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Parental care in relation to offspring sex and mate attractiveness in the Blue Tit

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Parental care in relation to offspring sex and mate attractiveness in the Blue Tit

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CHAPTER 1

General Introduction

Tobias Limbourg

Background

Parental investment is of major importance when it comes to enhancing offspring fitness. As all resources and behaviours directed to enhance the fitness of the offspring will inevitably incur costs to the parents, there will be a trade off between the offspring's fitness and the residual fitness of the parent (Williams 1966). High investment in offspring, for instance by feeding them intensely or by defending them against predators, will benefit their fitness by increasing their growth rate and survival probability, dominance status in social systems and/or access to mates later in life of their offspring. However, any resource given to the offspring is not available to the parents themselves, and the investment in their offspring is therefore costly to them. Not only will it limit the amount of resources that is available to themselves, parental investment will also incur other costs. For instance, parents may be exposed to a higher predation risk when they are searching for extra food to feed their offspring or when they are defending them against predators. All these actions may additionally decrease parental body reserves that are needed for overwinter survival and their future reproductive success in general. Therefore, to maximise lifetime reproductive success, a parent has to adjust the level of its investment into its current reproductive event in relation to the costs that it has to pay for that investment in terms of its future reproductive success (Trivers 1972). Only those parents that solve the trade-off between the offspring's fitness and the residual future fitness of the parent in the best manner will prosper in the population.

But how should parents solve this trade-off? It is the expected value of offspring that is the determining factor in parental investment decisions. To maximise lifetime fitness, parents need to adjust their investment to the expected value of their offspring. For instance, it will pay for a parent to invest heavily in offspring with high expected fitness values because the parent itself will (indirectly) gain fitness through its offspring. However, if the same amount of investment is spent on offspring of low expected value, the benefits through offspring fitness might not outweigh the costs incurred by the parents.

The expected fitness value of offspring is determined by a number of factors, many of which are likely to vary among breeding attempts (Andersson 1994). The

parents themselves are one factor, simply because there is much variation in the quality of parents, which will in turn affect the quality of their offspring. These differences in quality may have a genetic basis. For instance, some parents may possess good genes that will give rise to offspring of high genetic quality. But also, even without the existence of good genes, parents vary in their ability to raise high quality offspring. Furthermore, the cost of raising your offspring depends on the breeding environment. For example, a territory with sufficient resources provides a good breeding environment that ensures good growth of the young, while in poor environments it may for example be much more difficult to find sufficient food, which may lead to undernourished offspring. Lastly, characteristics of the offspring themselves, in particular their sex, determine their expected value. There may be situations in which one offspring sex will offer higher pay-offs than the other sex (Charnov 1982). For instance, in many species male offspring have a higher potential mating success than female offspring, and only the fittest sons are likely to out compete other males (Clutton-Brock et al. 1982).

Differential Allocation

Parental attractiveness is one of the important factors that determine the expected fitness value of offspring. In sexually reproducing species with mating systems that involve mate choice, being attractive can be a major fitness advantage over competitors (Andersson 1994). This is because the most attractive individuals have the highest mating success. Offspring will benefit from the attractiveness of their parent if it is passed on to them, and offspring of attractive mates will thereby have a higher fitness. As mate attractiveness usually varies in the population, individuals should maximise their fitness pay-offs by adjusting their reproductive output to the attractiveness of their current mate (Burley 1986; Sheldon 2000).

Fitness pay-offs for offspring sired by attractive mates are likely to be higher than average for a number of reasons. Not only do they inherit the attractiveness of the parent or other genes that will increase the fitness of offspring (indirect benefits), such mates might also provide environments that are particularly suitable for raising offspring (direct benefits) (Andersson 1994). For individuals that are

mated to relatively attractive mates, it will thus pay to invest more in the current reproductive event, as the fitness returns of investment are likely to be higher than those with future mates, which will probably be of lower quality. If mated to a less attractive mate, in contrast, it might pay to invest less in the brood, and save resources for future survival and reproduction (residual reproductive value), resulting in a higher reproductive output in the succeeding breeding attempt with a more attractive mate (Sheldon 2000).

Burley (1986) was the first to emphasise that selection might favour individuals that allocate resources depending on characteristics of their mates. Originally she used the term 'differential allocation' (DA) to describe the idea that individuals may be willing to invest more in the current breeding attempt because the offspring will be of higher value, but also to obtain and/or keep an attractive mate. Consequently, the attractive parent reduces its parental effort and thus gains a survival advantage, as found in an experimental test of the differential allocation hypothesis in zebra finches *Taeniopygia guttata* (Burley 1988). However, pair members do not need to maintain even short term associations in order for selection for differential allocation to occur, so reduction of parental investment by the more attractive parent may not necessarily be involved in differential allocation (Sheldon 2000).

Testing of DA has to involve experimental manipulations of the trait that is known to predict mate quality to exclude confounding factors associated with being mated to a high quality mate (e.g. high quality males might defend high quality territories hence females could provide more parental resources). The first experimental test of DA was conducted by Burley (1988) by manipulating the attractiveness of both sexes of zebra finches using coloured leg rings (red rings make males more attractive, and black rings make females more attractive). Males and females increased their parental effort when mated to an attractive mate, while the attractive mate reduced its parental effort. However, parental feeding effort may not be the only variable involved in differential allocation. For instance, female mallards *Anas platyrhynchos* laid larger eggs after copulating with preferred males and smaller eggs after copulating with less preferred males (Cunningham & Russell 2000). In another experiment, Gil and colleagues (1999) tested whether female

zebra finches allocated more testosterone to eggs when mated to attractive males. By using a similar manipulation of attractiveness as Burley, they showed that females did indeed deposit more testosterone in eggs sired by attractive fathers. Chicks hatching from eggs with relatively high concentrations of testosterone show higher growth rates and begging rates (Schwabl 1996) and have higher social rank after fledging (Schwabl 1993).

Sex-biased parental care

Besides parental attractiveness, the sex of the offspring also influences parental care. If male and female offspring vary in any way that is related to fitness, the benefits for investment in each of the sexes are different for the parents (Maynard Smith 1980; Charnov 1982; Lessells 1998, 2002). For instance, if the survival or reproductive success of one offspring sex is more dependent on parental provisioning, higher allocation to that sex is selectively advantageous. Factors that cause fitness differences between the sexes are, for instance, the relative size of male and female offspring (Teather 1987; Yasukawa et al. 1990; Westneat 1995) or differential dispersal patterns of the two sexes (Stamps 1990; Gowaty & Droge 1991). By varying the amount of parental effort in sons and daughters, parents can decrease or increase the survival and future fecundity of each offspring sex while safeguarding or sacrificing their own residual fitness. Sex-biased parental care might apply via each form of parental care that can be split between the offspring, for instance differential provisioning with food of male and female offspring or egg investment in relation to offspring sex (Cordero et al. 2000)

Differential Sex-biased parental care

As we have seen above, differential allocation refers to an individual's decisions to allocate different amounts of investment to the current breeding attempt in relation to the quality or attractiveness of the mate. In contrast to differential allocation, differential sex-biased parental care involves a change in the

relative allocation to sons and daughters in relation to mate attractiveness. The selection pressures that lead to both behaviours are, however, in principle the same.

When the quality of the mate affects the fitness of only one sex of offspring (e.g. Ellegren et al. 1996; Svensson & Nilsson 1996; Kölliker et al. 1999; Sheldon et al. 1999), it pays for parents to invest more in this sex. In this case we would expect that parents invest in offspring sex in relation to mate attractiveness. In this thesis, this is referred to as 'differential sex-biased parental care'. If the benefits of the attractive parents are expressed equally in both offspring sexes, investing more in the total brood would result in a higher fitness. When the attractiveness of the mate equally affects the fitness of both offspring sexes, we would expect differential allocation in relation to the attractiveness of the mate. When the value of offspring is not determined by the quality of the mate but instead by the breeding environment (Trivers & Willard 1973) the two hypotheses can be clearly separated. In the latter case, good genes or mate quality are not involved. Instead the expected quality of offspring is influenced by variables of the breeding situation, such as the abundance of food (Kilner 1998).

Blue Tits

Testing whether individuals adjust their parental effort in relation to mate attractiveness and/or offspring sex requires a species in which attractiveness, parental investment and the sex of the offspring can be recorded. Birds are generally very well suited for questions regarding parental care because a large part of their effort consists of parental feeding, which in many species can be easily quantified. Information on offspring sex can be obtained using established molecular techniques (Griffiths et al. 1998). Blue tits *Cyanistes caeruleus* are especially suited as a model species because they show extensive biparental care and possess a trait that indicates attractiveness, the UV coloration of their plumage (Andersson et al. 1998). Blue tits have UV reflecting plumage on various parts of the body – the crown, the yellow chest and the olive green back (Hunt et al. 1998). As most birds, blue tits are capable of perceiving wavelengths in the UV spectrum (200-400 nm) because they possess four types of visual pigments (tetrachromacy) – a long wavelength-sensitive,

a medium wavelength-sensitive, a short wavelength-sensitive, and an extreme short wavelength-sensitive "UV" or "violet" pigment (Hart et al. 2000). This sensitivity to UV light seems to be present in all major animal groups (Tovee 1995).

Blue tits have among the highest sexual colour dimorphism in the UV part of the spectrum (Andersson et al. 1998). Especially the crown shows significant between-sex differences with respect to the spectral shape and the wavelength of maximum reflection, with males having more intense UV coloration and a shorter wavelength of peak reflection than females (Hunt et al. 1998). The crown is presented during courtship by horizontal posturing and erected nape feathers (Andersson et al. 1998). In laboratory experiments, blue tits showed mutual mate choice in which both sexes preferred unmanipulated mates over UV-reduced mates (Hunt et al. 1999). In an earlier experiment females chose males with the brightest crown, i.e. the crown with the highest UV reflectance (Hunt et al. 1998).

The extent of UV-crown coloration seems to be an indicator of quality. High survival prospects were found to be correlated with high UV coloration in males (Sheldon et al. 1999), and survival prospects have, in turn, been shown to be correlated with high levels of extra pair paternity (Kempenaers et al. 1992). Hence males that have a crown with high UV coloration seem to have survival and mate choice advantages. In addition, bright UV males show a high heterozygosity (Foerster et al. 2003). For female blue tits on the other hand, the relationship between UV and fitness is unknown. However, attractive traits are thought to be honest signals of quality which are costly to produce and maintain (Andersson 1994). For instance, aspects of UV coloration depend on the precision of nanostructural arrangement (Shawkey et al. 2003), confirming the idea that the production of UV reflecting plumage is relatively costly. Therefore, UV reflectance is likely to be an indicator of quality in both sexes.

As there is ample evidence that UV-brightness is an aspect of attractiveness in blue tits, they are a suitable species in which to investigate differential allocation. Additionally, sex-biased parental care or 'differential sex-biased parental care' may also occur in blue tits, as is indicated by a number of studies. The first evidence came from Svensson and Nilsson's (1996) study of a Swedish blue tit population. They found that females mated to males that survived to the next breeding season had a

significantly higher proportion of male offspring in their broods than females mated to males that did not survive. This was evidence that paternal phenotypic quality contributed to sex ratio variation in a natural population. The authors could not, however, identify any phenotypic characters by which females could assess the quality of males.

Two years later, when mate choice experiments on the basis of male crown UV coloration were conducted (Hunt et al. 1998) and the role of UV crown coloration in mate choice in the field was revealed (Andersson et al. 1998), a potential clue for females to judge mate quality was found. To investigate this further, Sheldon and colleagues (1999) conducted an experiment to test for the influence of male crown UV coloration on the sex ratio of their offspring. They masked UV coloration by applying UV blocking chemicals to the crown of males (UV-reduced males) after the pair had formed and before the clutch was produced, and compared the sex ratio produced by females mated with UV-reduced males to those of females mated to UV-unreduced control males. Three different components of UV coloration were measured: "brightness" (total spectral reflectance between 320-700 nm), "hue" (wavelength of peak reflectance, $\lambda(R_{max})$), and "chroma" (= purity of a given colour, calculated as reflectance ratio $R_{320\text{-}400}/R_{320\text{-}700}$). In the following and throughout this thesis I will refer to birds with high UV chroma as being "UV bright". Females mated to control males, which were not reduced in their UV appearance, produced sex ratios that were positively correlated to the measures of male crown coloration (significant for "chroma" (UV brightness) and "hue", but not for "brightness"). In the UV-reduced group this effect was reversed with females producing female-biased sex ratios. Furthermore they could show that the UV crown of males acts as a clue for females to judge the quality of their mates. Males that survived until the next breeding season had on average higher UV chroma than non-survivors, and male survival was positively correlated to male-biased sex ratios. This demonstrated a causal effect of male UV ornamentation on offspring sex ratio. These results have been repeated by two different studies, one that conducted the experiment in exactly the same way (Korsten et al. 2006) and another which used a different UV-manipulation method (Delhey et al. 2007).

In summary, because blue tits possess a trait that clearly indicates attractiveness and there are studies showing that females adjust the sex ratio of their brood in relation to that trait, blue tits potentially adjust their parental care to either the attractiveness of their mates, to the sex of their offspring, or to both.

Aim of the thesis

The aim of this thesis is to determine whether differential allocation, sex biased care or differential sex-biased care occurs in blue tits. By videotaping feeding rates of blue tits and by determining the sex of offspring using molecular techniques, we can measure the amount of parental care by male and female blue tits in each sex of offspring. By measuring the UV-coloration of the parents, we are able to determine how much is invested in the total brood and in each offspring sex in relation to parental attractiveness.

Chapter 2 summarizes the results on male and female feeding rates in relation to brood sex ratio and offspring sex obtained over 4 years of study. I show that there is no correlation between feeding rate and sex ratio, and that parents do not preferentially feed one of the offspring sexes. However, there are significant year to year differences in the relationship between feeding rates and sex ratio for males but not for females. In some years male feeding rates correlate with sex ratio, but not in others. This may be due to differences in food abundance between years.

Chapter 3 examines correlations between paternal and maternal feeding effort on the one hand, and maternal and paternal UV-coloration on the other. Feeding rates of both sexes are correlated to mate UV coloration and not to own UV-coloration. Interestingly, the correlation between feeding rates and mate UV is reversed in the parental sexes: female feeding rates are positively correlated to male UV, but male feeding rates are negatively correlated to female UV. Moreover, the tarsus length, but not mass, of fledglings is related to male UV chroma. This chapter represents correlational support for differential allocation in blue tits.

Chapter 4 shows the temporal change in the effect of a UV-reduction method used to show causal relationships between parental care and UV-coloration (chapters 5 and 6). It shows that just after this treatment is applied, the UV-chroma of the treated birds is below the natural range but recovers quickly so that UV-chromas are back within the natural range within just a few days.

Chapters 5 & 6 are experimental tests of a causal relationship underlying the correlations found in chapter 3. These tests are necessary because patterns as differential allocation are easily created by confounding variables, and experimental testing is needed to confirm a causal relationship. Chapter 4 experimentally tests the positive relationship between female feeding rates and male UV-coloration, while chapter 5 tests the negative relationship between male feeding rate and female UV-coloration. We decreased UV-coloration of males (chapter 4) and females (chapter 5) in two consecutive years and compared feeding rates of their mates to those mated to unreduced control birds. We demonstrate causal relationships between feeding effort and mate UV-coloration. Birds with UV-reduced mates reacted as predicted by the results in chapter 3. Females had lower feeding rates while males had higher feeding rates, both compared to control pairs. In both cases we thus provide evidence for true differential allocation. Furthermore, offspring phenotype was also affected by UV-coloration, since the tarsus length of fledglings differed between treatment groups, with larger tarsi in the group with the higher feeding rates.

Finally, chapter 7 summarizes the most important results from this thesis and places them in a broader framework.

CHAPTER 2

Yearly variation in patterns of sex-biased parental care in blue tits *Cyanistes caeruleus*

T. Limbourg, A.C. Mateman & C.M. Lessells

Abstract:

Sex allocation by means of post natal sex-biased parental care in birds has received little attention compared to studies on avian sex ratio adjustment. By measuring feeding rates of male and female blue tits towards individual offspring of known sex in four successive breeding seasons we aimed to investigate whether parental care of either parent is biased in respect to offspring sex, or alternatively, to the sex ratio of the brood. Overall, we found no correlation between male or female investment and sex ratio or offspring sex. Males however, but not females, showed a significant sex ratio * year interaction caused by a significant positive correlation between feeding rates and sex ratio in one of the four years. Neither parental sex preferentially fed one of the offspring sexes, even in the year where male feeding rates were correlated with sex ratio. The sex ratio influenced the number of feeds per offspring of each sex: Daughters received on average less food than sons in male-biased broods, possibly because male offspring outcompeted their smaller female siblings. Our study indicates that sex-biased parental care may occur only occasionally and that food allocation may be partially influenced by the offspring.

1. Introduction

Parents are selected to vary investment in sons and daughters if the costs and benefits of parental care differ between the offspring sexes. If the sex ratio is under parental control parents may vary their total investment in each offspring sex by producing different numbers of sons and daughters (Fisher 1930; Trivers & Willard 1973). If the sex ratio is not under parental control, parents are still able to vary total investment in each offspring sex by providing different amounts of post-natal parental care to sons and daughters (Maynard Smith 1980; Lessells 1998; Lessells 2002).

In recent years, a large body of literature has dealt with sex allocation in the form of adaptive sex ratio adjustment, while the role of post natal sex-biased parental care has been largely ignored. Birds are a well suited taxa to study sex-biased parental care because most of the post natal care consists of provisioning nestlings and this is relatively easy to quantify compared to, for instance, suckling by mammals. Despite this, surprisingly few studies measuring the relative feeding investment in the offspring sexes have been conducted in birds, and the great majority of those which have been carried out have focussed on species with sexual size dimorphism. Most studies of size dimorphic species report negative results (Newton 1978; Fiala 1981; Roskaft & Slagsvold 1985), implying that sex-biased provisioning in birds may not simply be a function of sexual size dimorphism.

Sex-biased parental care in terms of food allocation may also occur in species with little or no sexual dimorphism: whenever the survival or reproductive success of one offspring sex is more dependent on parental provisioning, higher allocation to that sex should be selectively advantageous (Maynard Smith 1980; Lessells 1998). For instance, the reproductive success of male birds is often more dependent on body condition than that of females. Sons that receive more parental resources as nestlings and that are therefore potentially in better condition as adults may have advantages in competition for mates and territories and through high fertilization success. In addition, if one sex is more likely to disperse, the dispersing offspring sex might be needier than the philopatric sex (Gowaty & Droge 1991). Although in both cases parents might achieve a selective advantage when they increase investment for the more costly offspring sex, few studies have shown sex-biased parental care in

non-size dimorphic species (Stamps et al. 1987; Gowaty & Droge 1991; Clotfelter 1996). Overall there is little support for the idea that sex-biased parental care is a common behaviour in birds (Stamps 1990; Gowaty & Droge 1991).

If one or both parents preferentially feed one of the offspring sexes, this will result in a correlation between parental care and sex ratio because the total amount of care increases as the number of the preferred offspring sex increases. Alternatively, correlations between parental care and sex ratio might occur without higher allocation to one sex of offspring within the brood. For instance, a correlation between total begging intensity of the brood and sex ratio will arise if one of the offspring sexes is more vigorous at begging. Elevated begging intensity might then stimulate parents to raise feeding rates without actually discriminating between the offspring sexes. The difference between these two scenarios is that in the first case the parents control the amount of care, while in the second case the offspring themselves might have partial control over the amount of care.

The sex of offspring may not only influence the total amount of parental care but also the allocation of care between the offspring sexes. One sex might be more dominant than the other sex in competing for food, for instance, by increased begging behaviour or by positioning themselves closer to the feeding parent (Kölliker et al. 1998). As a result, one sex might generally receive more care. Alternatively, there might be an interaction between offspring sex and sex ratio on the amount of care individuals of each sex receive if sons receive more care and daughters are increasingly outcompeted as the sex ratio increases. In this scenario it is possible that total parental feeding investment either stays constant or increases with sex ratio.

In blue tits, the reproductive success of male offspring is likely to be more dependent on parental care than that of female offspring. Blue tits are a weakly size dimorphic passerine hole-breeding species (males are ca. 5% larger than females). Female blue tits engage in extra-pair copulations with high quality mates (Kempenaers et al. 1992) and polygyny occurs occasionally (Dhondt 1987; Kempenaers 1994). Sons in good condition might lose less within-pair paternity, gain more extra-pair paternity or attract additional mates and might thus provide greater fitness returns than daughters. Therefore, higher investment in male offspring might

pay for parents. Prevous studies on blue tit sex allocation have focused on sex ratio rather than parental care. A number of studies have shown correlations between sex ratio and subsequent overwinter survival of the male parent (Svensson & Nilsson 1996; Sheldon et al. 1999; Griffith et al. 2003). Furthermore, blue tits have shown sex ratio variation in response to experimentally decreased UV crown coloration, (Sheldon et al. 1999; Korsten at al. 2006), an indicator of male survival (Svensson & Nilsson 1996; Sheldon et al. 1999; Griffith et al. 2003) and attractiveness (Hunt et al. 1998; Hunt et al. 1999; Andersson et al. 1998). Here we contribute to studies on avian sex allocation in terms of sex-biased parental care. By recording feeding rates of male and female blue tits towards individual offspring of known sex in four different years we aimed to determine whether parental investment is related to either offspring sex or brood sex ratio.

2. Methods

General field procedures

The study was conducted from May to June during the years 2000 to 2004 in the Hoge Veluwe National Park in the Netherlands. The study site consists of 450 nest boxes occupied mainly by great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*) and to a smaller extent by blue tits. Nest boxes with blue tit pairs were monitored weekly from the beginning of April, and daily from shortly before the calculated hatching date to identify the exact day of hatching (day 0). To determine the brood sex ratio and the sex of individual offspring we marked each chick individually and took a 10 µl blood sample on day 3. Blood samples were sexed in the laboratory following a standard PCR-based protocol (Griffiths et al. 1998). To measure parental investment we video recorded feeding rates on days 10 and 14 (see below). We measured mass (to the nearest 0.1 g) and tarsus length (to the nearest 0.1 mm) of nestlings on day 15 ('fledgling mass/tarsus length').

Parental feeding rates

To measure feeding rates (number of feeds per 2 h) and the number of feeds per offspring of each sex (number of feeds per son (or daughter) per 2 h) we video

recorded feeding visits for 3 h on day 10 in 2000, and days 10 and 14 in 2001 to 2003. One day prior to video recording we placed a box to house the video camera on top of the nest box to accustom the birds to the equipment. Approximately one hour before video recording began, we marked the chicks individually with paint dots on their heads and put a Sony handycam with infra-red function in place. The last 2 h of the 3 h recordings were used to score the identity of the parent and chick fed at each parental feeding visit. If parents fed more than one chick at a single visit we estimated the proportion of food each chick received. Videos were not scored if one of the parents did not feed within the first 1.5 h of the 3 h video recording, most likely due to disturbance by the video equipment. In total we scored feeding rates at a total of 81 nests (2000: 20 pairs, 10 day = 20; 2001: 33 pairs, 10 day = 22 , 14 days = 33; 2002: 13 pairs, 10 days = 12, 14 day = 12; 2003: 15 pairs, 13 10 days = 13, 14 days = 14).

Data selection

In two of the 4 study years presented in this study we conducted an experiment to test the influence of blue tit UV crown coloration on feeding rates of the mate. We reduced the UV crown coloration of males in 2002 (Limbourg et al. 2004) and of females in 2003 (Chapter 4, this thesis), following a similar protocol using a mixture of duck preen gland fat and UV blocking chemicals. Additionally we swapped clutches between broods with similar brood size and hatching date. Here we use only data from the control groups of these years, which were treated with only duck preen gland fat, which did not alter the visual appearance of the birds. Experimental years do not differ in mean feeding rates from non-experimental years for males ($\chi^2 = 1.26$, d.f. = 1, $p = 0.26$) and females ($\chi^2 = 0.8$, d.f. = 1, $p = 0.37$).

Statistical analysis

To analyse feeding rates in relation to the sex ratio of the brood we used the number of feeds in 2h as the dependent variable in a repeated measure analysis using the Genmod procedure in SAS (SAS Institute Inc. 1996), assuming a Poisson distribution of feeding rates. We used a unique identifier for each brood of blue tits as the repeated measure subject. Male and female feeding rates were analysed

separately because some of our models containing the data for both sexes did not converge.

To select variables for consideration in a model for all four available years we first analysed each year separately by fitting the variable of interest 'Sex ratio' as well as other variables that might cause variation in feeding rates. These additional variables were 'Brood size' and 'Hatching date' as continuous variables, and 'Chick age' as a class variable. We also fitted the two-way interactions of sex ratio and the other main effects. We eliminated non-significant two-way interactions in a stepwise fashion followed by any non-significant main effects that were not part of a two-way interaction to select the final model for each year. Having done that we fitted any main effect or two-way interaction that was significant in any of the four years in the final analysis containing all data. Additionally we fitted the interactions of these terms with 'Year', and 'Year' as a main effect. From this model we eliminated all non-significant three-way interactions, followed by any non-significant two-way interactions that were not part of a three-way interaction and finally any non-significant main effects that were not part of a significant higher order term to obtain the final model. To test whether relationships between feeding rates and sex ratio differed between the parental sexes we used t-tests on the slopes and standard errors from the models for each sex separately.

To analyse feeding rates in relation to the sex of individual offspring we calculated the mean number of feeds per male and female offspring by each of the parents in 2 h and used this as the dependent variable in a repeated measure analysis using the Mixed procedure in SAS. The feeding rates of male and female parents were again analysed separately. Feeds per offspring sex were log-transformed to make this analysis comparable to the earlier analysis of feeding rates that assumed a poisson distribution. The variables of interest were again 'Brood sex ratio' and, additionally to the previous analysis, 'Offspring sex'. Model selection was carried out as described for the analysis of male and female feeding rates.

3. Results

We analysed the number of feeds by males and females recorded during the years 2000 to 2003 to determine the influence of the sex ratio on parental feeding rates. Table 1 summarizes these results.

Table 1. Repeated measures analysis (type III tests) of the number of male and female feeding visits scored during 2h of videos recordings (N = 126) made in 2000 to 2003 from a total of 81 nests on days 10 (N = 67) and 14 (N = 59).

	X^2	d.f.	*P*
No. Of feeding visits by the male:			
Brood size	15.03	1	0.0001
Brood sex ratio[†]	0.79	1	0.375
Hatching date[†]	0.16	1	0.690
Year[†]	6.38	3	0.095
Brood sex ratio * Year	7.85	3	0.049
Hatching date * Year	9.24	3	0.026
No. Of feeding visits by the female:			
Brood size	32.54	1	<0.0001
Chick age	5.61	1	0.018
Variables not in the model:			
Brood sex ratio[†]	0.51	1	0.474
Brood sex ratio * Year	6.07	3	0.108

Note: Estimates represent log-transformed values. The estimate for 'Chick age' represents the difference in intercepts between day 14 and 10.

[†] Estimates, χ^2- and p-values for these variables are given with all two-way interactions involving that main effect removed from the model.

