Sexual selection and blue tit (*Parus caeruleus*) crown coloration

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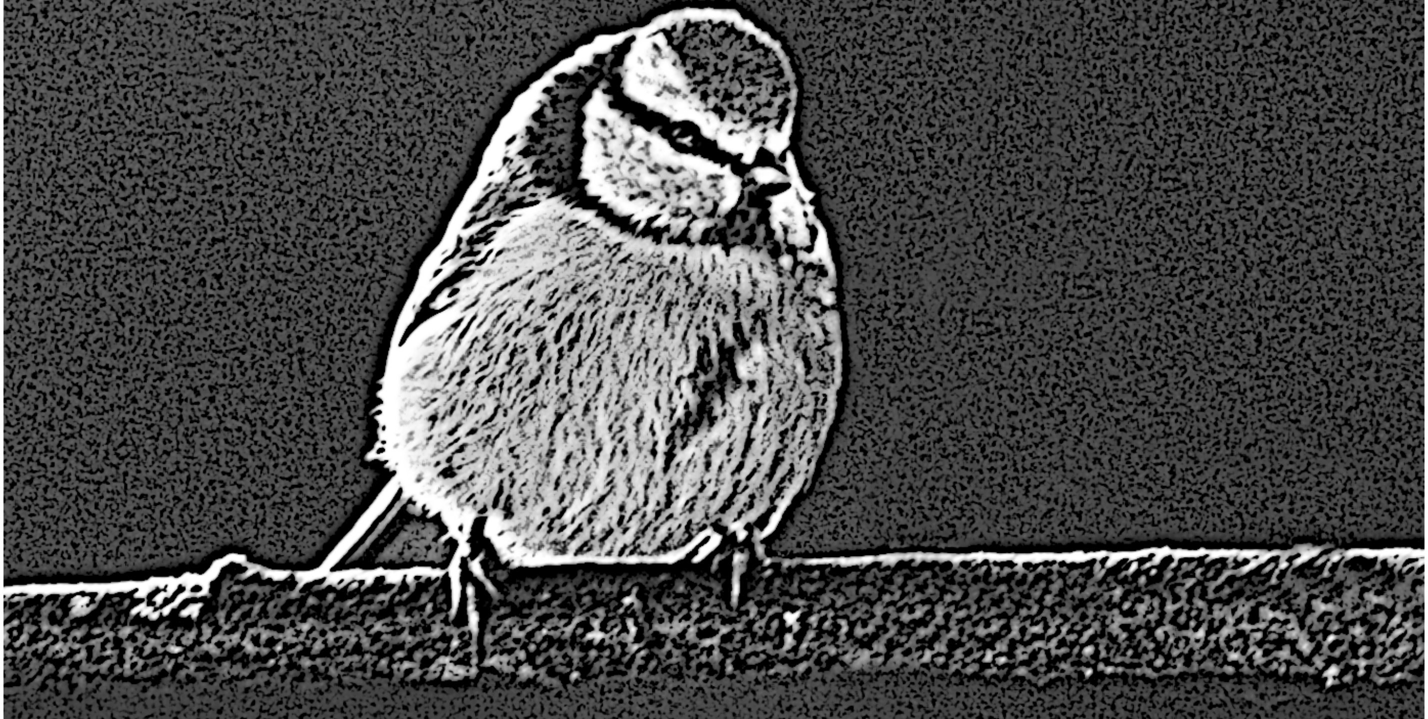

vorgelegt von Kaspar Delhey

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1. Gutachter: Prof. Dr. B. Kempenaers

2. Gutachter: Prof. Dr. S. Foitzik

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Erklärung

Hiermit erkläre ich, dass meine Dissertation “Sexual selection and blue tit (*Parus caeruleus*) crown coloration“ von Prof. Dr. Eberhard Gwinner und Prof. Dr. Bart Kempenaers betreut wurde.

Ich habe diese Dissertation selbständig, ohne unerlaubte Hilfe angefertigt.

Weder habe ich bereits anderweitig ohne Erfolg versucht, eine Dissertation einzureichen, noch ist diese Arbeit ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden.

Seewiesen, den 8. Juni 2005

(Kaspar Delhey)

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ABSTRACT

Conspicuous, sexually dimorphic plumage in birds is most likely a consequence of sexual selection favouring more ornamented males at obtaining a territory and/or a mate. Recent comparative analyses suggest that, among socially monogamous species, extra-pair paternity has also contributed to the elaboration of male ornaments. If females prefer more ornamented males as social or extra-pair mates this could translate into strong directional selection for ornament elaboration, since these males might sire more offspring in their own brood (within-pair success) or in broods of other males (extra-pair success).

In this thesis I study the expression of the UV/blue crown coloration in the blue tit (*Parus caeruleus*) and investigate whether this trait could be selected through increased male within- or extra-pair success. Blue tits are socially monogamous passerines with relatively high levels of extra-pair paternity, and males in this species display more ultraviolet(UV)/blue reflectant crown feathers than females.

Based on three years of data I found that crown coloration could be a cue used by females to assess male age since blue tits became more UV-ornamented as they aged. Crown coloration, however, did not correlate with survival to the next breeding season, suggesting that more UV-ornamented males are not necessarily of higher quality. While crown UV-ornamentation increased between years, it declined in the course of a year due to feather wear and dirt accumulation and this could affect female perception of male attractiveness. However, although the decline in UV ornamentation between winter and spring was large, it had no effect on male reproductive success, and winter and spring colour were still positively correlated.

Using genetic paternity analysis I could show that more UV-ornamented males do not benefit through increased within-pair or extra-pair success. On the contrary, less UV-ornamented, adult males sired most of the extra-pair offspring. Hence the most successful males of the population were adult males that resembled juveniles in their crown colour. Accordingly, females seem to recognise less UV-ornamented males as highly successful, since they biased brood sex ratio towards male offspring if paired to these males.

The causality of these patterns was tested in a colour manipulation experiment, where I treated males to become more (UV+) or less (UV-) ornamented within the natural range of variation. Against expectations UV(+) males sired significantly more extra-pair offspring than UV(-) males while the proportion of within-pair offspring was unaffected by the manipulation. Brood sex ratios did not differ between treatments but depended on male colour before manipulation. While these results do suggest that crown colour plays a role in

paternity and brood sex allocation, they do not provide experimental support for the observed correlational patterns. I discuss the discrepancy between observational and experimental data, emphasising potential problems with the experimental manipulation of structural plumage colour.

Finally, given that more UV-ornamented males did not sire more offspring, I explore the possibility that they would benefit by pairing with high quality females. High quality females in other species are often more ornamented, and birds of high quality pair assortatively based on ornament expression. This was not the case in this blue tit population, since female colour did not appear to indicate relevant female qualities (fecundity, seasonal reproductive success) and blue tits did not mate assortatively by crown colour in any of the three study years.

To conclude, selection seems to favour older, less UV-ornamented males in this population. Whether this is due to female preference is unclear. Alternatively I hypothesise that being less ornamented may enable males searching for extra pair copulations to intrude into other territories without eliciting aggression by territory owners, perhaps by mimicking juveniles. Detailed behavioural observations in the wild coupled with choice chamber experiments in captivity are necessary to test this idea.

GENERAL INTRODUCTION

The bizarre displays, bright colours, extravagant plumages and melodious song of birds have since long captured the attention of man. What is the function of these seemingly useless traits? Why are they normally more developed in males? Although these questions were probably asked repeatedly in the past, it was not until Darwin (1871) put forward his theory of sexual selection that a satisfactory hypothesis was offered. Sexual selection was Darwin's explanation for the existence of those traits that have no apparent function in the struggle for life and most likely impose survival costs to their bearers. Ornamental traits, Darwin hypothesised, may enable males to seduce females or deter rivals, ultimately increasing the reproductive success of the owner. Hence, sexual selection favours those individuals which are successful in the competition over mates.

Sexual selection acts mainly through two avenues: direct contests between males to obtain a female or female choice, where certain males are preferred over others as mates (Andersson 1994). Sexual selection usually acts more strongly on males since they show greater variance in reproductive success than females (Shuster and Wade 2003). In most birds female reproductive success is constrained by the number of eggs she can produce, while males can mate with multiple females and hence increase the number of offspring sired. The potential for sexual selection to shape male phenotype depends on the variance in male reproductive success, and this is strongly dependent on the extent that males can monopolise females, which is best described by the mating system.

Sexual selection and mating systems

Mating systems in birds are extremely variable, ranging from a few males mating with most females in a population (as in lekking or polygynous species) to life-long monogamy where each individual has only one mate. In agreement with the predictions of sexual selection theory, the variance in male reproductive success, or mating skew, is related to the degree of ornamentation and sexual dimorphism. Comparative studies revealed a significant association between the degree of sexual dimorphism in ornamentation and mating systems, with polygynous species being more sexually dimorphic than monogamous species (Oakes 1992, but see Höglund 1989). Nonetheless, much of the interspecific variation in ornamentation remained unexplained, in particular the observation that many monogamous species are strongly sexually dimorphic, despite an apparent lack of variation in reproductive success. This was already recognised by Darwin (1871) who proposed, as an alternative, that among monogamous species more ornamented males would pair with high quality females which produced more or better offspring. While this idea has received theoretical and empirical support (Kirkpatrick et al. 1990; McGraw et al. 2001; Dearborn and Ryan 2002), the advent of molecular parentage assignment uncovered a potentially more powerful source of variability in reproductive success: extra-pair paternity.

Extra-pair paternity is a consequence of males and/or females seeking copulations with mates other than their social mate (i.e. the mate with whom they share a nest or territory). To date more than 70% of the studied socially monogamous bird species show at least some offspring being sired by extra-pair fathers (Griffith et al. 2002) and this has the potential to greatly increase the variance in male reproductive success (Webster et al. 1995). Whether extra-pair behaviours are under male or female control is a debated issue. Both males and females in several species have been reported intruding on other territories, potentially seeking extra-pair copulations (Westneat and Stewart 2003). While it is easy to see what males gain from engaging in extra-pair copulations, the benefits for females are less clear and several hypotheses have been proposed (reviewed in Griffith et al. 2002). One set of hypotheses suggests that females obtain direct benefits from copulating with extra-pair males such as food or sperm as insurance against mate infertility. The alternative, indirect benefits, claims that females seek extra-pair copulations with specific “chosen” males and hence obtain genetic benefits inherited by her offspring (Griffith et al. 2002). Although conclusive evidence is still lacking, indirect benefit hypotheses are currently favoured by researchers in the field (Griffith et al. 2002). If females actively pursue copulations with certain males disregarding others, they should have means to identify the quality of these males. One possibility is that females identify male quality by the expression of ornamental traits.

If more ornamented males are more successful at securing extra-pair copulations and/or less cuckolded by their social mates, sexual selection through extra-pair matings may have a strong effect on ornament elaboration. Indeed, multivariate comparative analyses revealed stronger effects of the level of extra-pair paternity than social mating systems (degree of polygyny) on the degree of sexual dichromatism in birds (Møller and Birkhead 1994; Owens and Hartley 1998). This led to the conclusion that extra-pair paternity was the most important selective force behind the elaboration of male ornaments in birds. This view was recently challenged by a more exhaustive comparative analysis that included more than 1000 bird species (Dunn et al. 2001), where the degree of polygyny had a stronger effect on sexual dichromatism than the degree of extra-pair paternity, although the latter was still correlated to sexual dichromatism. Hence most probably both, the degree of polygyny and the level of extra-pair paternity have contributed to the evolution of male ornamentation in birds.

Extra-pair paternity and bird coloration

The possibility that extra-pair paternity may promote the evolution of male ornamentation in monogamous species, suggests that we should also expect a correlation between male secondary sexual traits and paternity within species. Indeed, several studies to date have found that more ornamented males are less cuckolded (within-pair success) and/or sire more extra-pair offspring (extra-pair success), although several others have failed to do so (**Table 1**). Traits involved in paternity success appear to span the entire range of bird ornaments, from elongated feathers, to the size of conspicuous badges as well as the intensity of different types of colours, but from this limited number of studies it is not obvious whether certain types of plumage ornaments are more likely to be involved in sexual selection through paternity success. Comparative analyses however, reveal that if sexual dichromatism is partitioned into the different types of feather coloration (mainly melanins, carotenoids and structural colours, see **Box 1**) only sexual dichromatism due to structural colours (i.e. shades of blue, purple, ultraviolet and iridescent colours) predicts interspecific variation in extra-pair paternity (Owens and Hartley 1998). This intriguing result suggests that for some unknown reason structural colours are more likely to have been selected through extra-pair paternity than carotenoid or melanin based colours.

Studies addressing the importance of the expression of structural colours on paternity success within species are scarce. Two species have been studied to date: the bluethroat (*Luscinia svecica*) and the black-capped chickadee (*Poecile atricapillus*). In both cases the level of ultraviolet reflectance of plumage patches (the throat in the bluethroat, and the black cap in the chickadee) correlated positively with the proportion of offspring a male sired in its own nest (**Table 1**) but not with a male's success at cuckolding other males. While this provides some support for Owens and Hartley's (1998) findings more studies are needed to evaluate whether this pattern is the same in other species with structural ornaments.

The main aim of this thesis is to examine whether the expression of structural-based coloration is selected through increased within-pair and/or extra-pair success, using the blue tit (*Parus caeruleus*) as a model species.

Box 1. The colour of birds

The plumage of birds attains its coloration either from the presence of chemical pigments, its microstructure, or a combination of both (Lucas and Stettenheim 1972). Pigments absorb light of certain wavelengths while reflecting at others. In birds three main types of pigments can be found: carotenoids, melanins and porphyrins. While the latter are rare, carotenoids and melanins are found throughout the Class Aves.

Melanins confer colours to feathers that range from black to buff, through shades of rusty reds and greys. Typical examples of melanin coloured feathers are the black bib of the house sparrow (*Passer domesticus*) or the rusty-red cheek patches of the zebra finch (*Taeniopigya guttata*) (McGraw and Wakamatsu 2004). Although melanins are probably the most common avian pigments, detailed studies of their effect on feather colours have just begun (Jawor and Breitwisch 2003; McGraw 2003).

Carotenoids, on the other hand, which colour feathers red, orange or yellow have received much more attention. Some of the best known examples of sexually selected traits involve carotenoid-based colours such as the plumage of the house finch (*Carpodacus mexicanus*) (Hill 1991; Hill 1992; Hill 2002). Carotenoids cannot be synthesized by birds (or other animals) and thus need to be ingested with food. Moreover, carotenoid intake is also dependent on individual condition and health status (McGraw and Hill 2000). It is thus not surprising that carotenoid-based colours feature as prime examples of honest signals of quality, reflecting foraging ability, disease resistance and overall condition (Blount et al. 2003).

Structural colours originate from the modification or separation of the components of white light by the structure of the feather. Roughly, structural colours can be divided into three categories: iridescent, non-iridescent and whites (Prum 1999). Iridescence, such as seen in the feathers of the starling (*Sturnus vulgaris*) or hummingbirds (Trochilidae) is produced by layers of scattering elements (granules of melanin and air vacuoles suspended in a keratin matrix) which are usually oriented parallel to the feather surface (Prum 1999). Iridescent feathers typically change hue (e.g. from red to blue) with changes in the angle of observation.

Non-iridescent structural colours like the crown feathers of the blue tit (*Parus caeruleus*) are produced by the ordered arrangement of nanostructural elements (a matrix of keratin rods and air vacuoles) beneath the feather cortex (the so-called "spongy layer", Prum et al. 2003). The size, regularity and distance between scattering particles, as well as the thickness of this spongy layer determine the colour produced (Andersson 1999; Shawkey et al. 2003; Shawkey et al. 2005). Smaller scattering elements, which are closely packed produce more shortwave hues (for example ultraviolet hues), while their regularity determines the saturation of a particular hue. This regular spatial arrangement at very fine scale reflects short-wavelength light through coherent scattering (Prum et al. 2003). It has been hypothesized that producing these highly ordered tissues is costly and could reflect genetic quality and/or condition during moult (Fitzpatrick 1998).

White feathers lack the ordered arrangement of scattering elements that characterize iridescent and non-iridescent structural colours, and hence reflect incident light across all wavelengths through incoherent scattering (Prum 1999).

Table 1. List of bird species (in alphabetical order) for which there are studies addressing the relationship between male feather ornaments and within-pair (proportion of offspring sired in the own nest or likelihood of being cuckolded) and extra-pair success (number of sired extra-pair offspring or likelihood of siring extra-pair offspring). The symbol "0" stands for no significant relationship with male ornamentation, "+" or "-" indicates whether there is a significant positive or negative relationship respectively, while "NS" stands for "not studied". "?" indicates weak statistical support and/or very small sample sizes.

Species	Male ornament	Within-pair	Extra-pair	Reference
Agelaius phoeniceus	Epaulette colour and size	0	0	(Weatherhead and Boag 1995)
Carpodacus mexicanus	Red colour	0	NS	(Hill et al. 1994)
	Red colour	0	NS	(Badyaev et al. 2001)
Dendroica petechia	Breast colour red-yellow	0	+	(Yezerinac and Weatherhead 1997)
Emberiza citrinella	Yellow colour	0	+	(Sundberg and Dixon 1996)
Ficedula albicollis	Front-patch size	+	+	(Sheldon and Ellegren 1999)
Ficedula hypoleuca	Dorsal black colour	0	NS	(Rätti et al. 1995)
	Dorsal black colour	-	+?	(Lifjeld et al. 1997)
Geothlypis trichas	Black face mask size	+	+	(Thusius et al. 2001)
Hirundo rustica	Tail length	+	NS	(Møller 1997)
	Tail length (manipulated)	-	NS	(Smith et al. 1991)
	Tail length (manipulated)	+	+	(Saino et al. 1997)
Luscinia svecica	UV/blue breast patch colour	+	0	(Johnsen et al. 2001)
	Width of chestnut band	+/0	0	(Johnsen et al. 2001)
Luscinia svecica	UV/blue breast patch colour (manipulated)	+?	+	(Johnsen et al. 1998)

Table 1-continued

Malurus cyaneus	Time of moult into blue plumage	0	+	(Dunn and Cockburn 1999)
Panurus biarmicus	‘Beard’ length	+	NS	(Hoi and Hoi-Leitner 1997)
Parus caeruleus	Crown UV/blue colour	+?	-	This thesis
Passer domesticus	Badge size	0	NS	(Whitekiller et al. 2000)
	Badge size	0	NS	(Cordero et al. 1999)
	Badge size	0[1]	NS	(Vaclav et al. 2002)
Poecile atricapillus	Black and white plumage colour	+	NS	(Doucet et al. 2005)
Setophaga ruticilla	Bib size	0	0	(Perreault et al. 1997)
Sturnus vulgaris	Hackle feather length	0	NS	(Smith and von Schantz 1993)

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[1] average badged males seemed to be more cuckolded.

"*The species used is the blue tit, which is fast becoming one of the 'lab rats' for studies of plumage coloration…*" Anonymous reviewer.

The 'lab rat'

The blue tit (*Parus caeruleus* L.), a small (10-13 g) hole-breeding bird belonging to the family Paridae, is probably one of the most intensively studied passerines to date. The main reasons for this popularity are its Europe-wide distribution and the fact that it readily breeds in nest boxes where researchers have easy access to adults and offspring. A simple search of the recent literature reveals that blue tits are suitable models for disparate research fields such as historical biogeography (Kvist et al. 2004), foraging ecology (Pulido and Diaz 2000), quantitative and population genetics (Dias et al. 1996; Merilä and Fry 1998; Charmantier et al. 2004b), environmental pollution (Eens et al. 1999), climate change (Sanz 2002) and others. Among these the study of sexual selection plays a prominent role.

