Avian Sex Allocation and Ornamental Coloration

A study on blue tits

Peter Korsten

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voor mijn opa, Jan Bosch

Contents

CHAPTER 1

General introduction

This thesis deals with the consequences of the two main problems that virtually all sexually reproducing organisms are facing: i) how to find a suitable mate; and once mated; ii) how much to invest in the production of male versus female offspring.

Sexual selection – finding a mate

In order to reproduce, organisms that reproduce sexually first need to find a suitable partner, generally of different sex. Individuals bearing genes that enable them to be more successful in attracting or finding mates will propagate their genes into future generations more successfully. This selective process which acts through variation in mating success was first recognised by Darwin (1871) and was named 'sexual selection' to distinguish it from ordinary 'natural selection' (Andersson 1994).

In general, males can potentially fertilize the egg cells of many females with cheaply produced sperm, whereas females have a much smaller reproductive potential. As a consequence, males are generally selected to maximize their number of offspring by maximizing the number of female mates they can obtain, whereas females mostly evolve to select only those males that leave them offspring of the highest quality (Andersson 1994). Note, however, that there are also cases in which these patterns are reversed between sexes (*e.g.* Berglund & Rosenqvist 1993; Delehanty *et al.* 1998). The most selective sex in choosing potential mates is often referred to as the 'choosy sex' (Andersson 1994).

Sexual selection can lead to the evolution of male (and female) exaggerated traits also named ornaments, which improve access to mates either by increased attraction ('inter-sexual selection') or better ability to withstand same-sex competitors ('intra-sexual selection') (Andersson 1994). Examples of sexually selected traits used by males to attract potential female mates are as varied as the chirping songs of crickets (Bentsen *et al.* 2006), pheromones of mice (Egid & Brown 1989) and the bright and conspicuous plumage colours of many species of bird (Hill 2006). Examples of traits selected by male-male competition over access to females or breeding territories are eye span in stalk-eyed flies (Panhuis & Wilkinson 1999), the enormous body size of male elephant seals (Le Boeuf 1974), antlers in deer (Kruuk *et al.* 2002, and references therein) and song in birds (Krebs *et al.* 1978). Often sexually selected traits have a dual function, both in female mate choice and male-male competition (Berglund *et al.* 1996).

Although evidence for female mate choice and male-male competition comes from a broad range of taxa, birds, with their often complex song and bright colours, have traditionally been key models in sexual selection research (Darwin 1871; Hamilton & Zuk 1982; Andersson 1994). The mating behaviour of birds continues to be intensively studied and birds are invariably important as models for testing general principles of sexual selection (*e.g.* Hadfield *et al.* 2006; Qvarnström *et al.* 2006; Figure 1.1). One particular issue, which has received much recent attention,

is the fact that avian colour vision differs in some important respects (*e.g.* ultraviolet [UV] vision) from human colour perception (Cuthill *et al.* 2000). This finding has great implications for the study of colour signalling and sexually selected coloration in birds (Bennett *et al.* 1994). Combined with the recent availability of affordable and light-weight spectrophotometers suitable for measuring plumage reflectance in the field (for a discussion see Andersson & Prager 2006), the recent appreciation of the particular properties of the avian visual system has stimulated a whole new area of research into the role of colour perception and the different colour-producing mechanisms (Box A) in avian sexual selection (reviewed in Hill & McGraw 2006a; Hill & McGraw 2006b).

BOX A. Mechanisms of plumage coloration

The great diversity in plumage colours of birds is produced by two basic mechanisms: 1) light absorption by pigments and 2) coherent light scattering by microscopic keratin structures in the feathers (Fox 1976; Prum *et al.* 1999; Hill & McGraw 2006a).

Pigment-based coloration: carotenoids and melanins

A wide variety of pigment types are used by birds to produce the diversity of plumage colours. The two most important classes of feather pigments are carotenoids (McGraw 2006) and melanins (Jawor & Breitwisch 2003). Carotenoids mostly cause bright yellow, orange and red colours. For example, carotenoids are responsible for the yellow coloration of the blue tit's (*Parus caeruleus*) breast feathers (Partali *et al.* 1987). Carotenoids cannot be synthesized *de novo* by birds and thus need to be derived from the food. Carotenoids for feather coloration may be limited, because they are often scarce compounds within the diet and also needed for other vital functions of the body such as a well-working immune system (Olson & Owens 1998). Therefore, birds face a trade-off between deposition of carotenoids in the plumage and reservation of these nutrients for important physiological activities. As a result of this trade-off carotenoid coloration may be a reliable signal of individual quality, revealing for example the general health or foraging efficiency of a particular individual (Lozano 1994; Olson & Owens 1998).

Melanins mostly cause rusty red, brown, grey, and black colours. For example, the blue tit's black eye stripe is probably caused by melanins. Birds can newly synthesise melanins in their bodies. It is unclear if this production is costly in terms of for example energy expenditure or use of valuable nutrients. The extent of melanin coloration is often related to individual aggressive behaviour and social dominance (Jawor & Breitwisch 2003).

Structurally based coloration
White, blue, purple, green, iridescent and UV-reflecting plumage, such as that of the blue tit's crown feathers (Andersson *et al.* 1998; Hunt *et al.* 1998), is mostly structurally based and dependent on the precise arrangement of the microscopic keratin structures in the feather barbs and barbules (Prum *et al.* 1999). Through their regular arrangements, these nano-scale structures cause coherent scattering of light, leading to the production of bright colours. Interest in the signalling function of structurally based UV-reflecting plumage coloration has rapidly grown after the broad recognition that birds generally have UV vision (*e.g.* Andersson & Amundsen 1997; Bennett *et al.* 1997; Andersson *et al.* 1998; Hunt *et al.* 1998; Johnsen *et al.* 1998; Sheldon *et al.* 1999; Limbourg *et al.* 2004; Siefferman & Hill 2005; Korsten *et al.* 2006).

Sex allocation – investing in male versus female offspring

After successful mating, sexually reproducing organisms must divide the investment of their resources between the production of male and female offspring. Differential allocation of parental resources to male and female offspring could influence either the relative *quality*, or lead to a change in the relative *number*, of male and female offspring (Charnov 1982). Here I will focus on the influence of parents on the relative number of male and female offspring they produce (referred to as offspring sex ratio).

Determination of offspring sex may often seem random, with males and females born in equal numbers. However, females of many species appear to have close control over the sex of their offspring, adaptively adjusting it to be the sex that gives them the highest fitness returns (*i.e.* leaving the mother most descendents) (Godfray & Werren 1996; Hardy 2002). Sex allocation theory predicts the proportion of male and female offspring that would maximize the parents' fitness (Charnov 1982). An important notion in sex allocation theory is that, at the population level, equal numbers of male and female offspring are generally expected (Fisher 1930). This arises because all individuals in a population must have both a father and a mother, and therefore the rarer sex at any given moment has a better chance of finding a mate to reproduce. This causes frequency dependent selection favouring the rarer sex, which will eventually lead to the evolution of a 50:50 sex ratio on the population level (Fisher 1930). Individual parents, however, may have higher fitness if they vary the sex ratio of their offspring according to their individual circumstances (Trivers & Willard 1973; Charnov 1982). For example, female parasitic wasps often lay a higher proportion of female eggs in large than in small hosts, because there is a strong positive relationship between the number of eggs subsequently produced by daughters and the size of the host from which they have

emerged, whereas in sons the relationship between mating success and host size and is weaker (Jones 1982).

Sex allocation theory has been particularly successful in explaining sex ratio patterns in some specific taxa, mostly invertebrates, such as haplodiploid insects (*e.g.* ants, bees, wasps), of which females can determine the sex of an egg by either fertilizing it or not (Godfray & Werren 1996; West *et al.* 2000). However, in vertebrates with chromosomal sex determination, like birds and mammals, sex ratio patterns are generally rather weak and less well understood (Williams 1979; Clutton-Brock 1986; Krackow 2002; Cassey *et al.* 2006; but see West and Sheldon, 2002; West *et al.* 2005). In birds, females are the heterogametic sex, which potentially gives them control over the sex of their eggs (referred as the *primary* sex ratio) (Krackow, 1995; Oddie, 1998). But the mechanism by which females could influence egg sex remains largely elusive (Krackow 2002; Pike & Petrie 2003; Pike 2005). There has been a surge of interest in adaptive sex ratio control in birds during the last decade (Hardy 2002; Figure 1.1), which was stimulated by both the publication of several well-documented cases showing adaptive primary sex ratio adjustment (Dijkstra *et al.* 1990; Daan *et al.* 1996; Ellegren *et al.* 1996; Komdeur *et al.* 1997; Nager *et al.* 1999; Pen *et al.* 1999; Sheldon *et al.* 1999) or extreme sex ratio biases (Heinsohn 1997) and by the advent of molecular methods that make it possible to use tiny blood samples for sexing young chicks (Griffiths *et al.* 1996; Griffiths *et al.* 1998) – which are impossible to sex based on morphology in most bird species. Studies on avian sex ratio are accumulating rapidly (Figure 1.1), but the

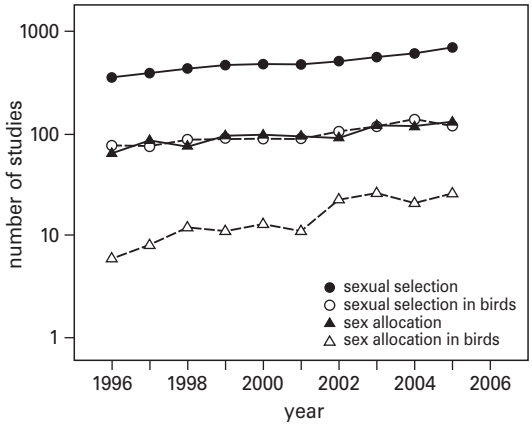


Figure 1.1 Rapidly increasing numbers of studies on sexual selection and sex allocation during the last decade. Numbers of studies are hits after a search in the ISI 'Web of Science' database. Lines indicate the different sets of search terms used: Sexual selection = 'sexual selection'; Sex allocation = 'sex allocation' OR 'primary sex ratio' OR 'offspring sex ratio' OR 'brood sex ratio'. Separate lines are plotted for cases including 'bird' as an additional search term (dashed lines, open symbols). Note exponential scale of vertical axis.

evidence for adaptive sex ratio adjustment in birds remains controversial (Palmer 2000; Radford & Blakey 2000; Hasselquist & Kempenaers 2002; Krackow 2002; Ewen *et al.* 2004; Cassey *et al.* 2006) and overall patterns in the literature appear to be rather weak and inconsistent (Komdeur & Pen 2002; West & Sheldon 2002; Cassey *et al.* 2006). It is currently unclear to what extent the relatively weak patterns in birds are due to constraints on the ability to bias sex ratios or to selective pressures which favour more modest adjustments in birds (*e.g.* Pen *et al.* 1999) compared to some other taxa (West & Sheldon 2002).

An idea that has raised particular interest, and which links theories of sex allocation and sexual selection, is that females should adjust the sex ratio of their offspring in response to the sexual attractiveness of their mate (Burley 1981, 1986). According to this idea, females paired to attractive males increase their fitness by producing a greater proportion of sons, given that sons inherit their father's attractiveness and consequently have high mating success, thereby yielding greater fitness returns than daughters. The validity of this verbal argument has recently been confirmed by analytical (Pen & Weissing 2000) and simulation models (Fawcett *et al.* 2006). Several empirical studies, mostly using birds as model species, have attempted to test the hypothesis, yielding mixed evidence for a link between offspring sex ratio and male attractiveness, with results varying between different years (*e.g.* Radford & Blakey 2000; Griffith *et al.* 2003; Korsten *et al.* 2006) and populations (*e.g.* Svensson & Nilsson 1996; Leech *et al.* 2001; Rosivall *et al.* 2004).

Sexual selection and sex allocation in the blue tit

The blue tit *Parus caeruleus* is a popular model species in studies of sexual selection and sex allocation in birds (*e.g.* Kempenaers *et al.* 1992; Kempenaers *et al.* 1997; Krokene *et al.* 1998; Svensson & Nilsson 1996; Andersson *et al.* 1998; Hunt *et al.* 1998; Sheldon *et al.* 1999; Leech *et al.* 2001; Delhey *et al.* 2003; Foerster *et al.* 2003; Griffith *et al.* 2003; Alonso-Alvarez *et al.* 2004; Limbourg *et al.* 2004, Johnsen *et al.* 2005; Dreiss *et al.* 2006; Korsten *et al.* 2006). There are a number of reasons for this popularity.

First, blue tits were among the first socially monogamous bird species in which the occurrence of extra-pair offspring was well-documented and linked to female preferences for high-quality males (Kempenaers *et al.* 1992; Kempenaers *et al.* 1997). In blue tits, as in most passerine birds, females do not receive any direct benefits, such as parental care, from their extra-pair mates. This strongly suggests that by their extra-pair mating behaviour, females gain genetic benefits for their offspring, such as more diverse, more compatible or simply 'better' genes.

Second, following recognition of UV vision in birds, it was discovered that the blue crown feathers of the blue tit – in which the two sexes appear closely similar to the human eye (Cramp & Perrins 1993) – are sexually dichromatic in the UV part of

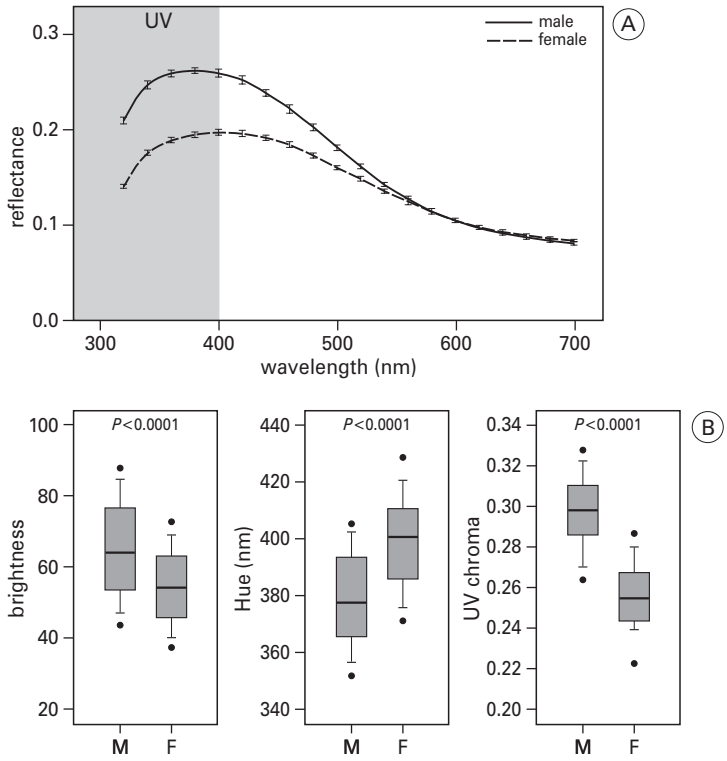


Figure 1.2 Sexual dichromatism of blue tit crown plumage. (A) Mean crown reflectance curves of 214 male and 207 females that were captured between 2001–2004, both during the breeding season and winter. Standard errors around means are depicted at 20 nm intervals. The shaded area indicates the UV part of the spectrum. (B) Indices of male and female crown coloration, calculated from reflectance measurements taken from the same 214 males (M) and 207 females (F). Brightness is total reflectance between 320–700 nm. Hue is the wavelength of peak reflectance. UV chroma is the proportion of UV reflectance (320–400 nm) relative to total reflectance (320–700 nm). Box plots give medians and the 5th and 95th, 10th and 90th, and 25th and 75th percentiles, which are indicated by black circles, whiskers and boxes respectively. Males had higher brightness, more UV-shifted hue and higher UV chroma (*t*-tests; brightness: $t = 7.785$, $P < 0.0001$; hue: $t = -12.368$, $P < 0.0001$; UV chroma: $t = 23.562$, $P < 0.0001$; all df = 419).

the spectrum (Andersson *et al.* 1998; Hunt *et al.* 1998). Blue tit males have more UV-shifted and more UV-chromatic crown plumage than females (Figure 1.2). As blue tits have UV vision (Hart *et al.* 2000) they will be able to detect variation in UV reflectance among conspecific individuals. The sexual dichromatism strongly suggests that the crown colour of blue tits is a sexually selected trait, important in mate choice or male-male competition. This idea was confirmed by a mate choice experiment with captive blue tits (Hunt *et al.* 1998), and by the finding of assortative mating for crown UV coloration in the field (Andersson *et al.* 1998; but see Box B).

Third, based on the finding that female blue tits biased offspring sex ratio towards sons when paired to a presumably high-quality male that had greater survival chances (Svensson & Nilsson 1996), Sheldon *et al.* (1999) designed an experiment in which the crown UV coloration of males was manipulated and found a striking effect on offspring sex ratio. This finding was the first experimental evidence for a causal link between male attractiveness and offspring sex ratio in a wild bird population. In addition, the probability of male over-winter survival was positively correlated with crown UV coloration, indicating UV coloration to be a signal of male viability (Sheldon *et al.* 1999).

From this starting point I set out to investigate further: i) the inheritance of the blue tit's sexually selected crown UV coloration; ii) the influence of male crown UV coloration on female reproductive decisions (sex allocation, hormone deposition in the yolk of eggs); and iii) the importance of the crown UV coloration as a signal in inter-individual competition (male-male territorial conflict, competition over food in winter). The project was carried out at the University of Groningen (Box C), which has a strong tradition in avian sex ratio research (*e.g.* Dijkstra *et al.* 1990; Daan *et al.* 1996; Komdeur *et al.* 1997; Pen *et al.* 1999; Komdeur & Pen 2002), and embedded into a larger research program, which aimed to further elucidate mechanisms and functions of avian sex allocation (for more details see Chapter 8, Background of the project).

Thesis outline

Part I. Inheritance of UV coloration

The inheritance of male sexual attractiveness is a key assumption underlying the verbal and mathematical models predicting the evolution of female sex ratio adjustment in response to the attractiveness of her mate (Burley 1981, 1986; Pen & Weissing 2000; Fawcett *et al.* 2006). In ***Chapter 2***, I investigated whether the blue tit's ornamental crown plumage is inherited from parents to offspring. Based on a four-year database of natural broods I show that offspring significantly resemble their parents with regard to crown coloration, which fulfils one of the main conditions for adaptive sex ratio adjustment in relation to male ornamentation to evolve. These findings pose a remarkable contrast to a recent study in another blue tit population where no significant heritable variation for crown colour was found (Hadfield *et al.* 2006).

Part II. Female reproductive adjustment to male plumage UV coloration

To establish a causal link between female reproductive decisions and male ornamental plumage coloration, it is essential to measure the female response to experimentally manipulated male plumage colour. The treatment of feathers with a mixture of UV-absorbing chemicals and fat is the most commonly used method for manipulating

plumage UV reflectance in studies of avian UV signalling (Andersson & Amundsen 1997; Johnsen *et al.* 1998; Sheldon *et al.* 1999; Siitari *et al.* 2002; Alonso-Alvarez *et al.* 2004; Limbourg *et al.* 2004; Korsten *et al.* 2006). However, the persistence of the treatment and the temporal changes in UV reflectance after the treatment's application are poorly known. Such knowledge is extremely useful in the design and interpretation of plumage UV manipulation experiments. ***Chapter 3*** demonstrates the temporal changes in plumage UV coloration after such a treatment of UV-absorbing chemicals mixed with fat. The measurements show that directly after treatment the UV reflectance of the crown feathers is reduced below the natural range. However, thereafter the UV reflectance initially increases rapidly again and returns to the natural range within two days. After that, the recovery of the UV coloration becomes much slower, and although within the natural range, the UV reflectance of manipulated individuals remains significantly reduced for up to *ca.* 10 days. After four weeks there was no longer a detectable difference between UV-reduced and control birds. The treatment had no effect on the probability of survival to the following breeding season.

In ***Chapter 4*** the causal link between offspring sex ratio and male ornamentation was tested experimentally by manipulation of the crown coloration of males before their females had started egg laying. The UV reflectance of the crown plumage of one group of males was reduced by application of a mixture of UV-absorbing chemicals and fat as described above, while another group of males served as a control, being treated with fat only. In contrast to the straightforward prediction of sex allocation theory (Trivers & Willard 1973; Burley 1981, 1986; Pen & Weissing 2000; Fawcett *et al.* 2006), females paired to UV-reduced – unattractive – males did not produce an overall lower proportion of sons. Instead, in one of the two years, the positive correlation between offspring sex ratio and natural male UV reflectance (after control treatment) disappeared. This rather unexpected result shows a remarkable similarity with the outcome of a previous experimental study of blue tit sex ratios in a different population (Sheldon *et al.* 1999), and indicates these complex sex ratio patterns to be partially repeatable.

In ***Chapter 5*** the UV-reduction treatment was used to test experimentally the effect of male plumage ornamentation on female hormone deposition in the eggs. There is large variation in the amount of various androgenic hormones, such as testosterone, that female birds deposit in the yolk of their eggs (Gil 2003; Groothuis *et al.* 2005a). This variation is believed to represent an adaptive maternal effect, which fine-tunes the offspring's development and behaviour to the prevailing environmental conditions (Groothuis *et al.* 2005). Recent studies have indicated that females of some bird species also vary yolk hormones in response to the sexual attractiveness of their mate (Gil *et al.* 1999, 2004, 2006; von Engelhardt *et al.* 2004; but see Saino *et al.* 2006). The results of Chapter 5 show that blue tit females indeed rapidly change patterns of yolk testosterone deposition in response to an experimental manipulation of male crown coloration.

Part III. UV coloration as a signal in inter-individual competition

Ornaments that are important as female mate choice cues often also play a role as signals in male-male competition and *vice versa* (Berglund *et al.* 1996). Several studies have found direct and indirect evidence for blue tit crown coloration being an important signal in female mate choice (*e.g.* Andersson *et al.* 1998; Hunt *et al.* 1998; Sheldon *et al.* 1999; Delhey *et al.* 2003; Limbourg *et al.* 2004). However, whether blue tit crown coloration also acts as a signal in male-male competition remains less well studied (but see Alonso-Alvarez *et al.* 2004).

In ***Chapter 6*** it was investigated whether male UV coloration plays a role in conflict between territory owners and intruders. Two taxidermic male blue tit mounts were presented in the territories of resident males during the female fertile period; one mount with natural crown UV reflectance and one mount with reduced crown UV. Territory owners did not direct their aggression specifically to either the UV-reduced or the control mount. Furthermore, the variation in natural crown UV reflectance of the resident males did not predict the intensity of their aggressive response. Contrary to previous findings (Alonso-Alvarez *et al.* 2004), we found no evidence for UV signals playing a role in male-male interactions during territorial intrusions.

There is large and continuous variation in blue tit crown UV coloration both within- and between the sexes (*e.g.* Delhey & Kempenaers 2006; Figure 1.2). Such plumage variation could be used by individuals to settle small conflicts, for example over food items, at a low cost (Rohwer 1975, 1982; for a recent review see Senar 2006). In this scenario individuals in fact 'signal' their dominance or social status to other individuals by their plumage coloration. Previous work on blue tits has shown that males with more intense UV coloration have a better chance of surviving the winter (Sheldon *et al.* 1999; but see Delhey & Kempenaers 2006). Possibly, such males with higher UV reflectance are better able to monopolize scarce food sources during winter. ***Chapter 7*** presents a test of this so-called 'status-signalling hypothesis'. It is shown that social dominance at a food source in winter is strongly dependent on the distance of individuals to their territories, their sex and their age. Crown coloration, however, does not influence the outcome of competition over food. Nor was crown colour related to the survival probability to the following breeding season. These results refute the idea that blue tit crown coloration acts as a signal of social status during winter.

Finally, ***Chapter 8*** provides a synthesis in which the most important results of this study are summarized and put into context of other research findings. I end this last chapter by setting out possible lines for future work.

BOX B. Assortative mating and UV coloration in blue tits

In a Swedish population, blue tits mated assortatively with respect to UV crown coloration (UV chroma), which suggests that mutual mate choice for UV crown coloration may occur in blue tits (Andersson *et al*. 1998). However, in our blue tit study population in the Netherlands (De Vosbergen) no such assortative mating was found (Figure B.1; Table B.1; for details on crown colour measurements see Chapter 4).

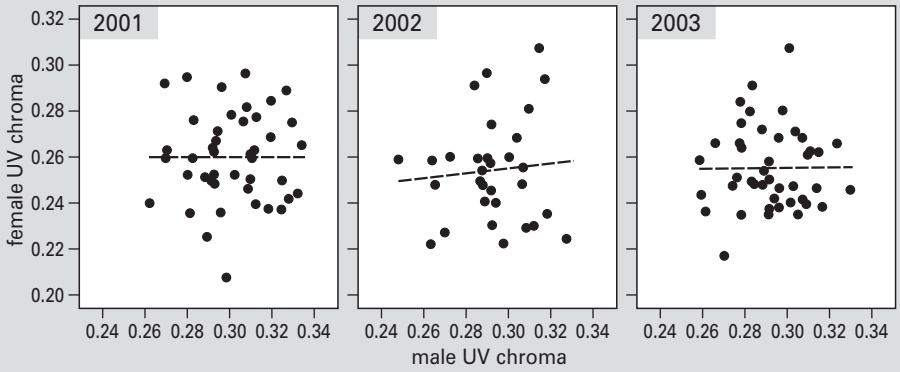


Figure B.1 No assortative mating for UV chroma in blue tits. See also Table B.1.

Table B.1 No correlations between crown coloration of paired male and female blue tits in 2001–2003. Males and females were captured for crown reflectance measurements during chick feeding.

	2001 (n = 43)		2002 (n = 31)		2002 (n = 43)	
Colour index	r	P	r	P	r	P
Brightness [a]	0.11	0.50	0.08	0.67	0.19	0.23
Hue (nm) [b]	–0.06	0.70	0.12	0.51	–0.03	0.88
UV chroma [c]	0.01	0.97	0.09	0.65	0.01	0.92

[a] Brightness is total reflectance between 320–700 nm.
[b] Hue is wavelength of peak reflectance.
[c] UV chroma is the proportion of UV reflectance; this is the reflectance between 320–400 nm divided by the reflectance between 320–700 nm.

BOX C. The Vosbergen blue tit population

In 2001 a blue tit study population breeding in nestboxes was established on the estate of 'De Vosbergen', in the north of the Netherlands near Groningen (53°08'N, 06°35'E; Figure C.1). The study area covers *ca*. 50 ha consisting of patches of both young and old mixed deciduous and coniferous forest interspersed by open grassland. The presence of many lanes of old oak trees makes the habitat ideal for blue tits. In January 2001 approximately 185 nestboxes designed for blue tits (with a 26.0 mm entrance hole excluding larger sized great tits *Parus major*) were put up in the area, which were readily used for breeding by blue tits. The Vosbergen blue tit population has since been continuously monitored. All breeding attempts have been followed and all breeding adults have been captured and individually marked with a numbered metal ring and a unique combination of colour rings. All nestlings were also ringed. This work has resulted in a valuable database on the demography of the Vosbergen blue tit population with data on individuals' resident status, age, survival and recruitment (Figure C.2).

Figure C.1 The study area, estate 'De Vosbergen'.

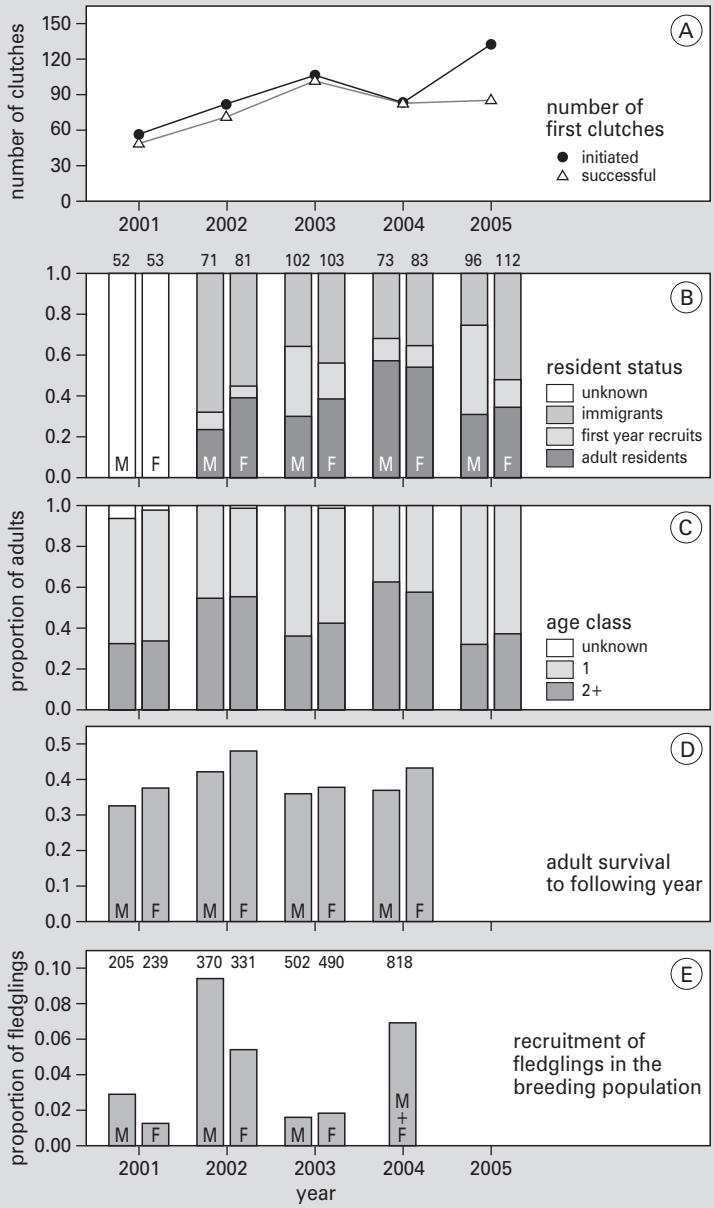
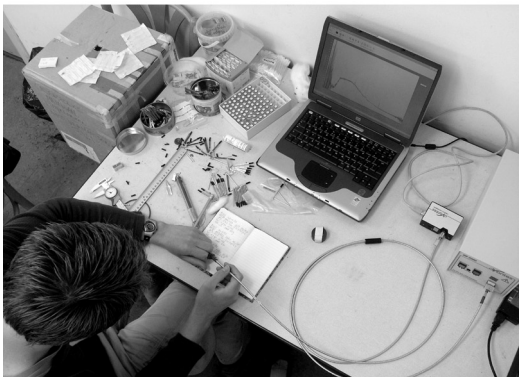


Figure C.2 Demographic parameters of the Vosbergen blue tit population (2001–2005): population size (number of clutches; A), resident status (B), age (C), survival (D) and recruitment (E). A clutch was designated successful if at least one young fledged. M: male; F: female. Numbers above bars indicate sample size.

PART I

Inheritance of plumage UV coloration



CHAPTER 2

Heritable variation in sexually selected structural coloration in blue tits

Peter Korsten & Jan Komdeur

ABSTRACT

Despite overwhelming evidence for the importance of ornamental plumage in avian mate choice, it is largely unclear what benefits drive the evolution of female preferences for bright male plumage colours. Most sexual selection models assume that females obtain genetic benefits, such as more attractive offspring, by mating with highly ornamented males. These models thus require the presence of heritable variation in ornament expression. However, evidence for heritable variation in sexually selected plumage coloration is scarce. We used a four-year data set from a population of wild blue tits *Parus caeruleus* to assess the repeatability and heritability of the blue tit's sexually selected ultraviolet/blue crown coloration. Previous studies on female extra-pair mating behaviour, sex allocation and parental investment in relation to male crown colour provide indirect evidence for a heritable component of the blue tit's structurally based crown coloration, but a recent study on the inheritance of blue tit crown coloration remarkably found no significant heritability ($h^2 < 0.11$). In contrast to this study, we found significant heritable variation for crown coloration in our blue tit population (h^2 values from 0.23–0.41; based on mid-parent–mid-offspring regression). It is unclear what causes this striking difference between populations, and future studies should aim at elucidating this.

INTRODUCTION

Bright plumage coloration in birds has long been a main focus in the study of sexual selection. (Darwin 1871; Hamilton & Zuk 1982; Andersson 1994; Hill & McGraw 2006b). There is overwhelming evidence for the importance of plumage characteristics, both morphological (*e.g.* tail feather length), and coloration, in promoting female mate choice and male-male competition (Andersson 1994; Hill & McGraw 2006b). However, it is much less clear what benefits drive the evolution of female preferences for exaggerated male ornaments (Kirkpatrick & Ryan 1991; Arnqvist & Kirkpatrick 2005). Derived benefits could either be direct, such as increased paternal care (Heywood 1989; Hoelzer 1989), or indirect inherited effects (Fisher 1930).

The majority of current sexual selection models is built on the assumption that, by mating with highly ornamented males, females obtain indirect genetic benefits through the production of more attractive or higher quality offspring. Thus, a prerequisite of these models is that variation in ornament expression is heritable (Mead & Arnold 2004). However, such evidence is scant, and restricted to only a few species (Hill 1991; Norris 1993; Møller 1989; Qvarnström 1999; for a review see Merilä & Sheldon 2001). Nevertheless, the existence of heritable variation for plumage coloration is commonly assumed, for example in the study of avian sex allocation in response to male sexual attractiveness (Ellegren *et al.* 1996; Sheldon *et al.* 1999; Korsten *et al.* 2006). Central to these studies is the idea that females mated to attractive males are selected to produce a higher proportion of sons because sons inherit their father's attractiveness (expression of plumage coloration) (Burley 1981; Fawcett *et al.* 2006). Similarly, studies on the differential allocation of parental care in response to mate attractiveness assume that plumage ornamentation has a heritable component (Limbourg *et al.* 2004; Johnsen *et al.* 2005). These studies hypothesize that a female should increase her level of parental investment when mated to a more attractive or higher quality male because, assuming that the properties of the male are inherited by the offspring, the reproductive value of her brood will be increased (Sheldon 2000).

The blue tit *Parus caeruleus* with its structurally based (*i.e.* based on the microstructure of the feather barbs instead of pigments; Prum *et al.* 1998) ultraviolet(UV)/blue ornamental crown plumage is extensively used as a model species in studies of avian sex allocation (*e.g.* Sheldon *et al.* 1999; Griffith *et al.* 2003; Dreiss *et al.* 2006; Korsten *et al.* 2006) and differential allocation of parental care (Limbourg *et al.* 2004; Johnsen *et al.* 2005). Recently, Hadfield *et al.* (2006) investigated the presumed inheritance of blue tit crown coloration in a large-scale cross-foster experiment on a UK population (Silwood Park, UK). Despite a rigorous experimental setup involving large-scale cross-fostering and good statistical power, they found no evidence for either a significant genetic or an environmental component that explained the phenotypic variation in blue tit crown coloration. The apparent lack of heritable variation is in contrast with previous studies on blue tits providing several lines of

evidence to suggest that the UV/blue crown plumage is a sexually selected ornament that plays an important role in mate choice. First, the blue tit's crown coloration is strongly sexually dichromatic, with males having more intensely coloured crown feathers, especially in the UV part of the spectrum (Andersson *et al.* 1998; Hunt *et al.* 1998). Second, captive females preferred bright UV partners in mate choice trials (Hunt *et al.* 1998) and assortative mating for crown coloration has been found in the field (Andersson *et al.* 1998). Third, patterns of extra-pair paternity in wild blue tits were found to be related to male UV coloration (Delhey *et al.* 2003). This latter finding in particular suggests that indirect, genetic benefits – such as the inheritance of attractive crown coloration by the offspring – are associated with variation in male crown colour, because it is highly unlikely that females obtain any direct benefits from extra-pair mates (Griffith *et al.* 2002). Finally, the occurrence of female adjustment of primary sex ratio and paternal care to male crown coloration found in several populations is highly indicative of a heritable component of blue tit crown coloration (Sheldon *et al.* 1999; Griffith *et al.* 2003; Limbourg *et al.* 2004; Johnsen *et al.* 2005; Korsten *et al.* 2006, but see Dreiss *et al.* 2006). Given the accumulated indirect evidence predicting a heritable component of blue tit crown coloration it is remarkable that no significant heritability of crown coloration was found in a UK population of blue tits (Hadfield *et al.* 2006). To improve our understanding of this apparent paradox, it is important to investigate whether the low heritability of blue tit crown coloration found by Hadfield *et al.* (2006) is also found in other blue tit populations and is therefore a general phenomenon. Knowledge of the variation in the heritability of crown coloration among populations may also help to explain some of the variability in sex ratio patterns found among different blue tit populations (Sheldon *et al.* 1999; Griffith *et al.* 2003; Dreiss *et al.* 2006; Korsten *et al.* 2006).

