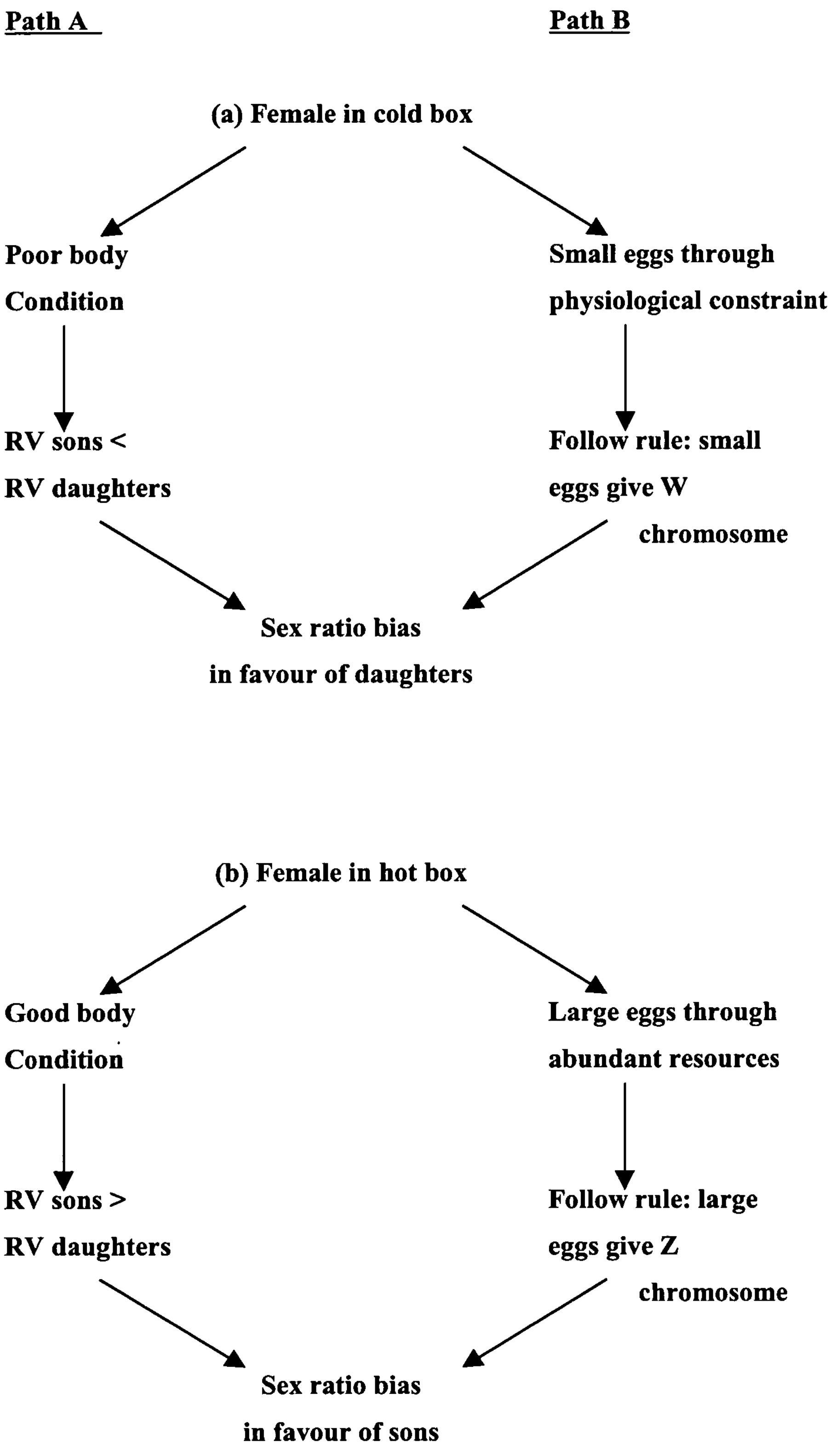
4. Does female condition affect egg sex ratio?

The previous chapter provided provoking results of a non-random pattern of egg sex with laying sequence in this population of tits, which suggests that females may indeed have some control over egg sex ratio. If females can bias egg sex ratios through manipulations of oocyte development (i.e. pre-laying), they may do so in response to either external factors such as prevailing environmental conditions and mate quality, or in relation to their own body condition. To examine the possibility of such primary sex ratio biasing, I experimentally manipulated the energy budgets of female tits and observed their responses in clutch sex ratio as well as other breeding parameters. Energetic constraints were imposed by heating and cooling nestboxes overnight for 2-3 weeks before, and during, egg-laying.

Egg production may be a particularly demanding phase of reproduction for female birds (reviewed in Thomson *et. al.* 1998). A typical female great tit will produce a clutch of nine eggs in as many days, the cumulative mass of which amounts to 82% of her own body mass (own data). Effects of energetic constraints in birds have been investigated previously with respect to a variety of traits: clutch size (Arcese & Smith 1988, Nilsson 1991), lay date (Källander 1974, Arcese & Smith 1988), egg mass (Högstedt 1981), incubation efficiency (Nilsson & Smith 1988, Bryan & Bryant 1999) and laying interruptions (Yom-Tov & Wright 1993). Although the notion that egg or offspring quality (and/or number) may be compromised by energetically-challenged females, no previous study has considered effects of maternal energy limitations on clutch sex ratios in wild birds.

It is possible to distinguish 2 pathways through which clutch sex ratios might be modified in response to the experimental manipulation (see figure 4.1). Both pathways assume female control over the sex ratio she produces and predict sex ratio biases in the same direction. However, the processes involved are quite different. In the 'Trivers and Willard' pathway, the experimental manipulation causes changes in the sex ratio through changes to female condition. Females in cold nestboxes are expected to use more energy reserves to keep warm and hence be in poor condition,

Figure 4.1: Pathways leading to modification of sex ratios by female tits nesting in (a) cooled and (b) heated nestboxes. Both Paths A ('Trivers and Willard') and B ('Egg volume constraint') predict the same outcomes.



whilst those in hot boxes can spend more time foraging and building up resources for investment in reproduction (good condition). Females modify investment in sons and daughters (at the egg stage) according to their own body condition, as Trivers and Willard (1973) originally proposed. Here the assumption is that males have higher variance in reproductive success than females, and hence that poor quality males will have lower reproductive value than their poor quality sisters (and vice versa for high quality males). A mother in poor condition would then benefit genetically by producing more female offspring, and if in good condition more males. Although there may be little difference to females in resources involved in production of a male and female eggs, after hatching she will be rearing nestlings of the sex ratio laid. Thus females may adaptively adjust clutch sex ratio according to their anticipation of their own condition during rearing, which is influenced by that at laying.

The second pathway involves female manipulation of clutch sex ratio in response to egg volumes, rather than to her own condition. I call this the 'egg volume constraint' hypothesis. Females follow a simple rule of thumb according to the volume of egg they lay: large eggs are allocated Z chromosomes and small eggs allocated W chromosomes. This theory assumes that egg volume is adjusted according to female condition, and that fitness returns are greater for males than for females hatching from larger eggs. Thus selection favours mothers who provision large eggs with Z chromosomes and small eggs with W chromosomes. Males have been found to hatch from larger eggs in three species (Mead *et al.* 1987, Anderson *et al.* 1997, Cordero *et al.* 2000), two of them also small passerines (Mead *et al.* 1987, Cordero *et al.* 2000). That females may be capable of 'choosing' constituents of eggs may not be so extraordinary as it first appears. Recent studies show differential transmission of maternal hormones into eggs, associated with size differences of offspring hatching from those eggs (Schwabl 1993, Schwabl 1996).

The idea also rests on the thermal manipulation resulting in changes in egg volume. In a previous study of great tits where nestboxes temperatures were manipulated, Nager & van Noordwijk (1992) found egg volumes to vary by 14% between females laying in heated and cooled boxes. Similarly, non-experimental variation in egg volume with temperature was recorded by van Noordwijk (1984), who found great tit egg size to correlate positively with the temperature three days before production of each egg. A number of other reproductive traits might be adjusted by females following manipulation of energy available before and during laying. Most experiments examining effects of energy limits on female breeding decisions have involved supplementary feeding of females (Martin 1987; Arcese & Smith 1988; Boutin 1990; Nilsson 1991; Nilsson & Svensson 1993). Modification of the thermal environment experienced by females may be a more effective manipulation of female condition. Such a design has three advantages: i) temperature can be manipulated in a bidirectional manner, that is it can be increased or decreased, whereas it is very difficult to experimentally restrict food consumption by wild birds; ii) through supplementary feeding, food quality as well as quantity is manipulated, and it is difficult to disentangle effects of each; iii) it is difficult to target individuals for supplementary feeding in wild groups.

Two studies have investigated effects of experimentally elevated temperatures experienced by breeding females (Yom-Tov & Wright 1993; Bryan & Bryant 1999) but only Nager & van Noordwijk (1992) examined effects of both increasing and decreasing temperatures. I repeated this experiment on the Gotland population of great tits to examine effects on clutch sex ratios. Concurrently I recorded effects on a number of other reproductive traits, which may be constrained by energy availability and which are unlikely to be independent. With sample sizes five times as large as those of Nager & van Noordwijk, I could examine whether their temperature effects were robust and consistent. Furthermore, I extended the experiment to investigate the effects of the same temperature manipulations on the closely related blue tit, *Parus caeruleus*.

Method

Experimental treatment

The study was conducted over two years in eight non-adjoining woodlands on the Swedish island of Gotland (57°10'N, 18°20'E). Field assistance was provided by A.Impey (1998) and C.Reim and A.Russell (1999). In each year, all nestboxes in four woodland areas were heated, and in another four woodlands were cooled. All woodlands were composed of mixed deciduous stands of hazel (*Corylus avellana*), ash (*Fraxinus excelsior*), birch (*Betula spp.*), oak (*Quercus robur*), and blackthorn (*Prunus spinosa*). Specially modified nestboxes were provided which allowed thermal manipulations through insertion of 'hot packs' (handwarmers, Grabber inc.) or 'cold packs' (freeze blocks, BDI) into a chamber at the rear of the box. A wire mesh separated the hot and cold packs from the nest-building chamber (figure 4.2).

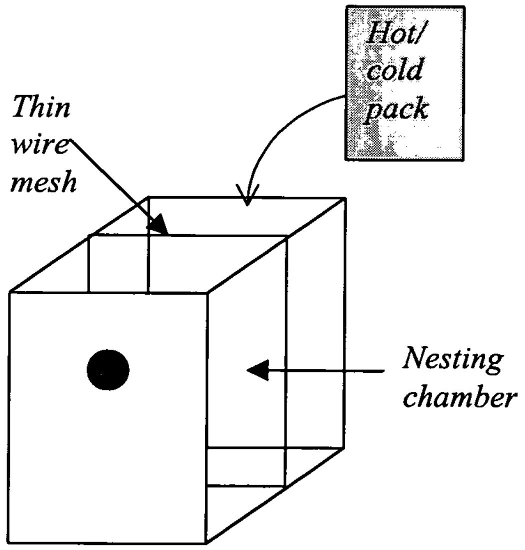


Figure 4.2: Modified nestbox with rear chamber for insertion of hot and cold packs

Experiments began in mid-April in 1998 and 1999. Each evening between 1500 and 1800 CET, a hot or cold pack was inserted into the rear nestbox chamber and the previous spent pack was removed. Females enter boxes around dusk to roost for the night (*per. obs.*; Dhondt & Eyckerman 1979, Yom-Tov & Wright 1993). New packs were thus inserted just before female birds entered boxes, so overnight nestbox temperatures were manipulated without disturbing the birds. All nestboxes within a woodland area were subjected to the same hot or cold treatment even when empty (i.e. no signs of nest-building). Following Nager and van Noordwijk (1992), I heated or cooled all boxes in a woodland to avoid females switching between heated and cooled boxes within the wood. This introduced the potential problem of confounding area and treatment effects, so in 1999 when the experiment was repeated, treatments were switched between areas. Thus a cold area in 1998 became heated in 1999 and vice versa. Overnight nestbox temperatures were determined from two thermometers placed in unoccupied nestboxes, one heated and one cooled. Each afternoon the thermometers were placed in new nestboxes to avoid pseudoreplication of temperatures from certain nextboxes. Temperature recordings were taken at around 2330 each night (i.e. >5 hours after sunset).

Following nest-building, great and blue tits usually lay one egg per day before beginning incubation of the whole clutch simultaneously. Nightly heating and cooling treatment continued throughout nestbuilding and egg-laying, but was discontinued as soon as a female began incubating eggs. Onset of incubation was easily determined from nest observations: prior to incubation eggs were covered with nesting material and cold; at incubation eggs were arranged neatly in the nest-cup, uncovered and warm.

Data collection

Each egg was numbered and weighed to the nearest 0.125g using a 5g pesola spring balance on the day it was laid, whilst visiting boxes to change hot and cold packs. Fresh egg weight was measured rather than weighing the whole clutch upon completion as egg weight may vary over time (e.g. due to evaporation, Ar & Rahn 1980; Carey 1986). Between 8 and 12 days after clutch completion, the length and breadth of each egg was measured to the nearest 0.5mm using dial callipers, and egg volume calculated according to the formula: volume = length x breadth^2 x π/6. To measure eggs, the whole clutch was removed from the nest and warmed dummy eggs were placed in the nest cup. This allowed careful egg measurements to be recorded without alarming female birds already incubating or returning to nests. Clutches were not out of the nest for longer than 30 minutes. In three great tit nests (4.5%) and one blue tit nest (2.9%), females began incubation before clutch completion and one unnumbered egg was discovered in the clutch at the time of measuring. These eggs must have been the last laid, and their weights were recorded when they were measured.

Nests were monitored daily for hatching, and a small (2-5μl) blood sample was taken from the brachial vein for sex determination when each nestling was one day old. Blood was stored in 98% ethanol and DNA extracted using 5% chelex. PCR amplification of Z- and W- linked copies of the CHD1 gene revealed the sex of an individual through the presence of two DNA fragments in heterogametic females and one in homogametic males (see Griffiths *et. al.* 1998).

Because reproductive traits may be affected by adult size (e.g. larger females lay bigger or more eggs), parents were caught provisioning nestlings on day 12 (blue tits) and 15 (great tits) and their mass, tarsus, wing measurements and age were recorded. At the time of measuring, females had been incubating eggs and provisioning nestlings for about 1 month after experimental treatment, thus these measurements were not intended to examine experimental effects on adults, but to control for parental body size in analyses. Measures of females were not attempted during laying or incubation because of the high rates of nest desertion if females are disturbed at these times.

Data Analysis

I compared box occupation between heated and cooled nestboxes using analysis of variance for great tits and blue tits combined, and then the 2 species separately. Area and year were included as factors to control for differences between woodlands and years. However, area and treatment effects were not independent of year, and so I also analysed effects of treatment independently for each year. I also used paired *t*-tests to see whether box occupation varied between years for a given area, i.e. whether a different proportion of boxes were occupied in each area when it was subjected to hot treatment compared to cold.

Analysis of variation in timing of breeding (laying and hatching dates), incubation duration, clutch sizes and egg sizes were carried out using ANCOVA models on great tits and blue tits separately. In all analyses I tested for effects of treatment, year and woodland area as well as variation due to female and male body size and mass. Additionally, for analyses of clutch size and incubation duration I included effects of laying date, and for analyses of egg sizes effects of clutch size and laying date. In all analyses I tested for effects of the mean ambient temperature at the time of laying. A single figure for mean ambient temperature was designated for each nest by determining the mean of temperatures 7 days before the first egg was laid to 9 days afterwards (9 is the average clutch size). Data for ambient temperatures were provided by the Swedish Meteorological and Hydrological Institute weather station at Hoburgen, approximately 20km south of the study woodlands.

Heated and cooled nestboxes were occupied by 35 pairs of blue tits and 67 pairs of great tits over the two year study period. Sample sizes for great tit analyses do not always equal 67 for the following reasons: For analysis of timing of breeding one outlier, representing an extremely late nest, was excluded. Nests where exact hatch dates were not known were not included in analyses of hatching dates and incubation times. For analysis of variation in clutch sizes, 2 females stopped laying mid-clutch; these nests are not included. For blue tits, one female stopped laying before clutch completion and the exact hatch date for one nest was not known. Not all female adults were caught, and therefore if a female body measure is included as a significant variable in a model, the degrees of freedom are reduced considerably. Analyses of egg sizes include measurements of eggs from 72 great tit ‘control’ nests that were neither heated or cooled. Results do not differ from those reported if only heated and cooled nests are included in analyses.

The proportion of eggs hatching and nestlings fledging in each nest, and the proportion of males in a clutch, were analysed using logistic regression analysis with binomial errors and a logit link (binomial denominators of clutch size, numbers hatching, and clutch size respectively). Explanatory variables (year, laying date, treatment, female tarsus length, female body mass residuals) were added to the model and removed stepwise. The ratio of the residual deviance to the residual degrees of freedom, the heterogeneity factor (HF), gives an estimate of how the actual variance compares to the expected variance of a binomial distribution. A heterogeneity factor of <1 indicates less than binomial variance, and >1 more than binomial variance. HFs >1 indicate overdispersed data which could lead to spurious results, so when HF>1 the deviance was scaled by the HF before testing, to give more conservative parameter estimates. The significance of the increase in deviance after the removal of a factor from the model was assessed using a χ^2 test or F test (HF>1) with appropriate degrees of freedom (Crawley 1993). Experimental nests which were deserted after laying began (great tits 4/67, 6.0%; blue tits 1/35, 2.9%) were excluded from analyses of the proportion of eggs hatching. Similarly, nests which were abandoned after hatching were excluded from analyses of proportion of nestlings fledging (great tits 8/63, 12.7%; blue tits 1/34, 2.9%).

Results

Thermal conditions experienced by roosting female great tits were manipulated for approximately 15.8 and 19.4 days before the mean laying date in 1998 and 1999 respectively. For blue tits, nests were heated and cooled 15.5 and 21.1 days before the mean laying date. There was a significant effect of experimental treatment on the temperature in the boxes, with a 4.89°C (1998) and 4.49°C (1999) difference in temperature between hot and cold boxes ($F_{1,55}=201.523, p<0.0001$). This effect was consistent throughout the treatment period, i.e. there was no effect of date ($F_{1,22}=0.782, p=0.3866$).

Ambient temperature patterns were quite different between the two years. In 1998 temperature increased steadily with lay date, but in 1999 there was a drop in temperature which coincided with the last days of laying and first week of incubation for most birds (figure 4.3).

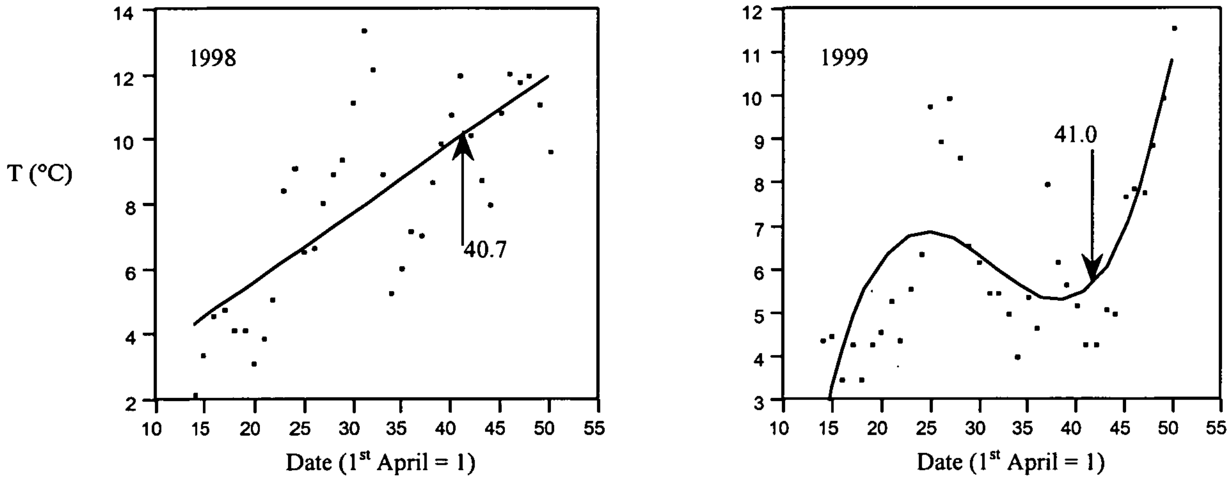


Figure 4.3: Ambient temperatures (daily means) on Gotland during the breeding seasons 1998 and 1999. Arrows represent onset of incubation, calculated as median lay date plus clutch size for each year.

Box occupation

Nestbox occupation was compared between the two treatment groups for both species combined as an overall measure of box preference. The proportion of nestboxes occupied in heated areas was greater than the proportion of cooled boxes occupied (table 4.1, figure 4.4). This was mainly due to the distribution of blue tits between treatment areas in 1999. Analysing separately for species, blue tits occupied heated boxes significantly more than cold, but great tits exhibited no nestbox preference (table 4.1). Repeating the analysis with 'boxes occupied by blue tits' included as an effect on great tit occupation and vice versa, to control for effects of interspecific competition, gave the same results. Analysing treatment effects for years separately reveals no difference in nestbox occupation for either species in 1998, but again a significant tendency for blue tits to occupy a greater proportion of heated boxes in 1999. This lead to an overall preference for heated boxes in 1999 when data for both species were combined (table 4.1)

Likewise, for a given area subjected to different treatments across years, box occupation was higher in years when the area was hot-treated when both species were combined (t=-3.42, p=0.0056, d.f.=7) and for blue tits (t=-2.74, p=0.014, d.f.=7) but not for great tits (t=-1.34, p=0.111, d.f.=7).

Table 4.1: Numbers of blue and great tits nesting in heated (H) and cooled (F) nestboxes in 1998 and 1999 (absolute numbers, 4.1a), and effect of treatment on the proportion of nestboxes occupied in each group (4.1b).

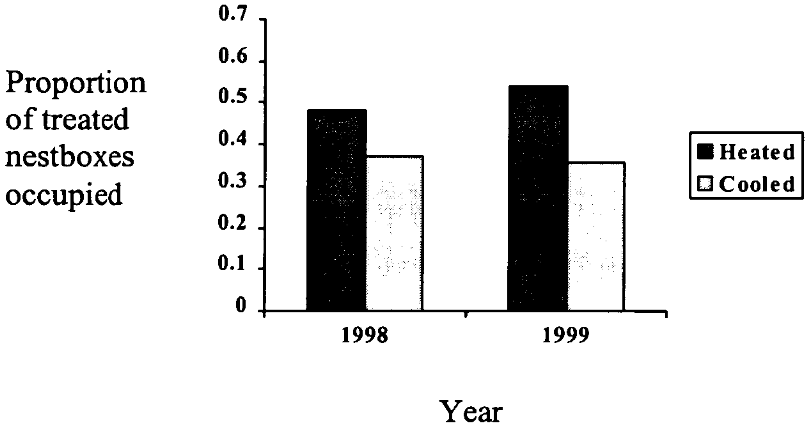
a) Numbers of nesting birds

Species	Number of occupied boxes			
	98		99	
	H	F	H	F
Blue tit	8	7	14	5
Great tit	18	16	19	14
Both	26	23	33	19

b) Difference in box occupation between heated and cooled boxes

Species	Factor	Effect		
		d.f.	F	*p*
	Including 'year' as a factor			
Blue tit	Area	7	0.871	0.575
	Yr	1	0.001	0.973
	Treatment	1	6.458	0.044*
Great tit	Area	7	3.469	0.075
	Year	1	0.036	0.855
	Treatment	1	1.546	0.260
Both	Area	7	1.714	0.264
	Year	1	0.399	0.551
	Treatment	1	10.697	0.017*
	Analysis for years separately			
Blue tit	Treatment 98	1	1.414	0.279
	Treatment 99	1	6.135	0.048*
Great tit	Treatment 98	1	0.823	0.399
	Treatment 99	1	0.087	0.779
Both	Treatment 98	1	1.976	0.209
	Treatment 99	1	6.443	0.044

Figure 4.4: Proportion of heated and cooled nestboxes occupied by great tits and blue tits combined for breeding attempts in 1998 and 1999



Timing of breeding and incubation

a) Great tits

In 1998 clutches were initiated earlier ($F_{1,38}$=5.111, p=0.0296) and hatched earlier ($F_{1,38}$=7.924, p=0.0077; see also table 4.2) than in 1999. Females which laid and hatched clutches later were in better body condition, measured as residual mass from a regression of mass on tarsus length (lay date: $F_{1,38}$=15.698, p=0.0003; hatch date: $F_{1,38}$=8.052, p=0.0073). Laying and hatching dates also varied between areas (lay date: $F_{7,38}$=2.925, p=0.0150; hatch date: $F_{7,38}$=2.924, p=0.0150). There was no effect of treatment on either laying or hatching dates (table 4.3).