Brood size was the most significant determinant of feeding rates for both parents. For females, the only other significant term besides brood size was chick age. Females fed less when chicks were 10 days old, compared to 14 day old chicks. Female feeding rates did not differ significantly between years and were unrelated to brood sex ratio. Male feeding rates changed with hatching date of the brood in

each year, indicated by the significant hatching date * year interaction. Overall, their feeding rates were unrelated to brood sex ratio, but we found a significant brood sex ratio * year interaction, i.e., the relationship between male feeding rates and sex ratio differed between the years (figure 1). Male feeding rates were positively related to sex ratio in 2001 ($b = 0.748 \pm 0.251$ (s.e.), $z = 2.98$, $p = 0.003$), but not in any of the other years (2000: $b = -0.292 \pm 0.290$ (s.e.), $z = -1.01$, $p = 0.314$; 2002: $b = -0.161 \pm 0.287$, $z = -0.56$, $p = 0.574$; 2003: $b = -0.518 \pm 0.380$, $z = -1.36$, $p = 0.172$).

Because male feeding rates were related to brood sex ratio in 2001 (but not in the other years) whereas female feeding rates were unrelated to brood sex ratio in any year, we tested whether the slope of feeding rates in relation to sex ratio differed between males and females in 2001. This difference was significant whether the male slope for 2001 was tested against the female slope for 2001 ($t = 2.56$, $df = 64$, $p = 0.013$) or the overall slope for females ($t = 2.94$, $df = 112$, $p = 0.004$).

One potential explanation for the sex ratio * year interaction is that sons are the more costly offspring sex and male parents increase investment in male-biased broods only in food abundant years when increased feeding rates incur lower costs. We tested this hypothesis by fitting general linear models with the slope of the relationship between male feeding rates and sex ratio for each year as the dependent variable versus some measures that directly or indirectly indicate the food abundance in a given year in (see fig. 2). The best but non-significant correlation was found when the direct measure of the food abundance, the peak caterpillar biomass per year, i.e. the largest amount of caterpillars measured on one day during a particular year (Visser et al. 2006), was entered as dependent variable ($b = 0.131 \pm 0.048$ (s.e.), $t = 2.74$, $p = 0.112$). However, statistical power was extremely low since this analysis comprises only four data points. Indirect measures of the food abundance such as mean offspring mass ($b = 1.314 \pm 0.989$, $t = 1.22$, $p = 0.316$), mean offspring tarsus length ($b = -0.197 \pm 1.254$, $t = -0.16$, $p = 0.889$) and mean male feeding rates ($b = -0.030 \pm 0.085$, $t = -0.35$, $p = 0.787$) showed less significant correlations.

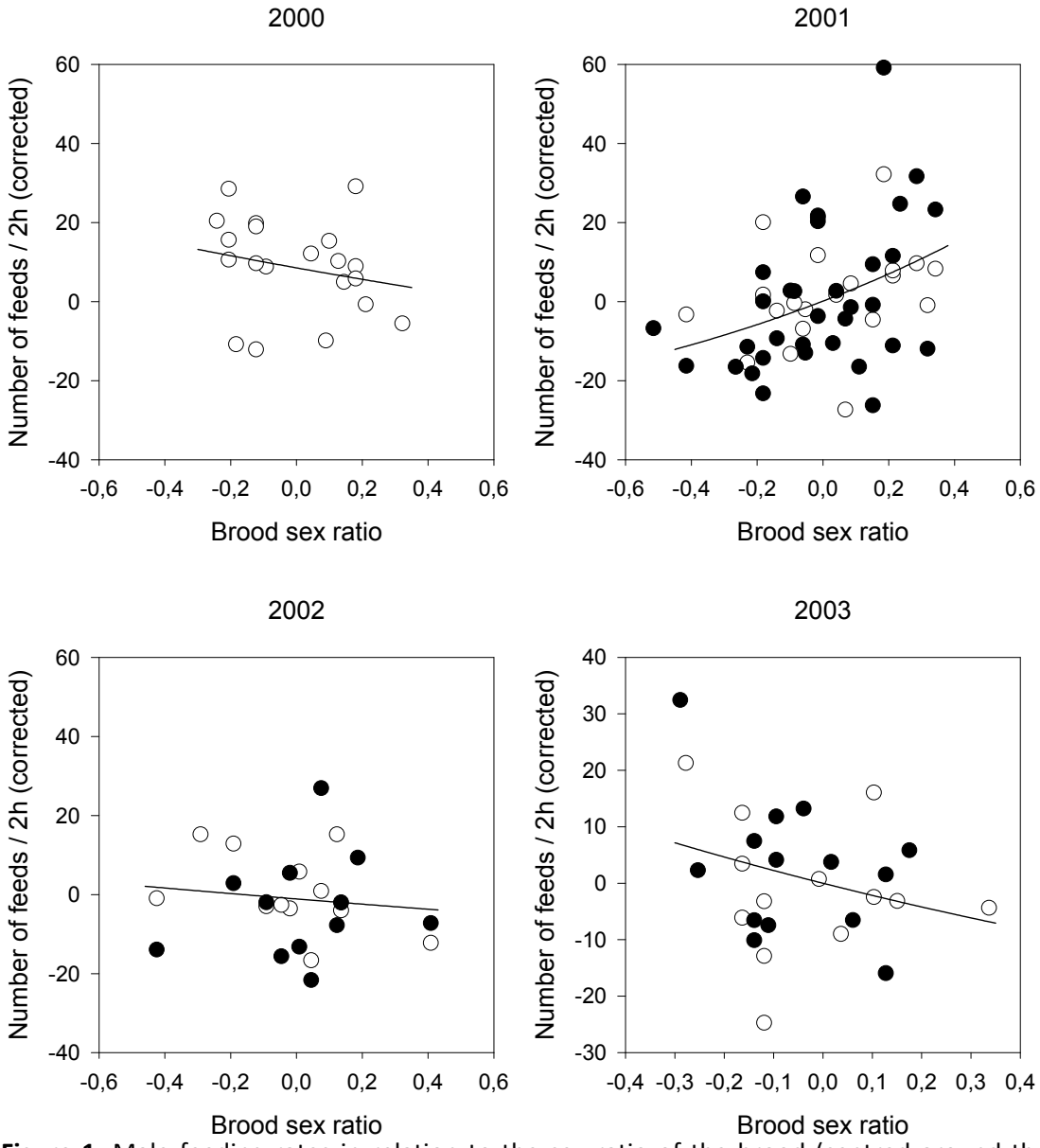

Figure 1: Male feeding rates in relation to the sex ratio of the brood (centred around the mean sex ratio in each year) scored from 2h video recordings on days 10 (open circles) and 14 (filled circles) during 2000 to 2003. Points shown are corrected for 'Brood size', 'Hatching date' and 'Hatching date * Year'. The lines shown are those given by the repeated measures model (Table 1).

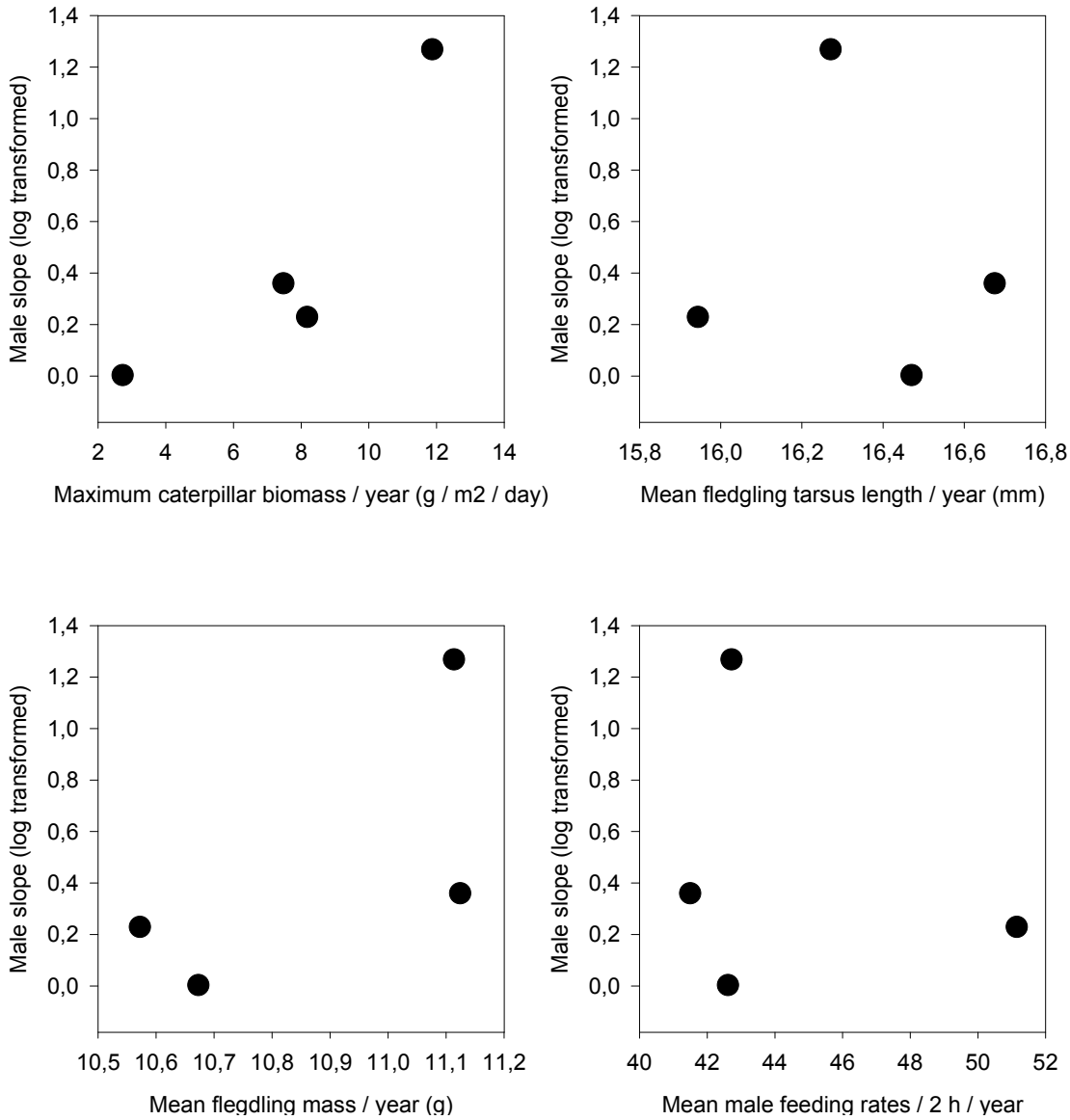

Figure 2: The slope of the relationship between male feeding rates and brood sex ratio for each year in relation to direct and indirect measures of the food abundance.

We also analyzed the number of feeds to individual offspring. The results of this analysis were consistent with the results of the previous analysis: for females, there was no relationship with brood sex ratio in any year, whereas for males, this relationship varied significantly between years (i.e. there was a significant brood sex ratio * year interaction; Table 2). In addition, both parental sexes showed a

significant sex ratio * offspring sex interaction (Table 2). Feeding rates to female offspring decreased relative to those to male offspring as sex ratio increased (fig. 3). Note that offspring sex did not significantly influence feeding rates per offspring of each sex of male and female parents (Table 2). Allocation was not generally higher to one of the offspring sexes and the absence of a significant offspring sex * year interaction showed that this also did not occur in any of the 4 years.

Table 2. Repeated measure analysis (type III tests) of the number of male and female feeds per offspring of individual sex scored during 2h.

	F	*df*	*P*
Male feeds per individual offspring:			
Brood sex ratio[†]	1.03	1, 81.1	0.314
Offspring sex[†]	0.67	1, 66.5	0.417
Year[†]	0.58	3, 68.9	0.631
Brood sex ratio * Offspring sex	13.91	1, 73.2	0.0004
Brood sex ratio * Year	3.70	3, 76	0.015
Female feeds per individual offspring:			
Brood sex ratio[†]	0.02	1, 69.1	0.889
Offspring sex[†]	0.14	1, 67.4	0.709
Year	3.20	3, 66.5	0.029
Chick age	5.44	1, 43.9	0.024
Brood sex ratio * Offspring sex	5.88	1, 75.2	0.018

Note: Estimates represent log-transformed values.

[†] Estimates and p-values for these variables are given with all two-way interactions involving that main effect removed from the model.

'Offspring sex' gives the difference in the estimates for daughters and sons.

4. Discussion

We recorded feeding rates of male and female blue tits during four successive breeding seasons to determine the influence of brood sex ratio and offspring sex on parental care. There was no correlation between feeding rates of males and females and sex ratio over four years. However, the relationship between

feeding rates and sex ratio differed significantly between years for males but not for females. Both sexes of parents invested equally in individual male and female offspring on average, even when males adjusted feeding rates to sex ratio, but investment in sons relative to daughters increased with the proportion of males in the brood.

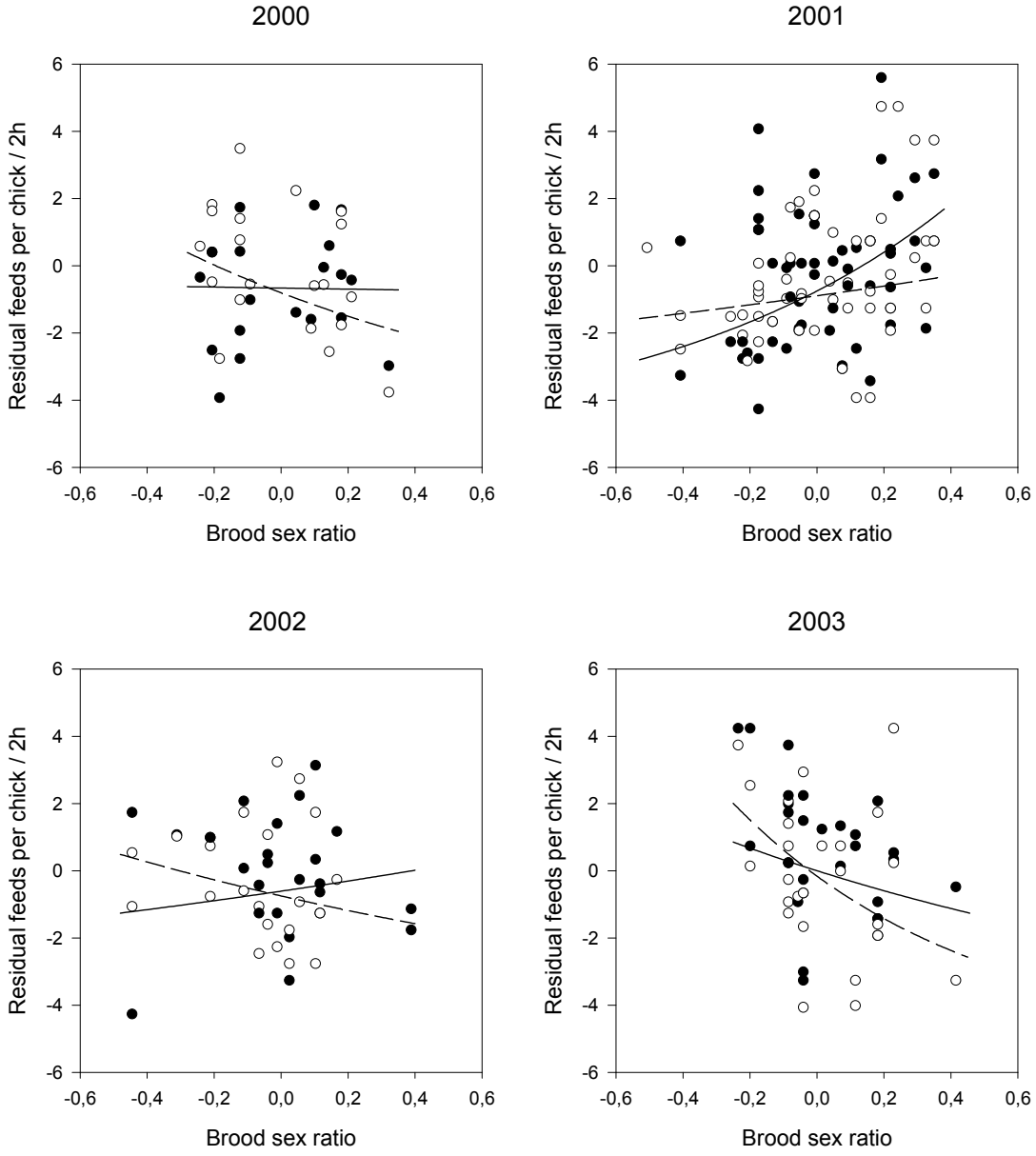

Figure 3a: See next page for figure legend.

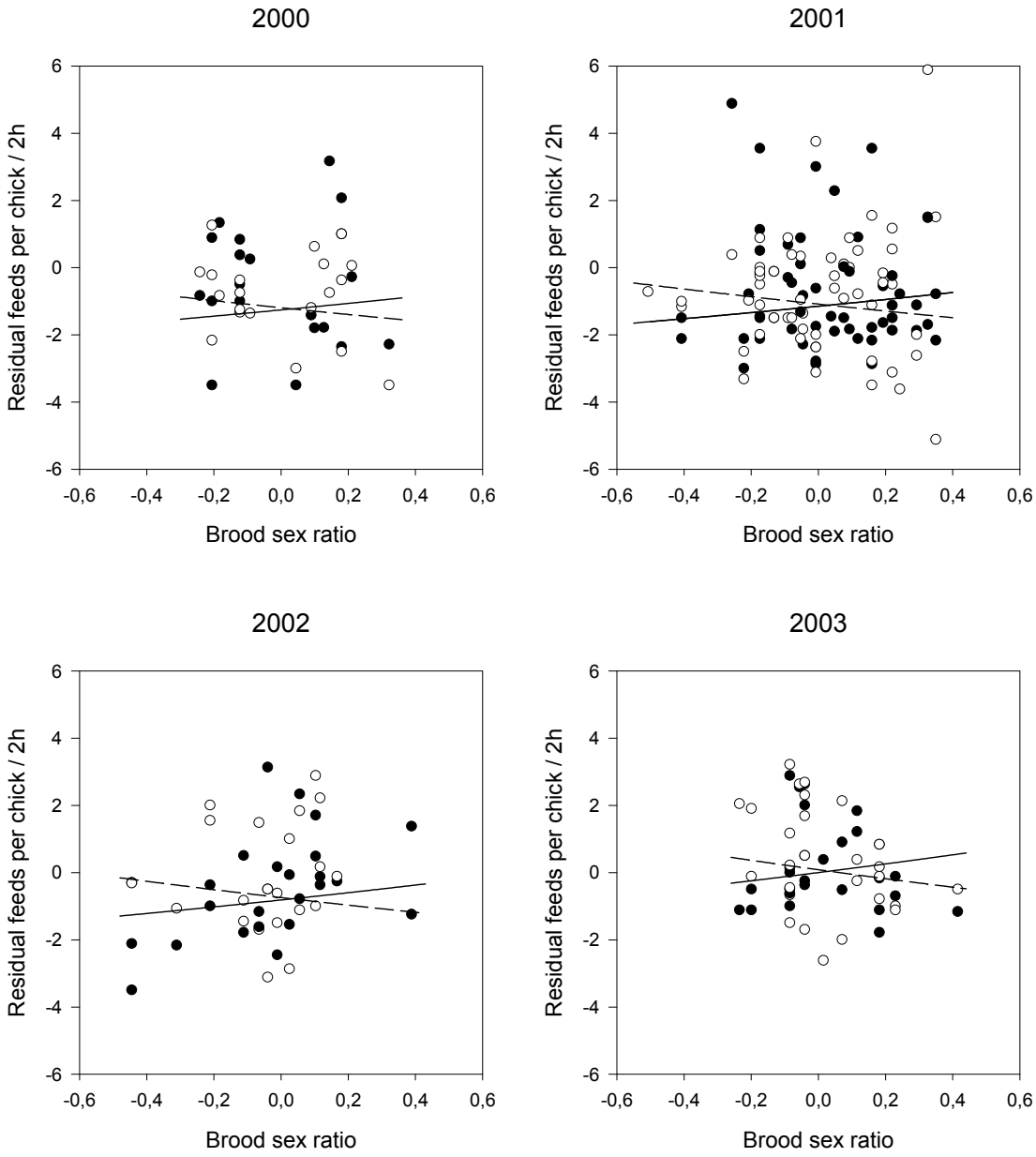

Figure 3b: Feeding rates per individual offspring for male parents (a, previous page) and female parents (b) in relation to the sex ratio of the brood (centred around the mean sex ratio in each year) scored from 2h video recorded during 2000 to 2003. Points shown are feeds per male offspring (filled circles) and female offspring (open circles) corrected for chick age (females only). Each plot shows two points for each nest for days 10 and 14 (if data were available for both days). The lines shown are those given by the repeated measures model (Table 2) for male offspring (full line) and female offspring (dashed line).

We found no overall relationship between blue tit feeding rates and brood sex ratio when all 4 available years were analysed. In the congeneric great tit, comparable results were found since neither sex of parent altered their parental care in relation to experimentally manipulated sex ratios (Lessells et al. 1998). Male blue tits, however, showed a significant sex ratio * year interaction in feeding rates in our study, due to a significant positive relationship between feeding rates and sex ratio in 2001 and no relationship in the other three years.

The question arises why we found sex-biased parental care in only one out of four years. Potentially, sex-biased investment might be rarely observed because it occurs only when the environment allows parents to show elevated levels of investment to one sex without risking their own survival or the survival of the other sex (Nishiumi et al. 1996), for instance when sufficient food for offspring provisioning is available or parents are in good condition to provide extra investment. We found *a osterior* that the slopes of the relationship between male feeding rates and sex ratio in each of the four years correlated best with the peak caterpillar biomass per year, a direct measure of the food abundance (caterpillars are the predominant food for nestlings). Moreover, in 2001, the year in which males positively adjusted feeding rates to sex ratio, we found the highest caterpillar biomass of all four years. Other indirect measures of the food abundance such as mean fledgling body mass and tarsus length, as well as mean male feeding rates per year did not correlate as well. Nevertheless, the correlation between male investment in relation to brood sex ratio and the caterpillar peak biomass suggests, although with low power, that males increased feeding rates for male-biased broods only in food-rich years. Higher investment in male offspring is potentially beneficial if selection acts more strongly on males than females. Sons in good condition may have advantages as adults in competition for (additional) mates (Dhondt 1987) and by losing less within-pair paternity and gaining more extra-pair paternity (Kempenaers et al. 1997). In contrast, the fitness benefit parents accrue through high quality sons may be outweighed by the fitness costs of increased parental investment, as for instance reduced chances of (overwinter-) survival, in years with low food abundance.

We analysed the feeds per individual offspring and found no higher allocation to one of the offspring sexes by either of the parents, either overall or in any

individual year. The correlation between male feeding rates and sex ratio that we found in 2001 is thus not due to higher allocation to sons. Instead both male and female chicks in broods with high sex ratio received higher feeding rates. One likely explanation for this pattern is that sons beg harder than daughters and males react to the increased begging intensity of the brood but do not or are not able to discriminate between individual offspring in terms of their begging intensity. Although we have no measure of the begging intensity of male and female nestlings in blue tits we have some indication that sons are the more competitive offspring sex in blue tits. We found a significant sex ratio * offspring sex interaction on male and female feeds per offspring of each sex. Both parents feed relatively more per individual son than daughter as the proportion of sons in the brood increases. A potential explanation is that sons are more competitive than daughters as was found in great tits (Oddie 2000). If sons are more successful in for instance positioning themselves in front of the feeding parent the chance of an individual daughter being fed will decrease as the number of sons increases. Our results are inconsistent with that theory since the lines of the relationship between feeding rates and sex ratio for each offspring sex cross around the mean sex ratio in each year (figure 2) while they should cross at a more female-biased sex ratio. However, our statistics merely tested whether the slopes of the relationships between feeding rates and sex ratio differ between the offspring sexes and provide no statistical test about the location at which the lines cross. The confidence intervals of the point where the sex ratio relationships for each sex cross are likely to be large. It is thus possible that our results fit the pattern of sons being more dominant than daughters so that sons receive more food when the sex ratio increases while daughters receive less food at the same time. Another explanation why parents adjust feeding rates to sex ratio but do not preferentially feed male offspring is that parents may not be selected to distinguish between sons and daughters when the costs of the time needed for discrimination outweighs the benefits of so doing, as was suggested by Lessells and colleagues (1998b).

Male care in relation to brood sex ratio differed significantly from female care in 2001. The slope of the relationship between male feeding rates and sex ratio in 2001 differs from the slope of the same relationship for females in the same year

and when all four years were analysed together. Parents are expected to differ in their investment in relation to sex ratio when the fitness returns differ between sons and daughters and the costs for parental investment differ between parents (Lessells 1998). We discussed earlier why the fitness benefits might differ between the offspring sexes in blue tits. Blue tit parents may also differ in the cost for parental provisioning investment. Female blue tits, in general, might not be selected to show high levels of investment in broods with high sex ratio, even when food is abundant. Since blue tits are small birds that produce relatively large clutches females might invest most of their resources in the production of the clutch and retain no reserves to show elevated investment in male-biased broods in food abundant years. Males usually show higher feeding rates than females in blue tits and might be selected to provide extra investment in food abundant years since they do not show high investment prior to chick feeding.

Our study shows that the relationship between parental care and sex ratio is subject to strong year to year variation. One potential explanation for the lack of positive results of sex-biased parental care in birds might be due to this strong variation. We found some indication that it only occurs when the environment allows this extra investment but we can merely speculate about the potential explanation. Experimental studies or studies that cover a range of environments, for instance by studying different years, may clarify whether sex-biased investment in birds is as uncommon as current studies suggest.

CHAPTER 3

Opposite correlations between male and female investment and mate's UV coloration in blue tits

T. Limbourg, A.C. Mateman & C.M. Lessells

Abstract:

Parental investment and sexually-selected signals can be intimately related, either because the signals indicate the effort that an individual is prepared to invest, and hence its value as a mate, or because individuals are selected to vary their own investment in relation to their mates' signals. Correlations between parental investment and the sexually selected signals of both an individual and its mate are therefore of central interest in sexual selection. We used blue tits *Cyanistes caeruleus* to investigate such correlations because they provide substantial amounts of biparental care and possess sexually-selected structural UV coloration that seems to signal attractiveness in both sexes. We investigated whether feeding rates of male and female blue tits are correlated with either their own or their mate's UV coloration, and whether any such correlation is associated with the sex ratio of the brood. We also investigated whether any such correlations were reflected in offspring phenotype. Feeding rates were not correlated with either parent's own UV coloration. They were correlated with the mate's UV coloration, but in opposite directions in males and females: females had higher feeding rates when mated to males with high UV chroma, while males had lower feeding rates when mated to females with high UV chroma. These relationships were unaffected by the sex ratio of the brood. In addition, fledgling tarsus length, but not mass, was related to male UV coloration. These results imply that both male and female attractiveness influence parental investment of the mate, and that this in turn affects offspring phenotype.

1. Introduction

Initial studies of sexually-selected signals focused on whether mate choice was, indeed, occurring, but quickly moved on to include the benefits of any mate choice, and whether sexual selection might be imposed in other ways than through mate choice (reviewed in Andersson 1994). This change of focus led to interest in relationships between parental investment and sexually-selected signals – both of an individual itself and of its mate: first, a reliable link between an individual's sexually-selected signals and the parental investment that it will make would provide a benefit for mate choice (the 'good parent process'; Hoelzer 1989; Heywood 1989). Second, an individual would be expected to increase its parental investment when its mate's sexual signals indicate a higher marginal return rate in terms of fitness on that investment. More specifically, individuals should invest more when their mate is sexually attractive if this signals some indirect or direct benefit to the offspring ('differential allocation'; Burley 1986; Sheldon 2000). Sexually-selected signals are therefore intimately related to parental investment.