We studied the inheritance of crown coloration in a blue tit study population in the Netherlands (De Vosbergen). A serious complication in the assessment of the inheritance of blue tit crown colour is posed by the large within-individual temporal variations in crown coloration, which could potentially bias heritability estimates downwards. First, crown coloration shows dramatic seasonal changes probably due to feather wear and the accumulation of dirt after the annual moult (Örnborg *et al.* 2002; Delhey *et al.* 2006). Second, crown colour varies with individual age (Andersson *et al.* 1998; Delhey & Kempenaers 2006; see Chapter 7). Third, population-wide between-year differences in crown coloration can occur (Delhey *et al.* 2006).

Therefore, we corrected our measurements of crown coloration for seasonal variation, and variation due to age and year effects. Subsequently, we estimated the repeatability of crown colour measurements over time within individuals, both between different seasons within a single moulted plumage and between plumages produced in different annual moults. The repeatability of a trait within individuals sets an upper limit to its heritability, and thus indicates the scope for heritable variation to be present (Falconer & Mackay 1996). Against these background data, we

estimated the heritability of crown coloration based on parent-offspring regressions. Finally, we tested if an experimental manipulation of the crown UV coloration of the male parent affected crown coloration of the offspring.

METHODS

Study population and general field methods

The work was carried out on a nestbox population of blue tits at 'De Vosbergen' (53°08'N, 06°35'E), near Groningen, the Netherlands from 2001–2004. We caught adults during three periods (seasons) of the year: 1) 'winter', between 19 November–12 February 2001/02 and 2002/03; 2) 'early spring', between 25 March–22 April 2002 and 2003, in the period before egg laying [males only]; and 3) 'late spring', between 6 May–10 June 2001-2004, during chick feeding. All captured adults were ringed and blood sampled. Adults were sexed by the presence (= female) or absence (= male) of an incubation patch during the breeding season or based on molecular markers (primers P2 and P8; Griffiths *et al.* 1998). Unringed adults were aged as first year or older based on the colour of the primary coverts (Svensson 1992). We measured the reflectance of the crown feathers (see below for details on the procedure). All nestlings were individually ringed and blood sampled for sex identification. Based on their age at first catching, we classified individuals according to the following age classes: 1) first year/moult (= age 1); 2) second year/moult (= age 2); 1) older than second year/moult (= age 2+). Most birds were ringed as nestlings or as first year adults, and therefore we knew their exact age. Birds that were older than one year when ringed were assumed to be second year at ringing. The age class of these birds is therefore a minimum estimate. For further details on field methods and catching procedures see Korsten *et al.* (2006).

As part of other studies testing the causal links between brood sex ratio, parental brood provisioning and male sexual ornamentation (see Korsten *et al.* 2006), the UV reflectance of the crown plumage of some of the males was manipulated (after their crown reflectance had been measured) during the breeding seasons of 2002–2003 (2002: 49 of 72 males manipulated; 2003: 49 of 102 males manipulated). Manipulated males were either UV-reduced by applying a mixture of preen gland fat and UV-absorbing chemicals to the crown feathers, or control-treated with preen gland fat only. We excluded all crown reflectance measurements of these males from the time of UV manipulation until they had undergone their next annual moult (which takes place after the breeding season, between late May and late August; Cramp & Perrins 1993). The present analyses are based on 791 crown reflectance measurements from 421 adults (214 males, 207 females). This data set included 140 measurements of 95 adults that were born in our study population (60 males and 35 females from 68 broods) from known parents, which we used for the analyses on the inheritance of the crown coloration.

Measurements of crown reflectance

The spectral reflectance of the crown feathers was measured with a USB-2000 spectrophotometer with illumination by a DH-2000 deuterium-halogen light source (both Avantes, Eerbeek, The Netherlands). The measuring probe was held at a right angle against the plumage such that both illumination and recording were at 90° to the feathers. During each crown reflectance measurement we took five replicate readings and smoothed each of these reflectance spectra by calculating the running mean over 10 nm intervals. Following previous studies of blue tit crown coloration (Andersson *et al.* 1998; Sheldon *et al.* 1999; Griffith *et al.* 2003; Delhey *et al.* 2003; Korsten *et al.* 2006) we calculated three indices describing the variation in crown coloration – 'brightness', 'hue', and 'UV chroma' – from each reflectance spectrum, and averaged these across the five replicate spectra. 'Brightness' was the sum of reflectance between 320–700 nm ($R_{320–700}$), which corresponds to the spectral range visible to blue tits (Hart *et al.* 2000). 'Hue' was the wavelength of maximum reflectance, $\lambda(R_{max})$. 'UV chroma' was the sum of reflectance between 320–400 nm divided by the sum of reflectance between 320–700 nm ($R_{320–400} / R_{320–700}$). Both the 'hue' and 'UV chroma' indices have previously been identified as predictors of male attractiveness and viability in blue tits (Andersson *et al.* 1998; Sheldon *et al.* 1999; Delhey *et al.* 2003; Griffith *et al.* 2003).

The UV chroma, hue and brightness indices were significantly inter-correlated (UV chroma–hue: $r = –0.73$, $P < 0.0001$; UV chroma–brightness: $r = 0.16$, $P < 0.0001$; hue–brightness: $r = 0.09$, $P = 0.016$; all $n = 791$). We used principal component analysis (PCA) to summarize the information contained in the three colour indices and to reduce the number of colour variables in our analyses (following Siefferman *et al.* 2005). We performed PCA on correlation matrices without factor rotation and we extracted the first two PCs, which both had eigenvalues greater than one. PC1, describing 57.9% of total variance, had strong loading on both UV chroma (0.937) and hue (–0.922) (loading on brightness: 0.091), and thus indicates more UV chromatic and more UV shifted spectra with greater values. PC2, describing 34.5% of total variance, had a particularly strong loading on brightness (0.990), and thus indicates spectra with higher overall reflectance (achromatic brightness) with greater values (loading on UV chroma: 0.106; and hue: 0.205). We used PC1 and PC2 in further analyses of crown coloration. Because PC1 was strongly dependent on the two indices describing variation in UV reflectance, while PC2 mainly depended on achromatic brightness, we will refer to PC1 and PC2 as 'UV coloration' and 'achromatic brightness', respectively.

Temporal variation in crown coloration

Our crown colour data set contained repeated measures on individuals in different seasons (winter, early and late spring) and in different years (2001–2004). Variation due to age (Andersson *et al.* 1998; Delhey & Kempenaers 2006; Chapter 7), season (Örnborg *et al.* 2002; Delhey *et al.* 2006) and year effects (Delhey *et al.* 2006) could

cause temporal variation in crown colour measurements within individuals. We used multilevel statistical models that included individual identity as a random variable to examine the effects on crown coloration of the following (fixed) variables: 1) age (first moult, second moult, and above second moult); 2) season (winter, early or late spring); 3) date of capture within these periods (centred for mean catching date of that particular period); and 4) year in which the last autumn moult took place (2000–2003). We constructed models for each of the two dependent variables PC1 ('UV coloration') and PC2 ('achromatic brightness') and the two sexes separately to avoid unbalanced models due to the fact that no females were captured in the early-spring period. Full models including all explanatory variables were reduced by excluding variables in order of decreasing significance until only significant ($P < 0.05$) variables remained in the model. The second and third-order interactions of age, capture period and date were initially also entered in the full models, but we report significant interaction terms only (see Table 2.1). Non-significant third-order interactions were removed before removing second-order interactions and non-significant interactions of explanatory variables were removed before their main effects were excluded. Estimates of residual PC1 and PC2 values of individuals, controlled for the significant temporal variations in crown coloration, were derived from these models and used for analyses on the inheritance of crown coloration.

We calculated the repeatability of crown coloration between the different seasons for a single moulted plumage and also across moults at different ages. Repeatability estimates were calculated based on residuals derived from the multilevel models described above. Calculation was according to Lessells and Boag (1987) with standard errors calculated following Becker (1984).

Heritability of crown coloration

Estimates of the narrow-sense heritability (h^2) of crown coloration were calculated using parent–offspring regression (Falconer & Makay 1996). The majority of the 95 surviving offspring of which we had crown colour measurements as adults fledged from natural broods: 79 individuals from 58 broods from 2001–2003. In 2002, however, we cross-fostered 14 broods by swapping pairs of clutches with similar clutch size and hatching date at around the day of hatching, as part of another experiment in which we also manipulated UV coloration of the male parent during the chick feeding period (either UV-reduced or control-treatment; the cross-fostering and UV manipulation procedures were similar to the procedures described in Limbourg *et al.* 2004). We recaptured 16 offspring from 10 of these cross-fostered broods as adults and measured their UV coloration. Unfortunately, this small sample gave us insufficient statistical power to draw any conclusions about the inheritance of crown coloration in these offspring. Although some of the calculated heritability values were substantially greater than zero, none of them was significantly different from zero, due to the large standard errors (mid-offspring–mid-parent value of foster parents ($h^2 \pm$ SE; PC1: 0.02 ± 0.53, PC2: 0.16 ± 0.34; mid-offspring–mid-parent

value of biological parents: PC1: 0.50 ± 0.61, PC2: 0.34 ± 0.66). Therefore, we excluded the cross-fostered broods from further analyses.

Parent-offspring relationships were established by capture of both the male and female parent in the nestbox while feeding their chicks 7–15 days after hatching. For three males and two females which had offspring surviving into adulthood from two separate breeding attempts we only included the offspring of their first brood, leaving 53 broods for the analysis. Of 2 of these 53 broods, we identified only the female parent. Coloration of the male and female parent were not correlated (PC1: $r = 0.07$, $P = 0.61$; PC2: $r = 0.03$, $P = 0.82$, both $n = 51$), and therefore we did not need to correct our heritability estimates for assortative mating. Colour scores of the two sexes (both parents and offspring) were standardized to zero mean and unit standard deviation. For parents that had more than one offspring recaptured as an adult, mean colour of the offspring (mid-offspring colour) was used (sexes combined). Of the broods included in the analysis, the crown colour of 21 males had been manipulated in the period before egg laying (UV-reduced: $n = 12$, control: $n = 9$; see Korsten *et al.* 2006 for details on experimental procedures). We tested the effect of this manipulation on offspring UV coloration.

Multilevel models were carried out with MLwiN 2.02 using the IGLS estimation procedure. The significance of the explanatory variables was tested using the Wald statistic, which follows a χ^2-distribution. We used SPSS 13.0 for all other statistical tests. P values < 0.05 were considered significant.

RESULTS

Temporal variation in crown coloration: effects of age, season and year

Males ($n = 214$) had more UV-chromatic and UV-shifted crown plumage than females ($n = 207$) as indicated by their significantly higher PC1 scores ($\chi^2 = 516.74$, df = 1, $P < 0.0001$; Figure 1). Males also had higher achromatic brightness than females (PC2: $\chi^2 = 70.32$, df = 1, $P < 0.0001$; Figure 2.1).

While controlling for significant year effects, older individuals had more intense UV coloration (PC1) in both males and females (Table 2.1; Figure 2.1). Furthermore, PC1 scores were strongly dependent on season in both sexes, with highest PC1 scores in winter and early spring and strongly reduced scores during late spring (although no females were measured during early spring) (Table 2.1; Figure 2.1). In both sexes, PC1 was negatively related to the date of measurement during the different seasons (Table 2.1). The temporal patterns of achromatic brightness (PC2) were less straightforward (Figure 2.1). While controlling for significant year effects, male PC2 was not significantly related to age (Table 2.1). Males had higher PC2 scores in winter and early spring than in late spring. In females, there was a significant main effect of season on PC2 (Table 2.1). In addition, the interaction of age x season was significant, which is a reflection of the presence of a

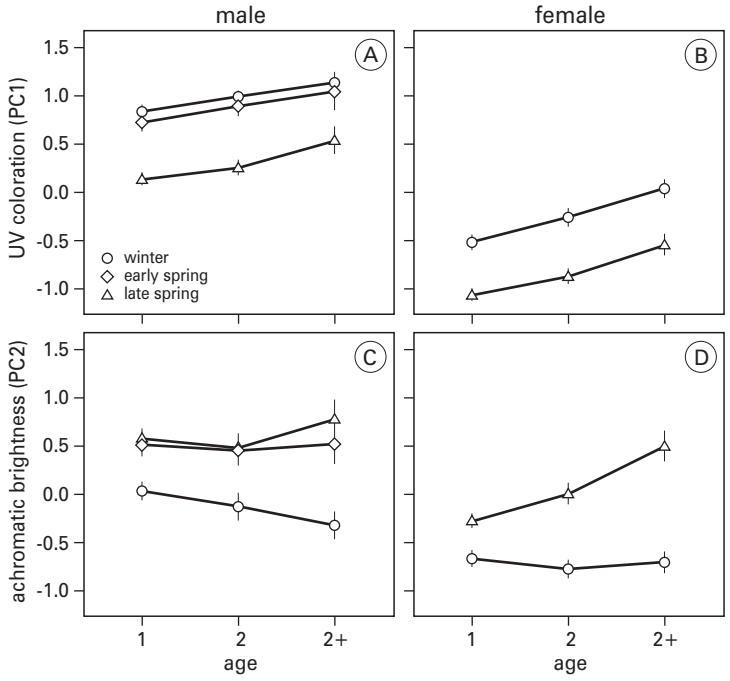


Figure 2.1 Blue crown UV coloration (PC1) and achromatic brightness (PC2) of males (A,C) and females (B,D) of different age classes measured during different periods of the year. Females were not measured in early spring. Graphs show mean values with standard errors. Based on 791 measurements of 421 individuals (214 males, 207 females). See also Table 2.1.

positive relationship of PC2 with age in late spring, but not in winter (Figure 2.1).

Residual values of PC1 and PC2 controlled for temporal variation, derived from the multilevel models described above (Table 2.1) were used to calculate estimates of repeatability and heritability.

Repeatability of crown coloration

The residual measures of male and female blue tit crown coloration were moderately repeatable, both between seasons within a single moulted plumage (Table 2.2) and across moults at different ages (Table 2.3). Repeatability values between seasons varied from 0.30–0.60 in males and from 0.18–0.35 in females (Table 2.2), which indicates that although part of the phenotypic variation is explained by consistent differences between individuals there remain large differences in within-individual changes in crown coloration over time. Repeatability estimates tended to be higher for crown UV coloration (PC1) than for achromatic brightness (PC2) and seemed to be higher for males than for females. Repeatability of crown coloration seemed also larger from winter to early spring, than from winter to late spring.

Table 2.1 Multilevel models of blue crown UV coloration (PC1; A,C) and achromatic brightness (PC2; B,D) of male (A,B) and female (C,D) blue tits as dependent variables and individual as random factor (based on 410 measurements of 214 males and 381 measurements of 207 females). See also Figure 1. Residuals derived from these models were used for calculation of repeatability and heritability estimates.

Variables	Wald (χ^2)	df	P
(A) Male UV coloration (PC1)			
Age	10.309	2	0.006
Season	143.540	2	< 0.0001
Day of season	19.381	1	< 0.0001
Year of last moult	41.682	3	< 0.0001
Explained variance: 31.2%			
(B) Male achromatic brightness (PC2)			
Season	76.810	2	< 0.0001
Day of season	110.748	1	< 0.0001
Year of last moult	37.365	3	< 0.0001
Explained variance = 27.8%			
(C) Female UV coloration (PC1)			
Age	34.261	2	< 0.0001
Season	67.695	1	<0.0001
Day of season	5.745	1	0.017
Year of last moult	31.687	3	< 0.0001
Explained variance = 28.6%			
(D) Female achromatic brightness (PC2)			
Age	2.571	2	0.28
Season	15.436	1	< 0.0001
Day of season	7.615	1	0.006
Year of last moult	13.333	3	0.004
Age x season	9.725	2	0.008
Explained variance = 20.9%			

Repeatability of crown coloration between plumages grown in different moults was of similar magnitude, and values varied between 0.12–0.52 in males and –0.08–0.47 in females; Table 2.3). Also the across-moult repeatability values tended to be higher for UV coloration (PC1) than for achromatic brightness (PC2; Table 2.3). Furthermore, repeatability values were higher in males than in females (Table 2.3). Furthermore, repeatability of achromatic brightness (PC2) was much higher from the first to the second year than from the second to the third year or older.

Table 2.2 Repeatability (*r*) of male and female blue tit crown colour measurements (UV coloration, PC1; achromatic brightness, PC2) between different seasons (winter, early and late spring) within one year, that is within a single moulted plumage. Repeatabilities were calculated for PC1 and PC2 scores controlled for within-individual variation due to effects of age, season, day of season, and year of last moult (see Table 2.1). Males measured in early spring were not re-measured in late spring. No females were measured in early spring. Multiple measurements of the same individuals in one season were averaged. Of individuals that were measured in more than one year, only measurements of the first year were included.

		r ± SE	*F*	df	*P*
Male	UV coloration (PC1)				
	Winter–early spring	0.60 ± 0.11	4.035	35, 36	< 0.0001
	Winter–late spring	0.47 ± 0.15	2.781	25, 26	0.006
	Achromatic brightness (PC2)				
	Winter–early spring	0.56 ± 0.12	3.528	35, 36	0.0001
	Winter–late spring	0.30 ± 0.18	1.844	25, 26	0.064
Female	UV coloration (PC1)				
	Winter–early spring	-	-	-	-
	Winter–late spring	0.35 ± 0.13	2.081	46, 47	0.007
	Achromatic brightness (PC2)				
	Winter–early spring	-	-	-	-
	Winter–late spring	0.18 ± 0.14	1.313	46, 47	0.18

Table 2.3 Repeatability (*r*) of male and female blue tit crown colour (UV coloration, PC1; achromatic brightness, PC2) at different ages (1–2+) across moults. Repeatabilities were calculated for PC1 and PC2 scores controlled for within-individual variation due to effects of age, season, day of season, and year (see Table 2.1). Multiple measurements of one individual at the same age were averaged.

		r ± SE	*F*	df	*P*
Male	UV coloration (PC1)				
	age 1–2	0.52 ± 0.13	3.145	29, 30	0.001
	age 2–2+	0.49 ± 0.15	2.885	26, 27	0.004
	Achromatic brightness (PC2)				
	age 1–2	0.42 ± 0.15	2.445	29, 30	0.009
	age 2–2+	0.12 ± 0.19	1.265	26, 27	0.26
Female	UV coloration (PC1)				
	age 1–2	0.41 ± 0.14	2.416	35, 36	0.005
	age 2–2+	0.26 ± 0.17	1.694	29, 30	0.08
	Achromatic brightness (PC2)				
	age 1–2	0.47 ± 0.13	2.763	35, 36	0.002
	age 2–2+	–0.08 ± 0.18	0.843	29, 30	0.68

Heritability of crown coloration

Crown UV coloration (PC1) showed significant heritable variation as indicated by the heritability estimate derived from mid-parent–offspring regressions (h^2 = 0.41 ± 0.17 SE, $F_{1,49}$ = 5.973, P = 0.018; Figure 2.2). The heritability estimate for the achromatic brightness (PC2) of crown coloration was somewhat lower, and bordered significance (h^2 = 0.23 ± 0.13 SE, $F_{1,49}$ = 3.317, P = 0.075; Figure 2.2). Male UV manipulation – either unmanipulated (n = 30), UV-reduced (n = 12), or control (n = 9) – had no effect on offspring UV coloration (PC1: $F_{2,48}$ = 0.919, P = 0.41) or achromatic brightness (PC2: $F_{2,48}$ = 0.224, P = 0.80).

We also derived heritability values from separate mother–offspring and father–offspring regressions to assess if the inheritance of crown coloration was sex-specific. Heritability values derived from single parent–offspring regressions are

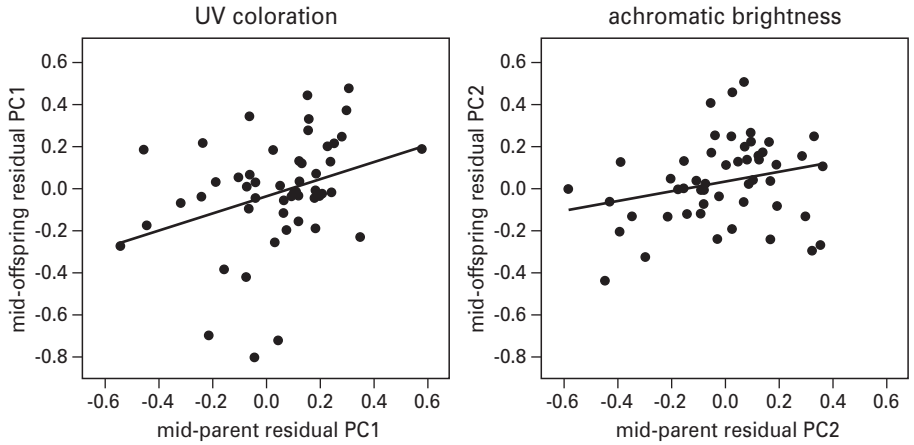


Figure 2.2 Mid-parent–mid-offspring regressions of residual blue crown UV coloration (PC1) and achromatic brightness (PC2). For calculation of residuals see Table2.1.

Table 2.4 Possible sex-linked inheritance or maternal effects influencing offspring crown coloration in blue tits.

		Father		Mother	
		h^2 ± SE	n	h^2 ± SE	n
UV coloration (PC1)	Son	0.44 ± 0.30	39	0.44 ± 0.38	40
	Daughter	0.23 ± 0.23	25	0.26 ± 0.30	28
Achromatic brightness (PC2)	Son	0.03 ± 0.18	39	1.06 ± 0.35[#]	40
	Daughter	0.11 ± 0.17	25	0.04 ± 0.30	28

[#] < 0.005

estimated as twice the coefficient (slope) of the regression and the accompanying standard errors are calculated by doubling the standard errors for the regression coefficient, which leads to relatively high standard errors for the h^2 estimates compared to estimates based on mid-parent–offspring regressions (Falconer & Mackay 1996; Table 2.4). Given the large standard errors, our sample size proved to be too limited to draw firm conclusions regarding sex-specific inheritance. Nevertheless we briefly comment on the results, which are presented in table 2.4.

Although, none of the h^2 estimates for UV coloration (PC1) was significantly different from zero, h^2 values were substantial (between 0.23–0.44; Table 2.4) and similar to the significant estimate derived from the mid-parent–mid-offspring regression ($h^2 = 0.41 \pm 0.17$ SE) presented above. Heritability estimates seemed to be higher for the variation in UV coloration in sons than in daughters, but did not differ between the male and female parent (Table 2.4). The pattern of inheritance of achromatic brightness (PC2) appeared more complex. There was a very high and significant heritability of achromatic brightness for the mother–son relationship ($h^2 = 1.06 \pm 0.35$ SE, $F_{1,38} = 9.371$, $P = 0.004$), whereas the mother–daughter, and father–son or daughter relationships all showed very low h^2 values (between 0.03–0.11), which were far from significant (all $P > 0.54$).

DISCUSSION

Our results show the presence of significant heritable variation for the sexually selected UV/blue crown plumage in our blue tit study population. Offspring resemble their parents in UV coloration (PC1; $h^2 = 0.41 \pm 0.17$ SE). In addition, there was a suggestion of heritable variation in achromatic brightness (PC2; $h^2 = 0.23 \pm 0.13$ SE), but the heritability estimate only bordered significance. This lack of significance could be due to the limited power of our analysis, which was based on relatively few broods (compare to *e.g.* Hadfield *et al.* 2006). Similar studies have shown heritable variation for pigment-based plumage ornaments (both for carotenoids and melanins) (*e.g.* Hill 1991; Norris 1993; Møller 1989; Qvarnström 1999; but see Griffith *et al.* 1999a), but the present study is, to our knowledge, the first to report heritable variation in a structurally based plumage ornament. This result is important as it confirms one of the main underlying assumptions of several studies on sex allocation and differential parental investment in relation to male attractiveness in the blue tit (Sheldon *et al.* 1999; Griffith *et al.* 2003; Limbourg *et al.* 2004; Johnsen *et al.* 2005; Korsten *et al.* 2006). We are aware that due to lack of cross-fostering in our study we are not able to separate genetic and early environmental effects, and therefore our heritability estimates may be inflated. However, it should be noted that the predictions of both verbal and mathematical models of sex allocation and differential parental investment in response to male attractiveness are not dependent on whether the resemblance between fathers and offspring is exclusively due to genetic

inheritance or is also caused by the presence of common environmental or parental effects (Sheldon *et al.* 2000; Fawcett *et al.* 2006). Additional experiments, using a cross-fostering design, would reliably separate the genetic and environmental components of the variation in crown coloration in our study population.

Another limitation of our study is that we have no information on extra-pair paternity for the full data-set. Extra-pair paternity may cause a downward bias of the heritability estimates for the male–offspring relationship. However, extra-pair paternity rates are generally relatively low in blue tits (10–15%; Kempenaers *et al.* 1997; Krokene *et al.* 1998; Leech *et al.* 2001, Delhey *et al.* 2003; P. Korsten, C. M. Lessells, A. C. Mateman, J. Komdeur, unpublished data; see also Box D) and such a low rate of extra-pair paternity should result in only a slight underestimation of heritability (Charmantier & Réale 2005).

Our results are in direct contrast to the outcome of a recent study also investigating the inheritance of blue tit crown coloration, which found no significant heritable variation in crown coloration (Hadfield *et al.* 2006; $h^2 < 0.11$). At this stage we can only speculate what factors could have been responsible for the difference. There are several differences in methodology between the studies that may have played a role. Most importantly, Hadfield *et al.*(2006) used large-scale cross-fostering (in combination with an 'animal model' approach for estimating heritability; see Kruuk 2004) to separate genetic and environmental effects, whereas we did not. Although we were therefore not able to separate genetic and early environmental effects, the lack of cross-fostering in our study may have increased the probability of detecting a significant resemblance in crown coloration between parents and offspring particularly if small-magnitude genetic and environmental effects act in the same direction.

Another difference between the two studies lies in the quantification of the variation in crown reflectance spectra. Hadfield *et al.* (2006) quantified this variation using 'colour contrasts' based on a biophysical model of photon catches by the blue tit visual receptors (Vorobyev *et al.* 1998; Endler & Mielke 2005), whereas we used more conventional 'objective colorimetrics' which describe specific variation in the shape of the reflectance curves (Andersson & Prager 2006; Montgomerie 2006). However, we believe that this difference in the type of estimation itself is unlikely to account for the different results, because we expect that the type of contrast values used by Hadfield *et al.* (2006) in practice largely capture the same variation in crown reflectance spectra as our 'UV chroma' and 'hue' indices (which were summarized by PC1). It should be noted, however, that we also calculated heritability estimates for the variation in achromatic brightness (PC2); Hadfield *et al.* (2006) removed this variation prior to their calculation of 'colour contrasts'. There is substantial variation in achromatic brightness of the blue tit crown plumage (Figure 2.1) and birds are likely to be able to perceive such variation (Schaefer *et al.* 2006), although probably not with the single cones used for colour discrimination, but with an additional type of receptor cells called double cones (Jones & Osorio 2004). Hadfield *et al.*

(2006) have therefore excluded a potentially important aspect of the blue tit's crown coloration from their analyses. Indeed we found a tendency for heritable variation in this component in our population.

After controlling for year effects, crown colour appeared to be extremely variable within individuals (males and females), and showed strong seasonal shifts, with decreasing intensity of UV coloration (PC1) and increasing achromatic brightness (PC2) with the progression of time after moult. This strong seasonal pattern in crown coloration, which was comparable in magnitude to the sexual dichromatism, was also found in other blue tit populations and is probably due to wear of the crown feathers (Örnborg *et al.* 2002; Delhey *et al.* 2006). Furthermore, colour variation related to individuals' age existed in our population. Older birds (males and females) had slightly more intense UV coloration (PC1) than young birds (*cf* Andersson *et al.* 1998; Delhey & Kempenaers 2006; Chapter 7). Achromatic brightness (PC2) was not related to age in males, but during late spring achromatic brightness was positively related to age in females. After correcting for the large within-individual variation in crown colour, both the intensity of UV coloration and achromatic brightness were moderately repeatable between different periods of the year within a single moulted plumage and between plumages of different moults, indicating the potential for a heritable component of crown coloration, as we indeed found. The fact that the repeatability of blue tit crown coloration was relatively low, however, also indicates the importance of collecting multiple measurements on the same individuals over a year as this will improve the accuracy of crown colour estimates for individual birds (Falconer & Mackay 1996). In general, the large within-individual variability of blue tit crown coloration could potentially obscure interesting patterns or even create spurious relationships and should thus be taken into account when studying blue tit crown coloration.

It is unknown what the functional significance of the large-within individual colour changes are, in particular between seasons. The decline of UV coloration over the year may be adaptive, and related to a shifting balance between benefits of a strong UV signal used in mate attraction during winter and early spring and costs such as the risk of detection by predators. For example, male rock ptarmigans (*Lagopus mutus*) soil their conspicuous white breeding plumage with dirt as soon as mating has taken place to increase crypsis (Montgomerie *et al.* 2001). On the other hand it has been hypothesised that the magnitude of the decrease in UV coloration after moult may be related to individual quality (Delhey *et al.* 2006). According to this idea high quality individuals are better able to prevent their plumage from degradation, because they can invest more time and energy in maintenance of their feathers (*e.g.* in the form preening behaviour). Indeed, the seasonal change in hue was negatively related to a measure of body size (tarsus length), whereas the loss of body mass between winter and spring was related to the magnitude of the decline in UV chroma, supposedly indicating a cost of feather maintenance (Delhey *et al.* 2006). However, female extra-pair mate choice was not related to variation in the

magnitude of the seasonal declines (Delhey *et al.* 2006). Clearly, further research is needed to better understand the causes and consequences of the large within-individual variation in blue tit UV coloration.

An important question is how the between-individual variation (which appeared heritable in our population) and in particular the sexual dichromatism in crown coloration is maintained. The sexual dichromatism indicates that selective pressures on crown coloration differ between the sexes. The sexual dichromatism could then be viewed as the result of a different balance in the costs (*e.g.* increased predation risk) and benefits (*e.g.* more successful mate attraction) of crown UV coloration in males and females. If selective pressures on male and female crown colour expression counteract each other to some extent, then the observed level of female UV coloration may be the result of a non-adaptive genetic correlation with male ornamentation, combined with incomplete sex-limitation of ornament expression (Kraaijeveld *et al.* submitted). Such a situation has been described for the ornamental red bill coloration of zebra finches (*Taeniopygia guttata*; Price & Burley 1993, 1994; Price 1996). In zebra finches, males with redder bills are more successful in mate attraction and have higher fitness, while in females individuals with more orange (less red) bills have higher fitness (Price & Burley 1994). This leads to a situation in which there is sexually antagonistic selection (Rice 1992) on bill coloration, with males selected to become more red and females to become more orange (Price & Burley 1994). A genetic correlation between bill coloration in males and females prevents the evolution of more pronounced sexual dichromatism (Price & Burley 1993, 1994; Price 1996). It is not unlikely that a similar situation exists for the blue tit's crown coloration. It is interesting in this respect that we found some suggestion of sex-linked inheritance (or a maternal effect) affecting one aspect of crown colour expression (Table 2.4), which might be expected in case of such sexually antagonistic selective pressures (Merilä & Sheldon 2001). The possibility that there is sexually antagonistic selection on blue tit crown coloration certainly deserves further attention, and this idea could be tested by a quantification of the fitness consequences of variation in crown coloration in both males and females.

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PART II

Female reproductive adjustment to male UV coloration


Echtes
Entenbürzel-
Fluegenfett

CHAPTER 3

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

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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.

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 & McGraw 2006a). Descriptive studies have implicated UV plumage and other coloration in sexual selection, through intra-sexual competition (*e.g.* Senar *et al.* 1993, Siefferman & 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 3.1). 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

Table 3.1 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 days)	Response to treatment	Description of response	Ref.
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.

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 days (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 days before hatching and again when the chicks were 7 days old. Subsequently, female provisioning behaviour was measured when the chicks were 10 and 14 days old (Limbourg *et al.* 2004; see 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 3.1).

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 days (8 UV-reduced males, 8 controls) or ≥ 28 days 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 x 4 x 3 [height] 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 & 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.

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 (Figure 3.1). The UV reduction treatment clearly reduced the reflectance between 320–400 nm, whereas the control treatment did not (Figure 3.1). So treatment reduced UV chroma by 38% compared to pre-treatment values (paired t-test: t = 28.90, df = 41, P < 0.001; Figure 3.2), a value 24% and 6% below the natural range of UV chroma for males and females, respectively (Figure 3.2). UV chroma was not affected by the control treatment (t = 1.63, df = 42, P = 0.11; Figure 3.2). The change in spectral profile (Figure 3.1) resulting from the UV-reduction treatment also increased the wavelength at peak reflectance (mean λ_{max} ± SE, before: 381 ± 2.2 nm, after: 418 ± 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} (λ_{max} ± SE, before: 377 ± 1.8 nm, after: 368 ± 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

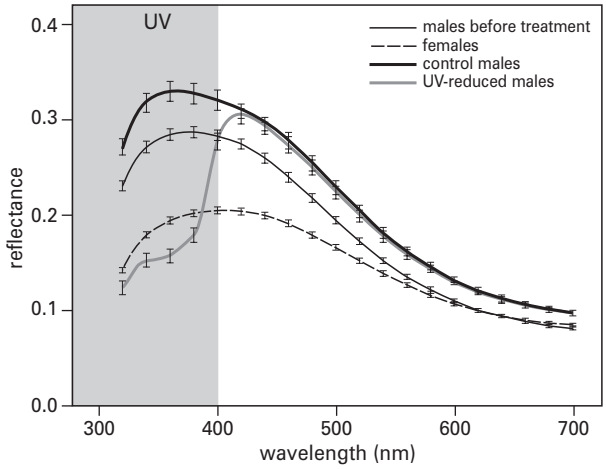

Figure 3.1 Mean reflectance curves of the crown plumage of wild male blue tits before manipulation (n = 85), after UV reduction (n = 42), and after control treatment (n = 43). The mean reflectance curve for unmanipulated females (n = 169) is shown for reference. Standard errors are depicted at 20-nm intervals. The shaded area indicates the UV part of the spectrum.

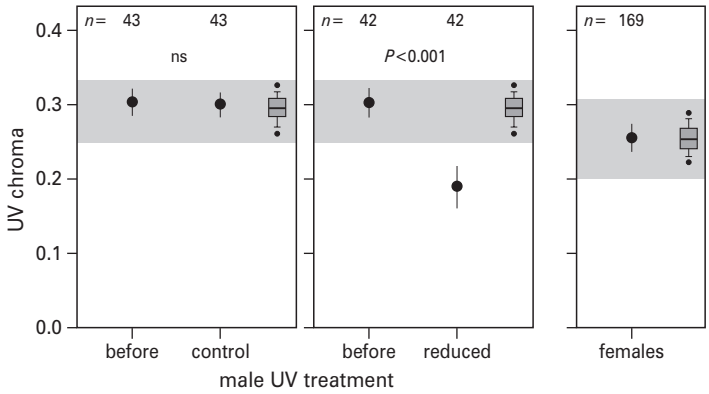

Figure 3.2 Mean UV chroma of crown plumage of wild male blue tits before and after UV reduction and control treatment. Mean UV chroma of unmanipulated females (n = 169) is shown for reference. Whiskers indicate standard deviations. Shaded areas indicate natural ranges of UV chroma of males (n = 111; range 0.2485–0.333) 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).

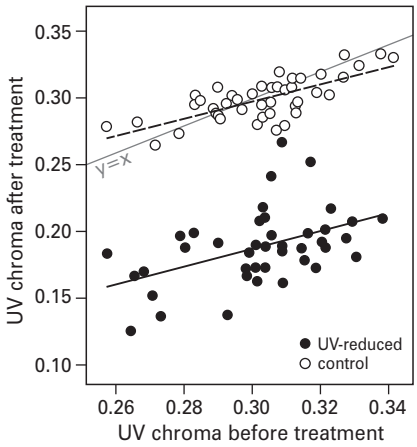

Figure 3.3 UV chroma of crown plumage of individual wild male blue tits before and after UV reduction (n = 42) or control treatment (n = 43). Lines are linear regressions on each treatment group separately.

(UV-reduced: r = 0.47, n = 42, P = 0.002; control: r = 0.72, n = 43, P < 0.001; Figure 3.3), and the slopes of the relationships did not differ between the groups (ANCOVA with UV chroma after treatment as response variable: UV treatment x UV chroma before treatment: $F_{1,81}$ = 0.007, P = 0.93; Figure 3.3).

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 (Figure 3.4A). 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 (Figure 3.4A), and the reduction in UV chroma (compared to pre-treatment values) was no longer significant 6 days after treatment (Figure 3.4A). Wild birds showed a similar pattern (Figure 3.4B), 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; Figure 3.4B), while both values were within the natural range (Figure 3.4B). 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; Figure 3.4B). 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).