Blue tits are socially monogamous although a certain proportion of males attract more than one female and become polygynous (10-20% males, Kempenaers 1994; **Chapter 4, 6**). Additionally this species shows relatively high levels of extra-pair paternity ranging from 33% to 68% of the broods and 5% to 25% of the offspring (**Table 2**). Extra-pair paternity increases the variance in male reproductive success (Kempenaers et al. 1992; **Chapter 1**), thus providing ample opportunity for sexual selection to act on male ornamentation.

Table 2. Levels of extra-pair paternity (EPP), represented as the proportion of broods that had at least one extra-pair young and the proportion of offspring that was sired by extra-pair fathers, in different blue tit populations. Note that southern populations seem to have higher levels of EPP.

Population	EPP $_{\text{broods}}$	EPP $_{\text{offsp.}}$	N_{broods}	$N_{\text{offsp.}}$	Reference
Norway					(Krokene and Lifjeld 2000)
Oslo	46%	13%	28	175	
Jomfruland	33%	5%	46	457	
Sweden (Uppsala)	29%	6%	7	51	(Gullberg et al. 1992)
Britain (Lancashire)	39.8%	11.7%	103	986	(Leech et al. 2001)
Belgium (Antwerp)	41.8%	12.5%	165	1443	(Kempenaers et al. 1997)
Germany (Bonn)	51.4%	13.4%	35	314	(Lubjuhn 2005)
Austria (Vienna)	58%	15%	172	1877	This thesis (**Chapter 4**)
France					(Charmantier et al. 2004a)
Rouviere	53.6%	16.1%	97	839	
Corsica, Pirio	68%	25.4%	50	288	
Corsica, Muro	50%	18.2%	30	205	

Blue tits are strikingly coloured (**Fig. 1**). Both sexes display several bright blue plumage patches, most notably the crown, wing coverts and tail, all used in sexual or agonistic displays (Cramp and Perrins 1993). Additionally they also have intense yellow coloured breast and belly feathers. Traditionally, blue tits were considered sexually monomorphic with only subtle differences in coloration between males and females (Svensson 1992). The rediscovery in the early 1990s that birds can perceive the human-invisible near-ultraviolet waveband (UV-A, 300-400 nm, see review in Cuthill et al.2000) prompted researchers, meanwhile equipped with UV-sensitive spectrometers, to search for examples of "hidden" sexual dichromatism in birds. The blue tit was one of these. Two research teams reported independently and simultaneously in 1998 that blue tits showed strong sexual dichromatism in the UV (Andersson et al. 1998; Hunt et al. 1998). This suggested that sexual selection on male plumage ornaments may have gone unnoticed in this species, and that UV reflectance of the crown feathers may play an important role in mate choice or male-male agonistic interactions. Indeed, Swedish blue tits paired assortatively based on the degree of crown UV-reflectance (Andersson et al. 1998), and in mate choice trials females seemed to prefer males with brighter crown feathers (Hunt et al. 1998). Follow-up studies confirmed apparent female preference for more UV-ornamented males both in captivity and in the wild (Hunt et al. 1999; Sheldon et al. 1999; Griffith et al. 2003; Limbourg et al. 2004; Johnsen et al. in press). However, the question whether crown reflectance relates to paternity success or even basic breeding parameters such as clutch size remained unanswered.

Fig. 1. A female blue tit while nest building. To the human eye both sexes look remarkably similar.

To fill this gap I studied blue tits in a nest box breeding population at Kolbeterberg (48º 13' N, 16º 20' E) in the periphery of Vienna, Austria. Field data for this thesis were collected between the years 2001 and 2003, but this work is part of an ongoing long term study of blue tit breeding biology which started in 1998 (Foerster et al. 2003). The study site (ca. 35 ha) is part of a large mixed-deciduous forest and the predominant tree species are beech (*Fagus sylvatica*), oak (*Quercus robur*) and ash (*Fraxinus excelsior*). This type of forest constitutes prime habitat for the blue tit (Cramp and Perrins 1993) and this is reflected in the high density of breeding pairs (between 57 and 84) and relatively large clutches (modal clutch size in each of the three study years was 12 eggs, range = 4-17). Blue tits were captured throughout the year, in winter while they roosted in the nest boxes at night, in early spring using mist nets and playback, and again at the nest box in late spring while feeding chicks. After capture birds were banded with a numbered metal band and a unique combination of plastic colour bands. In spring, birds were sexed based on the presence-absence of the brood patch; otherwise sex was determined by molecular methods (see below). A small (5-50 µl) blood sample was drawn from the brachial vein and stored in Queens Lysis buffer for genetic sex determination and paternity assignment. Bird age was determined by the colour of the primary coverts following Svensson (1992). Birds were classified as juveniles (hatched during the previous breeding season) or adults. Finally, I measured tarsus length (with callipers) and body mass (with an electronic balance) and crown coloration using reflectance spectrometry (see **Box 2**). In the breeding season of year 2003 I also carried out an experiment were male crown colour was manipulated before egg laying (see **Box 3**).

Data on basic breeding biology were collected every year starting in late March. Nest boxes were monitored at regular intervals throughout the whole breeding season from nest building to fledging. In this population egg laying peaks in early to mid-April, and after the clutch is completed females incubate during 12-13 days. After hatching chicks remain in the nest for 18-20 days, a period during which they are fed by both parents. For each nest we tried to obtain blood samples (5-20 µl) from all offspring, and most chicks were bled between day 9 and 14 after hatching. Embryos from unhatched eggs and dead chicks were also collected and stored in ethanol.

Paternity analysis was carried out by the genetic lab in Seewiesen as part of the database contributing to a long-term study of extra-pair paternity in the blue tit. Paternity of offspring (embryos and chicks) was determined using eight polymorphic microsatellite markers (*Pca3*, *7*, *8* and *9* (Dawson et al. 2000), *Pocc1* and *6* (Bensch et al. 1996), *Phtr3* (Fridolfsson et al. 1997) and *PK11* (Tanner SM, Richner H, Schuenperli D, unpublished; EMBL accession no: AF041465)). After standard PCR procedures amplified fragments were resolved on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Paternity was excluded if two or more loci showed mismatches between putative fathers and offspring. Microsatellites allow identifying the fathers of extra-pair offspring from the pool of sampled males. Hence, both the success of a male at avoiding being cuckolded (within-pair success) and its success at cuckolding other males (extra-pair success) could be estimated. Estimating both components of fertilisation success is crucial to understand variation in male fertilisation success. For example if there is a trade off between protecting within-pair paternity and pursuing extra-pair copulations, the benefits of siring extra-pair offspring may be offset by increased cuckoldry, and this may decrease the opportunity for sexual selection (Webster et al. 1995).

Sex of the offspring and winter-caught adults was determined either by using the sex-linked microsatellite marker *Phtr3* (Fridolfsson et al. 1997), or the specific primers *P2* and *P8* (Griffiths et al. 1998), which amplify the genes CHD1W and CHD1Z, located on the W and Z chromosomes respectively.

Box 2. Revealing the invisible: measuring blue tit colour

In this thesis I use crown coloration as a measure of male ornamentation. Why crown colour and not the colour of the other UV/blue body parts like tail or wing coverts? There are several reasons why the use of crown colour is desirable. First, the expression of the structural UV/blue colour is much more intense on the crown than on the tail or wing coverts (Örnborg 2002; Delhey et al. unpub. data) suggesting that crown colour constitutes the main signal. This is probably the reason why only crown colour has been used in other studies, which takes us to the second reason it being to allow comparisons between studies. Third the expression of structural colours is correlated between these different patches (Örnborg 2002), suggesting a certain degree of redundancy between colour patches.

Since blue tit crown feathers are maximally reflective in the UV (Andersson et al. 1998), and most birds - including the blue tit - are sensitive to UV light, I used UV/VIS reflectance spectrometry to measure crown colour. The set-up to measure feather reflectance consisted of a deuterium-halogen light source DH-2000 connected to a S-2000 spectrometer (Ocean Optics) through a bifurcated fibre optic cable. Light emitted by the lamp (spectral range 280 to 800 nm) is transmitted through the fibre optics cable and illuminates, at a 90° angle, a small spot (~11 mm^2) of the plumage. A plastic cylinder fixed at the end of the fibre optics enables to standardise measuring distance and exclude ambient light (Fig. 1). Light reflected by the feather is then collected by a different optic fibre (also at a 90° angle) and transmitted to the spectrometer. Finally, the raw data from the spectrometer are transformed into reflectance relative to a WS-2 white standard (Avantes) by the program Spectra-Win (Top Sensor Systems).

Each time a blue tit was captured I took five measurements on different but standardised spots of the crown (**Fig. 1**). I chose to measure different spots to obtain an overall estimate of the colour of the crown, since often the reflectance varies notably within the same plumage patch (personal observation, see **Chapter 2**). Reflectance spectra were imported into a spreadsheet program (Excel) for further analysis. Spectra were smoothed using a running average computed over a 10nm interval. Smoothing is a necessary step to avoid error

Figure 1. Measuring blue tit crown reflectance(left), note the plastic cylinder used to standardize measuring distance and exclude ambient light; and (right) approximate position of the five different spots measured to assess overall crown colour.

through the occasional spikes present in the raw reflectance spectra, which could influence the colour variables, especially hue (see below). For each of the five spectra collected per blue tit we computed the variables used to describe the colour (see below), and these were then averaged to obtain a single value per bird.

There exist several ways to obtain meaningful estimates of colour form reflectance spectra. For example the information contained in a reflectance spectrum can be summarized by principal component analysis which extracts a few (usually three) relevant variables (the principal components) that explain most of the variation in reflectance (Cuthill et al. 1999). Alternatively the wavelength spectrum can be subdivided into equally spaced (arbitrary) segments and colour can be estimated by computing the differences in relative intensity (reflectance) between them (Endler 1990). These two approaches have been found to yield very similar results although principal component analysis seems to be slightly better at revealing subtle differences between spectra, but has the drawback of being difficult to interpret and that their results are not directly comparable between data sets (Grill and Rush 2000). More recently visual models have been developed that use spectral sensitivities from the cones to predict psychophysical colour discrimination thresholds for different birds (Vorobyev et al. 1998) and this practice, which is becoming increasingly popular, has been recently applied to the study of blue tit colour (J. Hadfield pers. com.). Finally, a common approach consists of extracting the three basic descriptors relevant for colour perception (brightness, hue and chroma, Endler 1990) based on the geometric properties of the spectra (positions and ratios of peaks and through, slopes, etc.). These colour indices, although not always computed in exactly the same way, have been widely used in recent studies of avian coloration, and shown to be biologically relevant for a variety of colours (Keyser and Hill 1999; Pryke et al. 2001; Doucet and Montgomerie 2003; Siefferman and Hill 2005). Throughout this thesis I use this approach by computing a set of colour indices based on those introduced by Staffan Andersson in the study of blue tit coloration (Andersson et al. 1998; Sheldon et al. 1999). This was done not only to enable direct comparisons of results with already published work, but also with work in progress, since these same indices are used Europe-wide by most researchers studying blue tit coloration (pers. com.). Furthermore, the potential for comparing results and collaborative studies between research teams is maximized by the use of similar light sources, spectrometers, white standards and illumination angles.

Colour indices were computed for the spectral range between 300 and 700 nm which encompasses the entire visual sensitivity of the blue tit (Hart et al. 2000).

Brightness (**Fig. 2a**), which constitutes an estimate of the area under the curve or total light reflected by the feathers, can be estimated as the sum of reflectances (R) between 300 and 700 nm (Σ R300-700) or as the average reflectance (R_{av}), which is the total reflectance (Σ R300-700) divided by the number of data points. Given that studies usually differ in the number of data points contained in a reflectance spectrum, the latter measurement has the benefit to allow direct comparison among studies.

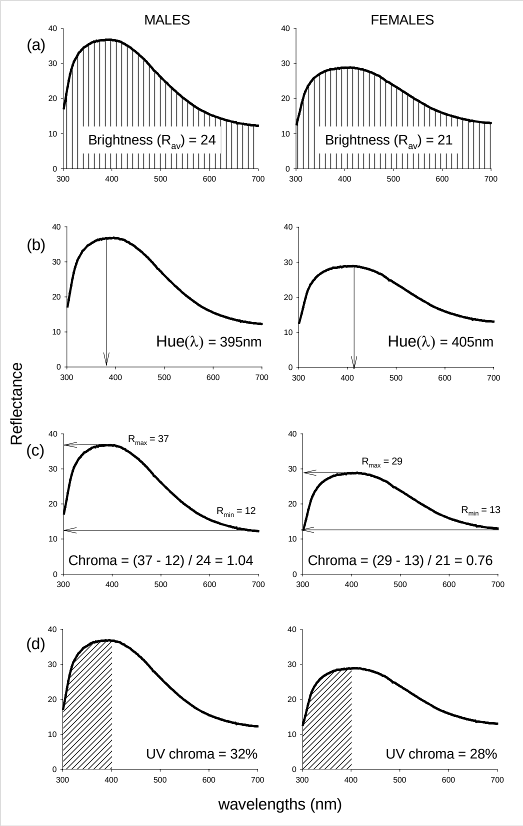


Figure 2. Typical male (left) and female (right) crown reflectance spectra. The different panels depict the calculations to obtain the different colour indices: (a) brightness, (b) hue, (c) chroma, and (d) UV chroma. See text for explanation.

Hue (**Fig. 2b**), in a simple single-peaked spectrum like that from the blue tit crown, is most easily approximated as the position on the x-axis (wavelengths) of the point of maximal reflectance (λRmax). This provides an estimate of which cone is stimulated the most. The lower the value of hue, the more ultraviolet the colour of the feathers.

Chroma (**Fig. 2c**), the degree of saturation or spectral purity, is higher in spectra that show larger differences in reflectance between different wavelength ranges (Endler 1990). Hence, chroma is estimated as the difference in reflectance between the highest and the lowest point of the spectrum divided by brightness.

Finally, I also computed UV chroma (**Fig. 2d**) or relative UV reflectance. This variable, which addresses specifically the contribution of UV wavelengths (300-400nm) to the reflectance spectrum, is computed as the ratio between reflectance in the UV (Σ R300-400) and total reflectance (Σ R300-700), and can be expressed as a proportion or percentage. All four indices show strong differences between the sexes (Andersson et al. 1998), **Chapter 2**). Hence, males are more UV ornamented (lower hue, higher UV chroma), more chromatic and brighter than females. Throughout this thesis more UV-ornamented birds are individuals with low hue (i.e. mort short-wave) and higher UV chroma while the converse is true for less UV-ornamented birds. These two variables are highly correlated and usually a bird with low hue also has high UV chroma. However, these two variables do not necessarily correlate in the same way with male mating success (see **Chapter 4**).

Box 3. Manipulating the invisible: crown colour manipulation methods

Manipulating feather coloration in the UV is not an easy task. To date researchers manipulating this trait in birds have used UV-absorbing chemicals, such as present in commercial sunblocks. This method, first introduced by Andersson and Amundsen (1997), has now been used to reduce UV reflectance in several bird species (Johnsen et al. 1998; Sheldon et al. 1999; Arnold et al. 2002; Siitari et al. 2002). Although initially a good way to identify UV reflectance as a cue in bird signalling, it has two disadvantages: (1) manipulating UV-reflectance with UV blocker can only reduce, and not enhance, a UV-blue ornament, (2) UV-blocked birds display unnatural reflectance spectra, with the UV portion of the spectrum 'shaved-off' (**Fig. 1a**). Such spectra do not exist in birds, so conspecifics might respond to the manipulation simply because the individual became "odd". To realistically examine the function of natural variation in colour, more subtle manipulations are necessary modifying colour within the natural range of variation. In an attempt to achieve this goal I developed a colour manipulation method using colour marker pens. To this effect I tried out different marker pens and assessed their effects on blue tit colour. From all tried types, Edding "T-shirt marker" pens produced the best results. I used two colours, light blue that increased reflectance in the UV and dark blue that strongly reduced it. Males allocated to the UV-enhanced treatment (UV(+)) were painted only with the light blue pen while UV-reduced males (UV(-)) were first painted with the dark blue pen and then with the light blue one. Both treatments were water-proofed by a coat of non-toxic silicone fly-dressing.

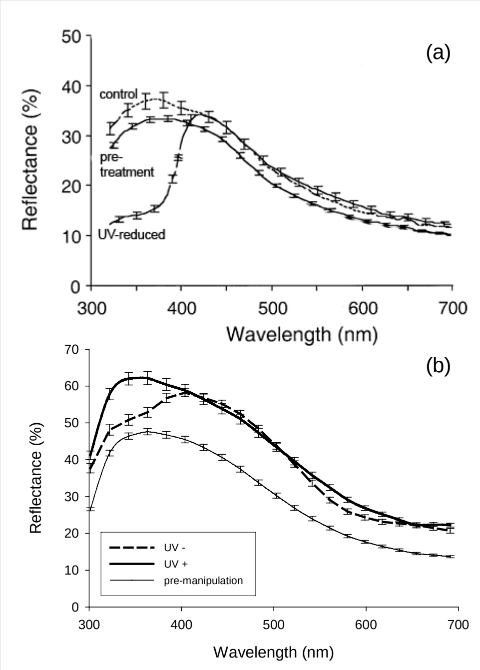
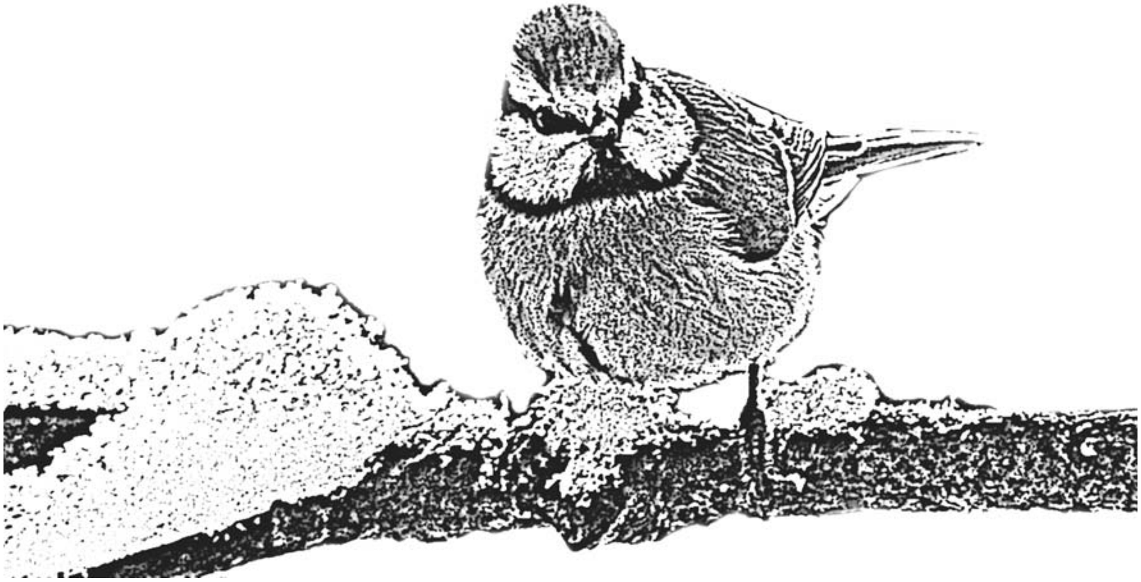


Figure 1. Average reflectance spectra of blue tit males corresponding to (a) control, pre-treatment and UV-reduced (sunblock), modified from Sheldon et al. 1999, and (b) pre-manipulation and UV(-) and UV(+) treated birds in this thesis (**Chapters 4 and 5**). Bars corresponds to standard errors in both (a) and (b).