Controlling for year differences, there was a significant effect of treatment on the number of days spent incubating by females ($F_{1,59}$=5.735, p=0.0198). This is apparent despite the lack of disparity between hot and cold nests in either lay or hatch date. Birds nesting in cold boxes incubated for longer than those in hot boxes in 1998 but not in 1999 (significant year*treatment interaction term: $F_{1,58}$=5.580, p=0.0215; see also table 4.2). Incubation duration also varied between woodland areas ($F_{8,51}$ = 2.664, p=0.0159), but the treatment effect remained even when controlling for area differences (table 4.3).

12 birds of 30 in the cool boxes had gaps in their laying sequence (i.e. missed laying an egg on one or more days), compared to 9 of 37 in the hot boxes. The difference is not significant (χ^2 likelihood ratio =1.697, p=0.1928). I also analysed the duration of gaps in days, rather than the presence or absence of gaps, using a two-sample t-test. I used a non-parametric test because 'number of gap days' were not normally distributed data (Shapiro Wilk W=0.527, p<0.0001). Comparing the mean days without laying between heated and cooled females reveals a non-significant trend for females nesting in cold boxes to stop laying for more days than those in hot boxes (t=1.677, p=0.0984, n=66).

b) Blue tits

Both laying and hatching dates of blue tits varied between years (lay date: $F_{1,33}$=12.021, p=0.0015; hatch date: $F_{1,32}$=9.477, p=0.0043), with birds breeding later in 1999, as for great tits (table 4.2). Timing of breeding was not affected by any other variable, including experimental treatment (table 4.3). No variable caused significant variation in the duration of the incubation period in blue tits. The number of nests where females had gaps in the laying sequence did not vary between hot and cold treatments (7/12 hot nests compared with 9/21 cold nests; χ^2 likelihood ratio =0.735, p=0.3914). Neither was there any difference in the mean number of days where laying was missed between females in cold boxes and females in hot boxes (t=-0.160, p=0.8739, n=33).

Table 4.2: Mean (± s.d.) laying dates, hatching dates and days of incubation for female a) great tits and b) blue tits nesting in heated and cooled nestboxes over 2 years.

Treatment	Lay date		Hatch date		Incubation time (days)	
	98	99	98	99	98	99
a) Great tits						
Cold	30.56 ± 3.63	32.10 ± 4.62	54.87 ± 3.60	56.79 ± 3.88	14.60 ± 1.72	15.93 ± 2.37
Hot	32.88 ± 3.66	32.97 ± 3.60	54.73 ± 3.83	56.18 ± 3.20	12.47 ± 1.68	15.32 ± 1.95
b) Blue tits						
Cold	30.57 ± 3.10	36.40 ± 2.79	57.00 ± 2.65	60.00 ± 4.00	14.29 ± 1.38	15.00 ± 2.94
Hot	32.22 ± 3.27	34.60 ± 2.82	57.88 ± 2.80	60.69 ± 2.59	14.38 ± 1.60	15.38 ± 1.71

Table 4.3: Heating and cooling treatment effects on laying parameters of great and blue tits after controlling for appropriate variables as below (see also results).

Response variable	Great tit			Blue tit		
	F	df	p	F	df	p
Timing of breeding						
Laying date	[1] 0.983	38	0.328	[3] 0.001	32	0.978
Hatching date	[1] 0.047	38	0.829	[3] 0.562	32	0.459
Incubation duration	[2] 4.782	51	0.036 *	2.052	31	0.162
Egg dimensions and number						
Egg mass	0.011	139	0.989	[4] 0.465	25	0.502
Egg length	0.331	134	0.719	[5] 0.000	25	0.994
Egg breadth	0.333	133	0.718	[5] 0.043	25	0.838
Egg volume	0.428	132	0.653	[5] 0.144	25	0.708
Clutch size	[2] 0.599	56	0.442	[6] 0.227	30	0.637

[1] Controlling for year and residual female mass effects
[2] Controlling for year and area effects
[3] Controlling for year effects
[4] Controlling for effects of female body size (tarsus length)
[5] Controlling for effects of female mass
[6] Controlling for year and lay date effects

Egg dimensions and number

a) Great tits

There were no effects of treatment on any egg measurements (table 4.3). There was a significant negative relationship between clutch size and egg length ($F_{1,136}$=6.5161, p=0.0118), suggesting a trade-off between the number and size of eggs produced. Clutch size varied between woodland areas and years (area: $F_{8,56}$=2.896, p=0.0090; year: $F_{1,56}$=11.364, p=0.0014), but was not affected by treatment (table 4.3).

b) Blue tits

All egg measurements (mass, length, bredth and volume) were associated positively with female size. A MANOVA including the dependent variables mean egg mass, volume, length and bredth demonstrates a significant effect of female mass ($F_{1,25}$=4.690, p=0.0401) and an almost significant effect of female tarsus length ($F_{1,25}$=3.893, p=0.0596) on egg sizes. Again there were no effects of treatment on any egg measurements (table 4.3). Clutch size was affected by year (larger clutches in 1998, $F_{1,33}$=5.339, p=0.0275) and lay date (decreasing clutch size as season progresses, $F_{1,33}$=6.408, p=0.0165) but not by treatment (table 4.3).

Hatching and fledging success

a) Great tits

There was a tendency for clutches in hot boxes to hatch a higher proportion of eggs compared to those in cold boxes (figure 4.4), but treatment did not explain significant variation in hatching success (ΔD=2.68, *n*=63, $p<0.15$, HF=2.04). Treatment did not effect fledging success (ΔD=0.28, *n*=55, ns, HF=2.10).

b) Blue tits

Hatching success in blue tits was affected by whether or not a female had gaps in her egg laying sequence (ΔD=4.25, *n*=34, $p<0.05$, HF=1.46). In 16 nests with no laying gaps the mean proportion of eggs hatching was 0.93 (±0.08), compared to 0.81 (±0.15) in nests where the female had laying gaps (*n*=15). No variables explained variation in fledging success. Treatment affected neither occurrence of laying gaps, nor hatching or fledging success (hatching: ΔD=0.06, *n*=31, ns; HF=1.46; fledging: ΔD=0.83, *n*=29, ns, HF=1.70).

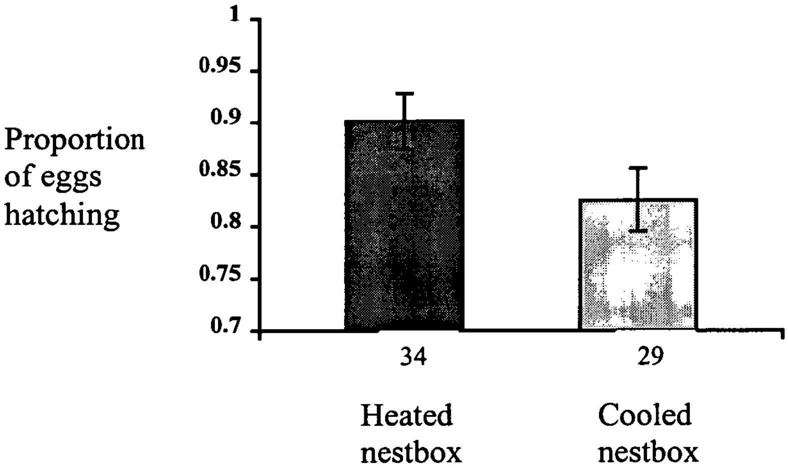


Figure 4.4: Mean hatching success (± s.e.) of great tit clutches laid by females in nestboxes that were heated and cooled prior to and during laying. Sample sizes for each group given under bars.

Sex ratio

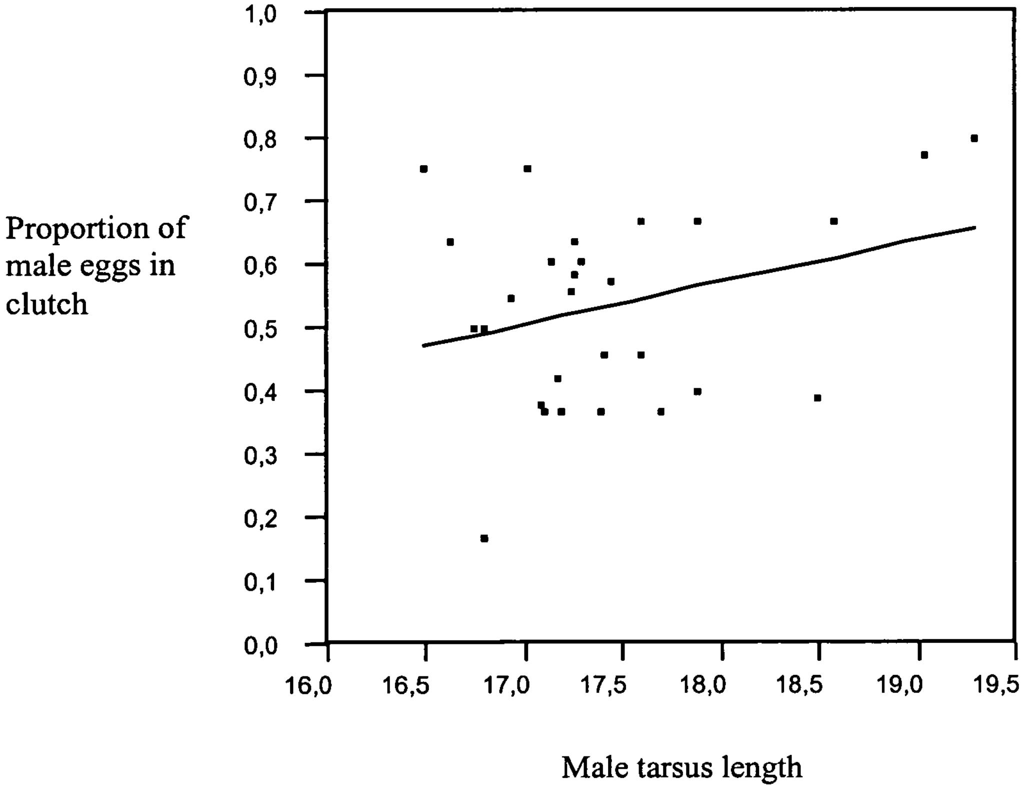
The sex ratio of both blue and great tit broods was not affected by treatment (table 4.4). For great tits, no other variable explained significant variation in egg sex ratios.

Although the same is statistically true for blue tits (table 4.4), there was a tendency for the proportion of males in a clutch to increase with male tarsus length. Performing a linear regression of proportion of male eggs laid in relation to male tarsus length suggests that 9% of the variation in clutch sex ratio in blue tits might be attributable to paternal body size measured as tarsus length (figure 4.5).

Table 4.4: Factors potentially explaining sex ratio variation in great and blue tits. Treatment refers to heating and cooling of nestboxes. Factors were added to the model and removed stepwise.

Factor	Great tits			Blue tits		
	d.f.	ΔD	*P*	d.f.	ΔD	*p*
Year	1	0.297	>0.50	1	0.009	>0.90
CS	1	0.641	>0.30	1	0.720	>0.30
LD	1	0.059	>0.80	1	0.643	>0.30
Male tarsus length	1	0.295	>0.50	1	3.371	>0.05
Male residual mass	1	0.001	>0.95	1	1.241	>0.20
Female tarsus length	1	0.018	>0.80	1	1.441	>0.20
Female residual mass	1	0.861	>0.30	1	0.275	>0.50
Treatment	1	0.690	>0.30	1	0.441	>0.50

Figure 4.5: Relationship between the proportion of male eggs in a clutch and paternal tarsus length in blue tits, *n*=28.



Discussion

Night-time temperatures experienced by roosting great and blue tits were experimentally manipulated during a two-year study on effects of energy available to egg-forming and laying females. I found no evidence of alterations to either timing of breeding or female investment in broods as measured by size and weight of eggs, clutch size and sex ratio of broods with respect to experimental treatment (table 4.3). This was true for both species. I did find that great tits in cooled boxes incubated these clutches for longer compared to those in warmed boxes.

These results suggest that egg volumes, clutch sizes, brood sex ratios and timing of breeding are traits which females do not trade-off against their own body maintenance. The fact that these traits are not compromised indicates their importance for successful production of young. Alternative explanations for the lack of variation in these traits include: a) the temperature manipulations were not sufficient to alter female energy budgets and consequently change breeding behaviour, or b) females did modify breeding parameters but statistical tests were not adequate to detect such alterations. I shall examine each in turn.

The first argument supposes that the experimental temperatures experienced by breeding female great tits did not effect their energy budgets and hence breeding decisions. However, changes in female behaviour suggest that this was probably not the case. Both great and blue tit females in heated nestboxes built their nestcups as close as possible to the heat source near the rear chamber, but against the front wall of the nestbox as far as possible from the cold packs in cooled boxes (*pers. obs.*). The same behaviour was noted by Nager & van Noordwijk (1992) in a similar experiment. Furthermore, female blue tits showed a preference for nesting in heated boxes and there was a trend (p=0.11) in the same direction for great tits. Quantitative evidence of heating and cooling effects on females was also found. Great tits in heated boxes incubated clutches for a shorter period and tended to have fewer laying gaps than those in cooled boxes. Increased incubation times were not observed in blue tits, perhaps because blue tit females avoided this physiological consequence of nestbox cooling by avoiding nesting in cooled boxes altogether (table 4.1). Blue tit incubation times may be less plastic than those of great tits, given the extra time constraint imposed through laying larger clutches of eggs. For great tits however, this result may reflect that fact that incubation duration and the presence of laying gaps are less important in determining fitness than clutch size, egg volume, and timing of breeding. As such these traits may be traded off against increased investment in the female's own body condition (and possible subsequent reproduction/survival), with less detriment to the reproductive value of the breeding attempt.

Two previous experimental manipulations of breeding great tits found changes in incubation times and lying gaps of female tits in heated nestboxes in the same direction as the present study. Bryan & Bryant (1999) demonstrated that female great tits nesting in heated boxes spend 55 minutes longer each day incubating eggs than those in unheated nests. They concluded that females are able to allocate this time to incubation rather than foraging because of reduced thermostatic demands. Applying their result over the whole incubation period would result in a reduction of incubation time of between 10.1 and 21.1 hours (minimum 11 days and maximum 23 days) for birds in heated compared to cooled boxes. This is similar to the difference in incubation times found for great tits in this study (table 4.2).

Yom-Tov & Wright (1993) found a significantly lower proportion of laying gaps amongst females in heated nestboxes compared to control nests. I found that female great tits in warm boxes tended to have fewer laying gaps, although this tendency was not statistically significant after controlling for female body condition. In blue tits, nests with more laying gaps had decreased hatching success. Increasing laying gaps and reduced hatching success of clutches may both reflect poor female body condition, although this could not be measured at the time of egg laying and incubation (see methods). If the cooling treatment did adversely effect female condition at the time of laying, these results would be consistent with the idea that females in cold boxes must allocate more time to self-investment at the expense of their clutch.

The second explanation as to why I did not find a differences egg volume, clutch size, brood sex ratio and timing of breeding among birds is that the sample sizes may not have been powerful enough to detect experimental effects with reasonable statistical power. Nager & van Noordwijk's (1992) great tit study also found no difference in laying dates between females in heated and cooled nestboxes. However, despite a smaller temperature manipulation than the present study (2.89°C c.f. 4.69°C), they did reveal a 14% reduction in egg volume. Using the mean egg volumes observed in their study, power analysis reveals the probability of my detecting a treatment effect in this study with 37 heated and 30 cooled nests to be 1 (effect size=0.8169, α=0.05). In other words, if my population were behaving in the same way as Nager & van Noordwijk's, I would be highly likely to have detected such an effect in my data. Further power analysis suggest that my experiment would have detected a 0.1mm^3 difference in egg volume with a power of 0.8.

Assuming that the Nager & van Noordwijk effect is not a Type I error, then the different results between the two studies must be explained. It may be that populations of great tits in Switzerland are particularly sensitive to temperature changes; certainly great tit populations throughout Europe are not consistent in their timing of breeding patterns and clutch sizes (e.g. see Lambrechts *et. al.* 1997 for review). It could also be true that whilst females of the Gotland population compensate for energetic demands by prolonged incubation times, females of the Swiss population may instead trade-off egg volume for self-investment and maintenance.

Results of the present study also highlight the importance of year differences, as these were the only factor to explain variation in timing of breeding. In 1998, ambient temperature rose steadily with date, but in 1999 a drop in temperature over the last days of laying and first days of incubation lead to most individuals abandoning incubation or delaying its onset (figure 4.3). This effectively standardised hatching dates, or led to abandonment of clutches. If 1990 and 1991 were particularly cold years in Switzerland, Nager & van Noordwijk could have observed a reduction in egg volumes which would have gone undetected in warmer years. A recent publication shows that the Nager and van Noordwijk experiment was repeated again in 1992, these data being analysed with respect to potential changes in timing of breeding (Nager *et al.* 2000). If egg volumes were also measured in this year, and year differences are indeed important, then perhaps addition of egg volumes from 1992 to the 1990 and 1991 data would result in no significant effects of nestbox heating on egg volumes?

It is evident from the present study that manipulation of a breeding female's thermal environment does affect some aspects of her reproductive behaviour. However, it is also clear that clutch sex ratios were not affected by the experimental temperature manipulation. It is possible that females were not responding to temperatures encountered within the nestbox at night, but instead may have been affected by ambient temperatures experienced during the day. As all females, regardless of their roosting nestbox conditions, experienced the same ambient daily temperatures on Gotland, this could account for the lack of difference in breeding parameters between those nesting in cooled and those nesting in heated boxes. Analysing variation in breeding measures in relation to ambient temperatures revealed no variation in any measure with respect to mean daily temperatures. Nevertheless, the fact that year differences alone explained variation in timing of breeding suggests that females might indeed be sensitive to ambient temperature differences between years.

Considering the two pathways which might lead to sex ratio biasing (figure 4.1), relatively high heritability of egg size may have nullified the 'egg volume constraint' pathway. Previous estimates of egg volume heritabilities in great tit populations have been high, estimating h^2 as 0.72 ±0.22 (Jones 1973), 0.86 .±0.29 (Ojanen *et. al.* 1979), 0.66 ±0.24 to 0.72 ±0.30 (van Noordwijk *et. al.* 1981) and 0.81 ±0.28 (Hõrak *et. al.* 1995), although this does not necessarily exclude common environmental effects acting on egg sizes in females. Hõrak *et. al.* (1995) conclude from their study that 'egg size in the Great Tit is highly heritable but also sensitive to the female condition', and substantial evidence does exist that environmental conditions during oogenesis at least in part determine avian egg size (Murphy 1986, Slagsvold and Lifjeld 1989, Potti 1993, Perrins 1996). In this study no egg measurements varied with treatment, thus we cannot expect sex ratios to vary following the egg volume constraint pathway. The only route by which sex ratios could have been manipulated is through a Trivers and Willard type argument.

The 'Trivers and Willard' mechanism may not have occurred because of inadequate selection for sex ratio biases if the reproductive value of sons and daughters did not differ. To test this principal in general would require knowledge of the breeding success of male and female offspring, i.e. production of grand-offspring through each sex, and how this varied with environmental gradients (see section 1.1.iii). Although female condition at the time of laying could not be measured (see methods), the fact that incubation times were affected by the temperature manipulations and female behaviour changed in response to heating and cooling, suggests that the treatment affected female energy budgets to some extent. Females in poor condition during laying are assumed to be in poor condition during rearing, as the demands of incubation allow females little time to recover condition. The relationship between mother's body condition and the differential value of male and female offspring needs to be established to test this hypothesis.

Interestingly, there was some suggestion that female blue tits might be able to modify egg sex ratios. There was a marginally significant effect of male tarsus length on clutch sex ratios in blue tits. A similar relationship has been demonstrated in great tits (Kölliker *et al.* 1999), and female blue tits have been found to modify their sex ratios in relation to male characteristics in two other studies (Svensson & Nilsson 1996, Sheldon *et al.* 1999, however see Leech *et al.*, submitted ms). These studies found the proportion of sons amongst broods to vary positively with the secondary sexual characteristic of plumage reflectance (Sheldon *et al.* 1999), and to increase with paternal survival prospects (Svensson & Nilsson 1996). The relationships are consistent with adaptive explanations of sex biases if male offspring benefit more from being fathered by larger or better quality males. Further investigations of this relationship with larger sample sizes, and estimates of sex-specific reproductive success of offspring, might uncover an interesting pattern of sex ratio manipulation and its potentially adaptive value.

II. POST-HATCHING SEX RATIO BIASING

5. Sex ratio biases during the nestling period: proximate and ultimate explanations

Manipulating egg sex ratio is just one way in which mothers may bias investment in sons and daughters. Differential sex allocation can also be achieved through biasing offspring sex ratios during the period of parental care, by investing more in one sex. In this case, both male and female parents have the potential to manipulate care given to nestlings, and some evidence already suggests asymmetries in feeding rates of parents with respect to the sex of their offspring (Stamps *et al.* 1987, Gowaty & Droge 1991, Clotfelter 1996, Nishiumi *et al.* 1996, see also Lessells 1998). Theories of sex allocation typically assume parental control of the sex ratio (see chapter 1). However nestling characteristics may also be important in determining the care each offspring receives, and such characteristics may be specific to sex. In this chapter I investigate the importance of sex-specific size differences amongst nestlings and the possible consequences of this for brood sex ratios.

In sexually dimorphic animals, two non-exclusive hypotheses have been proposed to explain sex ratio biases towards the smaller sex at the end of the period of parental care. Firstly, natural selection for equal allocation to male and female offspring will result in biased sex ratios when the cost of rearing each sex differs (as expected for dimorphic species; Fisher 1930). Secondly, sexual selection for larger size of one sex in adulthood may necessitate larger size of that sex during the period of parental investment. Consequent non-adaptive mortality of the larger sex due to higher energy demands which cannot be met by parents then results in sex-biased mortality (Clutton-Brock *et al.* 1985). Both processes can potanitally result in the overproduction of the smaller sex via the same mechanism, namely increased mortality of the larger sex.

Studies of altricial birds have tested for sex ratio biases in dimorphic species, considering both of the above theories as possible causes of sex ratio biases (e.g. Weatherhead & Teather 1991). However, although a few have documented sex ratio biases towards the smaller sex in stressful rearing conditions (Howe 1977, Cronmiller & Thompson 1981, Røskaft & Slagsvold 1985), most found no deviation from unity in sex ratios despite considerable sexual dimorphism of the study species (Selander 1960, Richter 1983, Weatherhead 1983, for reviews see Newton 1979, Clutton-Brock 1986). As it seems reasonable to expect that offspring size dimorphism results in different rearing costs of males and females or unequal nutritional requirements, the mismatch of empirical results with theoretical predictions points to other processes in the nest counterbalancing sex-biased starvation. The greater size of one sex may enable it to reach higher towards the parent, occupy favoured feeding positions, or push smaller nest-mates away from food deliveries. Through such competitive advantages, larger offspring may be able to gain additional food they require for growth and maintenance.

I investigate the importance of size and sex in competitive chick interactions in a large-brooded, synchronously hatching (within 2 days) species, the great tit *Parus major*, where competition for food is likely to be especially intense. Mean clutch size is 9.1 eggs (±1.16 s.d., n=273), and nestlings are provisioned for 17-22 days, with an average of 5.9 fledglings (±3.2 s.d., n=96) produced per nest. In this population adult great tits exhibit 7% sexual dimorphism in mass and 3.5 – 4% skeletal size dimorphism (tarsus and wing lengths; males>females), with size differences statistically apparent amongst nestlings from 5 days of age (this study). Males are expected to have higher nutritional requirements for maintenance and growth, and thus female-biased sex ratios are predicted if these requirements cannot be met. Studies measuring energetic requirements of sexually size-dimorphic blackbirds (Icteridae) have shown that in these species males (larger) do indeed have higher energy demands than females (Fiala & Congdon 1983, Teather & Weatherhead 1988). Similarly, greater energetic demands of the larger sex (females) have been directly measured in marsh harriers (Riedstra *et al.* 1998). In great tits, contrary to the expectation that under poor rearing conditions males perform relatively worse then their sisters, several studies have demonstrated male-biased sex ratios amongst nestlings reared in poor environments. Dhondt (1970) found that in both areas and periods unfavourable for nesting great tits significantly more males fledged. In a brood size manipulation experiment, Smith *et al.* (1989) found proportionally more males surviving from enlarged than reduced broods. Lessells *et al.* (1996) found that the proportion of males hatching in a clutch increases with laying date (later hatching broods are less successful and are expected to experience less food abundance). Furthermore, they found more males recruiting from nests with higher nestling mortality. An earlier study of the same population of tits similarly found a male-biased sex ratio amongst fledglings when nestling mortality was higher (Drent 1984).

These studies demonstrate a biased sex ratio contrary to that predicted by theory, and no study, to my knowledge, has reported the reverse pattern in great tits. Even if there is no difference in the cost of rearing male and female nestling great tits, as a recent study suggests (Lessells *et al.* 1998), we would expect to observe neither a male nor female biased sex ratio. I suggest that the observed male biases are due to the enhanced competitive ability of males due to their larger size. Using a cross-fostering experimental design to establish an obvious size difference within a brood, growth and survival of different sized nestlings of different sexes was investigated. By comparing nestling growth between 'small' males and females competing with 'large' males and females in the same nest, I test whether males indeed fare better than their sisters in conditions of extreme sibling competition.