In principle, differential allocation can apply to both sexes of offspring: parents whose mate's sexually-selected signals indicate some benefit to offspring fitness will usually gain by investing more in those offspring (Sheldon 2000). However, when the benefits of having an attractive parent are accrued disproportionately by one sex of offspring, parents should increase investment in that sex (Charnov 1982). For example, it would be advantageous for parents to increase investment in male offspring if sons, but not daughters, inherit the attractiveness of their father and therefore accrue advantages in mate choice. In these circumstances, provided parents can detect the sex of their offspring, we would expect to see differential sex allocation (in terms of parental feeding rates), in which there is an interaction between sex ratio of the brood and parental attractiveness.

Adaptive explanations for differential allocation are based on the idea that one parent brings fitness benefits to the offspring, signalled by its appearance, and that the other parent is then selected to increase its parental investment, thus further increasing offspring fitness. For both of these reasons, if differential allocation is occurring, a positive correlation is expected between measures of offspring fitness and sexual attractiveness of the parent. Whereas the benefits gained from the

sexually attractive parent may not be felt until adulthood (for example, indirect benefits such as the inheritance of sexual attractiveness), the increase in parental investment that constitutes differential allocation is expected to be manifest, at least in part, by immediate differences in offspring development. It is thus interesting to investigate whether any differential allocation is reflected in growth differences.

Differential allocation gives sexually attractive individuals a fitness benefit through the increased parental investment of their mate. This in turn implies that sexually-selected signals must be costly, because otherwise all individuals would display the maximum level of the signal in order to reap this fitness benefit. When the same signal is used by both sexes, it is a reasonable *a priori* assumption that they are underlain by the same physiological processes, and that costs will therefore change in the same direction in the two sexes with increasing levels of the signal. As a result, if both sexes show differential allocation to the same kind of signal in their mates, the relationship between parental investment and the signal is expected to be in the same direction (i.e. both positive or both negative).

Differential allocation implies a direct causal effect of sexual attractiveness of one individual and the parental investment of its mate. However, a correlation between these two variables is not unequivocal evidence for differential allocation. A relationship between the two variables might come about in other ways: for example, territory quality might have effects on both sexually-selected signals and on the rate at which individuals can provide parental care. Alternatively, a good-quality individual might be sexually attractive and be able to provide abundant parental care, so that its mate is able to reduce its investment. In the latter case, a negative relationship between an individual's investment and its mate's attractiveness is expected. Differential allocation can therefore only be unequivocally demonstrated through experiments manipulating the putative sexually-selected signal. Nevertheless, correlations provide a useful starting point in revealing possible cases of differential allocation. Moreover, when there is biparental care it is helpful also to know whether an individual's own investment is related to its own sexual attractiveness. Also when there is biparental care, differential allocation can be expected by both sexes of parents, but this has rarely been investigated (Amundsen 2000). Correlative studies have the advantage of the relative ease with which data can be simultaneously

collected on the parental investment by both sexes of parents in relation to both their own and their mate's sexual attractiveness.

We used blue tits *Cyanistes caeruleus* to study the relationship between parental investment and their own and their mate's attractiveness. Blue tits show extensive biparental investment in the form of parental feeding. Also, blue tit plumage features ultraviolet (UV) coloration with strong sexual dichromatism (Andersson et al. 1998; Hunt et al. 1998). In laboratory experiments, both male and female blue tits prefer individuals of the other sex with brighter UV coloration, i.e. with high UV chroma (Hunt et al. 1999). In males, UV coloration seems to signal individual quality, because males with bright UV coloration have higher survival (Sheldon et al. 1999; Griffith et al. 2003) and heterozygosity (Foerster et al. 2003). Evidence for females is, as yet, lacking. These relationships suggest that positive correlations between investment and an individual's own or its mate's attractiveness may occur in both males and females. In addition, female blue tits modify their brood sex ratios in response to manipulation of their mate's UV coloration (Sheldon et al. 1999; Korsten et al. submitted). This suggests that there may be selection for differential sex allocation in relation to male UV coloration.

This paper presents descriptive data on the relationships between parental investment and an individual's own and its mate's UV coloration in wild blue tits. In particular, we present data for both sexes of parents for the correlations between parental investment and both an individual's own and its mate's UV coloration. In addition, we test whether there is an interaction between the brood sex ratio and parental attractiveness on parental investment, which would suggest differential sex allocation. Finally we investigate whether any correlation between investment and attractiveness also affects offspring phenotype.

2. Methods

Study site and standard field procedures

The study was conducted in May and June 2001 in the Hoge Veluwe National Park in the Netherlands. The study site consists of 450 nest boxes in mixed woodland. We conducted weekly nest box checks to identify all blue tit nesting attempts, and daily checks around the predicted time of hatching to determine the exact hatching

date (day 0). For all blue tit pairs breeding in the study area we caught male and female parents in the nest box on day 7 and measured their UV crown coloration as a measure of parental attractiveness (see next section), and their tarsus length, to the nearest 0.1 mm, using callipers. Additionally, adult birds were classified as first year breeders or older, based on plumage characteristics. We monitored parental investment in the form of feeding rates on days 10 and 14 (see next section). To obtain information on the sex ratio of the broods, we took a 10 µl blood sample of all offspring on day 3 from the leg vain and sexed them using a standard PCR protocol (Griffiths et al. 1998). Taking blood samples from the leg vain is a standard method to obtain blood from young chicks and none of the offspring showed adverse effects (i.e. no visible injury, no mortality occurred). As an index of offspring fitness we measured mass, to the nearest 0.1 g, using a spring balance (Pesola), and tarsus length of all young on day 15 (*N* = 32 nests), shortly before the chicks fledge from day 16 onwards.

UV coloration

We measured the UV crown coloration of each individual bird by taking 5 replicate crown reflectance measures using an USB2000 Spectrometer with a DH2000 deuterium-halogen lamp as light source (both Avantes, The Netherlands) illuminating and measuring perpendicularly to the feathers. From these measurements we calculated the 'UV chroma' ($R_{320\text{-}400}/R_{320\text{-}700}$, the proportion of reflectance in the UV spectrum (wavelength 320-400 nm) in relation to the total reflectance within the visible spectrum of blue tits (320-700 nm)) which represents a measure of the purity of UV coloration. UV chroma has previously been identified as the main predictor of blue tit attractiveness (Sheldon et al. 1999).

Parental feeding rates

Parental effort measures were obtained by video recording feeding rates of males and females on days 10 and 14. We placed a second box to house the video camera on top of the nest box one day before making the video recording, and a Sony handycam with infra-red function inside this box one hour before starting a three hour recording. The birds resumed feeding after 5 – 15 minutes. This pause in feeding the offspring is within the naturally occurring range and does not have adverse effects

on the offspring. We scored the number of male and female feeding visits during the last two hours of the recording (feeding rates). We recorded UV chroma and feeding rates of 33 pairs (including one pair in which female UV chroma was not measured), but video recordings were not scored if one of the parents did not feed within the first 1.5 h of the 3 h video recording, most likely due to disturbance by the camera set up. This problem only occurred on day 10 (11 out of 33 videos excluded) and was also encountered in other years (Limbourg et al. 2004).

Statistical analysis

We analysed parental feeding rates (number of feeds during 2 h) for males and females separately in relation to their own and their mate's UV chroma in a repeated measures analysis with 'Nest box' as repeated measures subjects using the GENMOD procedure in SAS (SAS Institute Inc. 1996). We assumed a poisson distribution of feeding rates. We tested the variables of interest: 'Male UV chroma' and 'Female UV chroma', and also the interactions 'Male UV chroma * Sex ratio' and 'Female UV chroma * Sex ratio' and other variables likely to cause variation in feeding rates. These variables were 'Brood size', 'Sex ratio' (both at the time of recording) and 'Hatching date' as continuous variables, and 'Chick age' (i.e. days 10 and 14), 'Male age' and 'Female age' (i.e. 1st year breeder or older) as class variables. We also tested whether any interactions of these additional variables with an individual's own or its mate's UV chroma had a significant influence on feeding rates, except for 'Male age' and 'Female age' that were tested only as within sex interactions with UV chroma. Our general procedure involved backwards elimination of non-significant terms (judged from the type III *P*-value) from a model including all the terms to be tested. However, our maximum likelihood model did not converge with all the interactions fitted. We therefore tested the interactions in three separate groups and then constructed a single model containing all main effects and the significant interactions. From this model we first eliminated any interactions that were non-significant in this combined model and then any main effects that were not significant and not involved in any of the significant interactions.

Fledgling mass and tarsus length were analysed using a hierarchical mixed model using the MIXED procedure in SAS and assuming normally distributed errors.

We fitted 'Male UV chroma', 'Female UV chroma', 'Brood size', 'Hatching date' and 'Offspring sex' as fixed effects and 'Nest box' as a random effect. Model selection was carried out as described for the analysis of male and female feeding rates, including the tests of all two way interactions with male and female UV chroma.

Table 1. Repeated measures analysis (type III tests) of the number of male and female feeding visits scored during 2h of video recording from 33 nests on days 10 (N = 22) and 14 (N = 33).

	Estimate	s.e.	χ^2	*P*
Number of feeding visits by the male:				
Female UV chroma	- 4.190	1.913	3.86	0.049
Brood size	0.093	0.019	8.88	0.003
Sex ratio	0.711	0.264	4.47	0.035
Variables not in the model:				
Male UV chroma			0.00	0.962
Sex ratio * Male UV chroma			0.22	0.638
Sex ratio * Female UV chroma			0.34	0.560
Number of feeding visits by the female:				
Male UV chroma	9.390	2.133	5.59	0.018
Brood size	0.139	0.019	15.68	<.0001
Chick age	0.241	0.075	7.86	0.005
Sex ratio†	-0.394	0.198	3.51	0.061
Variables not in the model:				
Female UV chroma			0.51	0.474
Sex ratio * Male UV chroma			0.58	0.447
Sex ratio * Female UV chroma			0.73	0.395

Note: Estimates represent transformed values. The estimate for 'Chick age' represents the difference in intercepts between days 14 and 10.

† Although the type III p-value for 'Sex ratio' is marginally non-significant in the analysis of female feeding rates, we retained it in the model because the estimate of its slope is significantly different from zero (z = -1.99, p = 0.047). Excluding 'Sex ratio' does not change the final model.

3. Results

The strongest determinants of female feeding rates (number of feeds per 2h) were brood size and chick age (Table 1). Females increased feeding effort with increasing brood size and nestling age, but we cannot determine whether the latter effect was due to a change in female feeding rates with chick age or due to females being disturbed by the first appearance of the video equipment on day 10. Female feeding rates were not related to their own UV chroma (Table 1) but are positively correlated to their male's UV chroma (Fig. 1, Table 1). Hence females fed more when they are mated to bright UV males.

Male feeding rates were positively correlated to brood size (Table 1), but uninfluenced by chick age. As in the case of the feeding rates of females, there was no correlation between male feeding rates and their own UV chroma (Table 1). Like female feeding rates, male feeding rates were correlated to their mate's UV chroma (Figure 1, Table 1), but in contrast to female feeding rates, this relationship was negative. Males therefore invested more in their broods when their female had low UV coloration, although we expected the opposite to be true.

Recent studies have revealed a strong influence of male age on extra- and within-pair paternity in blue tits (Delhey et al. 2003), and of female age on parental feeding rates in relation to manipulated male UV coloration (Johnsen et al. 2005). We therefore tested any same-sex interactions between UV coloration and parental age on male and female feeding rates and found that neither male ('Male age' * 'Male UV chroma': $\chi^2 = 3.10$, $df = 1$, $P = 0.078$; 'Female age' * 'Female UV chroma': $\chi^2 = 1.28$, $df = 1$, $P = 0.257$) nor female feeding rates ('Male age' * 'Male UV chroma': $\chi^2 = 0.87$, $df = 1$, $P = 0.352$; 'Female age' * 'Female UV chroma': $\chi^2 = 0.12$, $df = 1$, $P = 0.73$) were affected by this.

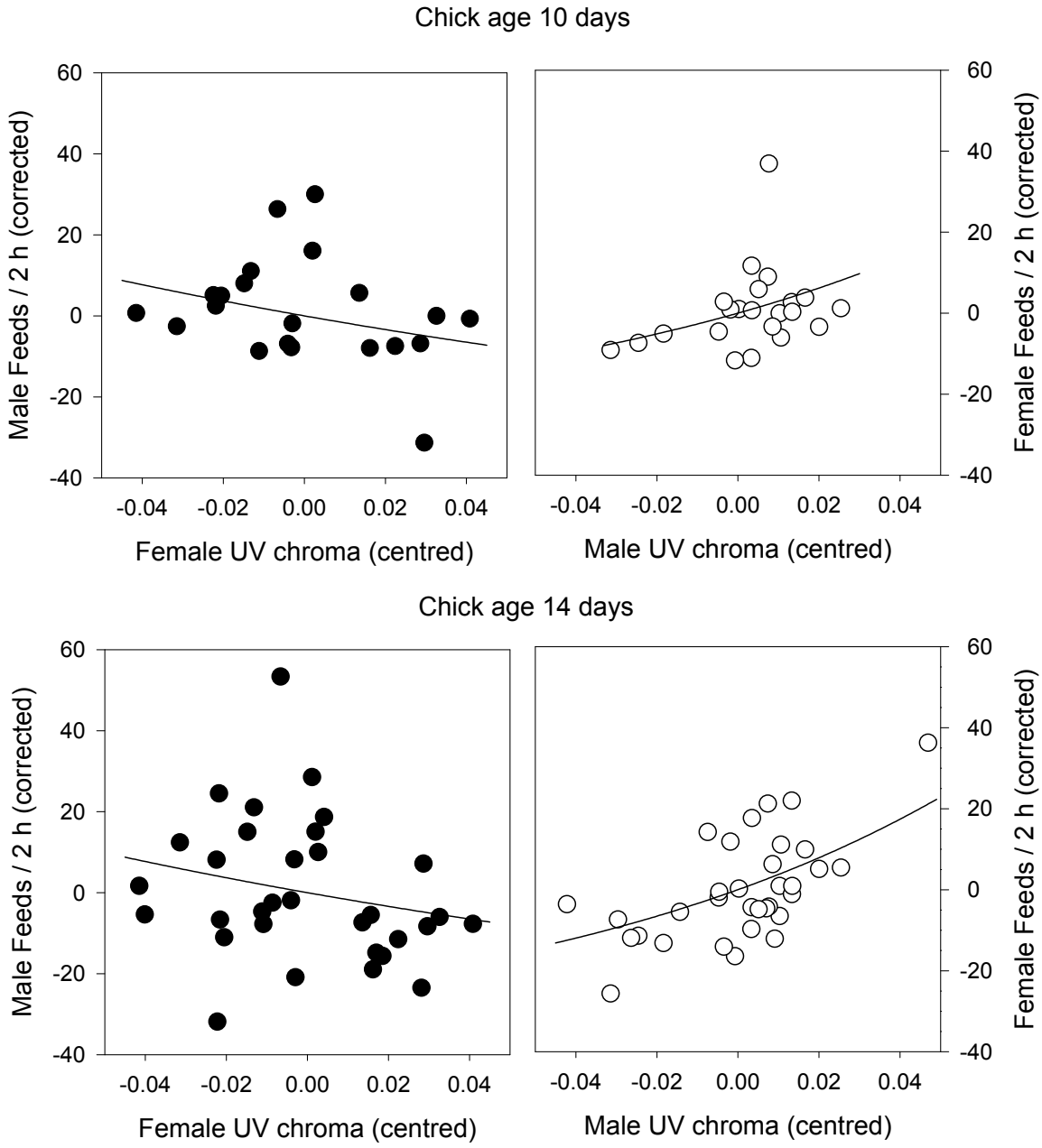

Figure 1. Feeding visits by male and female parents in relation to the UV chroma of their mates. The number of feeding visits by males and females was scored from 2h of video recordings made 10 (*N* = 22) and 14 days (*N* = 33) post-hatching from a total of 33 nests. Curves are based on repeated measure models (Table 1) and the values plotted are corrected for all terms in the model except mate UV chroma. UV chroma is centred around the mean UV chroma for males (0.321 ± 0.018 SD.) and females (0.291 ± 0.022 SD).

As reported above, both male and female blue tits showed no correlation between feeding rates and their own UV chroma and the estimates for own UV chroma did not differ significantly (T = 0.327, df = 63, P = 0.721). However, feeding rates of the two sexes were correlated in opposite directions with the mate's UV chroma. The slopes of these relationships between feeding rates and mate's UV chroma differed significantly (T = 4.09, df = 63, P = 0.0001).

Table 2. Hierarchical mixed model (type III tests) of fledgling tarsus length and mass (32 nests).

	Estimate	SE	*df*	*F*	*P*
Fledgling tarsus length:					
Male UV chroma	7.121	2.407	1, 22.7	8.76	0.007
Hatching date	0.008	0.013	1, 27.1	0.33	0.569
Offspring sex	0.487	0.055	1, 272	77.19	<0.0001
Male tarsus length	0.263	0.107	1, 28	6.00	0.021
Female tarsus length[†]	0.085	0.092	1, 24.3	0.85	0.365
Male UV chroma * Hatching date	1.723	0.694	1, 23.1	6.16	0.021
Female UV chroma (not in the model)				0.46	0.506
Fledgling mass:					
Hatching date	-0.080	0.024	1, 30.2	10.85	0.003
Brood size*	-0.066	0.035	1, 30.4	3.58	0.068
Offspring sex	0.441	0.065	1, 264	46.52	<0.0001
Male UV chroma (not in the model)				2.34	0.138
Female UV chroma (not in the model)				2.18	0.151

Note: The estimate for 'Offspring sex' represents the difference in intercepts between male and female offspring.

[†] Although non-significant, 'Female tarsus length' was retained in the model, because there was no a priori reason for expecting different heritabilities of tarsus length of the mother and father. The estimates for male and female tarsus length did not differ significantly (T = 1.258, df = 62, P = 0.213).

* Although 'Brood size' is marginally non-significant we retained it in the model. Removing this variable does not change the outcome of the model.

We did not find any significant interaction between brood sex ratio and own or mate's UV chroma on feeding rates of male and females (Table 1). Instead we found that males and females feeding rates were correlated to the brood sex ratio itself. Males increased feeding rates in broods with high sex ratio while females decreased feeding rates (Table 1). The slope of feeding rate on brood sex ratio differed significantly between the parental sexes (T = 3.348, df = 64, P = 0.0014). Sex ratio was not correlated to either male UV chroma (N = 33, R = 0.307, P = 0.082) or female UV chroma (N = 32, R = -0.042, P = 0.82).

The differential investment of parents in relation to mate UV chroma may affect offspring phenotype, measured as fledgling mass and tarsus length. Fledgling mass was strongly influenced by environmental variables like hatching date and brood size but was not related to the UV chroma of either parent (Table 2). Instead, we found a positive correlation between fledgling tarsus length and male UV chroma (Fig. 2, Table 2) but not with female UV chroma (Table 2). However, the estimates of the slopes for fledgling tarsus length in relation to male and female UV chroma did not differ significantly (T = 1.738, df = 62, P = 0.087). Further, a significant interaction between hatching date and male UV chroma implied that male UV chroma had a stronger positive effect on offspring tarsus length for fledglings that hatch late in the breeding season (Fig. 3, Table 2).

The opposite patterns of parental investment in relation to mate UV coloration in males and females might suggest that high UV coloration is attractive in males but unattractive in females. This might give rise to disassortative mating with respect to UV chroma in blue tits. However, there was no correlation between male and female UV chroma within pairs (N = 32, r = -0.117, P = 0.525).

4. Discussion

The aim of this study was to describe the relationships between parental investment and an individual's own and its mate's attractiveness in blue tits. Feeding rates of male and female blue tits are not correlated to their own UV coloration, but to the UV coloration of their mates. Female feeding rates are positively correlated to

male UV while male feeding rates are, unexpectedly, negatively correlated to female UV. Finally, we found that fledglings in nests with bright UV fathers had longer tarsi.

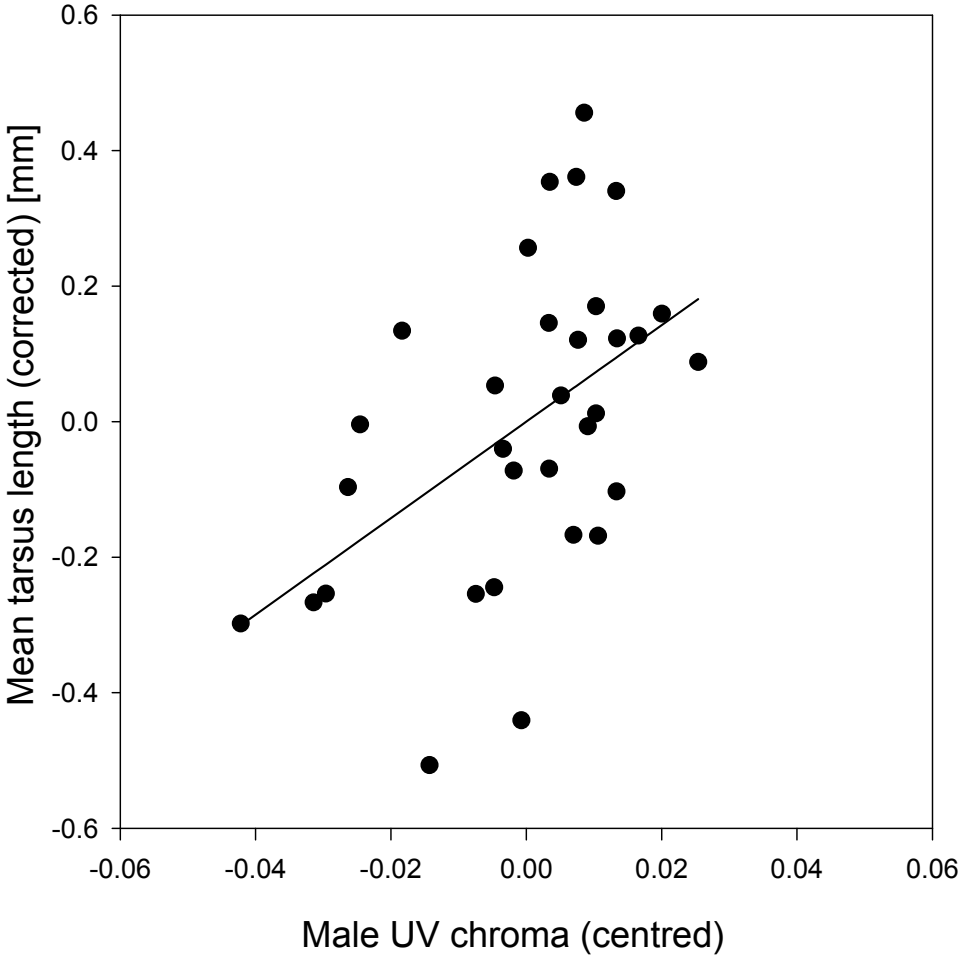

Figure 2. Fledgling tarsus length in relation to male UV chroma. The curve is based on a hierarchical mixed model (Table 2). The points represent the average per brood (*N* = 32) of all individual values corrected for all terms in the model (Table 2) except male UV chroma. UV chroma is centred around the mean male UV chroma (0.321 ± 0.018 SD).

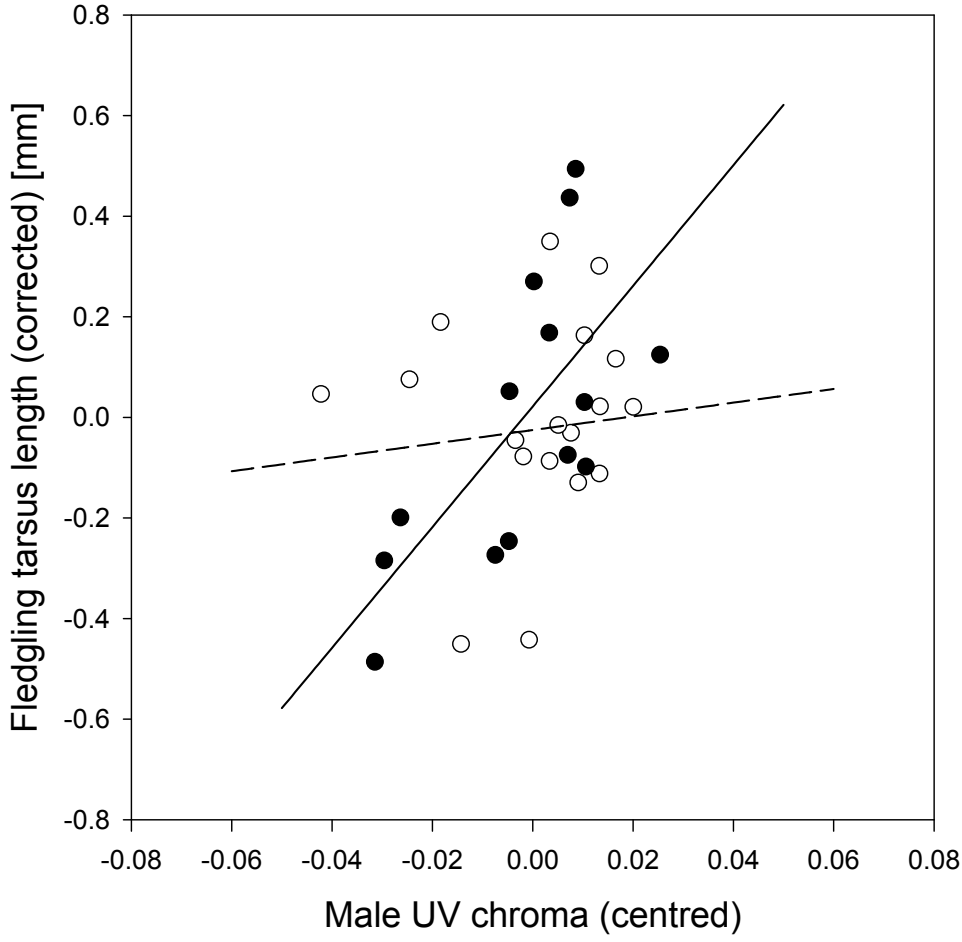

Figure 3. Tarsus length of early and late hatched fledglings in relation to male UV chroma. The points are the average per brood (*N* = 32) of all individual values, corrected for all terms in the model (Table 2) except male UV chroma, for broods hatched before (open dots) and after the medium hatching date (closed dots). UV chroma is centred around the mean male UV chroma (0.321 ± 0.018 SD). The curves are based on a hierarchical mixed model (Table 2) and represent the slope for early-hatched chicks (mean hatching date – 1 SD = 15th May; dotted line) and late-hatched chicks (mean hatching date + 1 SD = 21st May; solid line).