After treatment, reflectance spectra of both groups were different but still resembled those of untreated birds in overall shape (**Fig. 1b**). UV(+) birds reflected more in the UV (higher UV chroma) and had a more UV-shifted peak (hue) than UV(-) birds. Compared to pre-treatment colour, both treatment groups were brighter (had higher overall reflectance) and this effect was due to the use of silicone paste that increases feather gloss. Colour variables (see **Box 2**) for both treatments were largely within the natural range of variation, except for two UV(-) males which had a slightly more longwave hue than the maximum recorded. Both treatments faded with time, but UV(+) males faded more than UV(-). After two weeks UV(-) males were on average still less UV than before treatment. Although UV(+) birds no longer differed from pre-treatment, they still had a significantly more UV-shifted hue than UV(-) birds. Hence, although this treatment constitutes an improvement over the use of sunblock to manipulate UV-blue structural colour, it still shows the common problem that the effects of treatment greatly decline with time.

General Introduction



GENERAL RESULTS AND DISCUSSION

In this section I will summarize and discuss the results from the different chapters. I have chosen to include some of the relevant figures from each chapter to aid the reader, avoiding the need to consult it every time a figure is cited.

Briefly, in **Chapter 1,** I explore correlations between male colour and fertilisation success using data collected in the first year. **Chapters 2** and **3** are devoted to understanding how crown coloration varies between and within-individuals and between- and within-years. In **Chapter 4,** I first re-assess the patterns between male crown colour and fertilisations success described in **Chapter 1** using data from all three years before testing the causality of these patterns through a colour manipulation experiment. **Chapter 5** addresses female perception of male attractiveness studying the relationship between female sex allocation strategies and male colour, using both correlations and the colour manipulation experiment. Finally, in **Chapter 6** I study the potential signaling function of the crown colour in females and the possibility of mutual mate choice in the blue tit.

Chapter 1. Within- and extra-pair success correlate with male crown colour

The first necessary step to study the link between blue tit crown coloration and paternity success is to establish whether there is indeed a relationship between these two variables. This was the aim of the first chapter, where I explored correlations between the different colour variables and male within-pair and extra-pair success. To this effect I used data collected during the first year of my research (2001). My expectation was that more ornamented blue tits would have higher overall success by either being less cuckolded (within-pair success) and/or siring more extra-pair young with other females (extra-pair success), or both. This was based on the premise that females should be more faithful and/or seek extra-pair copulations with high quality males (Kempenaers et al. 1997) and that male quality could be signaled through crown coloration. Indeed, I uncovered a relationship between coloration and paternity, although not quite as expected.

More than half (65%) of the 47 studied males were cuckolded, and combined with extra-pair success, this increased actual variance in male fertilisation success more than three-fold compared to apparent variance. In agreement with my prediction, cuckolded males had a more long-wave hue (i.e. they were less UV-ornamented) than males that sired all offspring in their broods, and the proportion of offspring sired in the brood correlated negatively with hue (**Fig. 2a**) indicating that females were more faithful to males which show a more UV-shifted hue (i.e. more short-wave).

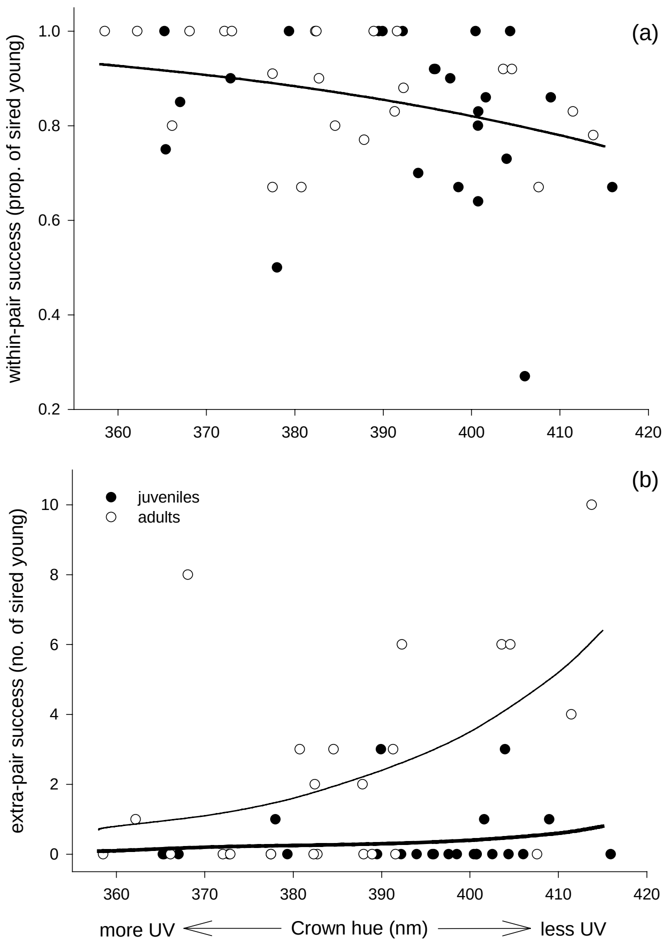


Fig. 2. Male crown hue correlates both with within-pair success (a) and extra-pair success (b).

Extra-pair paternity was mainly determined by male age, since adult males (≥2 years old) were much more successful at siring extra-pair offspring than juveniles, a common pattern among birds (see review in Griffith et al. 2002). However, after statistically accounting for male age, extra-pair success correlated positively with crown hue (**Fig. 2b**) and, against the expectations, less UV-ornamented males sired more extra-pair young.

Total fertilisation success, the sum of within- and extra-pair offspring, was higher for adults than juveniles and since selection through extra-pair success was stronger than selection through within-pair success, less UV-ornamented males tended to sire more offspring in total.

The main message from this first chapter is that sexual selection acting through within-pair and extra-pair success has the potential to shape the evolution of male crown coloration. However, it also suggests that more ornamented males cannot maximize both within- and extra-pair success at the same time, and that there are benefits of being less ornamented, at least for adult males, which sire most of the extra-pair offspring.

Chapter 2. Crown colour changes between years with male age but does not predict male overwinter survival.

Since adult males are often preferred by females as social mates or as extra-pair partners (Griffith et al. 2002; **Chapter 1**), the degree of ornamentation may be one cue that females use to identify male age. In many bird species older individuals are more ornamented than juveniles, and this pattern can arise through two mechanisms: either through within-individual changes as birds age or through differential mortality, where only the most ornamented individuals survive.

Differences in coloration between juveniles and adults are not well described in the blue tit (Örnborg et al. 2002). Hence, the first aim of this chapter was to establish whether there were indeed age differences in crown coloration in our study population. Additionally, by studying how colour changes within-individuals between years, and how colour relates to overwinter survival, I try to identify the mechanism behind potential age-differences.

Age differences in crown coloration were evident for all four colour variables and followed a similar pattern in both males and females. Older birds of both sexes were more UV (lower hue, higher UV chroma, **Fig. 3**), more chromatic and brighter than juveniles. These differences, more pronounced between 1st and 2nd year birds, were caused by within-individual changes in colour expression with age, since individuals measured in consecutive years became more UV, more chromatic and brighter as they aged. Despite these changes, crown colour expression in the same individual was repeatable between years. Survival, on the other hand, was largely unrelated to crown coloration both in males and females and in the different age-classes. Thereby this study provides the first evidence that individual changes rather than differential mortality lie at the basis of increased UV ornamentation in older males.

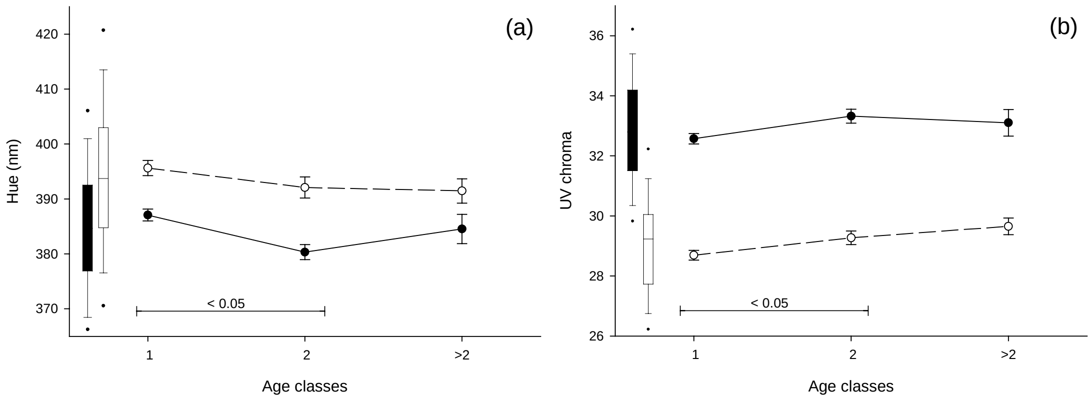


Fig. 3. The effect of age on male and female blue tit crown (a) hue and (b) UV chroma. Depicted are means and their corresponding standard errors. Significant differences between age classes based on post-hoc tests (sexes pooled, see Chapter 2 for statistical details) are indicated. Box plots on the left of each panel represent the degree of colour variability for each sex separately. Depicted are the 5th, 10th, 25th, 75th, 90th and 95th percentiles.

Females, if they are actively choosing their extra-pair partners, do not seem to use crown coloration to identify male age, since the most successful extra-pair males are adults that strongly resemble juveniles in their crown colour (**Chapter 1**, see also **Chapter 4**). Moreover, given that crown coloration was unrelated to overwinter survival, females cannot judge male viability based on the degree of UV-ornamentation as is the case in other blue tit populations (Sheldon et al. 1999; Griffith et al. 2003). Hence, extra-pair males in this population are not necessarily higher quality mates, at least as judged by their survival chances. Indeed, males that survived to the next breeding season were not more successful at siring extra-pair offspring (unpubl. data).

Chapter 3. Blue tit crown UV-reflectance declines dramatically during the year but this does not affect male reproductive success

Plumage colours, although often regarded as honest signals of quality are usually considered static traits unable to reflect short-term changes in condition or health (Hill et al. 1999). Hence, after moult is completed feather colours are assumed to be fixed and unable to change. However, feathers may deteriorate between moults and their colours fade (Test 1940; Johnson and Jones 1993; McGraw and Hill 2004). If these colours are used in agonistic or epigamic signalling, such a decline might have strong effects on individual fitness. Thus, the ability to prevent or reduce the loss in coloration may be adaptive and reflect individual quality (Fitzpatrick 1998; Zampiga et al. 2004).

Changes in the expression of crown colour have been recently reported for the blue tit (Örnborg et al. 2002). Data form two Swedish populations suggest that crown UV reflectance declines throughout the year after the annual moult in autumn due to feather wear and dirt accumulation. However, since this study was not based on repeated measures of the same individuals, sources of bias like colour-related mortality or dispersal, could not be ruled out (Örnborg et al. 2002). In this chapter I address this issue by measuring the same individuals repeatedly over the season. This enabled me to test whether the degree of colour change relates to individual quality and whether it has an effect on male fertilisation success.

Both males and females showed dramatic declines in crown UV-ornamentation between winter (November-December) and spring (May-June), as shown by an increase in hue (i.e. becoming more long-wave or less UV) and decrease in UV-chroma (see **Fig. 4** for males). The magnitude of the decline was similar for males and females and not significantly different between age classes, and there was considerable between-individual variation. Interestingly, changes in colour correlated with skeletal size and changes in body condition in males but not in females. This suggests that the degree of colour change between winter and spring could be indicative of or depend on male phenotypic quality.

Declines or changes in the expression of sexually selected traits have the potential to affect sexual selection and male fitness (Sullivan 1990). This is especially likely in this case since the

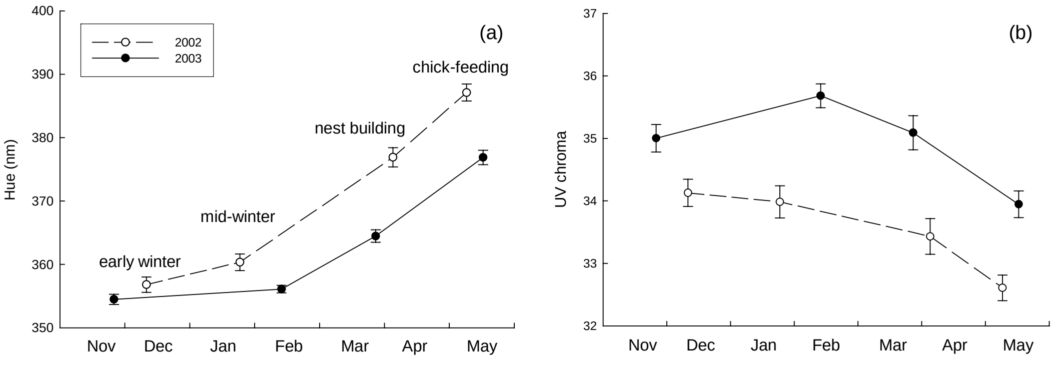


Fig. 4. Seasonal change in male blue tit crown (a) hue and (b) UV chroma between winter and spring in the years 2002 and 2003. Shown are means and their corresponding standard errors.

magnitude of the seasonal changes exceeded sexual dimorphism (change in hue, ~ 25 nm) or age-related differences (change in UV chroma, ~ -1.5%; see **Chapter 2**). It is thus surprising that such large changes had no effect on male seasonal reproductive success, as indicated by the lack of correlation with variables such as clutch size, proportion of offspring sired or male success at siring extra-pair offspring. This may be due to the fact that male ranking based on crown ornamentation does not change during the year, since winter and spring colour are moderately correlated. Moreover, these results suggest that measuring blue tit UV-ornamentation in late spring, as it is often done due to the ease of capturing adults while chick-feeding (Andersson et al. 1998; Delhey et al. 2003; Griffith et al. 2003; **Chapter 1, 4, 5, 6**), still constitutes a meaningful estimate of male crown coloration earlier in the year, when fertilisation success is determined.

Chapter 4. Do older, less UV ornamented males mimic juveniles to sire extra-pair offspring? Experimental colour manipulations provide no support.

Sexual selection, either through male-male competition or female choice, usually favours those individuals which show the more developed ornaments (Ryan and Keddy-Hector 1992; Andersson 1994). Cases where smaller or less ornamented males are better at attracting mates or outcompeting other males are rare (Griffith et al. 1999; Balmford et al. 2000; Badyaev and Hill 2002; Voigt et al. 2005).

In the first chapter of this thesis I found that while more UV-ornamented males sired a larger proportion of their own brood, older, less UV-ornamented males sired more extra-pair offspring. The aims of this chapter are twofold: (1) First to re-evaluate the results from **Chapter 1** which are based on data from only one breeding season. Given that selection pressures often vary among years we test for the generality of the patterns reported in **Chapter 1** by analysing data from two more breeding seasons. (2) Second, I experimentally test the relationship between ornament expression and within- and extra-pair paternity, by manipulating male crown coloration within the natural range of variation (see **Box 3**).

Based on a large sample collected over three breeding seasons I could confirm that older, less UV-ornamented males sire more offspring in total. This pattern was mainly due to the higher success at siring extra-pair paternity of less UV-ornamented adult males, since there was no correlation between crown colour and juvenile extra-pair success (**Fig. 5a**). Conversely, within-pair success was not significantly correlated with male colour when the three years of data were analysed together, since the pattern of more UV-ornamented males being less cuckolded was not consistent and only significant in 2001 (**Fig. 5b**).

Why are older, less UV-ornamented males more successful? There are at least two possible ways in which these males may benefit from being less ornamented: (1) that females prefer to copulate with less UV ornamented extra-pair males or (2) that being less UV enables males to intrude into nearby territories without eliciting aggression when seeking extra-pair copulations.

Direct female preference for less ornamented males has been reported for house finches (*Carpodacus mexicanus*) and house sparrows (*Passer domesticus*) (Griffith et al. 1999; Badyaev and Hill 2002). In these cases female preference is due to the fact that less ornamented males invest more in offspring provisioning and are therefore better fathers. This is not the case here since female blue tits do not obtain material benefits other than sperm from extra-pair males (Kempenaers et al. 1997). Independent evidence that females may consider older, less UV males more attractive comes from female sex-allocation strategies since females paired to such males overproduce sons (see **Chapter 5** for rationale). However, it is unclear then why, if considered more attractive, females are not also more faithful to these males.

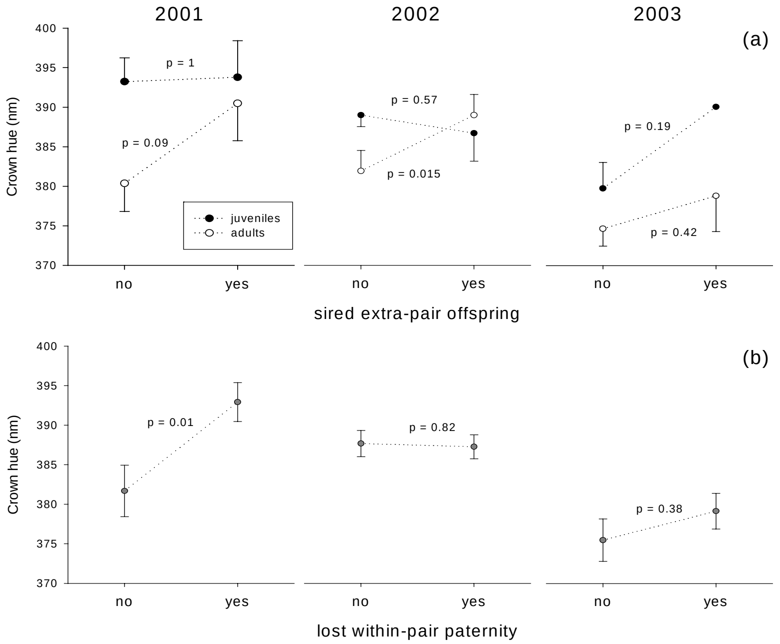


Fig. 5. Differences in crown hue between blue tit males that (a) did and did not sire extra-pair offspring (shown separately for juveniles and adults) and (b) did and did not loose within-pair paternity in each of the three study years. P-values are based on Mann-Whitney U-tests.

Alternatively, less UV-ornamented males, when roaming for extra-pair copulations, may avoid or minimize aggressive interactions with territory owners by mimicking juveniles. Juveniles are less UV-ornamented in this blue tit population (**Chapter 2**), and as in many other passerines (Griffith et al. 2002) less successful at siring extra-pair paternity (Kempenaers et al. 1997; **Chapter 1** and **4**). Hence, juveniles pose little danger of cuckoldry to territorial males and may be tolerated (Muehter et al. 1997; Greene et al. 2000). Accordingly, territorial males respond aggressively towards taxidermic mounts simulating an intruder, but aggressiveness is considerably reduced if mounts are manipulated to reduce crown UV reflectance (Alonso-Alvarez et al. 2004).