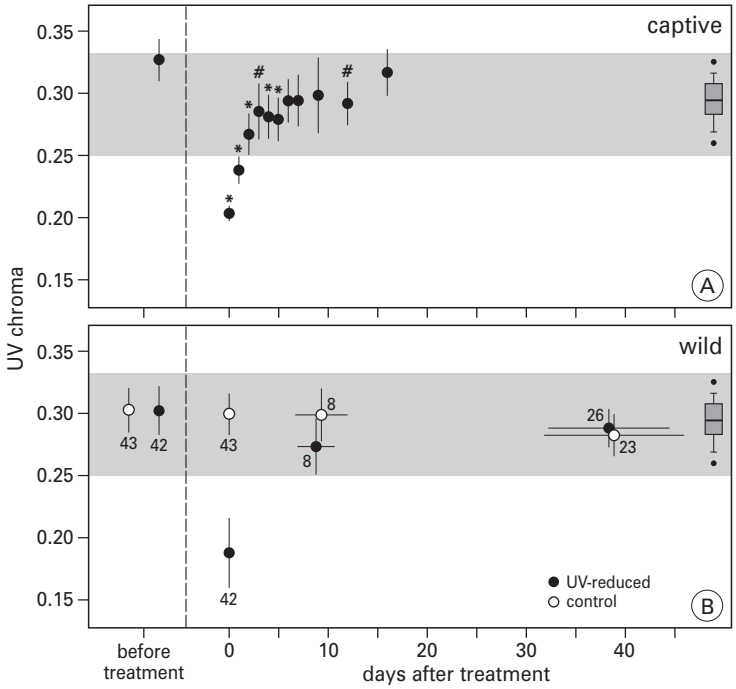

Figure 3.4 Temporal changes of crown UV chroma after UV-reduction treatment of (A) captive and (B) wild male blue tits. (A) Mean UV chroma (± SD) of 4 individual UV-reduced males who were repeatedly measured. Differences in UV chroma from pre-treatment values were tested with one-tailed paired t-tests (# $P < 0.05$; * $P < 0.01$). (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).

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 days in wild birds. Importantly, mean UV chroma values are outside the natural range for only a short period (less than 2 days 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, personal observation) and the treatment never led to divorce (see Korsten *et al.* 2006). The treatment was reversible (no UV reduction effect detectable after approximately 28 days) 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 & 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.

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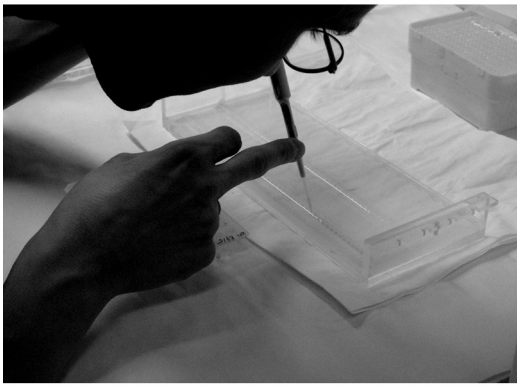

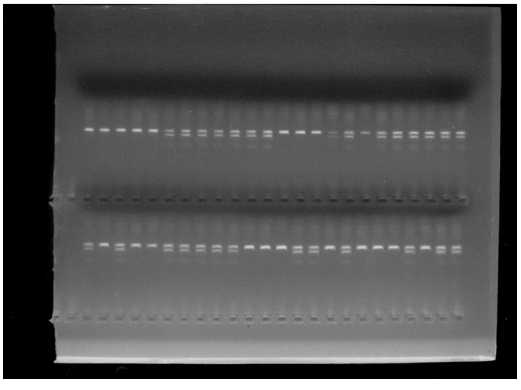

CHAPTER 4

Primary sex ratio adjustment to experimentally reduced male UV attractiveness

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A. Christa Mateman, Marco van der Velde &
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Behavioral Ecology (2006) 17: 539–546

ABSTRACT

The study of primary sex ratio adjustment in birds is notorious for inconsistency of results among studies. To develop our understanding of avian sex-ratio variation, experiments that test a *priori* predictions and the replication of previous studies are essential. We tested if female blue tits *Parus caeruleus* adjust the sex ratio of their offspring to the sexual attractiveness of their mates, as was suggested by a previous benchmark study on the same species. In two years we reduced the ultraviolet (UV) reflectance of the crown feathers of males in the period before egg-laying to decrease their attractiveness. In contrast to the simple prediction from sex allocation theory we found that the overall proportion of male offspring did not differ between broods of UV-reduced and control-treated males. However, in one year the UV-treatment influenced offspring sex ratio depending on the natural crown UV reflectance of males before the treatment. The last result confirms the pattern found in the previous blue tit study, which suggests that these complex patterns of primary sex ratio variation are repeatable in this bird species, warranting further research into the adaptive value of blue tit sex ratio adjustment to male UV coloration.

INTRODUCTION

Sex allocation theory has been very successful in explaining and predicting patterns of adaptive primary sex ratio variation especially in invertebrate taxa, for example haplodiploid insects (Godfray & Werren 1996; West *et al.* 2000). However, when applied to primary sex ratio variation in vertebrates with chromosomal sex determination, such as birds, the explanatory success of sex allocation theory seems modest (Williams 1979; Clutton-Brock 1986; Krackow 2002; Ewen *et al.* 2004; but see West & Sheldon 2002; West *et al.* 2005). In birds, females are the heterogametic sex, which potentially gives them control over the sex of the gametes they produce (Krackow 1995; Oddie 1998). However, although the results of many studies follow the predictions of sex allocation theory and therefore suggest adaptive primary sex ratio adjustment in several species of birds (*e.g.* Dijkstra *et al.* 1990; Daan *et al.* 1996; Ellegren *et al.* 1996; Komdeur *et al.* 1997; Nager *et al.* 1999; Pen *et al.* 1999; Kalmbach *et al.* 2001), there are also many studies not providing any evidence for adaptive primary sex ratio variation, despite adequate sample sizes (*e.g.* Newton & Marquiss 1979; Koenig & Dickinson 1996; Westerdahl *et al.* 1997; Leech *et al.* 2001; Budden & Beissinger 2004). Furthermore, results have been inconsistent between studies within the same species and may differ between different years or different populations (*e.g.* Lessells *et al.* 1996; Kölliker *et al.* 1999; Radford & Blakey 2000; Verboven *et al.* 2002). The notorious inconsistency among avian sex allocation studies has led to critical views on how general a phenomenon adaptive primary sex ratio adjustment is in birds (Radford & Blakey 2000; Krackow 2002; Komdeur & Pen 2002) and several authors have suggested the existence of a publication bias in favour of positive evidence (Hasselquist & Kempenaers 2002; Krackow 2002; Ewen *et al.* 2004).

Interestingly, two recent meta-analyses of the literature on avian sex ratio variation came to opposing conclusions on the generality of facultative primary sex ratio adjustment in birds. West and Sheldon (2002), who restricted their analysis to studies with clear *a priori* predictions, concluded that birds can show strong sex ratio shifts. However, Ewen *et al.* (2004) who conducted a more extensive meta-analysis, which also included studies with weaker *a priori* predictions, found no evidence for the general occurrence of avian primary sex ratio adjustment. Nevertheless, Ewen *et al.* (2004) identified a few influential case studies that showed particularly large effect sizes, but it is unclear if these individual studies represent rare biological exceptions in which the study species indeed exhibits sex ratio control, or whether these studies represent false positives (*i.e.* statistical type-I errors). The majority of published avian sex ratio studies to date is correlative (*e.g.* only 7 out of 40 studies used in the meta-analysis by Ewen *et al.* [2004] were experimental; see also Komdeur & Pen 2002), and the inclusion of correlative studies giving *post hoc* adaptive explanations is likely to lead to type-I statistical errors, publication bias and complications in the application of meta-analysis (Palmer 2000; Gurevitch *et al.* 2001; West & Sheldon 2002; Ewen *et al.* 2004). To gain further

insight into avian sex ratio variation, experimental studies that test clear *a priori* predictions concerning causal relationships between sex ratio and the variables under investigation are needed. Furthermore, it is crucial to replicate key studies to evaluate the robustness and generality of the patterns found (Palmer 2000; Griffith *et al.* 2003). However, real replicates (*i.e.* replicates in the same species) of existing studies are scarce (Palmer 2000).

In this paper we report a replicate of the highly influential benchmark study by Sheldon *et al.* (1999), which suggested facultative primary sex ratio adjustment in response to experimental variation in male attractiveness in a wild blue tit *Parus caeruleus* population. The study of Sheldon *et al.* (1999) was based on the hypothesis by Trivers and Willard (1973) that it would be adaptive for individuals to adjust the relative investment in offspring of the different sexes in response to any physiological or ecological variable influencing the relative fitness of sons and daughters. Blue tits mostly form socially monogamous pairs during breeding, but nevertheless regularly engage in extra-pair copulations leading to roughly 10–15% of all offspring being sired by an extra-pair male (Kempenaers *et al.* 1997; Leech *et al.* 2001; Delhey *et al.* 2003; P. Korsten, C.M. Lessells, A.C. Mateman & J. Komdeur, unpublished data). Therefore, female blue tits paired to sexually attractive males are predicted to bias the sex ratio of their offspring towards sons, because – given the option of pursuing extra-pair matings – sons would benefit more than daughters from inheriting their father's attractiveness. Conversely, females paired to less attractive males should produce female-biased sex ratios, as their sons may suffer from increased rates of cuckoldry (Sheldon *et al.* 1999; see also Burley [1981] for rationale).

The ultraviolet (UV) reflectance of the bright blue crown feathers of male blue tits is an important cue in both social and extra-pair mate choice (Andersson *et al.* 1998; Hunt *et al.* 1998; Delhey *et al.* 2003). Furthermore, male survival is positively correlated to male crown UV reflectance, whilst the proportion of male offspring is positively correlated to male survival (Svensson & Nilsson 1996; Sheldon *et al.* 1999; Griffith *et al.* 2003). In line with these findings, Sheldon *et al.* (1999) found that the proportion of male offspring was positively correlated to natural variation in male crown ultraviolet reflectance. However, an experimental reduction of the UV reflectance of males – making them unattractive – before their mates had started egg laying did not lead to a lower proportion of sons in broods of UV-reduced males compared to control males (Sheldon *et al.* 1999). Instead, the UV reduction reversed the positive correlation between the sex ratio and natural male UV reflectance so that the proportion of sons decreased with increasing pre-treatment UV reflectance. This experimental result was unexpected and lacks a good biological explanation as the most straightforward prediction from Trivers and Willard's (1973) hypothesis is that females paired to UV-reduced – unattractive – males should produce a lower overall proportion of sons than control females (Burley 1981). Sheldon *et al.*'s (1999) results, which were based on a single breeding season, were partly corroborated by correlative data collected in the same population

during two additional breeding seasons, but the UV-reduction experiment was not repeated (Griffith *et al.* 2003). In contrast to the studies above, Leech *et al.* (2001), who conducted a large-scale study in a different blue tit population and measured male survival, extra-pair mating success and offspring sex ratios – but not crown UV reflectance – found no significant relationships between offspring sex ratio and any of the variables measured. It is unclear whether these inconsistent results reflect genuine differences between blue tit populations or study years, or are caused by statistical type I or type II errors (Griffith *et al.* 2003).

The aim of this study was to exactly replicate the study by Sheldon *et al.* (1999) to evaluate the robustness and generality of the intriguing combined effect of the UV manipulation and male pre-manipulation UV reflectance on offspring sex ratio. Therefore, we reduced crown UV reflectance of male blue tits before their mates had started egg laying and determined the resulting offspring sex ratios in two years, while closely following the experimental protocol of Sheldon *et al.* (1999). We tested: 1) if overall sex ratio was more female-biased for the UV-reduced than for the control group as predicted by the Trivers and Willard (1973) hypothesis (Burley 1981); 2) if the effect of the male UV treatment on sex ratio depended on UV reflectance before treatment as was found by Sheldon *et al.* (1999).

METHODS

Study population and general field methods

We carried out the UV manipulation experiment in a nestbox population of blue tits in 'De Vosbergen' (53°08'N, 06°35'E), near Groningen, the Netherlands, during the breeding seasons of 2002 and 2003. The blue tit breeding population in De Vosbergen was monitored from 2001–2003. During this period all breeding adults were captured in their nestboxes when feeding nestlings, mostly between days 6 and 14 (where day of hatching of the first nestling = day 0). We sexed adults by the presence (= female) or absence (= male) of an incubation patch, and aged them as first year or older (see Svensson 1992). We also measured mass (± 0.1 g), length of tarsus (± 0.1 mm) and third primary feather (± 0.5 mm), and the reflectance of the crown feathers (see below for details on the procedure). We ringed and blood sampled all adults caught. We took small blood samples (*ca.* 10 μl) from the nestlings on day 4, and unhatched eggs and nestlings found dead before blood sampling were collected, for molecular determination of sex using Griffiths *et al.*'s (1998) P2 and P8 primers. Molecular sexes of 81 nestlings and 231 adults were confirmed (no mismatches) using field observations of the same individuals when breeding.

Measurements of crown reflectance

We measured the reflectance of the crown feathers using a USB-2000 spectrophotometer with a DH-2000 deuterium-halogen light source (both Avantes, Eerbeek,

The Netherlands). The measuring probe was held at a right angle against the plumage, *i.e.* both illumination and recording were at 90° to the feathers. During each crown reflectance measurement we took 5 replicate readings and smoothed each of these reflectance spectra by calculating the running mean over 10 nm intervals. Following previous studies of UV colour signalling in blue tits (*e.g.* Sheldon *et al.* 1999; Delhey *et al.* 2003; Griffith *et al.* 2003) we calculated indices of the three main dimensions of colour perception – brightness, hue, and chroma (Hailman 1977) – and averaged these across the 5 replicate spectra. Brightness (spectral intensity) was the sum of reflectance between 320–700 nm ($R_{320–700}$), which corresponds to the spectral range visible to blue tits (Hart *et al.* 2000). Hue (spectral location) was the wavelength of maximum reflectance, $\lambda(R_{max})$. As an index of chroma (spectral purity) we used 'UV chroma', which was the sum of reflectance between 320–400 nm divided by the sum of reflectance between 320–700 nm ($R_{320–400}$ / $R_{320–700}$). Crown colour measurements were repeatable within individuals between separate days of capture within a single breeding season (mean number of days between captures: 4.1 ± 2.4 SD; repeatability brightness = 0.60, $F_{14,15} = 4.04$, $P = 0.006$; repeatability hue = 0.65, $F_{14,15} = 4.74$, $P = 0.002$; repeatability UV chroma = 0.70, $F_{14,15} = 6.02$, $P < 0.001$; see Lessells & Boag 1987).

Manipulation of male crown UV reflectance

We manipulated the crown UV reflectance of male blue tits before their females started egg laying. Males were captured near nestboxes containing completed nests with mistnets using song playback and a mounted male blue tit specimen as a decoy. Males were assigned sequentially to the UV-reduction or control treatment. To reduce crown UV reflectance we used a previously developed method (Andersson & Amundsen 1997; Sheldon *et al.* 1999; Limbourg *et al.* 2004) in which a mixture of UV blocking chemicals (Parsol 1789 and MCX, Roche, Switzerland) and duck preen gland fat (fishing fly dressing, purchased at Euro-Fly, Paris, France) was applied to the males' crown feathers. As a control treatment we applied duck preen gland fat only. Crown reflectance was measured immediately before and after treatment. The UV-reduction treatment was effective in reducing UV reflectance (comparison with pre-treatment UV chroma: paired $t_{36} = 30.0$, $P < 0.0001$), whereas the control treatment did not affect UV reflectance (UV chroma: paired $t_{30} = 1.56$, $P = 0.13$; Figure 4.1). The gloss of the preen fat produced a slight uniform increase in reflectance in both treatments (Figure 4.1). The UV reduction caused by the UV reduction treatment is known to decrease with time, but was still detectable after about 10 days in a previous study on blue tits using the same technique (Limbourg *et al.* 2004). During nestling feeding (*ca.* 30–50 days after the treatment) there was no longer a difference between UV-reduced and control-treated males (P. Korsten & J. Komdeur, unpublished data). Because the effect of the UV-reduction treatment diminished over time we aimed to have a similar interval

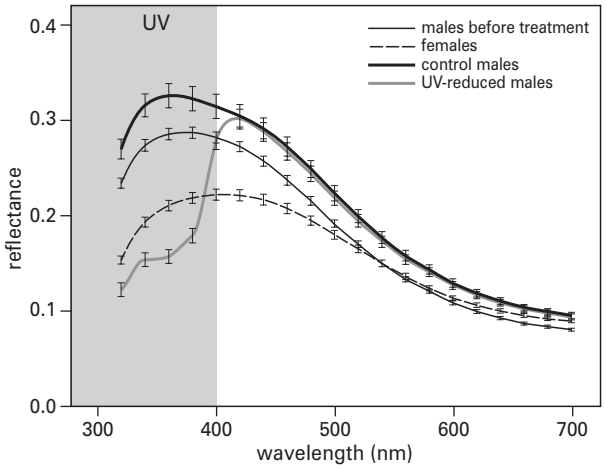

Figure 4.1 Mean crown reflectance curves of male blue tits before UV manipulation (n = 70), after UV reduction (n = 37) and after control treatment (n = 31), and of female blue tits captured during nestling provisioning (n = 70), for the years 2002 and 2003 combined. Crown reflectance of two control-treated males was not re-measured after treatment. Standard errors around the means are depicted at 20 nm intervals. The shaded area indicates the UV part of the spectrum.

between male UV treatment and laying of the first egg by their mates as Sheldon *et al.* (1999) (Sheldon *et al.* 1999: 10 days ± 5.2 SD; this study: 2002: 4.4 days ± 2.9 SD; 2003: 8.0 days ± 6.5 SD).

We manipulated crown UV reflectance of 84 males, 35 in 2002, 49 in 2003. Of these, 70 males were included in our analyses: 26 in 2002 (13 UV-reduced, 13 control) and 44 in 2003 (24 UV-reduced, 20 control). The other UV-manipulated males were excluded for the following reasons: seven males were not recaptured during nestling provisioning, and could therefore not be assigned with certainty to a specific brood, five males turned out to have been UV-manipulated after their female had started egg laying, one male was polygynous, and the clutch of one male was destroyed before hatching due to vandalism. Males of both treatment groups did not differ in age (Yates' corrected $\chi^2_1 = 0.826$, $P = 0.36$), body size (mass, and tarsus and third primary length: t-tests, all $P > 0.33$) or pre-treatment crown colour (brightness, hue, UV chroma: t-tests, all $P > 0.72$). There was also no difference between the treatment groups in subsequent clutch size ($t_{68} = 0.12$, $P = 0.90$) or laying date of the first egg ($t_{68} = 0.16$, $P = 0.87$). On eighteen occasions we also captured a female when mistnetting the male. Both members of all these putative pairs were recaptured in the same nestbox when provisioning the nestlings, indicating that pair formation had taken place before the experimental treatment, and that the treatment did not lead to divorce.

Data analyses

To test whether the distributions of sex ratios (*i.e.* the proportions of sons) over broods departed from binomial we conducted randomization tests. These were carried out by randomly redistributing the nestlings over the broods 10,000 times, while keeping the original distribution of brood sizes, and calculating the deviance each time. The *P* value was obtained from the proportion of the 10,000 runs in which the deviance was greater than for the real broods. We used multilevel mixed models with a binomial error distribution with a logit link function following Krackow and Tkadlec (2001) and Rasbash *et al.* (2004) to analyze sex ratio, with nestlings nested within broods (*i.e.* brood identity was fitted as a random effect). The models were implemented using restricted iterative generalized least squares (RIGLS) and second-order penalized quasi-likelihood approximation (PQL) (Rasbash *et al.* 2004). To test for a main effect of the UV treatment on sex ratio (Burley 1981; Trivers & Willard 1973) we fitted UV treatment, year (2002, 2003) and their interaction (see Figure 4.2). To test if the effect of UV treatment was dependent on pre-treatment male crown colour (Sheldon *et al.* 1999) we fitted UV treatment, year, pre-treatment crown colour (brightness, hue, UV chroma), and all interactions (see Table 4.1; Figure 4.4). As two of the three crown colour indices were strongly inter-correlated (hue versus UV chroma: $r_p = -0.76$, $n = 70$, $P < 0.001$) we fitted independent models for each of the crown colour indices. The significance of variables was tested using the Wald statistic, which follows a χ^2-distribution. Five males and nine females belonged to experimental pairs ($n = 10$ pairs) both in 2002 and 2003. Proportions of male offspring were not significantly correlated between years within these individuals either in males ($r_s = 0.67$, $n = 5$, $P = 0.22$) or females ($r_s = 0.29$, $n = 9$, $P = 0.44$), and we included these individuals in our analyses for both years. Multilevel models were carried out using MLwiN 2.0 and all other statistical tests using SPSS 12.01. Probability values are two-tailed.

RESULTS

Sex ratio variation at the population level

We sexed 95.3% of eggs laid ($n = 783$) in 70 experimental broods and 96.6% of eggs laid ($n = 292$) in 26 non-experimental broods used in within-individual comparisons (see below). In total we collected 44 of 71 unhatched eggs, of which we sexed 29 embryos. There was no visible embryo development in the majority of the eggs that were not sexed. We found no indication for sex-biased embryo mortality; 41.4% ($n = 29$) of sexed embryos were male versus 51.0% ($n = 999$) of all sexed nestlings (Yates' corrected $\chi^2_1 = 0.69$, $P = 0.41$). Therefore, we assumed that our data represent brood sex ratios at laying (*i.e.* the primary sex ratio).

Overall, 51.6% ($n = 746$) of offspring in experimental and 48.2% ($n = 282$) of offspring in non-experimental broods were male, which in neither case differed

from 50% (experimental broods: Yates' corrected $\chi^2_1 = 0.71$, P = 0.40; non-experimental: Yates' corrected $\chi^2_1 = 0.29$, $P = 0.59$). The distribution of male and female offspring over broods also did not depart from a binomial distribution in either experimental (randomization test: deviance = 74.97, df = 69, $P = 0.42$) or non-experimental broods (randomization test: deviance = 22.82, df = 25, $P = 0.65$).

Main effect of UV treatment on sex ratio

There was no difference in the overall proportion of male offspring between the UV-reduced and the control-treated group in either 2002 or 2003 (Figure 4.2). Neither was there any suggestion of a main effect of experimental treatment on sex ratio from the limited sample of individuals for which within-individual comparisons were possible (Figure 4.3; see Oddie and Reim (2002) for an explanation why such tests may be more powerful).

Effect of pre-treatment male UV reflectance on sex ratio

Sex ratios were not related to either pre-treatment crown brightness or hue of males (Table 4.1; Figure 4.4A,B,D,E). However, the interaction term 'UV treatment x pre-treatment UV chroma x year' had a significant effect on sex ratio (Table 4.1). This three-way interaction including 'year' indicates that the interacting effects of UV treatment and pre-treatment UV chroma differed between the two years. To analyze this effect in more detail we fitted the effects of UV treatment and pre-treatment UV chroma, and their interaction separately for each year. In 2003, but not in 2002, we found that the interaction of UV treatment with pre-treatment UV chroma

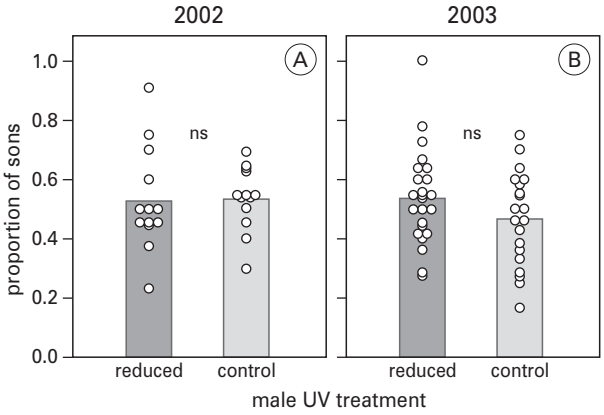

Figure 4.2 No difference in the proportion of male offspring in broods of UV-reduced and control-treated male blue tits in 2002 (A) or 2003 (B) ($n = 70$; UV treatment: Wald = 1.383, df = 1, $P = 0.24$; year: Wald = 1.418, df = 1, $P = 0.23$; UV treatment x year: Wald = 0.927, df = 1, $P = 0.34$). Bars indicate mean proportion of male offspring for each experimental group. Circles indicate proportions of male offspring of individual broods.

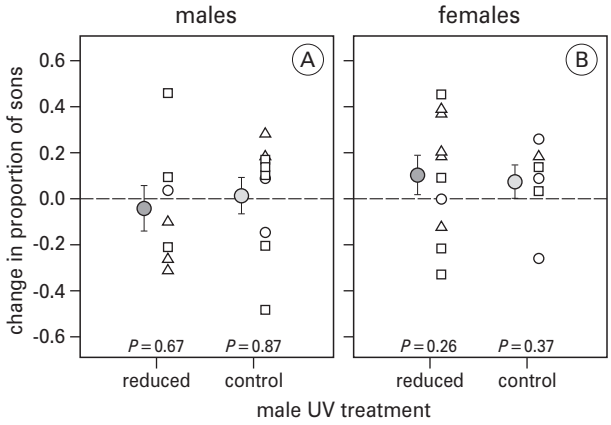

Figure 4.3 Within-individual comparisons of brood sex ratio for males (A) and females (B) recorded breeding in two years; one in which they were subject to the experiment, and one in which they were not (change in proportion of sons = proportion of sons in experimental brood – proportion of sons in non-experimental brood). Large circles are mean changes with standard errors. Small symbols indicate within-individual changes: circles: non-experimental brood in 2001, experimental brood in 2002; triangles: experimental brood in 2002, non-experimental brood in 2003; squares: non-experimental brood in 2002, experimental brood in 2003. The within-individual changes in sex ratio did not differ from 0 (one-sample *t*-tests: UV-reduced males: $t_6 = -0.445$, $P = 0.67$; control males: $t_8 = 0.165$, $P = 0.87$; females paired to UV-reduced males: $t_9 = 1.198$, $P = 0.26$; females paired to control males: $t_5 = 0.992$, $P = 0.37$). Furthermore, the within-individual change in sex ratio did not differ between UV-reduced and control broods (ANOVA: males: $F_{1,12} = 0.126$, P = 0.73; females: $F_{1,12} = 0.085$, P = 0.78) and the magnitude of the change was not dependent on the year of experimental treatment (ANOVA: males: $F_{1,12} = 0.009$, $P = 0.93$; females: $F_{1,12} = 0.018$, $P = 0.90$), or on the sequence of the experimental and non-experimental year (ANOVA: males: $F_{1,12} = 0.010$, $P = 0.92$; females: $F_{1,12} = 1.299$, $P = 0.28$).

Table 4.1 Multilevel models of brood sex ratios of blue tits. UV treatment, year (2002 or 2003), male colour index (brightness, hue, UV chroma), and all their interaction terms were retained in all models (all $n = 70$). The table shows Wald statistics and *P* values. All df = 1. See also Figure 4.4.

	Brightness		Hue		UV chroma	
Variables included in models	Wald	*P*	Wald	*P*	Wald	*P*
UV treatment	0.016	0.90	0.947	0.33	7.717	0.0055
Colour index	0.011	0.92	1.556	0.21	5.875	0.0154
Year	0.148	0.70	2.524	0.11	8.279	0.0040
UV treatment x year	0.814	0.37	0.767	0.38	7.365	0.0067
Colour index x year	0.046	0.83	2.647	0.10	7.939	0.0048
Colour index x UV treatment	0.090	0.76	0.995	0.32	7.444	0.0064
Colour index x UV treatment x year	1.144	0.28	0.836	0.36	7.006	0.0081

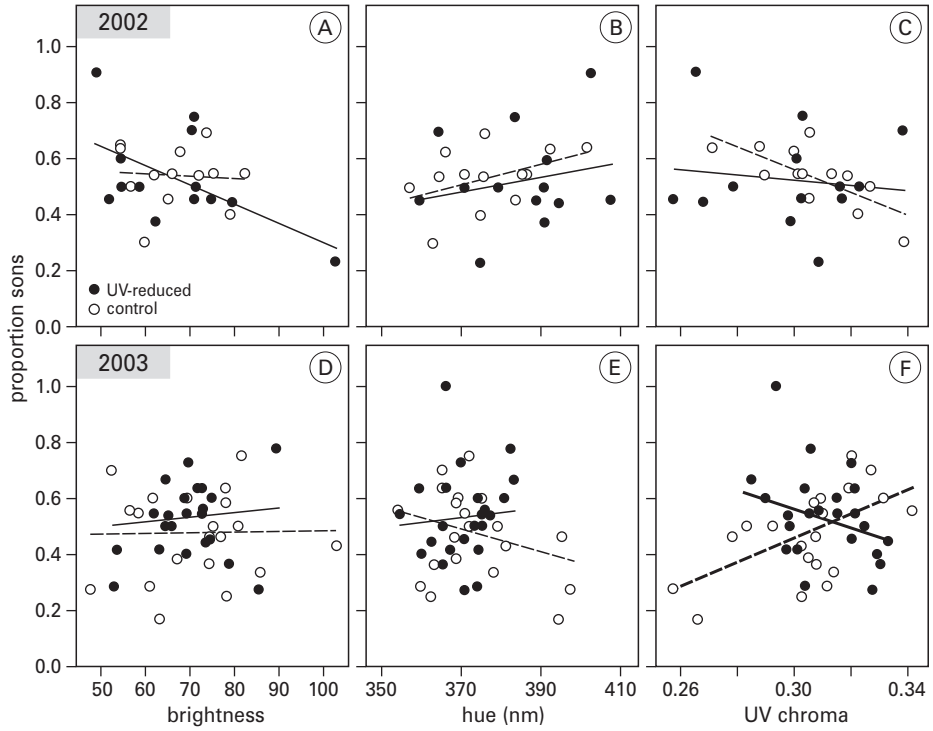

Figure 4.4 Relationships between brood sex ratio and male pre-treatment crown brightness (A,D), hue (B,E) UV chroma (A,B) for blue tits of two treatment groups (UV-reduced and control) in 2002 (A,B,C) and 2003 (D,E,F). Solid and dashed lines indicate the predicted proportions of sons in UV-reduced and control broods, respectively, depending on male pre-treatment brightness, hue and UV chroma. Non-significant relationships are indicated by thin lines (A,B,C,D,E), whereas thick lines indicate statistically significant relationships (F). See also Table 4.1.

had a significant effect on sex ratio (UV treatment x UV chroma: 2002: Wald = 1.16, df = 1, n = 26, P = 0.28; 2003: Wald = 7.44, df = 1, n = 44, P = 0.0064; Figure 4.4C,F). Subsequent analysis revealed that in 2003 pre-treatment UV chroma was positively related to the proportion of sons in the control group (Wald = 5.880, df = 1, n = 20, P = 0.015), but not related to the proportion of sons in the UV-reduced group (Wald = 2.177, df = 1, n = 24, P = 0.14) (Figure 4.4F).

DISCUSSION

No main effect of UV treatment

In contrast to previous suggestions from correlative data (Sheldon *et al.* 1999; Griffith *et al.* 2003) we found no experimental evidence for primary sex ratio adjustment in relation to male (UV) attractiveness in blue tits according to the simple prediction from sex allocation theory (Trivers & Willard 1973; Burley 1981). Although the UV reduction treatment we used has been shown to effectively lower attractiveness of male blue tits (Limbourg *et al.* 2004), we found no difference between the overall sex ratios produced by females paired to UV-reduced and control-treated males. This result confirms the result of Sheldon *et al.* (1999) who also found no difference in overall sex ratio between the UV-reduced and control-treated group. Likewise, Foerster and Kempenaers (2004) who experimentally enhanced the attractiveness of male blue tits by testosterone-releasing implants found no difference between overall sex ratio produced by females paired to testosterone-implanted – attractive – and control males. Only three experimental studies in other bird species in which some aspect of male attractiveness was manipulated have found an effect on primary sex ratio such as predicted by the Trivers and Willard (1973) hypothesis (collared flycatcher *Ficedula albicollis*, Ellegren *et al.* 1996; spotless starling *Sturnus unicolor*, Polo *et al.* 2004; peafowl *Pavo cristatus*, Pike & Petrie 2005). Several other experimental studies in different species failed to find an effect of male attractiveness on primary sex ratio (barn swallow *Hirunda rustica*, Saino *et al.* 1999; mallard *Anas platyrhynchos*, Cunningham & Russell 2000; dark-eyed junco *Junco hyemalis*, Grindstaff *et al.* 2001; zebra finch *Taeniopygia guttata*, Zann & Runciman 2003; Rutstein *et al.* 2004a).

Interaction of UV treatment and pre-treatment UV

Although there was no main effect of UV treatment on sex ratio, the interaction of pre-manipulation UV chroma with UV treatment had a significant effect on sex ratio in 2003, but not in 2002. This significant interaction effect was caused by the presence of a significantly positive relationship between the proportion of sons and male pre-manipulation UV chroma in the control group, whereas such a relationship was absent in the UV-reduced group. Our result is almost identical to the pattern found by Sheldon *et al.* (1999), although Sheldon *et al.* (1999) also found the interaction of pre-manipulation hue – which is negatively correlated with the UV chroma index – with UV treatment to be significant. As a causal explanation for the curious interaction effect of UV treatment and pre-treatment crown colour, which does not follow the initial prediction of an overall bias towards daughters in the UV-reduced group (Burley 1981), Sheldon *et al.* (1999) proposed that the UV reduction treatment might not merely make males unattractive, but may completely mask the variation in natural male UV reflectance. Possibly, this deprives females of cues on male UV attractiveness, leading to the absence of a relation between male

UV and sex ratio which is naturally present in the control group of males with unaffected UV reflectance. In addition, several other factors could be important for the female's perception of her mate's attractiveness/quality after the UV reduction treatment, such as the discordance between the reduced UV reflectance of the crown feathers and the UV reflectance of other plumage parts, *e.g.* the UV/blue wing coverts, which are correlated in unmanipulated birds (Sheldon *et al.* 1999; our study, crown versus wing coverts UV chroma: $r_p = 0.45$, $n = 48$, $P = 0.001$), or other male quality signals such as song performance. We also suggest that the UV reduction may interfere with the signalling function of the male crown plumage during male-male territorial conflicts (Alonso-Alvarez *et al.* 2004), which could also influence the female's perception of the quality of her mate. All of these possible explanations are speculative and carefully designed experiments and detailed behavioural observations of UV-manipulated individuals are needed to better understand the biological consequences of the UV treatment.

It is unclear why we found the interaction of pre-treatment UV chroma and UV treatment to have a significant effect on sex ratio in 2003, but not in 2002. Possibly, the interval between UV manipulation and laying of the first egg was too short in 2002 (4.4 days ± 2.9 SD), whereas in 2003 this interval was longer (8.0 ± 6.5 SD) and closer to the interval in Sheldon *et al.*'s (1999) study (10 days ± 5.2 SD). In both years we aimed to have a similar interval between treatment and egg laying as Sheldon *et al.* (1999), but due to the unpredictability of the onset of egg laying it is impossible to achieve a fixed interval between treatment and subsequent egg laying. Females may need a minimum amount of time to influence offspring sex ratios or there may be a time window during which females are particularly sensitive to the appearance of their mates. Given that the UV reducing effect diminishes over time, the timing of the experimental treatment with respect to subsequent egg laying is probably crucial for the treatment to influence the sex ratio. However, this idea was not supported by a significant effect of the interaction of the interval between UV treatment and laying of the first egg x UV treatment x pre-manipulation UV chroma in either 2002 (Wald = 0.328, $n = 26$, df = 1, all P values = 0.57) or 2003 (Wald = 1.152, $n = 44$, df = 1, $P = 0.28$), but statistical power was low in these analyses. At present, we have no alternative plausible explanations for the significant interaction with year. The two breeding seasons appeared very similar and were not different in for example mean laying date ($t_{68} = -1.286$, $P = 0.20$), clutch size ($t_{68} = 0.025$, $P = 0.98$), or fledging success (Wald = 0.170, $n = 70$, df = 1, $P = 0.68$). Also female body condition, which can also influence primary sex ratio (Nager *et al.* 1999), was not different between the two years (measured as body mass, controlled for tarsus length; ANCOVA: $F_{1, 67} = 0.162$, $P = 0.69$).

Inter-annual variation in patterns of primary sex ratio among individual broods has more often been encountered in birds, but most studies lack convincing biological explanations for such year effects (Lessells *et al.* 1996; Hartley *et al.* 1999; Korpimäki *et al.* 2000; Radford & Blakey 2000; Griffith *et al.* 2003; C.M. Lessells,

unpublished data). Population wide sex ratios, however, have previously been shown to vary among years in relation to food availability (*e.g.* Wiebe & Bortolotti 1992; Hipkiss & Hörnfeldt 2004), length of the breeding season (Weatherhead 2005) and the mean number of helpers at the nest (Dickinson 2004).

Adaptive sex ratio adjustment?