We cannot make unequivocal conclusions about the causation for the relationships between male and female investment and mate attractiveness based on the results presented here, although the lack of any correlation between feeding rates and an individual's own UV implies that they do not arise through adjustment by

each parent to the mate's investment. Differential allocation can only be unequivocally concluded from experiments manipulating the sexually-selected signals. Based on the results reported here, we have conducted an experiment on male blue tits (Limbourg et al. 2004). When we reduced male UV coloration, females decreased their feeding rates compared to females mated to unmanipulated controls. Johnsen and colleagues (2005) found a similar response in one year-old females mated to males whose UV was enhanced compared to those whose mate's UV was reduced, but in older females there was no difference between the experimental treatments. This differs from our descriptive results, since we did not find a significant 'Parental age x UV coloration' interaction for either sex. In other respects our descriptive results are entirely consistent with the experimental results demonstrating differential allocation in females.

The most surprising result of our studies is that females work harder when mated to high UV males, but that the reverse is true for males. The result for females is as we expected, because UV appears to signal male quality (bright UV males have higher survival (Sheldon et al. 1999; Griffith et al. 2003) and heterozygosity (Foerster et al. 2003)) and males with high UV chroma are attractive to females (Hunt et al. 1999). Male UV therefore seems to signal benefits for offspring fitness, and females might be expected to increase their investment accordingly. However, we expected these arguments also to apply to the response of males to their mates' UV: it is generally accepted that signals must be costly to be honest, and we expected similar physiological mechanisms to underlie the production of high UV in both sexes, so that high UV would be the costly signal of high quality in both sexes.

Why then do males work harder for low UV females? The first possibility is that we are mistaken in concluding that high UV in females is costly and hence attractive. However, this seems not to be the case because females prefer high UV males in laboratory tests (Hunt et al. 1999) and there is assortative mating for UV in wild blue tits (Andersson et al. 1998), although our study and several others (B. Kempenaers, Vienna / Austria; P.Korsten, Groningen / The Netherlands; S. C. Griffith, Oxford / Great Britain; pers. Comm.) failed to find assortative mating. Moreover, aspects of UV coloration depend on the precision of nanostructural arrangement (Shawkey et al. 2003), confirming the idea that the production of high UV is relatively costly.

If high UV is indeed attractive in females, our results imply that males work harder for less attractive females. This might be because attractive females increase the fitness of their offspring in such a way that male investment has less of an effect on offspring fitness so that males are selected to decrease, not increase, their investment. For example, attractive females might produce better quality eggs, or provide better care in terms of incubation, removing parasites from the nest or nestling provisioning. The results presented here suggest that the last of these is not the case because female feeding rates are unrelated to their own UV. The suggestion that individuals should work harder for unattractive mates seems directly at odds with the differential allocation hypothesis as it is usually stated (Sheldon 2000). This apparent contradiction occurs because verbal explanations of the differential allocation hypothesis incorrectly focus on the effects of the mate's phenotype or genotype on offspring fitness. In fact, it is the effect of these on the relationship between offspring fitness and an individual's own investment that determines optimal differential allocation. More specifically, it is the slope of this curve that is important, so that a higher curve ('fitter offspring') can be accompanied by selection for a decrease in an individual's own investment (see Maynard Smith (1980) and Lessells (2002) for examples where these curves depend on offspring phenotype rather than mate attractiveness).

Such an explanation does not avoid the problem that, if mates invest more in relation to low attractiveness, and if signals of low attractiveness are not costly, individuals would gain by falsely signalling low attractiveness. In this situation honesty of signalling may be enforced if there are compensating advantages of high attractiveness at other times. For example, attractive females may obtain better quality mates. This explanation requires that the signals of attractiveness cannot be rapidly modified, as is the case for blue tit crown feathers which are moulted only once a year (Jenni & Winkler 1994).

We found no evidence for differential sex allocation in terms of parental investment. Previous studies have shown differential allocation in terms of the sex ratio: females adjusted their sex ratio in response to experimental manipulation of male attractiveness in blue tits (Sheldon et al. 1999; Korsten et al. 2006). Why differential sex allocation is limited to sex ratio is unknown, but differential sex

allocation in investment may be difficult if parents cannot reliably and rapidly identify the sex of chicks.

We found that offspring tarsus length, but not mass, was related to male UV chroma. This is consistent with the results of an experiment manipulating male UV (Limbourg et al. 2004). There is positive selection on both tarsus and mass (Charmantier et al. 2004), implying that offspring of high UV males have higher fitness. We did not find a significant relation between tarsus length and female UV, but neither was there a significant difference between the relationships for female and male UV, so we cannot conclude whether differential allocation in response to female UV has a different effect on offspring tarsus growth than that in response to male UV.

In conclusion, we found that feeding rates by parental blue tits were related to their mate's UV, but not their own UV, thus adding to the limited number of studies that have investigated parental investment in both sexes in response to mate attractiveness (Amundsen 2000). Intriguingly, we found that whereas females have higher feeding rates when mated to high UV males, the reverse is true for males. This raises interesting questions, because we expected high UV to be either attractive, or unattractive, in both sexes. Whether the difference between the sexes occurs because high UV females are unattractive, or because males increase investment when mated to unattractive females, is an open question for further research.

5. Acknowledgments

We thank the Hoge Veluwe National Park for permission to conduct our field study. This study was funded by NWO. The study was approved by the Animal Experimental Committee of the Royal Dutch Academy of Arts and Sciences.

CHAPTER 4

Effectiveness of a commonly-used technique for experimentally reducing plumage UV reflectance

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& J. Komdeur

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Abstract:

Ultraviolet (UV) plumage is thought to be sexually selected through intra-sexual competition, female choice and differential allocation. Experimental manipulations of plumage UV reflectance are essential to demonstrate that mate choice or intra-sexual competition are causally related to UV coloration. The most widely-used technique for manipulating UV reflectance in wild birds is the application of a mixture of UV-absorbing chemicals and preen gland fat. However, although this UV reduction technique is commonly used, little is known about the persistence of the treatment and the temporal variation in UV reflectance that it causes. We manipulated the UV crown plumage of wild and captive blue tits *Parus caeruleus*, and took repeated photospectrometric measurements of both UV-reduced and control-treated individuals. Our results show that the UV reduction lasts for at least five days and that the treatment has no negative effects on the survival of wild birds.

1. Introduction

Recently there has been a surge of interest in avian colour vision and coloration, especially regarding the significance of plumage ultraviolet (UV) reflectance, which is visible to most bird species but not to their human observers (Hill and McGraw 2006). Descriptive studies have implicated UV plumage and other coloration in sexual selection, through intra-sexual competition (e.g. Senar et al. 1993, Siefferman and Hill 2005), female choice (e.g. Andersson et al. 1998, Hill et al. 1999) and differential allocation (Linville et al. 1998), but experimental manipulation of coloration is needed to unequivocally demonstrate a causal link between the behaviour of conspecifics and an individual's coloration (e.g. Hill 1991, Bennett et al. 1996, Johnsen et al.1998, Limbourg et al. 2004).

The most widely used technique for manipulating plumage UV reflectance in wild birds involves applying a mixture of UV-absorbing chemicals and duck preen gland fat to the feathers. This technique was first used by Andersson and Amundsen (1997) in bluethroats *Luscinia svecica* and has since been used on several species in both the field and captivity (Table 1). Although these experiments show that conspecifics respond to the treatment, and some studies have given approximate indications of how long the treatment lasts (Johnsen et al. 1998, Limbourg et al. 2004), there has, remarkably, been no detailed study of the time course of the UV reduction effect. Thus we have little idea of how the coloration varies through time after the treatment. This information is particularly pertinent in studies which aim to measure a response to the UV manipulation several days after the application of the treatment. For example, crown UV reflectance in male blue tits *Parus caeruleus* has been manipulated before the start of laying by the female after which the sex ratio of the subsequently-laid clutch was measured (Sheldon et al 1999, Korsten et al. 2006). In blue tits, successive eggs of a clutch are laid daily over a period of about 10 d (mean clutch size: 10.9 ± 1.7 SD; Korsten et al. 2006). Thus, depending on the temporal variation in UV reflectance, the sex of individual eggs in a clutch may have been determined when the male differed considerably in appearance. In another study on blue tits, UV reflectance of males was reduced 2 d before hatching and again when the chicks were 7 d old. Subsequently, female provisioning behaviour was measured when the chicks were 10 and 14 days old (Limbourg et al. 2004; see

Table 1. Studies manipulating plumage UV reflectance using mixtures of UV-absorbing chemicals and preen gland fat.

Species	Captive / wild	Time between treatment and measurement of response (in d)	Response to treatment	Description of response	Reference
Blue tit *Parus caeruleus*	Wild	10 ± 5.2 SD	Yes	Females adjust offspring sex ratio in response to male UV reduction	1
Blue tit	Wild	3 / 7	Yes	Females feed their young less when paired to UV-reduced males	2
Blue tit	Wild	8.0 ± 6.5 SD / 4.4 ± 2.9 SD	Yes / No	Females adjust offspring sex ratio in response to male UV reduction in 1 of 2 years	3
Bluethroat *Luscinia svecica*	Captive	< 1	Yes	Females discriminate against UV-reduced males in choice test	4
Bluethroat	Wild	Variable: ca 7–20	Yes	UV-reduced males have lower (extra-pair) mating success	5
Pied flycatcher *Ficedula hypoleuca*	Captive	< 1	Yes	Females discriminate against UV-reduced males in choice test	6

1. Sheldon et al. 1999; 2. Limbourg et al. 2004; 3. Korsten et al. 2006; 4. Andersson & Amundsen 1997; 5. Johnsen et al. 1998; 6. Siitari et al. 2002.

Johnsen et al. 2005 for a similar experiment using marker pens instead of UV-absorbing chemicals). Again, male coloration during the observations of female behaviour could have differed considerably from that immediately after treatment. Clearly, knowledge of the temporal changes in the effect of UV-reduction treatment would facilitate the successful application and correct interpretation of these kinds of experiment.

We therefore investigated how UV coloration varied with time after treatment in both wild and captive birds. We studied blue tits, because their crown UV coloration is one of the most extensively investigated UV-reflecting plumage ornaments (e.g. Andersson et al. 1998, Hunt et al. 1998, Sheldon et al. 1999, Delhey et al. 2003, Limbourg et al. 2004, Johnsen et al. 2005, Hadfield et al. 2006, Korsten et al. 2006) and the most frequent subject of manipulation using UV-absorbing chemicals (Table 1).

2. Methods

General

We caught wild male blue tits in the period from nest building to hatching at De Vosbergen, The Netherlands (see Korsten et al. 2006 for details) during 2002 and 2003, and manipulated their crown UV reflectance (42 UV-reduced males, 43 controls). Crown UV reflectance was measured immediately before and after treatment (= day 0). 70 males were recaptured and remeasured during chick provisioning, most of them (65 males) either 7–14 d (8 UV-reduced males, 8 controls) or $\geq$ 28 d after the initial treatment (26 UV-reduced males, 23 controls).

In addition, 4 male blue tits were captured at Westerheide (The Netherlands) in November 2002. They were held together in a large outdoor aviary (ca $2 \times 4 \times 3$ m) at the Netherlands Institute of Ecology (NIOO) in Heteren and fed *ad libitum* with standard bird food. Their crown UV reflectance was manipulated (all reduced) and measured immediately before and after treatment (= day 0), and on days 1–7, 9, 12 and 16. Males were subsequently released at the capture site.

Crown UV treatment and measurements

We reduced UV reflectance of the crown feathers using a 40/60% (by weight) mixture of duck preen gland fat (which is commercially available and used as fishing fly dressing; purchased at Euro-Fly, Paris, France) and UV-absorbing chemicals (Parsol 1789 and Parsol MCX (50% of each by weight; Roche, Basel, Switzerland; Andersson and Amundsen 1997, Johnsen et al. 1998, Sheldon et al. 1999, Limbourg et al. 2004, Korsten et al. 2006). As a control, we applied pure duck preen gland fat (Johnsen et al. 1998, Sheldon et al. 1999, Limbourg et al. 2004, Korsten et al. 2006).

We measured the reflectance of the crown feathers using a USB-2000 spectrophotometer and DH-2000 deuterium-halogen light source (both Avantes, Eerbeek, The Netherlands). For more details of measurement and processing of the reflectance spectra see Limbourg et al. (2004) and Korsten et al. (2006). We calculated 'UV chroma' as the sum of reflectance between 320–400 nm divided by the sum of reflectance between 320–700 nm ($R_{320–400}$ / $R_{320–700}$) following previous studies (e.g. Sheldon et al. 1999, Delhey et al. 2003, Limbourg et al. 2004, Korsten et al. 2006). UV chroma is an important predictor of male attractiveness in blue tits (Andersson et al. 1998, Sheldon et al. 1999, Limbourg et al. 2004).

We also measured crown reflectance of unmanipulated males (n = 111) and females (n = 169) at De Vosbergen during the 2001–2003 breeding seasons.

3. Results

Effect of UV manipulation on crown coloration of wild males

Both UV-reduced and control-treated feathers became slightly more glossy after the treatment, but otherwise looked the same to the human observer. The gloss caused a small uniform increase in reflectance for both treatments (Fig. 1). The UV reduction treatment clearly reduced the reflectance between 320–400 nm, whereas the control treatment did not (Fig. 1). So treatment reduced UV chroma by 38% compared to pre-treatment values (paired t-test: $t = 28.90$, $df = 41$, $P < 0.001$; Fig. 2), a value 24% and 6% below the natural range of UV chroma for males and females, respectively (Fig. 2). UV chroma was not affected by the control treatment ($t = 1.63$, $df = 42$, $P = 0.11$; Fig. 2).

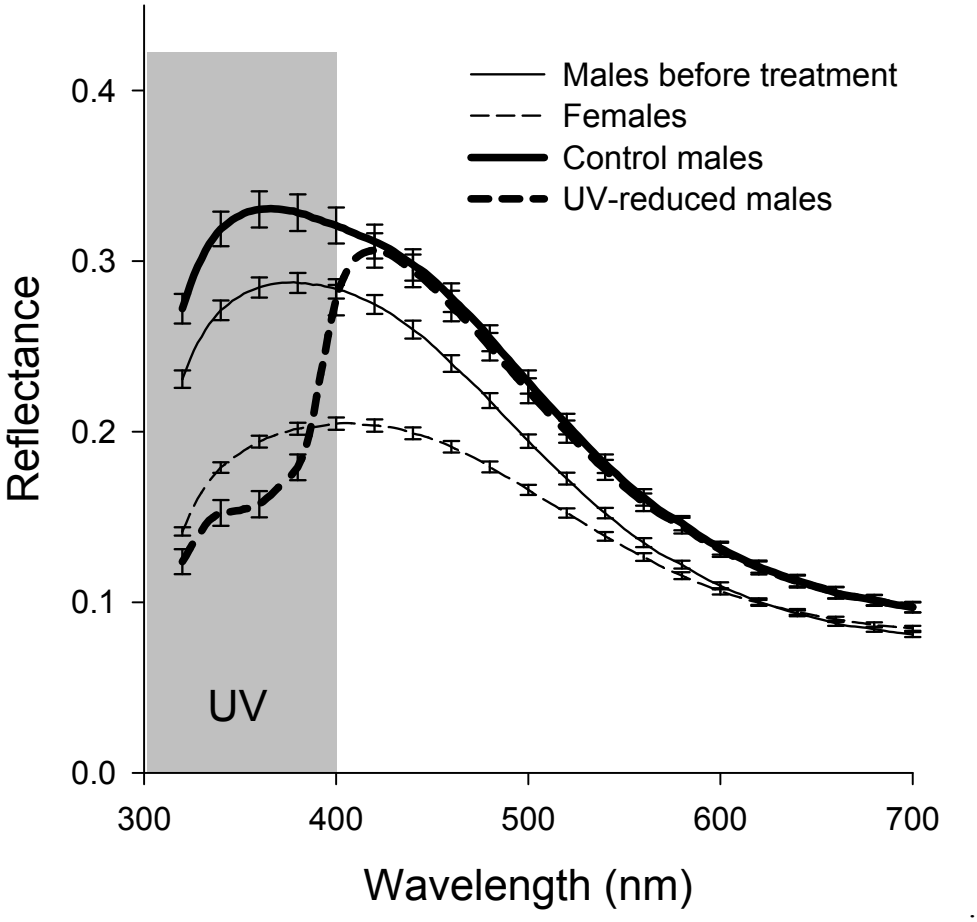

Figure 1: Mean reflectance curves of the crown plumage of wild male blue tits before manipulation (n = 85), after UV reduction (n = 42), and after control treatment (n = 43). The mean reflectance curve for unmanipulated females (n = 169) is shown for reference. Standard errors are depicted at 20-nm intervals. The shaded area indicates the UV part of the spectrum.

The change in spectral profile (Fig. 1) resulting from the UV-reduction treatment also increased the wavelength at peak reflectance (mean $\lambda_{max} \pm$ SE, before: 381 $\pm$ 2.2 nm, after: 418 $\pm$ 1.1 nm; paired t-test: t = -19.70, df = 41, P < 0.001). The control treatment caused a smaller but significant decrease in λ_{max} ($\lambda_{max} \pm$ SE, before: 377 $\pm$ 1.8 nm, after: 368 $\pm$ 2.0 nm, paired t-test: t = 4.85, df = 42, P < 0.001). Pre- and post-treatment UV chroma of individual males were strongly correlated in both treatment groups (UV-reduced: r = 0.47, n = 42, P = 0.002; control: r = 0.72, n = 43, P < 0.001; Fig. 3), and the slopes of the relationships did not differ between the groups

(ANCOVA with UV chroma after treatment as response variable: UV treatment × UV chroma before treatment: $F_{1, 81} = 0.007$, P = 0.93; Fig. 3).

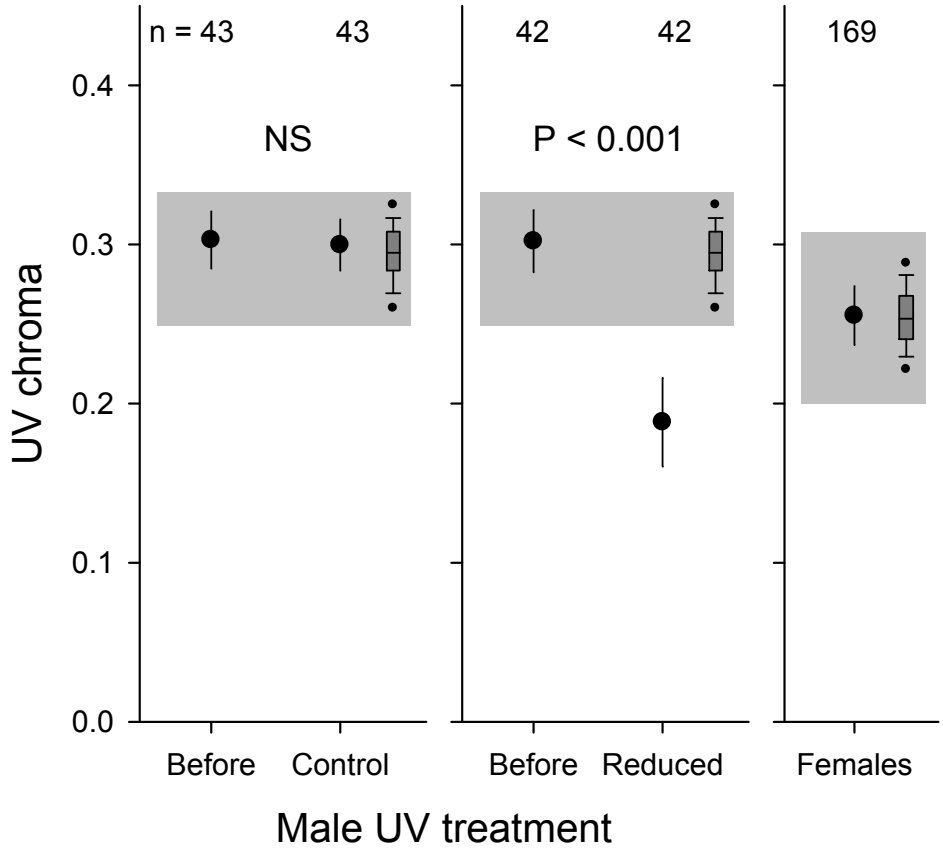

Figure 2. Mean UV chroma of crown plumage of wild male blue tits before and after UV reduction and control treatment. The mean UV chroma of unmanipulated females (n = 169) is shown for reference. Whiskers indicate standard deviations. Shaded areas indicate natural ranges of UV chroma of males (n = 111; range 0.249–0.333) and females (n =169; range: 0.200–0.307). Box plots on the right of each panel show the variability of natural UV chroma in males and females (depicted are the median and the 5th, 10th, 25th,75th, 90th and 95th percentiles).

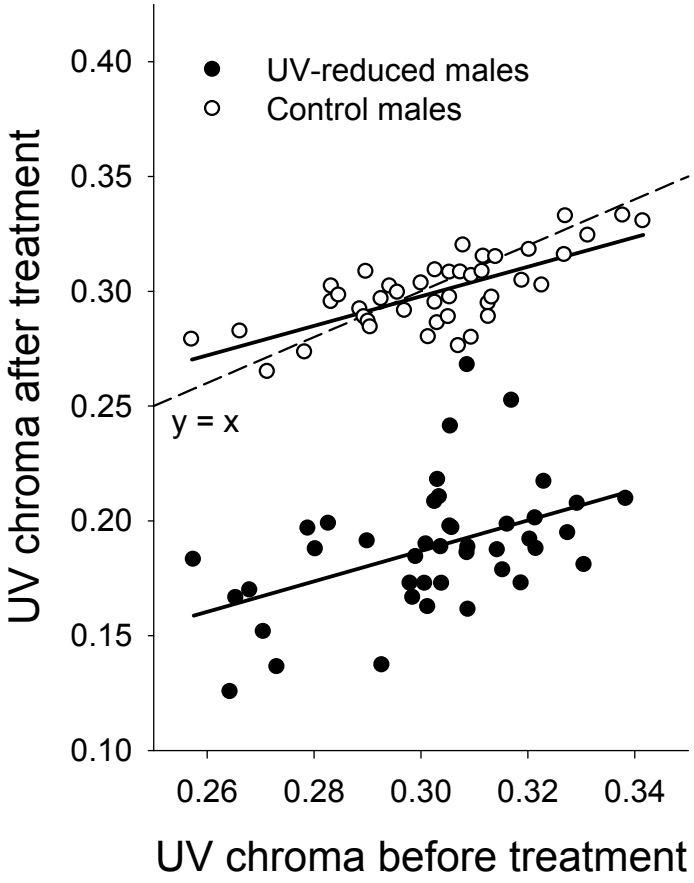

Figure 3: UV chroma of crown plumage of individual wild male blue tits before and after UV reduction (n = 42) or control treatment (n = 43). Solid lines are linear regressions on each treatment group separately.

Temporal change of UV reduction in captive and wild males

The effect of the UV-reduction treatment in captive birds diminished over time, being most rapid directly after application (Fig. 4a). Although the treatment initially decreased UV chroma to unnaturally low values, average UV chroma of UV-reduced males was already within the natural range again two days after treatment (Fig. 4a), and the reduction in UV chroma (compared to pre-treatment values) was no longer significant 6 days after treatment (Fig. 4a). Wild birds showed a similar pattern (Fig. 4b), although UV-reduced males still had significantly lower UV chroma than control males 7–14 days after treatment ($t = -2.36$, $df = 14$, $P = 0.034$; Fig. 4b), while

both values were within the natural range (Fig. 4b). The difference between the UV chroma of UV-reduced and control males had disappeared in individuals recaptured ≥28 days after treatment (t = 1.19, df = 47, P = 0.24; Fig. 4b). There was no difference in survival to the following breeding season between treated and untreated males (treated males: 37.6%, n = 85; untreated males: 39.2%, n = 74; Fisher's exact test: P = 1.0) or between UV-reduced and control males (UV-reduced: 35.7%, n = 42; control: 39.5%, n = 43; Fisher's exact test: P = 0.84).

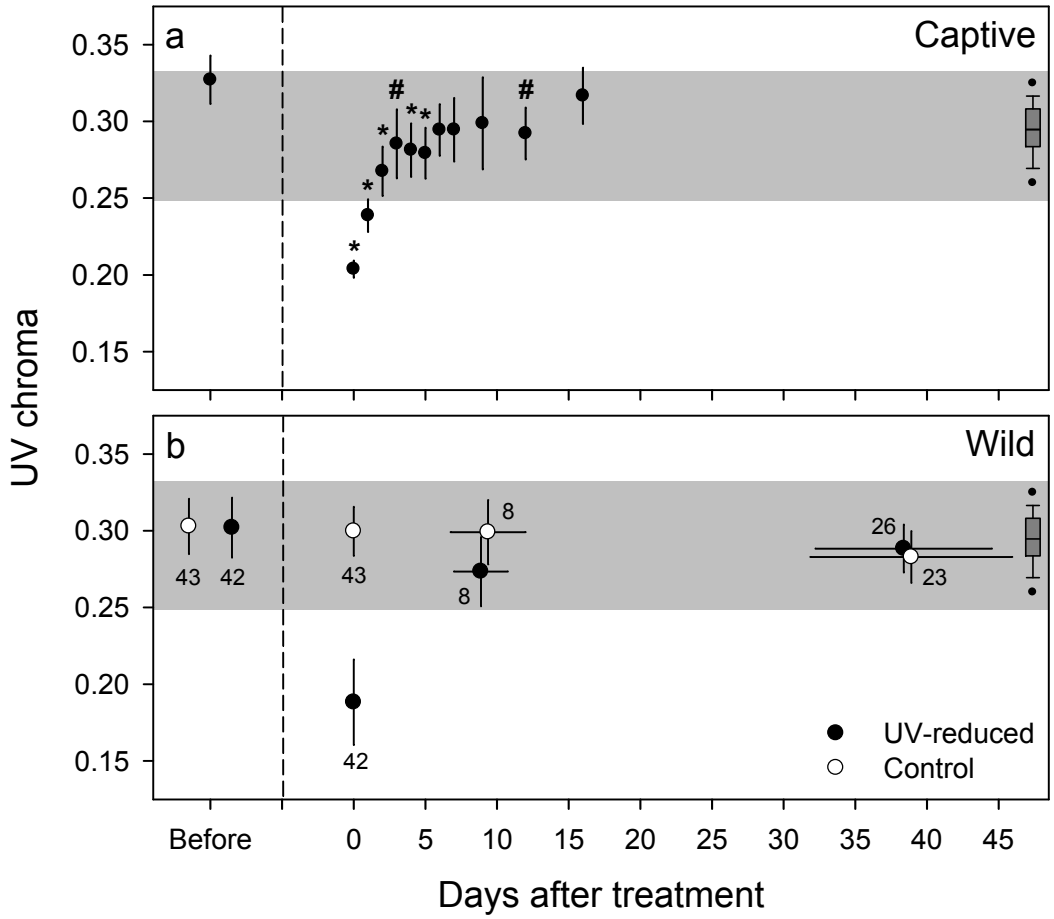

Figure 4: Temporal changes of crown UV chroma after UV-reduction treatment of (a) captive and (b) wild male blue tits. (a) Mean UV chroma ± SD of 4 individual UV-reduced males who were repeatedly measured. Differences in UV chroma from pre-treatment values were tested with one-tailed paired t-tests (# P < 0.05; * P < 0.01). (b) Mean UV chroma values ± SD of UV-reduced and control-treated males are shown before and immediately after treatment, and for manipulated males recaptured 7–14 days or > 28 days after treatment. Numbers indicate sample sizes. Shaded areas in both panels (a, b) indicate natural ranges of male UV chroma (n

= 111). Box plots on the right of each panel show the variability of natural UV chroma in males (depicted are the median and the 5th, 10th, 25th,75th, 90th and 95th percentiles).