Finally, a third alternative to explain why less UV-ornamented are more successful is that crown colour is not causally related to extra-pair success, but that this pattern is caused by an unknown correlated variable. If this were the case, manipulating male crown coloration should have no effect on extra-pair success. However, if there is a causal relationship between colour and extra-pair success, older males with experimentally reduced UV-ornamentation should sire more extra-pair offspring. These possibilities were tested in an experiment, where I manipulated male crown coloration within the natural range of variation during the nest-building period before egg-laying (**see Box 3**). Males were allocated to two possible treatments: UV(+) were crown UV-reflectance was increased and UV(-) were it was decreased (**Box 3**).

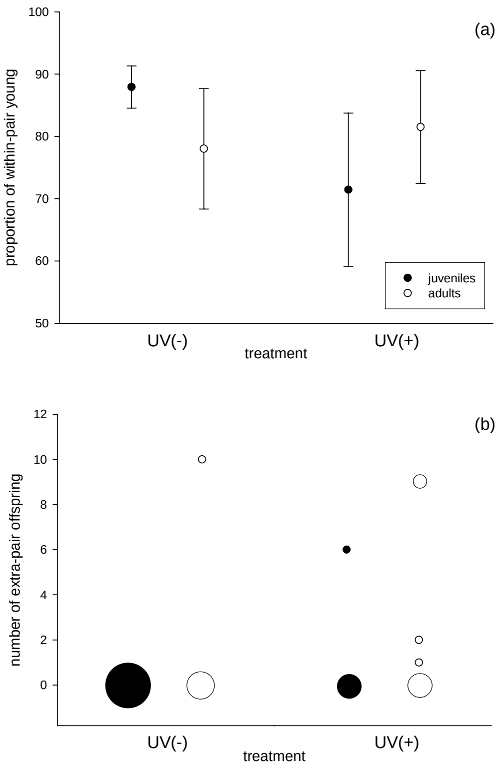


Fig. 6. Effects of plumage colour treatment (UV(+) or UV(-)) on (a) within-pair paternity (proportion of young sired) and (b) extra-pair success (number of extra-pair young sired). Shown are means and standard errors in (a). In (b) increasing dot size represents 2, 6, 7, 9 and 13 data points, respectively.

Manipulating male crown colour had no effect on within-pair success since both treatment groups were cuckolded to the same extent (**Fig. 6a**). Colour manipulation, however, affected extra-pair success and UV(+) males were more likely to sire extra-pair offspring than UV(-) males, irrespective of male age (**Fig. 6b**). This effect was exactly the opposite to that predicted from the observation that less UV-ornamented have higher extra-pair success.

How should we interpret these experimental results? If we trust the experiment our conclusion should be that, against all previous knowledge, females prefer more UV-ornamented males as extra-pair mates. While this remains a possibility, data from three breeding seasons indicate then that, *despite* a putative female preference, less UV-ornamented males still sired more extra-pair offspring. Hence, if it exists, a female preference for UV-ornamented extra-pair mates is unlikely to contribute much to selection for male ornamentation. Alternatively, we may consider the above result spurious and conclude that there is no causal relationship between male crown colour and extra-pair success. While this could be the case since the effect on UV(+) extra-pair success is not very strong, it was however stronger than the 'established' effect of age on extra-pair success. Finally another possibility is that the experiment did not manipulate male ornamentation properly, and hence the results are an artefact of disrupting normal male and/or female behaviours (see Concluding remarks for further discussion of this point).

Rather surprisingly, this is only the fourth study that attempted to confirm a correlation between male ornamentation and paternity success using experiments. Previous studies involved the barn swallow (*Hirundo rustica*), where tail length was manipulated (Smith et al. 1991; Saino et al. 1997) and bluethroats (*Luscinia svecica*) where throat patch UV reflectance was reduced with sunblock (Johnsen et al. 1998). Interestingly, while the results from Saino et al. (1997) corroborated previous correlational studies (male barn swallows with elongated tails sired both more within- and extra-pair offspring), neither the results from Smith et al. (1991) nor from Johnsen et al. (1998) fit the expected patterns (Møller 1988; Johnsen et al. 2001). In the latter two cases, the experiments were assumed to be flawed since male ornaments were manipulated beyond the natural range of variation. Difficulties in designing experimental procedures that realistically manipulate male ornaments are probably the main cause for the scarcity of experimental studies in this field, and to date experimental evidence linking male colours to extra-pair paternity is still wanting.

Chapter 5. Male attractiveness and female sex allocation: are old and ugly males sexy?

Sex allocation theory based on mate attractiveness predicts that females should bias the brood sex ratio towards male offspring if paired to a sexually attractive male, given that sons would benefit more than daughters by inheriting its father’s attractiveness (Trivers and Willard 1973; West and Sheldon 2002). Empirical evidence consistent with this theory is accumulating (West and Sheldon 2002; Ellegren et al. 1996; Polo et al. 2004; Pike and Petrie 2005) and indeed the blue tit constitutes one of the most cited examples of adaptive female sex allocation in birds (Sheldon et al. 1999). Data from a Swedish blue tit population showed that females bias brood sex ratios towards sons if paired to highly UV-ornamented males (Sheldon et al. 1999; Griffith et al. 2003), and these males show higher overwinter survival in this population and are thus assumed to be of high phenotypic quality. Here, based on the assumption that female blue tits are able to bias brood sex ratios according to male attractiveness, I try to gain insight into which males are considered attractive in the Viennese study population by analysing three years of correlational data between male ornamentation and brood sex ratio, and by a colour manipulation experiment as described above (**Box 3**).

As we saw in **Chapter 1** and **4**, in this study populations older, less UV-ornamented males are more successful at siring extra-pair offspring, an advantage that translates into higher overall seasonal fertilisation success. If crown colour is heritable in the blue tit (Johnsen et al. 2003), sons of these less UV-ornamented males may inherit, their father's success at obtaining extra-pair offspring, provided that they reach adulthood (≥2 years). Hence, if females recognize older, less UV-ornamented males as successful, they should bias brood sex ratios towards sons, at least when paired to adult males.

During the three years of this study I found a consistent trend for females to bias brood sex ratio towards males when paired to less UV-ornamented (higher hue and lower UV-chroma) adult males, while this relationship was reversed for females paired to juvenile males (Fig. 7). If female sex allocation is based on male UV-ornamentation this means that in the Viennese study population male attractiveness, as judged by its colour, reverses with age (Grahn and Schantz 1994). While the negative relationship between adult male colour and sex ratio may reflect their higher seasonal fertilisation success and therefore their higher quality, why females choose to reverse this pattern when paired to juveniles is not as intuitive. One possibility is that more ornamented juvenile males are better at obtaining and defending a territory (Siefferman and Hill 2005), while at least in some years more ornamented males are less cuckolded (Chapter 1).

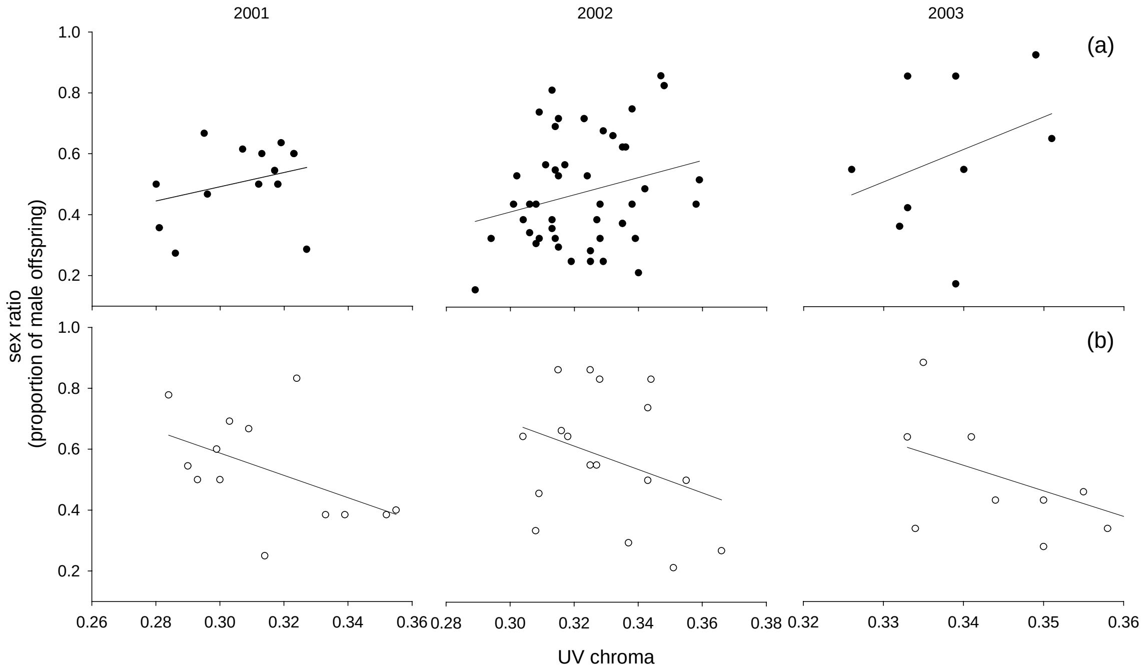


Fig. 7. Correlations between brood sex ratio (proportion of male offspring) and crown UV chroma in juvenile (a) and adult (b) blue tit males for each of the three study years. Least squares trend lines are given for visual aid.

The correlational pattern however, was not confirmed by the experimental manipulation of crown colour in adult and juvenile males. I expected to find a significant treatment*age interaction, with juveniles having a more male biased brood sex ratio than adults in the UV(+) treatment, and the opposite in the UV(-) treatment, However, I found no differences in sex ratios between groups or age classes. The treatment was not without effect however, but this effect depended on male colour before manipulation. Among males allocated to the UV(-) treatment the proportion of male offspring in the brood was negatively related to male colour prior to manipulation, while there was no such pattern among UV(+) males (**Fig. 8a**). Hence, females paired to UV(-) males which were highly UV-ornamented before treatment produced less male offspring.

This pattern strongly resembles the result from a similar experiment performed by Sheldon et al. (1999)(**Fig. 8b**), where male crown UV-ornamentation was either strongly reduced by applying sunblock (see **Box 3**) or relatively unchanged in control males. Sheldon's et al. (1999) interpretation of this rather paradoxical result was that females produced fewer sons the larger the decline in male colour due to the treatment. While such a mechanism could also explain the results presented in this thesis, it is important to remember that, despite similar experimental results, correlational patterns are not consistent between these two populations. In the Swedish population, were Sheldon and co-workers (1999) carried out their studies, females usually bias brood sex ratios towards sons when paired to highly UV-ornamented males, irrespective of their age (Griffith et al. 2003), quite unlike the reversal with male age that occurs in the Viennese population. Hence, similar responses to UV-reduction in both studies may simply reflect a common negative reaction of females towards unnaturally looking males.

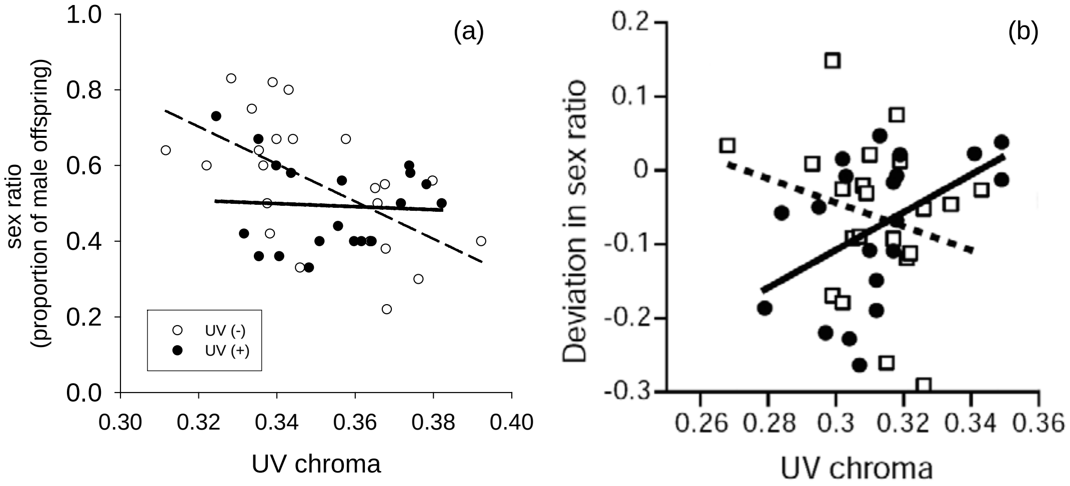


Fig. 8. Correlations between male pre-manipulation UV chroma and brood sex ratio (proportion of male offspring) for (a) UV(+) and UV(-) treatment groups in this thesis and (b) for UV-reduced and control males in Sheldon's et al. (1999) experiment. Closed symbols and solid line correspond to UV(+) or control males respectively, while open symbols and dashed line belong to UV(-) or UV-reduced males. Note that sex ratio in Sheldon et al. (1999) is given as deviation from 50:50.

Chapter 6. Female crown colour does not reflect female quality

The expression in females of ornaments which are usually more developed in males has been traditionally interpreted as a correlated response to sexual selection acting on males (Lande 1980). Recently however, several studies have revealed unexpected links between female ornamentation, female quality and male mate choice which suggest that these ornaments may play a similar role in females as in males (reviewed in Amundsen 2000), and therefore could be under direct sexual selection.

Since more UV-ornamented males in this study population do not seem to have an advantage by being less cuckolded or siring extra-pair offspring (**Chapters 1** and **4**), an alternative would be that they produce better offspring by mating with a high quality mate (Darwin 1871; Kirkpatrick et al. 1990). Assortative mating would then follow if more UV-ornamented females are of higher phenotypic quality and preferred by more UV-ornamented males. Females in the blue tit, although less ornamented than males (Andersson et al. 1998; Hunt et al. 1998), show similar levels of variability and age-related changes in crown coloration (**Fig. 4**, **Chapter 2**). Moreover, since males show a preference for UV-reflectant females in captivity (Hunt et al. 1999), and blue tits have been shown to pair assortatively by crown colour in the wild (Andersson et al. 1998) the possibility exists that female colour reflects female quality and that this trait may be used by males during mate choice. Here, using correlational data from the three study years, I assess whether female colour reflects female quality as measured by its reproductive output (clutch size, breeding success, investment in brood provisioning) and whether blue tits pair assortatively by crown coloration in this population.

Overall there was little evidence that female colour signalled female quality. Females with higher chroma and UV chroma tended to be in better condition at the end of the breeding season, but this did not translate in higher reproductive output of more ornamented females. More chromatic females started breeding earlier in one out of three study years but did not lay larger clutches or fledge more young. On the contrary less UV-ornamented females (higher hue, lower UV-chroma) tended to fledge more offspring. Moreover, female colour did not correlate with her investment in brood rearing, as measured by the food delivered to the nestlings. This suggests that males would gain little by pairing with more ornamented females. Accordingly, in the three study years there was no evidence that blue tits mated assortatively by crown colour. I conclude that female colour is most likely not an indicator of phenotypic quality under sexual selection in this blue tit population.

Concluding remarks and prospects for future work

The aim of this thesis was to test whether more UV-ornamented male blue tits would be favoured by sexual selection acting through extra-pair mating success. Now, having weighted the facts, there seems to be very little evidence in favour of this hypothesis. On the contrary, since less UV-ornamented (adult) males are so successful at siring offspring the main question for future work becomes: what do more UV-ornamented males gain in this population?

Some of the alternatives were examined and deemed unlikely: more UV-ornamented males do not live longer (**Chapter 2**), nor obtain mates of higher quality (**Chapter 6**). One possibility, however, remained unadressed, and that is that male crown ornamentation acts as a signal of fighting ability or status enabling males to obtain and defend territories. Little is known about which male (or female) traits determine the outcome of agonistic interactions or territorial disputes in blue tits. More importantly we have little information on the characteristics of the males that tried to settle and failed, since these are not present in the breeding population and thus remain unstudied. If being less UV-ornamented considerably lowers the chances of obtaining a territory, this would arguably exert a strong selection pressure for ornament elaboration, far beyond the subtle effects of extra-pair paternity. Future work should therefore address the function of crown ornaments in male-male contests. Promising avenues may be staged contests among captive blue tits where ornament expression and resources can be controlled by the experimenter. These methods have been used with success in other species (Senar and Camerino 1998; Pryke et al. 2002) but have not been applied to the blue tit so far.

The other point raised in this thesis that needs further study are the mechanisms through which less UV-ornamented males achieve their higher extra-pair success. We would assume that males that are highly successful at siring extra-pair offspring are those that are preferred by females, since extra-pair copulations are believed to be mainly female driven and under female control (Westneat and Stewart 2003). I hypothesized in **Chapter 4** that successful extra-pair males may be mimicking juveniles to sneak extra-pair copulations avoiding aggression from territorial males. Hence, instead of female choice, I invoke male-male interactions as the mechanism behind the success of less UV-ornamented adult males. Little is known about which traits or behaviours enable extra-pair males to overcome mate guarding by pair males (Westneat and Stewart 2003). However, it seems conceivable that extra-pair males could benefit by deceiving pair males about their true status when seeking extra-pair copulations and thus avoid escalated fights in case of being discovered. Males that mimic females (instead of juveniles) to steal copulations from other males have been described in various taxa (Oliveira et al. 2001; Neff et al. 2003; Hanlon et al. 2005) including birds (Lanctot et al. 1998). Nonetheless, at this stage this idea is purely speculative and detailed behavioural observations together with knowledge of how male colour influences the outcome of male-male contests (see above), are needed to test this possibility.

Moreover, choice tests in captivity (Andersson and Amundsen 1997; Hunt et al. 1999) are necessary to gain insight what kind of males females prefer. If females show a preference for more UV-ornamented males as the experiment in **Chapter 4** and other data suggest (Andersson et al. 1998; Griffith et al. 2003; Limbourg et al. 2004; Johnsen et al. in press) this could imply that female choice for extra-pair or social mates is constrained by male-male interactions (Andersson et al. 2002; Candolin 2004). These tests could also help to understand whether indeed females prefer adult males over juveniles. Higher success at siring extra-pair offspring has been traditionally considered evidence of female preference for adult males (Griffith et al. 2002). However, adult males are often cuckolded as much as juveniles (**Chapter 1**, **4**). This lack of consistency between within- and extra-pair success suggest that female choice alone cannot explain why adult males sire more extra-pair offspring.

Finally, to manipulate crown colour in a realistic way seems a difficult task in the blue tit or other birds with this type of structural colour (see **Box 3**). The effects of the experiment on both paternity and sex ratios did not fit the correlational data. This could be taken as evidence for the lack of a direct relationship between male colour and success or attractiveness. However, the experimental manipulation had significant effects on paternity or sex ratios, but these effects were unexpected, complex and differed whether we considered paternity success or sex allocation. The latter could be expected however, since paternity success depends on both male and female behaviour while sex allocation is solely under female control. Interestingly, in both instances, the experimental results parallel those of similar studies in bluethroats and blue tits.