Several studies reporting strong biases in primary sex ratio show that birds can have considerable control over offspring sex ratio (*e.g.* Heinsohn *et al.* 1997; Komdeur *et al.* 1997; Komdeur *et al.* 2002). Furthermore, patterns of avian primary sex ratio variation may be very flexible to variable selective pressures (Badyaev *et al.* 2002; Zann & Runciman 2003) and can be complex (Legge *et al.* 2001). Therefore, we believe that the complex relationship between blue tit sex ratio and male attractiveness that we found, which did not follow simple prediction from theory (Trivers & Willard 1973; Burley 1981) and seems rather inconsistent between study populations and years (Sheldon *et al.* 1999; Leech *et al.* 2001; Griffith *et al.* 2003; our study), does not necessarily indicate that blue tit primary sex ratio variation is non-adaptive or constrained by their chromosomal sex determination system. It rather suggests that simple verbal arguments (Burley 1981) predicting optimal sex ratio in relation to mate attractiveness may not generally be applicable (Pen & Weissing 2000). Patterns of optimal sex ratio variation may be subtle and vary between years and populations, depending on the local ecological circumstances.

The relationship between the optimal offspring sex ratio, yielding maximum fitness, and paternal attractiveness may be relatively weak and/or not straightforward in blue tits for several reasons. Firstly, the typical percentage of ca. 10–15% extra-pair offspring in blue tits (*e.g.* Kempenaers *et al.* 1997; Delhey et al 2003; P. Korsten, C.M. Lessells, A.C. Mateman & J. Komdeur, unpublished data for the present study population; see also Box D), is not particularly high when compared to other socially monogamous bird species (Griffith *et al.* 2002). The variance in reproductive success sets an upper limit to the strength of sexual selection, and hence of selection on sex ratio in relation to male sexual attractiveness (*cf* Griffin *et al.* [2005] for variation in the extent of sex ratio modification in relation to the strength of selection through benefits from helpers at the nest). The strength of selection on sex ratio variation may thus be relatively low in blue tits. This may especially be true when compared to, for example, ungulate mammals with harem systems, that often do show the patterns of sex ratio variation predicted from the Trivers and Willard (1973) hypothesis (Sheldon & West 2004). In many of these ungulate species there is extreme variation in reproductive success among males, with individual males being either very successful or unsuccessful, while most females have relatively similar reproductive output.

Another reason why the relationship between the optimal sex ratio and male crown colour may not be simple in blue tits, is that the relationship between male within and extra-pair mating success and UV crown coloration is not necessarily

straightforward. It has recently been found that males with more UV-shifted crown reflectance are less cuckolded, whereas males with less UV-shifted crown reflectance sire more extra-pair young (Delhey *et al.* 2003). However, this result is based on a single breeding season and seems to contrast with a previous study, in a different blue tit population, which suggests that males that are successful in gaining extra-pair paternity also have a higher share of paternity in their own broods (Kempenaers *et al.* 1997). Thus, it remains to be investigated how the relative reproductive values of sons and daughters depend on the crown UV reflectance of their fathers.

Finally, blue tits lay very large clutches (mean clutch size [2001–2003]: 10.9 ± 1.7 SD, $n = 249$). Until now most case studies in birds that found strong shifts in primary sex ratio involved species that lay only 1 or 2 eggs per clutch (Ewen *et al.* 2004), suggesting that selection for extreme sex ratio shifts is more constrained by the chromosomal sex determination system (Emlen 1997; Pike 2005; but see Komdeur *et al.* 2002) and/or weaker in species with large clutches such as the blue tit.

In conclusion, we found no experimental evidence for primary sex ratio adjustment in relation to male UV attractiveness according to the simple prediction from sex allocation theory (Trivers & Willard 1973; Burley 1981). However, remarkably, one of our year's results confirms the intriguing UV treatment x pre-treatment UV interaction effect on sex ratio previously found by Sheldon *et al.* (1999). This is an extremely important result because it demonstrates that the previously unexpected pattern that Sheldon *et al.* (1999) found is more than an anomalous result in a single year and population. Our replication of the result therefore provides the basis for moving on to the next step of elucidating the adaptive value of blue tit sex ratio variation and the proximate and ultimate causes of the variability of blue tit sex ratio patterns among years and populations.

ACKNOWLEDGEMENTS

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BOX D. Extra-pair paternity and male UV coloration in blue tits

Correlational data have shown that blue tit males with more UV-shifted crown coloration (lower $\lambda[R_{max}]$) are less frequently cuckolded, while less UV-shifted males (higher $\lambda[R_{max}]$) are more successful in gaining extra-pair paternity in other broods (Delhey *et al.* 2003). These correlations suggest that male UV coloration influences female extra-pair mating decisions. Females paired with more UV-shifted males may be more faithful. Alternatively, more UV-shifted males may be more successful in mate guarding. It is remarkable that less UV-shifted males were found to be more successful in siring extra-pair young. Possibly, high and low UV males follow alternative reproductive strategies, leading to approximately equal fitness pay-offs (Delhey *et al.* 2003). Patterns of extra-pair paternity were not related to male UV chroma ($R_{320–400}$ / $R_{320–700}$; Delhey *et al.* 2003), another index of UV coloration which is thought to be a generally important determinant of male attractiveness (Andersson *et al.* 1998; Sheldon *et al.* 1999).

To experimentally test the effect of male UV coloration on a male's share of paternity in his own brood, we determined the parentage of the broods included in the male crown UV manipulation experiment in 2003 (for more details on the experimental procedure and general field methods see Chapters 3 and 4). We determined the parentage of 23 broods of UV-reduced and 20 broods of control-treated males (1 UV-reduced brood was excluded, because the female was not captured). We genotyped the chicks and the putative parents of these broods with the microsatellite markers *Pca*3, *Pca*8, *Pca*9 (Dawson *et al.* 2000) and *Pocc*6 (Bensch *et al.* 1997) and determined the paternity (within-pair or extra-pair) of each chick by direct comparison of parent and offspring genotypes. None of the loci deviated significantly from Hardy-Weinberg equilibrium. Using the observed allele frequencies, we calculated total exclusionary power in CERVUS (version 2.0; Marshall *et al.* 1998): the probability of exclusion was 0.999 for assigning the father when the mother was known. A maximum of one mismatch was allowed when assigning paternity. We analysed the effect of UV treatment on the proportion of extra-pair offspring in MLwiN (2.02) with multilevel models with individual offspring nested within broods (broods: $n = 43$; offspring: $n = 452$). Models were implemented with a binomial error structure and logit-link function (for a similar statistical approach see Chapter 4).

The analysis showed that experimental treatment had no effect on the overall proportion of extra-pair offspring (UV reduced [$n = 23$] versus control broods [$n = 20$]: $\chi^2 = 0.135$, df = 1, $P = 0.71$; Figure D.1). There was no difference in the proportion of broods with at least one extra-pair offspring between the UV-reduced and the control group (Yates' corrected $\chi^2 = 0.00$; $P = 1.0$; Figure D.1A).

Nor was there a difference between the proportion of extra-pair offspring in the broods of UV-treated and control males, if only broods with at least one extra-pair young were included in the analysis (UV reduced [$n = 12$] versus control broods [$n = 10$]: $\chi^2 = 0.142$, df = 1, $P = 0.71$; Figure D.1B).

Hence, we find no evidence for an influence of UV crown coloration of males on their share of paternity in their own brood. This finding is in conflict with the hypothesis that females' extra-pair mate choice is dependent on the UV coloration of their mate. Possibly, naturally high UV males are of better overall quality and therefore also more effective in mate guarding, a characteristic which is not changed by the application of the UV reduction treatment. However, the proportion of extra-pair offspring was not related to natural UV coloration before treatment (brightness: $\chi^2 = 2.09$, $P = 0.15$; hue: $\chi^2 = 0.033$, $P = 0.86$; UV chroma: $\chi^2 = 0.488$, $P = 0.48$; all df = 1), which refutes this last idea. As a follow up on these results, it would be worthwhile to assign the paternity males gained in other broods than their own and to subsequently test the effect of treatment on gain of extra-pair paternity in other broods.

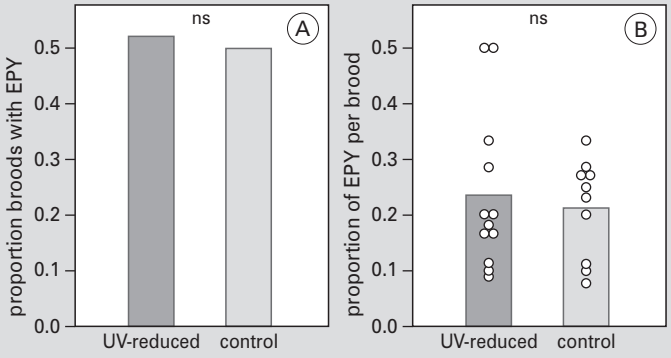
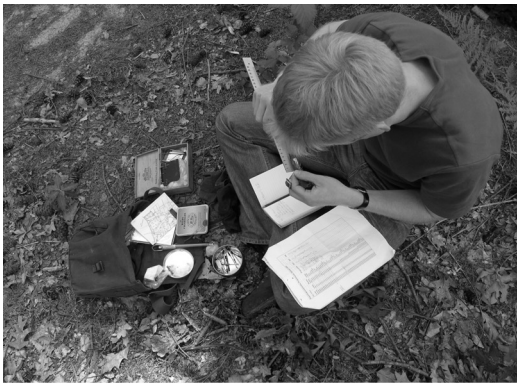


Figure D.1 Proportion of blue tit broods with at least one extra-pair young (EPY) (A) and the proportion of extra-pair young in these broods (B); both in relation to UV treatment of the putative father's crown coloration. Circles indicate proportions of extra-pair young of individual broods.



CHAPTER 5

Rapid changes in maternal yolk hormone deposition in response to manipulated male attractiveness

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ABSTRACT

Avian eggs contain substantial amounts of androgens of maternal origin that can have profound effects on chick development. Experiments on captive birds have shown that females deposit higher concentrations of androgens in the yolk of their eggs when mated with an attractive male. However, studies in wild bird populations have yielded less clear results with contradictory findings between correlational and experimental studies. We conducted a similar study in a wild bird population, using a correlational and experimental approach in the same individuals, and we applied a within-female design to control for possible between-female variation that could have confounded the results in previous field studies. We manipulated the sexually selected UV coloration of the blue crown feathers of male blue tits (*Parus caeruleus*) on the day their female had laid the second egg of the clutch, and subsequently measured the effect on androgen concentrations (testosterone and androstenedione) in the fifth, seventh, and ninth egg. We also measured androgen levels of the second egg that was laid before application of the treatment, both as a baseline measure for each clutch and to investigate the correlation between yolk androgen levels and natural male crown coloration. The concentration of testosterone, but not of androstenedione, in eggs laid after the manipulation was significantly higher in control (attractive) than in UV-reduced (unattractive) pairs. The effect diminished over the egg sequence, coinciding with the recovery of crown UV coloration after manipulation. This suggests that females are capable of very rapid adjustments of yolk testosterone levels in response to changes in an ornamental plumage character of their mate. However, we could not detect any correlation between the androgen concentration in the second egg and pre-treatment male crown coloration. We discuss this discrepancy and we suggest that the results of experimental studies on the relationship between yolk androgen deposition and male attractiveness should be treated with caution.

INTRODUCTION

Maternal effects may represent adaptive transgenerational phenotypic plasticity allowing organisms to optimally adjust offspring phenotype to the environment they are encountering (Mousseau & Fox 1998). Over the last decade, maternally derived hormones in the yolk of avian eggs – in particular androgens – have started to attract much attention as potential mediators of such adaptive maternal effects. Avian mothers deposit variable amounts of androgens in the yolk of their eggs that can have important effects on offspring development (reviewed in Groothuis *et al.* 2005a). These effects include an increase in begging behaviour, suppression of immune function, and both positive and negative effects on growth and survival (*e.g.* Schwabl 1996; Sockman & Schwabl 2000; Eising *et al.* 2001, 2003; Pilz *et al.* 2003; Groothuis *et al.* 2005b; Müller *et al.* 2005).

Although most studies have concentrated on explaining within-clutch variation in yolk androgen concentrations, which may be related to female strategies of brood reduction or compensation of hatching asynchrony, between-clutch variation is often even larger (Groothuis *et al.* 2005a; Reed & Vleck, 2001). One of the factors that have been indicated to induce this variation is the sexual attractiveness of a female's mate (Gil *et al.* 1999). Several Lab-based studies have demonstrated that females increase the deposition of androgens to the yolk of their eggs when paired with a more attractive male (see Table 5.1). In captive zebra finches (*Taeniopygia guttata*), females paired with males wearing 'attractive' red leg rings deposited higher concentrations of yolk androgens, than females with males wearing 'unattractive' green rings (Gil *et al.* 1999; but see Rutstein *et al.* 2004a). Captive zebra finch females also deposited higher levels of yolk androgens when paired to a mate that was found to be preferred in a preceding mate choice trial (von Engelhardt 2004). Furthermore, captive female canaries (*Serinus canaria*) deposited higher levels of androgens in their eggs when exposed to more attractive male song (Gil *et al.* 2004; Tanvez *et al.* 2004). The increase in female androgen deposition in response to male attractiveness found in these studies has been interpreted as a form of increased investment in offspring of which the mother expects higher fitness returns (*e.g.* Gil *et al.* 1999; 2004), as predicted by the differential allocation hypothesis (Burley 1988, Sheldon 2000). However, this interpretation assumes that enhanced hormone allocation to eggs is costly for the mother, for which no convincing evidence exists to date. Moreover, this view ignores the fact that increased yolk androgen levels can also have negative effects on the offspring, such as reduced immune function (Groothuis *et al.* 2005b) and survival (Sockman & Schwabl 2000). An alternative hypothesis is that females use yolk androgens to manipulate their male's feeding rate to the offspring. For example, if a female anticipates her male to feed her offspring at a low rate, she may increase the male's feeding rate through a stimulation of offspring begging, which could be induced by increased yolk androgen deposition (Navara *et al.* 2006). Positive relationships between yolk androgen deposition and

Table 5.1 Overview of studies on the effect of mate attractiveness on yolk androgen deposition, including which androgens were investigated (testosterone [T], androstendione [A4] and dihydrotestosterone [DHT]) and the relationship found. It is indicated whether studies were correlational (Corr) or experimental (Exp), and whether studies were conducted in wild or captive birds.

Species	Male characteristic	Effect	Androgens	Captive/Wild	Corr/Exp	Reference
Canary *Serinus canaria*	Song quality	Positive	T	Captive	Exp	Gil *et al.* 2004
Canary	Song quality	Positive	T, DHT (total)	Captive	Exp	Tanvez *et al.* 2004
Zebra finch *Taeniopygia guttata*	Ring colour	Positive	T	Captive	Exp	Gil *et al.* 1999
Zebra finch	Ring colour	Zero	T	Captive	Exp	Rutstein *et al.* 2004a
Zebra finch	Mate preference	Positive	total androgen level	Captive	Exp	v. Engelhardt *et al.* 2004
Barn swallow *Hirundo rustica*	Tail length	Positive	A4	Wild	Exp	Gil *et al.* 2006
Barn swallow	Tail length	Zero	A4	Wild	Exp	Saino *et al.* 2006
Collared flycatcher *Ficedula albicollis*	Forehead patch	Zero	T	Wild	Corr	Michl *et al.* 2005
House finch *Carpodacus mexicanus*	Plumage coloration	Negative	T, A4, DHT (total)	Wild	Corr	Navara *et al.* 2006
House sparrow *Passer domesticus*	Testosterone level	Zero	T	Wild	Exp	Mazuc *et al.* 2003

male attractiveness are then to be expected, because in some species attractive males tend to feed less (Groothuis *et al.* 2005a).

In wild bird populations, however, the overall direction of the relationship between yolk androgen levels and male attractiveness appears less straightforward (Table 5.1). Experimentally manipulated length of the sexually-selected tail streamers of barn swallows had a positive effect on maternal androgen deposition in one population (*Hirundo rustica*; Gil *et al.* 2006), but not in another (Saino *et al.* 2006). In a study on wild house sparrows (*Passer domesticus*) no effect of male testosterone implantation (which was assumed to increase male attractiveness) was found on yolk androgen levels (Mazuc *et al.* 2003). A correlational study on collared flycatchers (*Ficedula albicollis*) also found no correlation between yolk androgen levels and male attractiveness (size of the forehead patch; Michl *et al.* 2005). Finally, in a population of house finches (*Capodacus mexicanus*) a negative correlation between yolk androgen levels and male ornamental plumage was found (Navara *et al.* 2006). Since in this species unattractive males show lower paternal feeding rates, this result would be consistent with the hypothesis that avian mothers increase paternal feeding rate via androgen deposition in the egg if they anticipate a low male feeding rate.

The greater inconsistency in the results of field studies compared to lab studies may be caused by the fact that in addition to the single tested aspect of male attractiveness several other factors affect yolk androgen levels in the field. This may also explain why correlational studies, taking only one aspect of male quality into account, do not yield clear results. Furthermore, differences between studies could be due to differences in timing of the experimental treatments relative to egg laying. Therefore we conducted a field study which had the following aims: 1) analysing the effect of an experimental manipulation of mate attractiveness on egg androgen concentrations using a within-female design, which controlled for the confounding effect of between-female variation; 2) comparing correlational and experimental data in the same population; and 3) investigating on what time scale females adjust their hormone deposition in response to male quality.

The blue tit (*Parus caeruleus*) is an excellent model species to experimentally test the effect of male attractiveness on patterns of female yolk androgen deposition. Blue tits have sexually selected UV-reflecting crown feathers (Andersson *et al.* 1998; Hunt *et al.* 1998; Delhey *et al.* 2003) and the UV coloration of the crown plumage plays a role in female mate choice (Andersson *et al.* 1998; Delhey *et al.* 2003; Hunt *et al.* 1998) and may serve as male viability indicator (Griffith *et al.* 2003; Sheldon *et al.* 1999; but see Delhey & Kempenaers 2006). It has recently been found that female blue tits decrease their reproductive investment (in terms of nestling food provisioning) in response to experimentally reduced crown UV reflectance of males and consequently these females fledge significantly smaller chicks (Limbourg *et al.* 2004). In addition, yolk androgens have been suggested to be involved in avian primary sex ratio control (Petrie *et al.* 2001; but see Pilz *et al.* 2005) and female blue

tits were found to modify the primary sex ratio in response to manipulation of male crown UV reflectance (Sheldon *et al.* 1999, Korsten *et al.* 2006).

We manipulated UV coloration of the crown feathers of male blue tits and subsequently measured the effect on yolk androgens in their females' eggs. UV reflectance of males was first measured and thereafter manipulated on the day their female laid the second egg. The UV-reduction treatment was non-permanent, and UV reflectance is known to recover within days after treatment (Limbourg *et al.* 2004; Chapter 3). Therefore we collected the fifth, seventh, and ninth laid eggs, enabling us to test whether and at what time scale females adjusted yolk androgen levels to changes in male attractiveness. We also measured androgen levels of the second egg, which was laid before application of the treatment, in order to obtain a baseline measurement for the two treatment groups. Finally, we correlated androgen levels of the second unmanipulated egg to pre-manipulation crown coloration of males.

METHODS

Study area, bird handling and sample sizes

The experiment was carried out in the breeding season of 2005 (7 April–5 June) in a population of blue tits breeding in nestboxes at the 'Vosbergen' estate (*ca.* 50 ha; 53°08' N, 06°35' E), near Groningen, The Netherlands. This population has been intensively studied since 2001. The study area consists of patches of mixed deciduous and coniferous forest interspersed by patches of open grassland. Nestboxes were checked daily for presence of the first-laid egg.

Males (n = 36) were caught in front of occupied nestboxes on the day the second egg had been laid, using a mistnet and a decoy (a mounted male blue tit) with song playback. Males were subsequently transported in a dark bird bag to the nearby field station, where their age, body mass (to the nearest 0.1 g using a 30 gram spring balance), tarsus (to the nearest 0.1 mm using sliding callipers) and natural crown reflectance were measured. We determined age (1 year or >1 year) based on the colour of the primary coverts following Svensson (1992). Thereafter, we manipulated the males' crown UV coloration (see below). Birds were released in their own territory after treatment.

We caught the females of experimental pairs (n = 30; three females were not caught) in their nestboxes during chick feeding (6–10 days after hatching) using a spring trap, and we determined their age and measured their body mass, tarsus length and crown reflectance following the protocol described above. During the same period we also captured the males of all occupied nestboxes in the study area. Three males included in our experiment were also caught at another nestbox, indicating these males to be polygynous. All other males in the experiment were re-captured in the same territory as where they were initially caught, indicating that in all

territories we manipulated the resident male. The three polygynous males were excluded from further analyses, yielding a final sample size of 33 experimental clutches (16 controls and 17 UV-reduced). Of these, we could calculate the correlations between yolk androgen levels and natural male crown colour for 32 broods as the measurement of natural crown reflectance before manipulation failed in one male.

Crown reflectance measurements

Before the manipulation of the crown UV reflectance, the spectral reflectance of the crown feathers was measured with an USB-2000 spectrophotometer with illumination by a DH-2000 deuterium-halogen light source (both Avantes, Eerbeek, The Netherlands). The measuring probe was held at a right angle against the plumage, *i.e.* both illumination and recording were at 90° to the feathers. During each crown reflectance measurement we took 5 replicate readings of the same spot and smoothed each of these reflectance spectra by calculating the running mean over 10 nm intervals. Following previous studies of UV colour signalling in blue tits (Andersson *et al.* 1998; Sheldon *et al.* 1999; Griffith *et al.* 2003; Delhey *et al.* 2003; Korsten *et al.* 2006) we calculated three indices describing the variation in crown coloration – 'brightness', 'hue', and 'UV chroma' – from each reflectance spectrum, and averaged these across the 5 replicate spectra. 'Brightness' was the sum of reflectance between 320–700 nm ($R_{320–700}$), which corresponds to the spectral range visible to blue tits (Hart *et al.* 2000). 'Hue' was the wavelength of maximum reflectance (R_{max}). 'UV chroma' was the sum of reflectance between 320–400 nm divided by the sum of reflectance between 320–700 nm ($R_{320–400}$ / $R_{320–700}$). Both the 'hue' and 'UV chroma' indices have previously been identified as important predictors of male attractiveness and viability in blue tits (Andersson *et al.* 1998; Sheldon *et al.* 1999; Delhey *et al.* 2003; Griffith *et al.* 2003).

Males were captured within a relatively short period (10–21 April) leading to little variation in crown feather wear (Örnborg *et al.* 2002; Delhey *et al.* 2006), and consequently crown coloration was not significantly related to the date of capture (brightness: $r = –0.253$, $P = 0.16$; hue: $r = 0.250$, $P = 0.17$; UV chroma: $r = –0.319$, $P = 0.08$; all $n = 32$).

Crown UV manipulation

UV reflectance was reduced with a mixture of duck preen gland fat and UV blocking chemicals (50% Parsol 1789 and 50% Parsol MCX [by volume]; Roche, Basel, Switzerland) as used successfully in previous studies of wild blue tits (*e.g.*; Sheldon *et al.* 1999; Limbourg *et al.* 2004; Korsten *et al.* 2006). Control males were treated with the duck preen gland fat only. This treatment was smeared on the crown feathers and to measure its effect, three replicate crown reflectance measures were taken directly after the manipulation following the protocol described above. Males were assigned sequentially to either the UV-reduced or control treatment.

Egg collection

Nestboxes were visited daily and newly laid eggs were marked with non-toxic markers until the last egg was laid and clutch size was determined. We collected eggs 2, 5, 7 and 9 from each brood, on the day they were laid. Collected eggs were replaced with plastic dummy eggs. Collected eggs were incubated for 72 hours in an incubator at 35°C to induce embryonic development for DNA extraction to be used for molecular sexing. However, for unknown reasons incubation failed and eggs contained no embryos, so that eggs could not be sexed. After incubation, eggs were stored at –20°C until androgen analyses were conducted.

Androgen quantification

Androgens (testosterone [T] and androstenedione [A_4]) were measured by radioimmunoassay (RIA) after extracting them from the yolk with ether and on celite columns (Wingfield & Farner 1975; Schwabl 1993). The whole yolk was removed from the eggs when still frozen and weighed to the nearest 0.001 gram using an analytical balance. A weighed amount of yolk (150–300 mg) was homogenized in 200 μl of distilled water by vigorous mixing on a vortex facilitated by the addition of a few glass beads. A known amount of radioactive T and A_4 (*ca.* 2000 counts per minute) was added to a weighed subsample (150–280 mg) of the homogenate to assess extraction efficiency, and samples were kept for 1 h at 37 °C for equilibration. Batch 1 of samples was extracted three times with 3 ml of petroleumether: diethylether, 30:70 (vol:vol), batch 2 and 3 were three times extracted with 3 ml of diethylether (both methods extract T and A_4 from yolk and yielded similar recoveries, which were on average 56% en 50% for T and A_4 respectively). The three ether fractions were decanted from the snap-frozen egg yolk/water phase, combined, and dried under a stream of nitrogen. The dried extract was re-dissolved in 1 ml of 90% ethanol, stored overnight at –20°C and then centrifuged. The supernatant was dried under nitrogen, re-dissolved in 1 ml 2% ethylacetate in isooctane and transferred to diatomaceous earth chromatographic columns (Kieselgur, pro-analysi, Merck). Steroids were eluted with 4 ml of pure isooctane (discarded), 4.0 ml of 2% ethylacetate in isooctane (eluate containing A_4), 4.5 ml of 10% ethylacetate in isooctane (discarded) and 4.5 ml of 20% ethylacetate in isooctace (eluate containing T). The eluates were dried and re-dissolved in 200 μl of Tris-Buffer. T and A_4 levels were measured in duplicates of 50 μl of sample using DSL (Diagnostic System Laboratories, USA) radioimmunoassay kits.

Statistical analyses

To test the effect of UV treatment on yolk testosterone levels, we used a multilevel model that included a random effect for female identity to account for the non-independence of the eggs produced by a single female. We calculated the relative change of T and A_4 levels after the treatment was applied by dividing the levels of egg 5, 7 and 9 by the androgen levels of egg 2, the baseline level for each clutch before

treatment. We also tested the effect of the interaction of UV treatment and laying sequence on the relative change of yolk androgen concentration, to take into account a potentially diminishing treatment effect due to recovery of the UV reflectance with time after application of the UV reduction treatment. Significance was assessed using the increase in deviance (Δ deviance, which follows a χ^2 distribution) when a parameter was removed from the model.

Since egg 2 was not affected by male UV manipulation, we related androgen levels of egg 2 to natural male crown coloration as measured before manipulation to investigate the natural correlation between yolk androgen levels and male crown coloration. To account for possible confounding factors we additionally carried out two separate multiple regression analyses using a stepwise backward selection procedure to explain the variation in T and A_4. In the first class of models, we entered the three male crown colour indices (brightness, hue, UV chroma) together with male age, body mass and tarsus length as predictors of either T or A_4 levels (n = 32). In the second class of models, we entered the three male crown colour indices as well as female characteristics – female crown colour indices (brightness, hue, UV chroma), age, body mass, tarsus length and lay date – as predictors of either T or A_4 levels (n = 29). We chose not to run a single analysis including all male and female predictor variables at the same time, to avoid the risk of over-parameterisation of our models given the limited sample size. For the same reason we did not include interaction effects in the models. Analyses were conducted using MLwiN 2.02 for multilevel models and SPSS 12.0 for all other statistical tests.

RESULTS

Date of capture, body size (mass and tarsus), and crown coloration before manipulation did not differ between UV-reduced and control-treated males (Table 5.2). The UV-reduction treatment caused a large decrease in the UV reflectance of the crown plumage directly after manipulation (Figure 5.1). All three indices of crown coloration were significantly different between the two treatment groups directly after manipulation (Table 5.3). Clutch size did not differ between UV-reduced and control-treated pairs (Mean ± SE, UV-reduced: 11.8 ± 0.15, control treatment: 12.1 ± 0.25; t = 0.826, df = 31, P = 0.42).

T and A_4 levels of eggs were correlated (r = 0.378, P < 0.001, n = 138). There were significant negative effects of UV treatment on the change of yolk T concentrations (Δ deviance = 4.11, df = 1, P = 0.043) and also of the position in the laying sequence (egg number 7–9) (Δ deviance = 4.32, df = 1, P = 0.038), as well as a significant interaction effect of UV treatment x position in laying sequence (Δ deviance = 4.04, df = 1, P = 0.045; Figure 5.2). The data indicate that after treatment yolk testosterone levels were initially higher in the control group than in the UV-reduced group, which difference subsequently decreased over the laying sequence (Figure

Table 5.2 Pre-treatment characteristics of UV-reduced and control-treated male blue tits.

	UV-reduced (n = 17)		Control (n = 16)		Test	
	Mean	SE	Mean	SE	t	P
Capture date (April days)	16.35	0.69	16.25	0.81	0.10	0.92
Tarsus length (mm)	16.99	0.08	17.03	0.12	0.33	0.74
Body mass (g)	11.28	0.13	11.08	0.14	1.04	0.31
Brightness[1]	75.86	3.38	79.06	2.82	0.72	0.48
Hue (nm)[1]	386.2	2.12	387.4	2.59	0.35	0.73
UV Chroma[1]	0.29	0.004	0.29	0.005	0.20	0.85

[1] Pre-treatment crown coloration was measured for 15 males only in the control-treated group.

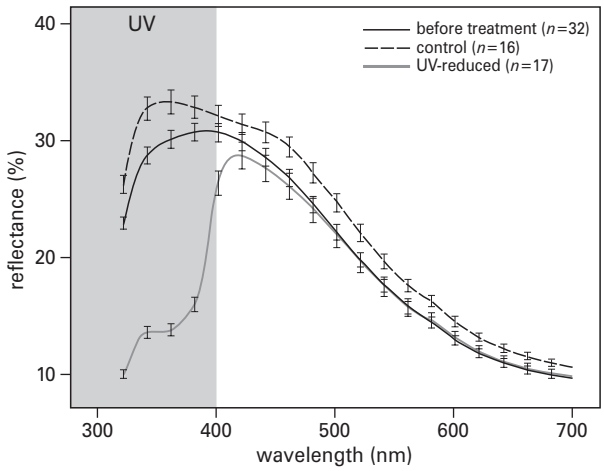

Figure 5.1 Mean reflectance spectra (± SE at 20 nm intervals) of crown plumage of male blue tits before treatment, and after UV-reduction or control treatment (the pre-treatment measurement of one control-male failed).

Table 5.3 Indices of male crown coloration after UV-reduced and control treatment.

	UV-reduced (n = 17)		Control (n = 16)		Test	
	Mean	SE	Mean	SE	t / U	P
Brightness	65.07	2.82	85.27	2.46	5.37[1]	< 0.001
Hue (nm)	416.2	0.67	363.7	3.58	0.00[2]	< 0.001
UV Chroma	0.18	0.004	0.29	0.004	19.60[1]	< 0.001

[1] t-test
[2] Mann-Whitney U test

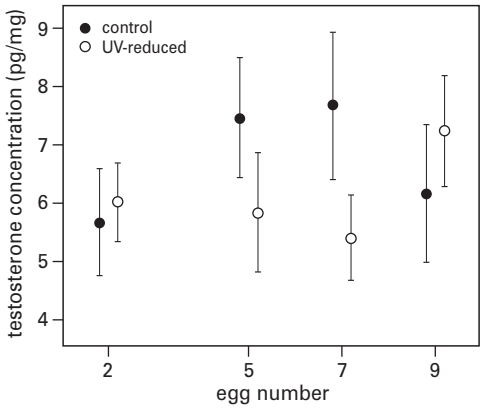

Figure 5.2 Mean (± SE) yolk testosterone concentration in eggs of UV-reduced (n = 17) and control-treated pairs (n = 16) plotted against position of the egg in the laying sequence (egg number).

5.2). Relative yolk A_4 concentrations did not differ with respect to treatment (Δ deviance = 0.002, P = 0.97) or laying sequence (Δ deviance = 0.58, P = 0.45). Also the interaction between treatment x laying sequence was not significant (Δ deviance = 2.28, P = 0.13).

Baseline concentrations of T and A_4 measured in the second egg did not correlate with natural male crown reflectance before manipulation (T, brightness: r = 0.103, n = 32, P = 0.58; hue: r = –0.036, n = 32, P = 0.84; UV chroma: r = 0.140, n = 32, P = 0.44; A_4, brightness: r = 0.164, n = 32, P = 0.40; hue: r = –0.079, n = 32, P = 0.69; UV chroma: r = 0.108, n = 32, P = 0.58). Likewise, none of the multiple regression analyses in which we used male and/or female characteristics in addition to male crown colour indices to explain the variation in either yolk T or A_4 concentrations in the second egg yielded a significant model (all P values > 0.05).

DISCUSSION

Maternal androgens in avian eggs represent an intriguing example of hormone-mediated maternal effects. One of the most frequently cited factors that may explain variation in androgen concentrations among clutches is male attractiveness. However, especially results of field studies are inconsistent (Table 5.1). The inconsistency among field studies may be due to a greater influence of confounding factors in the field compared to the controlled laboratory situation. Another reason for the inconsistency could be the differences between studies in the timing of experimental treatment or the measurement of attractiveness relative to egg laying. All studies until to date used a between-female experimental design. We used a more

sensitive within-female design, in which we manipulated male attractiveness after the second egg was laid, comparing the hormone concentrations of subsequently laid eggs with the second egg. We found that wild female blue tits quickly changed the deposition of testosterone, but not androstenedione, in the yolk of their eggs in response to manipulation of male crown UV coloration, a sexually selected trait.

Relative to the second egg, concentrations of testosterone were significantly lower in the subsequent eggs in clutches of UV-reduced – unattractive – males compared to males that received a control treatment. This effect diminished over the laying sequence, and had disappeared in the ninth egg. Possibly, the diminishing treatment effect was due to a rapid female response to recovery of male crown coloration after a few days in UV-reduced males (Chapter 3). To test this idea, one would need to compare the yolk testosterone pattern in clutches of singly UV-reduced males to males in which the UV reduction treatment is re-applied after some days. In any case, the fact that we observed a treatment effect from the fifth egg onwards demonstrates that female blue tits can adjust the level of androgen deposition in their eggs very rapidly in response to external stimuli.

There are several hypotheses that predict adjustment of female yolk androgen deposition to the characteristics of her mate. According to the differential allocation hypothesis females are expected to invest more in a current reproductive event at the cost of future reproduction when paired with an attractive or high-quality male (Sheldon 2000). In this scenario increased yolk androgen deposition by females in response to the mate's attractiveness could be viewed as greater maternal investment in the offspring (*e.g.* Gil *et al.* 1999, 2004, 2006). According to this idea maternal yolk androgens are viewed as a limited resource for the offspring. Indeed, increased maternal yolk hormones can have several beneficial effects on the offspring, including increased nestling growth (Groothuis *et al.* 2005a). But yolk androgens may also have negative effects on the offspring, such as reduction of immune function (Groothuis *et al.* 2005b). Moreover, it remains unclear whether the deposition of yolk androgens is costly to the female herself (Groothuis *et al.* 2005a).

Alternatively, yolk androgens may not act as limited resources, but as signals to the offspring, which fine-tune the balance of different trade-offs in the offspring (*e.g.* investing in growth versus immune function; Groothuis *et al.* 2005b), thereby maximising the fitness of the offspring depending on the prevailing circumstances. Females may, for example, change the yolk hormone deposition to adjust offspring begging behaviour to the parental care they expect from their male (Navara *et al.* 2006). If for example male food provisioning is correlated with his attractiveness or some other characteristic (either positively or negatively), then females could anticipate, and extract maximum parental investment from her male by influencing offspring growth and begging through adjustment of maternal hormones in the eggs. Consistent with this idea, female house finches (*Carpodacus mexicanus*) deposit higher levels of androgens in their eggs when paired to unattractive males, which

show reduced nestling feeding. Whether one of these adaptive explanations applies to our results in the blue tit remains an open question. Females decreased yolk testosterone deposition in response to an experimental reduction of male attractiveness (crown UV coloration), which seems consistent with the differential allocation hypothesis, but it remains to be demonstrated that such increased androgen levels benefit the offspring and are costly to produce for the female.

Although we found a significant effect of the male UV manipulation on yolk testosterone levels, there was no significant correlation between androgen levels in the second, base-line egg, and male pre-treatment coloration. The inclusion of additional male characteristics (age, body mass, tarsus) as explanatory variables in a multiple regression analysis, which accounted for the possibility that female androgen deposition was dependent on multiple male cues, did not reveal any significant effect either. Nor did the inclusion of female characteristics (age, body mass, tarsus, laying date). The UV reduction caused by our treatment is large and even reduces crown UV reflectance below the natural range (Chapter 3). Possibly, in the natural situation blue tit females only modify yolk androgen deposition if the UV reflectance of the crown plumage of their male shows a sudden and dramatic decrease, for example caused by damage due to disease or severe fighting. The magnitude of the manipulations of the male sexual signals applied in the studies listed in Table 5.1 was probably also large. It should be carefully considered to what extent the results of such studies indicate adaptive maternal androgen deposition in response to male attractiveness.