4. Discussion

Our results confirm that the application of a mixture of preen gland fat and UV-absorbing chemicals reduces UV reflectance, whilst pure preen gland fat can serve as an adequate control. The UV reduction effect diminishes rapidly shortly after the treatment, but is still detectable after 5 days in captive birds, and 7–14 d in wild birds. Importantly, mean UV chroma values are outside the natural range for only a short period (less than 2 d in captive males), partly refuting previously raised concerns that manipulated birds were outside the natural range (Johnsen et al. 2005, Hadfield et al. 2006). We do not know how conspecifics perceive the effect of the UV reduction treatment. For example, they might respond to discordance between the coloration of different areas of plumage (Sheldon et al. 1999) or to temporal variation in UV reflectance (Limbourg et al. 2004). Nevertheless, we wish to emphasize that these experiments demonstrate that information contained in the UV part of the spectrum is causally involved in intra-specific communication.

The treatment had no undesired side-effects in the wild (and captive) blue tits. In spite of their changed appearance, most manipulated wild males re-established contact with their mates immediately after release (P. Korsten, pers. Obs.) and the treatment never led to divorce (see Korsten et al. 2006). The treatment was reversible (no UV reduction effect detectable after approximately 28 d) and had no negative effects on the chances of survival to the following breeding season.

Marker pens have also recently been used to successfully manipulate UV plumage coloration (Ballentine and Hill 2003, Johnsen et al. 2005), and can produce an increase, as well as a decrease, in UV reduction, although duration of the treatment effects has not yet been investigated.

In conclusion, mixtures of UV absorbing chemicals and (preen gland) fat offer an excellent tool for manipulating the UV reflectance of plumage. Our results will add considerably to the usefulness of studies using this technique, by underlining the need for careful planning, possibly including re-application of the treatment (e.g. Limbourg et al. 2004), because of the short-term nature of the UV reduction, and by

revealing the time course of variation in UV coloration in relation to the behavioural responses that are measured.

5. Acknowledgements

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CHAPTER 5

Female blue tits adjust parental effort to manipulated male UV attractiveness

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Limbourg, T., Mateman, A.C., Andersson, S. & Lessells, C.M. 2004. Female blue tits adjust parental effort to manipulated male UV attractiveness. *Proc. R. Soc. Lond. B* **271**: 1903-1908.

Abstract:

The differential allocation hypothesis predicts that parents should adjust their current investment in relation to perceived mate attractiveness if this affects offspring fitness. It should be selectively advantageous to risk more of their future reproductive success by investing heavily in current offspring of high reproductive value but to decrease investment if offspring value is low. If the benefits of mate attractiveness are limited to a particular offspring sex we would instead expect relative investment in male versus female offspring to vary with mate attractiveness, referred to as 'differential sex allocation'. We present here strong evidence for differential allocation of parental feeding effort in the wild and show an immediate effect on a component of offspring fitness. By experimentally reducing male UV crown colouration, a trait known to indicate attractiveness and viability in wild breeding blue tits *Parus caeruleus*, we show that females, but not males, reduce parental feeding rates and that this reduced the skeletal growth of offspring. Differential sex allocation, on the other hand, did not occur. We conclude that blue tit females use male UV colouration as an indicator of expected offspring fitness and adjust their investment

1. Introduction

Parents face a trade-off between their current and future reproduction because any investment to increase the fitness of current offspring evokes costs in terms of their own future survival and reproduction (Trivers 1972). To maximise its lifetime reproductive success a parent should therefore adjust its level of parental investment to the value of the current offspring in each breeding attempt in relation to the expected value of future breeding attempts. One potential factor indicating the value of offspring is the attractiveness of the current mate. Attractive mates might provide either indirect benefits for offspring fitness by passing on 'good genes' for attractiveness or viability, or direct benefits via environmental correlates of attractiveness (Andersson 1994). Assuming that the current mate is attractive and there is sufficient variation in the attractiveness of future mates, the value of current offspring will be higher than the expected value of future offspring with different, probably less attractive mates. Mate attractiveness would thus affect the trade-off between current and future reproduction (Sheldon 2000) and we would expect differential allocation to occur (Burley 1986b). If the 'Differential Allocation hypothesis' (Burley 1986b; Sheldon 2000) is correct, individual parents are expected to adjust their investment to the perceived sexual attractiveness of their mates and, as a consequence, the higher investment in offspring of attractive mates should lead to increased offspring fitness, and equally, reduced investment in offspring of less attractive mates should lead to decreased offspring fitness.

The same logic applies if the benefits indicated by mate attractiveness for offspring fitness are sex-limited, but then increased investment should be restricted to the offspring sex affected (Charnov 1982). For instance, the future reproductive success of male offspring might be more strongly correlated to parental attractiveness than that of female offspring. In this case, the fitness of male offspring would vary strongly with parental attractiveness and we would expect that relative investment in male and female offspring should be adjusted to mate attractiveness. We refer to this as 'differential sex allocation'.

However, differential allocation requires by definition that parents adjust their investment to the attractiveness per se of their mates and not to environmental correlates of attractiveness, but attractive individuals might provide some direct

benefits, such as food rich territories, that enable their mates to show higher levels of parental investment. Thus, to establish a causal link between individual investment and mate attractiveness experimental testing is required to eliminate such confounding variables (Sheldon 2000).

Recent experimental tests of differential allocation have primarily focused on aspects of primary reproductive effort (egg number and quality). Parents adjusted clutch size and egg size or quality to manipulated mate attractiveness in various species, such as birds (Cunningham & Russell 2000; Gil et al. 1999), fish (Kolm 2001), amphibians (Reyer et al. 1999) and insects (Simmons 1987; Thornhill 1983). Equally, there is evidence for differential sex allocation in primary reproductive effort; that is, females adjusted brood sex ratio in relation to manipulated mate attractiveness (Burley 1986a; Sheldon et al. 1999).

In species with prolonged parental care, however, offspring provisioning is the major component of parental investment and should therefore be adjusted to mate attractiveness, but unequivocal experimental evidence from wild populations is lacking. The majority of experimental studies have not demonstrated differential allocation in provisioning rate (Mazuc et al. 2003; Pilastro et al. 2003; Rohde et al. 1999; Sanz 2001). Of the two studies that provide some support, Burley's (Burley 1988) pioneering study on zebra finches *Taeniopygia guttata* with artificial ornaments, was carried out on captive birds, and the other on barn swallows *Hirundo rustica* (De Lope & Møller 1993) cannot be unambiguously interpreted as differential allocation. This is because males with experimentally elongated tails (attractive males) reduced their feeding effort and the observed increase in female feeding rate may have been a response to this rather than the male's tail length (Witte 1995, but see also Møller & deLope 1995). We know of no experimental study that has measured feeding effort in the two sexes of offspring in relation to mate attractiveness, so differential sex allocation in feeding effort has not previously been investigated.

Structural ultraviolet colouration (UV) in blue tits *Parus caeruleus* offers an ideal study system to experimentally test whether females differentially allocate parental feeding effort. Blue tits are sexually dimorphic in the UV spectrum with males having brighter UV colouration with a peak at shorter wavelengths than

females (Andersson et al. 1998; Hunt et al. 1998). UV crown colouration indicates sexual attractiveness in both sexes (Hunt et al. 1999) and male viability (Griffith et al. 2003; Sheldon et al. 1999). Male UV colouration might thus act as an indicator of the expected value of current offspring and females adjust their feeding effort accordingly (i.e. show differential allocation). In addition, because of high levels of extra-pair paternity, sexual selection is likely to act more strongly in males than females (Kempenaers et al. 1992). The expected reproductive success of male offspring may therefore be more dependent on parental attractiveness than that of female offspring, favouring differential sex allocation. Moreover, the experimental test showing that female blue tits modify the sex ratio of the offspring that they produce in relation to manipulated male attractiveness (Sheldon et al. 1999) also implies that differential sex allocation in feeding rates would be selectively advantageous. Lastly, we have found (unpublished data) a correlation between female feeding rate and male UV colouration, but an experimental test is needed to determine whether this involves differential allocation.

The aim of this study was therefore to experimentally test whether female blue tits show either differential allocation or differential sex allocation in relation to male UV colouration. We also examined whether any differential allocation had apparent fitness costs for the female parent or fitness benefits for the offspring.

2. Methods:

(a) *General description of the experiment*

The experiment was performed in May and June 2002 on a Dutch blue tit population breeding in nest boxes in the Hoge Veluwe National Park. To study the influence of male sexual attractiveness on parental investment we manipulated the UV colouration of males' crown feathers shortly before hatching and again during the nestling stage, and video-recorded the feeding behaviour of the adults on days 10 and 14 (where day 0 is the hatching date).

To minimize maternal effects mediated via egg composition (Schwabl 1993) we cross-fostered whole clutches on the estimated day -3 between nests with similar

clutch sizes (± 2 eggs) and predicted hatching dates (± 2 days). We caught males on the following day in a random order independent of the cross-fostering, sequentially assigned them to a UV-reduced or control group and applied the respective treatment (see below). As a result, there should be no bias in egg quality with repect to experimental treatment. Moreover, the two treatment groups did not differ in hatching date ($F_{1,23}$ = 0.31, p = 0.58) or clutch size ($F_{1,23}$ = 0.01, p = 0.94) after cross-fostering.

On day 3 we individually marked all young and took 10 µl blood samples which were used to identify offspring sex using a molecular technique (Griffiths et al. 1998). On day 7 both parents were caught and weighed (to the nearest 0.1 g) and the UV-reduced or control treatment was reapplied to males. Offspring mass (to the nearest 0.1 g) and tarsus length (to the nearest 0.1 mm) were measured on day 15, shortly before the chicks fledge from day 16 onwards.

(b) *Measurements and manipulation of male UV reflectance*

The UV component of male colouration was reduced using a previously developed method (Andersson & Amundsen 1997; Sheldon et al. 1999) in which a mixture of UV blocking chemicals (Parsol 1789 and MCX) and duck preen gland fat was smeared on the males' crown feathers. Control males were treated with the duck preen gland fat only. Before and after the treatments we took 5 replicate crown reflectance measures using an USB2000 Spectrometer with a DH2000 deuterium-halogen lamp as light source (both Avantes) illuminating and measuring perpendicularly to the feathers. The reflectance measurements were used to derive the previously identified (Sheldon et al. 1999) main predictor of male attractiveness, 'UV chroma' ($R_{320\text{-}400}/R_{320\text{-}700}$, the proportion of reflectance in the for blue tits visible spectrum (between wavelength 320-700 nm) occurring in the UV (between 320-400 nm)). This is a measure of the purity of UV colouration. The treatment was successful in producing a difference in the UV chroma of UV reduced males compared to control males (mean UV chroma in the UV-reduced group = 0.258 ± 0.005 s.e.; mean UV chroma in the control group = 0.345 ± 0.004 s.e.; ANOVA, $F_{1,35}$ = 185.03, p < 0.0001). To ensure that males were UV reduced throughout the nestling period we re-applied the experimental and control treatments on day 7. Within the UV-reduced group the

blocking effect was diminished but still significant (comparison with pre-treatment chroma: paired-t = -2.44, n = 14, p = 0.03) while the UV-chromas of control males did not differ from pre-treatment chroma (paired-t = -1.47, n = 16, p = 0.162). The second application resulted in similar UV chromas to the first treatment. A small sample of UV-reduced males caught for a third time (mean days post-hatching = 15.2) showed a similar diminished but significant blocking effect (comparison with pre-treatment chroma: paired-t = -3.58, n = 5, p = 0.023). In summary, we reduced the UV chroma of UV-reduced males throughout the nestling period although the level of reduction varied considerably.

(c) *Parental feeding effort*

To measure parental investment, we made 3 hour infra-red video recordings within the nest boxes on days 10 and 14 and scored the last two hours of each recording. Chicks had been individually marked with paint spots on their crown and the sex of the parent and the identity of the fed chick was recorded for each feeding visit. Parental loads are normally (93 % of visits) given to a single chick but when parents distributed the prey among more than one chick we estimated the proportion that each chick received. Videos were not scored if one of the parents did not enter the nest box in the first 90 minutes of the video recording (UV-reduced group: 2 males, 2 females on day 10; 1 control female on days 10 and 14) or if females showed extensive (> 20 minutes) brooding behaviour (due to cold or wet weather) during the scoring period (1 control each on days 10 and 14).

(d) *Statistical Analysis*

We analysed parental feeding effort (the number of feeds during 2 h), relative investment in offspring sex and offspring tarsus length and mass using hierarchical mixed models in SAS (SAS Institute Inc. 1996). We fitted UV-Treatment and its interactions with Parental sex (analysis of parental feeding effort), Offspring sex (analysis of offspring tarsus length and mass) or both Parental sex and Offspring sex (analysis of relative investment in offspring sex). We tested additional variables and their interactions as appropriate (see tables 1 and 2) to control for their potential effects. These additional variables and their interactions were removed if they were

not significant, but the variables of specific interest (Parental and Offspring sex, UV treatment) were always retained in the model. Interactions were removed first followed by the main effects. Parental feeding effort was treated as a Poisson distributed variable using PROC GENMOD with nest as a subject with repeated measurements, and UV treatment and Brood size as between subject variables, and Parental sex and Chick age as within subject variables. The number of feeds to offspring of each sex were divided by the number of young of that sex and analysed using PROC MIXED assuming a normal distribution, with nest as a repeated measure subject and UV treatment and Brood size as between subject, and Parental sex, Offspring sex and Chick age as within subject variables. Offspring tarsus length and mass were analysed using PROC MIXED assuming a normal distribution, with individual offspring nested within broods. UV treatment and Brood size were fitted as between nest, and Offspring sex as within nest variables. For offspring mass, we additionally fitted offspring tarsus length as a within nest variable and the time of weighing as a between nest variable.

3. Results

Females of UV reduced males fed at a significantly lower rate than females in control pairs (figure 1). In contrast, UV-reduced males did not differ in feeding rate from control males (figure 1). There was no difference between control parents in feeding rates (figure 1). In our mixed model (table 1), these result are seen as a significant interaction between parental sex and UV-treatment, while both of these main effects (effectively the sex difference in control pairs and the effect of experimental treatment in males) are non-significant (table 1).

In contrast to the differential allocation in the feeding effort of females, we found no evidence for differential sex allocation. Our treatment did not affect relative investment in male and female offspring (Offspring sex x UV treatment, $F_{1,21} < 0.01$, $p = 0.95$; one brood contained sons only and was excluded from the analysis), and male and female parents did not differ in the number of feeds per offspring sex in relation to our treatment (Offspring sex x UV treatment x Parental sex , $F_{1,21} = 2.52$, $p = 0.127$).

Table 1. Hierarchical mixed model of the number of male and female feeding visits on 10 (*n* = 19) and 14 days (*n* = 23) post hatching in relation to UV treatment (11 UV-reduced pairs, 13 control pairs).

	z	d.f.	*p*
Variables included in model:			
Parental sex	-1.22	1	0.224
UV Treatment	0.17	1	0.869
Parental sex x UV Treatment*	-2.35	1	0.019
Brood size	1.84	1	0.066
Brood size x Parental sex	4.49	1	< 0.0001
Variables not included in model (all p > 0.05):			
Brood size x UV Treatment			
Male UV chroma			
Male UV chroma x Parental sex			
Male UV chroma x UV Treatment			
Male UV chroma x UV Treatment x Parental sex			
Chick age			
Chick age x Parental sex			
Chick age x UV Treatment			

Note: 'UV Treatment' tests the difference between males in the UV-reduced and control group. 'Parental sex' tests the difference between males and females in the control group.

* The final model was overdispersed (deviance = 234.14 with 78 d.f., $p < 0.001$), but the 'Parental sex x UV Treatment' interaction remained significant in a scaled likelihood ratio test (scaled change in deviance = 5.07 with 1 d.f., $p < 0.05$).

Young in broods of UV-reduced males had significantly shorter tarsi compared to young in the control group (figure 2, table 2). However, we found no difference in mass between the two groups (figure 2, table 2).

Parental mass taken on day 7 did not differ between the UV-reduced and control group in females (ANOVA, $F_{1,23} = 0.79$, $p = 0.39$) or males (ANOVA, $F_{1,23} = 0.58$, $p = 0.46$).

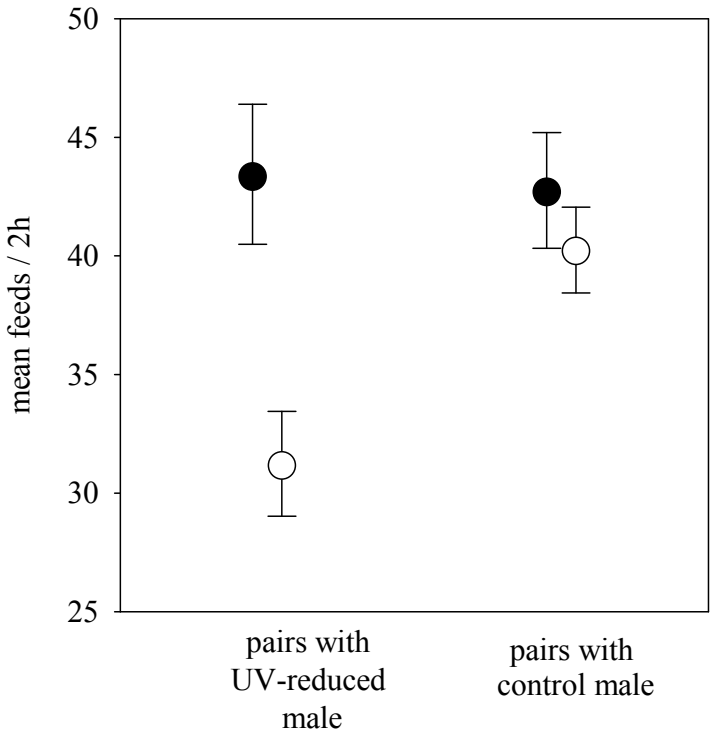

Figure 1: Feeding visits by male and female parents in pairs where the male's UV crown colouration was reduced or subjected to a control treatment. The number of feeding visits by males (filled circles) and females (open circles) was scored from 2h of video recordings made 10 (*n* = 19) and 14 days (*n* = 23) post hatching from a total of 24 nests. Points shown are the mean (± s.e.) number of feeding visits estimated for the mean brood size from a hierarchical mixed model (see table 1).

4. Discussion:

Our experiment demonstrates differential allocation in maternal feeding rates in a wild blue tit population. We reduced the sexual attractiveness of males by reducing their UV crown colouration throughout the nestling period and females of those males had lower feeding rates compared to females of UV-unreduced control males. Because the only difference between the two groups was in the amount of male UV colouration, and mate choice occurred before manipulation, our experimental approach demonstrates that females reduced feeding effort in response to reduced male UV colouration and not to phenotypic or environmental correlates. The interpretation of our result further benefits from the fact that males of both groups did not differ in feeding rates. This avoids the problems of

interpretation faced by the earlier experiment on barn swallows (De Lope & Møller 1993; Witte 1995).

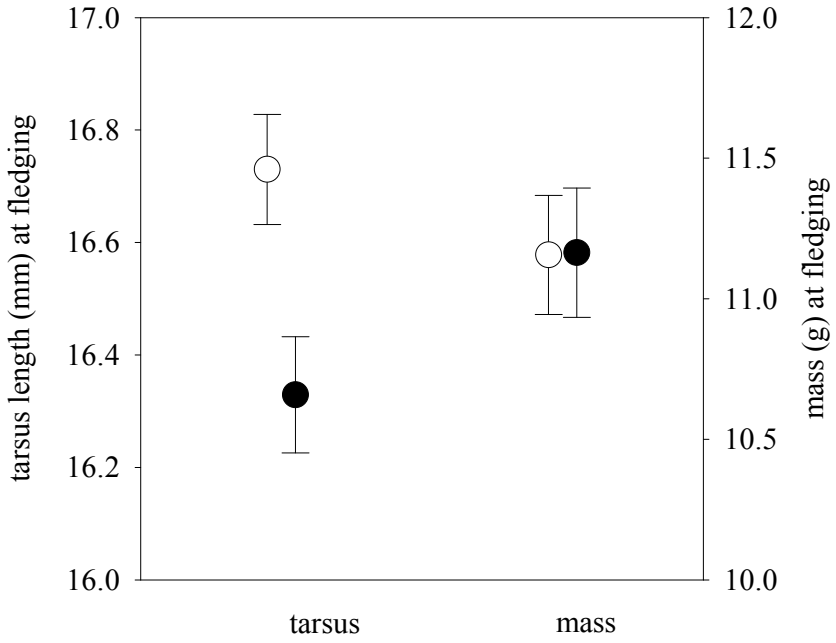

Figure 2: Fledgling tarsus length and mass of chicks with UV-reduced (filled circles, *n* = 11) and control male parents (open circles, *n* = 13). Points shown are the mean (± s.e.m.) estimated from hierarchical mixed models (see table 2).

Table 2. Hierarchical mixed model (type III tests) of tarsus length and mass of male and female fledglings (11 UV-reduced nests, 13 control nests).

	Tarsus length (mm)			Mass (g)		
	F	d.f.	*p*	*F*	d.f.	*p*
Variables included in model:						
Offspring sex	14.97	1,200	0.0001	53.55	1,189	< 0.0001
UV Treatment	6.58	1,17.8	0.0196	0.04	1,22	0.838
Offspring sex x UV Treatment	0.07	1,200	0.793	1.03	1,189	0.311
Variables not included in model (all $p > 0.05$):						
Brood size						
Brood size x UV Treatment						
Brood size x Offspring sex						
Brood size x UV Treatment x Offspring sex						
*Tarsus length						
*Time of weighing						

* 'Tarsus length' and 'Time of weighing' were used only for the analysis of offspring mass.

The experiment shows that differential allocation by females occurred, but not whether the females responded to the reduced colouration per se or to the rapid fluctuations in the UV colouration due to our treatment. Changes in attractiveness might indicate a drop in health status, pointing to a low genetic quality of males (e.g. in disease resistance (Hamilton 1982; Westneat & Birkhead 1998)) or a reduced ability to invest in the brood (Møller & Thornhill 1998).

We did not find evidence for differential sex allocation in maternal feeding rates, as might have been expected from the previous study on blue tit sex ratio (Sheldon et al. 1999). However, our negative result does not allow us to conclude that the offspring fitness benefits related to differential allocation are not limited to male offspring because parents may simply be constrained by an inability to identify offspring sex, for instance due to the relative darkness inside the nest cavity (Hunt et al. 2003).

The differential allocation hypothesis suggests that females adjust parental investment to male attractiveness to trade off their own future fitness with current offspring fitness. Consistent with this, we found that reduced investment of females in the UV-reduced group was accompanied by detrimental effects on nestling growth. Young in the UV-reduced group had shorter tarsi than young in the control group but there was no difference in body mass. The different effects on these two body measures might be due to different trajectories for skeletal growth and the accumulation of fat reserves (Kunz & Ekman 2000). To our knowledge this is the first time that decreased feeding effort in relation to manipulated mate attractiveness in birds has been shown to result in detrimental effects for the offspring. Small body size is known to have adverse effects on offspring survival post fledging and competitive ability later in life (Alatalo & Lundberg 1986; Garnett 1981), so reduced tarsus length is likely to result in reduced offspring fitness. Decreased provisioning might have further costs for the offspring if it hampers the development of sexually attractive traits. Indicator traits, including blue tit UV colouration, are thought to be condition dependent (Keyser & Hill 1999; Rowe & Houle 1996) and good condition at fledgling might be necessary to sufficiently develop attractive UV colouration as adults (Ohlsson et al. 2002). A recent blue tit study showed that fledgling colouration

(structural and carotenoid-based colours) is condition dependent (Johnsen et al. 2003) and a similar effect of early condition on adult colouration might exist.

The shorter tarsus length of offspring in the UV-reduced group compared to the control group seems to be attributable to the lower feeding rates of females of UV-reduced males. Our experiment therefore shows that the benefits the offspring gain by being sired by a male with an indicator trait of high value may not necessarily be due to 'good genes' for viability passed on from fathers to their offspring. Instead, differences in offspring viability between males could thus be driven purely by male genes for attractiveness, mediated by differential investment of their females.

We could show adverse effects on offspring growth but decreased female investment should also lead to benefits in terms of their own future reproduction (Sheldon 2000). We found no difference between the UV-reduced and control group in female mass on day 7, but females do not provision young extensively before that age, and other variables might well have revealed costs to females. Female blue tits with experimentally enlarged broods had reduced survival (Dhondt 2001) most probably because of an increase in feeding effort. We expect the decreased feeding rates of females of UV-reduced males to have the opposite effect.

We have experimentally demonstrated differential allocation in a wild blue tit population and that this affected offspring growth. Our result supports the idea that fitness benefits of attractive males are not only mediated by advantages in mate choice but also by differential maternal effects (Mousseau & Fox 1998) such as the differential allocation we have demonstrated. Attractive individuals might therefore gain a double benefit: first, through mate choice advantages and second because their mates show increased investment in their offspring. UV colouration has been recognised as a ubiquitous signal across avian families (Eaton & Lanyon 2003) and our finding underlines the importance of UV colouration to sexual selection in birds. Our study, in combination with a previous experimental study showing that female blue tits modified sex ratio in relation to male UV colouration (Sheldon et al. 1999), demonstrates that female blue tits may adjust their parental investment in multiple ways to mate attractiveness, and makes blue tit UV colouration one of the best understood signals of sexual attractiveness.

5. Acknowledgments:

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CHAPTER 6

Male blue tits increase parental effort when female UV coloration is reduced

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Abstract:

A number of studies have shown that females adjust parental effort to the attractiveness of their mates, a behaviour known as differential allocation. Fewer studies however, have examined male care in response to the attractiveness of their female mates, although in the large number of species with biparental care, differential allocation should apply equally to males and females. In an earlier descriptive study on blue tits we found a negative correlation between male feeding rates and female UV-chroma, an indicator of attractiveness and viability in this species. This was unexpected since, if anything, a positive correlation between male investment and female UV was predicted. In this study, we experimentally tested whether there is a causal relationship between male investment and female UV. We demonstrated that this relationship is causal because males of females with experimentally reduced UV had higher feeding rates than males with control females. In addition, offspring in nests with UV-reduced females grew larger tarsi thus showing a direct effect of our treatment on expected offspring fitness.

1. Introduction

Differential allocation occurs if individuals adjust their level of parental investment to the relative attractiveness of their current mate (Burley 1986; Sheldon 2000). If offspring sired by attractive mates are of higher quality it pays for a parent to raise its level of investment if mated to an attractive mate and thereby increase offspring fitness at a cost to their own residual fitness. However, if the current mate is unattractive, investment should be decreased to save their own residual fitness at a fitness cost to the current offspring. Differential allocation is hence the result of a trade-off between the residual fitness of parents and the fitness of current offspring, and is involved in the maximization of parental lifetime reproductive success (Sheldon 2000).