Experimental reduction of throat UV-reflectance in the bluethroat (Johnsen et al. 1998) led to reduced success at siring extra-pair offspring compared to control birds. This was then taken as evidence that female choice for extra-pair mates favoured males with more UV-ornamented throat patches. However, correlational studies carried out afterwards found no evidence that more UV-ornamented males sire more extra-pair offspring in the same population (Johnsen et al. 2001), a similar incongruence between experiment and correlation as described in **Chapter 4**. As detailed in **Chapter 5**, experimental manipulation of male crown colour led to similarly complex patterns of female sex allocation in two different blue tit populations (**Fig. 8**), even though correlational patterns were strikingly different between them (Griffith et al. 2003). These results suggest that females, males, or both, react to manipulations of male coloration but that these reactions do not necessarily reflect how birds would behave normally. Why is this so? One possibility is that manipulations look unnatural. This is more likely to be the case when using UV-blocking chemicals as when using marker pens. However, whatever the method employed both treatments cause sudden changes in male colour. These changes are very different to the relatively gradual changes over the year (see **Chapter 3**). Additionally, manipulating the colour of only one colour patch may cause a mismatch between otherwise correlated traits. In the blue tit the expression of structural colours is correlated between colour patches (crown, wing coverts and tail) within the same bird (Sheldon et al. 1999; Delhey et al. unpubl.data). Mismatches of different phenotypic traits are not necessarily restricted to morphological traits, and decoupling appearance and behaviour may cause manipulated birds to behave in ways at odds with their new phenotypes (Rohwer and Rohwer 1978). Finally, these colour manipulations fade with time (see Chapter 5 and Johnsen et al. 1998; Limbourg et al. 2004), again unlike in normal birds.

These problems limit the use of these methods to manipulate the phenotype of free living birds. Some of them can be addressed, for instance multiple colour traits can be manipulated simultaneously, while detailed behavioural observations may provide insight to behavioural changes or constraints. The “odd-looking birds effect” could be tested by manipulating male coloration in an obviously unnatural way (for example by painting male blue tit crown red or white). This would provide a yardstick against which to compare the results from more “natural” manipulations. Remarkably some studies have carried out this type of manipulations for other purposes and found no effect on female preference (see for example Rohwer and Røskaft 1989; Qvärnstrom et al. 2004). Fading of these treatments with time restricts their use to short term manipulations of male ornaments in the wild or in captivity (Siitari et al. 2002; Ballentine and Hill 2003; Pryke and Andersson 2003; Johnsen et al. in press). The developing of new and improved manipulation methods that could overcome some of the shortcomings pointed out above will allow us to gain better insight in the function and selection benefits of colourful male plumage in this and other species.

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Chapter 1 - Paternity analysis reveals opposing selection pressures on crown coloration in the blue tit (*Parus caeruleus*)

Kaspar Delhey[1], Arild Johnsen[1], Anne Peters[1], Staffan Andersson[2] and Bart Kempenaers[1]

[1] Max Planck Institute for Ornithology, Seewiesen.
[2] Department of Zoology, Göteborg University, Göteborg, Sweden.

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ABSTRACT

In socially monogamous species, extra-pair paternity can increase the variance in reproductive success and thereby the potential for sexual selection on male ornaments. We studied whether male secondary sexual ornaments are selected through within- and/or extra-pair reproductive success in the blue tit (*Parus caeruleus*). Male blue tits display a bright blue crown plumage, which reflects substantially in the ultraviolet (UV) and previously has been indicated to be an important sexual signal. Here we show that males with a more UV shifted crown hue were less cuckolded, which probably resulted from female preference for more ornamented mates. Conversely, however, older males and males with a less UV-shifted hue sired more extra-pair young. This probably did not reflect direct female preference, since cuckolders were not less UV ornamented than the males they cuckolded. Alternatively, a trade-off between UV ornamentation and other traits that enhance extra-pair success could explain this pattern. Our results might reflect two alternative male mating tactics, where more UV ornamented males maximize within-pair success and less UV ornamented males maximize extra-pair success. Since crown colour was selected in opposite directions by within-pair and extra-pair paternity, directional selection through extra-pair matings seemed weak, at least in this population and breeding season. Reduced intensity of sexual selection due to alternative mating tactics constitutes a potential mechanism maintaining additive genetic variance of male ornaments.

INTRODUCTION

Ornamental traits such as extravagant feathers and colourful plumage are widespread in birds. Usually, males are more ornamented than females, and this sexual dimorphism is associated with sexual selection (Darwin 1871; Andersson 1994). The potential for sexual selection to operate on a given trait is proportional to the variance in reproductive success (Arnold and Wade 1984) and extravagant ornamentation and sexual dimorphism are thus usually associated with polygynous mating systems (Darwin 1871). However, many monogamous species are sexually dichromatic despite an apparent lack of selection potential. Here, more ornamented males can increase their reproductive success by pairing with the most fecund females (Kirkpatrick et al. 1990) or through extra-pair matings (Trivers 1972). Genetic parentage analyses have shown that many socially monogamous species show a high incidence of extra-pair paternity (Petrie and Kempenaers 1998; Griffith et al. 2002), boosting the variance in male reproductive success (Møller and Ninni 1998).

Since comparative studies have found that sexual dimorphism in ornamentation correlates with the level of extra-pair paternity, it has been claimed that sexual selection through extra-pair matings is the main selective force behind the exaggeration of male ornaments in socially monogamous birds (Møller and Birkhead 1994; Owens and Hartley 1998; but also see Dunn et al. 2001). Given that male total fertilisation success can increase by siring a larger proportion of the offspring in the own nest (within-pair success) and/or by siring more extra-pair offspring (extra-pair success), both components need to be assessed to evaluate the net effect of sexual selection on male ornamentation.

We studied how the expression of male plumage ornamentation relates to fertilisation success in the blue tit (*Parus caeruleus*), a socially monogamous species in which extra-pair matings are known to increase the variance in male reproductive success (Kempenaers et al. 1992). Blue tits were traditionally considered to show only little sexual dichromatism (Svensson 1992). Recently, however, the use of spectroradiometry has shown that the crown and other plumage regions differ substantially between males and females in the ultraviolet (UV, 300-400 nm). Males have brighter and more chromatic crown plumage with reflectance peaking deeper into the UV (Andersson et al. 1998; Hunt et al. 1998). Several lines of evidence suggest that crown coloration is under directional sexual selection by female choice. In indoor mate choice experiments, females seemed to prefer males with brighter crowns (Hunt et al. 1998), and blue tits viewed through UV-blocking filters were less preferred than birds behind UV-transparent filters (Hunt et al. 1999). Free living birds mate assortatively with respect to crown coloration (Andersson et al. 1998), and females produce more male offspring when paired to males with a highly UV-chromatic crown (Sheldon et al. 1999). Since these colourful males also survived better (Sheldon et al. 1999), females may perceive males with highly UV reflectant crown plumage as high quality mates. Males with high survival prospects achieve increased seasonal reproductive success by being less cuckolded and siring more extra-pair offspring (Kempenaers et al. 1997). These results suggest that male plumage colour could play an important role in female choice of social and extra-pair mates.

We investigated, by means of spectroradiometric colour measurement and microsatellite parentage analysis, whether within-pair and/or extra-pair success select for male UV crown ornamentation. Here we show that, despite increased variance in male reproductive success and significant effects of male ornamentation on both components of male fertilisation success (within-pair and extra-pair paternity), the net directional selection on male UV crown colour is weak. Our results suggest that the effect of sexual selection on male ornamentation can be more complex than usually assumed.

METHODS

Study site and general methods

This study was carried out during March-June 2001, as part of a long-term research program on the reproductive biology of a blue tit *Parus c. caeruleus* population. The study area of about 35 ha, is located in Vienna, Austria (48° 13’ N, 16° 20’ E). It consists of a mixed deciduous woodland dominated by oak (*Quercus robur*), beech (*Fagus sylvatica*) and ash (*Fraxinus excelsior*) and contains around 220 nestboxes.

We captured blue tits in nestboxes, either in winter while they were roosting or in late spring during chick feeding. Unbanded birds were marked with a unique combination of plastic colour bands and a numbered metal ring. At capture we measured tarsus length with a caliper to the nearest 0.05 mm, and body mass with an electronic balance to the nearest 0.1 g. Chick feeding birds were sexed by presence or absence of a brood patch and aged according to Svensson (1992) as juveniles (second year birds) or adults (after second year birds). A small (5-50 µl) blood sample was taken for parentage analysis and colour measurements were performed (see below).

Basic data on reproduction were obtained by regular nestbox checks starting during courtship and nest-building (March-April) until fledging (May-June). Chicks were bled (5-25 µl) and banded 14 days after hatching. All unhatched eggs and dead chicks found in the nests were collected, and tissue samples were stored in 70% ethanol. As part of another project, ten clutches were collected after one day of incubation; eggs were opened and embryos stored in ethanol.

Colour measurements

Forty-nine males (25 juveniles and 24 adults) captured in the nestbox during chick feeding (days 8-9 after hatching), were colour measured. The reflectance of the crown was measured using a S-2000 spectroradiometer with a DH-2000-FHS Deuterium-Halogen light source (Ocean Optics, Eerbek, Netherlands). We used a bifurcated fiber optic probe on which a cylindrical plastic sheath was mounted to exclude ambient light and standardise measuring distance. The probe was held perpendicular to the feathers and measurements of five different and standardised spots (11.3 mm^2) of the crown were made. Reflectance was calculated relative to a white standard (WS-2) with the Spectrawin 4.2 software (Top Sensor Systems). Raw spectra were smoothed by a running average calculated on 10 nm intervals. We calculated colour coefficients from the smoothed spectra and averaged them for each individual. Three different coefficients (brightness, hue and chroma), describing the variation in spectral shape (Hailman 1977), were calculated for each spectrum. ‘Brightness’ (spectral intensity) was calculated as the average reflectance (R_{av}) in the 320 to 700 nm interval, which encompasses the entire visual sensitivity range of birds. ‘Hue’ (spectral location) was estimated as the wavelength of peak reflectance ($\lambda(R_{max})$), and ‘chroma’ (spectral purity) was calculated as $(R_{max} - R_{min})/R_{av}$, that is, the difference between peak and trough divided by brightness. Hue correlated with both chroma ($r = -0.43$, $p = 0.002$, $n = 49$) and brightness ($r = 0.37$, $p = 0.01$, $n = 49$), but the latter two were not correlated ($r = 0.01$, $p = 0.5$, $n = 49$).

Paternity analysis

We used eight polymorphic microsatellite markers (*Pca3*, *7*, *8* and *9* (Dawson et al. 2000), *Pocc1* and *6* (Bensch et al. 1996), *Phtr3* (Fridolfsson et al. 1997) and *PK11* (Tanner SM, Richner

H, Schuenperli D, unpublished; EMBL accession no: AF041465)) to determine the paternity of 574 offspring (nestlings and unhatched eggs) in 51 broods. We followed a standard PCR protocol using about 20 ng genomic DNA, 0.25 U of taq DNA polymerase (Promega) and 1.5 mM $MgCl_2$. The PCR profiles had the following annealing temperatures: 53˚C (*Pca8*, *Phtr3*) 55 ˚C (*Pca3*), 56˚C (*Pocc1*), 57˚C (*Pca9*, *Pocc6*) and 60˚C (*Pca7*, *PK11*). Amplified fragments were resolved on an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

The combined probability of exclusion (Jamieson 1994) for the marker set was >0.999. Paternity was excluded if two or more loci showed mismatches between putative fathers and offspring. In 485 cases, there was no (n = 475) or one mismatch (n = 10) with the social father. For these offspring, the average probability of false inclusion (Jeffreys et al. 1992) was $9.4 \times 10^{-5} \pm 3.5 \times 10^{-4}$ s.d. (range 1.9×10^{-9} - 4.8×10^{-3}). We therefore conclude that they were sired by the social male and that the single mismatches were due to mutations. The remaining 89 offspring showed two or more mismatches with the putative father and were thus sired by extra-pair males. For 66 of the extra-pair offspring, an alternative male matched the paternal genotype completely. One offspring showed a single mismatch with a putative extra-pair sire and since the same male was the unequivocal sire of another offspring in the same nest, we assigned both offspring to this male. The average probability of false inclusion was $8.2 \times 10^{-5} \pm 3.8 \times 10^{-4}$ s.d. (range 1.6×10^{-9} – 3.1×10^{-3}) for the 67 assigned extra-pair offspring.

Statistical analysis

Male total fertilisation success can increase by avoiding cuckoldry in the own nest (within-pair success) and/or by cuckolding other males (extra-pair success). Response variables to estimate within-pair success were: whether a male was cuckolded or not, the proportion of sired offspring in its nest and the number of cuckolding males siring young in its nest. The corresponding response variables for extra-pair success were: whether a male sired extra-pair young or not, the number of extra-pair young it sired and the number of males it cuckolded. Initially, univariate tests were performed to assess the relationship between male colour variables and within- and extra-pair paternity success. Parametric tests were used if the response variable was normally distibuted, otherwise the non-parametric equivalent was utilized. All tests were two-tailed. In addition to simple correlations, we constructed multiple regression models to investigate the effect of colour variation on male mating success. Since male age and morphometrics can affect paternity (Kempenaers et al. 1992; Kempenaers et al. 1997), and given that crown colour also differs between adults and juveniles (Andersson et al. 1998; own unpubl. data), we included these variables together with the three colour variables in Generalized Linear Models. The full models were reduced by sequentially excluding the variables that did not explain a significant part of the deviance. Variables with $p < 0.1$ were included in the final model. All excluded variables were included one-by-one in the final model to confirm that they did not explain a significant part of the variation. Reported statistics and probabilities correspond to the variables when included in the final model. For more details on individual models see Table 3. All analyses were performed with SPSS 11.0 and Genstat™ 6.0.

RESULTS

Patterns of within-pair and extra-pair paternity

Thirty-three of 51 nests (65 %) contained at least one extra-pair young. The number of extra-pair young in a nest varied from 0 to 8 (0 to 73 % of the clutch), and extra-pair offspring accounted for 15% of all typed eggs and young (n = 574). In most cases (24 out of 33) only one male sired all extra-pair offspring, while in 7 nests two extra-pair fathers were present, and in two cases three males cuckolded the same male. For 67 of the 89 extra-pair offspring (75 %) we could assign the father. All except two extra-pair males were birds found breeding in 2001; two males that were caught during winter and probably bred at the boundary of the study area also sired extra-pair offspring. Within-pair paternity and extra-pair paternity success were independent. Males that lost paternity in their own nest were neither more nor less likely to sire extra-pair young in other nests ($\chi^2_1 = 0.69$, $p = 0.41$, $n = 47$), and the proportion young sired in the nest was not correlated with the number of extra-pair young sired by a given male (Spearman rank correlation, $r_s = -0.08$, $p = 0.57$, $n = 47$). The opportunity for sexual selection (I_s = variance in male reproductive success divided by the square of mean success; (Arnold and Wade 1984)) was 3.5 times higher for male actual fertilisation success ($I_s = 0.123$) than expected for male apparent success ($I_s = 0.035$). Extra-pair success was assessed for all 49 colour measured males, while nest predation before blood sampling reduced our sample for within-pair and total success to 47 males.

Male coloration and within-pair success

Males that were cuckolded had a more long-wave shifted hue (Table 1) than those that achieved full paternity, but they did not differ in the other two colour variables. Similarly, males with a more long-wave hue sired a lower proportion of eggs in their own nest and were cuckolded by more males (Table 2, Fig. 1a). The number of cuckolding males and the proportion of lost paternity were highly positively correlated (Spearman rank correlation, $r_s = 0.88$, $p < 0.001$, $n = 47$), even when only nests with extra-pair young were considered (Spearman rank correlation, $r_s = 0.54$, $p = 0.002$, $n = 31$).

Multiple logistic regression analysis, including the three colour variables, male age and morphometrics, confirm crown hue as the only significant predictor of the likelihood of losing paternity (Table 3). Thus, males with long-wave shifted hue were more likely to be cuckolded. Accordingly, these males had a higher proportion of extra-pair young in their nests, but this effect was weaker ($p = 0.055$, Table 3).

Male coloration and extra-pair success

Males that gained extra-pair paternity did not differ significantly in coloration from males that did not sire extra-pair offspring (Table 1). Additionally, in the univariate analyses no colour variable correlated with the number of extra-pair young sired by a given male, nor with the number of nests a male cuckolded (Table 2). Multiple regression models indicate that male age was the main determinant of extra-pair success (Table 3). However, when age effects were accounted for, crown hue also predicted a significant part of the variation in extra-pair paternity (Table 3, Fig. 1b); males with less UV-shifted crown hue were more likely to gain extra-pair paternity ($p = 0.087$) and sired significantly more extra-pair young ($p = 0.004$). Although this last effect seemed more pronounced in adults (Fig. 1b), the interaction term was not significant (age*hue, $\chi^2_1 = 0.18$, $p = 0.67$). In conclusion, older males and males with a less UV-shifted crown hue sired more extra-pair young.

Table 1. Comparison of crown colour for a) males that did and did not lose within- pair paternity in their nests (n = 47), and b) males that did and did not gain extra-pair paternity (n = 49). Shown are means ± standard errors.

	(a) lost within-pair paternity				(b) gained extra-pair paternity			
	No (n=16)	Yes (n=31)	t	p	No (n=32)	Yes (n=17)	t	p
Hue	381.2 ± 3.4	393.1 ± 2.6	-2.70	0.01	387.8 ± 2.6	392.1 ± 3.6	-0.94	0.35
Brightness	0.23 ± 0.01	0.25 ± 0.01	-1.19	0.24	0.24 ± 0.01	0.26 ± 0.01	-1.18	0.24
Chroma	1.02 ± 0.03	0.99 ± 0.02	0.88	0.38	0.99 ± 0.02	1.01 ± 0.04	-0.34	0.73

Table 2. Correlations between crown colour and male within-pair, extra-pair and total fertilisation success. Shown are Pearson (r) or Spearman rank (r_s) correlation coefficients.

	Within-pair success (n = 47)		Extra-pair success (n = 49)		Total success (n = 47)
	% sired young	No. cuckolders	No. extra-pair young	No. cuckolded males	No. of sired offspring
Hue	r_s=-0.33, p=0.023	r_s=0.40, p=0.006	r_s=0.18, p=0.20	r_s=0.14, p=0.32	r=0.18, p=0.22
Brightness	r_s=-0.15, p=0.33	r_s=0.20, p=0.18	r_s=0.19, p=0.19	r_s=0.20, p=0.16	r=0.09, p=0.53
Chroma	r_s=0.12, p=0.42	r_s=-0.20, p=0.17	r_s=0.01, p=0.92	r_s=-0.02, p=0.91	r=0.07, p=0.65

Table 3. Generalized linear models (GLM) for male within-pair, extra-pair and total fertilisation success. Final models after sequentially dropping non-significant variables are depicted in bold. Statistics and probabilities of excluded variables are based on one-by-one inclusion in the final models.

	Within-pair paternity success				Extra-pair paternity success				Total fertilisation success[d]	
	Likelihood of losing within-pair paternity[a]		Proportion of sired young[b]		Likelihood of gaining extra-pair paternity[a]		Number of extra-pair young[c]			
	χ^2	p	χ^2	p	χ^2	p	χ^2	p	t	p
Age	0.00	0.947	0.72	0.400	**6.97**	**0.008**	**21.07**	**<0.001**	**2.92**	**0.005**
Hue	**6.81**	**0.009**	**3.88**	**0.055**	**2.93**	**0.087**	**9.11**	**0.004**	**2.01**	**0.05**
Brightness	0.13	0.721	0.32	0.576	0.01	0.939	0.19	0.665	-0.78	0.438
Chroma	0.05	0.828	0.52	0.474	0.89	0.345	0.94	0.338	1.16	0.252
Tarsus length	0.04	0.836	0.65	0.425	0.86	0.352	0.05	0.830	-0.46	0.643
Body mass	0.02	0.900	0.07	0.786	0.00	0.965	0.27	0.604	-0.88	0.384
Final Model	**6.81**	**0.009**	**3.88**	**0.055**	**3.96**	**0.019**	**12.88**	**<0.001**	**5.15**[e]	**0.01**
d.f.	**1**		**1**		**2**		**2**		**2**	

[a] Binary logistic regression; [b] GLM with binomial error distribution and logit link, binomial denominator = no. of typed offspring; [c] GLM with Poisson error disitribution and logarithm link; [d] GLM with normal error distribution and identity link. [e] F statistic.