Methods

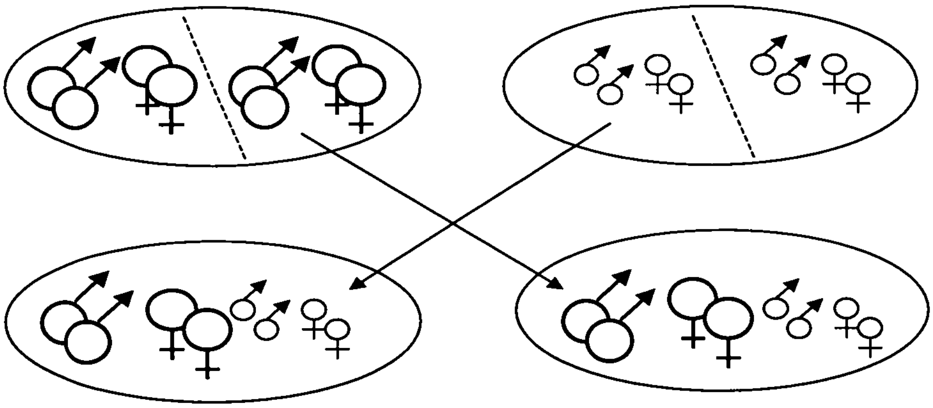
Cross-fostering manipulation

Nest-box breeding great tits on the Swedish island of Gotland (57°10'N, 18°20'E) were experimentally manipulated for this study in 1997 and 1998. Field data in 1997 were collected with the help of M.Incagli. Nest-boxes were monitored for nest establishment and egg-laying, and daily nest-box checks allowed exact hatching dates to be determined. Following hatching, a small amount of blood (1-5µl) was collected from the brachial vein of each chick on day 1 (day of first egg hatching = day 0) for sex determination using PCR-based molecular techniques (Griffiths *et al.* 1998). Blood was stored in 50µl SET buffer at -20°C (1997) or 98% alcohol (1998). Individual nestlings were matched to their blood samples by a unique identification code created by selective clipping of one or two of their six down tracts.

The size of nestlings within a brood was experimentally manipulated by moving chicks of different ages between nests. Nests with similar brood size (same or ± 1 nestling) at hatching were paired for cross-fostering generally when nestlings in one nest were two days old and in the other four days old. Half of the 'large' brood was then swapped with half of the 'small' brood, creating two nests each with half 'large' and half 'small' nestlings (see Fig. 1.) Sex of nestlings was not known at the time of swap, but the large brood size (mean = 8.33 at time of cross-fostering) in this species meant that in the majority of cases nestlings of both sexes would be present in each group. Chicks were selected for swapping on the basis of which down tract was clipped; for each manipulation I selected nestlings for moving starting with the next consecutive letter code to the last swap in order to randomise which nestlings were relocated. Brood sizes did not change except in two pairs of nests where brood size increased or decreased by one nestling in each nest. In total 42 pairs of nests were created (17 in 1997 and 25 in 1998).

Of importance when swapping was the size difference between nestlings rather than age *per se*. Therefore in six of the 42 pairs, nestlings of three and five days were swapped (especially at the beginning of the season when nestlings grew slowly) or three with four day olds etc. Nests with hatching asynchrony of more than 1 day were not used. In this population eggs sometimes hatch one or two days after the first hatching date. Size manipulations thus reflected possible natural variation in sibling size, and competitive situations which may be naturally encountered by last-hatching nestlings. If it was obvious that the size difference between nestlings was too extreme for smaller nestlings to survive, the swap was not carried out. As all nestlings were bled on day one, nestlings were not bled and transported on the same day.

Figure 5.1: Representation of cross-fostering design to create broods (n=84) of mixed size *Parus major* nestlings. Typically half a brood of four day old nestlings were swapped with half a brood of two day old nestlings. Sex of nestling was not known at swap, rather the proportion of one sex moved was determined by chance.



Morphometric measures

The following morphological measurements were recorded at the time of cross-fostering: mass (using 10g pesola spring balance accurate to 0.1g), tarsus length and gape length (using dial callipers to nearest 0.5mm). Gape length measures from one end of the mouth crease to the point of the bill, and increases with nestling age as the bill develops. Measures were repeated five days after experimental manipulation (n=80, i.e. nestlings aged 7 and 9 days), ten days after manipulation (n=39, i.e. nestlings aged 12 and 14 days), and 12 days after manipulation (n=72, nestlings aged 14 and 16 days). Nestling mass of great tits on day 15 after hatching is generally the same as that at fledging (van Balen 1973, Schifferli 1972), and the last set of measurements were taken to reflect condition at fledging. Originally measurements were taken 10 days following manipulation, but it was soon recognised that nestlings could be safely measured two days later without causing premature fledging. Because of time constraints, nestling measures at day 12 post manipulation were prioritised, explaining the small sample size for day 10 measures. Other sample sizes less than 84 represent whole brood failures or predation. On days 10 and 12 post-manipulation, wing development was substantial enough that wing length was additionally measured to the nearest 0.5mm. Nestlings were banded with aluminium rings, matching down clipping identification marks to ring number. Where nestlings were not aged 2 and 4 days when swapped, measures were not always taken five, 10 or 12 days after swapping, but on days when nestlings were 7/9, 12/14 or 14/16 days old (or as near as possible).

After fledging, boxes were checked for individuals failing to fledge. Birds recruiting to this population in 1998 and 1999 were recorded by catching pairs breeding in any of the ~1000 nestboxes in the surrounding woodlands using either nest-box traps or mist-nets. Between 170 and 240 pairs of great tits breed annually in the nest-boxes on Gotland. In 1998, 72.1% and 76.9% of all breeding males and females were caught respectively, and in 1999 83.5% males and 85.3% females were caught. Although it is possible that recruiting individuals may breed in natural holes as well as nestboxes, there is no reason to believe why nestlings of either sex from either experimental category should preferentially nest in natural cavities. Thus I expect no sampling bias in recruits from each treatment due to recruitment of individuals outside nestboxes, which could not be measured.

Sex identification

Nestling sex was determined by PCR amplification of two CHD genes located on the sex chromosomes. Two copies of the gene are present in females (CHD1W, present on the W chromosome and CHD1Z, present on the Z chromosome), whereas only one copy (CHD1Z) is present in homogametic males. After DNA extraction from blood samples (5% chelex extraction) these sex-specific fragments of the CHD gene were amplified using primers P2/P8 and PCR conditions as described in Griffiths *et. al.*, 1998. Products were run on 6% polyacrylamide gel and visualised using silver staining *(Promega)*. I lacked samples for 25/699 (3.6%) nestlings and failed to determine sex in a further 2 (0.3%) cases. From 54 blood samples of adults (28 males, 26 females) and 20 recruits (12 males, 8 females) where I determined phenotypic sex in the field, genetic sex determined by molecular methods matched in all 74 cases.

Data analysis

Comparison of male and female sizes (mass, tarsus length, wing length, gape length) among both small and large nestlings were made using paired t-tests of sib-group means. Analyses were carried out to look for effects of nestling sex and size ('large' vs. 'small' nestlings, according to box of origin) on survival and morphological measures. To determine whether there was a different effect of being large or small for each sex of individual on growth, the interaction term size*sex was entered in a general linear model with morphological measurements (tarsus, mass, gape length, wing) as dependent variables and size and sex as factors. The unit of analysis was each nestling, but because nestlings are reared non- independently in a common environment, box of rearing was included as a factor in the model to control for differences between nests due to parental/territory quality and year. Because sizes of nestlings at swapping would influence subsequent measures, initial mass and tarsus measures were included as covariates. Timing of breeding was found to have a significant effect on growth measures and this variation was removed from the model by including clutch initiation date as a covariate. Analyses were carried out separately for each set of chick measures (on days five, ten and twelve following manipulation) using procedure MANOVA in the statistical package JMP Statistical Discovery Software Version 3.1.

Logistic regression was used to assess whether survival of nestlings to day five, ten and twelve following brood manipulation was a function of their sex, size and the interaction of the two. The same analysis was used to examine effects of an individual's size and sex on survival to fledging. From 75 nests (excluding four predated nests and five total nest failures, total number of nestlings=633), 79% of nestlings fledged. As survival differs greatly between nest-boxes, the environmental factor 'box of rearing' was added to the model as a random effect. Likelihood ratio tests were used to determine the significance of a variable, entering variables 'sex', 'size', 'size*sex' and 'box of rearing' in the model simultaneously. Since controlling for timing of breeding (lay date) and cross-fostering had no effect on the outcome of tests, these variables were not included in results presented here in order to keep the model as simple as possible. The number of birds recruiting from experimental nests in 1998 and 1999 in relation to their size and sex was analysed similarly.

Results

The 699 nesltings involved in the study fell into the following categories: 161 small males, 175 small females, 156 large males and 182 large females (25 unsexed).

Nestling growth

Development of sexual dimorphism

For all measurements post cross-fostering where sexual dimorphism was detected, males were larger than females (Tables 5.1a and 5.1b). At the time of manipulation, there was no difference in any size measure between small (2 day old) male and female nestlings. Amongst small nestlings, by day five post-manipulation, males had larger tarsi (t=-2.06, d.f.=46, p=0.045). By day 10 post-manipulation sexual differences in tarsus length remained (t=-3.61, d.f.=20, p=0.002) and a difference in body mass was also apparent (t=-2.23, d.f.=20, p=0.038). Wing length was marginally non-significantly different in males and females (t=-2.02, d.f.=20, p=0.057). Near fledging (12 days post manipulation) male nestlings were larger than females for all measures (mass: t=-3.29, d.f.=35, p=0.002; tarsus: t=-5.77, d.f.=35, p=0.000; gape length: t=-2.32, d.f.=35, p=0.026; wing length: t=-2.20, d.f.=35, p=0.034).

Amongst large nestlings, females had longer gapes than males at swap (4 days old; t=2.93, d.f.=68, p=0.005) but other measures did not differ (mass: t=0.58, d.f.=68, p=0.561, tarsus: t=1.17, d.f.=68, p=0.248). By day five after manipulation, males were heavier (t=-2.6, d.f.=63, p=0.012) than females and gape length differences were no longer apparent. The sex-specific mass difference remained 10 days post-manipulation (t=-4.39, d.f.=27, p=0.000) when males also had longer tarsi (t=-4.56, d.f.=27, p=0.000). There was non-significant tendency for males to have longer wings than females at this time (t=-1.87, d.f.=27, p=0.072). Near fledging males were heavier (t=-5.54, d.f.=55, p=0.000) and had longer tarsi (t=-5.53, d.f.=55, p=0.000). There was still a tendency for males to have longer wings than females but this was not statistically significant (t=-1.91, d.f.=55, p=0.061), and there was no apparent sexual size dimorphism in gape length (t=-1.28, d.f.=55, p=0.206).

Table 5.1a. Small nestlings

Day post-manipulation	Age (days)	n	Mass	Tarsus	Gape length	Wing
0	2	65	-0.10	-0.10	-0.72	
5	7	47	+6.04	+2.60*	-0.25	
10	12	21	+8.03*	+5.78**	+0.01	+4.15
12	14	36	+5.80**	+3.69***	+0.92*	+2.66*

Table 5.1b. Large nestlings

Day post-manipulation	Age (days)	n	Mass	Tarsus	Gape length	Wing
0	4	69	-1.32	-1.33	-2.28**	
5	9	64	+3.82*	+1.14	-0.77	
10	14	28	+4.00***	+2.24***	+0.04	+1.82
12	16	56	+4.64***	+2.19***	+0.44	+1.50

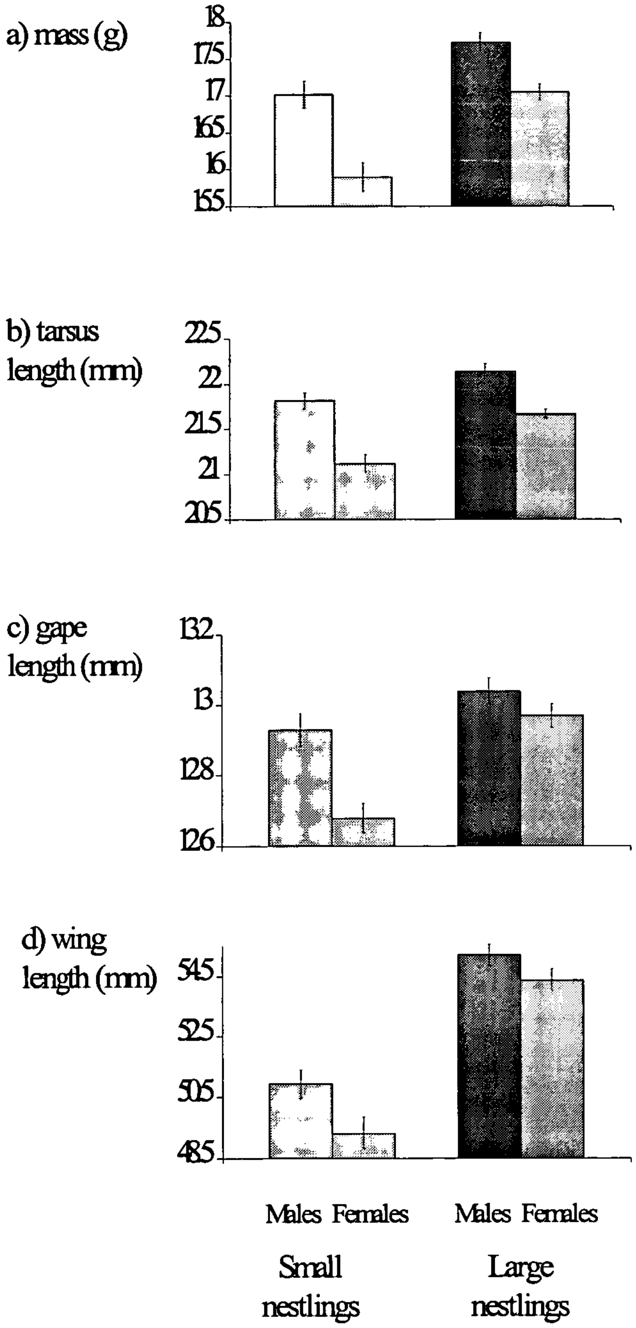
Tables 5.1a and 5.1b. Size dimorphism (%) between male and female great tit nestlings following cross-fostering manipulation of half a brood of small nestlings with half a brood of large nestlings. Values for small nestlings in Table 1a, large nestlings in Table 1b. Both nestlings which were moved and those which stayed in their natal nest are included. Positive values indicate males larger than females, negative values females larger than males. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$. For statistical tests see text). Sample sizes on day 0 deviate from the original 84 manipulated nests because not every nest was composed of nestlings from each category. Sample sizes on days five, 10 and 12 differ as nestlings died, leaving some categories empty, and because not all nests were visited on each measurement day to record nestling sizes (see methods: morphometric measures).

Comparison of size differences in large and small nestlings

In order to compare sexual size differences between large and small nestlings, the interaction term of the effects 'size category' and 'sex' was determined in a multiple analysis of variance of nestling measures. After five days of growth with size-manipulated nest-mates, the size difference between males and females was the same amongst small nestlings as that for large nestlings (size*sex, $F_{1,432}$=1.505, p=0.221). However, 12 days following manipulation, there was a significant difference between the sexual dimorphism amongst the large nestlings compared to the small nestlings (size*sex, $F_{1,354}$=5.434, p=0.020). Ten days following manipulation the size*sex term was marginally non-significant ($F_{1,195}$=2.822, p=0.095), perhaps due to smaller sample sizes. The significant effect on day 12 post-manipulation was mainly due to an effect on wing length (dropping wing length from analysis: size*sex, $F_{1,354}$=3.586, p=0.059). Significance remained when any other dependent variables were removed from the model (mass: $F_{1,354}$=5.900, p=0.016, tarsus: $F_{1,354}$=5.085, p=0.025, gape length: $F_{1,354}$=5.018, p=0.026).

Nestling measures both ten and twelve days following manipulation reveal a greater sexual size difference amongst small nestlings compared to the size difference in large nestlings. Amongst small nestlings, females are smaller than males to a greater degree than amongst large nestlings (Fig. 5.2).

Figure 5.2 (overleaf): Size differences between male and female great tit nestlings in cross-fostered groups of 'small' and 'large' offspring, 12 days following experimental manipulation. Small nestlings are thus typically 14 days old, large nestlings 16 days old. Graphs illustrate mean nestling a) mass (g), b) tarsus length (mm), c) gape length (mm) and d) wing length (mm) with standard errors.


a) mass (g)
18
17.5
17
16.5
16
15.5
b) tarsus length (mm)
22.5
22
21.5
21
20.5
c) gape length (mm)
13.2
13
12.8
12.6
d) wing length (mm)
54.5
52.5
50.5
48.5
Males
Females
Males
Females
Small nestlings
Large nestlings

Nestling mortality

Parental and/or territory quality was a strong influence on nestling survival, with nest of rearing a significant effect on survival of nestlings to all ages. The size category of nestlings 'large' or 'small' was also a predictor of survival, with larger nestlings clearly suffering less mortality (Table 5.2). Nestling survival was not affected by nestling sex, and there was no different effect of nestling sex among large and small young (non-significant size*sex interaction; Table 5.2).

Recruitment

20 birds recruited to the breeding population (3.1%), 15 from 1997 and five from 1998. The size category of a nestling in the experimental manipulation did not affect its chances of recruiting (χ^2=0.00, p=0.99, d.f.=1), nor did any morphological measure at fledging predict recruitment probability (mass: χ^2=2.250, p=0.134, d.f.=1; tarsus: χ^2=2.642, p=0.104, d.f.=1; wing: χ^2=0.627, p=0.429, d.f.=1; gape length: χ^2=1.143, p=0.285, d.f.=1). Body condition, measured as residual mass at fledging for a given body size (tarsus length) was almost a significant predictor of nestling recruitment (χ^2=3.038, p=0.081, d.f.=1). Comparing the masses of individuals recruiting to those not, recruiting nestlings had higher mean absolute body weights (recruits: 17.28 ± 0.40g; non-recruits 16.94 ± 0.08g) and higher weights relative to body size (recruits: 0.50 ± 0.29g; non-recruits: -0.02 ± 0.06g) although these differences are not statistically significant (day 12 mass: $F_{1,444}$=0.688, p=0.407, residual day 12 mass: $F_{1,444}$=3.008, p=0.083). The recruits consisted of 12 males and 8 females; this difference was not statistically significant (χ^2=1.322, p=0.245, d.f.=1). Finally, the interaction effect size*sex on recruitment was not significant (χ^2=0.020, p=0.881, d.f.=1).

Time post Manipulation	Proportion of nestlings dying		(n)	Predictors of nestling survival	d.f.	Likelihood Ratio χ^2	P ($>\chi^2$)
5 days	Large		312	Nest of rearing	73	184.04	<0.001
	males	.09	13	Size	1	50.006	<0.001
	females	.07	12	Sex	1	0.412	0.52
	Small		321	Size*Sex	1	0.145	0.70
	males	.29	45				
	females	.27	45				
10 days	Large		312	Nest of rearing	73	190.30	<0.001
	males	.09	13	Size	1	60.581	<0.001
	females	.07	12	Sex	1	0.462	0.50
	Small		321	Size*Sex	1	0.073	0.79
	males	.32	50				
	females	.29	48				
12 days	Large		312	Nest of rearing	73	208.32	<0.001
	males	.10	14	Size	1	56.271	<0.001
	females	.10	18	Sex	1	0.108	0.74
	Small		321	Size*Sex	1	0.177	0.67
	males	.34	53				
	females	.34	56				
Fledging	Large		312	Nest of rearing	73	201.63	<0.001
	males	.10	14	Size	1	61.012	<0.001
	females	.11	18	Sex	1	0.037	0.85
	Small		321	Size*Sex	1	1.016	0.31
	males	.35	55				
	females	.34	56				

Table 5.2 Proportion of great tit nestlings dying and predictors of survival. Logistic regression relating nestling mortality to nestling size and sex and size*sex interaction, following brood composition manipulation (see methods). Original number of nestlings in each treatment group in nests without total nest failure/predation: large males 143, large females 169, small males 156, small females 165.

Discussion

For a species where males are larger than females, one would expect a female sex ratio bias at the end of the period of investment, assuming the smaller sex to be less costly to rear (Fisher 1930, Maynard-Smith 1980). Several studies of great tits have reported the opposite to be true in poor conditions (Dhondt 1970, Drent 1984, Smith *et. al.* 1989, Lessells *et. al.* 1996), and this study highlights the importance of size dominance of larger offspring which could account for this observed discrepancy. In experimentally manipulated broods of great tits comprising half 'large' and half 'small' nestlings, sexual size dimorphism was less marked for large individuals than for small individuals. When competition for food is relaxed, i.e. amongst large nestlings, males and females attain more similar growth measures than when competition is pronounced (small nestlings). The advantage of 'being male' - attaining higher weights and larger biometric sizes than sisters - is particularly important for small nestlings who face an extremely competitive nest environment. Larger individuals may be more successful at obtaining food by reaching closer to parents when begging, pushing smaller competitors away from access to parents, and occupying preferred feeding positions in the nest (Rydén & Bengtsson, 1980, Bengtsson & Rydén 1983, Kölliker *et .al.* 1998).

The idea that females are at a competitive disadvantage with larger nestmates is supported by the sexual size differences observed between nestlings (Tables 5.1a and 5.1b). By fledging, small males and females differed significantly in every dimension measured (mass, tarsus length, wing length, gape length), but amongst large nestlings only in mass and tarsus length. Moreover, the degree of dimorphism is greater amongst small nestlings. Small females must compete with 3 classes of nestling, all larger than themselves: large males, large females and small males. Large females on the other hand must compete only with one class of nestling larger than themselves (large males).

It could be argued that size treatment in this experiment is confounded with age, and that sexual size dimorphism initially increases to a certain nestling age, but then declines. In this way it would be possible to generate the observed results of size dimorphism more apparent amongst smaller nestlings simply as a function of their age. However, it is biologically unlikely that sexual size differences would develop and then diminish, and the significant size*sex interaction recorded 12 days post-manipulation was also detected 10 days post-manipulation, though not statistically significant. Size dimorphism could initially increase then decrease if males were to reach their asymptotic size before females. However, in a review of sexually dimorphic species, Richner (1991) found no evidence for this, but that there is no difference between time for each sex to reach its asymptote or the smaller sex reaches asymptotic body mass quicker.

The effect reported here of males faring better than their sisters is opposite to that expected from a simple nutritional dependence. It is also contrary to studies of sexually dimorphic mammals demonstrating higher mortality of the larger sex (Clutton-Brock *et. al.* 1985, Clutton-Brock 1991). Studies revealing sex-biased mortality of sexually dimorphic mammals typically deal with organisms with a litter size of one (ungulates, primates, man), where there is no sibling competition. In multiparous, sexually size dimorphic organisms, sibling competition may represent a considerable influence on the sex ratio at the end of the period of care. The effect of sibling interactions on the relative reproductive value of sons and daughters has not received much attention in investigations of parental care and offspring sex ratios. However, such interactions may change sex allocation optima and our predictions about sex ratio biases. Discussion of this idea is expanded and presented in Appendix 4.

It is possible that hormonal differences between the sexes, their effects on behaviour (e.g. increased aggression), and immune responses of nestlings could account for male dominance in the feeding arena, rather than size as I have argued. The experimental design allows the effect of size to be teased apart from the effect of sex and other traits which covary with sex such as hormonal levels. If sex-specific hormone levels alone were responsible for increased male growth we would expect the same sexual dimorphism to be equally apparent in small and large nestlings.

However, this study reveals that that size *per se*, and not other traits specific to sex, is an important determinant of offspring growth.

There is one possible explanation for depressed growth in small females which cannot, however, be excluded on the basis of this study. In birds, females are the heterogametic sex, and environmentally-dependent expression of deleterious recessive alleles on the unguarded W chromosome could result in decreased female performance. However, investigations of sex-specific growth combined with brood size manipulations in the sexually monomorphic collared flycatcher, *Ficedula albicollis*, provide no evidence for this phenomenon in birds (Sheldon *et.al.* 1998).