ACKNOWLEDGEMENTS

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PART III

UV coloration as a signal in inter-individual competition

CHAPTER 6

UV signalling is not involved in male-male territorial conflict in the blue tit

Peter Korsten, Thomas H. Dijkstra &
Jan Komdeur

Behaviour, provisionally accepted

ABSTRACT

Structurally-based ultraviolet (UV) coloration of plumage can signal male quality and plays a role in female mate choice in many bird species. UV-reflecting badges could also be important signals in male-male competition. We tested if territorial blue tit (*Parus caeruleus*) males discriminate between conspecific male intruders which differ in the UV reflectance of their crown feathers. During the female fertile period, when male intrusions for extra-pair copulations occur, we simultaneously introduced two male blue tit taxidermic mounts in the territories of resident males; one mount with natural crown UV reflectance and one mount with reduced crown UV. The two mounts provoked strong aggressive reactions from resident males. Males specifically directed their aggression to conspecific intruders, as a male blue tit mount received substantially more aggression than a mount of a European robin (*Erithaca rubecula*) when these were simultaneously presented. However, aggression of resident males did not vary between the UV-reduced and the control mount. Furthermore, the variation in natural crown UV reflectance of the resident males did not predict the intensity of the aggressive response towards the mounts. Contrary to previous findings our results suggest that UV signals play only a limited role in male-male interactions during territorial intrusions.

INTRODUCTION

Competition between males over limited resources and potential mates occurs in many avian mating systems and can, together with female mate choice, drive the evolution of male plumage colours that have social signalling functions (Andersson 1994). Such colour badges of status allow competitors to assess each other's fighting ability from a distance, and hence to settle contests without risking injury during aggressive interactions (Rowher 1975, 1982; Whitfield 1987). In order to ensure reliability and to prevent cheaters providing dishonest information such status signals have to be associated with costs (Maynard Smith & Harper 1988; Johnstone & Norris 1993). Costs of the production and maintenance of bright plumage coloration could be socially induced, increased predation risk, direct energetic or nutrient limitation, or hormone mediated (Møller 1987; Folstad & Karter 1992; Andersson 1994; Slagsvold *et al.* 1995).

Plumage colours are either due to pigment deposition in the feathers (melanins or carotenoids; Olson & Owens 1998; Jawor & Breitwisch 2003) or to microstructures in the feather barbs (Fox 1976; Prum *et al.* 2003). These microstructures can not only produce blue, violet and iridescent colours, but also ultraviolet (UV), which is invisible to humans. UV-reflective plumage parts are widespread among many avian taxa (Burkhardt 1989; Eaton & Lanyon 2003; Hausmann *et al.* 2003), and many bird species can detect wavelengths in the UV portion of the spectrum (320–400 nm; Parrish *et al.* 1984; Cuthill *et al.* 2000). UV plumage colours can signal several aspects of male quality, such as viability (Sheldon *et al.* 1999), parasite load (Doucet & Montgomerie 2003), nutritional condition (Keyser & Hill 1999; McGraw *et al.* 2002) and territory quality (Keyser & Hill 2000). Furthermore, several studies have shown female preferences for males with high plumage UV reflectance over less UV-reflective males (Andersson & Amundsen 1997; Hunt *et al.* 1998; Johnsen *et al.* 1998).

Male secondary sexual characters that function as female mate choice cues often also play an important role in male-male competition (Berglund *et al.* 1996). However, few studies have investigated the role of UV ornaments in male-male competition. In the Eastern bluebird (*Sialia sialis*), males with higher plumage UV reflectance had a competitive advantage over less colourful males in the acquisition of vacant nestboxes (Siefferman & Hill 2005). In the blue tit (*Parus caeruleus*), in which both sexes have UV reflecting blue crown feathers, territorial males showed higher levels of aggression towards male taxidermic mounts with natural crown UV reflectance than towards mounts with reduced crown UV (Alonso-Alvarez *et al.* 2004). The UV crown plumage of both male and female blue tits is probably important in inter-sexual signalling during mutual mate choice, as indicated by the occurrence of assortative pairing with respect to crown UV reflectance (Andersson *et al.* 1998; Alonso-Alvarez *et al.* 2004). In addition, the UV crown plumage of male blue tits was found to play a role in inter-sexual signalling during extra-pair mate choice (Delhey *et al.* 2003), offspring sex allocation (Sheldon *et al.* 1999; Griffith *et al.*

2003) and allocation of maternal care (Limbourg *et al.* 2004). However, in our blue tit study population we observed no assortative pairing with respect to crown UV reflectance (Box B), which suggests that the signalling function of blue tit UV crown plumage may vary among populations. Furthermore, when we presented mounts of male and female blue tits – which are sexually dimorphic primarily with respect to the intensity of the crown UV reflectance – to territorial males both mounts were attacked (T.H. Dijkstra & P. Korsten, personal observation). This suggests that in contrast to the findings of Alonso-Alvarez *et al.* (2004), who conducted their territorial intrusion study in a population of the North-African blue tit subspecies (*Parus caeruleus ultramarinus*) differences in the UV reflectance of conspecific intruders may not influence the aggressive response of resident males in our study population of the nominate subspecies.

Therefore, we aimed to test if ultraviolet signalling of the crown plumage is involved in male-male agonistic interactions during territorial intrusions in our blue tit study population. We introduced two taxidermic male blue tit mounts – of which one had reduced crown UV reflectance and the other had natural crown UV reflectance after a control treatment – into territories of resident males during the female fertile period, and we measured if resident males varied their aggressive responses towards the two different mount types. Alonso-Alvarez *et al.* (2004) presented both mounts sequentially to territorial males, separated by at least one-hour intervals. To control for possible mount effects Alonso-Alvarez *et al.* (2004) used one single male's mounted body fitted with either one of two detachable male heads, of which one had reduced crown UV reflectance and the other had natural crown UV. We presented the two male mounts with the different UV treatments simultaneously. This experimental set-up has the advantage of a direct comparison of resident males' reactions to the different intruder types, while controlling for irrelevant variation in male reactions due to *e.g.* differences in the timing of the mount presentation, the motivation of the males, or the presence or absence of the female mate during the simulated intrusion. To control for possible mount effects we switched the UV-reduced and control treatment regularly between the two mounts.

In addition to the replication of the original mount intrusion experiment of Alonso-Alvarez *et al.* (2004) we measured the natural crown UV reflectance of the resident males to investigate the relation between the UV signals of focal males and their aggressive responses towards the 'intruding' male mounts. In birds, males searching for a territory probably mostly attack territory owners with relatively low intensity colour signals indicating lower fighting ability (Røskaft & Rohwer 1987; Pryke *et al.* 2002). Furthermore, male blue tits with more UV-shifted crown plumage are less cuckolded (Delhey *et al.* 2003), which may indicate that they are better mate guarders than less UV-shifted males. Therefore, we expected that males with more intense UV signals would show fiercer reactions towards the mounts than less UV-reflective males, which would be similar to the positive correlation between the intensity of pigment-based plumage coloration and resident male

aggression that was found in several other bird species (Studd & Robertson 1985; Järvi *et al.* 1987a; Pryke *et al.* 2001a). The function of structural/UV colour signalling in competitive interactions is relatively unexplored and this is one of the few studies (see also Pryke & Griffith 2006) in which the extent of male aggressive behaviour has been related to males' own UV coloration.

METHODS

Study area and population

We conducted this research from 14 March to 30 May, 2003, in a blue tit population of the nominate subspecies breeding on the estate of de Vosbergen (*ca.* 50 ha; 53°08' N, 06°35' E), near Groningen, the Netherlands. The study area contains *ca.* 185 nestboxes designed for blue tits and consists of patches of mixed deciduous and coniferous forest interspersed by patches of open grassland. The blue tit population was monitored during the breeding seasons of 2001–2003, and during this period we marked all breeding adults with a uniquely numbered metal ring and a unique combination of colour rings.

For the present study we regularly checked nestboxes for occupation from March 14 onwards. Once we discovered nest building activity, we recorded the stage of nest building, and the dates of laying of the first egg, clutch completion and hatching. We conducted territorial intrusion trials with male blue tit taxidermic mounts, both in territories where nests were completed, but laying had not commenced, and in territories where the female had started laying, but not completed her clutch. We captured parent birds during nestling feeding with spring traps in nestboxes. We sexed adults by the presence (= female) or absence (= male) of an incubation patch, and aged them as first year or older (see Svensson 1992). We measured body mass (± 0.1 g, using a Pesola spring balance) and the spectral reflectance of the crown feathers.

Measurements of crown colour

The spectral reflectance of the crown feathers of all but two resident males (n = 35) in whose territories we conducted successful intrusion trials was measured with an USB-2000 spectrophotometer with illumination by a DH-2000 deuterium-halogen light source (both Avantes, Eerbeek, The Netherlands). The measuring probe was held at a right angle against the plumage, *i.e.* both illumination and recording were at 90° to the feathers. During each crown reflectance measurement we took 5 replicate readings and smoothed each of these reflectance spectra by calculating the running mean over 10 nm intervals. Following previous studies of UV colour signalling in blue tits (*e.g.* Sheldon *et al.* 1999; Griffith *et al.* 2003; Delhey *et al.* 2003) we calculated three indices describing the variation in crown coloration – 'brightness', 'hue', and 'UV chroma' – from each reflectance spectrum and averaged these across the 5

replicate spectra. Brightness was the sum of reflectance between 320–700 nm ($R_{320–700}$), which corresponds to the spectral range visible to blue tits (Hart *et al.* 2000). Hue was the wavelength of maximum reflectance, $\lambda(R_{max})$. 'UV chroma' was the sum of reflectance between 320–400 nm divided by the sum of reflectance between 320–700 nm ($R_{320–400}$ / $R_{320–700}$). Both the 'hue' and 'UV chroma' indices have previously been identified as important predictors of male attractiveness and viability in blue tits (*e.g.* Sheldon *et al.* 1999; Delhey *et al.* 2003; Griffith *et al.* 2003). Males were captured for crown colour measurements within a relatively short period (14–29 May) leading to little variation in crown feather wear (Örnborg *et al.* 2002; Delhey *et al.* 2006), and consequently crown coloration was not significantly related to the date of capture (brightness: $r = 0.30$, $P = 0.08$; hue: $r = –0.072$, $P = 0.68$; UV chroma: $r = 0.27$, $P = 0.12$; all $n = 35$).

Territorial intrusion experiment

We used two taxidermic mounts of male blue tits (mount A and B), which had a neutral pose (body straight, wings down alongside the body and the tail following the line of the back). We reduced the original UV reflectance of the crown feathers of one of the two mounts by applying a 40%/60% (by weight) mixture of duck preen gland fat and UV-blocking chemicals, 50% Parsol 1789 and 50% Parsol MCX (by weight; Roche, Basel, Switzerland; Figure 6.1). As a control, preen gland fat only was applied to the crown feathers of the other mount, which left the original UV reflectance unaltered (Figure 6.1). The method of UV reduction followed the procedures of previous UV manipulations of wild male blue tits (Sheldon *et al.* 1999; Limbourg *et al.* 2004) and the procedure used by Alonso-Alvarez *et al.* (2004) to manipulate the UV of a male blue tit taxidermic mount. After variable numbers of trials (9.6 ± 6.0 SD) the mounts were switched in treatment (5x) to control for differences in reactions of resident birds based on other physical differences between the mounts (total number of trials: $n = 49$). We switched the treatment after a variable number of trials to be able to balance the number of trials in which either mount A was UV-reduced or mount B was UV-reduced for those trials in which the resident closely approached (< 1 m) or attacked at least one of the mounts ($n = 15$ trials; mount A UV-reduced: $n = 8$; mount B UV-reduced: $n = 7$; see below for more details on sample sizes). To switch the mounts in treatment both the UV-reducing chemicals and the duck preen gland fat were removed from the feathers by rinsing with 96% ethanol after which the crown feathers were blown until dry. The presence or absence of a UV-reduction effect caused by the treatment was invisible to the human eye, but spectrophotometric measurements proved that the cleaning method was effective and entirely restored the original UV reflectance of the mounts (Figure 6.1). After we ascertained that the original UV reflectance of the mounts was restored the treatments were re-applied. In summary, mount A and B each received both the UV-reduction and control treatment 3 times, and each mount was cleaned 5 times (Figure 6.1).

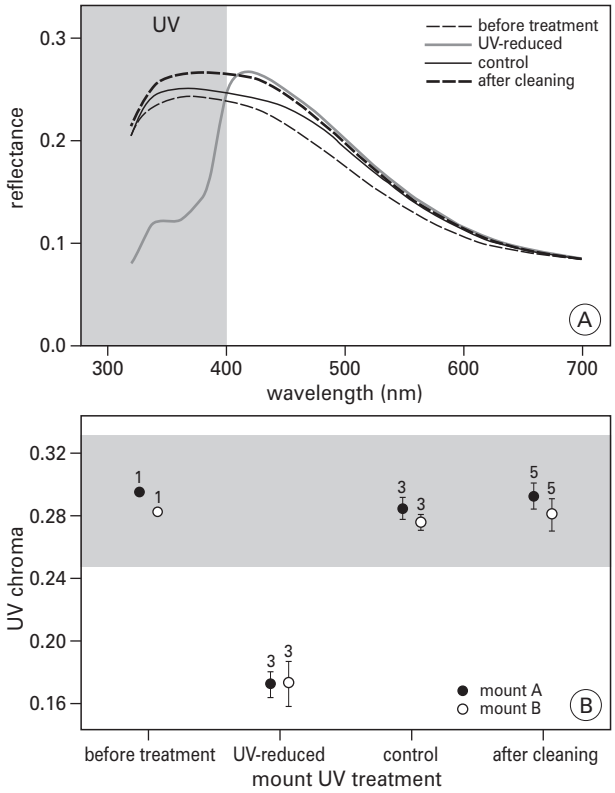

Figure 6.1 (A) Mean reflectance spectra and (B) UV chroma of the crown plumage of two male blue tit taxidermic mounts (A and B) used to simulate territorial intrusions calculated from crown reflectance measurements taken before the first UV treatment (n = 2), after each UV-reduction treatment (n = 6), after each control treatment (n = 6), and after each cleaning (n = 10). In (B), error bars indicate SD's and numbers indicate sample sizes. The shaded area indicates the natural range of UV chroma values of blue tit males captured during the breeding seasons of 2001–2003 (n = 111).

During the intrusion trials both mounts were simultaneously presented in the territory of a resident male on top of two T-shaped sticks (horizontal 2 x 1.0 m on a 1.5 m vertical stick; Figure 6.2). To prevent the mounts from damage due to the aggressive responses of the resident males each mount was put in a green wire cage (7.0 x 12.0 x 15.0 cm). The sticks with the mounts were placed alongside each other, at 7 meters from a tree with an occupied nestbox, in such a manner that both mounts were equally accessible from surrounding trees and nearby branches (Figure 6.2). The sticks including the empty wire cages were placed in position one day before each trial to habituate the resident birds to the foreign objects in their territory. At the start of each intrusion trial we placed both mounts in the cages

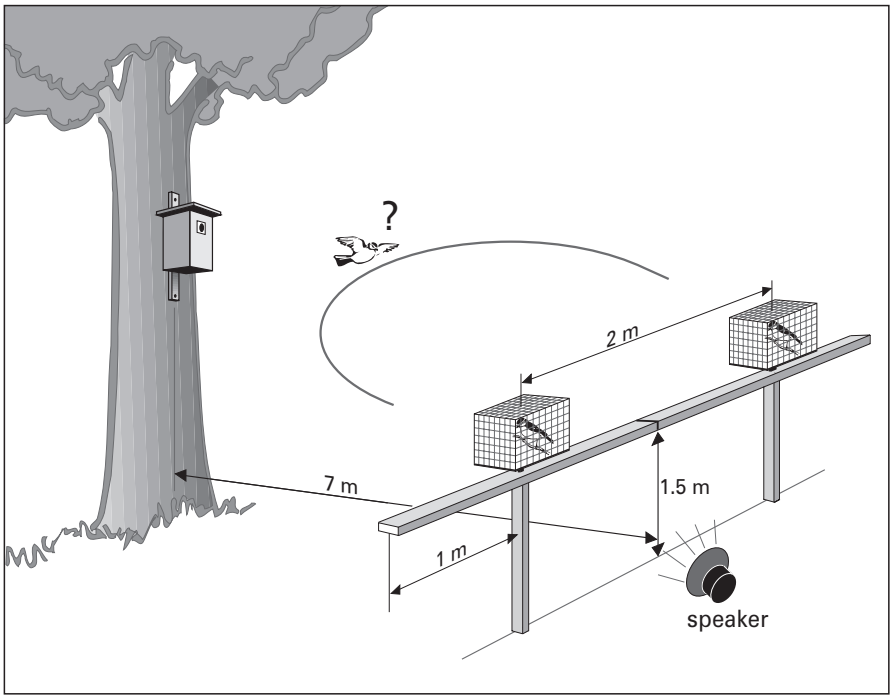

Figure 6.2 Experimental set-up of territorial intrusion trials with male blue tit taxidermic mounts.

having first ascertained that none of the resident birds were in the vicinity. In each trial the UV-reduced and control-treated mounts were randomly assigned to the left and right cages. To attract the resident birds, song of an unfamiliar blue tit male was played from a speaker placed on the ground in between the two mounts (Figure 6.2). The sexes of the resident birds were identified by their colour rings. If the resident male did not respond to the presence of the mounts and song playback, *i.e.* did not arrive within a distance of 10 m from one of the mounts within 15 minutes (recorded with a stopwatch) we stopped the intrusion trial. If the male responded within 15 minutes we measured the latency from the start of song playback until the moment of first arrival within 10 m (hereafter referred to as 'latency of response'). At the arrival of the male we started an observation protocol of five minutes (see Alonso-Alvarez *et al.* 2004). We measured the latencies from the moment of the male's arrival until his first approach within 1 m of one of the mounts (hereafter referred to as 'latency to approach'), and until the moment he first landed on one of the cages to physically attack the mount inside (hereafter referred to as 'latency to attack'). Because distance of resident birds to intruders is a good estimate of the intensity of aggression (Studd & Robertson 1985; Pryke *et al.* 2001a; Alonso-Alvarez *et al.* 2004) we recorded the distance between the focal male and

the nearest mount every five seconds on a voice recorder. We recorded the following distances: (1) contact with cage, hereafter referred to as 'attack'; (2) between 0–1 m, but no contact with cage, hereafter referred to as 'approach'; (3) > 1 m from cage. When the male was exactly in between the mounts only the distance to both mounts (0–1 m) but no nearest mount was recorded (Figure 6.2). Only one trial per nestbox was conducted. All trials were conducted without the observer (TD) knowing which type of treatment was used on each of the two mounts (treatment applied by PK).

We conducted intrusion trials at 49 nestboxes with nest stages ranging from 11 days before to 7 days after laying of the first egg (average clutch size: 11.0 ± 1.67 SD). The precise onset of the fertile period of females is unknown in most wild birds (Birkhead & Møller 1998). In blue tits, 5 to 6 days before laying resident males are frequently challenged by male territorial intrusions (Kempenaers *et al.* 1992) and intensify mate guarding. At this time the fertile period is likely to begin, which lasts until one day before completion of the clutch (Kempenaers *et al.* 1995). Therefore, we excluded eight trials which were conducted more than 6 days before the first egg was laid from the analyses. Four trials conducted in the territories of what turned out to be two polygynous males were also excluded from the analyses.

Of the 37 individual resident males included in the analysis, 23 males responded to the mounts and song playback and arrived within 10 m from the mounts in less than 15 minutes. Fifteen of these 23 males approached within 1 m of at least one of the mounts, and 13 of these males attacked one or both mounts. In 8 of these trials mount A was UV-reduced and mount B control-treated; in 7 trials mount B was UV-reduced and mount A control-treated. There was no effect of the different male mounts on the response of males, as they did not first approach or first attack one of the mounts more often (4 first approaches to mount A versus 11 to mount B, Yates' corrected χ^2 =2.40, df = 1, P = 0.12; 7 first attacks of mount A versus 6 of mount B, Yates' corrected χ^2 =0.00, df = 1, P = 1.0). Also the number of males that approached or attacked either mount A or mount B during the intrusion trials was not different, as 14 of 23 males approached mount A versus 15 of 23 males that approached mount B (Yates' corrected χ^2 =0.00, df = 1, P = 1.0) and 12 of 23 males attacked mount A versus 11 of 23 males that attacked mount B (Yates' corrected χ^2 =0.00, df = 1, P = 1.0). Males also spent a similar amount of time within 1 m from each mount (mean number of five-second intervals ± SE: mount A, 5.00 ± 0.87; mount B, 7.07 ± 1.18; paired t-test: t = -1.70, df = 14, P = 0.11) and attacking each mount (mean number of five-second intervals ± SE: mount A, 14.7 ± 2.89; mount B, 17.0 ± 2.16; paired t-test: t = -0.85, df = 12, P = 0.41). Therefore, we can exclude the possibility that differences in appearance between the two mounted blue tit males, other than manipulated crown UV reflectance, influenced our results.

To check if the aggression by the resident males towards the male blue tit mounts was specifically directed to conspecific intruders we presented a mount of a

European robin (*Erithaca rubecula*), which is also a common species in our study area, together with a male blue tit mount in the territories of 8 different resident males following the same protocol as described above.

Data analyses

To investigate if aggression of resident males was specifically directed to one of the two simultaneously presented mount treatment types we tested: (1) if one of the mounts was more often first approached (within 1 m) or first attacked by the resident males; (2) if a greater number of resident males approached or attacked one of the mounts during the five-minute intrusion trial (see Alonso-Alvarez *et al.* 2004); (3) if the time (= number of five-second intervals) resident males spent within 1 m from each mount or attacking each mount differed between the two mount types.

To investigate if the intensity of the aggressive reaction of resident males was related to their own UV coloration we tested: (1) if indices of crown colour differed between males that responded to the simulated intrusion, approached at least one of the mounts, or attacked at least one of the mounts, and males that did not; (2) if male crown colour indices were correlated with the latency of response to the simulated intrusion, or with the latencies to approach or attack; and (3) if male crown colour indices were correlated with the time males spent within 1 m from the mounts or attacking the mounts.

To investigate if resident males directed their aggression to either the UV-reduced or control-treated mount depending on their own UV coloration we tested if crown colour indices differed between males that first approached or attacked the UV-reduced or control-treated mount. Furthermore, we tested if male crown colour indices were related to the proportion of five-second intervals spent within 1 m from the UV-reduced mount or attacking the UV-reduced mount when compared to the total number of five-second intervals spent within 1 m and attacking respectively. Distributions of the proportions of time spent within 1 m from the UV-reduced mount and attacking the UV-reduced mount did not deviate from normal and we used weighted linear regression for this analysis. The linear regression models were weighted for the total number of intervals spent within 1 m of either mount or attacking either mount respectively, because these numbers varied considerably among males (Figure 6.3). All statistical tests were two-tailed and *P* values smaller than 0.05 were regarded significant. We carried out all analyses using SPSS 12.1.

RESULTS

Blue tit mount versus European robin mount

Five of eight resident blue tit males responded within 15 minutes when we simultaneously presented mounts of a male blue tit and a European robin in their territories. Resident males specifically directed their aggression to the blue tit mount; four

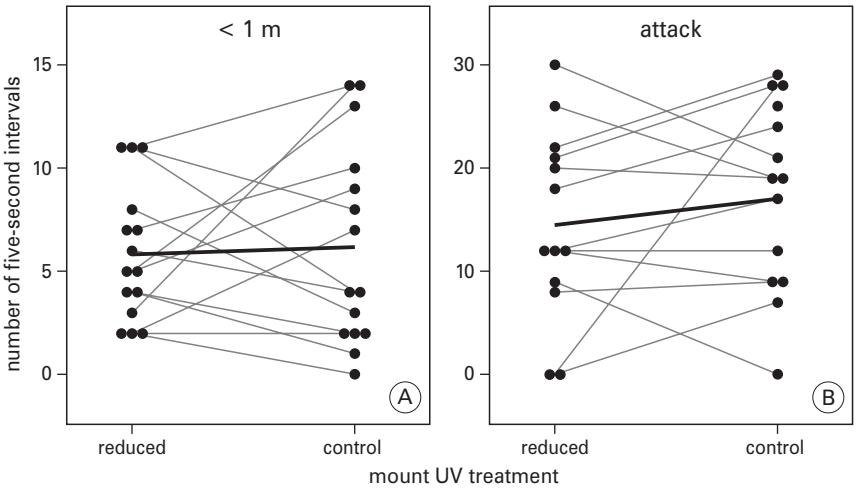

Figure 6.3 Number of five-second intervals spent by territorial male blue tits (A) within 1 m of (n = 15) or (A) attacking (n = 13) each of the two simultaneously presented intruder mounts (UV-reduced and control) during five minute trials. Dots connected by thin lines indicate paired observations of individual resident males. Thick lines indicate average slopes. Paired t-tests of UV-reduced versus control: Approach < 1 m, t = –0.25, df = 14, P = 0.81; Attack, t-test: t = –0.91, df = 12, P = 0.38.

of five males first approached the blue tit mount and four males landed on the cage with the blue tit mount, whereas only one male landed on the cage with the robin mount. Although males did not spend more time within 0–1 m ('approach') from the blue tit mount than within 0–1 m from the robin mount (mean number of five-second intervals ± SE: blue tit, 1.80 ± 1.56; robin, 1.40 ± 0.51; paired t-test: t = 0.27, df = 4, P = 0.80), males spent substantially more time on the cage ('attack') with the blue tit mount, than on the cage with the robin mount (mean number of five-second intervals ± SE: blue tit, 39.6 ± 10.8; robin, 0.80 ± 0.80; paired t-test: t = 3.66, df = 4, P = 0.022).

UV-reduced versus control-treated blue tit mounts

Resident males did not preferentially first approach within 1 m from either the UV-reduced (9 males) or control-treated mount (6 males) (Yates' corrected χ^2 = 0.27, df = 1, P = 0.61), or more often first attack either the UV-reduced (6 males) or control-treated mount (7 males; Yates' corrected χ^2 = 0.00, df = 1, P = 1.0). Also, the number of males that approached or attacked either the UV-reduced or control-treated mount during the intrusion trials was not different, as 15 of 23 males approached the UV-reduced mount versus 14 males that approached the control-treated mount (Yates' corrected χ^2 = 0.00, df = 1, P = 1.0) and 11 of 23 males attacked the UV-reduced mount versus 12 males that attacked the control-treated

mount (Yates' corrected $\chi^2 = 0.00$, df = 1, $P = 1.0$). Males spent similar amounts of time within 1 m of and attacking the two mount types (Figure 6.3).

Influence of resident male crown colour

There was no difference in the crown colour of resident males that responded within 15 minutes to the presentation of the blue tit mounts and males that did not respond (Table 6.1). Similarly, there was no difference between the crown colour of males that approached (< 1 m) or attacked at least one of the mounts and males that did not (Table 6.1).

Crown colour of males was unrelated to: 1) their latency of response after we presented the male blue tit mounts in their territories; 2) their latency to approach and their latency to attack; 3) the amount of time they spent within 1 m from the mounts or attacking the mounts (Table 6.2).

Crown colour did not differ between males that first approached the UV-reduced or control mount (Table 6.3). Similarly, crown colour did not differ between males that first attacked the UV-reduced or control-treated mount (Table 6.3). Male crown colour was unrelated to the proportions of five-second intervals spent within 1 m of the UV-reduced mount or attacking the UV-reduced mount (Table 6.4).

Table 6.1 Comparisons of crown colour indices of blue tit males with different aggressive reactions to male blue tit mounts.

Colour indices	Response			No response			Independent samples *t*-tests		
	Mean	SE	n[1)]	Mean	SE	n[1)]	t	df	P
Brightness	71.1	2.46	21	74.4	2.79	14	–0.86	33	0.40
Hue	394.4	2.57	21	394.1	3.04	14	0.07	33	0.94
UV chroma	0.289	0.003	21	0.288	0.006	14	0.12	33	0.91
	Approach < 1 m			No approach < 1 m					
Brightness	71.1	2.36	13	71.2	5.46	8	–0.01	19	1.0
Hue	397.5	2.51	13	389.3	5.11	8	1.42	10.4[2)]	0.18
UV chroma	0.286	0.003	13	0.294	0.005	8	–1.38	19	0.18
	Attack			No attack					
Brightness	72.0	2.38	12	70.0	4.96	9	0.37	11.6[2)]	0.72
Hue	397.5	2.73	12	390.2	4.59	9	1.43	19	0.17
UV chroma	0.287	0.004	12	0.293	0.005	9	–0.95	19	0.35

1) Note that sample sizes are reduced, because crown reflectance of 2 males included in the analyses was not measured.
2) Adjusted for unequal variances.

Finally, male age and body mass were unrelated to the likelihood of response to the simulated territorial intrusion or any measure of aggressive behaviour (both P values > 0.11). Neither were male age or body mass related to the UV treatment of the mount to which the males directed their aggression (both P values > 0.46).

Table 6.2 Relationships between crown colour indices of blue tit males, and latency and intensity of aggressive reactions to male blue tit mounts.

	Brightness			Hue			UV chroma		
	r	n[2)]	P	r	n[2)]	P	r	n[2)]	P
Latency of response	0.04	21	0.86	–0.06	21	0.79	0.18	21	0.45
Latency to approach (< 1 m)	0.46	13	0.12	0.12	13	0.70	0.20	13	0.52
Latency to attack	0.33	12	0.30	0.09	12	0.78	0.14	12	0.66
Time spent[1)] within 1 m	–0.07	21	0.77	0.08	21	0.72	–0.02	21	0.95
Time spent[1)] attacking	0.03	21	0.90	0.35	21	0.12	–0.31	21	0.17

1) Number of five-second intervals.
2) Note that sample sizes are reduced, because crown reflectance of 2 males included in the analyses was not measured.

Table 6.3 Comparisons of crown colour indices of blue tit males that first approached (< 1 m) or attacked either the UV-reduced or control mount.

Colour indices	Response			No response			Independent samples t-tests		
	Mean	SE	n[1)]	Mean	SE	n[1)]	t	df	P
First approach									
Brightness	69.5	2.94	8	73.7	4.03	5	–0.86	11	0.41
Hue	398.4	1.98	8	396.0	6.11	5	0.44	11	0.67
UV chroma	0.285	0.004	8	0.288	0.006	5	–0.43	11	0.68
First attack									
Brightness	71.1	4.40	5	72.7	2.89	7	–0.32	10	0.76
Hue	395.5	6.04	5	398.8	2.31	7	–0.58	10	0.58
UV chroma	0.288	0.008	5	0.286	0.004	7	0.33	10	0.75

1) Note that sample sizes are reduced, because crown reflectance of 2 males included in the analyses was not measured.

Table 6.4 Relationships between crown colour indices of blue tit males and the proportions of five-second intervals these males spent near (within 1 m) and attacking the UV-reduced male blue tit mount.

	Brightness			Hue			UV chroma		
	F	df[2)]	*P*	*F*	df[2)]	*P*	*F*	df[2)]	*P*
Proportion time spent < 1 m of UV-reduced mount[1)]	0.18	1, 11	0.68	0.04	1, 11	0.85	0.04	1, 11	0.84
Proportion time spent attacking UV-reduced mount[1)]	0.28	1, 10	0.61	0.14	1, 10	0.72	0.15	1, 10	0.71

1) Proportion of five-second intervals spent near (< 1 m) or attacking the UV-reduced mount. Linear regression models were weighted for total number of five-second intervals spent near (< 1 m) or attacking either the UV-reduced or control mount.
2) Note that sample sizes are reduced, because crown reflectance of 2 males included in the analyses was not measured.

DISCUSSION

In contrast to a previous study by Alonso-Alvarez *et al.* (2004) in a blue tit population of the *ultramarinus* subspecies, we found no difference in the likelihood of territorial blue tit males of the nominate subspecies attacking a conspecific male mount with natural (after control treatment) or reduced crown UV reflectance, when we simultaneously introduced these different mount types in males' territories. Power analysis shows that, given the effect size that we found – 12 of 23 males attacked the control-treated mount, whereas 11 of 23 males attacked the UV-reduced mount – we would have needed a sample size of > 2 x10^3 trials to reach 80% power of rejecting the H_0-hypothesis of no difference in the likelihood of males attacking the two mount types with $\alpha = 0.05$ (Buchner *et al.* 1997). Therefore, we are confident that the absence of a significant effect was not due to lack of statistical power, but that UV coloration of the intruder mount in reality had no or a negligibly small effect on the aggressive response of the territorial males. This clearly negative result was confirmed by the absence of an effect of mount UV treatment on all other parameters of resident male aggression we measured, such as which mount type was first approached or attacked, and the time that males spent near (< 1 m) or attacking the differently treated mounts. Furthermore, we found that resident males' own crown UV coloration was unrelated to the intensity of their aggressive reaction to the mounts, which is another indication that crown UV signalling is of little importance in male-male conflict between territory owners and intruders in our study population.

The UV reduction treatment distorted the spectral profile of the crown reflectance and reduced UV chroma values to below the natural range (Figure 6.1). This may lead to an unnatural appearance of the UV-reduced mount to the resident

blue tit males, which could potentially lead to artifactual results. Nevertheless, we believe that our conclusions are valid for the following reasons. Firstly, the UV-reduced blue tit mount was certainly recognised as a conspecific, since resident males directed their aggression equally to the UV-reduced and control-treated mount. Furthermore, it is very unlikely that resident males would discriminate between intruders that vary more subtly in their crown colour (*e.g.* within the natural range), given that they did not vary their responses in relation to the extreme difference in crown coloration between the two intruder mounts in our experiment. Lastly, we used the same UV-reduction treatment as Alonso-Alvarez *et al.* (2004), who did find an effect of the treatment on resident male aggression in their study population.

At this stage we can only speculate why the importance of crown UV reflectance as a signal in owner-intruder conflict varies between different blue tit subspecies or populations. Interestingly, Alonso-Alvarez *et al.* (2004) both found evidence for a role of crown UV coloration in male-male conflict, and in male and female mate choice, as indicated by the occurrence of assortative mating with respect to crown UV reflectance, whereas in our population we did not find evidence for a role of crown UV reflectance in either male-male conflict or in mutual mate choice as indicated by the absence of assortative pairing (Box B). There is a remarkable parallel here with the studies on the signalling function of the melanin-pigmented black bib feathers of the house sparrow (*Passer domesticus*), where the role of the black bib in male-male competition and female mate choice also varies between populations, possibly in relation to the intensity of male-male competition over suitable nest sites (Griffith *et al.* 1999b). It is possible that differences in the local ecological circumstances between blue tit populations, such as for example differences in light environment, breeding densities, food situation, opportunity for extra-pair copulations, and abundance of suitable breeding territories and nest cavities, also cause variation in the signalling function of the crown UV coloration among blue tit populations.

Some of these factors may indeed be different between the population of the *ultramarinus* subspecies studied by Alonso-Alvarez *et al.* (2004) and our study population of the nominate subspecies, for example: (1) the light environment in the natural habitat of the *ultramarinus* subspecies, whose natural range is at lower latitudes than the nominal subspecies' range, is more luminant (C. Alonso-Alvarez, personal communication). In this context it seems interesting that the *ultramarinus* subspecies has darker crown plumage with lower overall reflectance (see Alonso-Alvarez *et al.* 2004). (2) Furthermore, the study population of Alonso-Alvarez *et al.* (2004) was breeding on a small island (83 km^2) in the Mediterranean Sea (Pantelleria, Italy; 36°82' N, 11°97' E), whereas we studied a mainland population. In passerine birds, rates of extra-pair paternity are generally lower in island than in mainland populations (Griffith 2000; but see Krokene & Lifjeld 2000). If extra-pair paternity rates are indeed lower in Alonso-Alvarez *et al.*'s (2004) study population

than in ours, male territorial intrusions may pose less of a paternity threat and a relatively greater territorial threat in Alonso-Alvarez *et al.*'s (2004) population. Male blue tits with low crown UV were found to sire at least as many extra-pair offspring as high UV males (Delhey *et al.* 2003), but low UV males may be less successful in territory take-overs than high UV males. Therefore, variation in the relative importance of the territorial and paternity threat posed by intruding males may lead to different defence strategies of territory owners based on the intruder's crown colour in the different study populations.

Prior residency is generally the main determinant of the outcome of aggressive interactions during territorial intrusions (Davies 1978; Krebs 1982) often overruling indicators of fighting ability, such as colour badges of status (Holberton *et al.* 1990) or body size (Chellappa *et al.* 1999; Dale & Slagsvold 1995; Turner 1994). This could explain why we found no effect of the residents' own crown colour, body mass or age on their aggressive response. Also several other studies found no evidence for structurally-based plumage colour signalling during conflicts between territory owners and intruders. Territorial male purple martins (*Progne subis*) with experimentally lightened structural plumage coloration did not defend their territories more or less successfully than males that were control-treated (Stutchbury 1992). Territorial bluethroat males (*Luscinia svecica*) also did not differ in their ability to retain their territories when either UV-reduced or control-treated (Johnsen *et al.* 1998). Nevertheless, structural plumage signals could still be important in male-male competition when the ownership of vacant territories has to be established and the competing males have similar aggressive motivation. For example, in purple martins, subadult males that were dyed to appear adult-like obtained a territory more quickly than control subadults (Stutchbury 1991). Also, male eastern bluebirds (*Sialia sialis*) with high UV reflectance were more successful during the acquisition of nest sites than males with low UV reflectance (Siefferman & Hill 2005).