In socially monogamous species with biparental care, differential allocation should equally apply to males and females. In such bird species, both parents contribute equally to raising their offspring so one would expect similar responses to the attractiveness of the mate in males and females. However, while a number of bird studies show that females differentially allocate investment in relation to male attractiveness (e.g. Burley 1988; Møller & deLope 1995; Limbourg et al. 2004), only a small proportion of studies on differential allocation have investigated the responses of males to their females' appearance (reviewed in Amundsen 2000). As yet the role of female attractiveness plays in male parental investment decisions in species with biparental care is unclear.

We have studied differential allocation in blue tits (*Cyanistes caeruleus*), which exhibit extensive and roughly equal amounts of parental effort and possess UV-plumage coloration which acts as an indicator of sexual attractiveness (Andersson et al. 1998; Hunt et al. 1999), and male genetic quality (Sheldon et al. 1999). Male blue tits do not only have brighter UV coloration, but male UV is also shifted towards shorter wave lengths than female UV. This sexual dimorphism is most likely due to sexual selection (Hunt et al. 1998; Andersson et al. 1998). However, in mate choice experiments both sexes preferred individuals with their natural UV-coloration over individuals whose UV coloration was removed (Hunt et al. 1999). UV coloration thus seems to be an attractive trait in both sexes and we would expect similar responses in parental investment in relation to mate attractiveness.

Recently we showed a causal relationship between female investment and male UV because females reduced feeding rates when male UV was experimentally reduced. This also led to reduced growth of the young (Limbourg et al. 2004). Thus we not only demonstrated differential allocation by female blue tits, but also an immediate effect of our treatment on offspring condition. This was consistent with the positive correlation between female feeding rates and male UV that we had found in an earlier descriptive study (see Chapter 2, this thesis). In the latter study however, we unexpectedly found a negative correlation between male feeding rates and female UV. It appears that males and females react in an opposite fashion to the UV coloration of their mates. However, the intriguing negative correlation between male investment and female UV still needs experimental confirmation before conclusions about the role of female UV coloration in male investment decisions can be drawn.

The aim of this study is to test for a causal relationship between male feeding rates and female UV in blue tits by reducing female UV and subsequently comparing the feeding rates of their males to those of males of control females. In addition we test for an effect of our treatment on expected offspring fitness by analyzing whether offspring growth parameters differ between the two groups.

2. Methods

To test for a causal relationship between male feeding rates (number of feeding visits / 2 h) and female UV coloration (UV-chroma = $R_{320\text{-}400}/R_{320\text{-}700}$) we used an identical experimental setup to our experimental test of female feeding rates in relation to male UV coloration (see Limbourg et al. 2004, chapter 3). To summarize our methods: the study was conducted in May and June 2003 in the Hoge Veluwe National Park in the Netherlands. We swapped all clutches with similar hatching date and clutch size on day -3 (with day 0 as the expected day of hatching). We caught females on days -2 and 7 and treated their crowns with a mixture of UV-blocking chemicals and duck preen gland fat (UV-reduced group) or duck-preen fat only (control group) (Andersson & Amundsen 1997; Sheldon et al. 1999; Korsten et al. 2006). This resulted in significantly lower UV chroma of females in the UV-reduced

group compared to the control group (mean female UV-chroma in the UV-reduced group: 0.166 ± 0.006 s.e.m.; mean female UV-chroma in the control group: 0.256 ± 0.009 s.e.m.; Anova, $F_{1,33} = 112.49$, $p < 0.0001$). On days 10 and 14 we videotaped feeding rates of males and females towards their nestlings for 2 h. On day 3 we took a blood sample of all offspring for sexing, and measured their tarsus length and mass on day 15.

We analysed parental feeding rates (number of feeds during 2 h) for males and females separately in relation to our treatment in a repeated measures analysis with 'Nest box' as repeated measures subjects using the GENMOD procedure in SAS (SAS Institute Inc. 1996). We assumed a poisson distribution of feeding rates. In addition to 'UV Treatment' we included some additional explanatory variables that were known to significantly influence feeding rates in blue tits (Chapter 1, Chapter 2, Limbourg et al. 2004). These variables were 'Chick Age', 'Brood sex ratio', 'Date of hatching' and 'Mate UV chroma'. We also tested any two-way interaction involving 'UV-treatment'. Non-significant interactions were removed from the model in a backwards fashion, followed by any non-significant main effects.

Offspring tarsus length and mass were analyzed using hierarchical mixed models using the MIXED procedure in SAS and assuming normally distributed errors. We fitted 'UV treatment', Offspring sex', as fixed effects and 'Nest box' as a random effect. Model selection was carried out as described for the analysis of male and female feeding rates, including the tests of all two way interactions with male and female UV chroma.

Statistical tables show all variables that remained in our models after the model selection process, except for 'UV Treatment', and 'Offspring sex' (the latter was used only for the analysis of offspring tarsus length and mass) which were always retained in the models.

3. Results

Males with UV-reduced females had higher feeding rates than males with control females, as shown by the significant UV-Treatment effect on male feeding

rates (Table 1a, Figure 1) while there was no difference in female feeding rates between groups (Table 1b, Figure 1).

Table 1. Repeated measure analysis of the number of male (a) and female (b) feeding visits on 10 (*n* = 29 nests) and 14 days (*n* = 30 nests) post hatching in relation to female UV treatment (15 UV-reduced pairs, 18 control pairs).

	estimate	χ^2	d.f.	*p*
a) Male feeds / 2h:				
UV Treatment	0.173	6.52	1	0.011
Brood size	0.0899	5.92	1	0.015
Brood sex ratio	-0.487	4.32	1	0.038
Hatching date	-0.043	0.28	1	0.594
Hatching date x UV Treatment	0.076	10.97	1	0.0009
b) Female feeds / 2h:				
UV Treatment*	0.045	0.33	1	0.563
Brood size	0.087	8.08	1	0.005
Brood sex ratio	-0.571	5.51	1	0.019
Hatching date	-0.029	4.54	1	0.033
Chick Age	-0.1898	7.65	1	0.006

Note: Estimates are for log-transformed values. The estimate for 'Hatching date' is the slope for the Control group and the estimate for 'Hatching date x UV Treatment' is the slope of the UV-reduced group relative to the control group.

* UV treatment in the analysis of female feeding rates was retained in the model, although the effect is not significant.

For males, in addition, there was a strong interaction between hatching date and UV-treatment, showing that feeding rates of males in the control-group decreased with hatching date while feeding rates in the UV-reduced group increased with hatching date (Figure 2).

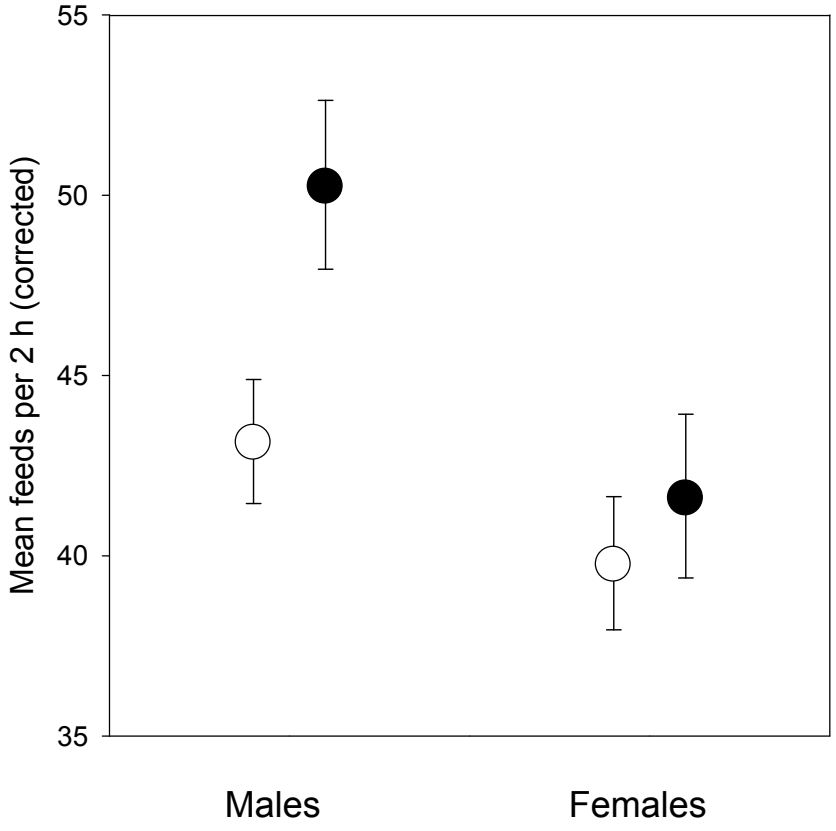

Figure 1: Mean feeds / 2h for male and female blue tits for pairs with UV-reduced females (filled circles) and control females (open circles). The points shown are estimated means (± s.e.m.) for the mean brood size from the repeated measure analysis shown in table 1.

Females feeding rates on the other hand decreased with hatching date and did not show an interaction with UV-treatment but differed between chick ages (Table 1b). As is usually found, brood size was a strong determinant of feeding rates, which increased with increasing brood size for males and females (Table 1). Another variable related to male and female parental effort was the sex ratio of the brood which negatively influenced feeding rates (Table 1).

Chicks of UV-reduced foster-mothers had longer tarsi (Table 2, Figure 2), but the offspring did not differ in mass between treatment groups after controlling for tarsus length (Table 2).

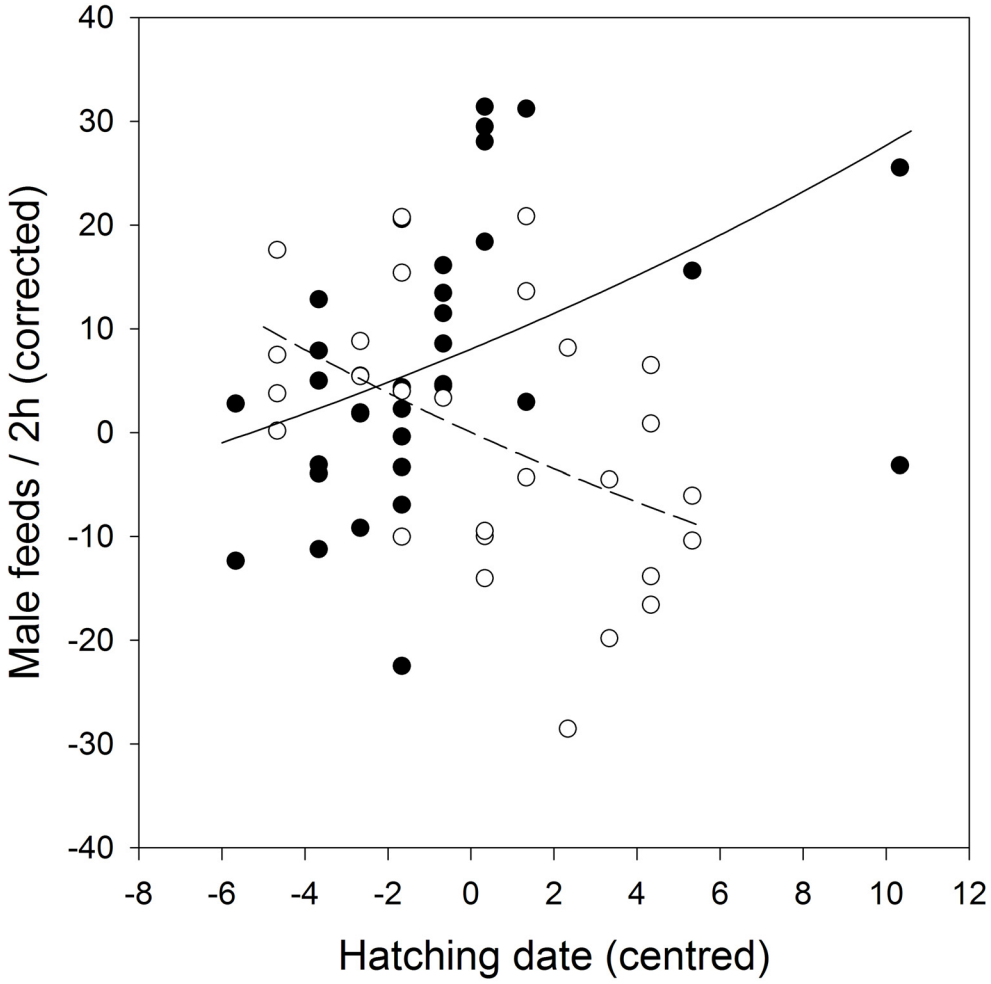

Figure 2: Number of feeds by male blue tits in relation to hatching date in nests with UV-reduced females (filled circles and solid lines) and control females (open circles and dashed lines). Curves are based on repeated measure models (Table 1) and the points plotted are corrected for all terms in the model except UV-treatment, hatching date and the interaction between the two variables. Hatching date is centred around the mean hatching date.

4. Discussion

By manipulating female UV-crown coloration we have shown experimentally that male blue tits increase their feeding rates when female UV is reduced. Females did not change their feeding rates in response to the treatment. Further, the treatment also affected offspring growth since offspring in nests with UV-reduced

mothers had longer tarsi, but did not differ in mass after controlling for this difference.

Table 2. Hierarchical mixed model (type III tests) of tarsus length and mass of male and female fledglings (21 UV-reduced nests, 18 control nests).

	Tarsus length (mm)			Mass (g)		
	F	d.f.	*p*	*F*	d.f.	*p*
Variables included in model:						
Offspring sex	57.82	1, 270	<0.0001	17.1	1, 264	<0.0001
UV Treatment	4.29	1, 36.4	0.046	1.32	1, 35.8	0.256
Tarsus length*				86.46	1, 276	<0.0001

*Tarsus length was used only for the analysis of offspring mass.

Because we manipulated female UV shortly before hatching, hence after pair formation and clutch completion, and additionally swapped whole clutches at random between pairs, we can rule out other confounding factors that might be responsible for the difference in feeding rates between the groups. Such factors might be differences in territory quality, offspring quality or high quality females attracting high quality mates. Our results therefore show unequivocally that the negative correlation between male feeding rates and female UV chroma we found in our descriptive study (see chapter 3) is due to a causal relationship.

The results on feeding rates presented here are largely consistent with the results of our previous experiment to test for female differential allocation in relation to male UV (Limbourg et al. 2004, chapter 5 in this thesis). Feeding rates of the manipulated sex do not differ between groups while feeding rates of their mates differ as expected from the descriptive results (chapter 3). Some findings do differ from the results we obtained in our test of female differential allocation (Limbourg et al. 2004).

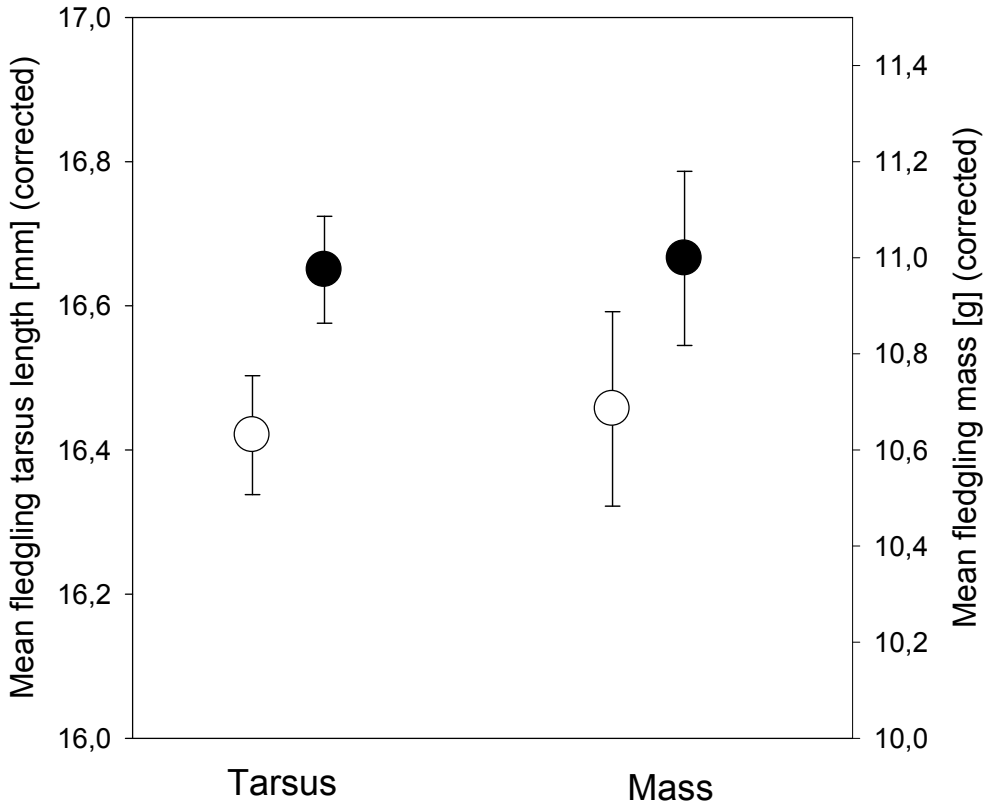

Figure 3: Mean fledgling tarsus length and mass of broods with UV-reduced (filled circles) and control foster mothers (open circles). The points shown are estimated means (± s.e.m.) for the mean brood size from the repeated measure analysis shown in table 2.

The first of these differences is the interaction between hatching date and UV-treatment on male, but not female, feeding rates. This difference between the studies is most likely due to year differences in the food availability in relation to hatching date. In 2002, the year in which we conducted the test of female differential allocation, the weather and probably also food availability, remained good throughout the season. In contrast, 2003, the year in which we conducted the current study, was dominated by heavy rainfalls in the first half of the season and warm weather in the second half of the season. It is likely that these changing weather conditions in 2003 affected either food availability or the ability of the parents to search for or find food.

Second, feeding rates of both parents were correlated to the sex ratio of the brood. Both parents decrease feeding rates with increasing sex ratio. In chapter 2 we found that correlations between feeding rates and sex ratio differed significantly between years and are possibly dependent on food availability. Feeding rates are positively correlated to sex ratio in food-rich years but negatively in years with low food abundance. Since food availability was low in 2003, a negative correlation between investment and sex ratio might have been expected. For further discussion of sex ratio related correlations see chapter 2.

Fledglings in the UV-reduced group have longer tarsi, most likely due to the higher male feeding rates in this group, but offspring in both groups did not differ in mass after controlling for tarsus length. Comparable results were found in both our correlational study on the relationship between parental care and UV-chroma (chapter 3), and our experimental test of female differential allocation (chapter 5), although in the first study tarsus lengths were related to male UV chroma only. The fact that only the tarsus length and not mass seems to be affected by feeding rates in relation to mate UV-chroma is thus a consistent effect and is most likely due to different growth trajectories of these two traits (Kunz & Ekman 2000). In summary, our studies show that offspring phenotype is directly affected by the differential allocation of their parents. It is likely that a larger size at fledging will provide some fitness benefit during adulthood (Garnett 1981; Alatalo & Lundberg 1986).

We showed a causal negative relationship between male investment and female UV. However, the question remains why males, against general expectations, negatively adjust investment to female UV coloration. There are two potential sets of explanations which are also discussed in chapter 3.

First, low UV females are more attractive than bright UV females and males increase investment due to the expectation of high quality offspring. This case would be the clearest form of differential allocation since males trade off their own residual fitness with the fitness of their current offspring. However, this explanation is unlikely because traits are thought to need to be expensive to posses and maintain in order to be attractive. UV coloration is likely to be costly to produce because the intensity of UV is dependent on the nanostructural arrangements of feather barbs (Shawkey et al. 2003).

Second, bright UV females are attractive and increase offspring fitness in such a way that males are selected to decrease, rather than increase, care for offspring of such females (see chapter 3). Although this does not seem to be differential allocation at first glance, it still represents an effect of the mate's phenotype or genotype on the relationship between an individual's parental care and offspring fitness as required by the differential allocation hypothesis (Sheldon 2000). However, we only tested for a causal relationship between male investment and female UV-coloration and are unable to distinguish between these two sets of explanations.

Our study adds to the small number of reports on male investment decisions in relation to the appearance of their females. The fact that males negatively adjust feeding rates to female UV in blue tits shows that males react differently to attractive females than current theory suggests. Further research is required to clarify whether female attractiveness plays a comparable role to male attractiveness in species with biparental care.

5. Acknowledgments

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CHAPTER 7

Summarizing discussion

Tobias Limbourg

Summarizing discussion

A. The aim of this thesis

Parental investment is a major part of life history and understanding which environmental factors influence parental investment decisions is crucial in determining the lifetime fitness of individuals (Andersson 1994). This thesis investigates parental investment in relation to some common aspects of each sexually reproducing individual – these aspects are the sex of offspring, the parent's own attractiveness and the attractiveness of its mate. As part of the NWO programme 'Avian sex allocation', the goal of my thesis was primarily to examine the relationship between parental investment and offspring sex. Other parts of the programme focussed on the physiological mechanism to bias the primary sex ratio in birds (Von Engelhardt, Groningen, The Netherlands, thesis 2004), on modelling adaptive sex ratio adjustment in vertebrates (T. Fawcett and I. Pen, project, University of Groningen, see Fawcett et al. 2007) and - most closely related to my thesis – primary sex ratio adjustment in blue tits (Korsten, Groningen, The Netherlands, thesis 2006)

Reproduction is costly. In many species, parents have to spend resources that benefit the growth and survival of their offspring. These resources will not be available to the parents themselves or other offspring. As a result, parental effort incurs cost for parents. These cost are called parental investment. Trivers (1972) defined parental investment as any action that raises offspring fitness at the cost of the parent's ability to invest in other offspring. Clutton-Brock (1991) further included costs to any other component of parental fitness. By definition, parental investment thus implies a trade off between current and future reproduction, because any investment in current offspring reduces residual fitness of the parent (Trivers 1972; Clutton-Brock 1991). This means that if the value of the current reproductive attempt varies, parents should make higher investment when the current attempt is worth more, and invest more in individual offspring that are of higher value (Trivers 1972).

Sex allocation theory extends this line of argument to include differences in the reproductive value of offspring of the two sexes and deals with the allocation of

parental resources to male and female offspring (Charnov 1982). Generally, parents should vary investment in sons and daughters if the costs and benefits of parental care differ between the offspring sexes (Maynard Smith 1980). This is because, if the sexes differ in any way that is related to fitness, the benefits of producing or investing in each sex will be different for parents. Theoretically, sex allocation can act at two different stages of reproduction. The first stage is the primary sex ratio produced at fertilisation (Fisher 1930). The second stage is the amount of parental investment each offspring sex receives post fertilisation (Maynard Smith 1980). If the sex ratio is under parental control parents may vary their total investment in each offspring sex by producing different numbers of sons and daughters (Fisher 1930; Trivers & Willard 1973; Fawcett et al. 2007). However, if the sex ratio is not under parental control, parents can still vary total investment in each offspring sex by showing sex-biased care, i.e. providing different amounts of post-natal parental care to sons and daughters (Maynard Smith 1980; Lessells 1998; Lessells 2002). A large number of studies have focused on sex allocation in the form of adaptive sex ratio adjustment (Alonso-Alvarez 2006; Fawcett et al. 2007), while post natal sex-biased parental care has been largely ignored (but see Magrath et al. 2007). The aim of this thesis was therefore to add to the few studies in sex-biased parental care and to fill some gaps in knowledge in this field.

Sex-biased care might occur in two different ways. First, offspring of each sex might receive different amounts of care. This will inevitably lead to a correlation between parental care and sex ratio because, as the number of the preferred offspring sex changes the amount of care changes. However, correlations between sex ratio and care are not always due to one sex receiving more care. For instance, if one of the offspring sexes begs harder than the other, parents might be stimulated to increase feeding rates for the whole brood without actually discriminating between the offspring sexes (Stamps et al. 1987). Parental investment would then be related to the sex ratio of the brood, but not differ between the sexes of offspring within a brood.

Another hypothesis which is strongly related to parental investment is differential allocation. Differential allocation (Burley 1986) refers to individual decisions to vary parental investment in the current breeding attempt in relation to

the quality or attractiveness of the mate. The idea behind differential allocation is that life history decisions are associated with the reproductive value of offspring produced in the current reproductive attempt. The attractiveness of the mate, which is expected to influence the reproductive value of offspring, therefore affects the optimal trade-off between current and future reproduction (Sheldon 2000). Fitness pay-offs of offspring sired by high quality mates should be higher than average because such mates might provide environments that are particularly suitable for raising offspring or transmit genes that will increase the fitness of offspring. If mated to an attractive mate, it will pay to invest more in the brood because the current fitness returns are higher than the expected future fitness returns, i.e., from offspring of future mates that are probably of lower quality. However, if mated to a less attractive mate, in contrast, it should pay to invest less in the brood. Instead, the parent should save resources for future survival and reproduction (residual reproductive value) to have a higher reproductive output in the succeeding breeding attempt with a potentially more attractive mate. In contrast to sex allocation, differential allocation does not necessarily involve higher investment in one particular offspring sex but the selection pressures that lead to both behaviours are in principle the same.

Potentially, differential allocation and sex-biased investment may occur at the same time. If the value of each offspring sex varies differently with parental attractiveness, one would expect investment in each sex to be adjusted differently to parental attractiveness. For example, if sons benefit more than daughters by the attractiveness of their fathers, mothers should increase investment in sons relative to daughters when mated to an attractive male. Throughout this thesis I have termed this complex form of sex allocation 'differential sex allocation', simply to describe the special case when parental attractiveness influences sex-biased investment.

Recent years have brought new insights into sexual selection in birds, in particular as a result of increased interest in UV vision and UV plumage reflectance. As is the case for most vertebrates, birds are capable of perceiving wavelengths in the UV spectrum (200-400 nm) because they possess four types of visual pigments (tetrachromacy) – a long wavelength-sensitive, a medium wavelength-sensitive, a short wavelength-sensitive, and an extreme short wavelength-sensitive "UV" or

"violet" pigment (Hart et al. 2000). This sensitivity to UV light seems to be present in all major animal groups (Tovee 1995). Although there is also a short wavelength sensitive pigment in the human retina, we are not capable of perceiving UV light, largely because the lens of human eyes absorbs wavelengths of the UV spectrum. The ocular media in birds have relatively high transmission of short wave lengths and thus permit wavelengths of the UV spectrum to pass (Vorobyev et al. 1998). The perception of colours by birds is therefore very different from the human perception of colours.

Besides the ability to perceive in the UV spectrum a large number of bird species possesses plumage that reflects UV light (Cuthill et al. 2000), which might act as a special waveband for signalling in sexual selection (Hunt et al. 2001b). In general, two primary mechanisms produce brightly coloured plumage – pigments deposited in growing feathers and reflective or disruptive physical structures in feather barbs (Cuthill et al. 2000). The latter are responsible for short wavelength reflection and produce UV-colours, blue, purple, green and iridescence by scattering incident light in the reflective keratin of the feathers that causes reflections in the shortest wavelengths (Keyser & Hill 1999). Because UV spectrometers have become affordable in recent years, more and more researchers were able to measure UV reflectance in birds, including myself. Commonly, three different components of UV coloration are measured: "brightness" (total spectral reflectance between 320-700 nm), "hue" (wavelength of peak reflectance, $\lambda(R_{max})$), and "chroma" (= purity of a given colour, calculated as reflectance ratio $R_{320\text{-}400}/R_{320\text{-}700}$). Only the latter is used in this thesis and birds with high UV chroma are called as being of "bright UV" or high "UV brightness" throughout my thesis.