Although this study demonstrates that both size and sex of offspring affect fledging measures, the results showed nestling mortality to be a function of size category only, and not predicted by nestling sex. Increased mortality amongst small nestlings was not surprising given that they had to compete with half a brood of considerably larger sibs. In naturally hatching clutches, the longest interval between hatching of the first and last egg is commonly two days, in one case four (personal observation; also true for other great tit populations). In nests with such extreme hatching asynchrony, it is usual that most eggs hatch on day 0 and only one, or two at most, hatch up to 2 days later. Often these last-hatched nestlings die within a day or two, unable to compete with such large sibs. In such asynchronously hatching nests, this mortality could be interpreted as an adaptive parental strategy to increase chances of survival in remaining offspring (e.g. Lack 1954). Although in great tits synchronous broods produce slightly more (but not significantly so) offspring, fledglings from asynchronous broods are heavier (see Amundsen & Slagsvold 1998). Given the discrepancy in competitive ability of male and female offspring, non-random allocation of sex between eggs may present a mechanism by which female birds could exert some control in breeding decisions, according to environmental conditions at time of rearing (see Slagsvold *et al.* 1992). For example, if male nestlings are at a competitive advantage, females on high quality territories may produce larger broods by laying and hatching 'male eggs' last; females on low quality territories could follow a bet-hedging strategy more likely to result in brood reduction by laying 'female eggs' last. While there are numerous studies reporting associations between laying or hatching order and the sex ratio (e.g. Ankney 1982, Cooke & Harmsen 1983, Ryder 1983, Weatherhead 1985, Bortolotti 1986, Clotfelter 1996, Dzus *et al.* 1996, Kilner 1998, see also Chapter 3), at present there is no clear understanding of what explains such patterns.

In this study, the unequal competition experienced in the nest can be interpreted as a sub-lethal fitness effect, resulting in nestlings of unequal quality. Increased size and mass at fledging has often been found to have important fitness consequences for great tits. Mass of fledglings correlates positively with post-fledging survival in several great tit populations (Perrins 1965, Dhondt 1971, Garnett 1981, Tinbergen & Boerlijst 1990, however see Lindén *et al.* 1992) and a previous study (Verboven & Visser 1998) found fledglings of higher mass to have higher recruitment probability. Skeletally larger individuals at fledging may be able to monopolise parental feeds outside the nest cavity and obtain higher dominance rank in hierarchical winter flocks (see Hinde 1952, Garnett 1981). Kluijver (1957) found female great tits to be subdominant to males in competition for food and roosting places. Larger body size at fledging could therefore promote mass gain in large individuals, in turn enhancing overwinter survival prospects.

The reproductive value of offspring (Williams, 1966) is a more important measure of offspring fitness than overwinter survival, and therefore it is more critical to consider offspring recruitment to the breeding population. It would seem reasonable to assume that the positive relationship between fledging mass and survival demonstrated for tits would result in greater recruitment probability of heavier nestlings. Indeed, Verboven & Visser (1998) demonstrated such a relationship in two different populations of great tits. Although the present study found no significant effect of fledging mass or size on recruitment, the trend was in the expected direction but tests lacked power due to small numbers of birds recruiting (only 20 nestlings from 635; c.f. Verboven & Visser 1998). Individuals which recruited weighed more than non-recruits in both absolute terms and relative to body size, although differences were not statistically significant (see results). There was no evidence of sex-biased recruitment based on these small samples.

Thus, although no effects of nestling size and sex on mortality and recruitment were detected in this study, it is possible that the significant size differences observed at fledging could have important consequences for these two fitness parameters. This result is consistent with the explanation that a size advantage of male nestling great tits can account for observations of male-biased sex ratios. All previous reports of unbalanced sex ratios in great tits have recorded male-biased fledging and recruitment sex ratios when young were reared in poor environments (Dhondt 1970, Drent 1984, Smith *et.al.*1989, Lessells *et.al.* 1996). Similarly, Heeb *et. al.* (1999) found a greater proportion of male nestlings recruiting from experimentally flea-infested broods. These observed male-biased ratios could even be enhanced by parents altering sex ratios themselves to account for lower fitness values of daughters in some conditions. If this indeed occurs, it could amplify the sex bias effects recorded in unmanipulated brood studies (Dhondt 1970, Drent 1984, Lessells *et al.* 1996). Regardless, the present study points to competition as an important selective force in the nest affecting quality of males and females raised. It cannot, of course, offer a proximate explanation for correlations involving hatching sex ratios (e.g. Lessells *et. al.* 1996), but may offer an explanation for the evolution of such patterns.

Fisher's (1930) theory of equal parental investment in the sexes has been the motivation for most avian studies of sex-specific growth and mortality. Most of these studies have concentrated on the differential cost to parents of rearing offspring to fledging, searching for adaptive explanations for biased sex ratios. Less attention has been paid to proximate explanations for biased fledging/ recruitment sex ratios (although see Schifferli 1980, Teather 1992). Such mechanistic explanations of sex biases at fledging may be important, and not in conflict with ultimate explanations. This and previous studies of great tits highlight the importance of considering both approaches in sex ratio studies. Although natural selection is the ultimate cause of sex ratios biases, constraints due to an organism's life-history (i.e. sexual size dimorphism driven by sexual selection) should not be ignored. Recent molecular technology allowing sexing of young birds should facilitate further studies of sex-allocation and sex-biased parental care. However, the outstanding problem in avian sex allocation studies remains that of quantifying fitness returns of sons versus daughters, and even in delimiting the period of parental care.

III. Evidence of biased sex allocation in a wild bird population

6. Primary sex ratio biases in Gotland great tits over three years

Natural selection favours individuals that modify investment in male and female offspring when fitness benefits from producing each sex differs. Frequently studies use the sex ratio of offspring produced as a measure of investment in males and females. If sex ratios are indeed representative of the division of resources between sons and daughters, then sex ratio biases are expected when the reproductive value of male offspring exceeds that of females, or vice versa. The reproductive value of male and female offspring might differ with certain breeding conditions, and accordingly sex ratio biases are expected. In birds, these conditions include timing of breeding, intensity of brood competition (reflected in clutch sizes, brood sizes or hatching asynchrony), male quality or attractiveness, female quality, environmental or territory quality, presence or absence of 'helper offspring' and brood status.

Recent studies of primary sex ratio biases have searched for correlational evidence of sex ratio variation across individuals in relation to a number of breeding gradients or traits (Koenig & Dickinson 1996, Lessells *et al.* 1996, Svensson & Nilsson 1996, Appleby *et al.* 1997, Bradbury *et al.* 1997, Cooch *et al.* 1997, Westerdahl *et al.* 1997, Nishiumi 1998, Hartley *et al.* 1999, Kölliker *et al.* 1999, Pagliani *et al.* 1999, Torres & Drummond 1999, Hörnfeldt *et al.* 2000, Korpimaki *et al.* 2000, Questiau *et al.* 2000, Radford & Blakey 2000a, Radford & Blakey 2000b,Westerdahl *et al.* 2000, Leech *et al.*, submitted ms). Whilst there is clearly a need for experimental manipulations in further investigations of avian sex allocation, observations of natural sex ratio variation and sex ratio skews can offer a view of natural investment patterns of parents in male and female offspring, and the degree to which we can expect sex ratios to be biased in the wild. Furthermore, any particularly consistent, significant trends within species, genera, or even the taxon, should be recognisable.

Evidence for sex ratio biases at the egg stage, generated by the recent interest in sex ratios in birds, has proved variable. In some wild bird populations, hatchling sex ratios have been found to vary in relation to resource abundance (Komdeur *et al.* 1997, Appleby *et al.* 1997, Korpimaki *et al.* 2000), timing of breeding (Howe 1977, Weatherhead 1983, Dijkstra *et al.* 1990, Zijlstra *et al.* 1992, Daan *et al.* 1996, Lessells *et al.* 1996, Sheldon *et al.* 1999), clutch size (Lessells *et al.* 1996), hatching asynchrony (Lessells *et al.* 1996), brood status (Patterson *et al.* 1980, Nishiumi 1998, Westerdahl *et al.* 2000), paternal traits (Svensson & Nilsson 1996, Ellegren *et al.* 1996, Westerdahl *et al.* 1997, Kölliker *et al.* 1999, Sheldon *et al.* 1999), maternal traits (Blank & Nolan 1983, Gowaty & Lennarz 1985, Heg *et al.* 1999, Nager *et al.* 1999, Wittingham & Dunn. 2000), harem size (Nishiumi 1998) and helping activity (Ligon & Ligon 1990, Gowaty & Lennartz 1985). In laboratory studies, sex ratios variation has been associated with parental attractiveness (Burley 1981, 1986) and diet and maternal quality (Kilner 1998, Bradbury & Blakey 1998). Many studies, however, report no significant sex ratio biases in relation to a number of variables, even when such associations are sometimes expected (yellow-headed blackbird: Patterson & Emlen 1980, lesser snow goose: Harmsen & Cooke 1983, Cooch *et al.* 1997, western bluebird: Koenig & Dickinson 1996, european starling: Bradbury *et al.* 1997, corn bunting: Hartley *et al.* 1999, yellowhammer: Pagliani *et al.* 1999, barn swallow: Saino *et al.* 1999, bluethroat: Questiau *et al.* 2000, great tit: Radford & Blakey 2000a, blue tit: Leech *et al.* submitted ms). It is impossible to estimate how many more studies could remain unpublished due to preferential publication of significant results. One study which does uncover a strong primary sex ratio bias in Tengmalm's owl broods can offer no explanation for their observation (Hörnfeldt *et al.* 2000). Consistent sex ratio trends do not appear to be emerging, though this may be due to inappropriate assumptions on which our expectations of sex ratio biases are based. At present it is difficult to make any generalisations about causes of avian sex ratio variation, or the adaptive nature of skews.

Within species, reports of sex ratio variation have also been inconsistent. In two Swedish blue tit populations, clutch sex ratios have been shown to vary in an adaptive manner with paternal sexual characteristics (Sheldon *et al.* 1999) and probability of survival (Svensson & Nilsson 1996, Sheldon *et al.* 1999). However, no effect of paternal quality or extra-pair paternity on clutch sex ratio has been found in a British population of the same species (Leech *et al.* submitted ms). Similarly in great tits, a positive relationship between hatchling sex ratio and male body size has been demonstrated in one population (Kölliker *et al.* 1999) but not in two others (Radford & Blakey 2000a, Lessells *pers. comm.*). Furthermore, hatchling sex ratio biases within the same populations have sometimes proved inconsistent, with significant sex ratio biases in some years but not others (Koenig & Dickinson 1996, Korpimaki *et al.* 2000, Radford & Blakey 2000a).

When studies involve data collected over more than one year, such data are typically analysed for all years pooled. Each breeding individual in each year is considered as a single case, and 'year' is entered into a general linear model as a factor explaining variance in the sex ratio as would be other morphometric or environmental traits (e.g. territory quality, lay date, male size, female age etc.). I suggest that looking for differences within recaptured breeding individuals across years may be a fruitful alternative approach to analysing sex ratio variation in wild populations. A within-individual analysis permits identification of factors influencing clutch sex ratio having removed nuisance variables specific to individual birds. Although this method may provide valuable insight as to individual decisions concerning investment in young of different sexes, few published studies have examined the variance in sex ratio due to different factors within individuals. Westerdahl *et al.* (2000) examined primary sex ratio variation within female great reed warblers breeding in different years in relation to one specific factor - breeding status, and found that individual females had a higher the proportion of sons in their brood when of primary rather than secondary status. Likewise, Komdeur *et al.* (1997) revealed a strong shift in sex ratios of individual females translocated to territories of different quality.

Analysing sex ratio variation across different breeding attempts also allows investigation of the effect of partner fidelity and divorce, which cannot otherwise be examined. Females might be expected to increase the proportion of sons in their brood if male offspring have higher reproductive values than their sisters when fathered by a high quality male. Females may remain faithful to their mate if he is of particularly high quality (Lindén 1991), divorcing in order to 'upgrade' their partner. Hence, faithful females may benefit from producing a higher proportion of sons in their brood than those divorcing. For an island population of great tits I first analysed primary (egg) sex ratio variation in the traditional between-individual manner and then used this within-individual approach from a set of birds which were caught breeding in more than one year. Employing a 'within individual' approach yields interesting results for a sample of great tits in which no variables explain significant variance in observed sex ratios using a traditional analysis with year as an explanatory variable. I also explore whether clutches of different sex ratios are of different value to females by looking for associations between incubation time, hatching success and nest desertion and sex ratio. Here I report these results and encourage similar analyses in other bird populations.

Methods

Field work

Data were collected from great tits breeding in nest-boxes in 13 separate woodlands on the Swedish island of Gotland (57°10'N, 18°20'E) between 1997 and 1999. Field data were collected with the help of A. Impey, D. Salkeld, A. Russell and C. Reim. For each nest I recorded lay date (first egg), clutch size, hatch date (first egg) and the number of eggs hatching. Parents were caught and ringed (if not already) whilst provisioning broods between 8 and fourteen days post-hatching. I measured parental tarsus length (to nearest 0.5mm), wing length (to nearest 0.5mm), mass (to nearest 0.5g) and recorded age as either 1 year old or 2+ years. Body condition index of adults was calculated as the residual from a linear regression of body mass on tarsus length. Although male breast band stripe is believed to be a sexually selected and heritable trait in this species (Norris 1990) it was not measured because of time constraints of fieldwork. A small 2-10μl blood sample was taken from 1or 2 day old nestlings by puncturing the brachial vain and collecting in a capillary, which was then stored in SET buffer at 4°C (1997) or 98% ethanol (1998 & 1999). Unhatched eggs were collected four days or more after the hatch date of the first egg. (Only on very rare occasions do eggs hatch more than two days after the first hatch date). Blastocysts (seen as white spots on the yolk surface) and undeveloped embryos were dissected out immediately and stored the same way as blood samples.

Molecular sexing

Clutch sex ratios were determined using a PCR-based molecular technique from DNA extracted from blood samples or embryonic tissue from unhatched eggs. I used primers P2 and P8 (Griffiths *et al.* 1998) to amplify introns within the CHD1 gene. PCR products were run on 6% polyacrylamide gel for between 1 and 3 hours at 75W, and visualised using silver staining (Promega). Female nestlings/embryos possessed two, different-length copies of the PCR products – CHD1-W from the W chromosome and CHD1-Z from the Z chromosome. Homogametic males possessed only the CHD1-Z fragment. C. Reim helped sex a considerable number of clutches from 1999.

Analysis between individuals in three years

I analysed clutch sex ratio variation in relation to a number of factors from pooled breeding data collected over three years. Individuals breeding in more than one year were included only once to avoid pseudoreplication. In each such case the breeding attempt that was included was selected randomly. Sex ratio was measured as the proportion of males in a clutch. Because of non-normally distributed error variance and unequal sample sizes, I analysed the proportional data with a general linear model analysis of deviance, assuming binomial errors, and a logit link function. The response variable was the number of males in a clutch, with the number of eggs sexed as the binomial denominator. Using clutch size as the denominator would lead to over-representation of females as not all eggs were sexed and those I failed to sex would be categorised as 'not male' in the analyses. Analyses presented here were weighted according to the amount of information I had for each clutch, i.e. the proportion of a clutch sexed (total sexed/clutch size). Results did not differ if analyses were repeated on clutches only with complete sex ratio data.

A model including several predictor variables and their second order interactions with 'year' was first fitted to the data. The significance of a term in the model was determined by assessing the change in deviance following removal of that term, using a χ^2 test with appropriate degrees of freedom (Crawley, 1993). Reported in this paper are changes in deviance values following removal of each variable alone rather than sequentially because of the presence of missing cases within the whole data set (see Table 6.2). A new general linear model was made for each factor, still complete with all other terms and interactions, but excluding cases with missing values for the factor in question. If cases with missing values are not excluded in analyses, the change in degrees of freedom when the variable in question is dropped from the model is inflated. Results did not differ when variables were removed first from the model, or later following step-wise exclusion of other variables. A heterogeneity factor (HF), the ratio of residual deviance to the residual degrees of freedom, was calculated to examine the data for overdispersion. HF<1 indicates the variance in the data to be less than that expected for a binomial distribution, and HF>1 more than that expected. Here HF=1.06, and so fitting the data to a binomial model was justified. Analyses were carried out using the statistical package GLMStat (Beath 2000, http://www.ozemail.com.au/~kjbeath/glmstat.html).

The number of eggs sexed for each clutch is associated with clutch size. Clutch size was therefore not included in the model fitting data to a binomial error structure, because 'number of eggs sexed' had been included as the binomial denominator. Instead I tested for effects of clutch size on sex ratio with a general linear regression with the response variable 'proportion males'. Data were normally distributed (Shaprio Wilks W=0.981, p=0.356) and so were not transformed prior to analyses.

Associated questions: analyses with sex ratio as independent variable

I examined the effect of clutch sex ratio on incubation time, hatching success and nest desertion to test whether females with different clutch sex ratios invest differently in those clutches. I calculated incubation time by subtracting the lay date plus clutch size from the hatch date, assuming that females began incubating the day after laying their last egg, as is usually the case for great tits (*pers. obs.*). I fitted a general linear model to determine whether year, area, lay date, clutch size, female body condition index, female tarsus length and number of males/total number eggs sexed (and interactions) could explain variation in incubation times of females. To test whether hatching success varied significantly with clutch sex ratio, I analysed the proportion of eggs hatching per clutch with a GLM with binomial errors and logit link. The number of eggs hatching was used as the response variable, with clutch size as the binomial denominator. Sex ratio was included as a predictor variable along with year, area, lay date, clutch size, female body condition index and female tarsus length. Model deviance was scaled by the HF (2.81) because the data were overdispersed. Again terms fitted were removed stepwise, and whether this caused a significant increase in deviance was assessed with an F test. To test whether nest desertion was associated with clutch sex ratio, I carried out a logistic regression analysis of brood desertion in relation to the proportion of males in a clutch. I included year, lay date, clutch size, and male and female characteristics as predictor variables.

Analysis within individuals across two years

Birds were identified which nested in 1998 following 1997, and in 1999 following 1998. First I determined whether clutch sex ratio was repeatable within individuals across years, using a simple regression of sex ratio in the second year against the first. Sex ratio data were first arcsin square root transformed because of their proportional nature and a non-parametric Spearman's rank test was also carried out to give a conservative test for a relationship. To find the variance in clutch sex ratio due to individual birds I also ran a general linear model with binomial error structure and logit link including 'bird ID' as a factor, with number of males as the response variable and number of eggs sexed as the binomial denominator. The change in deviance when 'bird ID' was removed from the model indicated the proportion of the variance attributable to between individual differences. Analyses were carried out for 23 males and 23 females where sex ratios over two years were known.

I analysed the change in egg sex ratio from one year to the next for females, in relation to the change in their own body condition, with a linear regression. In the same way I analysed the change in females' sex ratio in relation to the change in their partners' quality relative to that of their previous years' mate. Similarly for males I analysed the change in clutch sex ratio in relation to the change in own and partner's body condition. Over two breeding attempts, a female may mate with either the same male in both years (remain faithful), or change her partner (divorce). I compared the clutch sex ratios of those females who paired with the same male in the second breeding attempt to those with a new partner. I repeated these analyses for 'faithful' and 'divorced' males. A female who is mated to a high quality male is expected to lay a relatively high proportion of male eggs and to remain faithful. Female's mated to poorer males are expected to lay proportionally fewer male eggs and pair with a different male the following year. Hence, I also examined whether there was a difference in the clutch sex ratio of females in the first breeding attempt according to their future mate fidelity. I tested whether females that subsequently divorce have lower sex ratios than those who remain faithful. Proportional sex ratio data were arcsin square root transformed in all analyses.

Results

Analysis between individuals in three years

A total of 238 nests with known sex ratios were included in the analysis. Of these, 139 nests had complete sex ratio data (i.e. proportion of eggs sexed = 1). If data were missing, it was usually not more than one egg per nest; for 204 nests (86%), the proportion of eggs sexed was greater than 0.8. In total I examined 26, 90 and 122 nests from pairs breeding in 1997, 1998, and 1999 respectively, a total of 2,034 eggs/nestlings. Overall population sex ratios analysed at the level of the nestling show no deviation from a 1:1 sex ratio in any year (Table 6.1). For the three years pooled, the overall sex ratio of broods showed binomial distribution of the sex ratio (residual deviance/residual degrees of freedom =251/237 ~1).

Year	n nests	Number males	Total sexed	Proportion males	G_1	P
1997	26	93	206	0.45	1.944	>0.10
1998	90	389	788	0.49	0.127	>0.70
1999	122	536	1040	0.52	0.985	>0.30

Table 6.1: Proportion of male eggs laid in each of three years and analysis of whether this differed significantly from 0.5 using *G* tests for goodness of fit. Sample sizes are greater for 1998 and 1999 because of increased sampling effort in the field.

I analysed variation in nest sex ratios in relation to a number of factors listed in table 6.2 with sample sizes for each. Analysis of the proportion of males per clutch for all three years pooled provided no evidence of systematic biases in sex ratio in relation to any factors included in a general linear model with binomial errors. Table 6.3 lists variables fitted to the model and the change in deviance and related *p*-value when each term was removed individually from the full model. No variables explained significant variation in the proportion of males in a brood, whether they were removed first from the model, or later following step-wise exclusion of other variables which contributed least to the variance.

Similarly, the proportion of males in a clutch was not associated with the total number of eggs laid in that clutch (no effect of clutch size in a general linear regression: $F_{1,226}=1.5219, p=0.219$).

Table 6.2: Samples sizes for variables included in analysis of clutch sex ratios for 1997-1999 pooled

Variables	Cases	Variable	Cases
Year	238	Lay date	197
Male age	143	Female age	138
Male tarsus length	173	Female tarsus length	175
Male body condition index	164	Female body condition index	162
Woodland area	238		

Table 6.3: Analysis of proportion of males in broods of great tits from 1997-1999 pooled. ΔD is the change in deviance in the model when each factor potentially affecting clutch sex ratio is excluded first from a GLM with binomial errors and logit link. Similarly, Δd.f. is the change in degrees of freedom when each factor is removed. The associated χ^2 test assesses the significance of the change in deviance for each explanatory variable when it was removed from the model.

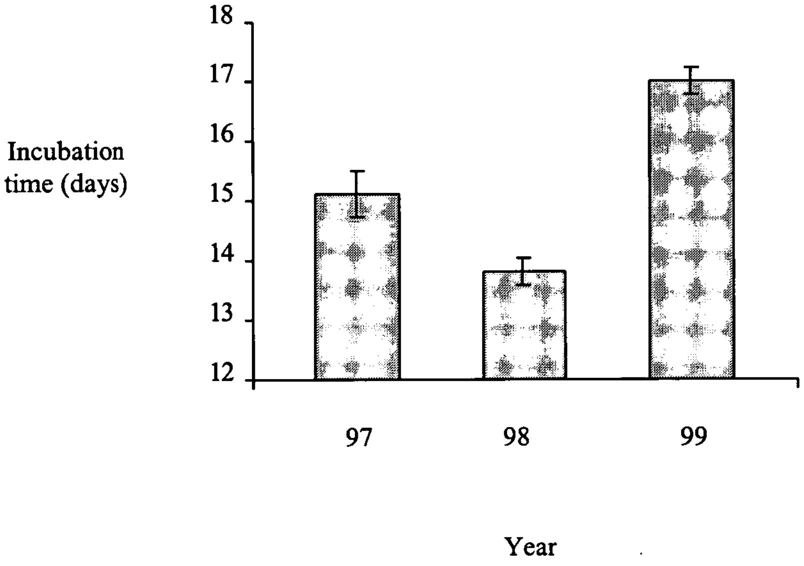
Factor	ΔD	Δd.f.	*P*
Single factors			
Year	4.139	2	>0.10
Woodland area	9.684	12	>0.50
Lay date	0.090	1	>0.70
Male age	0.010	1	>0.90
Male tarsus length	2.099	1	>0.10
Male body condition index	0.490	1	>0.30
Female age	0.334	1	>0.50
Female tarsus length	0.005	1	>0.95
Female body condition index	0.604	1	>0.30
Interactions with year			
Year * area	13.090	11	>0.20
Year * lay date	0.074	2	>0.95
Year * male age	0.978	2	>0.50
Year * male tarsus length	0.419	2	>0.80
Year * male body condition index	0.253	2	>0.80
Year * female age	1.571	2	>0.30
Year * female tarsus length	0.280	2	>0.80
Year * female body condition index	0.526	2	>0.70

Associated questions: analyses with sex ratio as independent variable

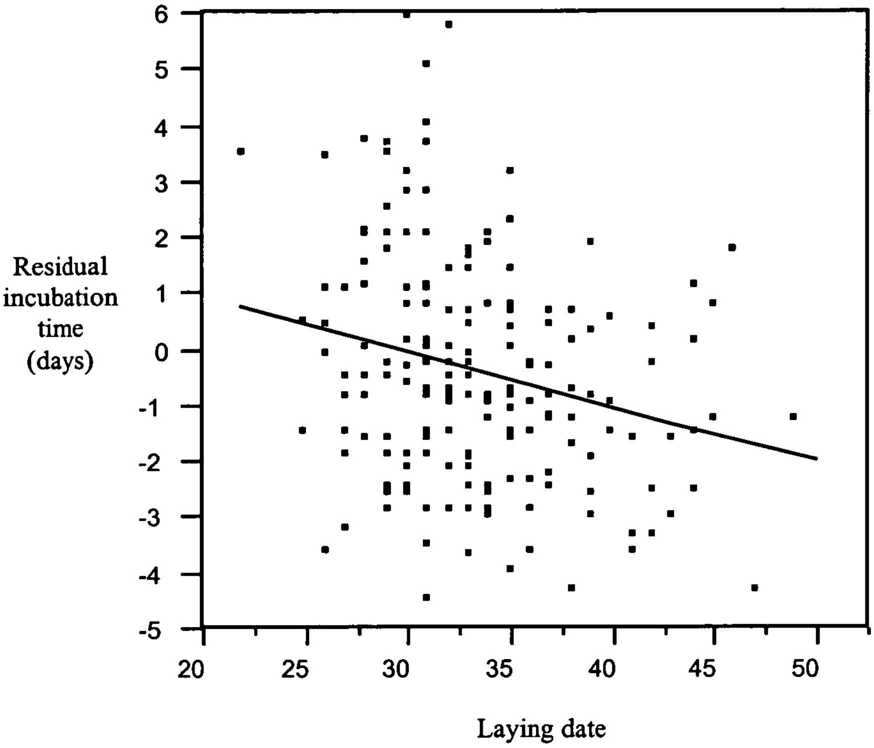
Time spent incubating a clutch by females was not related to the proportion of male eggs in that clutch ($F_{1,182}$=0.038, p=0.845, minimised model containing year, lay date and clutch size as factors). There were highly significant year differences in incubation times ($F_{2,183}$=50.680, p<0.0001, Fig 6.1a), mainly due to long incubation periods in 1999 when a cold week with bad weather prolonged incubation (see also chapter 4, figure 4.3). There was also a highly significant effect of lay date, with later clutches being incubated for fewer days ($F_{1,183}$=32.508, p<0.0001, Fig 6.1b). There was also a strong negative relationship between clutch size and incubation times ($F_{1,183}$=8.695, p=0.004, Fig 6.1c). Relationships were consistent, with no interaction terms in the model being significant.