Pairwise comparisons between cuckolder and cuckolded males

Neither colour, nor morphometric variables differed between cuckolded and cuckolder (paired t-test, all p > 0.14, n = 26). However, cuckolders were usually older than the males they cuckolded (Wilcoxon signed rank test, Z = -2.50, p = 0.012, n = 26). In four cases, males reciprocally exchanged paternity; excluding these cases did not change the conclusions (colour and morphometrics: paired t-test, all p > 0.10; age: Wilcoxon signed rank test, Z = -2.88, p = 0.004, n = 18).

Total fertilisation success and male coloration

The total number of eggs a male sired did not correlate with crown colour (Table 2). In the multiple regression including the three colour variables, morphometrics and age (Table 3), age was the main predictor of total success, while there was a trend for males with more long-wave shifted hue to sire more offspring. Thus, older males, and to a lesser extent males with less UV-shifted crown hue, achieved greater seasonal fertilisation success.

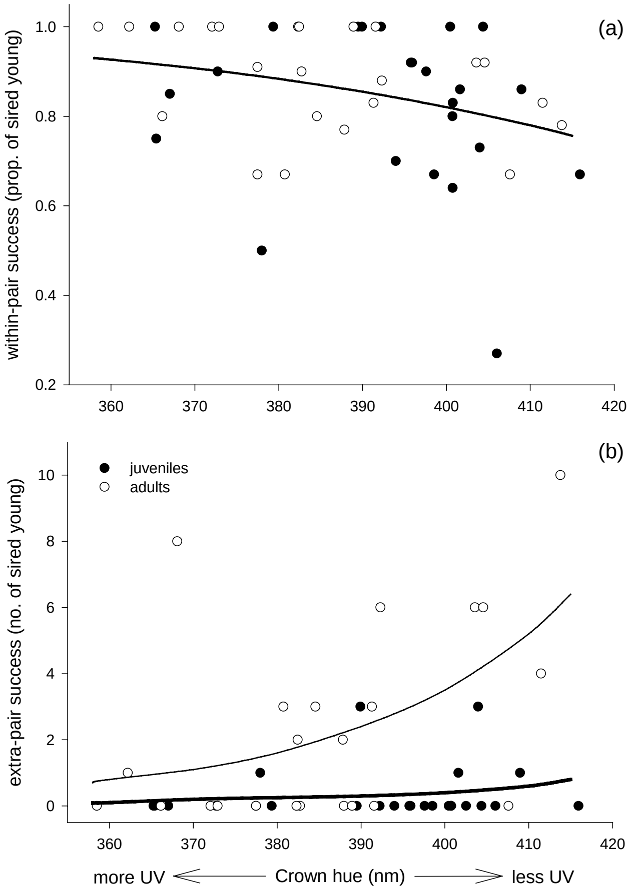

Figure 1. Male crown hue correlates with (a) within-pair and (b) extra-pair paternity. Filled circles represent juvenile and open circles adult males. Trend lines are as predicted by Generalized Linear Models in Table 3. In (b) the bold line corresponds to juvenile and the thin line to adult males

DISCUSSION

Crown colour of male blue tits correlated with both components of male seasonal reproductive success: within- and extra-pair paternity. Of the three variables we used to describe crown colour, only hue was related to fertilisation success. Hue, or spectral location, describes the spectral position of peak reflectance (Andersson et al. 1998). An increasingly UV-shifted reflectance peak is a likely form of exaggeration of an ultraviolet colour signal (Andersson 1999; Keyser and Hill 1999; Keyser and Hill 2000). Hence, we assume that males with a more UV-shifted crown plumage (hereafter ’more UV’ males) display a more extreme ornament than males with a less UV-shifted hue (hereafter ’less UV’ males).

Previous studies highlighted the importance of brightness (Hunt et al. 1998) and chroma (Sheldon et al. 1999) as sexually selected signals in blue tits. We found these two variables to be uncorrelated with fertilisation success. However, since hue correlates with brightness as well as chroma, it is apparent that the colour variables are not independent. This is also evident in the study by Sheldon et al. (1999) where both chroma and hue correlated with the proportion of male offspring in the brood, although only chroma predicted overwinter survival. Interestingly, in blue tit nestlings the chroma of the UV/blue tail feathers is strongly condition-dependent, while hue, although also influenced by condition, seems to be mainly genetically determined (Johnsen et al. 2003), suggesting that these two components could be signalling different aspects of quality.

’More UV’ males achieved higher within-pair fertilisation success independent of age: they were less likely to be cuckolded, sired a larger proportion of their broods (Fig. 1a), and lost paternity to fewer extra-pair males. More elaborate ornaments are usually produced by higher quality males, and such males should therefore be preferred by females (Andersson 1994). Accordingly, higher within-pair fertilisation success of more ornamented males has been shown in many bird species (for a recent review see Griffith et al. 2002). In the blue tit, crown UV reflectance has been related to survival (Sheldon et al. 1999), and males with higher survival suffered less from cuckoldry (Kempenaers et al. 1997). Thus, female blue tits can use male crown coloration to assess the quality of their mate, and our observation that females were more faithful to males with more UV-shifted crown reflectance supports this idea. Alternatively, ‘more UV’ males might be better at protecting paternity or could have ejaculates that better outcompete the sperm of extra-pair males, but our data do not support this. Although the association between paternity assurance behaviours (like mate guarding) and coloration in the blue tit is unknown, mate guarding seems to be ineffective at preventing cuckoldry in this species (Kempenaers and Dhondt 1995). Likewise if colourful males have more competitive sperm we would expect them to sire more extra-pair young as well, which was not the case (see below).

Extra-pair success was mainly determined by male age (Fig. 1b): older (adult) males were more likely to gain extra-pair paternity and sired a greater number of extra-pair offspring. Since most (72 %) of the identified extra-pair fathers were adult birds, cuckolders were usually older than the males they cuckolded. Higher extra-pair fertilisation success of older males has been observed before in blue tits (Kempenaers et al. 1997), and in several other bird species (Griffith et al. 2002). This might be due to older males being more experienced and skilled in sexual display or in the pursuit of extra-pair copulations (Johnsen et al. 2001), but it is usually considered a result of direct female preference for older males because of their proven survival ability (Brooks and Kemp 2001). Females can use the more developed ornaments of older males to assess their age. Therefore, since adult blue tits are on average more colourful than juveniles (Örnborg 2002; our unpublished data), we might expect that UV ornamentation is one cue by which females assess male age. However, this is not the case: the adults that were most successful at siring extra-pair offspring were those that most strongly resembled juveniles (Fig. 1b).

The (unexpectedly) higher extra-pair success of less UV ornamented males (Fig. 1b) is difficult to explain but very interesting. Several studies have failed to find a relationship between secondary sexual traits and extra-pair paternity (Hill et al. 1994; Cordero et al. 1999; Johnsen et al. 2001), but to our knowledge this is the first evidence of a negative correlation between ornament expression and extra-pair success to date. This is, however, unlikely to be due to active female choice for 'less UV' males as extra-pair mates. If females would indeed seek less ornamented males as extra-pair partners, we would expect cuckolders to be less UV than the males they cuckold, which was not the case.

A negative correlation between male UV ornamentation and extra-pair success can arise if crown coloration correlates negatively with other male characteristics that increase their extra-pair success. Here, we showed that less ornamented birds are at higher risk of being cuckolded, and since mate guarding appears ineffective in blue tits (Kempenaers and Dhondt 1995), an alternative tactic would be to increase the effort in pursuing extra-pair copulations. 'Less UV' males might thus devote more time and resources in intruding on other territories, or invest more in other sexual displays, such as song. Song is a sexually selected trait in the blue tit (Kempenaers et al. 1997; Poesel et al. 2001), but there are no published data on the relationship between coloration and song in this species. However, a recent comparative analysis found that elaboration of plumage ornamentation was negatively related to song complexity in cardueline finches, suggesting a trade-off between the expression of different sexual traits (Badyaev et al. 2002). That trade-offs between multiple sexual ornaments can operate intraspecifically, has recently been shown for tail length and carotenoid coloration in a widowbird (Andersson et al. 2002). In blue tits, a similar trade-off between UV ornamentation and some other trait(s) that enhance(s) male extra-pair success, may set the stage for a conditional mating strategy with two alternative tactics (Gross 1996): either investment in crown UV ornamentation and thereby reduce the likelihood of losing paternity, or investment primarily in traits or behaviours that increase extra-pair copulation success, maybe at the expense of crown coloration. Depending on their phenotypes, individual males should adopt the tactic that maximizes individual fitness, either through within- or extra-pair paternity. Detailed behavioural observations and experiments are needed to test this idea.

Irrespective of the explanation, the observation that the same male ornamental trait correlates in opposite directions with the two components of male fertilisation success has important implications. Earlier studies have found that more ornamented males achieve either higher within-pair success (Johnsen et al. 2001), or higher extra-pair success (Sundberg and Dixon 1996; Yezerinac and Weatherhead 1997; Thusius et al. 2001), or both (Saino et al. 1997; Sheldon and Ellegren 1999), but to our knowledge this study is the first indication that these two components of male fertilisation success exert selection on the same trait in opposite directions. Our results point at the critical importance of estimating both components of male fertilisation success to evaluate the effect of sexual selection through extra-pair matings on male ornaments. Conclusions on directional selection on male ornament exaggeration based on studies relying only on within-pair paternity (e.g. Møller and Ninni 1998) should thus be treated with caution.

In summary, we have shown that sexual selection, acting through within-pair and extra-pair paternity, has the potential to shape the evolution of male crown coloration in the blue tit. Since these two selective forces almost cancel each other out (Table 3), net directional selection on male UV ornamentation seems weak. However, our study did not assess potential selective forces other than fertilisation success. For example, more UV ornamented males might have an advantage in male-male competition and therefore settle on the best territories, have higher initial pairing success or pair with higher quality females producing better quality offspring, or show higher survival. Future studies should address these issues to get a more complete understanding how crown UV colour is selected in the blue tit. Meanwhile, our results suggest that the effect of sexual selection on male ornaments through extra-pair matings can be more intricate than usually assumed (Møller and Birkhead 1994;

Møller and Ninni 1998). The existence of alternative male mating tactics linked to ornament expression can reduce the intensity of directional selection on a given trait, and this could help maintain additive genetic variance of sexually selected ornaments.

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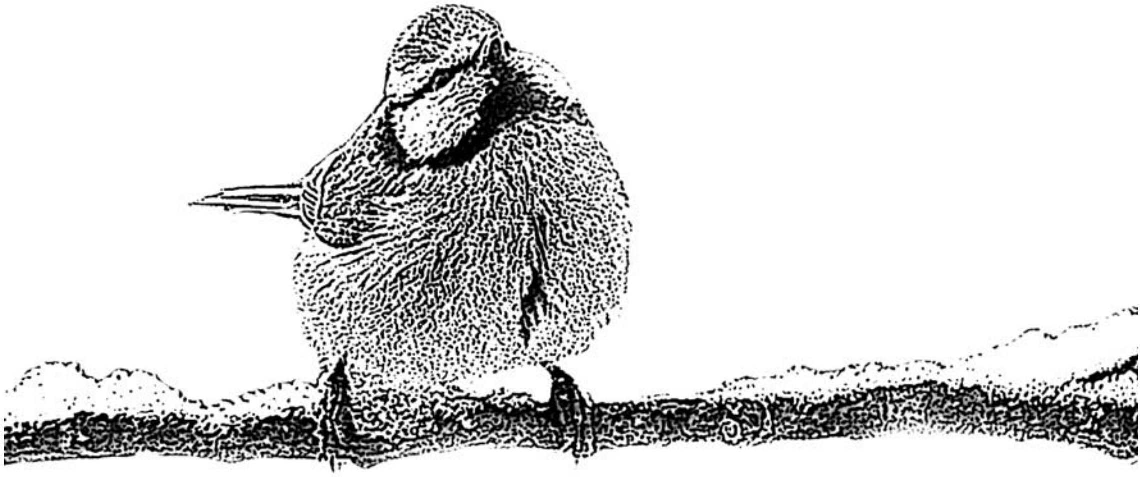

Chapter 2 - Age differences in blue tit plumage colour: within-individual changes or colour-biased survival?

Kaspar Delhey and Bart Kempenaers
Max Planck Institute for Ornithology, Seewiesen

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ABSTRACT

In many species of passerine birds yearlings display a less elaborate version of the adult secondary sexual traits, but the causes of such differences in ornamentation are not always well understood. We studied age-related changes in blue tit (*Parus caeruleus*) UV/blue structural crown coloration, a sexually selected trait. In our Austrian study population, older blue tits, irrespective of sex, displayed on average a more ultraviolet (lower hue, higher UV chroma), more chromatic and brighter crown coloration than yearlings. This age dichromatism was caused by within-individual changes in the expression of crown coloration between years since males and females became more UV, more chromatic and brighter as they aged. Colour biased survival did not contribute to the observed pattern of age dichromatism since crown coloration was largely unrelated to overwinter survival. Between-year repeatability of crown colour was significant for most colour variables but low in general, and lower for females than for males. In the blue tit, yearling males might benefit from being less ornamented by avoiding adult aggression but at the expense of sexual attractiveness. Adaptive explanations of blue tit age dichromatism should however take into account that age effects were of similar magnitude in males and females. This suggests that both male and female yearlings could benefit from being less ornamented and hence that sexual selection might be acting on both sexes simultaneously in this species.

INTRODUCTION

Older males (and sometimes females) of many species of passerine birds display more elaborate secondary sexual traits than juveniles and are often preferred as mates. The degree of age dimorphism in ornamentation shows great variability between species and according to Hill (1996) two main groups can be distinguished. In some species yearling males (first-time breeders born in the previous year) display a distinct "dull" female-like plumage, before acquiring the definitive adult plumage in a subsequent season. This particular type of age-specific variation in plumage pattern, known as "delayed plumage maturation", has attracted a great deal of attention and several hypotheses have been formulated to explain its occurrence (reviewed in Lyon and Montgomerie 1986 and Beauchamp 2003). In most species however, age-related differences in ornamentation are not categorical but more a matter of degree with yearling birds displaying a similar but subdued version of the full adult plumage. This has been termed "age-related" plumage variation, in contrast to the "age-specific" differences in species with delayed plumage maturation (Hill 1996). In contrast to most cases of delayed plumage maturation, age-related differences in the expression of secondary sexual traits are not necessarily restricted to males, although they are usually more pronounced in males than in females (Møller 1991; Hill 1996).

While age-specific differences in plumage coloration are due to a relatively fixed schedule of within-individual changes, age-related differences in the expression of secondary sexual traits could be brought about by either within-individual increases in ornamentation, by differential survival of more ornamented individuals, or by a combination of both. Indeed, often the most ornamented, presumably high quality individuals, show higher survival (see meta-analysis in Jennions et al. 2001), thus becoming over-represented in older age-classes (Forslund and Pärt 1995). On the other hand, if investment in sexual ornaments is costly, ornament expression could correlate negatively with survival (Brooks 2000; Hunt et al. 2004) and this could obscure the effect of within-individual changes (Gil et al. 2001). Hence, to assess the contribution of these two mechanisms to the observed pattern of age-related differences it is necessary to perform a longitudinal analysis on ornament expression using individuals of known age, in combination with an analysis of the relationship between ornament expression and survival.

We studied age-related variation in structural plumage coloration in male and female blue tits (*Parus caeruleus*). This species displays several conspicuous structurally coloured plumage patches which show pronounced sexual dichromatism in the near ultraviolet (UV), with males being more UV reflectant than females (Andersson et al. 1998; Hunt et al. 1998).

The appeal of this “hidden” sexual dichromatism and the large amount of knowledge about blue tit life-history have made this species an increasingly popular model for the study of sexual selection on structural colours (Sheldon et al. 1999; Delhey et al. 2003; Griffith et al. 2003; Johnsen et al. 2003; Limbourg et al. 2004). However, despite our increasing understanding of blue tit sexually selected traits, basic questions like age-related colour expression are still largely unexplored. For some populations there is limited evidence suggesting that age differences in plumage coloration exist (Andersson et al. 1998; Örnborg et al. 2002), but its causes and consequences have not been studied. Interestingly, since more UV ornamented males show increased over-winter survival in a Swedish population (Sheldon et al. 1999; Griffith et al. 2003), some of these age differences could arise through differential survival. Here, we attempt to disentangle the relative importance of differential survival and within-individual changes to explain age-dependent colour signalling in our Austrian study population. Using a large data set collected over three years, we perform both cross-sectional and longitudinal analyses of age-related colour variation and assess the relationship between coloration and survival.

METHODS

Study site and general methods

Fieldwork was carried out between March 2001 and December 2003, in a nestbox breeding, colour-banded blue tit population at Kolbeterberg, Vienna, Austria (48º 13’ N, 16º 20’ E). The study site of 35 ha is part of a large mixed deciduous woodland and contains 250 nestboxes. Blue tits were captured at the nestbox either during the night while roosting (in winter) or while feeding 8-14 day old chicks (in spring). Unbanded birds were fitted with a metal band and a unique combination of plastic colour bands. A small (5-50 μl) blood sample was drawn from the brachial vein for genetic analyses, and colour measurements were performed (see below). Birds were aged as adults or yearlings based on the colour of the wing coverts (Svensson 1992) and sexed in spring by presence/absence of the brood-patch. If birds were only captured during winter we determined their sex by molecular analysis (see Johnsen et al. 2003). Since this blue tit population has been closely monitored since 1998 (Foerster et al. 2003), most birds present from 2001 onwards had been first banded as juveniles and thus their exact age was known. Some birds (12 %) were captured for the first time as adults, and were assumed to be 2 years old then. This uncertainty did not bias the results however, since the patterns of age related colour variation remained qualitatively unchanged if these birds were excluded from the analyses (not shown). For the analyses, we classified birds as being first year (1 yr), second year (2 yr) or more than two years (>2 yr) old. Older age classes were combined to obtain an adequate sample size.

Blue tits undergo only one moult per year. The first moult, which takes place at *circa* two months of age, is limited to the head, body and a variable number of tertials, tail feathers and secondary coverts (Cramp and Perrins 1993). Thus, the plumage worn during a blue tit's first breeding season (juvenile or 1 yr birds) consists of a mixture of feathers grown in the nest (wing feathers and most tail feathers) and newly moulted feathers. After the first breeding season, blue tits undergo a complete post-breeding moult every year between late May and late August (Cramp and Perrins 1993).