If structurally-based coloration acts as a signal during territory acquisition, individuals that are more colourful may be able to obtain territories of higher quality. To date the relationship between territory quality and structural plumage coloration remains largely unstudied, but Keyser & Hill (2000) found in blue grosbeaks (*Guiraca caerulea*) that males with brighter structural coloration defend larger territories of higher quality than duller males. Territory quality is an important determinant of breeding success in blue tits (Blondel *et al.* 2000; Przybylo *et al.* 2001) and it would be interesting to investigate if a relationship exists between crown UV coloration and territory quality in this species. The existence of such a relationship would support the idea that the intensity of the UV signal influences the outcome of competition over vacant territories.

Another context in which blue tit UV plumage may act as a signal of competitive ability is in foraging winter flocks. Continual fighting over every small food item would be very costly. Status signalling through a colour badge could be an energy-saving solution. Male over-winter survival was found to be positively related

to crown UV reflectance in a Swedish population (Griffith *et al.* 2003; Sheldon *et al.* 1999), which may indicate that crown UV coloration plays a role in competitive interactions over scarce food sources or safe roosting sites during winter. However, in our study population first evidence suggests no influence of crown UV coloration on the outcome of competitive interactions over food (see Chapter 7).

In summary, contrary to a previous study in the same species we found no evidence for UV signalling playing a role in the agonistic interactions between male territory owners and intruders. This emphasizes the need for the replication of studies in behavioural and evolutionary ecology in different populations of the *same* species to evaluate the robustness and generality of the patterns found. Future research in avian visual communication should aim at improving our understanding of the relative importance of structural/UV colour signalling during competitive interactions in different bird populations and (sub)species and in different contexts such as during owner-intruder conflict, territory establishment and in winter foraging flocks.

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CHAPTER 7

Absence of status signalling by structurally based ultraviolet plumage in wintering blue tits

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ABSTRACT

Structurally based ultraviolet-reflective plumage parts can be important cues in mate choice. However, it remains largely unknown if ultraviolet (UV) plumage variation can also function as a signal of social status during competitive interactions. In blue tits (*Parus caeruleus*), the UV-reflective crown plumage functions as a female mate choice cue that probably indicates male quality, as males with higher UV reflectance have been shown to have higher chances of over-winter survival. Possibly, the UV crown plumage acts as a status signal in competition over scarce food sources during winter. To test this idea, we related dominance of individuals at an artificial food source during adverse winter conditions to spectrophotometric measurements of their crown plumage. However, while controlling for the confounding effects of sex, age and distance from territory, we found no significant effect of crown UV reflectance on dominance. Consistent with this result we also found no relation between crown UV reflectance and over-winter survival. We conclude that the structurally based UV reflectance of the blue tit crown feathers plays little role in competition between individuals during winter despite its importance as a cue in mate choice.

INTRODUCTION

Bright and conspicuous plumage colours in birds can function as cues in mate choice (Hill 1991; Andersson 1994) and as signals of social status, which individuals use to settle conflicts over food, territories and mates (Rohwer 1975; Møller 1990; Pryke *et al.* 2001a). The use of coloured plumage patches ('badges of social status') to settle conflicts over limited resources is thought to be beneficial to both dominant and subordinate individuals, because competing individuals of unequal fighting ability do not need to waste time and energy, or risk injury in assessing each other's fighting ability (Rowher 1982). To maintain the honesty of such status signals, individuals that express a signal of high dominance without actually having superior competitive ability ('cheaters') should pay some cost (*e.g.* Maynard Smith & Harper 1988; Johnstone & Norris 1993). Such costs of producing and maintaining bright plumage coloration could be increased predation risk, direct energetic or nutrient limitation, hormone-mediated immunosuppression, or an increase in the frequency of aggressive encounters with high-status individuals (Møller 1987; Folstad & Karter 1992; Slagsvold *et al.* 1995; Olson & Owens 1998; Buchanan *et al.* 2001).

Bright plumage colours in birds are produced by pigments, mostly carotenoids or melanins, which are deposited in the feathers, or by the nanostructure of the feather barbs (Hill & McGraw 2006a). In contrast to pigment-based plumage colour variation, which is primarily in the human-visible part of the spectrum, structurally based plumage coloration can also vary in the ultraviolet (UV) (Andersson 1999; Prum *et al.* 2003; Shawkey *et al.* 2003). Traditionally, studies of avian colour communication have mainly focused on pigment-based colour variation that is visible to human observers (Bennett *et al.* 1994). However, many bird species, including most passerine species tested to date, are capable of detecting wavelengths in the UV section of the spectrum (320–400 nm) (Cuthill 2006) and structurally based, UV-reflective plumage parts are widespread among many avian taxa (Eaton & Lanyon 2003; Hausmann *et al.* 2003). These findings make it very likely that birds use UV plumage colours both as cues in mate choice and as signals of social status during conflicts. UV colour variation has indeed been shown to act as a mate choice cue in several bird species (Andersson & Amundsen 1997; Bennett *et al.* 1997; Andersson *et al.* 1998; Hunt *et al.* 1998; Siitari *et al.* 2002; Delhey *et al.* 2003; Komdeur *et al.* 2005). However, until now the role of UV plumage colours in avian communication other than in a mate choice context, such as in inter-individual conflicts, or outside the breeding season remains largely unknown.

To evaluate if structurally based UV plumage coloration can function as a signal of social status during competitive interactions among non-breeding birds, we investigated if natural variation in UV reflectance of the crown feathers of blue tits (*Parus caeruleus*) indicates social dominance during winter. Blue tits often erect or flatten their crown feathers during agonistic interactions with conspecifics (Stokes 1962; Scott & Deag 1998), indicating a signalling function of the crown feathers in

the context of competition between individuals. The crown feathers, which appear bright blue to human observers, also reflect substantially in the UV. Blue tits are sexually dimorphic with respect to this UV component of the crown reflectance, *i.e.* males reflect more UV than females (Andersson *et al.* 1998; Hunt *et al.* 1998). Furthermore, the UV reflectance of the crown plumage is an important cue in both social and extra-pair mate choice (Andersson *et al.* 1998; Hunt *et al.* 1998; Delhey *et al.* 2003). The UV reflectance may also function as a signal in male-male territorial conflicts during the breeding season, as breeding males were shown to react more aggressively towards a mounted conspecific male with natural crown UV reflectance than towards a mount with reduced crown UV reflectance (Alonso-Alvarez *et al.* 2004, but see Chapter 6). Possibly, blue tit crown UV reflectance signals individual viability or quality, as in a Swedish population males with higher UV reflectance during the breeding season were found to have higher subsequent over-winter survival (Sheldon *et al.* 1999; Griffith *et al.* 2003).

We hypothesize that blue tit UV coloration has not only a signalling function in mate choice and male-male territorial conflicts during the breeding season, but also functions as a signal of social status within flocks of wintering birds. During winter, blue tits (and some other *Parus* species) aggregate in loosely organized foraging flocks ('basic flocks') that roam the area in search for food (Colquhoun 1942; Ekman 1989). We suggest that within these flocks highly UV-reflective individuals may be more successful at monopolizing food sources, which could be a proximate explanation for their demonstrated greater survival chances (Sheldon *et al.* 1999; Griffith *et al.* 2003). To evaluate if highly UV-reflective individuals have higher dominance and priority in access to food sources we related the dominance of individuals at an artificial food source to spectrophotometric measurements of their crown plumage. Furthermore, we measured the distances that these individuals had to travelled from their territories to the food source. This enabled us to control our analyses for the potentially confounding effects of site-dependent dominance, *i.e.* the phenomenon that individuals are more dominant at sites closer to their own territory (Colquhoun 1942; de Laet *et al.* 1984; Oberski & Wilson 1991; Dingemanse & de Goede 2004; Hansen & Slagsvold 2004). While controlling for sex, age and distance to territory, we tested if: (1) social dominance was related to crown UV reflectance; (2) individuals with higher UV reflectance had a greater probability of survival to the following breeding season, as reported previously (Sheldon *et al.* 1999; Griffith *et al.* 2003).

METHODS

Study area and population

This research was conducted during the winter of 2002/2003 on the estate of 'De Vosbergen', near Groningen, The Netherlands (53°08'N, 06°35'E). The study area of approximately 50 ha contains *ca.* 185 nestboxes designed for blue tits and consists

of patches of mixed deciduous and coniferous forest interspersed by patches of open grassland. The blue tit population breeding at the Vosbergen estate was monitored during the breeding seasons of 2001–2004, and during this period all breeding adults were routinely captured with mistnets or in nestboxes when feeding the nestlings. All captured adults and nestlings were marked with a uniquely numbered metal ring. In addition, all adults were marked with a unique combination of colour rings.

For the present study we provided a continuous food supply in the form of balls of seeds and fat at a feeding table near a fieldstation in the centre of the study area from October 2002–January 2003. We captured blue tits at the feeding table with baited cage traps from 26 November 2002–13 January 2003, and while roosting in nestboxes at night during two periods: from 19 November–4 December 2002 and from 20–27 January 2003. Individuals were aged as first-winter birds or older (see Svensson 1992). We measured body mass (± 0.1 g) and tarsus length (± 0.1 mm). Spectrophotometric measurements of crown colour were made and blood samples (*ca.* 20 μL) were taken by puncture of the brachial vein. DNA extracted from these blood samples was used to identify the sex of individuals using sex specific molecular makers (P2 and P8; Griffiths *et al.* 1998). Following Sheldon *et al.* (1999) and Griffith *et al.* (2003) we defined over-winter survivors as birds that were recaptured when breeding in the study area the following spring; nonsurvivors were defined as birds that were not recaptured.

Measurements of crown UV reflectance

We captured 166 individual blue tits, 91 males and 75 females, of which we made spectrophotometric measurements of crown colour. Of these, 95 individuals were captured and measured once, 55 were captured and measured on two separate days, 14 on three days, and 2 on four days, yielding a total of 255 measurements. Mean number of days (± SD) between first and second, second and third, and third and fourth captures were 44 ± 17, 22 ± 11, and 25 ± 10 days, respectively.

The spectral reflectance of the crown feathers was measured with an USB-2000 spectrophotometer with illumination by a DH-2000 deuterium-halogen light source (both Avantes, Eerbeek, The Netherlands). The measuring probe was held at a right angle against the plumage, *i.e.* both illumination and recording were at 90° to the feathers. During each crown reflectance measurement we took 5 replicate readings and smoothed each of these reflectance spectra by calculating the running mean over 10 nm intervals. See Figure 7.1 for mean reflectance spectra of the crown plumage of first-year and older males and females. Following previous studies of UV colour signalling in blue tits (Andersson *et al.* 1998; Sheldon *et al.* 1999; Griffith *et al.* 2003; Delhey *et al.* 2003) we calculated three indices describing the variation in crown coloration – 'brightness', 'hue', and 'UV chroma' – from each reflectance spectrum, and averaged these across the 5 replicate spectra. 'Brightness' was the sum of reflectance between 320–700 nm ($R_{320–700}$), which corresponds to the spectral

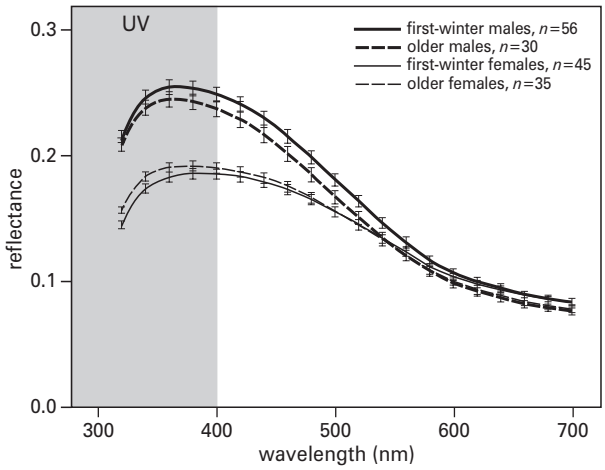

Figure 7.1 Mean crown reflectance spectra of first-year and older male and female blue tits during winter. Multiple measurements of same individuals taken on different days were averaged before calculation of the mean reflectance spectra. Standard errors around the means are depicted at 20 nm intervals. The shaded area indicates the UV part of the spectrum.

range visible to blue tits (Hart *et al.* 2000). 'Hue' was the wavelength of maximum reflectance, $\lambda(R_{max})$. 'UV chroma' was the sum of reflectance between 320–400 nm divided by the sum of reflectance between 320–700 nm ($R_{320–400}$ / $R_{320–700}$). Both the 'hue' and 'UV chroma' indices have previously been identified as important predictors of male attractiveness and viability in blue tits (Andersson *et al.* 1998; Sheldon *et al.* 1999; Delhey *et al.* 2003; Griffith *et al.* 2003). Hue and UV chroma values were significantly correlated (Table 7.1).

In accordance with previous findings (Örnborg *et al.* 2002; Delhey *et al.* 2006), we found that blue tit crown colour changed over time, as indicated by significant regressions of all colour indices on date of capture (brightness: $r = 0.52$, $P < 0.001$; hue: $r = 0.26$, $P < 0.001$; UV chroma: $r = –0.20$, $P = 0.001$; all $n = 255$). This pattern was also present within individuals that were captured on at least two separate days (paired *t*-tests comparing crown colour of first and last capture: brightness: $t = –10.51$, $P < 0.001$; hue: $t = –4.67$, $P < 0.001$; UV chroma: $t = 3.04$, $P = 0.003$; all df =70), and is probably due to feather wear or the accumulation of dirt or fat (Örnborg *et al.* 2002). Therefore we used the residuals of the regressions of crown colour indices on capture date in our further analyses. Residual crown colour measurements were repeatable within individuals between separate days of capture (brightness: repeatability = 0.50, $F_{70,89} = 3.26$, $P < 0.001$; hue: repeatability = 0.60, $F_{70,89} = 4.40$, $P < 0.001$; UV chroma: repeatability = 0.75, $F_{70,89} = 7.86$, $P < 0.001$; Lessells & Boag 1987). When crown colour of an individual had been measured on more than one day we used the average values of these separate measurements in our analyses.

Table 7.1 Matrix of correlations between dominance, sex, age (first-winter versus older birds), crown colour indices (brightness, hue and UV chroma), distance from territory and survival to the following breeding season of wintering blue tits.

	Sex			Age			Brightness			Hue			UV chroma			Distance			Survival		
	r	*n*	*P*	*r*	*n*	*P*	*r*	*n*	*P*	*r*	*n*	*P*	*r*	*n*	*P*	*r*	*n*	*P*	*r*	*n*	*P*
Dominance	**–0.39**	**36**	**0.018**	0.15	36	0.37	0.02	35	0.91	**–0.41**	**35**	**0.015**	**0.45**	**35**	**0.006**	**–0.52**	**31**	**0.003**	0.27	36	0.12
Sex				0.02	172	0.80	**–0.49**	**166**	**<0.001**	**0.59**	**166**	**<0.001**	**–0.72**	**166**	**<0.001**	0.01	143	0.87	–0.04	172	0.64
Age							–0.05	166	0.49	**–0.21**	**166**	**0.006**	0.15	166	0.053	0.12	143	0.16	**0.25**	**172**	**0.001**
Brightness										–0.05	166	0.49	0.09	166	0.26	–0.02	138	0.82	–0.06	166	0.44
Hue													**–0.82**	**166**	**<0.001**	–0.05	138	0.56	–0.14	166	0.06
UV chroma																–0.06	138	0.52	0.13	166	0.10
Distance																			–0.14	143	0.09

Dominance is the proportion of all other individuals encountered over which an individual was dominant. Colour indices are residuals controlled for seasonal change in crown reflectance. In total 172 individuals were captured and/or observed at the feeding table during the 2002/2003 winter; crown coloration was measured for 166 individuals; distance to territory could be calculated for 143 individuals and dominance for 36 individuals. Correlations for different combinations of these subsets can have different sample sizes. Significant correlations ($P < 0.05$) are in bold.

Competitive interactions and estimation of social dominance

We observed competitive interactions between blue tits competing for food at the feeding table from a distance of 5 m from inside the field station. The observations were made from 8–13 January 2003 during a single short period of snow cover and frost during that winter, which led to increased visitation rate and competition at the feeding table. During observation periods only a single ball of seeds and fat was provided to increase the competition among the feeding blue tits. Observations were made between 9:00 and 15:00 h when the largest numbers of birds were visiting the feeding table.

We recorded pairwise interactions between colour-banded individuals at the feeding table and inferred dominance when an individual (1) actively displaced another bird at the food source, either through a simple supplant, or by means of a postural display or attack, or (2) fed while an opponent waited to approach the food. Most of the observed conflicts were resolved with low intensity displays, and we rarely observed physical attacks (*cf* Scott & Deag 1998).

We observed 390 interactions between 55 colour-banded individuals, 31 males and 24 females. As a measure of social dominance we calculated the proportion of other individuals over which an individual was dominant (number individuals dominated / total number of individuals encountered; Hein *et al.* 2003). In total 350 interactions of 36 individuals, 25 males and 11 females, of which we observed 5 or more interactions, were included in this calculation (de Laet 1984). We chose to use the proportion of individuals dominated as a measure of dominance instead of calculating a ranking of individuals based on a win-loss matrix (de Vries 1998), because we believe this measure of dominance better reflects the site-dependent, and therefore spatially very dynamic, dominance relationships in the blue tit social system (Colquhoun 1942; Oberski & Wilson 1991; Hansen & Slagsvold 2004). Furthermore, the continuous scale nature of this measure of dominance allowed us to use parametric statistics for our analyses, which would not have been possible if we had used an ordinal dominance ranking of individuals. The measure of dominance we used was highly correlated with both the proportion of fights that an individual had won of all the fights in which it was involved ($r = 0.98$, $n = 36$, $P < 0.001$), and its dominance rank calculated according to de Vries (1998) ($r_s = 0.93$, $n = 36$, $P < 0.001$).

Calculation of travel distances

To control our analyses for the site-dependency of social dominance (*e.g.* Hansen & Slagsvold 2004), we calculated the travel distances (metres) from an individual's territory to the feeding table using GPS coordinates of the feeding table and the nestbox used for breeding and/or winter roosting. Territorial blue tits show high site fidelity and roost and breed in nestboxes inside their territories (Colquhoun 1942; P. Korsten & J. Komdeur, unpublished data). Therefore, the distance from an individual's territory to the feeding table was calculated as the average distance

between the feeding table's location and the locations of all nestboxes that an individual used for roosting during the winter of 2002/2003 and/or for breeding during the preceding (2002) or subsequent spring (2003). In this way we were able to estimate the travel distance for 143 individuals which were present in the study area during the winter of 2002/2003 (84 males, 59 females). Individual males and females were recorded at up to two breeding locations (one in 2002 and one in 2003) and three roosting locations (during the winter of 2002/2003). Based on these repeated individual recordings, we calculated the repeatability of travel distances. This analysis showed that distances were highly repeatable within individuals, both in males (repeatability = 0.94, $F_{59,116} = 46.25$, $P < 0.001$) and in females (repeatability = 0.90, $F_{40,76} = 26.15$, $P < 0.001$; Lessells and Boag 1987).

Statistical analyses

Body mass was significantly related to time of day, both for captures at the feeding table during daytime ($r = 0.58$, $n = 76$, $P < 0.001$) and captures in the nestboxes at night ($r = –0.22$, $n = 179$, $P = 0.003$). Therefore, we used residual body mass controlled for the time and period of day (day or night) in our further analyses. Multiple residual measures of body mass of single individuals were averaged. Also tarsus length (which is constant over life; P. Korsten & J. Komdeur, unpublished data) was averaged over repeated measurements within individuals. We used the ^{10}log values of the calculated distances from the individuals' territories to the feeding table in our analyses, because the distance-related decrease in dominance diminished at larger distances (see also Hansen & Slagsvold 2004). For a correlation matrix of the main variables included in our analyses see Table 7.1. *P* values <0.05 are considered significant and significance tests are two-tailed throughout. The analyses were carried out with SPSS version 13.0.

RESULTS

Influence of sex and age on crown coloration

Crown colour indices – brightness, hue and UV chroma – showed rather continuous frequency distributions with considerable overlap between the sexes and the two age classes (Figure 7.2). Nevertheless, males had on average brighter, more UV-shifted (lower hue), and more UV-chromatic crown plumage than females (Table 7.2; Figure 7.2). First-winter birds had less UV-shifted and UV-chromatic crown colour than older birds (Table 7.2; Figure 7.2). The age difference for crown hue was more pronounced in females than in males, as indicated by the significant interaction term (Table 7.2; Figure 7.2). Brightness of first-winter and older birds did not differ (Table 7.2; Figure 7.2). Canonical discriminant analysis with the three crown colour indices as predictor variables classified the individuals' sexes with an overall accuracy of 95.8% (84 of 91 males and 75 of 75 females were correctly

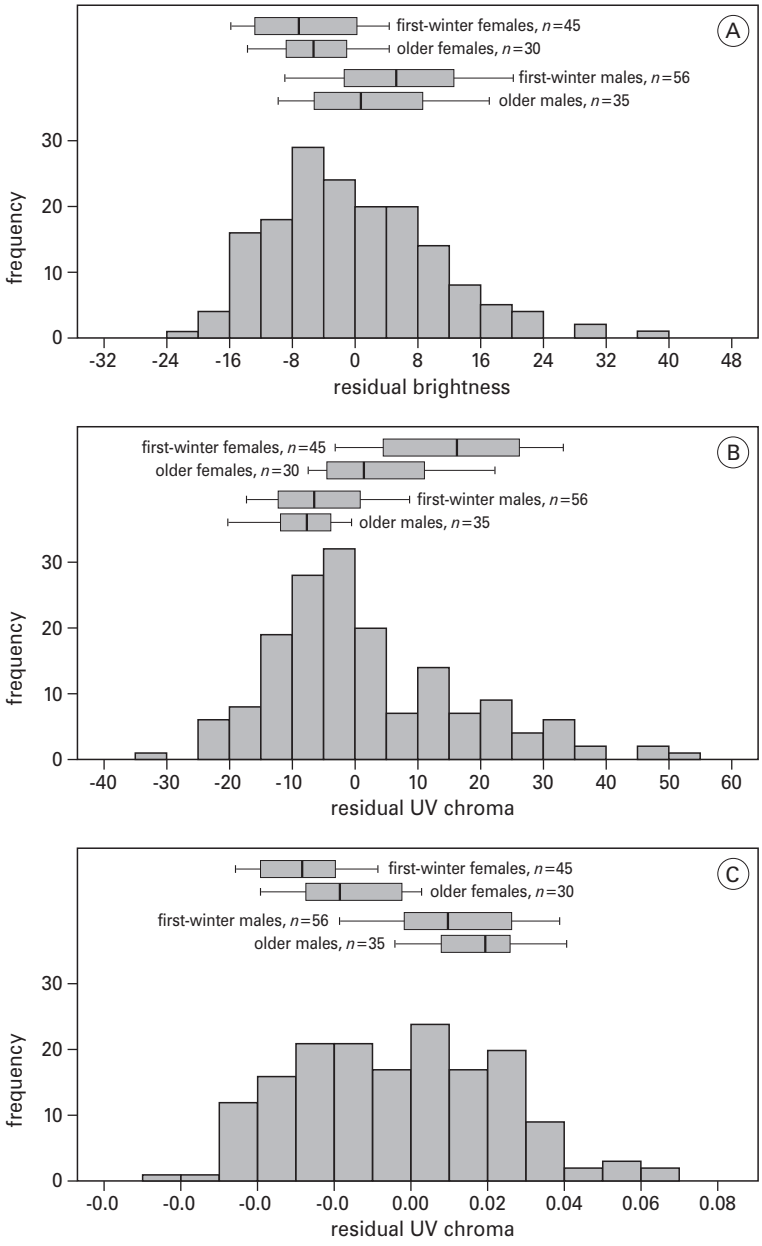

Figure 7.2 Frequency distributions of crown colour indices of wintering blue tits: residual brightness (A), hue (B), and UV chroma (C), controlled for the seasonal change of crown reflectance (n = 166; males and females, and different age classes combined). Horizontal box plots show brightness (A), hue (B), and UV chroma (C) for males and females, and for first-winter and older birds. Boxes indicate the 25th and 75th percentiles and whiskers indicate the 10th and 90th percentiles of the median. See also Table 7.2.

classified). The age of 56.0% of males (51 out of 91) and 72.0% of females (54 out of 75) could be classified correctly.

Influence of crown coloration on dominance

Dominance was strongly correlated with both hue and UV-chroma, with more dominant individuals having more UV-shifted and more UV-saturated crown coloration (Table 7.1). However, crown coloration was correlated with both sex and age (Tables 7.1 and 7.2), making it difficult to separate the effects of crown colour versus the effects of sex and age on dominance. Therefore, we used a stepwise forward multiple regression analysis in which sex, age, and indices of crown coloration were all entered to test which of these predictors best explained the variation in dominance (Table 7.3). Dominance was also strongly correlated with distance from the

Table 7.2 Influence of sex and age on crown coloration in wintering blue tits (n = 166).

Crown colour indices	Sex		Age		Sex x age	
	$F_{1,162}$	P	$F_{1,162}$	P	$F_{1,162}$	P
Brightness	43.98	< **0.001**	0.29	0.59	2.02	0.16
Hue	83.80	< **0.001**	14.87	< **0.001**	4.68	**0.032**
UV chroma	170.93	< **0.001**	9.48	**0.002**	0.39	0.53

Results from ANOVAs with sex and age (first-winter versus older birds) as factors. Colour indices are residuals controlled for seasonal change in crown reflectance. Significant P values are in bold. See also Figure 7.2.

Table 7.3 Influence of sex, age, crown coloration, body size and distance from territory on dominance in wintering blue tits (n = 30).

Explanatory variables	Coefficient	t	P
Included			
Distance	–0.47	–4.79	<0.001
Sex	0.28	3.55	0.001
Age	0.22	2.75	0.011
Excluded			
Brightness		–0.28	0.78
Hue		0.11	0.91
UV chroma		–0.54	0.60
Body mass		–1.42	0.17
Tarsus length		0.03	0.97

Results from a stepwise forward linear regression analysis with dominance as dependent variable and sex, age (first-winter versus older birds), indices of crown colour (brightness, hue, and UV chroma), body mass, tarsus length, and distance from territory as explanatory variables. Colour indices are residuals after controlling for seasonal change in crown reflectance. Final model including sex, age and distance: $F_{3,26}$ = 12.16, P < 0.01 (r^2 = 0.58). See also Figures 7.3 and 7.4.

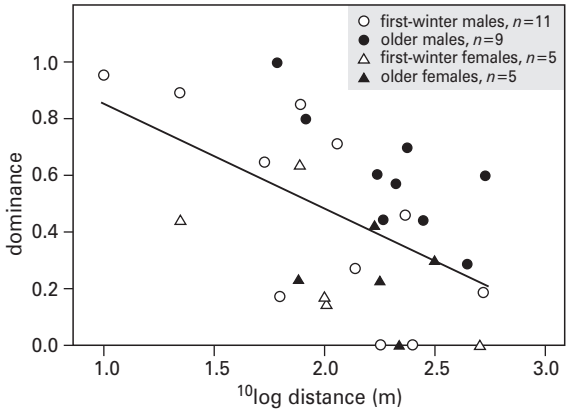

Figure 7.3 Influence of distance from territory on dominance of wintering blue tits. Overall regression line was added for visual purposes only. See also Table 7.3.

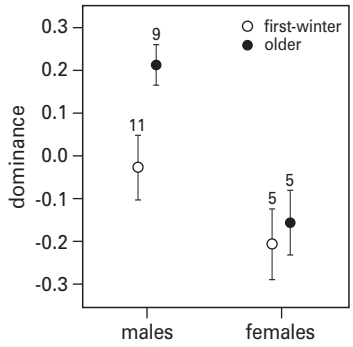

Figure 7.4 Influence of sex and age (first-winter or older) on residual dominance (controlled for distance from territory) of wintering blue tits. Means with standard errors. Numbers indicate sample size. See also Table 7.3.

territory (Table 7.1), which was therefore also entered as an explanatory variable. In addition, we entered body mass and tarsus length to control for the potential effects of body size on dominance. The regression analysis showed that distance from territory (Figure 7.3), and sex and age (Figure 7.4) together explained a large part of the total variation in dominance ($r^2 = 0.58$), whereas body mass, tarsus length and the three crown colour indices dropped from the final model as non-significant (Table 7.3). Adding the three crown colour indices as explanatory variables to this final model only led to a marginal and non-significant increase in the proportion of explained variation in dominance ($r^2 = 0.59$). An alternative model including distance and the crown colour indices, but not sex and age, explained considerably less variation in dominance ($r^2 = 0.44$) than the final model.

Influence of crown coloration on over-winter survival

Individuals with more UV-shifted and more UV-saturated crown coloration tended to have a higher probability of over-winter survival as indicated by their greater tendency for breeding in the study area the following spring (logistic regression, all $n = 166$: brightness: $\chi^2 = 0.60$, df = 1, $P = 0.44$; hue: $\chi^2 = 3.37$, df = 1, $P = 0.066$; UV chroma: $\chi^2 = 2.75$, df = 1, $P = 0.097$). However, as age and crown coloration were intercorrelated (Tables 7.1 and 7.2), it was difficult to separate the effects of age versus crown coloration on survival. A stepwise forward multiple logistic regression analysis in which age, crown colour indices, and also sex, body mass and tarsus length were entered as explanatory variables showed that only age had a significant effect on survival, whereas sex, crown coloration, body mass and tarsus length dropped from the model as non-significant terms (Table 7.4; Figure 7.5).

Table 7.4 Influence of sex, age, crown coloration and body size on survival to the following breeding season in wintering blue tits (n=166).

Explanatory variables	Coefficient	Wald (χ^2)	P
Included			
Age	1.14	11.10	0.001
Excluded			
Sex		0.15	0.70
Brightness		0.37	0.54
Hue		1.42	0.23
UV chroma		1.46	0.23
Body mass		1.81	0.18
Tarsus length		0.06	0.81

Results from logistic regression analysis with survival to the following breeding season as dependent variable and sex, age (first-winter versus older birds), indices of crown colour (brightness, hue, UV chroma), body mass and tarsus length as explanatory variables. Colour indices are residuals after correction for seasonal change in crown reflectance. See also Figure 7.5.

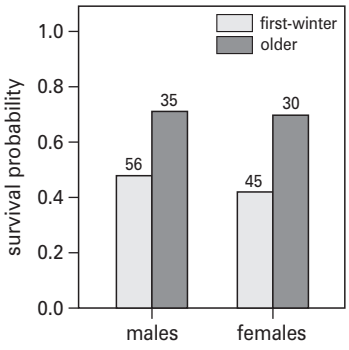

Figure 7.5 Probability of survival to the following breeding season for blue tit males and females of two age classes, first-winter and older birds. Numbers indicate samples sizes. See also Table 7.4.

DISCUSSION

Previous studies of social status signalling have mainly focused on pigment-based plumage colours and there is good evidence of a status signalling function for both melanin and carotenoid-pigmented plumage patches (*e.g.* Møller 1987; Pryke *et al.* 2001a, respectively). The present study has investigated if structurally based plumage coloration can also function as a signal of social status in the non-breeding season. Contrary to our prediction, the results show that the structurally based UV coloration of the blue tit crown feathers plays no apparent role in competition between individuals during winter and is not significantly related to winter survival, despite its importance as a cue in mate choice.

UV coloration and dominance

Blue tits in our study population were clearly sexually dimorphic for crown UV coloration, which confirms previous studies in other populations and supports the idea that the UV crown plumage is a sexually selected trait important in female mate choice (Andersson *et al.* 1998; Hunt *et al.* 1998; Delhey *et al.* 2003). Furthermore, we found that older birds of both sexes were somewhat more UV reflective than first-winter birds (*cf* Delhey & Kempenaers 2006), which may be caused by age-dependent ornament expression (as we found no evidence for differential survival according to plumage colour), and is typical for condition-dependent sexually selected characters (Andersson 1994; Siefferman *et al.* 2005). Given that the blue tit's crown UV coloration is an important mate choice cue, crown colour is also expected to act as a signal of social status in agonistic interactions, because ornamental traits mostly have dual signalling functions in both mate choice and intrasexual competition (Berglund *et al.* 1996). We found considerable and continuous variation in plumage UV reflectance (Figure 7.2), also within sex and age classes, suggesting that there is scope for UV status signalling in wintering blue tits. However, we found no evidence for UV status signalling, either within, or between, sex age classes. After controlling for the effects of sex, age and distance from territory, which together explained a remarkably large part of the variation in winter dominance ($r^2 = 0.58$), crown UV coloration did not significantly explain any additional variation in dominance. As males and older birds were both more dominant and more UV reflective than females and first-winter birds respectively, we cannot exclude the possibility that differences in UV crown plumage between sex and age classes have some influence on the outcome of aggressive interactions between birds of different sex and age, but this seems unlikely because sex and age class categories predicted individual dominance considerably better than did UV coloration. Furthermore, although crown UV coloration was a good predictor of sex (95.8% of individuals correctly classified based on crown coloration), it was a rather poor predictor of age, especially in males (56.0% of males and 72.0% of females correctly classified). Consequently, crown UV coloration would be an unreliable indicator of

age in competitive interactions. Although our correlational results strongly suggest that UV coloration does not function as a signal of social status, ultimately experimental manipulations of crown UV coloration are necessary to unequivocally refute any link between winter dominance and crown UV coloration in blue tits. Another possibility that still needs to be excluded is that between-individual variation in the *size* of the area of the UV reflecting crown plumage relates to individual dominance (for examples of such badge area related status signalling see *e.g.* Järvi *et al.* 1987b; Møller 1987; Pryke *et al.* 2001a)

UV coloration versus other determinants of dominance

Interestingly, we found distance from territory to be an important determinant of dominance (Figure 7.3), indicating that dominance was strongly site-dependent, which is in line with previous studies on winter dominance in blue tits and other *Parus* species (*e.g.* Dingemanse & de Goede 2004; Hansen & Slagsvold 2004). Thus the relative dominance of individual birds within highly-mobile flocks will be continuously changing depending on proximity to their territories, while obviously the appearance of their plumage or actual fighting ability does not. Therefore, an important part of the variation in dominance among individuals could not be caused by variation in plumage characteristics, and inter-individual conflicts must have been resolved in different ways. As we rarely observed escalated fights we suggest that the birds may use subtle behavioural cues to assess a competitor's motivation and likeliness to escalate a conflict (*cf* Scott & Deag 1998). Such behavioural cues may also play a role in the resolution of conflicts between individuals of different sex and/or age classes.

Sex, age and site-related effects are well-documented and generally important determinants of social dominance in birds (reviewed by Piper 1997). In addition, many other factors have been identified that may influence the outcome of contests between individuals. These include contestants' prior experiences with each other (Lemel & Wallin 1993), their relative durations of food deprivation (Lemel & Wallin 1993), prior residency (Krebs 1982), early social experiences (Hansen & Slagsvold 2004), personality (Dingemanse & de Goede 2004), and hormonal status (Järvi *et al.* 1987b). The use of status signalling through coloured plumage badges in the resolution of conflicts may in fact be limited to some quite specific contexts, given the large number of other factors which can potentially influence social dominance. It has indeed been suggested that status signalling through 'badges of social status' would only be evolutionary stable if the competing individuals are unfamiliar with each other, and if there are no asymmetries between individuals in for example territorial status at the location of the conflict (Maynard Smith & Harper 1988; Wilson 1992). This may also explain why studies of status signalling have often found equivocal or inconsistent results, probably depending on the exact circumstances during the observations and/or the experimental design, leading to an ongoing debate on the generality of badge status signalling in birds (Wilson 1992; Senar 1999).

Similarly in blue tits, status signalling by the crown UV coloration may only be important under specific circumstances. For example, Alonso-Alvarez *et al.* (2004) showed that during the breeding season territorial males reacted more aggressively towards male taxidermic mounts with natural UV reflectance than towards mounts with reduced UV. In this experiment territory owners could obviously not have used behavioural cues to assess the fighting ability or intentions of the (model) intruders, and apparently adjusted their aggressive behaviour to the relative UV reflectance of the models, which was the only perceivable difference between them. Note that a similar experiment in our study population which also involved model intruders, but had a slightly modified experimental design, showed no evidence for a role of crown UV coloration in territorial conflict during the breeding season (Chapter 6).