Blue tits were chosen as the species to study sex-biased parental care. They seem well suited for this task because as in many species, male offspring are likely to have higher variation in mating success than daughters. In blue tits this is due to the widespread occurrence of extra pair copulation (Kempenaers et al. 1992; Kempenaers et al. 1997) and only the fittest sons are likely to outcompete other males over access to mates (Clutton-Brock et al. 1982). Further, there are reports of sex ratio skews in relation to male overwinter survival (Svensson & Nilsson 1996) and UV coloration (Sheldon et al. 1999; Korsten et al. 2006; Delhey et al. 2007). These

results encouraged the idea that blue tits might adjust their sex ratio in response to variables that could indicate higher fitness benefits for male offspring.

In addition, blue tits also offer the potential for differential allocation. The years preceding the start of this research brought some interesting results and insights into sexual selection in blue tits. In particular, a group of studies showed that parts of the plumage of blue tits display UV coloration (Andersson et al. 1998; Sheldon et al. 1999; Hunt et al. 1998; Hunt et al. 1999), and that UV acts as an indicator of overwinter survival (Svensson & Nilsson 1996) and attractiveness (Sheldon 1999; Hunt 1998 and 1999). UV coloration thus seems to indicate individual quality and potentially affects the value of offspring if this is passed on to the offspring. Moreover, experimentally manipulated UV-coloration of blue tit males caused their females to adjust the brood sex ratio (Sheldon et al. 1999), a result that was experimentally repeated by Korsten et al. (2005) and Delhey et al. (2007). These results led to a number of predictions.

The first prediction is that investment in blue tit offspring should be dependent on UV coloration, as described by the differential allocation hypothesis (Burley 1986). Blue tits are especially suited to the study of differential allocation. This is because UV is a known indicator or attractiveness and varies strongly within blue tit populations (Andersson et al. 1998), the latter being necessary for differential allocation to occur. It should pay individuals to optimise their fitness pay-offs by adjusting their reproductive output to the variable attractiveness (UV) of their mates.

The second implication of Sheldon et al.'s study (1999) on sex ratio adjustment in relation to manipulated blue tit UV chroma is that investment in each offspring sex might be affected differentially by the UV colour of their parents. Because variation in paternal UV affected the numbers of each offspring sex produced, it seems likely that this also applies to parental investment post fertilisation. Sex-biased parental care might thus be affected by mate attractiveness and "differential sex allocation" might occur.

The aim of this thesis was to investigate sex-biased investment and differential allocation (including differential sex allocation) in blue tits. To this end, I video recorded feeding rates of male and female blue tits and sexed all offspring. Therefore I was able to determine how much each sex of parent fed each individual

son and daughter, and also how much each sex of parent invested in the brood in relation to the sex ratio. In addition, I measured and manipulated the UV-coloration of males and females to determine whether feeding rates were indeed affected by the attractiveness of the mate (differential allocation). With this data I was also able to determine whether any sex allocation was dependent on parental attractiveness (differential sex allocation). Lastly I measured the growth of the offspring, to estimate the fitness effects of parental investment on the chicks. In the following I summarize my findings.

B. Parental investment in relation to offspring sex

Sex-biased parental care is uncommon in blue tits

I obtained mixed results related to sex-biased provisioning in blue tits. Generally, parents are selected to vary investment in sons and daughters if the costs and benefits of parental care differ between the offspring sexes (Maynard Smith 1980). Blue tits are candidates for sex-biased parental care because the mating success of sons should be more variable than that of daughters, for instance due to extra pair copulations (Kempenaers et al. 1997). Also, there are reports of sex ratio adjustment in relation to parental traits (Svensson & Nilsson 1996; Sheldon et al. 1999; Korsten et al. 2006; Delhey et al. 2007). It was thus reasonable to expect differences in the provisioning of male and female offspring in blue tits.

Chapter 2 shows that there is no correlation between feeding rates and sex ratio over the four years of data that are covered in this thesis. However, the relationship between male feeding rates and sex ratio differs significantly between years. Males show a positive correlation between feeding rates and sex ratio in 2001 (see also Chapter 3), but not in 2000, 2002 and 2003. The relationship between male feeding rates and sex ratio is also significantly different from that of females either in 2001, as well as in all 4 years taken together. In contrast, even in 2001, when male feeding rates are related to sex ratio, there is no difference in the amount of care received by each sex of offspring, although there is a sex ratio * offspring sex

interaction on feeding rates of males and females. This interaction results in relative higher allocation to sons when the sex ratio increasesd (see chapter 2, fig. 3).

There are a number of conclusions from these results. I never found higher allocation to one offspring sex, either over all 4 years taken together, or in any individual year. This might occur either because blue tits are not selected to invest differently in each offspring sex or, alternatively, they might simply be unable to do so, perhaps because they cannot distinguish the sexes. There is some sexual dichromatism in UV coloration of blue tit chicks (Hunt et al. 2003), but it is questionable whether there is sufficient light in nest cavities for parents to distinguish the offspring sexes based on UV coloration.

The fact that neither offspring sex ever received more food than the other also shows that higher feeding rates to male biased broods are not because sons receive more care. The correlation with brood sex ratio might occur because sons beg more vigorously than daughters (Kilner & Johnstone 1997; Kilner 2002) and the increasing number of sons causes the overall begging intensity of the brood to increase. Males could simply react to the begging intensity by feeding the whole brood more without distinguishing the sexes. The fact that both parents feed relatively more per individual son than daughter as the proportion of sons in the brood increases (as shown by the sex ratio * offspring sex interaction on feeding rates) might support the idea that sons are more competitive than daughters.

Another result is that correlations between feeding rates and sex ratio are not a general pattern in blue tits. Instead it seems that these correlations occur in certain situations only. One explanation that I can offer, based on a non-significant correlation between male investment in relation to brood sex ratio and the caterpillar peak biomass over 4 years (measured by Visser et al. 2006), is that the correlation between male investment and brood sex ratio only occurs in food rich years. Although this test was performed a posteriori and is based on only 4 data points (years), it seems reasonable that parents are only able to provide extra care for broods with higher needs, as might be the case for male biased broods, when resources are abundant.

When males adjusted their feeding rates to the sex ratio in one of the four years, they were behaving significantly differently than females. This kind of difference

between the sexes of parents is predicted when the fitness returns differ between sons and daughters and the costs for parental investment differ between parents (Lessells 1998). However, another explanation could be that each parent fulfills a different role in raising the offspring. Females spend considerable amounts of time brooding the chicks and cleaning the nest cavities (which frequently occurred during the video observations). Such different parental roles might mean that males react to the brood's food requirements while females increase their care in other ways.

No differential sex allocation in blue tits

In contrast to the mixed results on sex biased parental care, the results on differential sex allocation are relatively straightforward. Blue tits were candidates for differential sex allocation (in terms of parental care) because of Sheldon's results on sex ratio adjustments in response to manipulated UV (1999) which lead to the conclusion that the value of male offspring is dependent on male UV coloration and that investment in the sexes is adjusted accordingly. My data allow me to rule out any influence of mate (or own) UV coloration on the relationship between blue tit feeding rates and sex ratio or offspring sex: I never found an interaction between either unmanipulated (chapter 3) or manipulated UV coloration (chapter 5 & 6) and sex ratio or offspring sex on feeding rates. Blue tits seem to adjust their sex ratio to UV-coloration, but this is not true for their feeding investment.

C. Parental care in relation to mate UV brightness

Feeding rates are correlated to mate UV brightness

The differential allocation hypothesis proposes that individuals should adjust parental investment to the sexual attractiveness of their mates, if this attractiveness signals some indirect or direct benefit for offspring (Burley 1986; Sheldon 2000). UV plumage coloration in blue tits acts as such an indicator of quality and /or attractiveness (Svensson & Nilsson 1996; Sheldon et al. 1999). Individuals of each sex also choose mates in relation to UV brightness (Hunt et al. 1999), which varies strongly in blue tits (Andersson et al. 1998) and individuals are therefore expected to

maximize their fitness pay-offs by adjusting their reproductive output in relation to the UV reflectance of their current mate (Burley 1986; Sheldon 2000).

I have found strong evidence for differential allocation in blue tits. Chapter 3 shows that correlations between feeding rates and UV-coloration appear in the wild for both males and females. Female feeding rates were positively correlated to male UV chroma, while male feeding rates were negatively related to female UV chroma. Feeding rates were never correlated to an individual's own UV chroma. However, these correlations found in the wild needed experimental testing to show that they are due to a causal relationship between investment and mate UV coloration. Chapters 5 and 6 provide such experimental tests, and demonstrate that the correlations found are not due to confounding factors.

One important finding is that feeding rates were not correlated in either sex to the individual's own UV coloration. Potentially, this might advertise the amount of parental care that an individual is willing to invest in the brood. According to the "good parent process" (Hoelzer 1989; Heywood 1989) attractive individuals are preferred in mate choice, because their attractiveness advertises some phenotypic quality, such as good parenting ability, which makes them especially suited to raising offspring. However, because feeding rates are not correlated to an individual's own UV this seems not to be the case. Instead, UV in blue tit seems to signals some quality to which it is beneficial for mates to adjust their investment.

Female differential allocation

The results for female differential allocation, i.e. female investment in relation to male UV, are straightforward. Females positively adjust feeding rates to male UV chroma, i.e. the more attractive a male blue tit the more its female invests in the young. This is shown in the correlational data (chapter 3) but also when male UV was experimentally reduced, which lead to decreased female feeding rates (chapter 5). This experimental test was crucial in demonstrating that this behaviour is indeed differential allocation. Correlations between UV and feeding rates could be caused by a potentially large number of confounding variables, which needed to be eliminated to show a causal relationship between mate attractiveness and investment. For instance, bright UV individuals might attract high quality mates that feed more per

se, or, bright UV males possess high quality territories that enable their females to show high feeding rates, due to the high food abundance (Sheldon 2000). I avoided such problems because the randomized manipulation of UV took place long after territories were established and pair bonds were formed. In addition, whole clutches were swapped between broods of similar clutch sizes before hatching (only in the experimental studies, chapters 5 & 6), to further account for other confounding variables like maternal effects that could have distorted the interpretation of the results. By removing all these confounding variables it is safe to conclude that the difference in feeding rates between the experimental groups was caused by the manipulation of UV.

To experimentally reduce blue tit UV brightness we applied a mixture of duck preen gland fat and UV blocking chemicals to the crown feathers of the birds. This method was first used before to test UV colour vision and ornamentation in bluethroats (Andersson & Amundsen 1997) and the same method was used by Sheldon and colleagues (1999) to test sex ratio adjustment in relation to male UV coloration in blue tits. However, although the method had been widely used, there had not been a systematic study of the effect of the treatment on UV coloration. Chapter 4 fills that gap and shows that the method is effective in reducing UV brightness. The UV chroma of the control birds, which were treated with preen gland fat only, was unchanged, whereas the treatment including sun-blocking chemicals reduced the UV chroma below the natural range in wild blue tit populations. However, due to a rapid decrease of the blocking effect, the UV of manipulated birds was back within the natural range within 2 days, but remained lower than the UV of control birds for 7-14 days post treatment.

Common criticisms on the experimental design were that a) our manipulation caused a (too) rapid change in the UV brightness of the manipulated birds, and b) that our treatment decreased the UV below the natural range of the population. Potentially, a sudden change in UV brightness, as was induced by our experimental treatment, might be perceived as a decrease in the health status of the manipulated males. The decrease in feeding rates of females could then also be interpreted as a reaction to the supposed change in health status rather than to the reduction of UV colour per se. However, chapter 4 shows that, although our UV-reduction method

reduces the UV brightness substantially, the treatment wears off over a relatively short time span. The strong decrease in UV brightness lasts only for two days and UV brightness is well back within the natural range by the time the videos were taken to score the feeding rates (10 and 14 days post hatching). Even more important, the fact that males actually increase feeding rates when their female mates are UV-reduced (chapter 6, see below), makes this interpretation of the data less reasonable.

Another problem for the interpretation of the results would have occurred if the feeding rates of the manipulated sex had differed between groups. This happened in an earlier experiment when some male swallows received artificially elongated tail feathers to make them more attractive while the tail feathers of others were shortened (Møller & deLope 1995). Females of males with elongated tail feathers had increased feeding rates which pointed to differential allocation, while females of shortened males had lower feeding rates. However, because males differed in feeding rates between groups it was also possible that males with increased tail feathers were hampered in their ability to hunt for food. The increase of female feeding rates could have been merely because they were forced to compensate for the decreased feeding rates of their mates, as argued by Witte (1995). It was thus questionable whether the results could be interpreted as differential allocation. However, feeding rates did not differ between the control and treatment birds in either of the experiments that I conducted. The advantage of manipulation of color (as opposed to tail length), as in my experiments, is that it does not hamper the manipulated birds in caring for their young and therefore does not cause this problem in the interpretation of the data.

With these potential problems out of the way, there is sufficient evidence for a causal relationship between investment and mate attractiveness, i.e. differential allocation, in blue tits. Similar results were found in another experiment (Johnsen et al. 2005). This latter study showed an interaction between female age and male UV treatment on feeding rates, which did not occur in my study (see chapter 3). Nevertheless, Johnsen and colleagues (2005) came to the same conclusion as discussed here. Females adjust their parental care to male UV and thus sacrifice an extra proportion of their residual fitness to invest in offspring of attractive males. This result underlines that fitness effects of sexual selection go beyond mate choice. First,

attractive individuals are preferred in mate choice, and second, their mates are also willing to invest more in the brood, which results in fitter offspring (as discussed later).

However, my results do not allow me to determine the beneficial (or detrimental) effects of male (or female) UV on offspring fitness. Young of attractive males may be more attractive themselves as adults and thus accrue advantages in mate choice or other contexts. Alternatively, they might survive better. For instance, it has been shown that UV brightness is correlated with survival prospects (Sheldon et al. 1999) and increased heterozygosity (Foerster et al. 2003), so that bright UV birds should have higher fitness than birds with lower UV brightness. However, although it is likely that this is also true for their offspring, two studies found contradicting results on the heritability of UV-plumage coloration in blue tits. Hadfield et al (2006) showed that there is no significant heritability for UV-plumage coloration, while Korsten and colleagues (Groningen, The Netherlands, thesis 2006) found the contrary. This might be due to the different populations of blue tits that were studied. A number of discrepancies in the methods between the two studies make it difficult to determine the true source of the difference in the results.

Male differential allocation

The most surprising result of this thesis is that males invest *less* in the brood when mated to bright UV females. Chapter 3 shows a negative correlation between male feeding rates and female UV. Chapter 6 confirms this result by showing experimentally that males increase care when the UV of their females is reduced. This experiment was conducted in the same way as the test of female differential allocation (Chapter 5) and provides evidence for a causal negative relationship between male investment and female UV. The direction of the relationship is opposite to that of female differential allocation and to what was expected. Males and females therefore behave significantly differently. Also, the slopes of the relationships between feeding rates and mate's UV chroma differ significantly when both sexes are analyzed (chapter 3).

The question arises whether a negative correlation between parental care and UV in blue tits is still differential allocation. The differential allocation hypothesis

makes assumptions about how the relationship between mate phenotype on offspring fitness affects individual investment decisions, but does not state the direction of the effect (Burley, Sheldon). In that sense, male blue tits clearly show differential allocation when they adjust their feeding rate to female UV, as shown in chapters 3 and 6. However, it seems to make less sense when males negatively adjust parental care to female UV. If males increase parental care for low UV females they sacrifice a higher proportion of their residual fitness to offspring born to these females, compared to offspring of brighter UV females. Low UV females, on the other hand, seem to gain some benefit through the increased parental investment of their mates. The attractive trait, to which investment is adjusted to, must be honest and costly for such behaviour to evolve; otherwise all individuals would express the trait in its maximum level (Andersson 1994). UV plumage reflectance seems to be such a costly and reliable trait. The UV brightness is produced by nanostructures in the barbs of feathers and the repeat frequency of the nanostructures correlates positively with UV brightness (Shawkey et al. 2003). There is no reason to assume that the physiological processes that build the barb nanostructures during feather development should differ between the sexes. I therefore have to assume that it is equally costly for females to display high amounts of UV coloration as it is for males. This implies however, that, for each sex, differential allocation in response to the same kind of signal of their mates is expected to be in the same direction (in this case, based on the likely cost of producing UV coloration, positive).

Why do males increase care for offspring of, as it seems, unattractive females with low UV coloration? One explanation is that, while UV coloration is attractive in males, the opposite is true for females. This seems unlikely, because, as discussed above, UV coloration seems to be a typically costly trait and being of bright UV coloration should therefore be perceived as attractive. Also, laboratory mate choice experiments imply that both sexes prefer mates with high UV reflectance (Hunt et al. 1999). However, if both sexes prefer bright UV mates than we should expect to find assortative mating in respect to UV chroma in wild blue tits. Although there is one report that blue tits mate assortatively (Andersson et al. 1998), I (see chapter 3) and others (B. Kempenaers (Vienna, Austria), pers comm; P. Korsten (Groningen, The Netherlands, thesis 2006); S. C. Griffith (Oxford, Great Britain), pers comm) have not

found a correlation between male and female UV chroma within breeding pairs. I therefore assume that assortative mating does not generally occur. Even more confusingly, if both sexes prefer mates with high UV coloration, it is difficult to explain how the strong sexual UV dichromatism in blue tits (Andersson et al. 1998) could have evolved. Dimorphism usually evolves when a trait is preferred in only one sex. If both sexes prefer the same trait then the sexes should not differ in this trait as strongly as in blue tits. In Hunt's (1999) test of preference for UV coloration, the appearance of potential mates was altered by placing a UV blocking filter between the birds being tested. It is possible that the results merely demonstrate a preference for the existence (as opposed to total absence) of UV coloration in conspecifics and does not properly test for a sexual preference that matters in mate choice (Hunt et al. 2001a); so that Hunt's study does not provide unequivocal evidence that UV is attractive in both sexes.

Generally, the literature on sexual selection is focused on male traits. For blue tits, a recent study by Doutrelant et al. (2008) measured coloration of female blue tits and correlated it to fitness traits. They found that yellow carotenoid-based coloration is linked to clutch size and recruitment success. UV coloration, on the other hand, was linked to overwinter survival, as was found in blue tit males (Sheldon et al. 1999), but not to variables related to reproductive success. Another study, on the other hand, indicated that bright UV female blue tits produce larger eggs (Szigeti et al. 2007). These findings provide some evidence that female UV coloration is positively correlated to female quality and reproductive sucess, but does not answer the question why males increase care for low UV females.

Another set of explanations involves the idea that high UV is indeed attractive in females and that blue tit males work harder for *unattractive* females. Attractive females might increase the fitness of their offspring in such a way that male investment has less of an effect on offspring fitness so that males are selected to decrease, not increase, their investment. Bright UV females could, for instance, produce better quality eggs or provide other benefits for offspring, such as better care in terms of incubation or removing parasites from the nest. Szigeti and colleagues (2007), for instance found an indication that bright females produce larger eggs. Potentially eggs of higher quality by bright females could render high male

investment less effective and males thus decrease investment, but this is mere speculation. In the end, my study shows that bright UV females do not provide more care in terms of feeding investment (chapter 3) and this is consistent with Doutrelant's (2008) study, in which female UV was not linked to clutch size, fledging success or recruitment.

Even if males increase care for unattractive mates, this behaviour still represents differential allocation. It is the effect of mate phenotype or genotype on the relationship between offspring fitness and individual investment decisions that matters. When not being attractive leads to higher investment by the mate, individuals would gain by falsely signaling low attractiveness. However, there might be compensating advantages of high attractiveness at other times, for instance that attractive females obtain better quality mates.

Whatever explanation seems more likely, I cannot provide evidence to support one or the other theory to explain why males increase care for low UV females. Hopefully, future research will focus more on the role of female ornamentation in sexual selection, in blue tits and in birds in general.

The offspring benefit by differential allocation of their parents

To examine how the feeding investment of the parents affects offspring phenotype (and fitness), I measured fledgling tarsus length and body mass on day 15 post hatching throughout the study. In all years in which I measured feeding rates in relation to UV coloration, I found that tarsus growth of fledglings was related to the differential allocation of their parents.

Chapter 3 shows a positive correlation between fledgling tarsus length and male UV chroma, but not with female UV chroma. A relationship with both, male and female UV brightness was expected because both males and females adjust their feeding rates to their mate's UV coloration. However, since there is no significant difference between the relationships between fledgling tarsus length and either female or male UV (chapter 3), I cannot conclude whether differential allocation in response to female UV has a different effect on offspring tarsus growth than that in response to male UV.

In chapter 5, when male UV was manipulated, young in the group with UV-reduced males had shorter tarsi, a result consistent with the correlation found in chapter 3. In chapter 6, on the other hand, fledglings in the UV-reduced group had larger tarsi. Hence in both chapters 5 and 6, fledglings in the experimental groups with higher feeding rates showed increased tarsus growth. To exclude parental genetic effects on the tarsus length of the young, I swapped whole clutches randomly between breeding pairs in the two experimental studies (Chapter 5 & 6). It thus seems that the differences in feeding rates, which are caused by the UV-blocking treatment (or related to natural variation in UV coloration (chapter 3)), are responsible for the difference in fledgling tarsus length. Other environmental variables strongly affected tarsus length. For instance, I found a significant interaction between hatching date and male UV chroma which implies that male UV chroma has a stronger positive effect on offspring tarsus length for fledglings that hatch late in the breeding season (chapter 3). Nevertheless, it is noteworthy that the UV of the parent indirectly affects offspring growth. This is an important finding, because attractiveness seems to offer benefits beyond being preferred in mate choice as adults and the results described here are a good example that sexually-selected signals can be intimately related to parental investment. It demonstrates the strength of differential allocation as a driving force for sexual selection.

Originally, I expected to find a correlation between offspring mass and parental UV (in chapter 3), and differences in offspring mass between the treatment groups in the two UV-experiments (chapter 5 & 6). However, fledgling mass was strongly influenced by environmental variables such as hatching date and brood size, but was not related to the UV chroma of either parent (chapters 5 and 6). The fact that only tarsus length, and not mass, is affected by feeding rates in relation to mate UV-chroma is a consistent effect and might be attributed to different growth trajectories of these two traits (Kunz & Ekman 2000). However, there is positive selection on both tarsus and mass (Charmantier et al. 2004), implying that offspring of high UV males have higher fitness, since it is likely that a larger size at fledging will provide some fitness benefit during adulthood (Garnett 1981; Alatalo & Lundberg 1986).

D. Conclusions

In this thesis I found that blue tits show a year-dependent pattern of sex-biased investment. Investment did not generally differ between the sexes of offspring and differential sex allocation did not occur. I then focused on differential allocation, and demonstrated, using a field experiment, that there is indeed a causal relationship between investment and mate attractiveness. Differential allocation affected the growth of offspring, and thereby, probably also their fitness expectations. The difference in the response of males and females to experimental manipulation of their mate's UV coloration raised some important new questions, in particular why males should increase their care when mated to low UV females. This question remains to be answered by future research.

Appendix

Samenvatting

Je voortplanten is verre van gemakkelijk! In veel soorten smeken nakomelingen hun ouders continu om het eten en de aandacht die ze nodig hebben voor hun groei, hun ontwikkeling en simpelweg om te kunnen overleven. Het is dan ook niet verwonderlijk dat de meeste ouders het grootste deel van hun tijd kwijt zijn aan het voeren, grootbrengen en beschermen van hun jongen. De tijd en energie die ze in hun jongen investeren, gaat echter ten kosten van de tijd die ouders aan zich zelf kunnen besteden, en daarmee indirect aan hun toekomstige nakomelingen. Tot deze zogenaamde *ouderlijke investering* kunnen we in principe al het ouderlijk gedrag rekenen dat het succes van hun huidige jongen verhoogt, maar dat ten kosten gaat van de nakomelingen die ze in de toekomst nog zullen krijgen (Trivers 1972). Dit betekent dus dat ouders voor een dilemma staan, waarbij ze moeten beslissen hoeveel ze investeren in hun huidige jongen, en hoeveel energie ze bewaren voor later (Clutton-Brock 1991).

Wat de optimale verdeling van zorg is, en dus wat de verdeling is waarbij een ouder zijn of haar reproductieve succes maximaliseert, hangt af van het verwachte reproductieve succes, of de waarde, van zijn of haar nakomelingen. In andere woorden, alleen jongen die overleven en zelf veel nakomelingen produceren, en daarmee dus jou genen verspreiden in de populatie, zijn het waard om veel moeite in te investeren. Het zijn de ouders die de kosten en baten die verbonden zijn aan de voortplanting het best tegen elkaar af weten te wegen, die gedurende hun leven de meeste nakomelingen groot zullen brengen, en daarmee hun genen het snelst in de populatie weten te verspreiden.

Vanuit het oogpunt van de ouders zijn niet alle nakomelingen even veel waard. Zo zullen sommige jongen wanneer ze volwassen zijn bijvoorbeeld sterker, gezonder of aantrekkelijker zijn dan andere. Het zijn deze jongen die er in zullen slagen om zelf meer nakomelingen te produceren, en daarmee dus meer kleinkinderen voor hun ouders. We verwachten dus dat ouders de beslissing hoeveel ze investeren in een bepaald jong of een bepaald broedsel af laten hangen van hun waarde, of hun verwachte succes, waarbij ze meer investeren in jongen die meer waard zijn, en minder in de jongen die

waarschijnlijk minder succesvol zullen worden (Trivers 1972). Een van de dingen die ik in dit proefschrift test, is of dit inderdaad het geval is.

Een van de factoren die het verwachte reproductief succes, en daarmee de waarde, van een nakomeling bepaalt is bijvoorbeeld zijn of haar geslacht. Het is de vraag naar wat de optimale verdeling van zorg over zonen en dochters is, waar *seksealloca*tie *theorie* zich mee bezig houdt (Charnov 1982). Deze theorie voorspelt dat ouders hun investering in zonen en dochters variëren als zonen en dochters verschillen in hun (toekomstige) reproductieve succes, en daarmee de relatieve voordelen van het investeren in zonen en dochters verschillen voor de ouders (Maynard Smith 1980; Charnov 1982; Lessells 1998, 2002). Ouders kunnen hoeveel ze investeren in zonen en dochters variëren door de verhouding van het aantal zonen en dochters (de sekse ratio), of de relatieve hoeveelheid zorg die zonen en dochters ontvangen te variëren. Het is deze laatste strategie, die van *geslachts-specifieke ouderzorg*, die een van de onderwerpen vormt van dit proefschrift.

In een soort die zich geslachtelijk voortplant en waar individuen kieskeurig zijn in hun partner keuze, levert aantrekkelijk zijn je een belangrijk voordeel op ten opzichte van je concurrenten. Het zijn immers de meest aantrekkelijke individuen die het hoogste parings succes hebben (Andersson 1994). Wanneer aantrekkelijkheid erfelijk is, en nakomelingen dus de aantrekkelijkheid van hun ouders erven, dan vormt de aantrekkelijkheid van de ouders een goede voorspeller voor het succes van hun nakomelingen. Omdat aantrekkelijkheid over het algemeen sterk varieert in een populatie, betekent dit dat individuen hun fitness kunnen optimaliseren door hun investering aan te passen aan de aantrekkelijkheid van hun partner, zoals beschreven wordt door de *differentiële allocatie hypothese* (Burley, 1986; Sheldon, 2000).