Colour Measurements

Coloration of the crown was measured in spring between mid-May and early June and in early winter between late November and early December. To measure feather reflectance we used a S-2000 spectrometer and a DH-2000-FHS deuterium halogen lamp (Ocean Optics, Eerbek, The Netherlands). Spectrometer and lamp were connected through a bifurcated fiber optic probe, fitted at the end with a plastic cylinder to standardise measuring distance and shield out ambient light. The probe was held perpendicular to the surface of the feathers, and we took readings of five standardized spots (each 11.3 mm^2). Reflectance (R) was calculated relative to a WS-2 white standard using the program Spectra-Win. Raw spectra were imported into a spreadsheet program, smoothed using a running average calculated over a 10nm interval, and the following variables were computed. (1) Brightness, which constitutes an estimate of the area under the curve or total light reflected by the feathers, was calculated as the average reflectance (R_{av}), between 300 and 700 nm. (2) Hue or spectral location was determined as the wavelength of peak reflectance (λR_{max}). (3) Chroma or spectral purity was calculated as the difference between peak and trough divided by total reflectance ($(R_{max}-R_{min})/R_{300-700}$). (4) The relative amount of UV reflectance or "UV chroma" was calculated as reflectance in the UV range (300-400nm) divided by total reflectance ($(R_{300-400}/R_{300-700}) \times 100$) and expressed as a percentage (Andersson et al. 1998). We computed an estimate of measurement repeatability (Lessells and Boag 1987) using the five measurements taken per individual for a subsample of measured males. Repeatability estimates were moderate but highly significant for all colour variables (hue, $R = 0.50$, $F_{44,180} = 6.03$; UV chroma, $R = 0.64$, $F_{44,180} = 10.12$; chroma, $R = 0.56$, $F_{44,180} = 7.49$; brightness, $R = 0.40$, $F_{44,180} = 4.43$; all $p < 0.001$). These repeatability values are lower than previously reported for blue tit crown colour (see Örnborg et al. 2002). The difference is due to the fact that we measured five different spots on the crown to get an overall estimate of its colour, while Örnborg et al. (2002) repeatedly measured the same spot in the center of the crown. Hence our repeatability estimate combines variation from measurement error (estimated as 0.6 - 0.9 by Örnborg et al. 2002) and spatial variability within the crown. For a similar example focusing on repeatability of cell-mediated immune response see Granbom et al. (2005).

Data analysis

To assess whether coloration changes with age we performed both cross-sectional and longitudinal analyses. In the blue tit, crown feathers become progressively less UV reflectant as the plumage gets soiled and worn with use (Örnborg et al. 2002). Thus, age effects observed at the end of the breeding season might differ from those in freshly moulted plumage, especially if there is an age-related bias in the degree of seasonal change in colour. Therefore, we report the results of analyses using crown colour measurements taken both in early winter (late November to early December) and in late spring (May) when birds were feeding chicks. Changes in UV coloration are minimal during winter (Örnborg et al. 2002), and colour measurements during this period should thus provide an adequate estimate of colour after moult. For the cross-sectional analysis we used restricted maximum likelihood models (REML), with colour as dependent variable, age, sex, study year and the interaction between sex and age as fixed factors and individual identity as a random factor. A different model was built for each colour variable. For the longitudinal analysis we used birds that had been measured in two consecutive years during the same period (i.e. either winter or spring). The change in colour between years was analysed using repeated-measures ANOVA, with colour in year_x and colour in year_{x+1} (measured on the same individual), as within-subjects factor. A significant within-subjects factor would indicate that individual crown coloration changed between consecutive years (i.e. whether the change differed from zero). To test whether changes in colour were influenced by initial age (i.e. age in year_x), sex or season (either 2001 to 2002 or 2002 to 2003), we included the interaction between these factors and the within-subjects factor (i.e. colour change) in the model. The interaction between the within-subjects factor and age reflects differences in the way colour changes during life (e.g. colour expression could increase more between the first and second year than later on in life). Similarly, the interactions with sex or season indicate whether colour changes are different between males and females or depending on the years when the colour was measured.

The relationship between survival and coloration (measured in spring) was assessed using logistic regression with survival (yes/no) as dependent variable, and year, age, and sex as factors and colour as a covariate. The models also included the interaction terms age x colour and sex x colour. Models were simplified using a backward elimination procedure, where factors or variables were excluded in order of decreasing significance until only terms with $p < 0.05$ remained in the model. Lack of significance of excluded terms was confirmed by including them one-by-one in the final model. Survival was estimated as the probability of recapture, where individual birds were assumed to have died if they were not recorded nesting in the next breeding season (Sheldon et al. 1999; Griffith et al. 2003). The accuracy of this survival estimate would depend on the incidence of dispersal in our population and on the relationship between crown colour and dispersal. In our study population females are more likely to disperse after having established a territory than males (24% of surviving females disperse vs. only 3% of the males, $\chi^2_1 = 16.74$, $p < 0.001$, $n_{females} = 102$, $n_{males} = 88$; M. Valcû and B. Kempenaers, unpubl. data), and crown colour is unrelated to dispersal distance (for all colour variables: females, $p > 0.5$, $n = 40$; males $p > 0.2$, $n = 38$, M. Valcû, K. Delhey and B. Kempenaers, unpubl. data). Moreover if dispersal is biasing our survival estimate we would expect that females have a lower recapture probability than males which was not the case (see Results). Hence we believe that our survival estimate is reasonably accurate and, more importantly, unbiased with respect to the hypothesis being tested (i.e. correlation between crown colour and survival).

RESULTS

Cross-sectional analysis

After controlling for sex and year, age had a significant effect on all four crown colour variables measured in spring (Table 1). The interaction between age and sex was non-significant in all cases, although for brightness it approached significance. This suggests that in general age effects on crown colour are similar for males and females (but see below). On average, yearlings (1yr) were less UV-coloured (higher hue and lower UV chroma), and had lower chroma and brightness than older birds (Fig. 1). Among adults, two-year old and older birds did not differ significantly in crown colour (Figure 1), although the sample size for older birds was low.

The analyses based on colour measured in winter showed in general a similar pattern but with some differences. For hue and UV chroma, but not chroma or brightness, the interaction between sex and age was significant (sex x age; hue, $p < 0.001$; UV chroma, $p = 0.028$; chroma, $p = 0.11$; brightness, $p = 0.21$). This reflects the fact that in these two variables age effects in winter were more marked in females than in males. Finally, age differences in

Table 1. Age, sex and year effects on blue tit crown spring coloration. Terms in bold were included in the final model. Effect size (β, relative to the last category in each factor) and their corresponding standard errors (SE) are given for sex and age. Analysis carried out with restricted maximum likelihood models, based on 323 individuals, 11 measured in the three study years, 63 in two and the rest in only one.

		Hue			UV chroma			Chroma			Brightness		
	N	**β (SE)**	**F**	**p**	**β (SE)**	**F**	**p**	**β (SE)**	**F**	**p**	**β (SE)**	**F**	**p**
intercept		385.3 (1.7)			30.2 (0.2)			0.91 (0.02)			27.7 (0.7)		
Year		-	**56.17**	**<0.001**	-	**37.55**	**<0.001**	-	**28.22**	**<0.001**	**-**	**2.09**	**0.125**
Sex			**64.74**	**<0.001**		**504.1**	**<0.001**		**463.9**	**<0.001**		**113.0**	**<0.001**
male	205	-9.9 (1.2)			4.0 (0.2)			0.24 (0.01)			5.1 (0.5)		
female	204	-			-			-			-		
Age			**8.32**	**<0.001**		**7.89**	**<0.001**		**4.35**	**0.014**		**7.36**	**0.001**
1 yr	229	3.3 (1.7)			-0.7 (0.2)			-0.04 (0.02)			-2.4 (0.7)		
2 yr	123	-1.5 (1.7)			-0.1 (0.2)			-0.02 (0.02)			-1.1 (0.7)		
>2 yr	57	-			-			-			-		
Sex x Age		-	0.16	0.85	-	0.17	0.84	-	0.56	0.57	-	2.61	0.075

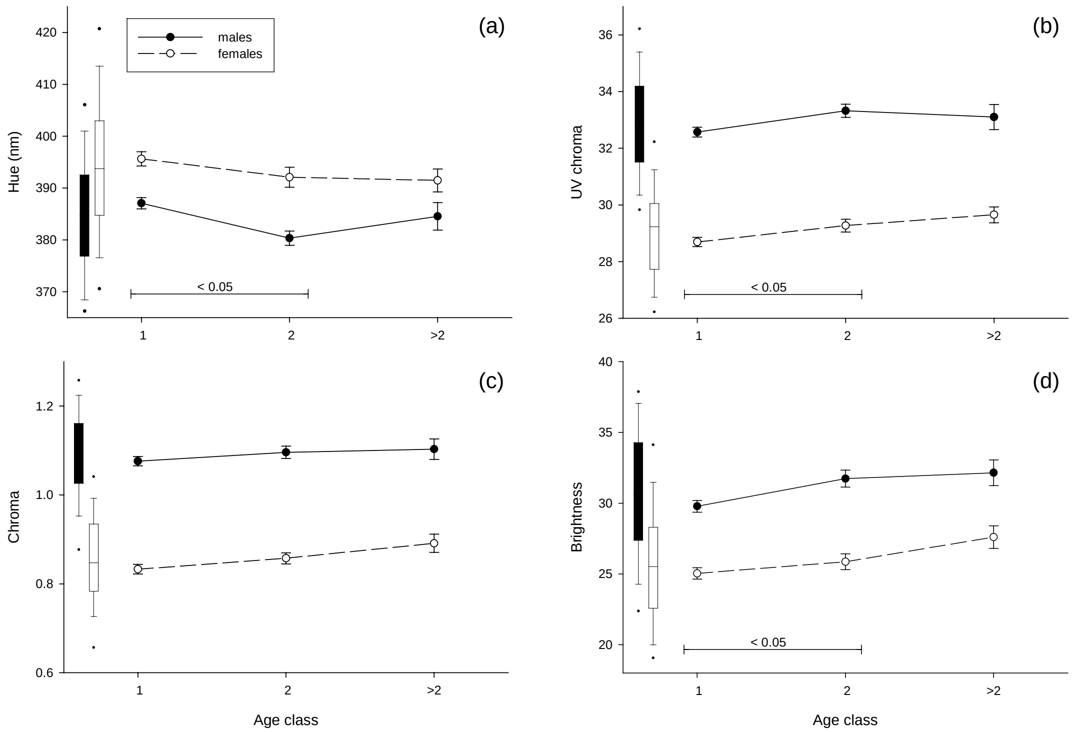

Fig. 1. The effect of age on male and female blue tit crown (a) hue, (b) UV chroma, (c) chroma, and (d) brightness. Depicted are means and their corresponding standard errors. Significant differences between age classes based on post-hoc tests (sexes pooled, see Table 1) are indicated. Box plots on the left of each panel represent the degree of colour variability for each sex separately. Depicted are the 5th, 10th, 25th, 75th, 90th and 95th percentiles.

winter brightness, although similar to spring differences, were not statistically significant ($p = 0.24$). Year effects were highly significant for all colour variables measured in winter (all $p < 0.001$).

Longitudinal analysis

The crown colour of individual blue tits became more UV (lower hue, higher UV chroma), and showed increases in chroma and brightness with age, as revealed by the significant effect of the within-subjects factor (colour change) on all colour variables (Table 2). The interaction between the colour change and age was significant for crown hue and UV chroma (Table 2), indicating that changes with age are more pronounced between the first and second year than later in life (Fig. 2a, b). Chroma increased constantly irrespective of age (Fig. 2c), although changes were small. Brightness on the other hand seemed to increase earlier in life in males

Table 2. Repeated measures ANOVA for age-related colour changes in the blue tit. The within-subject factor (i.e. the repeated measures) is coded as colour change (see Methods). Terms in bold are included in the final model.

	Hue			UV chroma			Chroma			Brightness		
	F	df	p	F	df	p	F	df	p	F	df	p
Colour change	**22.97**	**1,69**	**<0.001**	**19.33**	**1,69**	**<0.001**	**18.81**	**1,70**	**<0.001**	**6.25**	**1,67**	**0.015**
Colour change x year	1.02	1,68	0.314	0.17	1,68	0.673	2.10	1,69	0.151	1.04	1,66	0.311
Colour change x age	**15.61**	**1,69**	**<0.001**	**6.90**	**1,69**	**0.011**	0.00	1,69	0.988	0.14	1,67	0.709
Colour change x sex	0.01	1,68	0.893	0.03	1,68	0.864	1.33	1,69	0.252	0.06	1,67	0.797
Colour change x age x sex	0.62	1,67	0.432	0.05	1,67	0.815	0.00	1,67	0.938	**4.02**	**1,67**	**0.049**

(transition between 1 yr to 2 yr) and later in females (Fig 2d), as suggested by the significant interaction between the colour change, sex and age (Table 2). Overall however, there were no sex differences in the magnitude of age-related changes (Table 2, Fig. 2). Repeating the longitudinal analysis with winter colour confirmed that crown colour changes with age (within-subjects factor, $p < 0.001$ for all crown colour variables except brightness, where $p = 0.041$). However, there was no significant interaction with age for hue and UV chroma as with spring colour (both $p > 0.5$). All other interactions were non-significant (all $p > 0.10$), except for a significant effect of colour change x year on brightness (colour change x year, $p = 0.008$).

Crown colour within-individuals was generally correlated between years (Table 3, Fig. 3), but correlations were weak. If computed separately for each sex, repeatabilities (following Lessells and Boag 1987) were generally higher in males than in females, except for hue, which showed the lowest repeatability (Table 3, Fig. 3). Between year repeatabilities were generally lower for colour measured in winter than in spring (Table 3).

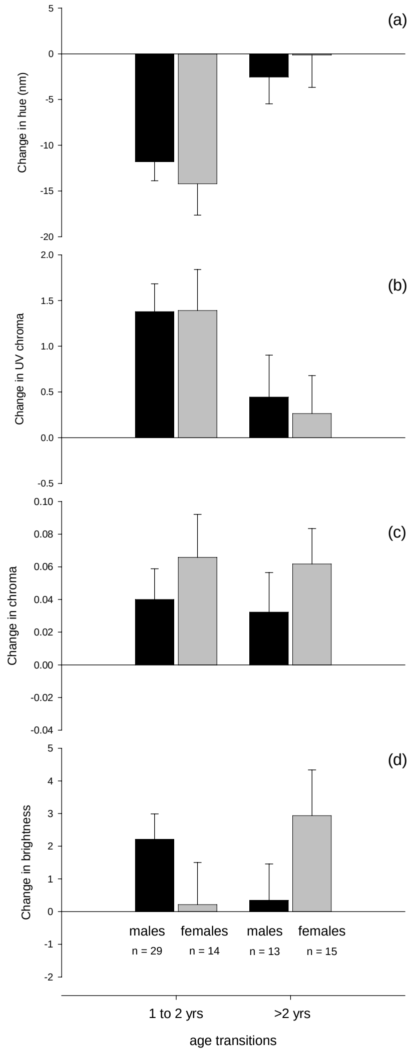

Fig. 2. Within-individual changes ($\text{colour}_{(\text{year } x+1)} - \text{colour}_{(\text{year } x)}$) in crown (a) hue, (b) UV chroma, (c) chroma, and (d) brightness of male and female blue tits due to age. Depicted are mean changes and their corresponding standard errors.

Table 3. Between-year repeatability of male and female blue tit crown colour measured in spring or winter in consecutive years (see Methods).

	Spring colour						Winter colour					
	Males[1]			Females[2]			Males[3]			Females[4]		
	R (SE)	$F_{42,47}$	p	R (SE)	$F_{29,37}$	p	R (SE)	$F_{34,46}$	p	R (SE)	$F_{12,15}$	p
Hue	0.18 (0.14)	1.47	0.099	0.25 (0.15)	1.76	0.053	-0.20 (0.12)	0.60	0.936	0.03 (0.23)	1.09	0.432
UV chroma	0.35 (0.12)	2.13	0.006	0.33 (0.14)	2.14	0.015	0.27 (0.13)	1.88	0.023	0.28 (0.22)	3.00	0.024
Chroma	0.41 (0.12)	2.74	<0.001	0.23 (0.15)	1.73	0.059	0.26 (0.13)	1.90	0.022	0.45 (0.19)	3.02	0.023
Brightness	0.54 (0.10)	3.53	<0.001	0.29 (0.15)	1.94	0.029	0.43 (0.12)	2.78	0.001	0.17 (0.23)	1.48	0.234

[1] n = 39 measured in two years, n = 4 measured in three years; [2]n = 27 measured in two years, n = 7 measured in three years; [3]n = 24 measured in two years, n = 11 measured in three years; [4]n = 11 measured in two years, n = 2 measured in three years

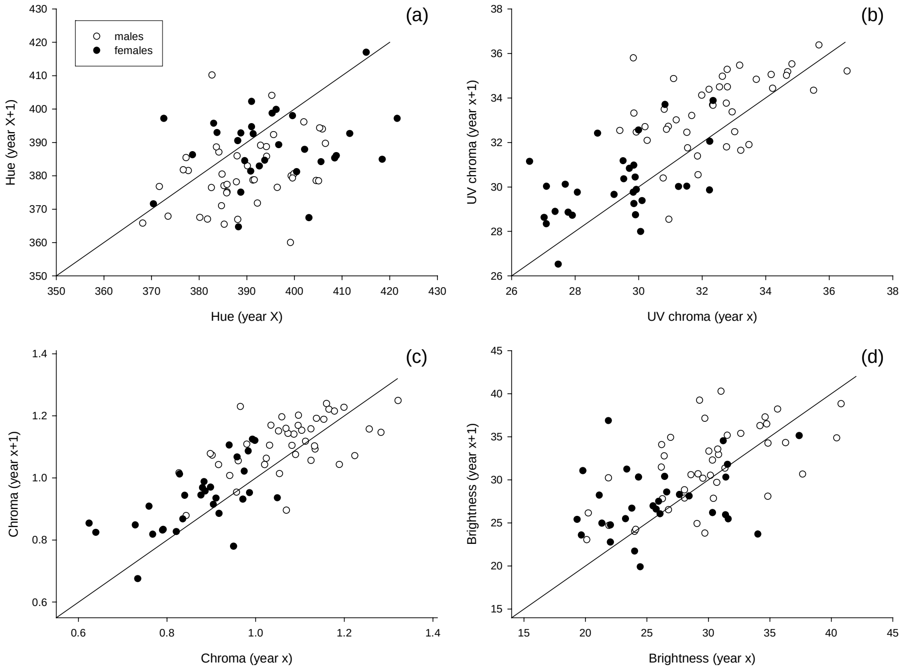

Fig. 3. Between-year repeatability of male and female blue tit crown (a) hue, (b) UV chroma, (c) chroma, and (d) brightness based on spring colour measurements. The diagonal line corresponds to Y = X; note that most data points lie only on one side of the line (below for hue, and above for the other variables), indicating significant changes in colour between years.

Colour and survival

Survival was independent of study year ($\chi^2_2 = 1.75$, $p = 0.42$), sex ($\chi^2_1 = 0.73$, $p = 0.39$), age ($\chi^2_2 = 4.51$, $p = 0.10$) or the interaction sex x age ($\chi^2_2 = 1.18$, $p = 0.55$). Crown coloration was overall unrelated to survival, although there was a trend for brighter birds of both sexes to show higher over-winter survival (Table 4).

Table 4. Blue tit crown colour and survival. Presented are means and standard errors (SE) for crown colour variables of male and female blue tit that did and did not survive to the next breeding season, and the results from the logistic regression models (see Methods for more details).