The proportion of eggs hatching was not dependent on the proportion of male eggs in the clutch (ΔD=2.122, p>0.05, d.f.=1). Neither was there any indication of the proportion of male eggs affecting the likelihood of nest desertion (χ^2_1= 0.635, p=0.426, n=238).

6.1 a)



6.1 b)



6.1 c)

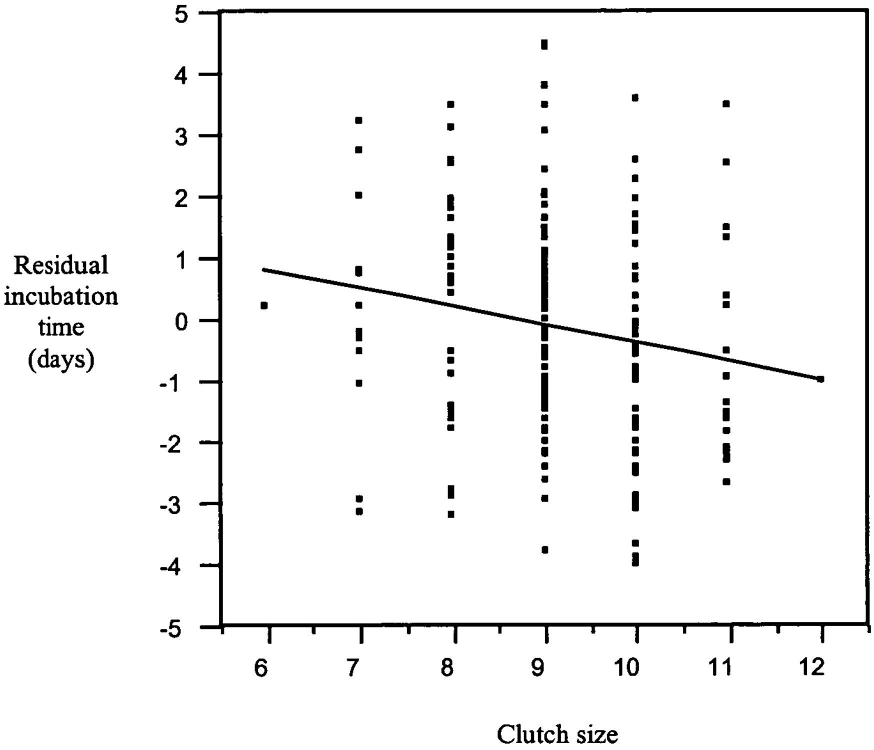


Figure 6.1: Changing incubation duration with (a) year, (b) lay date and (c) clutch size. Figure 6.1a plots mean and s.e. incubation time for each year. Figure 6.1b plots residual incubation time given clutch size and year effects for lay dates. Likewise 6.1c plots residual incubation time given lay date and year effects for clutches of different sizes.

Analysis within individuals across two years

There were 51 cases of female birds nesting in one year being recaptured in a subsequent breeding attempt, and 44 cases of male breeding recaptures (see table 6.4). Of these, sex ratios of broods in both years were known for 23 females and 23 males. One female and two males were caught breeding in all three years. In this case the individual was included in the analyses as a separate independent data point breeding in 1998 following 1997, and then in 1999 following 1998. Obviously these data are not independent and introduce the problem of pseudo-replication to the data set. However, results are reported for data analysed in this way because inclusion of each recapture allows larger sample sizes in a small data set, and does not change the outcome of any tests if they are carried out excluding either breeding attempt from the analyses. Not all data (e.g. exact lay dates, adult measures) were available for breeding attempts in both years, therefore sample sizes in analyses may not equal those in table 6.4.

Year 1	**Year 2**	***N***	**Year 1**	**Year 2**	***n***
Recaptured females			*Recaptured males*		
1997	1998	9	1997	1998	11
1997	1999	1	1997	1999	2
1998	1999	41	1998	1999	31
Total		*51*	*Total*		*44*

Table 6.4: Sample size of birds caught breeding in more than one year on Gotland. Numbers of recaptures in 1999 following 1998 are much greater because of greater ringing and trapping effort during these years.

A simple linear regression of offspring sex ratio of the second breeding attempt against the first suggested that sex ratio was not repeatable across years either for males ($F_{1,21}$=0.152, p=0.701) or females ($F_{1,21}$=1.059, p=0.315). Non-parametric rank correlation tests also revealed no association between sex ratio year 1 and sex ratio year 2 (males: Spearman's Rho=-0.019, p=0.933, females: Spearman's Rho=-0.234, p=0.282). This was confirmed by examining the variance in sex ratios due to individual birds in a general linear model with binomial error structure, essentially testing whether differences within an individual were smaller than differences between individuals. An insignificant proportion of the variance in sex ratio was accounted for by between subject differences (males: $\Delta D=25.29$, $\Delta d.f.=22$, $p>0.20$, HF=1.2; females: $\Delta D=24.41$, $\Delta d.f.=22$, $p>0.30$, HF=1.3).

The following results involve analyses of the change in sex ratio within individual birds (i.e. transformed sex ratio year 2 – sex ratio year 1) with respect to the change in predictor variables from one year to the next. The change in primary sex ratio of female birds was positively related to the change in body condition of the male to which she was mated ($F_{1,9}=9.270$, $p=0.014$, also table 6.5, figure 6.2a). Females did not alter the sex ratio they produced in response to changes in any other variables - either to their own body condition or environmental factors (Table 6.5). Contrary to females, clutch sex ratios of males breeding over successive years did not change consistently in with regards to their mate's quality (Figure 6.2b). Neither was there any relationship between the change in sex ratio of their broods and their own breeding condition or environmental factors (Table 6.5).

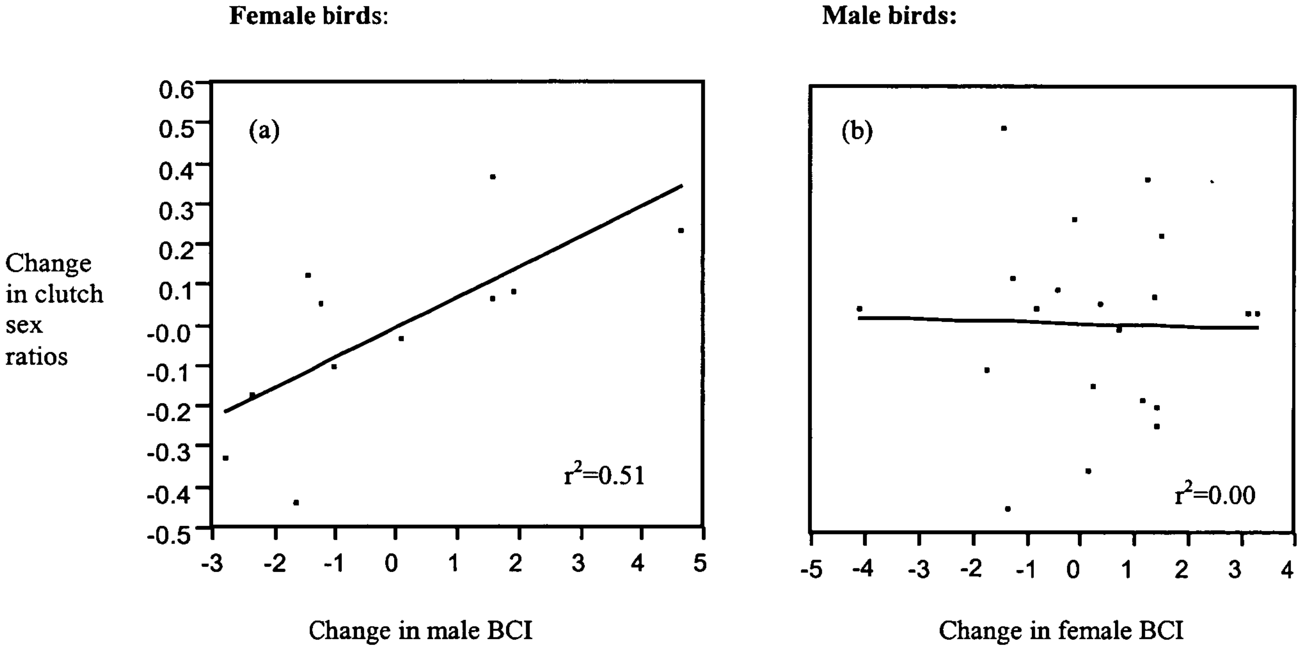


Figure 6.2 Change in clutch sex ratio measured as the proportion of sons in a clutch (year 2) minus the proportion of sons in a clutch (year 1) in relation to the change in partner's body condition index, for recaptured breeding females (a) and males (b). Body condition index is the residual mass from a correlation of mass on tarsus length. Figures show points for both divorced and faithful individuals pooled.

Variable	***N***	**d.f.**	***F***	***P***
Recaptured females				
Change in laying date	18	1	0.011	0.917
Change in clutch size	22	1	1.461	0.241
Change in partner's (males) body condition index	11	1	9.270	**0.014
Change in own body condition index	13	1	0.825	0.383
Recaptured males				
Change in laying date	17	1	0.971	0.340
Change in clutch size	22	1	0.465	0.503
Change in partner's (males) body condition index	19	1	0.013	0.911
Change in own body condition index	21	1	1.198	0.290

Table 6.5 Comparisons of changes in clutch sex ratio in relation to changes in breeding situation, mate's condition and own condition in successive breeding attempts of great tits on Gotland. Results of individual regressions.

Finally, I compared the sex ratios of divorced and faithful males and females. Females mating with the same male in a subsequent year showed little change in their clutch sex ratio, but those pairing with a new male showed a decrease in the proportion of males in their clutch (Figure 6.3a). The difference between the proportion of males in broods of faithful and divorced females was not statistically significant (t_1=1.779, p=0.091, n=21). Clutch sex ratios of divorced and faithful males did not differ (t_1=--0.185, p=0.855, n=21, fig. 6.3b).

There was no difference in clutch sex ratios in the first breeding attempt between females that subsequently divorced and those which kept the same partner (t_1=-1.159, p=0.256, n=30).

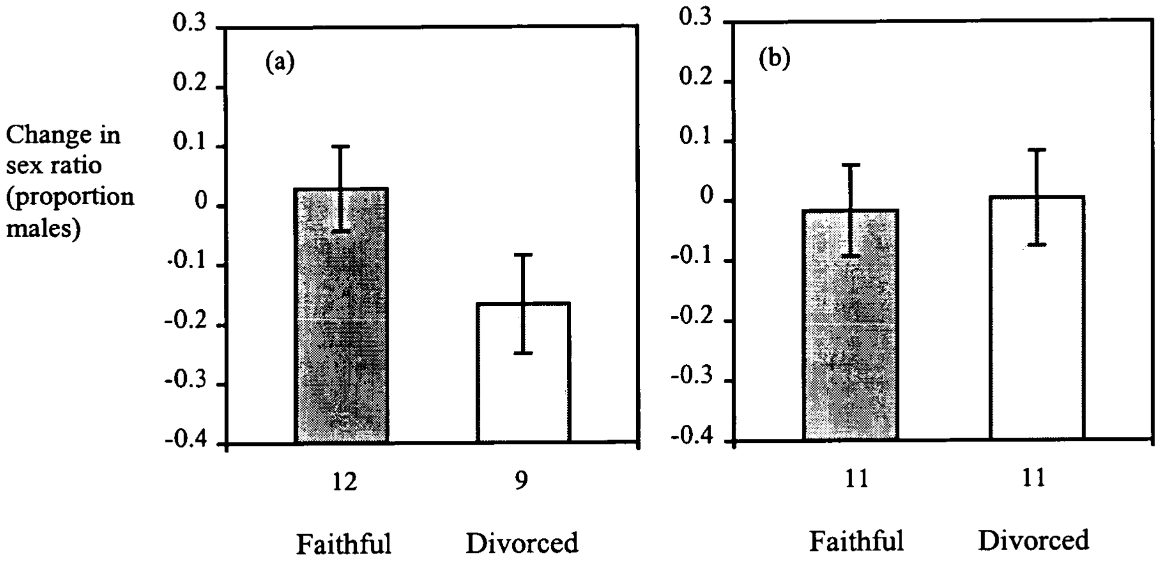


Figure 6.3: Mean change in clutch sex ratio (± S.E.) between first and second breeding attempts amongst divorced and faithful (a) females and (b) males. Sample sizes for each category are given below bars.

There was no difference in the body condition index of partners of divorced and faithful females (t_1=1.292, p=0.204, n=37), where change in body condition is a continuous variable. Categorising females as paring with males in better or worse condition than their previous mate revealed no association between divorce and relative mate quality (χ^2=0.483, p=0.487, n=23; table 6.6).

	Female breeding status	
	Divorced	*Faithful*
Paired with better condition male	2	5
Paired with worse condition male	7	9
Total	9	14

Table 6.6: Numbers of divorced females pairing with better and worse condition mates, relative to the condition of their partner in the previous year.

Discussion

Despite abundant theories and recent interest in primary sex ratio studies in birds, empirical evidence of consistent sex ratio biasing by females is proving elusive. Identifying factors responsible for sex ratio biases through a correlative approach across individuals is a commonly employed method for identifying relationships between variables and primary sex ratio variation. However, such an approach does not demonstrate causality, and experimental manipulations of traits potentially causing sex ratio biases are advocated in future research. Like many other investigations, I found no correlative evidence of primary sex ratio biasing by great tits over three years, from a large data set of over 270 broods. Brood sex ratio did not vary in relation to several environmental or parental variables (table 6.3).

Previous investigations of heterogeneity in great tit brood sex ratios at the egg stage have been published from three different populations. In a Dutch population, brood sex ratios increased with hatching date and hatching asynchrony, whilst decreasing with increasing clutch size (Lessells *et al.* 1996). The proportion of sons in broods of a Swiss population of tits increased significantly with increasing male tarsus length, and there was a similar, though non-significant, trend with increasing breast stripe size (Kölliker *et al.* 1999). Great tit females prefer males with larger breast stripes (Norris 1990), and male tarsus length correlates with breeding success (Blakey 1994, Verboven & Mateman 1997). The relationship between sex ratio and male traits was interpreted as a female response to either male genetic quality or body-size related territory quality (or both). This relationship with tarsus length was not apparent in the present study, despite a much larger sample size (n=173 compared to n=57), nor in the Dutch population.

The third great tit study suggested one potential explanation for the discrepancy in these findings. Radford & Blakey found significant predictors of brood sex ratio from a correlational analysis based on five years breeding data from British great tits. These included lay date (1993), male age (1998), male tarsus length (1991), female tarsus length (1991 and 1998) and female condition (1991). However, no relationships were consistent across years, and no variables predicted sex ratios when data for all years were combined (Radford & Blakey 2000a). They argued that breeding data from several years needs to be examined in order to identify consistent sex ratio biasing and confirm whether sex ratio manipulation is truly a female breeding strategy. The Swiss and Dutch studies reporting significant results both examined sex ratio biases over one year. The relationships described for both populations have since proved unrepeatable in analyses of subsequent years breeding data (Lessells *pers comm,* Kölliker & Brinkhof *pers comm*).

These previous studies have all examined sex ratio variation between individuals. Results of the present study suggest that examining variation within individuals could provide some interesting information as to whether females can bias egg sex ratio skews, and in relation to which factors. I found that females mated to better condition males relative to their last breeding attempt tended to increase their sex ratios compared to females mated with males in relatively worse condition, which adjusted their brood sex ratios negatively (figure 6.2). This relationship between sex ratio and mate's body condition was evident for recaptured females but was absent for recaptured males, because in birds the female is heterogametic and thus responsible for egg sex ratios. The function relating the change in sex ratio to change in male body condition passed through zero (figure 6.2), suggesting bidirectional female control of sex ratios, with no adjustment amongst females mated to males of similar condition to their last breeding attempt. The reliability of this result (table 6.6) could be questioned given that with multiple comparisons, there is a one in 20 chance of one test revealing a significant relationship with an alpha level of 0.05. However, tests of the effects of changes in breeding conditions on female's brood sex ratios were only carried out for four variables. One of these, change in male body condition, proved significant. With the reported p value of 0.01 one would expect a significant result from only one of ten variables by chance, rather than one of four. These results are complementary to those found by Kölliker *et al.* if females are adjusting sex ratios to increase the proportion of males in their brood with increasing male quality. A female 'upgrading' from her previous breeding partner to a male in better condition increased the proportion of sons in her brood.

This could be considered adaptive if variance in male reproductive success is greater than that of females (Trivers & Willard 1973), and sons of high quality (good body condition) males are likely to have a mating advantage of those fathered by poorer males. Females mating with males in better condition may increase grand-offspring production through rearing more male offspring in their broods.

If females modify sex ratios in respect to changing partner condition, one would expect a positive relationship between absolute male body condition and absolute brood sex ratios, which was not evident from a much larger data set (n=164). The only explanation for this discrepancy (apart from that the relationship is a Type I error) is that females are responding to relative body condition based on that of previous mating events, and without a 'benchmark', females are unable to respond to partner condition. Female control of the sex ratio could then only be expected to evolve if a significant proportion of the female population bred in more than one year. Results of the present investigation show that between 35% and 46% of female birds breed in more than one year.

Although the body condition of a male was not associated with the likelihood of a female remaining faithful to him or pairing with another male subsequently, 'faithful' females tended to have higher clutch sex ratios than 'divorced' females. Unfortunately sample sizes in this analysis are very small and the result is not statistically significant (p=0.09). However the data are provocative and it would be interesting to examine effects of partner fidelity on sex ratios in a much larger data set. Clutch sex ratios in the first breeding attempt did not differ between faithful and divorced females. This indicates that the suggestion of laying proportionally fewer male eggs in relation to breeding status is a result of decreasing sex ratios amongst divorcing females compared to faithful females (see also fig 6.3). In great tits, newly formed pairs have lower breeding success than those birds previously breeding together (Perrins & McCleery 1985) and pairs that enjoy high reproductive success together are more likely to remain together (Lindén 1991). Hence one might expect faithful females to maintain similar sex ratios across breeding attempts. Females with a new partner may be less sure of his parenting abilities (e.g. food provisioning) than those paired to the same male, and consequently produce more of the relatively smaller sex (females, e.g. Perrins 1963, Oddie 2000) which require less food.

The different findings from great tit sex ratio studies mirror the general variability of published results from investigations of avian sex ratio variation. Amongst those studies that do find effects, there is variability in both magnitude and direction of relationships and no factor that is consistently identified as influencing sex ratios. No formal meta-analysis has been carried out on published studies of sex ratio variation, or at least not published. Explanations for the lack of detection of consistent sex ratio skews to date can be split broadly into two categories: either the phenomenon does not exist biologically, or the methods we use for its detection are not sufficiently sensitive.

If the former is the case, it may be that Williams was correct in 1979 when he stated that 'parents....have no control of sex-chromosome recombination' and that adaptive control of offspring sex ratio has not evolved because of random Mendelian inheritance apportioning sex chromosomes to oocytes. However, Williams himself states that he 'finds it rather mysterious that adaptive control of progeny sex ratio seems not to have evolved' (Williams 1979). We expect adaptive sex ratio biases to occur because we assume an obvious fitness advantages to parents capable of sex ratio modifications. However, such an assumption may not always be true. In order for natural selection to favour females capable of offspring sex ratio control, fitness differences from producing a daughter versus a son must first be demonstrated. Only one avian study reporting a sex ratio skew has shown clear fitness advantages to females from producing unequal numbers of male and female offspring (Komdeur 1998).

If females are constrained by mechanistic processes to adjust egg sex ratios, perhaps any adaptive advantages of sex ratio manipulation are realised through adjustments of secondary sex ratios (post-hatching). Apart from this prompting the question of why produce the eggs in the first place, examples of egg sex ratio biasing exist which suggest this is not the case. Several studies have identified sex ratio skews at the egg stage (e.g. Kölliker *et al.* 1999, Nager *et al.* 1999, Sheldon *et al.* 1999, Nishiumi 1998, Westerdahl *et al.* 2000). Furthermore, biasing sex ratios post-hatching would be an impossible strategy of sex allocation for species which lay a single egg. Yet the most convincing evidence of female control of offspring sex ratio in an adaptive manner comes from a species that lays usually a single egg, the Seychelles warbler, *Acrocephalus sechellensis* (Komdeur *et al.* 1997). The strong sex ratio skews observed in this species must be present at the egg stage; these females would not have the opportunity to select offspring sex post-hatching with a clutch size of only one. The present investigation of primary sex ratio variation within individuals over more than one breeding attempt provides evidence that females may indeed manipulate primary sex ratios (figure 6.2, table 6.5).

The phenomenon of biased primary sex ratios may exist undetected if skews are small and require very large sample sizes to uncover. (However, if they are so small, their biological significance might be questioned). Perhaps there is not adequate selection for parents to manipulate investment in male and female offspring through primary sex ratio skews? It may be that particularly unusual situations are required to produce selective pressures strong enough to result in evolution of primary sex ratio skews, and investigations of species with peculiar breeding circumstances could be more likely to demonstrate interesting allocation patterns. In the case of the Seychelles warbler, the limited territories on an island habitat may provide the ecological extremities needed to provoke selection for strong sex ratio biasing. In this species, helping offspring remain on parental territories to raise subsequent young, and are usually female. On high quality territories they increase their parent's reproductive success, but on low quality territories reduce it (Komdeur *et al.* 1997). Hence parents on low quality territories benefit from production of dispersing sons, reducing competition in the natal territory. In other circumstances, parents could reduce such competition by expanding their territories; on an island however such expansions are impossible. The fitness consequences of producing a son versus a daughter on a low quality territory may be exceptional, providing a strong selective force for biased sex ratios (see Komdeur 1998).

Similarly, life-history as well as environmental characteristics may predispose a species to adjusting sex ratios if they influence the RVs of offspring of different sexes. For example, sex ratio biases have been demonstrated in particularly sexually dimorphic species (*Agelius phoeniceus* – 35% mass dimorphism, Weatherhead 1985; *Quiscalus mexicanus* – 44% mass dimorphism, Teather & Weatherhead 1989), reverse sexually size dimorphic species (e.g. *Circus pygargus* – 42% mass dimorphism, Leroux & Bretagnolle 1996; *Parabuteo unicinctus* – 45% mass dimorphism, Bednarz & Hayden1991) and those with helpers predominantly of one sex (Gowaty & Lennartz 1985, Legge *et al.* 2000).

The second explanation for the lack of observed primary sex ratio skews in birds is that our methods of detection are not sophisticated or sensitive enough to uncover systematic biases that exist. At the level of the taxon, we may be unable at present to make general statements concerning sex ratio skewing in response to certain factors because there are simply not enough appropriate studies yet for such widespread trends to be identified. The only statement that could be made is that common patterns among bird species do not exist. It seems sensible to consider the possibility of primary sex ratio manipulation independently for each species, as expectations concerning sex ratio biases from one species are unlikely to apply to another in a large group with such diverse ecology and life-histories.

Within species, our approach to sex ratio investigations may not improve our chances of detecting skews. Statistical tests may lack power because of small sample sizes unavoidable in field studies, or because of the small magnitude of effects. There may be adequate selection for sex ratio biasing, yet sex ratio trends go undetected because of multiple effects of a variety of factors on sex ratio. For example, imagine a species where males hatching early in the season have greater breeding success in the subsequent year than those hatching later (as is the case for some raptors, Daan *et al.*1996). Selection favours male biased early clutches. In this species, there is also selection for females in good condition to produce more sons, following Trivers and Willard's (1973) argument. Now imagine that females gain condition as the season progresses and food availability increases. Those reproducing at the beginning of the season are under selection for male biased sex ratios, whilst at the same time would benefit by producing female biased sex ratios considering their own body condition. Thus two variables influence offspring sex ratios, but with relationships in opposing directions. Methods to determine which selective factors, or combinations of factors, are most important in causing sex ratio biases need to be developed.