In addition to the blue tit study of Alonso-Alvarez *et al.* (2004) two other recent studies in different species have suggested that structurally based coloration could be important as a status signal in male-male competition during the breeding season. Siefferman and Hill (2005) found a negative correlation between date of nestbox occupation and UV/blue coloration in male Eastern bluebirds (*Sialia sialis*) and Keyser and Hill (2000) found a positive correlation between territory quality and blue coloration of the owners in male blue grosbeaks (*Guiraca caerulea*).

UV coloration and over-winter survival

The absence of a status signalling function of UV plumage in our population of wintering blue tits is consistent with the lack of a relation between survival to the following breeding season and UV coloration. Instead, over-winter survival was strongly dependent on age, with older birds having higher chances of survival (or acquisition of a breeding territory in the study area) than first-winter birds. This result is in contrast with two previous studies on a more northerly population in Sweden (Gotland) that reported higher chances of over-winter survival for more UV-reflective males (Sheldon *et al.* 1999; Griffith *et al.* 2003). In these previous studies survival was measured from one breeding season to the next, whereas for the present study we measured survival of birds that were present in winter to the following breeding season, which could have caused a discrepancy.

Alternatively, plumage-based status signalling may be more important in northerly blue tit populations, possibly because these are less sedentary during winter. In these populations individuals often leave their breeding areas when there is low food availability during cold weather (Smith & Nilsson 1987, and references therein). Under these circumstances encounter rates between non-territorial, unfamiliar birds will be greater and UV status signalling might be used to settle conflicts over food and shelter. This may eventually lead to higher over-winter survival of more UV-reflective individuals, as reported by Sheldon *et al.* (1999) and Griffith *et al.* (2003).

Mate choice cues versus signals of social status

The present study indicates that the UV coloration of the blue tit crown feathers may primarily function as a cue in mate choice, and not as an indicator of dominance during winter. This would be similar to the situation in the well-studied house finch (*Carpodacus mexicanus*) (*e.g.* Hill 1991; McGraw & Hill 2000a, 2000b), where females prefer mates with bright red carotenoid-based plumage coloration (Hill 1991), but red coloration does not reliably indicate male social status during the non-breeding season (McGraw & Hill 2000a). Remarkably, during the breeding season bright males are even subordinate to drab males in competition over food (McGraw & Hill 2000b). Comparable results have been found in the red-collared widowbird (*Euplectes ardens*), in which long male tail feathers are selected by female choice, but males do not use tail length as a signal in agonistic interactions (Pryke *et al.* 2001a, 2001b). In addition to their long tail, red-collared widowbird males have a red carotenoid collar badge which they do use as a status signal in male-male competition, but which is in turn not favoured by female choice (Pryke *et al.* 2001a, 2001b). These studies, together with our findings in the blue tit, show for several types of ornamentation (elongated tail feathers, structural and pigment-based plumage colours) that male ornaments selected through female mate choice are not necessarily always important as signals of social status in competitive interactions, or *vice versa*.

Interestingly, a recent comparative study suggested that the occurrence of sexually dimorphic structural plumage coloration among socially monogamous birds is related to especially sexual selection through extra-pair fertilizations, whereas this is not the case for melanin and carotenoid-based coloration (Owens & Hartley 1998). Instead, melanin-pigmented plumage seems to be mainly important in agonistic signalling (Badyaev & Hill 2000; Jawor & Breitwisch 2003), while carotenoid-based plumage coloration may be important in both mate choice (Hill 1991; Badyaev & Hill 2000) and agonistic signalling (Pryke *et al.* 2001a). These findings suggest that the different types of plumage coloration may have been largely selected by different forms of social and/or sexual selection. This idea is consistent with our results that show that a structurally based and sexually selected plumage character plays little role in inter-individual signalling in competitive interactions during winter.

ACKNOWLEDGEMENTS

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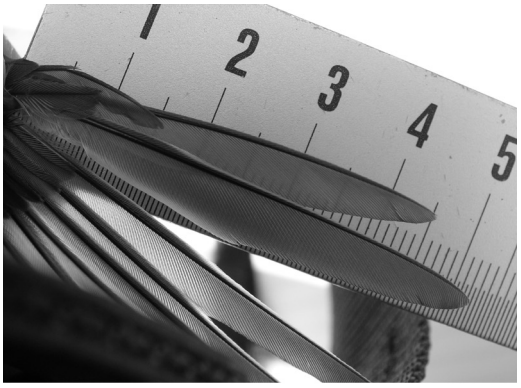
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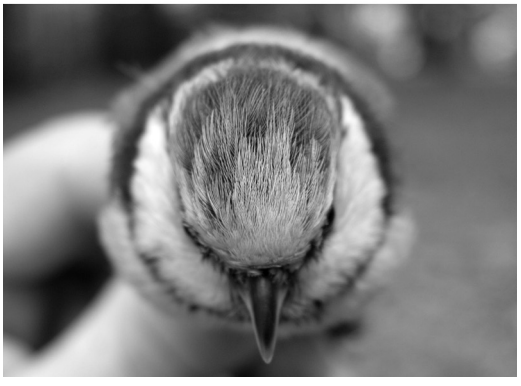

CHAPTER 8

General discussion

Background of the project

The research on blue tit sex ratio adjustment and male plumage coloration described in this thesis was part of a larger research program on avian sex allocation, which included four subprojects in total. The research program, which was funded by the Netherlands Organisation for Scientific Research (NWO) and coordinated by Serge Daan (University of Groningen), aimed at elucidating functions and mechanisms of avian sex allocation. Each subproject focussed on different aspects of sex allocation in birds. One project (PhD research by Nikolaus von Engelhardt, supervision by Cor Dijkstra and Ton Groothuis, University of Groningen) concentrated on revealing the physiological and hormonal mechanisms generating shifts in the primary and secondary sex ratio (von Engelhardt 2004). This research was carried out on zebra finches (*Taeniopygia guttata*), which seem ideal for studying the proximate control of avian sex allocation. Zebra finches are easy to keep and breed in captivity and shifts of primary or secondary sex ratio in response to manipulated male attractiveness (Burley 1981, 1986) and feeding regime (Kilner 1998; Bradbury & Blakey 1998; Rutkowska & Cichoń 2002; Rutstein *et al.* 2004b) have previously been described, which makes sex ratio variation in zebra finches open to experimentation. A second project (by Tim Fawcett, Ido Pen and Franjo Weissing, University of Groningen) took a modelling approach to further develop the theory on adaptive sex allocation, with an emphasis on vertebrates with complex life-histories such as birds and mammals (*e.g.* Pen & Weissing, 2000; Fawcett *et al.* 2006). The two remaining projects studied the functional aspects of sex allocation in response to male attractiveness in wild bird populations. The blue tit (*Parus caeruleus*) was chosen as a model species because male sexual attractiveness in the form of UV coloration of the crown plumage is easy to measure and manipulate in the field and shifts in sex ratio in relation to male attractiveness (Sheldon *et al.* 1999) and quality (measured in terms of over-winter survival; Svensson & Nilsson 1996) had been previously shown. In addition, the blue tit is a very abundant bird species in western Europe including the Netherlands and readily accepts nestboxes for breeding (Perrins 1979), making it a very practical species for study. One of the two blue tit projects (PhD research by Tobias Limbourg, supervision by Kate Lessells, Netherlands Institute of Ecology, Heteren) investigated sex-biased parental care as a strategy to adjust parental investment in male versus female offspring (Limbourg *et al.* 2004). The other project (supervision by Jan Komdeur, University of Groningen) investigated facultative adjustment of the sex ratio at laying (the primary sex ratio), which results are presented in this thesis.

In this last chapter, I first briefly review recent developments in avian sex allocation research and discuss our results in the light of these developments. Thereafter, I discuss the progress that has been made in understanding the role of the ornamental UV/blue crown plumage in sexual selection and sex allocation in the blue tit and compare our results to the findings in other blue tit populations. Finally, I suggest some directions for future research.

Avian sex allocation: function and mechanism

Mechanisms of primary sex ratio adjustment

It has long been thought that the chromosomal sex determination system of birds (and mammals) with random segregation of the sex chromosomes during meiosis poses a strong constraint that precludes facultative sex ratio adjustment at laying (Williams 1979; Clutton-Brock 1986). In the 1990s, however, a number of studies was published that showed significant, and probably also adaptive, shifts in the primary sex ratio (*e.g.* Dijkstra *et al.* 1990; Wiebe & Bortolotti 1992; Daan *et al.* 1996; Ellegren *et al.* 1996; Komdeur 1996; Svensson & Nilsson 1996; Appleby *et al.* 1997; Komdeur *et al.* 1997, Kilner 1998; Nager *et al.* 1999; Pen *et al.* 1999; Sheldon *et al.* 1999). These studies have stimulated a surge of interest in facultative primary sex ratio adjustment in birds and since then the number of published studies on the subject has been expanding continuously (see Figure 1.1, Chapter 1). Despite this large research effort, there is ongoing controversy about the generality of facultative primary sex ratio adjustment in birds (Koenig & Dickinson 1996; Radford & Blakey 2000; Krackow 2002) and recent meta-analyses have come to opposing conclusions on the general sex ratio patterns in the literature (West & Sheldon 2002; Ewen *et al.* 2004; Cassey *et al.* 2006). One important issue which is hindering further progress in this debate is the lack of a known genetic or physiological mechanism that birds use to influence the primary sex ratio (Krackow 1995; Pike & Petrie 2003; Krackow 2002).

In birds, in contrast to mammals, females are the heterogametic sex (females are ZW; males are ZZ). Consequently, female birds produce male (Z) and female (W) ova, which potentially gives them control over the sex of their offspring (Krackow 1995; Oddie 1998). Although it is far from clear how birds could bias the sex ratio at laying, several mechanisms are conceivable (Krackow 1995; Emlen 1997; Komdeur *et al.* 2002; Pike & Petrie 2003). These can broadly be separated into pre- and post-ovulation control mechanisms. Pre-ovulation, females could possibly influence the sex of the oocyte shed from the ovary either by non-random segregation of sex chromosomes during the first division of meiosis shortly before ovulation, by atresia of follicles of the 'wrong' sex, or by differential growth of follicles destined to become a specific sex (Krackow 1995; Komdeur *et al.* 2002; Pike & Petrie 2003). Post-ovulation, oocytes of the 'wrong' sex could be re-absorbed at various stages before and after fertilisation. Such re-absorption of a 'wrong' sex oocyte after ovulation would probably lead to a gap in the laying sequence (Emlen 1997), given the strong hierarchy in the development of follicles in the ovary with a fixed interval between the maturation of the successive follicles (Sturkie 1986). Female manipulation of egg sex by post-ovulation absorption would probably be more costly than pre-ovulation control, because of the loss of time and invested resources (Pen & Weissing 2002). It is not unlikely that different sorts of mechanisms of sex ratio adjustment are at work in different avian taxa (Pike & Petrie 2003).

Recently, several findings have been published that may bring us a step closer to understanding the physiological basis of avian primary sex ratio adjustment. In several species, the sex and size of eggs have been found to be related (Mead *et al.* 1987; Anderson *et al.* 1997; Cordero *et al.* 2000, 2001; Magrath *et al.* 2003), which indicates that females may be able to discriminate between eggs of different sex in their reproductive tract and allocate resources accordingly. Moreover, studies have found egg sex-specific allocation of maternal hormones to the yolk (Petrie *et al.* 2001; Müller *et al.* 2002; but see Pilz *et al.* 2005), which must be the result of differential hormone allocation to the developing oocyte by the cells of the follicular wall. This suggests either that females are able to discriminate between follicles destined to become a specific sex, or that the maternal hormones allocated to the developing oocyte influence the segregation of the sex chromosomes during the first meiotic division shortly before ovulation. Furthermore, several studies have shown endocrine influences on primary sex ratio (Correa *et al.* 2005; Pike & Petrie 2006; but see von Engelhard *et al.* 2004). Particularly strong evidence for pre-ovulation control of egg sex was presented by Komdeur *et al.* (2002) who showed egg sex of Seychelles warblers (*Acrocephalus sechellensis*) to be significantly biased without a preceding laying gap, which implies that the bias already existed before ovulation. Very recently, the first evidence has been found for atresia of 'wrong' sex oocytes by pigeons (Pike 2005; pre-ovulatory follicle selection) as well as sex-specific growth patterns of oocytes of house finches that exhibit sex-biased laying orders (Young & Badyaev 2004). This long-needed progress in the elucidation of the physiological basis of primary sex ratio adjustment is both very exciting and promising, and will hopefully give the study of avian primary sex ratio adjustment a new impulse.

Primary sex ratio adjustment to male attractiveness

A wide array of factors have been found to influence primary sex ratio in birds, including for example position in the laying order (Badyaev *et al.* 2002), season (Dijkstra *et al.* 1990), prey abundance (Appleby *et al.* 1997), habitat quality (Komdeur *et al.* 1997), feeding regime (Kilner 1998), maternal condition (Nager *et al.* 1999), male quality (Svensson & Nilsson 1996) and male sexual attractiveness (Sheldon *et al.* 1999). Although these studies show that birds modify the primary sex ratio in response to a wide variety of factors, it is often difficult to make a clear *a priori* prediction on the relationship between primary sex ratio and a specific factor. The sex ratio shift to be expected often depends on the exact details of an animal's life-history, which are often not precisely known in birds (Pen & Weissing 2002; West & Sheldon 2002). One factor for which it is possible to give a clear *a priori* prediction of the expected effect on sex ratio is male sexual attractiveness (West & Sheldon 2002). Females are expected to produce more sons when mated to an attractive male (Burley 1981, 1986; Pen & Weissing 2000; Fawcett *et al.* 2006). This is predicted because in birds males show generally greater variation in reproductive success than females (Møller & Ninni 1998). Therefore, sons will benefit more

from having an attractive father, given that attractiveness is heritable. Burley (1981, 1986) originally developed this verbal argument, and she showed that offspring sex ratio at fledging was related to manipulated male attractiveness in zebra finches. The validity of the verbal argument has recently been confirmed by analytical (Pen & Weissing 2000) and simulation models (Fawcett *et al.* 2006).

Several correlational studies have attempted to find a relationship between primary sex ratio and male attractiveness or quality, yielding mixed evidence for such a link, with results varying between different years (*e.g.* Radford & Blakey 2000; Griffith *et al.* 2003) and populations (*e.g.* Svensson & Nilsson 1996; Leech *et al.* 2001; Rosivall *et al.* 2004; Griffith *et al.* 2003; Dreis *et al.* 2006). Relatively few experimental studies in which some aspect of male attractiveness was manipulated have found an effect on primary sex ratio (collared flycatcher [*Ficedula albicollis*], Ellegren *et al.* 1996; blue tit, Sheldon *et al.* 1999; spotless starling [*Sturnus unicolor*], Polo *et al.* 2004; peafowl [*Pavo cristatus*], Pike & Petrie 2005). The results on blue tit primary sex ratio variation in relation to manipulated male UV coloration presented in this thesis add another experimental example of primary sex ratio adjustment to male attractiveness. Several other experimental studies in different species, have failed to find an effect of male attractiveness on primary sex ratio (barn swallow [*Hirunda rustica*], Saino *et al.* 1999; mallard [*Anas platyrhynchos*], Cunningham & Russell 2000; dark-eyed junco [*Junco hyemalis*], Grindstaff *et al.* 2001; zebra finch [*Taeniopygia guttata*], Zann & Runciman 2003; Rutstein *et al.* 2004a). Note that Burley (1981, 1986) in her seminal study on offspring sex ratio and male attractiveness in zebra finches investigated the sex ratio of surviving offspring after fledging (*i.e.* the secondary sex ratio).

Given the clear *a priori* prediction that females paired to attractive males should produce more sons, the inconsistency between studies is difficult to explain. It is interesting in this respect that the modelling study of Fawcett *et al.* (2006) showed that, although females should indeed produce more sons when mated with an attractive male, the selective pressure for such sex ratio adjustment to evolve is very weak. This may explain why studies find such different patterns in different years, populations and species. Given the generally weak selection pressure for sex ratio adjustment to evolve, the occurrence of sex ratio adjustment to male attractiveness may be very sensitive to the specific circumstances of each particular population and study species. Their specific ecological and social environment may easily shift the balance of the costs and benefits of sex ratio control in relation to male attractiveness. Further study of the fitness consequences of sex ratio adjustment would be needed to better predict the patterns of sex ratio variation to be expected (Komdeur & Pen 2002).

Primary sex ratio adjustment in the blue tit

Several correlational and experimental studies have now investigated the relationship between the primary sex ratio and male attractiveness or quality in blue tits (Table 8.1). The overall picture that emerges from these studies is rather mixed, with some evidence for primary sex ratio adjustment, but patterns vary among

study years and populations. Svensson and Nilsson (1996) provided the first evidence for primary sex ratio adjustment in the blue tit. In a Swedish population, they found females to bias the sex ratio of their broods towards sons if paired to a supposedly high-quality male that survived to the following breeding season. As a follow up on this finding, Sheldon *et al.* (1999) carried out their influential study (> 150 citations) in a Gotland population of blue tits that provided both correlational and experimental evidence for primary sex ratio adjustment in relation to male crown UV reflectance – which is probably an important determinant of male attractiveness (Andersson *et al.* 1998; Hunt *et al.* 1998; Delhey *et al.* 2003). Sheldon *et al.*'s (1999) correlational data showed that females produced a greater proportion of male eggs if paired with a male with higher natural UV reflectance, which is consistent with the straightforward prediction by sex allocation theory (Trivers & Willard 1973; Burley 1981, 1986; Fawcett *et al.* 2006).

The experimental data of Sheldon *et al.* (1999) were more difficult to interpret. Experimental reduction of male UV reflectance – making males less attractive – did not result in a lower proportion of male offspring, as predicted by sex allocation theory (e.g. Fawcett *et al.* 2006). Instead, the positive correlation between the sex ratio and natural male UV reflectance was reversed so that the proportion of sons decreased with increasing pre-treatment UV reflectance, which was also indicated by a significant 'UV treatment x pre-treatment UV reflectance' interaction effect on sex ratio (Sheldon *et al.* 1999). To test the generality of this unexpected pattern we replicated the Sheldon *et al.* (1999) study in two years in our Vosbergen blue tit population, while closely following Sheldon *et al.*'s (1999) experimental procedures (Korsten *et al.* 2006; Chapter 4). Like Sheldon *et al.* (1999), we found no difference in the overall sex ratio between the UV-reduced and control group, whereas in one of two years, the 'UV treatment x pre-treatment UV reflectance' interaction was significant (Korsten *et al.* 2006; Chapter 4). This result is important, because it shows the unexpected sex ratio pattern found by Sheldon *et al.* (1999) to be repeatable among populations, which provides the basis for moving on to the next step of elucidating the adaptive value of these complex patterns of blue tit sex ratio variation.

After the benchmark study of Sheldon *et al.* (1999), several correlational studies on blue tit primary sex ratio, male attractiveness and survival have been carried out in different populations (Table 8.1). The positive correlation between the proportion of sons and male UV reflectance reported by Sheldon *et al.* (1999), which was based on data of a single breeding season, was confirmed by additional data of the same population collected in a second year, but not by the data of a third year (Griffith *et al.* 2003). Furthermore, these data showed that males surviving to the following breeding season tended to have a higher proportion of sons in their broods than males that did not survive (Griffith *et al.* 2003), which confirms the study by Svensson and Nilsson (1996). In contrast, in a large dataset (comprising three study years) on a blue tit population in the UK no correlations among primary sex ratio, (extra-pair) paternity and male survival were found (Leech *et al.* 2001). Unfortunately, no data on

Table 8.1 Overview of published results on blue tit sex ratio variation in relation to male attractiveness and survival. The number of study years on which the study was based is indicated, as well as whether the study was experimental or correlational.

Population	Study years	Corr/ Exp	Results	Reference
Sweden	1	Corr	Broods of males that survive the winter are male-biased	Svensson & Nilsson 1996
Gotland	1	Corr	Broods of males that survive the winter are male-biased	Sheldon *et al.* 1999
	1	Corr	Positive correlation between sex ratio and male UV reflectance	Sheldon *et al.* 1999
	1	Exp	Females adjust sex ratio to manipulated male UV reflectance	Sheldon *et al.* 1999
	2	Corr	Broods of males that survive the winter tend to be male-biased; 1 year was previously included in the study of Sheldon *et al.* (1999)	Griffith *et al.* 2003
	3	Corr	Positive correlation between sex ratio and male UV reflectance in 2 of 3 years; 1 year was previously included in the study of Sheldon *et al.* (1999)	Griffith *et al.* 2003
UK	3	Corr	No correlations among brood sex ratio, (extra-pair) paternity and male survival	Leech *et al.* 2001
France	2	Corr	No correlation between sex ratio and male UV reflectance	Dreiss *et al.* 2006
	2	Corr	Positive correlation between sex ratio and quality of male song	Dreiss *et al.* 2006
Vosbergen	2	Exp	Females adjust sex ratio to manipulated male UV reflectance in 1 of 2 years	Korsten *et al.* 2006

male crown coloration are available for this population. Finally, a recent study on a French blue tit population found no significant correlation between offspring sex ratio and male crown coloration. Instead, the proportion of male offspring was positively correlated with a quality aspect of male song (Dreiss *et al.* 2006). It is unclear why correlational patterns of blue tit sex ratio differ between populations and more work is needed to understand the proximate and ultimate causes for this variability. A first step would be to compare the role of crown UV coloration in mate choice and competition, as well as its heritability, between the different populations.

Ornamental plumage coloration in the blue tit

The popularity of the blue tit and its structurally based UV/blue ornamental crown plumage as a model system in sexual selection and sex allocation research leads to a very favourable situation for behavioural ecologists. In this situation there is a large,

and still growing, amount of data on a single model system, while a number of investigations and experiments have also been replicated in different populations (*e.g.* Andersson *et al.* 1998; Hunt *et al.* 1998; Sheldon *et al.* 1999; Örnborg *et al.* 2002; Delhey *et al.* 2003; Foerster *et al.* 2003; Griffith *et al.* 2003; Alonso-Alvarez *et al.* 2004; Limbourg *et al.* 2004, Johnsen *et al.* 2005; Dreiss *et al.* 2006; Delhey *et al.* 2006; Delhey & Kempenaers 2006; Korsten *et al.* 2006). Here, I will summarize the present knowledge of this well-studied plumage ornament and compare the results presented in this thesis with findings by other studies.

Sexual dichromatism of UV coloration

Blue tits have long been regarded as largely monochromatic (Cramp & Perrins 1993), with males having perhaps only slightly brighter blue crown plumage than females (Perrins 1979). In 1998, however, two studies using spectrophotometry showed independently that the reflectance of the blue crown feathers was markedly different between males and females in the UV part of the spectrum (Andersson *et al.* 1998; Hunt *et al.* 1998; see also Figure 1.2, Chapter 1). It was shown that males have on average more UV-shifted and UV-chromatic crown plumage. This finding has been confirmed in a number of other populations (Delhey *et al.* 2006; Hadfield *et al.* 2006; Chapters 1 and 7). Moreover, it has now become clear that UV reflecting plumage as well as sexual dichromatism in the UV is in fact present in numerous bird species (Eaton & Lanyon 2003; Eaton 2005).

Blue tits have UV vision (Hart *et al.* 2000), like most passerine birds (Cuthill *et al.* 2000), and are thus able to perceive variation in UV reflectance of the plumage of conspecifics. The clear sexual dichromatism in blue tits must be the result of sex-specific selection on crown UV coloration, which strongly suggests that the blue tit crown plumage is a sexually selected ornament that functions as a signal in female mate choice or male-male competition. Interestingly, the sexual dichromatism is far from complete, and there is large individual variation in crown UV coloration (Chapter 7), with substantial overlap of the distributions of male and female crown colour (Chapters 1 and 7). This suggests that the UV crown plumage may have a signalling function in females as well, and play a role in male mate choice or inter-individual competition. The sexual dichromatism could then be viewed as the result of a different balance of the costs (*e.g.* increased predation risk) and benefits (*e.g.* more successful mate attraction) of crown UV coloration in males and females.

Alternatively, female UV coloration may be the result of a non-adaptive genetic correlation with male ornamentation, while sex-limitation of ornament expression is incomplete (Kraaijeveld *et al.* submitted). According to this scenario selective pressures on male and female crown colour expression may be counteracting each other to some extent. Such a situation has been described for the ornamental red bill coloration of zebra finches (Price & Burley 1993, 1994; Price 1996). In zebra finches, males with redder bills are more successful in mate attraction and have higher fitness, while in females individuals with more orange (less red) bills have

higher fitness (Price & Burley 1994). This leads to a situation in which there is sexually antagonistic selection (Rice 1992) on bill coloration, with males selected to become more red and females to become more orange (Price & Burley 1994). A genetic correlation between bill coloration of males and females subsequently prevents the evolution of more pronounced sexual dichromatism (Price & Burley 1993, 1994; Price 1996). It is not unlikely that a similar situation exists for the blue tit's crown coloration. It is interesting in this respect that we found a preliminary hint of sex-linked inheritance (or a maternal effect) affecting crown colour expression (Chapter 2), which might be expected in case of such sexually antagonistic selective pressures (Merilä & Sheldon 2001). The possibility that there is sexually antagonistic selection on blue tit crown coloration certainly deserves further attention, and this idea could be tested by a quantification of the fitness consequences of variation in crown coloration in both males and females.

Seasonal variation in UV coloration

An extremely important source of variation in blue tit crown coloration is the time of year (Örnborg *et al.* 2002; Delhey *et al.* 2006; Chapters 2 & 7). The reflectance of the crown plumage is maximally UV-shifted just after the yearly moult in autumn (Örnborg *et al.* 2002). Thereafter, over the year, crown coloration becomes gradually less UV-shifted, and towards the next breeding season also UV chroma decreases, while overall brightness increases (Örnborg *et al.* 2002). Changes in crown coloration are especially rapid during the breeding season between nest building and chick feeding (Örnborg *et al.* 2002). The decline in UV reflectance is probably due to feather wear as well as accumulation of fat and dirt (Örnborg *et al.* 2002). In particular the abrasion of the feather barbs and barbules containing the microscopic light-scattering structures could lead to a change in feather colour (Örnborg 2002). Also the action of feather-degrading bacteria and other ecto-parasites may play a role in the deterioration of the feathers (Kose & Møller 1999; Shawkey & Hill 2004).

The magnitude of the seasonal colour changes is surprisingly large (Örnborg *et al.* 2002; Delhey *et al.* 2006; Chapter 2). For example, Delhey *et al.* (2006) showed that changes in UV chroma and hue between early winter and late spring were similar to the differences in UV coloration due to sex and age respectively. It is unknown what the functional consequences of these striking colour changes are. The decline in UV coloration may be adaptive, and related to a shifting balance between benefits of a strong UV signal used in mate attraction during winter and early spring and costs such as the risk of detection by predators. For example, male rock ptarmigans (*Lagopus mutus*) soil their conspicuous white breeding plumage with dirt as soon as mating has taken place to increase crypsis (Montgomerie *et al.* 2001). On the other hand it has been hypothesised that the magnitude of the decrease in UV coloration may be related to individual quality (Delhey *et al.* 2006). According to this idea high quality individuals are better able to prevent their plumage from degradation, because they can invest more time and energy in main-

tenance of their feathers (*e.g.* in the form preening behaviour). Indeed, a measure of body size (tarsus length) was negatively related to the seasonal change in blue tit hue, whereas the magnitude of the decline in UV chroma was related to the loss of body mass between winter and spring, supposedly indicating a cost of feather maintenance (Delhey *et al.* 2006). However, female extra-pair mate choice was not related to variation in the magnitude of the seasonal declines (Delhey *et al.* 2006). Clearly, further research is needed to better understand the causes and consequences of the sharp seasonal decline in blue tit UV coloration.

Whether or not there is an adaptive explanation for the seasonal changes in crown coloration , it is obviously extremely important to control for this variation in analyses of crown colour variation. Especially, when estimates of crown coloration collected over long time frames are included or when comparisons of crown coloration are made between individuals measured at different periods (Chapter 2). If not adequately controlled, the large seasonal variation could easily obscure interesting patterns or lead to spurious relationships.

Age-related variation in UV coloration

A small part of the individual variation in blue tit UV coloration is related to age, both in males and females (Chapters 2 and 7). Older birds have significantly more UV-shifted and UV-chromatic crown plumage (Chapter 2). This supports the idea that the UV coloration is an indicator of individual quality, as condition dependent ornaments are typically more developed in older individuals (Andersson 1994; Siefferman *et al.* 2005). However, the age differences in UV coloration appear to be very subtle when compared to the variation due to sex differences as well as the seasonal changes (Delhey *et al.* 2006; Chapters 2 and 7). Indeed, we could assign the correct age based on crown UV coloration of only 56.0% of males and 72.0% females measured during winter (Chapter 7), which makes it a rather poor indicator of age.

The increase in UV coloration with age could have two different causes: 1) individuals may increase in UV coloration during their life; 2) individuals with less intense UV coloration may have a lower survival probability, leading to a positive correlation between age and UV colour due to selective disappearance of low-UV individuals at older ages (Siefferman *et al.* 2005). So far, there is evidence that both processes could be responsible for the positive correlation between UV coloration and age in blue tits. In the Gotland population, males, but not females, with more UV-chromatic crown plumage had a higher chance of surviving to the following breeding season (Sheldon et at. 1999; Griffith *et al.* 2003). In a blue tit population in Austria, however, over-winter survival was not dependent on crown coloration. Instead, in this population UV coloration was shown to increase with age within individuals (Delhey & Kempenaers 2006). In conclusion, evidence suggests that the sexually dichromatic UV coloration can function as a signal of individual quality or age, but it remains to be explained why the observed age effects are very subtle in comparison to overall individual variation.

Consequences of UV colour variation for mate choice and sexual selection

The greater expression of the UV coloration in males than in females (Andersson *et al.* 1998; Hunt *et al.* 1998) is a strong indication that the UV/blue crown plumage is a sexually-selected ornament in blue tits (Andersson 1994). However, the direct evidence for a role of UV coloration in mate choice is not as strong and straightforward as is sometimes assumed (Sheldon *et al.* 1999; Korsten *et al.* 2006).

A mate choice experiment with a relatively small number of captive females (n = 7) has shown that females preferred males with greater brighter (achromatic brightness), but not more UV-chromatic, crown feathers (Hunt *et al.* 1998). In another mate choice experiment both males and females were allowed to choose between two potential mates of the other sex that were each placed behind a transparent screen; one screen was transparent to UV light (UV+), whereas the other screen was opaque to UV (UV–) (Hunt *et al.* 1999). In this experimental set-up males and females had a preference for the UV+ individual. However, this result does not necessarily demonstrate that blue tits specifically prefer potential mates that have more UV-reflecting crown feathers, because the entire appearance and background are different between UV+ and UV– birds (*cf* the effect of looking through blue sunglasses which filter out red light; see also Hunt *et al.* 2001 for further discussion). In fact, this experiment only demonstrates that blue tits are capable of visual discrimination in the UV part of the spectrum, and that they select against odd looking (UV–) mates.

In a wild blue tit population in Sweden assortative mating for crown UV chroma was found (Andersson *et al.* 1998), which implicates mutual mate choice with both males and females preferring more UV-chromatic mates. However, in some other populations including ours (Box B) no such assortative mating was found (T. Limbourg, personal communication; but see Alonso-Alvarez *et al.* 2004). It is unclear what causes this difference between populations. A possible explanation for the difference may be the fact that northerly populations of blue tits in Sweden are less sedentary than temperate populations and often leave their breeding areas during harsh conditions in winter. This may lead to increased 'freedom' of mate choice during pair-formation in early spring when the birds return to the breeding area in northern populations, whereas in more temperate populations pairs tend to remain in the breeding area all year round, thereby restricting the choice options for unpaired individuals.

Analyses of genetic parentage in several blue tit populations have revealed that ca. 10–15% of all offspring are sired by a male outside the social pair bond, which is normally formed by a single male and female (Kempenaers *et al.* 1997; Krokene *et al.* 1998; Leech *et al.* 2001; Delhey *et al.* 2003; Box D). The occurrence of extra-pair paternity increases the variance in male reproductive success in socially monogamous bird species, such as the blue tit, and thereby the potential for sexual selection on male ornaments. Comparative analyses have suggested that the evolution of structurally based plumage coloration, such as the blue tit's crown coloration, is associated with high extra-pair paternity rates on the species level (Owens &

Hartley 1998). At present only one published study has investigated the relationship between extra pair paternity and male crown plumage in blue tits in more detail (Delhey *et al.* 2003; see also Delhey *et al.* 2006). This study showed that males with a more UV-shifted crown hue are less cuckolded (Delhey *et al.* 2003), which is consistent with a female preference for more ornamented males. Remarkably, however, males with less UV-shifted hue sired more extra-pair young (Delhey *et al.* 2003). This may indicate a potential trade-off between guarding the paternity of the own brood and the pursuit of extra-pair copulations (Kokko & Morrell 2005). In that case, the opposite correlations between UV coloration and within- and extra-pair paternity may reflect alternative male mating strategies, where more UV-ornamented males maximize within-pair success and less UV-ornamented males maximize extra-pair success (Delhey *et al.* 2003). However, an experimental UV reduction of males did not result in the predicted lower within-pair paternity in our study population, which refutes such a causal relationship between within-pair paternity and UV coloration (Box D). Assignment of extra-pair males would be an adequate next step to further investigate the causal links between UV coloration and male extra-pair success in our blue tit population.

Although the direct evidence for a role of blue tit crown UV coloration in (extra-pair) mate-choice is still not particularly strong, two recent studies on differential allocation of parental investment in blue tits provide additional evidence for female-driven sexual selection of male crown UV coloration ((Limbourg *et al.* 2004; Johnsen *et al.* 2005). These studies show that females decrease their reproductive investment in terms of nestling feeding or nest defence against predators (Johnsen *et al.* 2005) if the UV reflectance of their male is experimentally reduced. One of these studies shows that the decrease in female parental investment in response to male UV-reduction had a negative effect on the growth of the chicks (Limbourg *et al.* 2004) and may thus reduce male fitness. These results are predicted by the 'differential allocation hypothesis' (Burley 1988; for a review see Sheldon *et al.* 2000). According to this hypothesis a decrease in maternal investment in response to decreased male UV reflectance would be expected if broods of low UV males are of lower reproductive value to the female, for example because the young inherit the male's low UV reflectance, making them less successful in (extra-pair) mate attraction when adults.

While there is growing evidence for a role of blue tit crown UV coloration in mate-choice, the results presented in this thesis clearly suggest that UV coloration is not important in male-male competition during the breeding season (Chapter 6; but see Alonso-Alvarez *et al.* 2004) or competition for food during winter (Chapter 7).

Future perspectives

Adaptive sex ratio variation in birds

The very recent small steps towards a revelation of the physiological mechanism

underlying primary sex ratio adjustment in birds are promising. First evidence suggests that female birds are able to bias egg sex ratio before ovulation (Komdeur *et al.* 2002), either through biased segregation of sex-chromosomes (Correa *et al.* 2005), differential growth patterns of oocytes destined to become either male or female (Young & Badyaev 2006), or through atresia of 'wrong' sex oocytes (Pike 2005). The female's endocrine system may play an important role in the regulation of these processes and it is even possible that steroid hormones deposited by the follicular wall cells into the developing oocyte influence the segregation of sex chromosomes during the first meiotic division, shortly before ovulation (Petrie *et al.* 2001; Correa *et al.* 2005; but see Pilz *et al.* 2005). Elucidation of the physiological basis for primary sex ratio adjustment would greatly facilitate new progress in the study of adaptive primary sex ratio variation in birds. Knowledge about the underlying mechanism will help to define the costs and constraints that are involved in facultative primary sex ratio modification in birds, which would make it possible to develop more realistic models explaining and predicting avian sex ratio variation (Pen & Weissing 2002).

Although the number of case studies on avian sex ratio variation is quickly expanding, there remains an urgent need for experimental studies testing *a priori* predictions. Despite several calls for such experimental work (*e.g.* Sheldon 1998; Komdeur & Pen 2002), the majority of published sex ratio studies is still correlational. In addition, it is extremely important that well-documented and influential case studies (*e.g.* Sheldon *et al.* 1999), are replicated to assess the generality of the patterns found. Another approach that will help to get a grip on the generality of adaptive sex ratio variation is the use of meta-analyses, especially since a large enough number of case studies has now been accumulated. The chromosomal sex determining system may not pose such a strong constraint on the evolution of adaptive primary sex ratio adjustment in birds as was previously thought (West & Sheldon 2002; Griffin *et al.* 2005). It would be a fruitful future approach to try to understand patterns of avian sex ratio adjustment based on the relative costs and benefits of sex ratio manipulation, together with the constraints.

Blue tit crown coloration as a model in sexual selection research

We are just starting to get a grip on the significance of the blue tit's UV crown plumage as a sexually selected ornament. The basic patterns of sex, age, and season-related variation in UV coloration are now well-described. Their significance in mate-choice and competition, as well as the inheritance of crown colour, need now to be further investigated in the several blue tit populations studied. There is no doubt that the blue tit will continue to serve as an excellent model species for many studies of sexual selection and sex allocation still to come.