Het doel van dit proefschrift was om geslacht-specifieke ouderzorg en differentiële allocatie te onderzoeken in pimpelmezen (*Cyanistes caeruleus)*. Hiertoe heb ik video opnamen gemaakt terwijl ouders hun jongen voerden, en op deze manier heb ik kunnen bepalen hoeveel en hoe vaak vader en moeder hun jongen voerden. Vervolgens heb ik met behulp van moleculaire methoden het geslacht van alle jongen bepaald. Op deze manier kon ik zien hoeveel beide ouders hun zonen en dochters voerden, en daarmee dus hoeveel tijd en energie ze in hen investeerden, en hoe deze

investering afhing van het aantal zonen en dochters in hun broedsel (of te wel de sekse ratio)

Eerder onderzoek heeft laten zien dat pimpelmezen veren op hun kop hebben die ultraviolet licht (UV) reflecteren (Andersson et al., 1998; Sheldon et al., 1999; Hunt et al., 1998; Hunt et al., 1999). Het is gebleken dat de mate waarin deze kruinveren UV reflecteren, samenhangt met hoe waarschijnlijk het is dat ze de winter zullen overleven (Svensson & Nilsson 1996), zowel als met hun aantrekkelijkheid (Sheldon 1999; Hunt 1998 and 1999). Bovendien hebben, zoals verwacht, vrouwelijke pimpelmezen wiens partner een kruin heeft die veel UV reflecteert, en die dus waarschijnlijk ook aantrekkelijke zonen zal krijgen, meer zonen produceert dan een vrouwtje met een minder aantrekkelijke partner (Sheldon 1999).

In mijn onderzoek heb ik de kruinkleur van mannetjes en vrouwtjes gemeten, zowel als gemanipuleerd. Op deze manier heb ik getest of de hoeveelheid die een ouder voert, afhangt van de aantrekkelijkheid van zijn of haar partner. Daarnaast heb ik de groei van de jongen gemeten, om te kijken wat de consequenties zijn van de zorgzaamheid van de ouders op de groei en ontwikkeling van hun jongen, en daarmee op hun reproductieve succes.

Over een periode van vier jaar genomen, bleek dat ouders hun zonen en dochters even veel voerden. Daarnaast heb ik ook geen bewijs gevonden voor de voorspelling dat hoeveel de ouders investeren afhankelijk is van het relatieve aantal zonen en dochters in hun broedsel. Echter, hoewel er wanneer we alle vier jaren samen namen geen effect bestond tussen de sekse ratio van het broedsel en de voerfrequentie van de ouders, bleek het verband tussen de voerfrequentie van de vader, maar niet die van de moeder, en de sekse ratio van het broedsel, van jaar tot jaar te variëren. Mannetjes gedroegen zich in dat opzicht dus anders dan vrouwtjes. Interessant genoeg leek het er op dat mannetjes alleen meer investeerden in broedsels met veel zonen in jaren waarin er veel rupsen beschikbaar waren, welke het hoofdbestanddeel van het pimpelmees dieet vormen. Dit suggereert dus dat hoewel sex-specifieke investering geen algemeen fenomeen lijkt te zijn in pimpelmezen, het onder bepaalde omstandigheden wel degelijk plaats kan vinden, bijvoorbeeld wanneer er voldoende voedsel beschikbaar is.

Vervolgens heb ik onderzocht of ouders inderdaad meer investeren in hun jongen wanneer zij een aantrekkelijke partner hebben. Zoals verwacht blijken zowel mannetjes als vrouwtjes hun voer frequentie aan te passen aan de aantrekkelijk van hun partner. Het bleek dat vrouwtjes hun jongen meer voerden wanneer de kruin van hun partner veel UV licht reflecteerde, hetgeen hem relatief aantrekkelijk maakte. Mannetjes daar en tegen bleken juist minder te voeren wanneer de kruin van hun partner veel UV reflecteerde, hetgeen op het eerste gezicht suggereert dat mannetjes dit onaantrekkelijk vinden.

Deze twee resultaten heb ik vervolgens beide kunnen bevestigen in een serie van experimenten waarbij ik de UV reflectie van de kruinveren van het mannetje, respectievelijk het vrouwtje, verlaagd heb met behulp van een UV blokkerende crème. In beide experimenten bleken vogels te reageren op de manipulatie van hun partner: terwijl vrouwtjes minder voerden nadat ik de UV reflectie van hun partner verlaagd had, voerden mannetjes juist meer. Hiermee heb ik dus aangetoond dat pimpelmezen de investering in hun jongen aanpassen aan het uiterlijk van hun partner, en daarmee dat er bij pimpelmezen inderdaad sprake is differentiële allocatie.

Het feit dat mannetjes, in plaats van meer, juist minder investeerden wanneer ik de UV reflectie van de kruin van hun partner verlaagde, is verwarrend, maar betekent niet dat er hier geen sprake is van differentiële allocatie. Mannetjes pasten hun investering immers nog steeds aan aan de kleur van hun partner. Hoewel dit suggereert dat vrouwtjes met minder UV aantrekkelijker zijn dan vrouwtjes met een kruin die meer UV reflecteert (hetgeen dus het omgekeerde is als bij mannetjes het geval is), kunnen we dit met deze studie niet eenduidig aantonen. Het roept echter wel een aantal nieuwe vragen op over vrouwelijke aantrekkelijkheid in vogels die zeker meer aandacht verdienen.

Tot slot heb ik nog kunnen laten zien dat hoeveel de ouders beslissen te investeren in hun jongen er daadwerkelijk toe doet. Zo bleken de jongen in de groep waar een van de ouders frequenter voerde groter te zijn op het moment waarop ze het nest verlieten. Dit laat dus zien dat differentiële allocatie er toe leidt dat jongen die een vader hebben met een kruin die veel UV reflecteert, of jongen die een moeder hebben met veren die weinig UV reflecteren, beter groeien. Dit is een belangrijk resultaat gezien het feit dat het laat zien dat aantrekkelijk zijn niet alleen het vinden van een partner

vergemakkelijkt, maar dat het ook andere voordelen met zich mee kan brengen. Deze resultaten vormen dan ook een goede illustratie van het feit dat seksueel geselecteerde eigenschappen nauw kunnen samenhangen met ouderlijke investering, en ze laten zien hoe de sterkte van differentiële allocatie als motor van de seksuele selectie kan functioneren.

References

Alatalo, R. V. & Lundberg, A. 1986 Heritability and selection on tarsus length in the Pied Flycatcher (*Ficedula hypoleuca*). *Evolution* **40**, 574-583.

Alonso-Alvarez, C. 2006. Manipulation of primary sex-ratio: an updated review. *Avian and Poultry Biology Reviews*, **17,** 1-20.

Amundsen, T. 2000. Why are female birds ornamented? *Trends in Ecology & Evolution*, **15,** 149-155.

Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.

Andersson, S. and Amundsen, T. 1997. Ultraviolet colour vision and ornamentation in bluethroats. – Proc. R. Soc. B 264: 1587–1591.

Andersson, S., Ornborg, J. & Andersson, M. 1998. Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265,** 445-450.

Ballentine, B. and Hill, G. E. 2003. Female mate choice in relation to structural plumage coloration in blue grosbeaks. – Condor 105: 593–598.

Bennett, A. T. D., Cuthill, I. C., Partridge, J. C. and Maier, E. J. 1996. Ultraviolet vision and mate choice in zebra finches. – Nature 380: 433–435.

Burley, N. 1986 (a) Sex-ratio manipulation in color-banded populations of zebra finches. *Evolution* **40**, 1191-1206.

Burley, N. T. 1986 (b). Sexual selection for aesthetic traits in species with biparental care. *American Naturalist*, **127,** 415-445.

Burley, N. T. 1988. The differential allocation hypothesis: An experimental test. *American Naturalist*, **132,** 611-628.

Charmantier, A., Kruuk, L. E. B., Blondel, J. & Lambrechts, M. M. 2004. Testing for microevolution in body size in three blue tit populations. *Journal of Evolutionary Biology*, **17,** 732-743.

Charnov, E. L. 1982. The theory of sex allocation. *Monogr Popul.Biol.*, **18,** 1-355.

Clotfelter, E. D. 1996. Mechanisms of facultative sex-ratio variation in Zebra Finches (Taeniopygia guttata). *Auk*, **113,** 441-449.

Clutton-Brock, T. H., Albon, S. D. & Guinness, F. E. 1982. Parental investment in male and female offspring in polygynous mammals. *Nature*, **289,** 487-489.

Clutton-Brock, T. H. 1991. *The evolution of parental care*. Princeton, N.J.: Princeton University Press.

Cordero, P. J., Griffith, S. C., Aparicio, J. M. & Parkin, D. T. 2000. Sexual dimorphism in house sparrow eggs. *Behavioral Ecology and Sociobiology*, **48,** 353-357.

Cunningham, E. J. A. & Russell, A. F. 2000. Egg investment is influenced by male attractiveness in the mallard. *Nature*, **404,** 74-77.

Cuthill, I. C., Partridge, J. C., Bennett, A. T. D., Church, S. C., Hart, N. S. & Hunt, S. 2000. Ultraviolet vision in birds. In: *Advances in the Study of Behavior,* Vol. **29**, pp. 159-214.

De Lope, F. & Møller, A. P. 1993 Female reproductive effort depends on the degree of ornamentation of their mates. *Evolution* **47**, 1152-1160.

Delhey, K., Johnsen, A., Peters, A., Andersson, S. & Kempenaers, B. 2003. Paternity analysis reveals opposing selection pressures on crown coloration in the blue tit (Parus caeruleus). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270,** 2057-2063.

Delhey, K., Peters, A. Johnsen, A. & Kempenaers, B. 2007. Brood sex ratio and male UV ornamentation in blue tits (Cyanistes caeruleus): correlational evidence and an experimental test. *Behavioral Ecology and Sociobiology*. **61**, 853-862.

Dhondt, A. A. 1987. Polygynous blue tits and monogamous great tits: Does the polygyny-threshold model hold? *American Naturalist*, **129,** 213-220.

Dhondt, A. A. 2001. Trade-offs between reproduction and survival in Tits. *Ardea* **89**, 155-166.

Doutrelant, C., Gregoire, A., Grnac, N., Gomez, D., Lambrechts, M. M. & Perret, P. 2008. Female coloration indicates female reproductive capacity in blue tits. *Journal of Evolutionary Biology*, **21,** 226-233.

Eaton, M. D. & Lanyon, S. M. 2003. The ubiquity of avian ultraviolet plumage reflectance. *Proc. R. Soc. Lond. B* **270**, 1721-1726.

Ellegren, H., Gustafsson, L. & Sheldon, B. C. 1996. Sex ratio adjustment in relation to paternal attractiveness in a wild bird population. *Proc.Natl.Acad.Sci.U.S.A*, **93,** 11723-11728.

Fawcett, T. W., Kuijper, B., Pen, I., Weissing, F. J. 2007. Should attractive males have more sons? Behavioral Ecology, **18**, 71-80.

Fiala, K. L. 1981. Sex ratio constancy in the red-winged blackbird. *Evolution*, **35,** 898-910.

Fisher, R. A. 1930. *The genetical theory of natural selection*. Clarendon: Oxford.

Foerster, K., Delhey, K., Johnson, A., Lifjeld, J. T. & Kempenaers, B. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature*, **425,** 714-717.

Garnett, M. C. 1981. Body size, its heritability and influence on juvenile survival among Great Tits, *Parus major*. *Ibis* **123**, 31-41.

Gil, D., Graves, J., Hazon, N. & Wells, A. 1999. Male attractiveness and differential testosterone investment in zebra finch eggs. *Science*, **286,** 126-128.

Gowaty, P. A. & Droge, D. L. 1991. Sex ratio conflict and the evolution of sex-biased provisioning in birds. *Proceedings of the 20th International Ornithological Congress***,** 932-945.

Griffith, S. C., Ornborg, J., Russell, A. F., Andersson, S. & Sheldon, B. C. 2003. Correlations between ultraviolet coloration, overwinter survival and offspring sex ratio in the blue tit. *Journal of Evolutionary Biology*, **16,** 1045-1054.

Griffiths, R., Double, M. C., Orr, K. & Dawson, R. J. G. 1998. A DNA test to sex most birds. *Molecular Ecology*, **7,** 1071-1075.

Hadfield, J. D., Burgess, M. D., Lord, A., Phillimore, A. B., Clegg, S. M. & Owens, I. P. F. 2006. Direct versus indirect sexual selection: genetic basis of colour, size and recruitment in a wild bird. *Proceedings of the Royal Society B-Biological Sciences*, **273,** 1347-1353.

Hamilton, W. D. Z., M. 1982 Heritable true fitness and bright birds: a role for parasites. *Science* **218**, 384-387.

Hart, N. S., Partridge, J. C., Cuthill, I. C. & Bennett, A. T. 2000. Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: the blue tit (Parus caeruleus L.) and the blackbird (Turdus merula L.). *J.Comp Physiol [A]*, **186,** 375-387.

Heywood, J. S. 1989. Sexual Selection by the Handicap Mechanism. *Evolution*, **43,** 1387-1397.

Hill, G. E. 1991. Plumage coloration is a sexually selected indicator of male quality. – *Nature* **350**: 337–339.

Hill, G. E., Nolan, P. M. and Stoehr, A. M. 1999. Pairing success relative to male plumage redness and pigment symmetry in the house finch: temporal and geographic constancy. *Behavioural Ecology,* **10**: 48–53.

Hill, G. E. and McGraw, K. J. (eds). 2006. *Bird coloration*. Volume **1**: Mechanisms and measurements. – Harvard University Press, Cambridge.

Hoelzer, G. A. 1989. The Good Parent Process of Sexual Selection. *Animal Behaviour*, **38,** 1067-1078.

Hunt, D. M., Wilkie, S. E., Bowmaker, J. K. & Poopalasundaram, S. 2001a. Vision in the ultraviolet. *Cellular and Molecular Life Sciences*, **58,** 1583-1598.

Hunt, S., Bennett, A. T. D., Cuthill, I. C. & Griffiths, R. 1998. Blue tits are ultraviolet tits. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265,** 451-455.

Hunt, S., Cuthill, I. C., Bennett, A. T. D. & Griffiths, R. 1999. Preferences for ultraviolet partners in the blue tit. *Animal Behaviour*, **58,** 809-815.

Hunt, S., Cuthill, I. C., Bennett, A. T. D., Church, S. C. & Partridge, J. C. 2001. Is the ultraviolet waveband a special communication channel in avian mate choice? *Journal of Experimental Biology*, **204,** 2499-2507.

Hunt, S., Kilner, R. M., Langmore, N. E. & Bennett, A. T. D. 2003. Conspicuous, ultraviolet-rich mouth colours in begging chicks. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270,** S25-S28.

Jenni, L. & Winkler, R. 1994. *Moult and Ageing of European Passerines*. London, San Diego, New York, Boston, Sydney, Tokyo, Toronto: Academic Press.

Johnsen, A., Andersson, S., Örnborg, J. and Lifjeld, J. T. 1998. Ultraviolet plumage ornamentation affects social mate choice and sperm competition in bluethroats (Aves: *Luscinia s. svecica*): a field experiment. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **265**: 1313–1318.

Johnsen, A., Delhey, K., Andersson, S. & Kempenaers, B. 2003 Plumage colour in nestling blue tits: sexual dichromatism, condition dependence and genetic effects. *Proc. R. Soc. Lond. B* **270**, 1263-1270.

Johnsen, A., Delhey, K., Schlicht, E., Peters, A. and Kempenaers, B. 2005. Male sexual attractiveness and parental effort in blue tits: a test of the differential allocation hypothesis. *Animal Behaviour*, **70**: 877–888.

Kempenaers, B., Verheyen, G. R., vandenBroeck, M., Burke, T., vanBroeckhoven, C. & Dhondt, A. A. 1992. Extra-pair paternity results from female preference for high quality males. *Nature*, **357,** 494-496.

Kempenaers, B. 1994. Polygyny in the blue tit - Unbalanced sex ratio and female aggression restrict mate choice. *Animal Behaviour*, **47,** 943-957.

Kempenaers, B., Verheyren, G. R. & Dhondt, A. A. 1997. Extrapair paternity in the blue tit (Parus caeruleus): female choice, male characteristics, and offspring quality. *Behavioral Ecology*, **8,** 481-492.

Keyser, A. J. & Hill, G. E. 1999. Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **266,** 771-777.

Kilner, R. & Johnstone, R. A. 1997. Begging the question: Are offspring solicitation behaviours signals of needs. *Trends in Ecology & Evolution*, **12,** 11-15.

Kilner, R. 1998. Primary and secondary sex ratio manipulation by zebra finches. *Animal Behaviour*, **56,** 155-164.

Kilner, R. M. 2002. Sex differences in canary (Serinus canaria) provisioning rules. *Behavioral Ecology and Sociobiology*, **52,** 400-407.

Kolliker, M., Richner, H., Werner, I. & Heeb, P. 1998. Begging signals and biparental care: nestling choice between parental feeding locations. *Animal Behaviour*, **55,** 215-222.

Kolliker, M., Heeb, P., Werner, I., Mateman, A. C., Lessells, C. M. & Richner, H. 1999. Offspring sex ratio is related to male body size in the great tit (Parus major). *Behavioral Ecology*, **10,** 68-72.

Kolm, N. 2001 Females produce larger eggs for large males in a paternal mouthbrooding fish. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 2229-2234.

Korsten, P. 2006. Avian sex allocation and ornamental plumage coloration. A study on blue tits. Groningen: University of Groningen.

Korsten, P., Lessells, C.M., Mateman, A.C., von der Felde, M. & Komdeur, J. 2006. Primary sex ratio adjustment to experimentally reduced male UV attractiveness in blue tits. *Behavioral Ecology* **17**: 359-346.

Kunz, C. & Ekman, J. 2000. Genetic and environmental components of growth in nestling blue tits (*Parus caeruleus*). *Journal of Evolutionary Biology*, **13,** 199-212.

Lessells, C. M. 1998. A theoretical framework for sex-biased parental care. *Animal Behaviour*, **56,** 395-407.

Lessells, C. M. 2002. Parental investment in relation to offspring sex. In: The evolution of begging behaviour. Competition, cooperation and communication (Eds J Wright & M Leonard), pp. 65-85.

Lessells, C. M. 2002. Parentally biased favouritism: why should parents specialize in caring for different offspring? *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **357,** 381-403.

Lessells, C. M., Oddie, K. R. & Mateman, A. C. 1998. Parental behaviour is unrelated to experimentally manipulated great tit brood sex ratio. *Animal Behaviour*, **56,** 385-393.

Limbourg, T., Mateman, A. C., Andersson, S. & Lessells, C. M. 2004. Female blue tits adjust parental effort to manipulated male UV attractiveness. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **271,** 1903-1908.

Linville, S. U., Breitwisch, R. and Schilling, A. J 1998. Plumage brightness as an indicator of parental care in northern cardinals. *Animal Behaviour*, **55**: 119–127.

Magrath, M. J. L., Van Lieshout, E., Pen, I., Visser, G. H. & Komdeur, J. 2007. Estimating expenditure on male and female offspring in a sexually size-dimorphic bird: a comparison of different methods. *Journal of Animal Ecology*, **76,** 1169-1180.

Maynard Smith, J. 1980. A new theory of parental investment. *Behavioral Ecology and Sociobiology*, **7,** 247-251.

Mazuc, J., Chastel, O. & Sorci, G. 2003 No evidence for differential maternal allocation to offspring in the house sparrow (*Passer domesticus*). *Behav. Ecol.* **14**, 340-346.

Møller, A. P. & deLope, F. 1995 Differential allocation and sexual ornamentation. *Evolution* **49**, 1290-1292.

Møller, A. P. & Thornhill, R. 1998 Male parental care, differential parental investment by females and sexual selection. *Anim. Behav.* **55**, 1507-1515.

Mousseau, T. A. & Fox, C. W. 1998 *Maternal Effects as Adaptation*. Oxford: Oxford University Press.

Newton, I. 1978. Feeding and development of sparrowhawks *Accipter nisus* nestlings. *Journal of Zoology*, **184,** 465-487.

Nishiumi, I., Yamagishi, S., Maekawa, H. & Shimoda, C. 1996. Paternal expenditure is related to brood sex ratio in polygynous great reed warblers. *Behavioral Ecology and Sociobiology*, **39,** 211-217.

Oddie, K. R. 2000. Size matters: competition between male and female great tit offspring. *Journal of Animal Ecology*, **69,** 903-912.

Ohlsson, T., Smith, H. G., Raberg, L. & Hasselquist, D. 2002 Pheasant sexual ornaments reflect nutritional conditions during early growth. *Proc. R. Soc. Lond. B* **269**, 21-27.

Pilastro, A., Griggio, M. & Matessi, G. 2003 Male rock sparrows adjust their breeding strategy according to female ornamentation: parental or mating investment? *Anim. Behav.* **66**, 265-271.

Radford, A. N. & Blakey, J. K. 2000. Is variation in brood sex ratios adaptive in the great tit (Parus major)? *Behavioral Ecology*, **11,** 294-298.

Reyer, H. U., Frei, G. & Som, C. 1999 Cryptic female choice: frogs reduce clutch size when amplexed by undesired males. *Proc. R. Soc. Lond. B* **266**, 2101-2107.

Rohde, P. A., Johnsen, A. & Lifjeld, J. T. 1999 Parental care and sexual selection in the bluethroat, *Luscinia s. svecica*: A field-experimental test of the differential allocation hypothesis. *Ethology* **105**, 651-663.

Roskaft, E. & Slagsvold, T. 1985. Differential mortality of male and female offspring in experimentally manipulated broods of the rook. *Journal of Animal Ecology*, **54,** 261-266.

Rowe, L. & Houle, D. 1996 The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263**, 1415-1421.

SAS Institute Inc. 1996. *SAS/STAT User's guide*. Cary, NC: SAS Institute Inc.

Sanz, J. J. 2001 Experimentally reduced male attractiveness increases parental care in the pied flycatcher *Ficedula hypoleuca*. *Behavioural Ecology* **12**, 171-176.

Schwabl, H. 1993. Yolk is a source of maternal testosterone for developing birds. *Proceedings of the National Academy of Sciences of the United States of America*, **90,** 11446-11450.

Schwabl, H. 1996. Maternal testosterone in the avian egg enhances postnatal growth. *Comparative Biochemistry and Physiology A-Physiology*, **114,** 271-276.

Senar, J. C., Camerino, M., Copete, J. L. and Metcalfe, N. B. 1993. Variation in black bib of the Eurasian siskin (*Carduelis spinus*) and its role as a reliable badge of dominance. *Auk*, **110**: 924–927.

Shawkey, M. D., Estes, A. M., Siefferman, L. M. & Hill, G. E. 2003. Nanostructure predicts intraspecific variation in ultraviolet-blue plumage colours. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270,** 1455-1460.

Sheldon, B. C. 2000. Differential allocation: tests, mechanisms and implications. *Trends in Ecology & Evolution*, **15,** 397-402.

Sheldon, B. C., Andersson, S., Griffith, S. C., Ornborg, J. & Sendecka, J. 1999. Ultraviolet colour variation influences blue tit sex ratios. *Nature*, **402,** 874-877.

Shawkey, M. D., Estes, A. M., Siefferman, L. M. & Hill, G. E. 2003. Nanostructure predicts intraspecific variation in ultraviolet-blue plumage colours. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270,** 1455-1460.

Siefferman, L. and Hill, G. E. 2005. UV-blue structural coloration and competition for nestboxes in male eastern bluebirds. *Animal Behaviour*, **69**: 67–72.

Siitari, H., Honkavaara, J., Huhta, E. and Viitala, J. 2002. Ultraviolet reflection and female mate choice in the pied flycatcher, *Ficedula hypoleuca*. *Animal Behaviour*, **63**: 97–102.

Simmons, L. W. 1987 Female choice contributes to offspring fitness in the field cricket *Gryllus bimaculatus* (De Geer). *Behavioral Ecology and Sociobiology* **21**, 313-321.

Stamps, J., Clark, A., Kus, B. & Arrowood, P. 1987. The effect of parent and offspring gender on food allocation in budgerigars. *Behaviour*, **101,** 177-199.

Stamps, J. 1990. When should avian parents differentially provision sons and daughters. *American Naturalist*, **135,** 671-685.

Svensson, E. & Nilsson, J. A. 1996. Mate quality affects offspring sex ratio in blue tits. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **263,** 357-361.

Szigeti, B., Torok, J., Hegyi, G., Rosivall, B., Hargitai, R., Szollosi, E. & Michl, G. 2007. Egg quality and parental ornamentation in the blue tit Parus caeruleus. *Journal of Avian Biology*, **38,** 105-112.

Teather, K. L. 1987. Intersexual differences in food consumption by hand-reared great-tailed grackle (*Quiscalus mexicanus*) nestlings. *Auk*, **104,** 635-639.

Teather, K. L. & Weatherhead, P. J. 1988. Sex-specific energy requirements of great-tailed grackle (*Quiscalus mexicanus*) nestlings. *Journal of Animal Ecology*, **57,** 659-668.

Thornhill, R. 1983 Cryptic female choice and its implications in the scorpion fly *Harpobittacus nigriceps*. *Am. Nat.* **122**, 765-788.

Tovee, M. J. 1995. Ultra-violet photoreceptors in the animal kingdom - Their distribution and function. *Trends in Ecology & Evolution*, **10,** 455-460.

Trivers, R. L. 1972. Parental Investment and Sexual Selection. In: *Sexual selection and the descent of man.* (Ed. by Campbell, B.), pp. 136-179. London: Heinemann.

Trivers, R. L. & Willard, D. E. 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science*, **179,** 90-92.

Visser, M. E., Holleman, L. J. M. & Gienapp, P. 2006. Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird. *Oecologia* **147,** 164–172.

Von Engelhardt, N. 2004. Proximate control of avian sex allocation. A study on zebra finches. Groningen: University of Groningen.

Vorobyev, M., Osorio, D., Bennett, A. T. D., Marshall, N. J. & Cuthill, I. C. 1998. Tetrachromacy, oil droplets and bird plumage colours. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology*, **183,** 621-633.

Westneat, D. F. 1995. Paternity and paternal behavior in the red-winged blackbird, *Agelaius-phoeniceus*. *Animal Behaviour*, **49,** 21-35.

Westneat, D. F., Clark, A. B. & Rambo, K. C. 1995. Within brood patterns of paternity and paternal behavior in red-winged blackbirds. *Behavioral Ecology and Sociobiology*, **37,** 349-356.

Westneat, D. F. & Birkhead, T. R. 1998 Alternative hypotheses linking the immune system and mate choice for good genes. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 1065-1073.

Williams, G. C., 1966. Natural Selection, the Costs of Reproduction, and a Refinement of Lack's Principle. *The American Naturalist*, **100**, 687-690.

Witte, K. 1995. The differential-allocation hypothesis: Does the evidence support it? *Evolution*, **49,** 1289-1290.

Yasukawa, K., Mcclure, J. L., Boley, R. A. & Zanocco, J. 1990. Provisioning of nestlings by male and female red-winged blackbirds, *Agelaius-phoeniceus*. *Animal Behaviour*, **40,** 153-166.

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