Amongst mammals, variations in primary sex ratios seem as inconsistent as in birds. Recently it has been shown that in red deer, an established relationship between maternal dominance and offspring sex ratio was in fact dependent on another factor, environmental condition. At high population density, the tendency for dominant females to produce more males disappeared. Kruuk *et al.* (1999) postulated that 'multiple factor' effects may be a reason for notoriously inconsistent patterns of sex ratio variation in deer. To produce direct unequivocal evidence that a single trait can explain a significant proportion of variation in the sex ratio requires experimental manipulation of such a trait resulting in an observed sex ratio shift. The ease with which new molecular techniques can be utilised to assign sex to young nestlings or eggs has encouraged a large number of post-hoc studies of primary sex ratio variation in birds. This approach has several limitations:

1. Covariation between traits may mask the 'real' factor explaining any sex ratio variation. Experimental manipulation of any one variable allows causal relationships between single factors and sex ratios to be established.
2. Modifications of sex ratio may only occur in extreme environments (e.g. large brood sizes, poor years) which are not represented in the sample under investigation.
3. Data may be lacking for several variables, which would be interesting to include in the analyses but were not measured in the field.
4. Without empirical information about selection on sex allocation, assumptions about the form of such selection may be unjustified (see Chapter 5 and Appendix 4).

Unfortunately only six published sex ratio studies have so far involved trait modifications in wild populations (lay date: Svensson & Nilsson 1996, paternal condition: Ellegren *et al.* 1996; territory quality: Komdeur *et al.* 1997, paternal sexually selected character: Saino *et al.* 1999, Sheldon *et al.* 1999, maternal condition: Nager *et al.* 1999). In future, experimental studies are advocated to identify traits determining sex ratio variation.

Results of the present study suggest that investigating sex ratio variation within individuals over different breeding attempts may provide a more powerful means of identifying factors causing sex ratio skews in birds. Two previous studies have examined repeatability of sex ratios of individual females (Appleby *et al.* 1997, Westerdahl *et al.* 1997), and two others have found sex ratios to vary within individuals according to breeding territory (Komdeur *et al.* 1997) and breeding status (Westerdahl *et al.* 2000). No studies have considered within individual variation in relation to more than one determining factor. The present study demonstrated that great tit sex ratios of individual birds were not repeatable across years, suggesting that the proportion of males in a brood is not fixed for each individual, but varies between breeding attempts. Hence birds may potentially adjust sex ratios according to environmental or mate characteristics. Females appeared to adjust the sex ratio of their brood in relation to the change in condition of their partner, and in the direction expected (table 6.6, figure 6.2).

One significant limitation of within-individual comparisons is that sample sizes will inevitably be limited, due to low numbers of birds caught rebreeding between years. Data presented here suggest there may be some interesting adjustments by female birds to primary sex ratios in relation to mate quality (also Burley 1981, Burley 1986, Svensson & Nilsson 1996, Ellegren *et al.* 1996, Kölliker *et al.* 1999, Sheldon *et al.* 1999). It would be very interesting to confirm these results using within-individual comparisons of primary sex ratios from much larger data sets.

7. Concluding remarks and future directions

Although a number of studies have documented cases of sex ratio biases in birds, consistent evidence of sex ratio adjustment is lacking. Investigations of primary sex ratio biases, that is at the laying or hatching stage, usually assume parental control over the proportion of male and female offspring produced, and that any deviations from parity are adaptive in nature. However, in order to demonstrate that any sex ratio manipulation is adaptive, increased fitness to parents from biased offspring production should be demonstrated (e.g. Komdeur 1998). For example, changes in diet which lead to sex ratio modifications could be considered adaptive if, on high quality or unrestricted diets, females produced more of the conceived 'expensive' sex. Being in better physiological condition, these offspring would enjoy increased mating success relative to that of others of the same sex in the population. Hence a female capable of such a modification may expect increased genetic returns. However, has been suggested that such sex ratio biasing is a direct physiological response out-with the female's control (Russell & Griffith, submitted ms). This alternative explanation for observed sex ratio biases seems unlikely for three reasons. Firstly, it is difficult to imagine how selection would lead to the evolution and maintenance of a process causing females to skew sex ratios as a simple physiological response. In some cases such a response is likely to produce sex ratios which do not maximise parental fitness. Secondly, in the majority of cases where primary sex ratio skews have been found with the pattern of biasing demonstrated to be consistent with an adaptive reason, biases have been found in the direction predicted (e.g. Ellegren, *et al.* 1996, Svensson & Nilsson 1996, Komdeur *et al.* 1997, Nishiumi 1998, Kilner 1998, Nager *et al.* 1999, Kölliker *et al.* 1999, Sheldon *et al.* 1999, Westerdahl *et al.* 2000, see also Table 2.1). Thirdly, although it is possible to imagine that females may respond to environmental factors that can directly affect their condition, it is more difficult to imagine how such an explanation may apply to sex ratio biases correlated with mate characteristics.

Future studies of sex allocation would benefit from accurate knowledge of the reproductive value of a son versus a daughter, in order that predictions of sex ratio biases can be made based on these fitness payoff estimates to parents from producing male and female offspring. Many studies have investigated factors affecting offspring fitness but few have considered the sexes separately in their analyses (e.g. Merilä *et al.* 1997). Molecular sexing techniques mean that it is now possible to sex offspring and investigate sex-specific responses to changing environmental gradients or parental quality. This will give some idea of the cost of producing male and female offspring. From these estimates, predictions can be made about when sex ratio skews are expected, the direction and perhaps even the magnitude of skews, and whether such skews can represent adaptive responses. Estimates of the reproductive value of sons and daughters can be made from following individual reproductive events of offspring in the years following fledging. Here the advantage of large, long-term data sets is evident. Long-term studies also provide data suitable for analysis of the heritability of sex ratios, estimates of which have not yet, to my knowledge, been published.

Just as for egg sex ratios, secondary sex ratio biases have the potential to be either adaptive in nature, or occur as a result of other processes not controlled by either parent. Sex allocation theories work on the basis that selection acts to maximise fitness returns to parents, and as such assume parental control of sex ratio decisions (see Charnov 1982, Frank 1990). However, biases may also be shaped by food availability and sex-specific food requirements, unequal competitive abilities and/or growth rates of offspring of different sexes, or increased susceptibility of one sex to disease or parasites (Clutton-Brock *et al.* 1985, Howe 1977, Cronmiller & Thompson 1981, Røskaft & Slagsvold 1985, Potti & Merino 1996, but see also Selander 1960, Richter 1983, Weatherhead 1983, Clutton-Brock 1986). In birds, the role of sex-specific offspring characteristics in determining fledging sex ratios has not been given as much attention as parental decisions, - however both may be important determinants of sex ratios. Chapter 5 and Appendix 4 illustrate how important it may be to consider both processes acting to produce variation in sex ratios. In great tits, fledging sex ratios in three populations were consistently different from those predicted by theory. Male biased sex ratios were observed amongst families nesting in poor areas, breeding at unfavourable times, or subjected to an experimentally increased brood size. The experiment presented in Chapter 5 demonstrates that these biases may be explained by enhanced competitive ability of male offspring, despite the fact that as the larger sex they may require more resources than their sisters (e.g. Fiala & Congdon 1983, Teather & Weatherhead 1988).

Many recent publications investigating sex allocation in birds consider only manipulation of egg sex ratios. However, sex allocation theories apply to selective forces acting over the whole of the period of parental care. Altering numbers of offspring originally produced is just one way in which parents can bias investment in males and females (see Chapter 1). Predicted sex ratio biases may not be observed at hatching but could occur alter during the nestling phase. Studies of egg and fledging sex ratios both merit attention for research. The new interest in primary sex ratio skews has come about as a response to simple-to-use molecular sexing techniques. Typically studies search for correlative evidence of sex ratio biasing in relation to environmental or parental characteristics. However, experiments are required to test adequately for causal relationships between any variable and offspring sex ratios (Chapter 6). Many factors of different magnitudes and acting in different directions could potentially effect offspring sex ratios, and observed sex ratios may reflect the additive effect of all contributing factors, or interactions between factors. Once effects of factors have been identified, methods to incorporate these individual effects into one holistic model can be designed.

In the earlier chapters of the thesis, I examined evidence for female control of primary (egg) sex ratio. For great tits, there was a suggestion that females may indeed have some control over the sex of their eggs because egg sex ratio appeared to increase with laying order (Chapter 3). However, this effect was not robust and further investigations with larger sample sizes are advisable. Unfortunately this might require the sacrificing of whole clutches, unless another method of matching individual hatched nestlings to their eggs can be found. Comparative analysis across species also suggested interesting patterns of egg sex with regards to laying or hatching order, and that patterns may vary between groups of species according to brood size (Chapter 3). This data set should expand as further relationships are reported for different species, and firmer conclusions could then be made concerning sex ratio variation and the laying sequence. A bigger data set would also allow analyses controlling for phylogenetic differences between species, because the problem of loss of degrees of freedom this analysis introduces would be reduced. Within species, concurrent studies of egg sex ratio with laying sequence and sex-specific nestling interactions would provide evidence as to why sex ratio skews with laying sequence could be beneficial. Such investigations could provide support for the argument that brood reduction is a parental strategy to increase brood fitness, with mothers biasing egg sex to enhance the possibility of brood reduction. Further cross-species comparisons would be useful as a means to identify groups of birds for which such a strategy is prevalent.

The possibility of female manipulation of egg sex was also investigated by testing for differences in sex ratios with female body condition. To achieve this experimentally, nestboxes of female great and blue tits were heated and cooled before and during egg laying (Chapter 4). Although there were indications that the experimental treatment affected females, neither great nor blue tits appeared to alter brood sex ratios in response to female condition. Unfortunately, female condition could not be directly measured at the time of experimental manipulation. Other factors, i.e. incubation time, which are less important determinants of the reproductive value of a brood, appear to be modified before egg sex ratio is manipulated. It is possible that the experimental manipulations were not extreme enough to cause variation in female condition and consequently brood sex ratio. Alternatively, females may simply not adjust sex ratios in response to their own body condition (however, see Kilner 1998, Nager *et al.* 1999). In this population there was some evidence that female great tit's brood sex ratios did not vary with their own body condition, but in relation to the relative body condition of their partners (Chapter 6). There was also the suggestion of female manipulation of egg sex ratio in relation to male size in blue tits (Chapter 4). Clutch sex ratios increased with male tarsus length, and although the effect was not statistically significant, it was consistent with other studies of blue tits finding significant correlations between paternal characteristics and offspring sex ratios (Svensson & Nilsson 1996, Sheldon *et al.* 1999).

The mechanism by which sexual differentiation of eggs occurs within the female, either randomly, in response to her own condition, or any external factor, is as yet unknown. Physiological investigations have been, for the most part, limited to domestic fowl in the hope of producing female biased sex ratios. The importance of understanding the mechanism of sex determination for evolutionary predictions of sex ratio biases is discussed in Chapter 2 and Appendix 3. Physiological studies are required to determine whether female birds have the capacity for selective oocyte release or structures necessary for oocyte reabsorption, and whether such mechanisms are widespread throughout the taxon. At the same time the potential for variation in primary sex ratios through genetic mechanisms is being investigated. At present, a few genes specific to the W (and Z) chromosome in birds have been identified, but a gene analogous to the *Sry* gene in mammals (which triggers synthesis of proteins involved in male development) has yet to be found (Ellegren 2000).

Any general conclusions about sex ratio biases may be difficult to determine taxon-wide because of the problem of selective publication of investigations that find significant effects. Also, effects may not be consistent within species, for example sex ratio biases observed in one year may not be present in the next (e.g. Radford & Blakey 2000). Investigation of sex allocation in birds is a relatively new field, because prior to the identification of molecular markers to determine sex, it was not possible to sex nestlings. This thesis has investigated both primary sex ratios in great and blue tits, and secondary sex ratio biases in great tits. The results suggest that female birds may have limited control over primary sex ratios, but increased sample sizes are advisable for both within-species studies and across-species comparisons before any general trends can be identified. The thesis also shows that biases in the secondary sex ratio may as likely be influenced by nestling interactions as active parental choice.

Appendix 1

Lab protocol for sexing birds from small blood or tissue/embryo samples

A. Chelex extraction

1. Label tubes
2. Add 200: 5% chelex (ie 5g in 100ml dd $H_2 0$)
3. Add small piece of blood to each tube
4. Heat 56°C for 20mins
5. Vortex 10s
6. Pierce lid with needle
7. Heat 90+°C for 8 mins
8. Vortex 10s
9. Spin 13000 rpm for 3 mins
10. Transfer supernatent to microtitre tray

    Keep tray in fridge

B. PCR recipe and reaction conditions

1. Arrange PCR microtray on ice and add 3µl DNA suspension to each well.
2. Make PCR mix according to recipe:

Water	525µl
Buffer	110
P2/P8	55/55
dNTPs	11
enzyme	11

Mix above in 2ml eppendorf, on ice. Transfer 96.25µl of mixture into each of 8 smaller stock tubes.

3. Transfer 7µl of mix to each well using micropipette, on ice.
4. Remove immediately to PCR machine

5. Run programme:
   - 94°C for 3 min
   - then 35 times the following cycles:
     94°C 30 s
     48°C 30 s
     72°C 30 s
   - 72°C for 5 min
   - 4°C hold

C. Running polyacrylamide gels (Promega 1996)

i) Pouring gels

Glass plates are scored with 'TR' (=top right). This side of the plate should be treated for use.

1. Clean small plate with detergent
2. Clean then with 95% ethanol
3. In eppendorf mix 1ml 0.5% acetic acid in 95% ethanol with 3µl methacrylox.....silane (small fridge) *in fume cupboard*
4. Wipe mix over plate surface with small piece of Kimwipe.
   N.B. This sticks gel to plate, so only get it on this back plate or your gel could stick together – throw gloves away after use
5. Leave 5 mins.
6. Wash large plate with water. DO NOT use detergent!
   Water should bead on surface. If it does not, the plate needs recoating with repellent (i.e. rainex)
7. Wash large plate in 95% ethanol
8. Clean spacers and put in place on large plate

9. Rub small plate well with 95% ethanol. This takes residue off plate and MUST be repeated 3 times
10. Rub over small plate with dry tissue
11. Place small plate on spacers, with bottom ends of plates flush
    Push foam of spacers down onto plate to create a seal
    Do not push down in middle of small plate – glass is flexible and plates could stick
12. Clip sides with 2 bulldogs
13. Put 75 ml 6% polyacrylamide in beaker (for recipe see Promega 1996 p24)
14. Add 500μl 10% ammonium persulphate (from freezer) and 50μl TEMED (cupboard under gel bench)
15. Mix solutions with syringe. Time limit here
16. Slowly squirt polyacrylamide mix into gel plates. With ruler under plate frame, tilt plates to allow acrylamide to run down
17. Fit flat end of comb between plates into acrylamide so that the holes line up with the edge of the small plate
18. Cover end in clingfilm and clip with 4 bulldogs
19. Write time on plate; leave to set 1.5 hrs
20. Pour excess gel into beaker in fume cupboard; clean bench thoroughly

ii) Loading gel

1. Remove comb (care not to bend points), clean up plates
2. Put gel in rig and tighten screws in this order: top left, top right, bottom left, bottom right
3. Turn side screw to close plug
4. Fill reservoir with 50ml 10xTBE buffer/950ml water
   Fill top reservoir to 1cm above small plate
5. Clean top of gel
6. Push comb in so teeth just push into gel
7. Pre-run: Connect to power (manual, constant, 75 W, go). Leave for 45 mins
8. Loading samples: Add 7.5μl loading buffer to each 10μl sample
9. Denature samples (on hot block at 96°C or in PCR machine prog. 27)

Remove immediately to ice (Ok to load for 1 hr, otherwise denature again)

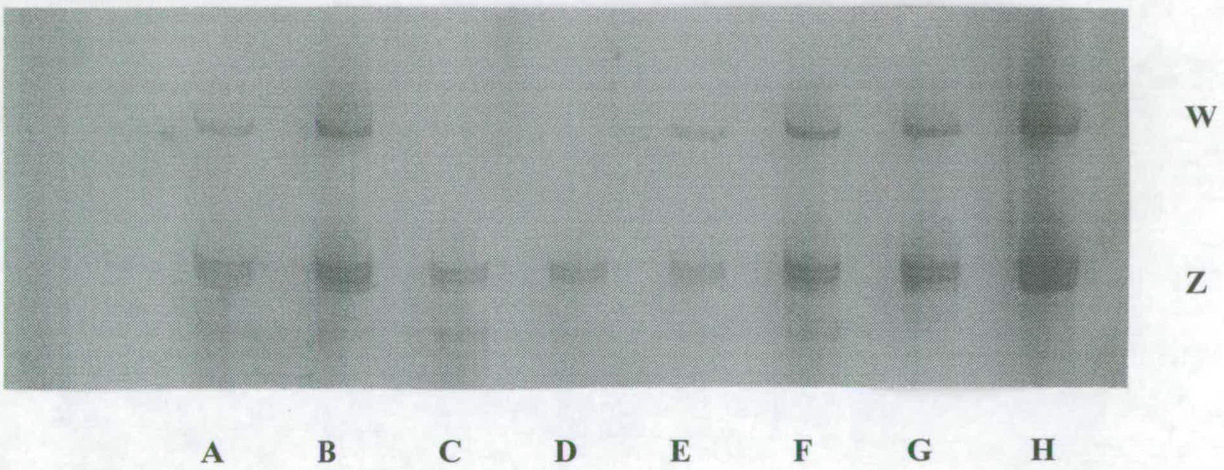
10. Load front 1 samples, 3-4μl sample (manual, constant, 70W, go)
    For sex primers, loading intervals 15-20 mins
11. Before loading each front, clean wells with pipette to remove urea
12. After loading last samples leave to run 45 mins-1hr (enough to ensure last samples pass heavy DNA garbage)
13. Prepare developer – dissolve 60g sodium carbonate in 2l water and refrigerate (24g in 800ml).

iii) Visualising

1. Open tap on gel rig
2. Unscrew gel plates, remove spacers and comb and part plates using wedge
3. Pour on fix/stop (200ml glacial acetic acid in 1.8l water – make in fume cupboard) CARE! (88ml in 800ml)
4. Leave 20 mins, moving occasionally
5. Rinse 3 times in water, 2 mins only each time
   Keep fix/stop in fume cupboard for later use
6. Put in stain (2g silver nitrate/ 3ml 37% formaldehyde in 2l water; or 0.8mg AgNO/1.2ml form. in 800ml)
7. Leave 30 mins
8. Remove developer soln from frigde and add 3ml formaldehyde/400μl sodium thiosulphate (1.2ml form. and 160μl sodium thiosulphate)
9. Remove gel, rinse in water 4-5 s ONLY before transferring immediately to developer
10. Put stain soln. in flask, adding sodium chloride to precipitate silver
11. Keep gel moving in developer until bands clear, then pout on stop soln from 5
12. Rinse gel and dry

Appendix 2

Gel products from PDR amplification of CHD1 gene introns



Products were visualised using silver staining procedures after PCR amplification as in Appendix 1. Z represents the CHD1 intron present on the Z chromosome that is amplified in both males (ZZ) and females (ZW). W represents the CHD1 intron present on the W chromosome and therefore distinguishes female birds. Hence individuals A, B, E, F, G and H are females, and C and D are males. The different strengths of bands reflects the amount of PCR products, e.g. individual E is a sample with little DNA, whereas sample H contains more concentrated DNA.

Appendix 3

Published paper:

Sex discrimination before birth. (1998*). Trends in Ecology and Evolution* 13 (4): 130-131.

we may see precursors of the avian air-sac system in the non-vascularized sections of the septate lung that are set aside to act as pumps and ventilate the vascularized sections of the lung, and one has to wonder whether the primary axis of cartilage development in the unique circumstances of the three fingered limb of a theropod, lacking the proximal elements of digit IV would look exactly like that in birds.

The debate over the phylogenetic position of birds seems far from any conclusion – the two camps seem as implacably opposed as ever. Why? Perhaps because where natural selection meets the strict constraints of biomechanics, convergence is inevitable, and separating common inheritance from common function may be near-impossible in a system so highly derived.

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Sex discrimination before birth

Sex-allocation theory describes how parents should bias investment in sons and daughters when male and female offspring give different fitness returns[1]. Often, studies of sex allocation are based on species where a mechanism influencing sex allocation is known to exist, for example haplodipoidy in some insects. Among birds, unusual physiological or genetic mechanisms of sex determination are not known; however, accumulating studies attribute skews to primary sex-ratio adjustment[2,3].

Although very little is known about the sex-determining mechanism (other than that it is chromosomally based), female birds have the potential to control the primary sex ratio, as they are the heterogametic sex. The few papers reporting skews in avian sex ratios often refer to secondary sex ratios and concentrate on adaptive rather than the proximate explanations for such biases. Now three[4–6] new papers indicate that female birds can detect offspring sex in the reproductive tract. Furthermore, females are capable of making decisions concerning investment in sons and daughters based on this information[6].

Heinsohn and colleagues report that in the parrots *Eclectus roratus* females are capable of extreme skewing of the sex ratio[4]. Data from aviculturalists' records of fledglings from successive breeding attempts showed that females produce much longer runs of one sex than expected if sex was determined by chance; in one case, 20 sons were produced before a daughter. *Eclectus* females usually lay two eggs but fledge only one young. Although it is possible that both eggs hatch and females then selectively kill chicks according to sex, the authors use statistical models to show that infanticide alone could not account for the observed sex biases. Moreover, where two young do fledge, they are very likely to be of the same sex: only six of 41 two-chick broods comprised young of both sexes. Rejected eggs were not found in the aviaries (R. Heinsohn, pers. commun.). In the absence of dump laying of 'wrong'-sexed eggs, or infanticide, the mechanisms generating these sex ratios must involve manipulation of sex before egg-laying.

Female zebra finches (*Taeniopygia guttata*)[5] similarly adjust the sex ratio of their eggs. In an elegant study, Kilner showed that females with experimentally restricted food intake produced significantly more male-biased sex ratios than when experiencing high food availability. The reduction in proportion of daughters produced was predicted, since some studies show that the reproductive success of females reared when food is scarce is more adversely affected than that of males. In addition to this overall sex ratio adjustment, Kilner found that early-laid eggs, which tended to hatch first, produced significantly more daughters than sons, regardless of food availability. Hence, females were manipulating not the overall ratio of sons to daughters, but also the order in which they produced males and females.

A description of avian reproductive physiology is needed to put these results into context. Female birds have only a single functional oviduct and ovary. In the ovary of a newly hatched chick there are millions of oocytes, most of which die during growth. Of the surviving fraction, a few develop each breeding attempt when layers of yolk are deposited sequentially around them. At maturity, the ovary contains a hierarchy of follicles, the largest being next to ovulate. Once released from the ovary, the follicle passes to the end of the oviduct and is fertilized by sperm waiting in folds at the top of this structure. The fertilized egg then spends a day passing through the oviduct, where albumen and shell is secreted around it before laying.

In principle there are at least three ways in which female birds could alter offspring primary sex ratio. They might either determine the sex of the follicle they ovulate through pre-ovulation control of chromosome segregation, differentially provision oocytes of different sexes to determine the order in which they will be released from the ovary, or detect the sex of the ovulated follicle and reabsorb follicles of the 'wrong' sex. The last explanation would result in delays in production of eggs (possibly reflected in the pronounced hatching asynchrony recorded in *Eclectus* parrots). Whatever the mechanism, these papers report strong evidence that females can recognize the sex of a follicle before laying.

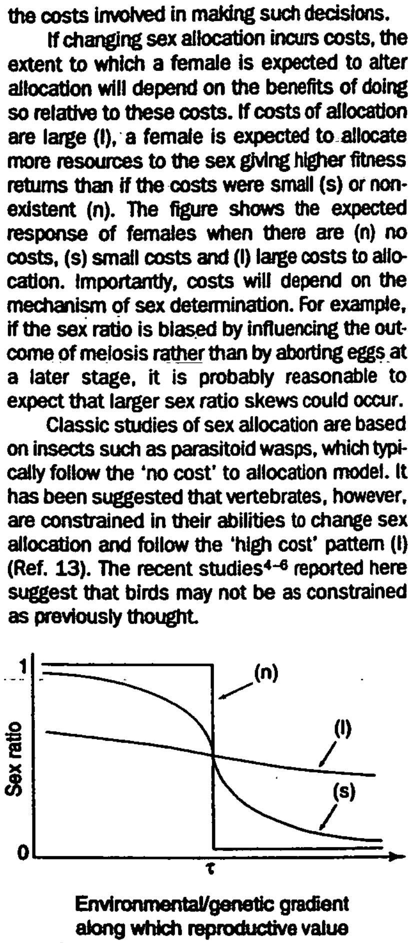
Research on American kestrels (*Falco sparverius*)[6] demonstrates that females are not only capable of recognizing the sex of a follicle, but also of provisioning the egg accordingly. Anderson and colleagues found that eggs producing males were larger than eggs producing females, irrespective of laying order. The authors explain this in terms of an adaptation to enhance the competitive ability of sons competing with larger sisters in the nest. In this case, the female may either determine which size of egg she is preparing to lay and then adjust the sex accordingly, or perhaps more likely detect the sex of the

Box 1. The importance of the cost of sex allocation

Imagine a changing environmental or genetic gradient across which male and female offspring give different fitness returns. Parents benefit by biasing investment in males in some situations and females in others. If there are no costs to adjusting sex allocation then up to some threshold value (τ), at which the reproductive values of males and females are equal, there is selection for always allocating to one sex. Above this threshold value there is selection for production of the other sex (n) (Ref. 12). One way to bias investment is to alter the primary sex ratio. However, whether a female changes allocation to males and females depends on the costs involved in making such decisions.