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Nederlandse samenvatting – Dutch summary

Seksеallocatie en felle kleuren bij vogels – een onderzoek aan de pimpelmees

'Is het inderdaad zo dat er meestal evenveel mannelijke als vrouwelijke nakomelingen worden geboren? En wanneer is dit wel of juist niet het geval?'

'Wat is de functie van de felle en opvallende kleuren die je ziet bij sommige vogels en ook andere dieren?'

Deze vragen vormen de basis van mijn proefschrift. Interessante vragen, maar zo op het eerste gezicht hebben ze niet veel met elkaar te maken. Of toch wel?

Dilemma's in de voortplanting

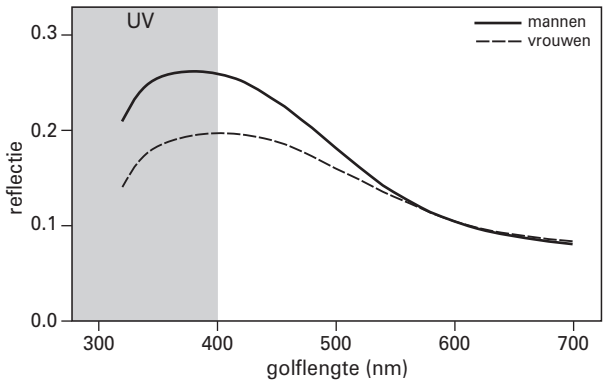
De meeste dieren (en ook planten en andere organismen) vermenigvuldigen zich door middel van seksuele voortplanting. Dat betekent dat er twee verschillende geslachten zijn, mannelijk en vrouwelijk, en dat een individu een partner van het andere geslacht nodig heeft om zich te kunnen voortplanten. Het nageslacht ontvangt daarbij een combinatie van erfelijke eigenschappen van beide ouders. Dieren die zich seksueel voortplanten staan bij hun voortplanting voor twee belangrijke dilemma's: 1) ze moeten een partner van het andere geslacht kiezen – en die vervolgens ook nog tot gezamenlijke voortplanting verleiden; en 2) ze moeten hun beperkte tijd en energie verdelen tussen mannelijk en vrouwelijk nageslacht. Elke investering in de ene sekse gaat ten koste van de andere sekse. Bijvoorbeeld, een oudervogel die na lang zoeken een voedzame rups vindt, kan deze rups of aan een zoon of aan een dochter voeren, maar niet aan allebei. Mijn onderzoek zoals beschreven in dit proefschrift richt zich op de evolutionair-biologische gevolgen van deze twee dilemma's.

Het voortplantingssucces van een individu zal sterk afhangen van de beslissingen[1] die het in deze dilemma's maakt. Potentiële partners kunnen bijvoorbeeld verschillen in hun kwaliteit als ouder, of in hun genetische kwaliteit, waardoor betere of juist minder goede genen aan het gezamenlijke nageslacht worden doorgegeven. Ook het te verwachten voortplantingssucces van zonen en dochters kan sterk verschillen, wat uiteindelijk het aantal kleinkinderen bepaalt. Bij een overschot aan mannen in de populatie zal een dochter bijvoorbeeld meer kans maken op het vinden van een partner, en dus op voortplanting, dan een zoon. Individuen die tijdens hun voortplanting de strategie volgen die de meeste nakomelingen oplevert (gemeten over heel veel generaties), zullen uiteindelijk de overhand in de populatie krijgen. Anders gezegd, ze worden geselecteerd ten koste van individuen met minder succesvolle strategieën. Dit is 'natuurlijke selectie' in actie.

Het zoeken van een partner – Seksuele selectie

Individuen met genen die tot meer succes leiden in het vinden of aantrekken van voortplantingspartners zullen hun genen vaker doorgeven aan toekomstige generaties. Darwin[2] onderkende dit speciale selectieproces en noemde het 'seksuele selectie', om het te onderscheiden van andere vormen van natuurlijke selectie. Het aantal nakomelingen dat vrouwen kunnen krijgen is meestal beperkt, terwijl mannen potentieel juist enorm veel nakomelingen kunnen voortbrengen, doordat ze de eicellen van meerdere vrouwen kunnen bevruchten. Hierdoor leidt seksuele selectie bij mannen vaak tot het ontstaan van kenmerken die hen succesvol maken in het 'veroveren' van zoveel mogelijk vrouwen. Klassieke voorbeelden van zulke kenmerken zijn de opvallende staartveren van de pauw en het gewei van edelherten. Deze kenmerken zijn aantrekkelijk voor vrouwen (de pauwenstaart), of ze worden gebruikt in de concurrentiestrijd tussen mannen (het hertengewei). Bij vrouwen leidt seksuele selectie vaak tot het ontstaan van een sterke voorkeur om alleen met de beste en meest aantrekkelijke mannen te paren.

Vogels met hun vaak opvallende zang en felle kleuren zijn in het onderzoek naar seksuele selectie een veelgebruikt onderzoeksmodel. Onlangs is aangetoond dat vogels ook een deel van het voor mensen onzichtbare ultraviolette (UV) spectrum kunnen zien. Verder is door metingen met behulp van fotospectrometers ontdekt dat veel vogelsoorten veren hebben die reflecteren in het UV. Mogelijk heeft seksuele selectie bij vogels geleid tot het ontstaan van signaalkleuren in het UV die een rol spelen in de partnerkeuze of concurrentiestrijd. Zo ook bij de pimpelmees, de vogelsoort in mijn onderzoek. De felblauwe kruinveren van pimpelmezen reflecteren sterk in het UV en deze reflectie is hoger bij mannen dan bij vrouwen (Figuur 1).



Figuur 1 Het gemiddelde reflectiespectrum van de blauwe kruinveren van mannelijke en vrouwelijke pimpelmezen. Vooral in het ultraviolette deel van het spectrum is de reflectie van de kruinveren hoger bij mannen dan bij vrouwen.

Dit wijst erop dat deze ultraviolette reflectie een belangrijk signaal is in de partnerkeuze of (mannelijke) concurrentiestrijd. In dit proefschrift wordt deze mogelijkheid verder onderzocht.

Het verdelen van de ouderlijke investering tussen zonen en dochters – Sekseallocatie

Wanneer dieren eenmaal een voortplantingspartner hebben gevonden staan ze voor een volgend dilemma. Hoe hun ouderlijke investering in de vorm van tijd en energie te verdelen tussen mannelijk en vrouwelijk nageslacht? De verdeling van de ouderlijke investering kan zowel de kwaliteit (bijv. grootte, gezondheid) van zonen ten opzichte van dochters als hun aantallen beïnvloeden. De verdeling van de ouderlijke investering tussen zonen en dochters wordt sekseallocatie genoemd. Mijn onderzoek concentreerde zich op de invloed van de ouders op het aantal zonen en dochters. De verhouding tussen het *aantal* zonen en dochters wordt ook wel de geslachtsverhouding of sekseratio genoemd. De sekseratio lijkt gewoonlijk gemiddeld 1:1 te zijn, met een even grote kans op de geboorte van een zoon of een dochter. Maar schijn bedriegt, want bij veel soorten zijn vrouwen in staat om de sekse van de door hen geproduceerde nakomelingen te beïnvloeden. Hierbij passen ze de sekseratio op zo'n manier aan, dat ze hun genen zo succesvol mogelijk doorgeven aan volgende generaties.

Seychellenzangers bijvoorbeeld, die leven op een eilandengroep in de Indische Oceaan (de Seychellen), leggen vooral vrouwelijke eieren als ze in een voedselrijk territorium (met veel insecten) broeden, terwijl ze vooral mannelijke eieren leggen in slechte territoria[3]. De evolutionaire verklaring hiervoor is dat dochters, in tegenstelling tot zonen, vaak in het broedterritorium van de ouders blijven om mee te helpen met het grootbrengen van volgende broedsels. De aanwezigheid van hulp van dochters kan de moeder een voordeel opleveren bij het grootbrengen van volgende broedsels, maar alleen in een voedselrijk territorium. In een slecht territorium betekent de aanwezigheid van helpende dochters dat er nog meer monden gevoed moeten worden met het toch al schaarse voedsel. Vrouwen die dus de aantallen door hen geproduceerde zonen en dochters af laten hangen van de voedselomstandigheden zullen hun genen succesvoller doorgeven aan volgende generaties. Het is overigens nog niet precies bekend hoe vogels het geslacht van hun eieren kunnen beïnvloeden.

Een idee dat sterk in de belangstelling staat, is dat vrouwen de verhouding tussen zonen en dochters ook zouden moeten aanpassen aan de aantrekkelijkheid van hun partner, om zo hun genen met meer succes te kunnen doorgeven aan volgende generaties. Ook bij pimpelmezen zou je dit verwachten om de volgende redenen. Bij pimpelmezen brengen mannen en vrouwen hun jongen gewoonlijk samen groot (ongeveer 10 per nest). De man is echter niet altijd de vader van alle jongen in zijn

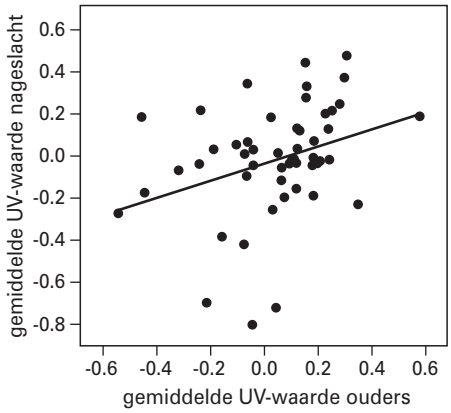
nest. Pimpelmeesvrouwen (en mannen) gaan vreemd, en zo'n 10–15% van de jongen is van een andere man. Daardoor is er meer verschil in voortplantingssucces tussen de mannen in de populatie dan tussen de vrouwen. Pimpelmeesvrouwen hebben immers altijd ongeveer 10 jongen, of die nu van hun eigen man afkomstig zijn of niet. Pimpelmeesmannen daarentegen kunnen naast de jongen in hun eigen nest nog extra jongen krijgen bij andere vrouwen, of ze kunnen juist jongen in hun eigen nest kwijtraken, doordat hun vrouw is vreemdgegaan met een andere man. Een aantrekkelijke man kan door vreemd te gaan met andere vrouwen potentieel veel meer nakomelingen krijgen dan een onaantrekkelijke man, terwijl er bij vrouwen niet zo'n duidelijk verband is tussen het aantal nakomelingen en hun aantrekkelijkheid. Als aantrekkelijkheid (bijvoorbeeld de intensiteit van de UV-reflectie van de blauwe kruinveren) deels erfelijk is, zouden we verwachten dat vrouwen die gepaard zijn met een aantrekkelijke man meer zonen produceren. Vrouwen die gepaard zijn met een onaantrekkelijke man zouden meer dochters moeten krijgen. De zonen van aantrekkelijke mannen zullen namelijk zelf ook weer aantrekkelijk zijn en hun moeder daardoor veel kleinkinderen geven, terwijl de zonen van onaantrekkelijke mannen juist minder kleinkinderen geven omdat ze waarschijnlijk vaker bedrogen worden.

Het onderzoek: Seksuele selectie en seksealloca tie bij pimpelmezen

Het onderzoek werd uitgevoerd aan een populatie van in nestkasten broedende pimpelmezen (*ca*. 50–110 broedpaartjes per jaar) op het vlakbij de stad Groningen gelegen landgoed 'De Vosbergen'. Vanaf 2001 zijn alle in nestkasten broedende pimpelmezen op het landgoed en hun jongen elk jaar geringd, waardoor we de beschikking hebben over een uitgebreide 'burgerlijke stand' van deze populatie. Met deze achtergrondinformatie als basis hebben we allerlei metingen en experimenten gedaan, om de erfelijkheid van de ultraviolette reflectie van de kruinveren vast te stellen, om de rol van de UV-reflectie in de partnerkeuze en de concurrentie tussen individuen te onderzoeken, en om de invloed van de UV-reflectie op bijvoorbeeld de sekseverhouding van het nageslacht te meten.

Erfelijkheid van UV-reflectie

Het overerven van aantrekkelijkheid van vaders op zonen is een essentiële voorwaarde voor het optreden van vrouwelijke aanpassing van de geslachtsverhouding aan de aantrekkelijkheid van haar partner. Daarom onderzoeken we in ***Hoofdstuk 2*** of de mate van de ultraviolette kleuring van de blauwe kruinveren erfelijk is. Dit doen we door de UV-reflectie van de in onze studiepopulatie geboren jongen, waarvan we de kruinreflectie op volwassen leeftijd hebben gemeten, te vergelijken met de UV-reflectie van hun ouders. Hieruit blijkt dat de mate van UV-reflectie van



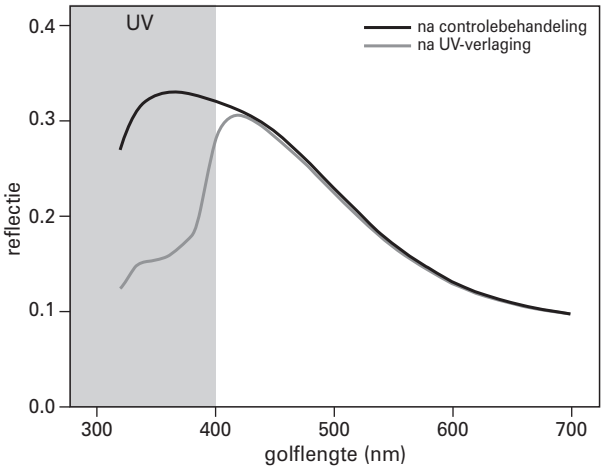
Figuur 2 Erfelijkheid van de UV-waarde van de blauwe kruinveren van pimpelmezen. Elk datapunt staat voor een nest. De UV-waarde van het nageslacht lijkt op die van hun ouders.

jongen inderdaad lijkt op die van hun ouders (Figuur 2). Hiermee wordt voor het eerst aangetoond dat de UV-reflectie van vogelveren deels erfelijk bepaald is. Dit resultaat is zeer belangrijk, omdat een groot deel van het onderzoek in dit proefschrift gebaseerd is op de aanname dat de UV-reflectie van pimpelmeesouders overerft op hun nageslacht. Het is bovendien een opvallend resultaat, omdat het afwijkt van de bevindingen in een parallel onderzoek aan een Engelse pimpelmeespopulatie[4] – een verschil dat nog moet worden opgehelderd.

Mannelijke UV-reflectie en vrouwelijke voortplantingsbeslissingen

Vervolgens onderzoeken we of pimpelmeesvrouwen de geslachtsverhouding van hun legsel inderdaad aan de UV-reflectie van de kruinveren van hun partner aanpassen. Voor het vaststellen van een *oorzakelijk* verband tussen de door pimpelmeesvrouwen geproduceerde geslachtsverhouding en de kruinreflectie van hun partner moet de kruinreflectie van mannen experimenteel worden gemanipuleerd. In ***Hoofdstuk 3*** testen we de werking van een methode om de UV-reflectie van veren tijdelijk te manipuleren. De kruinveren worden ingesmeerd met een mengsel van UV-absorberende chemicaliën (die ook gebruikt worden in anti-zonnebrandcrème) en veerwas[5], om zo de UV-reflectie te verlagen. Als controlebehandeling wordt alleen de veerwas aangebracht. Uit onze test blijkt dat deze behandeling de UV-reflectie van de kruinveren inderdaad effectief verlaagt (Figuur 3). Wel trad binnen enkele dagen al geleidelijk herstel van de natuurlijke UV-reflectie op, maar deze bleef toch nog ongeveer 10 dagen verlaagd vergeleken met de UV-reflectie van de controlegroep. Er was geen nadelig effect van de behandeling op de individuele overleving naar het volgende broedseizoen.

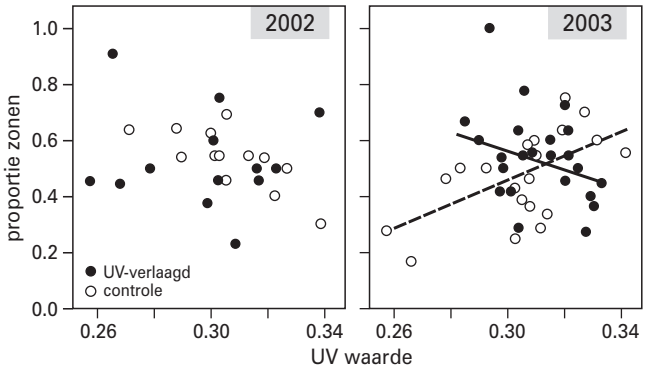
In ***Hoofdstuk 4*** passen we de UV-verlagende behandeling toe om de invloed van de aantrekkelijkheid van pimpelmeesmannen op de geslachtsverhouding van de



Figuur 3 Het effect van een UV-verlagende behandeling van de blauwe kruinveren bij mannelijke pimpelmezen. De reflectie in het UV-gebied van het spectrum is duidelijk lager bij de UV-verlaagde mannen dan bij de controle-mannen.

door hun vrouwen geproduceerde eieren te meten. We verlaagden de UV-reflectie van één groep mannen om ze onaantrekkelijker te maken terwijl een andere groep een controlebehandeling (alleen veerwas) kreeg. We deden dit experiment in 2002 en 2003. Uiteraard behandelden we de mannen voordat hun vrouwen begonnen waren met het leggen van eieren (gemiddeld 5–10 dagen voor het eerste ei). Vóór de behandeling hebben we bovendien hun natuurlijke UV-reflectie gemeten. Vervolgens hebben we het effect van de behandeling op het geslacht van de gelegde eieren gemeten. Om vast te kunnen stellen of de sekseverhouding van een broedsel al voor de eileg door vrouwen wordt gemanipuleerd is het noodzakelijk zo snel mogelijk na het uitkomen de sekse van de jongen te bepalen; namelijk voor er sterfte optreedt. Bij pimpelmezen zijn de beide geslachten op jonge leeftijd nog niet te onderscheiden. We namen daarom een druppel bloed af om met een DNA-techniek de sekse te bepalen. Dit deden we op de vierde dag na uitkomen, als er nog nauwelijks sterfte was geweest.

In 2002 vonden we geen significante invloed van het experiment of van de natuurlijke UV-reflectie van mannen op de geslachtsverhoudingen. In 2003 daarentegen vonden we zoals verwacht dat de mannen in de de controlegroep meer zonen kregen naarmate ze een hogere natuurlijke UV-reflectie hadden. Verder kregen de mannen met een hoge natuurlijke UV-reflectie na de UV-verlagende behandeling minder zonen. Dit zou je verwachten als de UV-verlaging ze inderdaad onaantrekkelijker maakt. Opvallend genoeg kregen de mannen die voor de UV-verlaging van nature al een lage UV-reflectie hadden na de behandeling juist meer zonen (Figuur 4). Een verklaring hiervoor zou kunnen zijn dat de vrouwen die gepaard zijn met



Figuur 4 De proportie zonen in de nesten van pimpelmeesmannen uitgezet tegen de natuurlijke UV-waarde van hun blauwe kruinveren, gemeten voordat ze een UV-verlagende of controlebehandeling kegen. Elk datapunt staat voor een nest. Er is een positief verband tussen de proportie zonen en de natuurlijke UV-waarde in de controlegroep, dus meer UV betekent meer zonen. In de UV-verlaagde groep hadden mannen met een hoge natuurlijke UV-waarde minder zonen in hun nest. Mannen met een lage natuurlijke UV-waarde kregen juist meer zonen.

deze 'extra-onaantrekkelijke' mannen meer vreemd gaan, met andere, meer aantrekkelijke mannen uit de populatie. Misschien dat ze daarom het aantal zonen in hun nest niet verlagen. Dit idee wordt echter niet ondersteund door een verschil in het aantal buitenechtelijke jongen tussen de UV-verlaagde en de controlegroep. Onze resultaten bevestigen de bevindingen van een eerdere studie in een Zweedse populatie[6] en tonen aan dat pimpelmezen in staat zijn om de sekse van hun eieren te beïnvloeden. Om beter te begrijpen waarom we niet, zoals voorspeld, een lagere proportie zonen in de nesten van alle UV-verlaagde mannen vonden, is nog verder onderzoek nodig. Ook is nog niet duidelijk waarom de uitkomst van ons experiment verschilt tussen de twee jaren.

Nog maar 10 jaar geleden is ontdekt dat vogels grote hoeveelheden geslachtshormonen aan hun eieren afgeven. Dit zijn vooral mannelijke geslachtshormonen, zoals testosteron. Deze hormonen kunnen grote invloed hebben op de ontwikkeling en overleving van de jongen nadat ze zijn uitgekomen. Verhoogde hormoonniveau's kunnen het bedelgedrag stimuleren en daarmee de groei van jongen versnellen en hun kansen om uit te vliegen vergroten, maar er zijn ook negatieve effecten gevonden, bijvoorbeeld op het immuunsysteem. Onlangs is bij verschillende vogelsoorten gevonden dat vrouwen de hoeveelheid hormonen ook variëren afhankelijk van de aantrekkelijkheid van hun partner. In ***Hoofdstuk 5*** onderzoeken we of dit bij pimpelmezen ook ook het geval is. We maken hiervoor nogmaals gebruik van de hierboven beschreven UV-verlagende behandeling. Uit dit experiment blijkt dat pimpelmeesvrouwen inderdaad de testosteronafgifte aan hun eieren binnen korte tijd kunnen variëren in reactie op een verandering van de aantrekkelijkheid (manipulatie van de

UV-reflectie van de kruinveren) van hun partner. Er zijn hiervoor verschillende verklaringen mogelijk. Eén mogelijkheid is dat met aantrekkelijke mannen (hoge UV-reflectie) gepaarde vrouwen de investering in hun jongen vergroten door meer hormonen in hun eieren af te zetten. Volgens dit scenario investeren vrouwen meer in hun huidige broedsel ten koste van hun eigen overleving en eventuele toekomstige broedsels als hun huidige jongen van grotere waarde zijn doordat deze de aantrekkelijkheid (UV-reflectie) van hun aantrekkelijke vader erven. Een alternatieve verklaring is dat mannen met veel UV de jongen meestal minder voeren en dat vrouwen daarvoor compenseren door het bedelgedrag van de jongen te stimuleren met extra hormonen in de eieren. Er is nog verder onderzoek nodig om te bepalen welke van deze verklaringen juist is.

UV-reflectie als signaalkleur in de strijd

Signaalkleuren die een belangrijke rol spelen tijdens de partnerkeuze zouden ook belangrijk kunnen zijn als signaal tijdens conflicten tussen individuen. Er zijn nu verschillende studies die laten zien dat de UV-reflectie van de kruinveren bij pimpelmezen en andere vogelsoorten belangrijk is in de partnerkeuze, maar het is nog vrijwel onbekend of de UV-reflectie van veren ook belangrijk kan zijn als signaal tijdens conflicten.

In ***Hoofdstuk 6*** wordt onderzocht of de UV-reflectie van de kruinveren een rol kan spelen tijdens conflicten tussen territoriumeigenaren en indringers. We presenteerden twee opgezette pimpelmezen in de territoria van pimpelmeesmannen tijdens de vruchtbare periode van hun vrouw. In die periode dringen vreemde pimpelmeesmannen vaak het territorium binnen, mogelijk op zoek naar buitenechtelijke paringen. De UV-reflectie van de kruinveren van één opgezette pimpelmees was experimenteel verlaagd; de andere had nog een normale reflectie na slechts een controle-behandeling te hebben gekregen. De territoriumeigenaren vielen de nepindringers vaak aan en hierbij benaderden ze beide modellen gemiddeld even agressief. Ook hun eigen UV-reflectie had geen invloed op hun agressieve reactie. Deze resultaten suggereren dat de UV-reflectie van de kruinveren geen rol van belang speelt als signaalkleur tijdens conflicten tussen territoriumeigenaren en indringers.
Er is veel variatie in de UV-reflectie van de kruinveren tussen individuele pimpelmezen, ook binnen de twee seksen. Het is eerder bij andere vogelsoorten aangetoond dat dit soort variatie in de kleur van het verenkleed de uitkomst van kleine conflicten tussen individuen kan beïnvloeden, bijvoorbeeld om voedsel in de winter. Individuen geven dan met hun veerkleur een signaal af over hoe dominant ze zijn. Het zou kunnen dat de UV-reflectie van pimpelmezen tijdens de winter ook zo'n functie heeft. In een Zweedse pimpelmeespopulatie is gevonden dat mannen met een hogere UV-reflectie een grotere kans op overleving naar het volgende voorjaar hadden[6]. Dit suggereert dat de UV-reflectie een rol kan spelen tijdens de concurrentie om schaarse voedselbronnen of veilige slaapplaatsen. In ***Hoofdstuk 7*** toetsen we die mogelijkheid. Het blijkt dat de uitkomst van conflicten om voedsel sterk afhangt

van de afstand van individuen tot hun territorium. Hoe dichter bij hun eigen territorium, hoe dominanter ze zijn. Verder zijn pimpelmeesmannen dominant over vrouwen en oude dieren over jonge. De UV-reflectie blijkt echter niet van belang voor de uitkomst van conflicten om voedsel. Ook de overleving van de pimpelmezen naar het volgende voorjaar was niet gerelateerd aan hun UV-reflectie. Een verklaring voor deze opvallende verschillen met de uitkomsten van de Zweedse studie zou kunnen zijn dat veel individuen uit onze studiepopulatie de hele winter in de buurt van hun broedterritorium te blijven. Dit in tegenstelling tot pimpelmezen uit meer noordelijke Scandinavische populaties die hun broedgebied 's winters vaak verlaten als de omstandigheden door koude en voedselgebrek te bar worden. De territoriale structuur, die in onze studiepopulatie de uitkomst van conflicten sterk bepaalt, verdwijnt dan en er zullen ook vaker conflicten zijn tussen voor elkaar onbekende individuen. Het is mogelijk dat kleursignalen alleen in zo'n situatie een rol spelen bij het oplossen van conflicten.

Tot slot

De resultaten in dit proefschrift wijzen er op dat de UV-reflectie van de kruinveren van de pimpelmees een belangrijk signaal is tijdens de voortplanting. Vrouwen hebben waarschijnlijk een voorkeur voor mannen met een hogere UV-reflectie. De UV-reflectie van de kruinveren blijkt deels erfelijk bepaald te zijn en pimpelmeesvrouwen laten bepaalde voortplantingsbeslissingen afhangen van de UV-reflectie van hun partner. Zo is bijvoorbeeld het aantal zonen en dochters dat ze produceren afhankelijk van de mate van UV-reflectie van hun man. We hebben in onze studiepopulatie geen aanwijzingen gevonden voor een rol van de UV-reflectie van de kruinveren in de (mannelijke) concurrentiestrijd.

De signaalfunctie van de UV-reflectie van de kruinveren van de pimpelmees wordt nu in een aantal populaties verspreid over Europa onderzocht. Een eerste vergelijking van de resultaten van deze onderzoeken wijst erop dat het belang van de UV-reflectie als signaal in de partnerkeuze en concurrentiestrijd verschilt tussen deze populaties. Ook de mate van erfelijkheid van de UV-reflectie blijkt te verschillen tussen populaties. Toekomstig onderzoek zou zich moeten richten op het verklaren van deze populatieverschillen.

Voetnoten

[1] Met de term 'beslissing' bedoel ik in deze context niet dat het om een 'bewuste' keuze hoeft te gaan. Met beslissing bedoel ik hier dat een dier verschillende, elkaar uitsluitende, opties heeft waarvan het er één kiest (of dit bewust of onbewust gebeurt doet niet ter zake).

[2] Darwin, C. 1871. The descent of man, and selection in relation to sex. John Murray, London.

[3] Komdeur, J., Daan, S., Tinbergen, J.M. & Mateman, A.C. 1997. Extreme adaptive modification in sex ratio of the Seychelles warbler's eggs. *Nature* 385: 522–526.

[4] 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 of London B* 273: 1347–1353.

[5] Veerwas is de wasachtige substantie die vogels produceren in hun stuitklier. Deze zit vlak boven de staart. Ze smeren hier hun verenkleed mee in om het waterafstotend en in goede conditie te houden.

[6] Sheldon, B.C., Andersson, S., Griffith, S.C., Örnborg, J. & Sendecka, J. 1999. Ultraviolet colour variation influences blue tit sex ratios. *Nature* 402: 874–877.

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Dit proefschrift markeert de afsluiting van mijn promotieonderzoek. Een onderzoeksproject dat ik voor een groot deel zelf heb kunnen vormgeven en invullen – wat ik als een groot voorrecht beschouw. Toch hebben vele anderen een belangrijke en vaak zelfs onmisbare rol gespeeld bij het tot stand komen van dit proefschrift.

Joost Tinbergen en Ton Groothuis lieten me tijdens mijn afstudeeronderzoeken voor het eerst kennis maken met gedragsbiologisch en ecologisch (veld)onderzoek aan vogels. Zij staan daarmee aan de basis van mijn vorming als onderzoeker. Na mijn afstuderen in Groningen maakte ik een jaar lang deel uit van de onderzoeksgroep van Jacques van Alphen in Leiden om de levensloop en het foerageergedrag van Asobara sluipwespen te bestuderen. Dit heeft mijn wetenschappelijke blik aanzienlijk verruimd. Het veldonderzoek aan vogels hield echter zijn aantrekkingskracht en Jacques liet me vrij om weer naar Groningen te vertrekken.

Mijn promotores Jan Komdeur, Rudi Drent en Serge Daan hebben het fundament gelegd waarop ik mijn onderzoek kon bouwen. Jan Komdeur zorgde voor de dagelijkse begeleiding. Jan gaf me veel vrijheid bij het vormgeven van mijn onderzoek en stond tegelijkertijd steeds achter me. Zijn werkkamer heeft een bijzonder lage drempel. En zijn optimisme, enthousiasme en werklust vormen een bron van inspiratie. Geduldig heeft hij me op weg geholpen bij het schrijven van mijn eerste artikelen en hoofdstukken. Rudi Drent zorgde in een later stadium voor een kritische beschouwing van de door mij geschreven hoofdstukken en hij hield de voortgang en de grote lijn van het onderzoek goed in de gaten. Serge Daan initieerde en coördineerde het 'Avian sex allocation' onderzoeksprogramma van NWO, waar mijn project een onderdeel van was, en hij vormde een kritisch gehoor en belangrijk inspirator tijdens de regelmatige bijeenkomsten waarin de laatste onderzoeksresultaten van de verschillende deelprojecten besproken werden.

Ik heb het als een groot voordeel ervaren dat mijn onderzoek deel uitmaakte van een breder onderzoeksprogramma. De uitwisseling van ideeën en expertise met de mensen van de andere deelprojecten, Cor Dijkstra, Tim Fawcett, Kate Lessells, Tobias Limbourg, Ido Pen, Nikolaus von Engelhardt en Franjo Weissing, was zeer vruchtbaar. Dit moge blijken uit het feit dat meerdere van hen als co-auteur een bijdrage hebben geleverd aan verschillende hoofdstukken in dit proefschrift. Tobias wil ik speciaal noemen. Hij werkte bij het Nederlands Instituut voor Ecologisch Onderzoek (NIOO) in Heteren aan een 'zuster'-pimpelmezenproject en was mijn 'sparring partner' voor het bespreken van nieuwe plannen en ideeën, en het oplossen van (technische) problemen. Ook Kate wil ik speciaal bedanken. Ik heb veel geleerd van haar scherpe probleemanalyses en haar commentaren op de door mij geschreven stukken.

De Dierecologiegroep in Groningen vormde een levendige en stimulerende werkomgeving door het grote aantal collega's met vergelijkbare onderzoeksinteresses, de goede faciliteiten en de hoge standaard van het onderzoek. Ik besef dat ik hierdoor wel wat verwend geraakt ben. Ik wil alle dierecologen en ook de collega's van de Gedragsbiologiegroep enorm bedanken voor de samenwerking en goede

sfeer, zowel binnen als buiten werktijd. Enkelen van hen wil ik uitdrukkelijk noemen. Maaike, die haar onderzoek – aan koolmezen – tegelijk met mij begon en haar proefschrift vorige maand verdedigde, zorgde tijdens mijn gehele Groningse 'aio-tijd' voor gezelligheid en feedback op het onderzoek. The first years I shared my office with Mathew, who was always ready for a good discussion and gave, together with Michael, a bit of an Australian flair to the atmosphere. Later nam Karen zijn plaats in en bleek een gezellige kamergenoot die graag meedacht over voorkomende (wetenschappelijke) vraagstukken. Martijn hielp regelmatig mee bij het vinden van statistische oplossingen. Together with István I spent many hours to extract the maximum amount of information from the data set on which I based Chapter 7. Wendt, sinds jij in Aken/Antwerpen, en ik in Leiden zit, heb ik nog geen goed alternatief gevonden voor onze weekelijkse klimavond samen met Simon. Super dat je nu paranimf bent. Suus Bakker-Geluk zorgde voor de goede afhandeling van administratieve en financiële zaken. Bij Leon Steijvers en Jan van de Burg kon ik steeds terecht voor computervragen. Wim en Roelie van de timmerwerkplaats maakten 200 nestkasten die nog jaren weer en wind zullen doorstaan.

Mijn eerste stappen in het DNA-lab heb ik gezet onder het toeziend oog van Cor Dijkstra, Guido Meeuwissen, Wendt Müller en Mathew Berg. Zij hielpen mij op weg met de eerste seksebepalingen van pimpelmezen aan de hand van DNA uit bloedmonsters. Later heb ik in het NIOO-lab in Heteren bij Christa Mateman en Kate Lessells de fijne kneepjes van de genetische ouderschapsanalyse geleerd. Terug in Groningen hielp Marco van der Velde met het verwerken van de laatste bloedmonsters.

Het jaarlijkse veldwerk vond plaats op het mooie landgoed De Vosbergen, waar ik mijn onderzoek in 2001 begon met het ophangen van bijna 200 gloednieuwe nestkasten. Het landgoed was een ideale onderzoekslocatie door de grote aantallen broedende pimpelmezen, de aanwezigheid van het kleine veldstation en de goede bereikbaarheid. Ik bedank de Kraus-Groeneveld Stichting voor de toestemming die zij mij gaven om mijn onderzoek in De Vosbergen uit te voeren. De heren Ten Pas en Palsma hebben mij tijdens het eerste veldseizoen wegwijs gemaakt op het landgoed, waarvoor ik ze hartelijk wil bedanken. Ik bedank ook de bewoners van het landgoed die goede buren voor ons waren, en die zich af en toe vast verbaasd hebben over het grote aantal studenten met ladders in het bos. Het veldwerk was onmogelijk geweest zonder de grote inzet van biologiestudenten, collega's en vrienden. Ingrid Heersche heeft naast haar eigen werk elk veldseizoen opnieuw vele weekenden en vrije dagen in De Vosbergen besteed.

Verschillende doctoraalstudenten hebben hun afstudeeronderzoeken uitgevoerd binnen het pimpelmezenproject. Susanne Schultz, Marieke Ninaber van Eijben, Thomas Dijkstra, Esther Bouma, Oscar Vedder en Sjouke Kingma hebben zo een belangrijke bijdrage aan dit onderzoek geleverd. Ik heb met groot plezier met jullie samengewerkt en daar veel van geleerd. Ik hoop dat dat voor jullie ook zo was. Ik vind het geweldig dat Oscar en Elske Schut, die kennis maakte met het pimpelme-

zenonderzoek tijdens de dierecologiecursus, het werk nu voortzetten met hun eigen promotieonderzoeken. It is great that Michael Magrath is also carrying out part of his research on the Vosbergen blue tit population.

De laatste hoofdstukken heb ik in Leiden geschreven, maar ik kwam regelmatig voor overleg naar Groningen. Daar kon ik voor een slaapplek altijd terecht bij Oscar in Bunne, met elke ochtend weer een goed ontbijt met verse cappuccino. Or I stayed at the fine little house of Michael and Theresa in 'De Oosterpoort'. Comments by Tim Fawcett, Ingrid Heersche and Theresa Jones improved the general introduction and discussion of the thesis. Nikolaus von Engelhardt las en corrigeerde het gehele manuscript. Dick Visser verzorgde met veel toewijding en vakmanschap de figuren en vormgeving van dit proefschrift. Sander van der Werf bedacht het omslagontwerp.

Naast een goede werkomgeving zijn ontspanning en afleiding onontbeerlijk. De Groningse huisgenoten in de Hoornsemeer boden een fijne plek om thuis te komen. Ik bedank mijn vrienden voor hun belangstelling, steun en relativering. Dat er nog maar veel mooie gezamenlijke avonden en dagen mogen volgen. Ook bij mijn familie vond ik een veilige thuishaven. Harie en Hilde hebben mij vrijgelaten in mijn keuzes en zijn er toch altijd als het nodig is. Femke, Maarten en Marjanne, ik ben trots op jullie. Niemand heeft de wording van dit proefschrift van zo dichtbij meegemaakt als Ingrid. Jij gaf me rust, ruimte en enorm veel vertrouwen. Ik zie uit naar alle nieuwe avonturen die we samen tegemoet zullen gaan.

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