If changing sex allocation incurs costs, the extent to which a female is expected to alter allocation will depend on the benefits of doing so relative to these costs. If costs of allocation are large (l), a female is expected to allocate more resources to the sex giving higher fitness returns than if the costs were small (s) or nonexistent (n). The figure shows the expected response of females when there are (n) no costs, (s) small costs and (l) large costs to allocation. Importantly, costs will depend on the mechanism of sex determination. For example, if the sex ratio is biased by influencing the outcome of meiosis rather than by aborting eggs at a later stage, it is probably reasonable to expect that larger sex ratio skews could occur.

Classic studies of sex allocation are based on insects such as parasitoid wasps, which typically follow the 'no cost' to allocation model. It has been suggested that vertebrates, however, are constrained in their abilities to change sex allocation and follow the 'high cost' pattern (l) (Ref. 13). The recent studies[4–6] reported here suggest that birds may not be as constrained as previously thought.



follicle and alter egg size. Interestingly, recent work has shown that female canaries (*Serinus canaria*)[7] and cattle egrets (*Bubulcus ibis*)[8] were able to influence offspring attributes by provisioning eggs with varying amounts of steroid hormones depending on laying sequence. Although this allocation of steroids was not sex-specific, it is further evidence that female birds can adjust the provisioning of eggs on a remarkably fine scale.

These papers provide striking evidence that birds bias sex allocation at very early stages of development. Further genetic and physiological studies are clearly required to investigate the mechanisms allowing detection or determination of egg sex. Of crucial importance is knowledge of the exact timing of reproductive processes, such as when the sex-determining meiotic division occurs in relation to ovulation. Typically, however, assumptions about such events are based on data from domestic species where biases in the sex ratio are rarely observed and which are phylogenetically very distant from most species studied by behavioural ecologists interested in avian sex ratios.

In a recent *TREE* article[9], Emlen reported the case of Seychelles warblers (*Acrocephalus sechellensis*) studied by Komdeur and colleagues[10,11], which exhibit extreme biases in the primary sex ratio (up to 87% females). He suggested this control over which sex of egg is laid may be particularly prevalent amongst birds which lay single-egg clutches. Recognizing sex and biasing allocation accordingly would be an option for these birds, as they are not constrained by the costs of hatching asynchrony. Certainly this explanation could apply to *Eclectus* parrots, where selective reabsorption is a potential mechanism given that there is a long time interval before the laying of the second egg[4]. Emlen suggested that a possible 'compromise' for species laying multiple eggs was to bias the sex of the first egg and leave the rest to chance. However, Kilner's results with zebra finches suggest that even in birds laying quite large clutches, there may be some control over sex of later laid eggs.

These and other recent studies demonstrate the ability of female birds to skew sex ratios. Although of interest in its own right, a detailed understanding of the mechanisms involved might allow more refined understanding of adaptive sex allocation. If the mechanism of adjustment has implications for the reproductive value of that breeding attempt (through changes in clutch size, for example), then optimal sex allocation could be considerably affected[1]. For instance, suppose the number of follicles to be ovulated in a breeding attempt is predetermined (e.g. by available food resources). A female that selectively reabsorbed a follicle to change the sex ratio of her brood would have decreased the size of her clutch; the same would be true were she to dump lay an egg to select for the 'correct' sex. The range of sex allocation strategies expected from females will thus depend on the cost of changing allocation of resources between sons and daughters (Box 1). Knowledge of the mechanisms involved will play a key role in determining how large these costs are.

Recently-developed methods of identifying sex in birds are allowing widespread studies of avian sex ratios[14,15]. Hitherto, most studies have concentrated on the adaptive nature of observed sex-ratio patterns, and there are many avian systems which could potentially provide interesting data. However, the proximate mechanisms behind avian sex ratio adjustment are equally deserving of investigation. These recent results[4–6] demonstrate that the study of these mechanisms has the potential to reveal surprising information about the control parents have over investment in their offspring.

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Appendix 4

Implications of sibling competition for sex allocation in birds

Of recent studies of sex ratio variation in birds, some are suggestive of adaptive sex allocation strategies, while others are not. The expectation against which naturally occurring patterns of sex ratio bias is tested is based on the assumption that parents control the sex ratio. However, interactions among siblings may play an important role. One explanation for the inconsistent empirical observations is that sibling competition changes the optimum sex ratio. Recent studies support the view that sibling competition can change the relative fitness of male and female offspring. If sibling competition is important in changing sex allocation optima, there are two consequences for future studies of sex allocation in birds. First, correlational studies of species with marked sexual size dimorphism may be expected to produce inconsistent results. Second, experimental manipulations are urgently needed in order to determine how selection may act on sex allocation in natural populations.

When the reproductive value of the two sexes is influenced differently by some aspect of the environment, parents are selected to adjust their relative investment in the two sexes in response to that variable (Charnov 1982). Strong empirical evidence supporting this prediction comes from studies of haplodiploid parasitoid wasps responding to variation in host size (reviewed in Godfray 1994), but the general prediction applies to all taxa. Birds provide an interesting vertebrate taxon in which to study sex allocation because the sex of offspring is potentially determined by the heterogametic female, and because it is relatively easy to determine the sex ratio at oviposition. Although some recent studies of avian sex ratios are consistent with adaptive biases in sex allocation (e.g. Dijkstra et al. 1990; Komdeur et al. 1997; Sheldon et al. 1999), there are also a number of published studies (and perhaps more unpublished) which have failed to detect any biases, or for which biases are present in only some years (reviewed in Sheldon 1998).

In this note I argue that one explanation for this failure is that interactions between siblings will commonly alter the optimal sex allocation strategy in birds. This contention is supported by recent experimental work on birds which indicates that sibling competition can have differential effects on the fitness of the two sexes of offspring. I argue that a proper understanding of selection for avian sex allocation requires an experimental approach, and suggest an approach that may prove fruitful.

Models of adaptive sex allocation make many assumptions which, if violated, can influence the optimal sex allocation strategy (reviewed in Antolin *et al.* 1993). In the present case the relevant assumption is that parents have control over the sex ratio. If offspring are raised in families together, compete for parental investment, and the competitive abilities of offspring depend on their sex, this can potentially influence the reproductive value of the offspring to the parents.

Consider a species of bird in which one sex is considerably larger than the other at sexual maturity, and in which the sexual size dimorphism develops during the nestling growth phase (as is generally the case for sexually dimorphic birds: Richner 1991). Nestlings compete with each other for food that the parents bring to the nest and, all else equal, larger nestlings are better competitors than smaller nestlings. Imagine the species breeds in a range of environments which vary in the amount of food available for offspring. If one considered food requirements in the absence of sibling competition, one would expect the larger sex to be more adversely affected by a shortage of food, and hence that the reproductive value to parents of the larger sex would be less than that of the smaller sex when food was scarce. However, now allow for the fact that sexual dimorphism develops during the nestling growth phase (generally before the peak of food demand for the brood: Richner 1991). The sex which is normally larger at sexual maturity will gain a competitive advantage over the smaller sex, and the consequences of this competitive advantage may be relatively greater under conditions of relative food shortage than when food is common.

If this scenario is correct, the relative reproductive value of the sexes under poor conditions may be the opposite of the traditional prediction that greater returns will be obtained by investing in the smaller sex. Hence, parents may be selected to allocate more to the larger sex when conditions are very poor, as well as when they are very good (Figure A). This biased allocation could be in the form of a bias in the primary sex ratio, or sex-biased parental investment. The relationship between the difference in reproductive value of the two sexes and environmental conditions may be complex and non-linear, suggesting that it will be very difficult to predict how parents should divide resources among the sexes. Whether unequal competitive ability of male and female siblings influences the optimal sex ratio may depend on whether parents have complete control over the sex ratio. If control is complete, then a likely response to poor conditions would be to produce a brood composed entirely of the smaller, less competitive sex, since it is only in the presence of larger sibs that they would suffer a competitive disadvantage. However, empirical studies of avian sex ratios suggest that single sex broods are rare when brood sizes are large. An additional layer of complexity may be added by the possibility of a trade-off between the sex ratio and brood size (Williams 1979).

The effects of sibling competition on avian sex ratios have received little attention from empirical studies; experimental investigations are conspicuously lacking. Interest has been limited to size dimorphic siblicidal species with small brood sizes, where the decrease in reproductive value of an individual of one sex through competitive interactions with its nest-mates is obvious and extreme (i.e. death). Results from these investigations have been inconsistent. Amongst raptors, biased sex ratios in favour of larger females have been recorded for hen harriers (Balfour & Cadbury 1979, Picozzi 1980), although further investigation is necessary to determine whether this was due to higher mortality of small males. Olsen & Cockburn (1991) recorded significantly female-biased sex ratios in 12 falconiforme species. Statistical re-evaluation suggests this to be the case for only two species – the hen harrier and peregrine falcon (Krackow 1993). Harris's hawk nests which hatched females first fledged significantly less young than those which hatched males first (Bednarz & Hayden 1991). A potential consequence amongst such nests

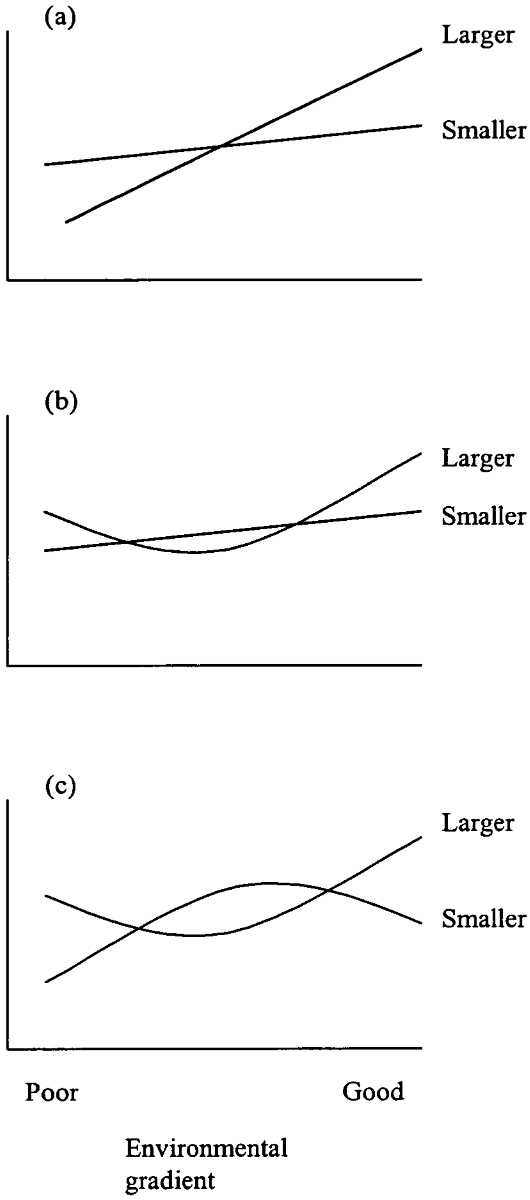

Figure A: Reproductive value of male and female offspring across a changing environmental gradient, in a sexually dimorphic species. Often RV is assumed to be linearly related to the resource environment, with the smaller sex having a higher RV in a poor environment and the larger sex a higher RV in a rich environment (Fig. Aa). However, RV may be affected by competition in the nest, with the larger sex able to outcompete smaller sibs. This may distort the functions relating RV to the environment (for either one, Fig. b, or both sexes, Fig. c) so that the larger sex is favoured in both poor and rich environments. In the case of sibling competition, we assume that competitive ability of one sex is reflected by increased RV of that sex (certainly the case for siblicidal species).

is heavily female biased fledging sex ratios. Two studies of eagles (golden eagles, Edwards & Collopy 1983; bald eagles, Bortolotti 1986) suggest greater mortality of smaller males, but neither produce evidence that brood sex composition affected offspring fitness or mortality. One study of siblicidal blue-footed boobies provides no evidence of a daughter's growth or survival advantage, despite the larger size of female offspring (Drummond *et. al.* 1991). In late-hatching nests they even recorded greater mortality amongst last-hatched females in three-chick broods (Torres & Drummond 1997).

Apart from the case of the blue-footed boobies, these studies hint at a positive relationship between body size and survival in reverse sexually size dimorphic birds. None, however, include experimental evidence to support correlational data. Several studies of wild great tits have revealed associations between environmental conditions and sex-specific survival in the opposite direction to that expected based on simple nutritional dependency (e.g. Dhondt 1970; Drent 1984; Smith *et al.* 1989; Lessells *et al.* 1996). A recent experimental test showed that when the degree of competition between sibling great tits Parus major was manipulated experimentally, males, although the larger sex, were less affected by increased competition than were females, supporting the suggestion that their larger size gave them a competitive advantage over their siblings (Oddie 2000, chapter 5).

If sibling competition frequently influences the reproductive value of the sexes in sexually dimorphic bird species it may have a number of consequences. Firstly, parents may be selected to influence the prospects of offspring of particular sexes, either by changing their position in the laying (and hence hatching) order, or by selectively provisioning eggs of the disadvantaged sex. Associations between laying (and hence hatching) order and sex ratio may be quite frequent in birds (e.g. Kilner 1998), and Anderson et al. (1997) have reported sex-associated variation in the size of American kestrel Falco sparverius eggs consistent with the suggestion that parents give smaller sons an initial advantage in sibling competition. Alternative explanations for associations between laying order and sex ratio are, of course, possible.

A second consequence of competition between sibs is that it makes it difficult to assess whether the failure of correlational studies to uncover relationships between environmental variation (e.g. laying or hatching date, or measures of food availability) and sex ratios in birds reflects an absence of adaptation in the parents or inappropriate expectations as regards selection on sex allocation. One way to solve this problem is to try to assess the fitness of male and female offspring in response to experimental manipulations that mimic an environmental gradient (e.g. Sheldon *et al.* 1998). Such experiments can potentially provide evidence as to whether there is actually selection for parents to bias sex allocation, something for which there is virtually no relevant empirical evidence from wild vertebrate populations. Experiments simultaneously manipulating the brood sex ratio (e.g. Lessells *et al.* 1998) and offspring rearing conditions would also provide a valuable means to investigate the way in which the sex ratio of the brood interacts with the conditions under which it is reared to determine fitness of male and female offspring. Studies of sex allocation in birds (and other vertebrates, such as mammals) need to move beyond the production of an ever-increasing number of correlations (or lack of correlation) with the sex ratio if we are to advance our understanding of adaptation and constraints on sex allocation in these organisms.

Appendix 5

Published paper:

Size matters: competition between male and female great tit offspring. (2000). Journal of Animal Ecology

Journal of Animal Ecology 2000,
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Size matters: competition between male and female great tit offspring

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Summary

1. Studies of sex allocation in birds have traditionally centred on Fisher's (1930) theory of equal parental investment in male and female offspring. They concentrate particularly on sexually dimorphic species, where costs of rearing offspring are assumed to vary between male and female young because of body size differences.

2. Higher mortality of the larger sex (males), particularly in poorer conditions, is expected to result in female-biased sex ratios in the great tit, *Parus major*. Several studies have found the contrary, reporting male-biased fledging and recruiting sex ratios when conditions are poor. One reason why this may be the case is that males can gain more food resources than their sisters because of the competitive advantage afforded by their larger size. They may thus suffer less mortality in the nest or fledge in better condition, thereby enhancing their survival prospects.

3. This study investigates the importance of size in competitive interactions between nestlings of different sexes. A cross-fostering design was employed to create broods of mixed size through swapping half a brood of 2-day-old 'small' and half a brood of 4-day-old 'large' nestlings. Nestling morphometrics and mortality were measured during the nestling period. Nestlings were sexed by PCR amplification of sex-linked genes. To test for a male advantage in competitive environments, size and mortality measures were compared between 'small' males and females, and 'large' males and females (i.e. the interaction term 'size treatment' and 'sex').

4. There was greater sexual dimorphism between small nestlings than large nestlings at fledging. This is interpreted as revealing enhanced competitive ability of male offspring under stressful conditions. Offspring from the 'large' group suffered lower mortality, but there was no difference in mortality according to sex, and no significant size*sex interaction. Similarly, no difference in recruitment was found, although this may be due to the small overall proportion of birds recruiting (3·1%).

5. The study suggests that male biased fledging/recruitment sex ratios in great tits may be explained by enhanced competitive ability of male nestlings in poor rearing environments.

Key-words: asynchrony, sex allocation, sex-specific growth, sexual size dimorphism, sibling competition.

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Introduction

In sexually dimorphic animals, two non-exclusive hypotheses have been proposed to explain sex ratio biases towards the smaller sex at the end of the period of parental care. First, natural selection for equal allocation to male and female offspring will result in biased sex ratios when the cost of rearing each sex differs (as expected for dimorphic species; Fisher 1930). Secondly, sexual selection for larger size of one sex in adulthood may necessitate larger size of that sex during the period of parental investment. Consequent non-adaptive mortality of the lar-

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ger sex due to higher energy demands which cannot be met by parents then results in sex-biased mortality (Clutton-Brock, Albon, & Guinness 1985). Both processes may result in the over-production of the smaller sex via the same mechanism, namely increased mortality of the larger sex.

Studies of altricial birds have tested for sex ratio biases in dimorphic species, considering both of the above theories as possible causes of sex ratio biases (e.g. Weatherhead & Teather 1991). However, although a few have documented sex ratio biases towards the smaller sex in stressful rearing conditions (Howe 1977; Cronmiller & Thompson 1981; Røskoft & Slagsvold 1985), most found no deviation from unity in sex ratios despite considerable sexual dimorphism of the study species (Selander 1960; Richter 1983; Weatherhead 1983; for reviews see Newton 1979; Clutton-Brock 1986). As it seems reasonable to expect that offspring size dimorphism results in different rearing costs of males and females, or unequal nutritional requirements, the mismatch of empirical results with theoretical predictions points to other processes in the nest counter-balancing sex-biased starvation. The greater size of one sex may enable it to reach higher towards the parent, occupy favoured feeding positions or push smaller nest-mates away from food deliveries. Through such competitive advantages, larger offspring may be able to gain additional food they require for growth and maintenance.

I investigate the importance of size and sex in competitive chick interactions in a large-brooded, synchronously hatching (within 2 days) species, the great tit *Parus major*, where competition for food is likely to be especially intense. Mean clutch size is 9·1 eggs (± 1·16, $n = 273$), and nestlings are provisioned for 17–22 days, with an average of 5·9 fledglings (± 3·2, $n = 96$) produced per nest. In this population, adult great tits exhibit 7% sexual dimorphism in mass and 3·5–4% skeletal size dimorphism (tarsus and wing lengths, males > females), with size differences statistically apparent amongst nestlings from 5 days of age (this study). Males are expected to have higher nutritional requirements for maintenance and growth, and thus female-biased sex ratios are predicted if these requirements cannot be met. Studies measuring energetic requirements of sexually size-dimorphic blackbirds (Icteridae) have shown that in these species males (larger) do, indeed, have higher energy demands than females (Fiala & Congdon 1983; Teather & Weatherhead 1988). Similarly, greater energetic demands of the larger sex (females) have been directly measured in marsh harriers (Riedstra, Dijkstra & Daan 1998). In great tits, contrary to the expectation that under poor rearing conditions males perform relatively worse then their sisters, several studies have demonstrated male-biased sex ratios amongst nestlings reared in poor environments. Dhondt (1970) found that both in areas and periods unfavourable for nesting great tits significantly more males fledged. In a brood size manipulation experiment, Smith, Kallander & Nilsson (1989) found proportionally more males surviving from enlarged than reduced broods. Lessells, Mateman & Visser (1996) found that the proportion of males hatching in a clutch increases with laying date (later hatching broods are less successful and are expected to experience less food abundance). Furthermore, they found more males recruiting from nests with higher nestling mortality. An earlier study of the same population of tits similarly found a male-biased sex ratio amongst fledglings when nestling mortality was higher (Drent 1984).

These studies demonstrate a biased sex ratio contrary to that predicted by theory and no study, to my knowledge, has reported the reverse pattern in great tits. Even if there is no difference in the cost of rearing male and female nestling great tits, as a recent study suggests (Lessells, Oddie & Mateman 1998), we would expect to observe neither a male nor female biased sex ratio. I suggest that the observed male biases are due to the enhanced competitive ability of males due to their larger size. Using a cross-fostering experimental design to establish an obvious size difference within a brood, growth and survival of different sized nestlings of different sexes was investigated. By comparing nestling growth between 'small' males and females competing with 'large' males and females in the same nest, I test whether males indeed fare better than their sisters in conditions of extreme sibling competition.

Methods

CROSS-FOSTERING MANIPULATION

Nest-box breeding great tits on the Swedish island of Gotland (57°10′N, 18°20′E) were experimentally manipulated for this study in 1997 and 1998. Nest-boxes were monitored for nest establishment and egg-laying, and daily nest-box checks allowed exact hatching dates to be determined. Following hatching, a small amount of blood (1–5 μL) was collected from the brachial vein of each chick on day 1 (day of first egg hatching = day 0) for sex determination using PCR-based molecular techniques (Griffiths, Double, Orr & Dawson 1998). Blood was stored in 50 μL SET buffer at −20°C (1997) or 98% alcohol (1998). Individual nestlings were matched to their blood samples by a unique identification code created by selective clipping of one or two of their six down tracts.

The size of nestlings within a brood was experimentally manipulated by moving chicks of different

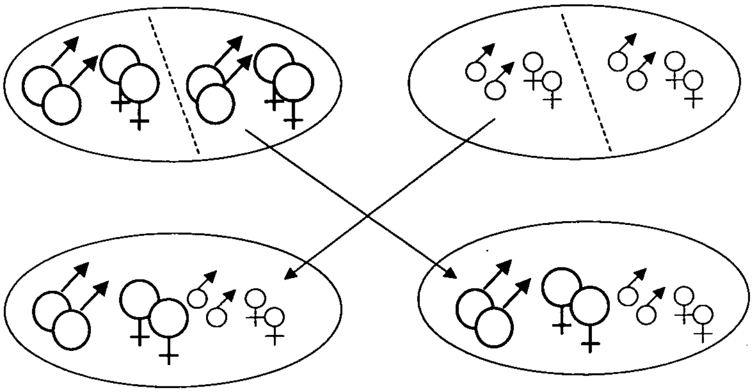

Fig. 1. Representation of cross-fostering design to create broods ($n = 84$) of mixed size *Parus major* nestlings. Typically half a brood of 4-day-old nestlings were swapped with half a brood of 2-day-old nestlings. Sex of nestling was not known at swap, rather the proportion of one sex moved was determined by chance.

ages between nests. Nests with similar brood size (same or ± 1 nestling) at hatching were paired for cross-fostering generally when nestlings in one nest were 2 days old and in the other 4 days old. Half of the 'large' brood was then swapped with half of the 'small' brood, creating two nests each with half 'large' and half 'small' nestlings (see Fig. 1.) Sex of nestlings was not known at the time of swap, but the large brood size (mean = 8·33 at time of cross-fostering) in this species meant that in the majority of cases nestlings of both sexes would be present in each group. Chicks were selected for swapping on the basis of which down tract was clipped; for each manipulation I selected nestlings for moving starting with the next consecutive letter code to the last swap in order to randomise which nestlings were relocated. Brood sizes did not change except in two pairs of nests where brood size increased or decreased by one nestling in each nest. In total, 42 pairs of nests were created (17 in 1997 and 25 in 1998).

Of importance when swapping was the size difference between nestlings, rather than age *per se*. Therefore, in six of the 42 pairs, nestlings of 3 and 5 days were swapped (especially at the beginning of the season when nestlings grew slowly) or 3- with 4-day-olds, etc. Nests with hatching asynchrony of more than 1 day were not used. In this population eggs sometimes hatch 1 or 2 days after the first hatching date. Size manipulations thus reflected possible natural variation in sibling size and competitive situations which may be naturally encountered by last-hatching nestlings. If it was obvious that the size difference between nestlings was too extreme for smaller nestlings to survive, the swap was not carried out. As all nestlings were bled on day 1, nestlings were not bled and transported on the same day.

MORPHOMETRIC MEASURES

The following morphological measurements were recorded at the time of cross-fostering: mass (using 10-g pesola spring balance accurate to 0·1 g), tarsus and gape length (using dial callipers to nearest 0·5 mm). Gape length measures from one end of the mouth crease to the point of the bill and increases with nestling age as the bill develops. Measures were repeated 5 days after experimental manipulation ($n = 80$, i.e. nestlings aged 7 and 9 days), 10 days after manipulation ($n = 39$, i.e. nestlings aged 12 and 14 days), and 12 days after manipulation ($n = 72$, nestlings aged 14 and 16 days). Nestling mass of great tits on day 15 after hatching is generally the same as that at fledging (Schifferli 1972; van Balen 1973), and the last set of measurements were taken to reflect condition at fledging. Originally, measurements were taken 10 days following manipulation, but it was soon recognized that nestlings could be safely measured 2 days later without causing premature fledging. Because of time constraints, nestling measures at day 12 were prioritized, explaining the small sample size for day 10 measures. Other sample sizes less than 84 represent whole brood failures or predation. On days 10 and 12 post-manipulation, wing development was substantial enough that wing length was additionally measured to the nearest 0·5 mm. Nestlings were banded with aluminium rings, matching down clipping identification marks to ring number. Where nestlings were not aged 2 and 4 days when swapped, measures were not always taken 5, 10 or 12 days after swapping, but on days when nestlings were 7/9, 12/14 or 14/16-day-old (or as near as possible).

After fledging, boxes were checked for individuals failing to fledge. Birds recruiting to this population in 1998 and 1999 were recorded by catching pairs breeding in any of the ~1000 nestboxes in the surrounding woodlands using either nest-box traps or mist-nets. Between 170 and 240 pairs of great tits breed annually in the nest-boxes on Gotland. In 1998, 72·1 and 76·9% of all breeding males and females were caught, respectively, and in 1999, 83·5% males and 85·3% females were caught. Although it is possible that recruiting individuals may breed in natural holes, as well as nestboxes, there is no reason to believe why nestlings of either sex from either experimental category should preferentially nest in natural cavities. Thus, I expect no sampling bias in recruits from each treatment due to recruitment of individuals outside nestboxes, which could not be measured.

SEX IDENTIFICATION

Nestling sex was determined by PCR amplification of two *CHD* genes located on the sex chromosomes. Two copies of the gene are present in females (*CHD1W*, present on the W chromosome and *CHD1Z*, present on the Z chromosome), whereas only one copy (*CHD1Z*) is present in homogametic males. After DNA extraction from blood samples (5% chelex extraction) these sex-specific fragments of the *CHD* gene were amplified using primers P2/P8 and PCR conditions as described in Griffiths *et al.* (1998). Products were run on 6% polyacrylamide gel and visualized using silver staining *(Promega).* I lacked samples for 25/699 (3·6%) nestlings and failed to determine sex in a further two (0·3%) cases. From 54 blood samples of adults (28 males, 26 females) and 20 recruits (12 males, eight females) where I determined phenotypic sex in the field, genetic sex determined by molecular methods matched in all 74 cases.

DATA ANALYSIS

Comparison of male and female sizes (mass, tarsus length, wing length, gape length) among both small and large nestlings were made using paired *t*-tests of sib-group means. Analyses were carried out to look for effects of nestling sex and size ('large' vs. 'small' nestlings, according to box of origin) on survival and morphological measures. To determine whether there was a different effect of being large or small for each sex of individual on growth, the interaction term size*sex was entered in a general linear model with morphological measurements (tarsus, mass, gape length, wing) as dependent variables and size and sex as factors. The unit of analysis was each nestling, but because nestlings are reared non-independently in a common environment, box of rearing was included as a factor in the model to control for differences between nests due to parental/territory quality and year. Because sizes of nestlings at swapping would influence subsequent measures, initial mass and tarsus measures were included as covariates. Timing of breeding was found to have a significant effect on growth measures and this variation was removed from the model by including clutch initiation date as a covariate. Analyses were carried out separately for each set of chick measures (on days 5, 10 and 12 following manipulation) using procedure MANOVA in the statistical package JMP Statistical Discovery Software Version 3·1).

Logistic regression was used to assess whether survival of nestlings to day 5, 10 and 12 following brood manipulation was a function of their sex, size and the interaction of the two. The same analysis was used to examine effects of an individual's size and sex on survival to fledging. From 75 nests (excluding four predated nests and five total nest failures, total number of nestlings = 633), 79% of nestlings fledged. As survival differs greatly between nest-boxes, the environmental factor 'box of rearing' was added to the model as a random effect. Likelihood ratio tests were used to determine the significance of a variable, entering variables 'sex', 'size', "size*sex' and 'box of rearing' in the model simultaneously. Since controlling for timing of breeding (lay date) and cross-fostering had no effect on the outcome of tests, these variables were not included in results presented here in order to keep the model as simple as possible. The number of birds recruiting from experimental nests in 1998 and 1999 in relation to their size and sex was analysed similarly.

Results

The 699 nestlings involved in the study fell into the following categories: 161 small males, 175 small females, 156 large males and 182 large females (25 unsexed).

NESTLING GROWTH

Development of sexual dimorphism

For all measurements post-cross-fostering where sexual dimorphism was detected, males were larger than females (Tables 1a and 1b). At the time of manipulation, there was no difference in any size measure between small (2-day-old) male and female nestlings. Amongst small nestlings, by day 5 post-manipulation, males had larger tarsi ($t = -2{\cdot}06$, d.f. $= 46$, $P = 0{\cdot}045$). By day 10 post-manipulation sexual differences in tarsus length remained ($t = -3{\cdot}61$, d.f. $= 20$, $P = 0{\cdot}002$) and a difference in body mass was also apparent ($t = -2{\cdot}23$, d.f. $= 20$, $P = 0{\cdot}038$). Wing length was marginally non-significantly different in males and females ($t = -2{\cdot}02$, d.f. $= 20$, $P = 0{\cdot}057$). Near fledging (12 days post- manipulation) male nestlings were larger than females for all measures (mass: $t = -3{\cdot}29$, d.f. $= 35$, $P = 0{\cdot}002$; tarsus: t

Table 1. (a) and (b) Size dimorphism (%) between male and female great tit nestlings following cross-fostering manipulation of half a brood of small nestlings with half a brood of large nestlings. Values for small nestlings in Table 1a, large nestlings in Table 1b. Both nestlings which were moved and those which stayed in their natal nest are included. Positive values indicate males larger than females, negative values females larger than males (* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$; for statistical tests see text). Sample sizes on day 0 deviate from the original 84 manipulated nests because not every nest was composed of nestlings from each category. Sample sizes on days 5, 10 and 12 differ as nestlings died, leaving some categories empty, and because not all nests were visited on each measurement day to record nestling sizes (see Methods: morphometric measures)

(a) Small nestlings

Day post-manipulation	Age	*n*	Mass	Tarsus length	Gape (days)	Wing
0	2	65	−0·10	−0·10	−0·72	
5	7	47	+6·04	+2·60*	−0·25	
10	12	21	+8·03*	+5·78**	+0·01	+4·15
12	14	36	+5·80**	+3·69***	+0·92*	+2·66*

(b) Large nestlings

Day post-manipulation	Age	*n*	Mass	Tarsus length	Gape (days)	Wing
0	4	69	−1·32	−1·33	−2·28**	
5	9	64	+3·82*	+1·14	−0·77	
10	14	28	+4·00***	+2·24***	+0·04	+1·82
12	16	56	+4·64***	+2·19***	+0·44	+1·50

= −5·77, d.f. = 35, P = 0·000; gape length: t = −2·32, d.f. = 35, P = 0·026; wing length: t = −2·20, d.f. = 35, P = 0·034).

Amongst large nestlings, females had longer gapes than males at swap (4-day-old; t = 2·93, d.f. = 68, P = 0·005), but other measures did not differ (mass: t = 0·58, d.f. = 68, P = 0·561, tarsus: t = 1·17, d.f. = 68, P = 0·248). By day 5 after manipulation, males were heavier (t = −2·6, d.f. = 63, P = 0·012) than females and gape length differences were no longer apparent. The sex-specific mass difference remained 10 days post-manipulation (t = −4·39, d.f. = 27, P = 0·000) when males also had longer tarsi (t = −4·56, d.f. = 27, P = 0·000). There was non-significant tendency for males to have longer wings than females at this time (t = −1·87, d.f. = 27, P = 0·072). Near fledging males were heavier (t = −5·54, d.f. = 55, P = 0·000) and had longer tarsi (t = −5·53, d.f. = 55, P = 0·000). There was still a tendency for males to have longer wings than females, but this was not statistically significant (t = −1·91, d.f. = 55, P = 0·061), and there was no apparent sexual size dimorphism in gape length (t = −1·28, d.f. = 55, P = 0·206).

Comparison of size differences in large and small nestlings

In order to compare sexual size differences between large and small nestlings, the interaction term of the effects 'size category' and 'sex' was determined in a multiple analysis of variance of nestling measures. After 5 days of growth with size-manipulated nestmates, the size difference between males and females was the same amongst small nestlings as that for large nestlings (size*sex, $F_{1,432}$ = 1·505, P = 0·221). However, 12 days following manipulation, there was a significant difference between the sexual dimorphism amongst the large nestlings compared to the small nestlings (size*sex, $F_{1,354}$ = 5·434, P = 0·020). Ten days following manipulation the size*sex term was marginally non-significant ($F_{1,354}$ = 2·822, P = 0·095), perhaps due to smaller sample sizes. The significant effect on day 12 post-manipulation was mainly due to an effect on wing length (dropping wing length from analysis: size*sex, $F_{1,354}$ = 3·586, P = 0·059). Significance remained when any other dependent variables were removed from the model (mass: $F_{1,354}$ = 5·900, P = 0·016, tarsus: $F_{1,354}$ = 5·085, P = 0·025, gape length: $F_{1,354}$ = 5·018, P = 0·026).

Nestling measures both 10 and 12 days following manipulation reveal a greater sexual size difference amongst small nestlings compared to the size difference in large nestlings. Amongst small nestlings, females are smaller than males to a greater degree than amongst large nestlings (Fig. 2).

NESTLING MORTALITY

Parental and/or territory quality was a strong influence on nestling survival, with nest of rearing a significant effect on survival of nestlings to all ages. The size category of nestlings 'large' or 'small' was

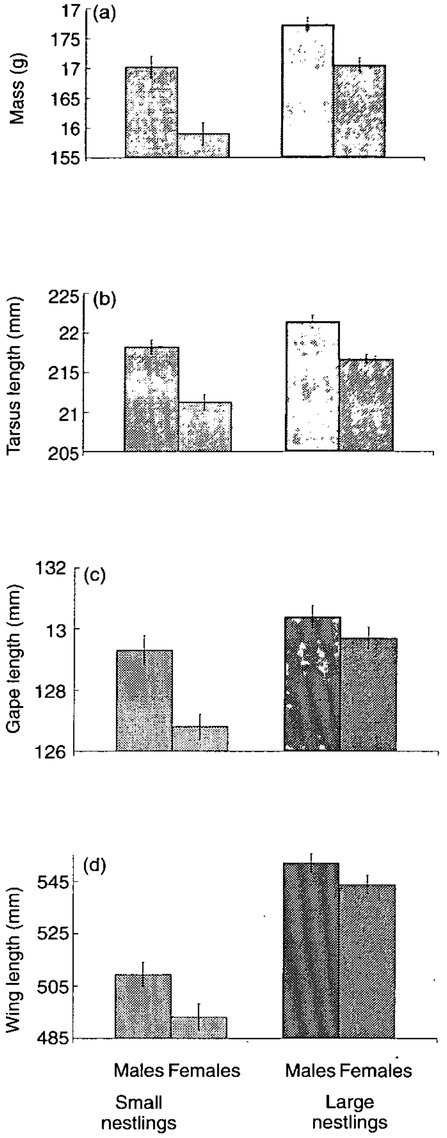

Fig. 2. Size differences between male and female great tit nestlings in cross-fostered groups of 'small' and 'large' offspring, 12 days following experimental manipulation. Small nestlings are thus typically 14-day-old, large nestlings 16-day-old. Graphs illustrate mean nestling (a) mass (g) (b) tarsus length (mm) (c) gape length (mm) and (d) wing length (mm) with standard errors.

also a predictor of survival, with larger nestlings clearly suffering less mortality (Table 2). Nestling survival was not affected by nestling sex, and there was no different effect of nestling sex among large and small young (non-significant size*sex interaction; Table 2).

RECRUITMENT

Twenty birds recruited to the breeding population (3·1%), 15 from 1997 and five from 1998. The size category of a nestling in the experimental manipulation did not affect its chances of recruiting ($\chi^2 = 0{\cdot}00$, $P = 0{\cdot}99$, d.f. = 1), nor did any morphological measure at fledging predict recruitment probability (mass: $\chi^2 = 2{\cdot}250$, $P = 0{\cdot}134$, d.f. = 1; tarsus: $\chi^2 = 2{\cdot}642$, $P = 0{\cdot}104$, d.f. = 1; wing: $\chi^2 = 0{\cdot}627$, $P = 0{\cdot}429$, d.f. = 1; gape length: $\chi^2 = 1{\cdot}143$, $P = 0{\cdot}285$, d.f. = 1). Body condition, measured as residual mass at fledging for a given body size (tarsus length) was almost a significant predictor of nestling recruitment ($\chi^2 = 3{\cdot}038$, $P = 0{\cdot}081$, d.f. = 1). Comparing the masses of individuals recruiting to those not, recruiting nestlings had higher mean absolute body weights (recruits: 17·28 ± 0·40 g; non-recruits 16·94 ± 0·08 g) and higher weights relative to body size (recruits: 0·50 ± 0·29 g; non-recruits: −0·02 ± 0·06 g), although these differences are not statistically significant (day 12 mass: $F_{1,444} = 0{\cdot}688$, $P = 0{\cdot}407$, residual day 12 mass: $F_{1,444} = 3{\cdot}008$, $P = 0{\cdot}083$). The recruits consisted of 12 males and eight females; this difference was not statistically significant ($\chi^2 = 1{\cdot}322$, $P = 0{\cdot}245$, d.f. = 1). Finally, the interaction effect size*sex on recruitment was not significant ($\chi^2 = 0{\cdot}020$, $P = 0{\cdot}881$, d.f. = 1).

Discussion

For a species where males are larger than females, one would expect a female sex ratio bias at the end of the period of investment, assuming the smaller sex to be less costly to rear (Fisher 1930; Maynard-Smith 1980). Several studies of great tits have reported the opposite (Dhondt 1970; Drent 1984; Smith *et al.* 1989; Lessells *et al.* 1996) and this study highlights the importance of size dominance of larger offspring which could account for this observed discrepancy. In experimentally manipulated broods of great tits comprising half 'large' and half 'small' nestlings, sexual size dimorphism was less marked for large individuals than for small individuals. When competition for food is relaxed, i.e. amongst large nestlings, males and females attain more similar growth measures than when competition is pronounced (small nestlings). The advantage of 'being male' – attaining higher weights and larger biometric sizes than sisters – is particularly important for small nestlings who face an extremely competitive nest environment. Larger individuals may be more successful at obtaining food by reaching closer to parents when begging, pushing smaller competitors away from access to parents, and occupying preferred feeding positions in the nest (Rydén & Bengtsson 1980; Bengtsson & Rydén 1983; Kolliker *et al.* 1998).

The idea that females are at a competitive disadvantage with larger nest mates is supported by the sexual size differences observed between nestlings (Tables 1a and 1b). By fledging, small males and females differed significantly in every dimension measured (mass, tarsus length, wing length, gape

Table 2. Proportion of great tit nestlings dying and predictors of survival. Logistic regression relating nestling mortality to nestling size and sex and size*sex interaction, following brood composition manipulation (see Methods). Original number of nestlings in each treatment group in nests without total nest failure/predation: large males 143, large females 169, small males 156, small females 165

Time post-manipulation	Proportion of nestlings dying		*n*	Predictors of nestling survival	d.f.	Likelihood ratio χ^2	$P (> \chi^2)$
5 days	Large		312	Nest of rearing	73	184.04	< 0.001
	Males	0.09	13	Size	1	50.006	< 0.001
	Females	0.07	12	Sex	1	0.412	0.52
	Small		321	Size*Sex	1	0.415	0.70
	Males	0.29	45				
	Females	0.27	45				
10 days	Large		312	Nest of rearing	73	190.30	< 0.001
	Males	0.09	13	Size	1	60.581	< 0.001
	Females	0.07	12	Sex	1	0.462	0.50
	Small		321	Size*Sex	1	0.073	0.79
	Males	0.32	50				
	Females	0.29	48				
12 days	Large		312	Nest of rearing	73	208.32	< 0.001
	Males	0.10	14	Size	1	56.271	< 0.001
	Females	0.10	18	Sex	1	0.108	0.74
	Small		321	Size*Sex	1	0.177	0.67
	Males	0.34	53				
	Females	0.34	56				
Fledging	Large		312	Nest of rearing	73	201.63	< 0.001
	Males	0.10	14	Size	1	61.012	< 0.001
	Females	0.11	18	Sex	1	0.037	0.85
	Small		321	Size*Sex	1	1.016	0.31
	Males	0.35	55				
	Females	0.34	56				

length), but amongst large nestlings only in mass and tarsus length. Moreover, the degree of dimorphism is greater amongst small nestlings. Small females must compete with three classes of nestling, all larger than themselves: large males, large females and small males. Large females, on the other hand, must compete only with one class of nestling larger than themselves (large males).

It could be argued that size treatment in this experiment is confounded with age and that sexual size dimorphism initially increases to a certain nestling age, but then declines. In this way, it would be possible to generate the observed results of size dimorphism more apparent amongst smaller nestlings simply as a function of their age. However, it is biologically unlikely that sexual size differences would develop and then diminish, and the significant size*sex interaction recorded 12 days post-manipulation was also detected 10 days post-manipulation, although not statistically significant. Size dimorphism could initially increase then decrease if males were to reach their asymptotic size before females. However, in a review of sexually size dimorphic species, Richner (1991) found no evidence for this, but that there is no difference between time for each sex to reach its asymptote or the smaller sex reaches asymptotic body mass quicker.

The effect reported here of males faring better than their sisters is opposite to that expected from a simple nutritional dependence. It is also contrary to studies of sexually dimorphic mammals demonstrating higher mortality of the larger sex (Clutton-Brock *et al.* 1985; Clutton-Brock 1991). Studies revealing sex-biased mortality of sexually dimorphic mammals typically deal with organisms with a litter size of one (ungulates, primates, man), where there is no sibling competition. In multiparous, sexually size dimorphic organisms, sibling competition may represent a considerable influence on the sex ratio at the end of the period of care.

It is possible that hormonal differences between the sexes, their effects on behaviour (e.g. increased aggression) and immune responses of nestlings could account for male dominance in the feeding arena, rather than size as I have argued. The experimental design allows the effect of size to be teased apart from the effect of sex and other traits which co-vary with sex such as hormonal levels. If sex-specific hormone levels alone were responsible for increased male growth we would expect the same sexual dimorphism to be equally apparent in small and large nestlings. However, this study reveals that that size *per se*, and not other traits specific to sex, is an important determinant of offspring growth.

There is one possible explanation for depressed growth in small females which cannot, however, be excluded on the basis of this study. In birds, females are the heterogametic sex, and environmentally dependent expression of deleterious recessive alleles on the unguarded W chromosome could result in decreased female performance. However, investigations of sex-specific growth combined with brood size manipulations in the sexually monomorphic collared flycatcher, *Ficedula albicollis*, provide no evidence for this phenomenon in birds (Sheldon *et al.* 1998).

Although this study demonstrates that both size and sex of offspring affect fledging measures, the results showed nestling mortality to be a function of size category only and not predicted by nestling sex. Increased mortality amongst small nestlings was not surprising given that they had to compete with half a brood of considerably larger sibs. In naturally hatching clutches, the longest interval between hatching of the first and last egg is commonly 2 days, in one case 4 (personal observation; also true for other great tit populations). In nests with such extreme hatching asynchrony, it is usual that most eggs hatch on day 0 and only one, or two at most, hatch up to 2 days later. Often these last-hatched nestlings die within a day or two, unable to compete with such large sibs. In such asynchronously hatching nests, this mortality could be interpreted as an adaptive parental strategy to increase chances of survival in remaining offspring (e.g. Lack 1954). Although in great tits synchronous broods produce slightly more (but not significantly so) offspring, fledglings from asynchronous broods are heavier (see Amundsen & Slagsvold 1998). Given the discrepancy in competitive ability of male and female offspring, non-random allocation of sex between eggs may present a mechanism by which female birds could exert some control in breeding decisions, according to environmental conditions at time of rearing (see Slagsvold, Husby & Sandvik 1992). For example, if male nestlings are at a competitive advantage, females on high quality territories may produce larger broods by laying and hatching 'male eggs' last; females on low quality territories could follow a bet-hedging strategy more likely to result in brood reduction by laying 'female eggs' last. While there are numerous studies reporting associations between laying or hatching order, and the sex ratio (e.g. Ankney 1982; Cooke & Harmsen 1983; Ryder 1983; Weatherhead 1985; Bortolotti 1986; Clotfelter 1996; Dzus, Bortolotti & Gerrard 1996; Kilner 1998), at present there is no clear understanding of what explains such patterns.

In this study, the unequal competition experienced in the nest can be interpreted as a sublethal fitness effect, resulting in nestlings of unequal quality. Increased size and mass at fledging has often been found to have important fitness consequences for great tits. Mass of fledglings correlates positively with post-fledging survival in several great tit populations (Perrins 1965; Dhondt 1971; Garnett 1981; Tinbergen & Boerlijst 1990; however, see Lindén, Gustafsson & Pärt 1992) and a previous study (Verboven & Visser 1998) found fledglings of higher mass to have higher recruitment probability. Skeletally larger individuals at fledging may be able to monopolize parental feeds outside the nest cavity and obtain higher dominance rank in hierarchical winter flocks (see Hinde 1952; Garnett 1981). Kluijver (1957) found female great tits to be subdominant to males in competition for food and roosting places. Larger body size at fledging could therefore promote mass gain in large individuals, in turn enhancing overwinter survival prospects.

The reproductive value of offspring (Williams 1966) is a more important measure of offspring fitness than overwinter survival and therefore it is more critical to consider offspring recruitment to the breeding population. It would seem reasonable to assume that the positive relationship between fledging mass and survival demonstrated for tits would result in greater recruitment probability of heavier nestlings. Indeed, Verboven & Visser (1998) demonstrated such a relationship in two different populations of great tits. Although the present study found no significant effect of fledging mass or size on recruitment, the trend was in the expected direction, but tests lacked power due to small numbers of birds recruiting (only 20 nestlings from 635; cf. Verboven & Visser 1998). Individuals which recruited weighed more than non-recruits in both absolute terms and relative to body size, although differences were not statistically significant (see Results). There was no evidence of sex-biased recruitment based on these small samples.

Thus, although no effects of nestling size and sex on mortality and recruitment were detected in this study, it is possible that the significant size differences observed at fledging could have important consequences for these two fitness parameters. This result is consistent with the explanation that a size advantage of male nestling great tits can account for observations of male-biased sex ratios. All previous reports of unbalanced sex ratios in great tits have recorded male-biased fledging and recruitment sex ratios when young were reared in poor environments (Dhondt 1970; Drent 1984; Smith *et al.* 1989; Lessells *et al.* 1996). Similarly, Heeb *et al.* (1999) found a greater proportion of male nestlings recruiting from experimentally flea-infested broods. These observed male-biased ratios could even be enhanced by parents altering sex ratio themselves to account for lower fitness values of daughters in some conditions. If this indeed occurs, it could amplify the sex bias effects recorded in unmanipulated brood studies (Dhondt 1970; Drent 1984; Lessells *et al.* 1996). Regardless, the present study points to competition as an important selective force in the nest affecting quality of males and females raised. It cannot offer a proximate explanation for correlations involving hatching sex ratios (e.g. Lessells *et al.* 1996), but may offer an explanation for the evolution of such patterns.

Fisher's (1930) theory of equal parental investment in the sexes has been the motivation for most avian studies of sex-specific growth and mortality. Most of these studies have concentrated on the differential cost to parents of rearing offspring to fledging, searching for adaptive explanations for biased sex ratios. Less attention has been paid to proximate explanations for biased fledging/recruitment sex ratios (although see Schifferli 1980; Teather 1992). Such mechanistic explanations of sex biases at fledging may be important, and not in conflict with ultimate explanations. This and previous studies of great tits highlight the importance of considering both approaches in sex ratio studies. Although natural selection is the ultimate cause of sex ratios biases, constraints due to an organism's life-history (i.e. sexual size dimorphism driven by sexual selection) should not be ignored. Recent molecular technology allowing sexing of young birds should facilitate further studies of sex-allocation and sex-biased parental care. However, the outstanding problem in avian sex allocation studies remains that of quantifying fitness returns of sons vs. daughters, and even in delimiting the period of parental care.

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