	Males - Survived (means ± SE)		Females - Survived (means ± SE)		Logistic regression
	No (N = 110)	Yes (N =49)	No (N = 122)	Yes (N = 44)	
Hue	383.7 (1.1)	385.2 (1.6)	394.6 (1.3)	392.7 (2.1)	Hue, $\chi^2_1 = 0.17$, $p = 0.68$
					Hue x sex, $\chi^2_1 = 1.08$, $p = 0.30$
					Hue x age, $\chi^2_2 = 0.22$, $p = 0.89$
UV chroma	32.9 (0.2)	32.8 (0.2)	28.8 (0.2)	29.0 (0.2)	UV chroma, $\chi^2_1 = 0.50$, $p = 0.48$
					UV chroma x sex, $\chi^2_1 = 0.68$, $p = 0.41$
					UV chroma x age, $\chi^2_2 = 0.88$, $p = 0.64$
Chroma	1.08 (0.01)	1.09 (0.02)	0.84 (0.01)	0.85 (0.01)	Chroma, $\chi^2_1 = 1.20$, $p = 0.27$
					Chroma x sex, $\chi^2_1 = 0.36$, $p = 0.55$
					Chroma x age, $\chi^2_2 = 0.53$, $p = 0.77$
Brightness	30.4 (0.4)	31.0 (0.7)	24.4 (0.4)	26.6 (0.7)	Brightness, $\chi^2_1 = 3.22$, $p = 0.07$
					Brightness x sex, $\chi^2_1 = 0.40$, $p = 0.53$
					Brightness x age, $\chi^2_2 = 1.69$, $p = 0.43$

DISCUSSION

The main conclusion from this study is that the observed age differences in blue tit crown coloration are due to within-individual changes and not to differential survival. Older blue tits in our study population had brighter, more chromatic and more UV (higher UV and lower hue) crown feathers than younger birds (Fig. 1 and Table 1). Age differences in crown colour were small compared to sexual dichromatism (Fig. 1) and differed somewhat from other published studies. In one of the first papers on blue tit crown coloration, Andersson et al. (1998) reported significant age effects on brightness (adult males being brighter than juveniles) but not on hue or UV chroma in a Swedish blue tit population (sample size n = 41). This result was confirmed in a follow-up study, which also included blue tits from a different Swedish population and had a larger sample size (n = 339; Örnborg et al. 2002). No age differences were found for crown chroma in a Spanish population (brightness and hue were not studied), although plumage colour was measured with a colorimeter that did not take reflectance in the UV into account (Figuerola et al. 1999). At the moment it is unclear whether between-populations differences in age dichromatism are common in the blue tit and what is the cause for this discrepancies. One source of variation could be between-population differences in selection pressures on colour expression through either survival, male-male interactions or female choice. More studies are necessary to understand the extent and significance of between-populations differences in age dichromatism.

Age effects on structural colours have also been reported in other bird species. Adult males had more UV-chromatic feathers than juveniles in bluethroats (*Luscinia svecica*, UV-blue throat patch; Johnsen et al. 2001) and in pied flycatchers (*Ficedula hypoleuca*, iridescent-black dorsal coloration; Siitari et al. 2002). On the other hand, no age-related differences were found in the iridescent bluish-black dorsal coloration of barn swallows (*Hirundo rustica*; Perrier et al. 2002).

Age changes in males and females

Age differences seemed to be similar for both sexes (Fig. 1), despite the pronounced sexual dimorphism in crown colour (Andersson et al. 1998; Hunt et al. 1998). However, in the winter sample, where colour was measured before the seasonal decline in UV reflectance (Örnborg et al. 2002), age dichromatism was more pronounced in females than in males. The reason for this is unclear, although it could be due to differences in the rate of crown colour decline between young and old males and females. However, the degree of decline in UV reflectance between winter and spring does not differ between sexes or between adult and juvenile males (this thesis, Chapter 3). This suggests that differential effects of feather wear or dirt accumulation are unlikely causes of age differences in colour, at least among males. The fact that age differences in crown coloration were at least as large in females as in males was confirmed by longitudinal analyses, which show for spring and winter, that sex had no effect on the magnitude of age-related colour change (Table 3). Thus, sexual dichromatism did not increase with age, in contrast to what is observed in most species with delayed plumage maturation (Lyon and Montgomerie 1986). Lack of sexual differences in the way ornamentation changes with age in addition to assortative mating (Andersson et al. 1998) and male preference for UV-reflectant females in captivity (Hunt et al. 1999) suggest that crown ornamentation may be selected through mutual mate choice in the blue tit.

Age changes in juvenile and older birds

The magnitude of within-individual changes in coloration depended on individual age, except for crown chroma which increased steadily and irrespective of age. Hue and UV chroma followed the usual pattern of age-related ornamentation, where the greatest increase in ornamentation takes place between the first and second year (Møller 1991; Dale et al. 1999; Hegyi et al. 2002; Török et al. 2003). Age-related increases in brightness, on the other hand, showed a different timing in males and females: changes in this variable were more pronounced early in life in males and later in females (Fig. 2d).

What underlies the different ways in which colour variables respond to age remains unclear. Non-iridescent structural colours, like the crown of the blue tit, are created through coherent scattering of light by the internal microstructure of the feather (Prum et al. 2003). While chroma and UV chroma might depend on the regularity and number of scattering particles respectively (Shawkey et al. 2003), hue is probably determined by the size of these particles (Andersson 1999; Shawkey et al. 2003). Brightness on the other hand might depend on the thickness of the feather cortex or the amount of melanin deposited in the feathers (Shawkey et al. 2003). Thus, given that different mechanisms are responsible for different colour variables, it is possible that they change independently of each other with age. For example the regularity of the keratin rods could increase steadily as birds get older but their size may become fixed once a certain age is reached. Studies addressing the proximate mechanisms involved in the expression of structural ornaments in adult blue tits are needed to test this idea.

Between year repeatability of colour

Blue tit crown coloration seems to be a rather plastic trait able to change within a given year (Örnborg et al. 2002) and also between years with individual age. Nonetheless the expression of crown colour in the same individual tended to be correlated in consecutive years (Fig. 3). Between year repeatability of crown coloration was slightly higher in males than in females, but low in general. Low repeatabilities are probably due to a combination of different factors. (1) Repeatability of colour measurements of live birds using spectrometry is moderate (0.4-0.6) setting an upper limit to between-year repeatability (see Figuerola et al. 1999; Perrier et al. 2002 and Methods). (2) Changes in coloration due to age as described here (Fig. 2), especially between the first and the second year also contribute to lower repeatability. (3) Blue tit crown colour shows a high degree of within-year changes, with pronounced declines in UV reflectance, but also changes in chroma and brightness, after moult is completed (Örnborg et al. 2002; this thesis Chapter 3). Thus, colour at the moment of measurement might differ to an unknown extent from the colour produced at moult. Surprisingly, however, repeatabilities of winter colour were lower than those for spring colour (Table 3), although winter and spring colour are positively correlated (this thesis Chapter 3). (4) Structural colours show condition-dependence (Keyser and Hill 1999; Doucet 2002; McGraw et al. 2002; Johnsen et al. 2003), hence between-year differences in environmental and individual conditions at moult could affect the colour of the growing feathers. Indeed, study year had a significant effect on all crown colour variables except for brightness (Table 1). Relatively low between-year repeatability in the expression of condition-dependent plumage coloration has also been reported for carotenoid- or melanin-based ornamental plumage (Hill 1992; Veiga and Puerta 1996; Griffith and Sheldon 2001; Senar et al. 2002).

Age effects and survival

Previous studies on a Swedish blue tit population showed that more UV-chromatic males had a higher probability of survival (Sheldon et al. 1999; Griffith et al. 2003). However, in our population, survival was unrelated to the expression of crown colour in males or females, except for a very weak effect of brightness (Table 4). This suggests that, unlike Swedish blue tits, females in our study population cannot assess future survival prospects of males by their crown colour. Differences in the relationship between ornamentation and survival across populations have been reported for other intensively studied species like house sparrows (*Passer domesticus*, Møller 1989; Griffith 2000), great tits (*Parus major*, Hõrak et al. 2001;

Fitze and Richner 2002) and house finches (*Carpodacus mexicanus*, Hill 1991; Badyaev et al. 2001). Current models of sexual selection, which take life history trade-offs into account, predict positive, no, or even negative relationships between survival and the expression of condition-dependent ornamentation depending on the intensity of sexual selection (Kokko et al. 2002). If the intensity of sexual selection is high (i.e. only the few most ornamented males sire most offspring), investing resources in the production of costly ornaments at the expense of future survival (negative correlation between survival and ornamentation) becomes the most successful strategy. On the other hand if mating skew is lower these models predict no, or even a positive correlation, between viability and ornamentation. Differences in the intensity of sexual selection could be brought about by differences in the costliness of female choice, which could in turn be caused by different environmental or social conditions (Kokko et al. 2002). While it would be tempting to speculate that differences across blue tit populations in the attractiveness-survival relationship are due to differences in the intensity of sexual selection, we presently lack the necessary information to support this claim. Nonetheless, in the future the blue tit could become a suitable model to test some of the predictions of the "sexual selection continuum" model (Kokko et al. 2002). In this species between-population differences in the incidence of extra-pair paternity and polygyny (Kempenaers 1994; Kempenaers et al. 1997; Krokene and Lifjeld 2000; Charmantier and Blondel 2003) may provide a gradient of mating skew, which could be related to the correlation between crown UV ornamentation and survival in each population.

The function of age-related colour expression in the blue tit

Adaptive explanations of age differences in ornamentation are mainly based on the idea that juvenile plumage, despite its lower sexual attractiveness, is advantageous by reducing adult aggressiveness (Lyon and Montgomerie 1986; Beauchamp 2003). This could indeed be the case in the blue tit since juvenile males might avoid male aggression by being less UV ornamented (Alonso-Alvarez et al. 2004), although they may then have lower breeding success as a result of female choice (Sheldon et al. 1999; Delhey et al. 2003; Griffith et al. 2003; Limbourg et al. 2004). However, even if on average yearlings could signal their lower status or age to adults, age-related differences in crown colour were small (Fig. 1), suggesting that the discriminative power of this trait might not be very high. Indeed, while discriminant analysis functions based on crown coloration (including all four colour variables) correctly classified more than 95% of the birds according to sex, aging proved to be more difficult with only 70% of males and 60% of females being correctly identified as juvenile or adult (our unpubl. data). Thus, the degree of age-related differences in ornamentation in the blue tit does not provide receivers with an unambiguous signal of age as in species with true delayed plumage maturation.

Another important difference with delayed plumage maturation is that male and female blue tits display comparable levels of age-related changes in crown coloration (Fig. 1 and 2). This suggests that similar mechanisms are responsible for age differences in both sexes (Mountjoy and Robertson 1988), and argues against the idea that age-effects in females are just a correlated response to selection on males (Amundsen 2000). Whether female colour indicates attractiveness to males, as some studies suggest (Andersson et al. 1998; Hunt et al. 1999), or whether it could help mediate agonistic interactions between competing females (Kempenaers 1995), remains an open question. Little is known about the function of female coloration in the blue tit, and future studies focusing on the relationship between female crown coloration and breeding success might help to shed light on this issue.

Finally, an alternative, non-adaptive explanation for age differences in ornamentation is that juvenile birds are less ornamented simply because developmental constraints or lack of resources prevent them from acquiring full adult plumage (Landmann and Kollinsky 1995; Hill 1996). Experiments have shown that juvenile and adult birds attain similar levels of melanin- and carotenoid- based ornamentation if moulting in captivity under the same conditions (Hill 1996; Veiga and Puerta 1996). Given that the expression of blue tit structural coloration is partly condition-dependent (Johnsen et al. 2003), similar experiments using captive blue tits might provide insight into this possibility.

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Chapter 3 - Seasonal changes in blue tit crown colour: do they signal individual quality?

Kaspar Delhey, Anne Peters, Arild Johnsen and Bart Kempenaers

Max Planck Institute for Ornithology, Seewiesen,

ABSTRACT

Plumage coloration is generally perceived as a static trait and therefore not a good indicator of current condition. However, fading of feather colours after moult does occur and could affect signalling functions and sexual selection. We studied longitudinal changes in blue tit (*Parus caeruleus*) crown UV/blue colour, a sexually selected trait, by repeatedly measuring the same individuals between early winter and late spring. While crown UV reflectance (UV chroma and hue) decreased dramatically over time, brightness and chroma did not show consistent patterns of change. The magnitude of the decline in coloration exceeded sexual and age dichromatism in hue and UV chroma respectively. Hence, seasonal colour changes could have strong effects on blue tit sexual signalling. Between-individual variation in the decline in UV coloration was large, and related to attributes of male, but not female, quality, such as size and condition. Thus, conspecifics could potentially gain information about male phenotypic quality by assessing colour change over the year. However, the degree of decline in male UV colour did not affect breeding success, since neither the number of within-pair nor extra-pair offspring produced correlated with changes in crown colour. Seasonal changes in the expression of plumage coloration are probably widespread in species with highly UV reflective plumage such as the blue tit. Maintaining plumage coloration could thus constitute an additional honesty-enforcing mechanism after moult is completed.

INTRODUCTION

Conspicuous plumage coloration, when acting as a secondary sexual trait, is often considered an honest signal of male quality in birds (Andersson 1994). Indeed, producing many, intensely colored feathers may entail substantial costs (see for example Hill and Montgomerie 1994; Doucet 2002; McGraw et al. 2002; Johnsen et al. 2003), and only high quality individuals can afford producing the most exaggerated colours. Intriguingly, production costs of plumage coloration are paid only during the relatively short period of moult. After moult is completed feather colours are assumed to be fixed and unable to change with the physiological state (condition, disease, hormonal levels, etc.) of the bird (Hill et al. 1999; McGraw and Hill 2004). Despite the presumed static nature of plumage colours, feather coloration can change considerably between moults due to wear and bleaching. In some cases, feather wear leads to increased conspicuousness of pigmented patches (Lucas and Stettenheim 1972), for example when buff-brown feather tips that conceal the underlying coloration abrade away. Usually, however, plumage coloration deteriorates between moults and the colours fade (Michener and Michener 1931; Test 1940; Johnson and Jones 1993). In those cases, and if more ornamented individuals enjoy an advantage either in agonistic or epigamic signalling, a decline in the expression or intensity of coloured feathers might have detrimental effects on individual fitness. Thus, the ability to prevent or reduce the decline in coloration may be adaptive and reflect individual quality (Fitzpatrick 1998; Zampiga et al. 2004).

Alternatively, declines in the expression of ornaments may be beneficial, especially if the degree of change can be modulated, to a certain extent, by the bird itself. Decreasing ornamentation after pairing may reduce conspicuousness to predators, increasing the chances of survival. Male ptarmigans (*Lagopus mutus*), for example, actively soil their white plumage after mating as a sort of camouflage before moulting into their cryptic summer plumage (Montgomerie et al. 2001). In other cases reducing the degree of ornamentation may help to reduce intraspecific aggressive interactions. This might be particularly important in cases where the level of display no longer matches the level of competitive ability due to loss of condition or disease. Indeed, pied flycatchers (*Ficedula hypoleuca*) experimentally challenged with a novel antigen decreased the size of their white, sexually selected, forehead patch (Kilpimaa et al. 2004), while house sparrows (*Passer domesticus*) with experimentally reduced testosterone levels kept their throat badge small through reduced abrasion of the buff feather tips concealing the black feathers (Gonzalez et al. 2001).

Changes in plumage coloration have been described for several species (Johnson and Jones 1993; Örnborg et al. 2002; McGraw and Hill 2004) but the causes and consequences of such changes are not well understood. Here we analyse the seasonal changes in crown structural coloration of individual blue tits (*Parus caeruleus*). This species shows sexual dichromatism in crown colour, with males being more ultraviolet (UV) than females (Andersson et al. 1998; Hunt et al. 1998), and crown UV reflectance seems to be important in both intra- and intersexual signalling (Sheldon et al. 1999; Delhey et al. 2003; Alonso-Alvarez et al. 2004). The blue tit is a good model to study changes in coloration since pronounced seasonal changes in the UV crown coloration have recently been reported for two populations in Sweden (Örnborg et al. 2002). In a cross-sectional study, Örnborg et al. (2002) showed that crown feathers became less UV reflectant with time, while overall brightness increased. However, since this study was not based on repeated measures of the same individuals, sources of bias like colour-related mortality or dispersal, even though they seemed unlikely, could not be ruled out. Our aims were, (1) to determine whether within-individual seasonal changes in crown colour follow the same pattern as suggested by Örnborg et al.(2002) by repeatedly measuring the same birds over the year, (2) to assess between-individual variability in colour change and explore correlations between attributes of phenotypic quality and the degree of individual colour change. And (3) to test whether the magnitude of colour changes has an effect on male breeding success. In the blue tit variance in male reproductive success is increased through extra-pair matings and crown colour correlates with fertilisation success in our study population (Delhey et al. 2003, Chapter 4). Hence we assessed the relationship between the degree of male colour change and within- and extra-pair paternity.

METHODS

This study was carried out between December 2001 and May 2003 in a colour banded blue tit population at Kolbeterberg (48° 13' N, 16° 20' E) in the outskirts of Vienna, Austria. The study site encompasses ca. 35 ha of a large tract of mixed deciduous woodland and contains 250 nest boxes.

Each study year (2002 and 2003), we captured blue tits at four times (periods) after the annual moult (which takes place between July and September; Cramp & Perrins, 1993): early winter (December 2001, November 2002), mid winter (January 2002, February 2003), early spring during the nest building phase (late March to early April, males only) and late spring while the birds were chick feeding (May). In winter and early spring, birds were captured in the nest box during the night. In early spring we also captured males using mist nets, decoy and playback while in late spring males and females were captured in the nest box when feeding 8-14 day old chicks. Unbanded birds were fitted with a unique combination of colour plastic bands and a numbered metal ring, and a small blood sample (5-50 μl) was taken from the brachial vein. For every captured bird we measured tarsus length with callipers to the nearest 0.05 mm and body mass with an electronic balance to the nearest 0.1 g. Birds were aged as juvenile (first year) or adult based on the colour of the greater coverts (Svensson 1992), and sexed based on the presence of a brood patch (late spring) or using molecular markers if only captured during winter (see details in Johnsen et al. 2003). Breeding pairs were monitored at regular intervals during the whole reproductive cycle, and chicks were blood sampled (5-25 μl) for paternity analysis between day 9 and 14 post-hatch.

Colour measurements

Each time we captured a bird, crown colour was measured by reflectance spectrometry with a S-2000 spectrometer and a DHS-2000 deuterium halogen light source connected through a bifurcated fibre optic probe. The probe was fitted with a plastic cylinder at the end to standardise measuring distance and exclude ambient light. Five readings per bird were taken in different but standardised spots on the crown. Feather reflectance was calculated between 300 and 700 nm relative to a WS-2 white standard (Avantes, Eerbek, The Netherlands). Reflectance spectra were imported into a spreadsheet program, each spectrum was smoothed with a running average computed over a 10nm interval and the following variables were calculated: average reflectance or brightness (R_{av}), spectral location or hue (wavelength of peak reflectance), spectral saturation or chroma (R_{max}-R_{min}/ $R_{300\text{-}700}$) and relative amount of UV reflectance or UV chroma (($R_{300\text{-}400}$/$R_{300\text{-}700}$) x 100) expressed as a percentage of total reflectance. For each bird, the coefficients from the five spectra were subsequently averaged. See Delhey et al. (2003) for more details on colour analysis.

Statistical analysis

Given that during winter and especially early spring (nest-building) few or no females were captured, statistical analyses were conducted separately for each sex to avoid unbalanced models. In all cases the full models (either REML or ANOVA, see below) were reduced by excluding variables in order of decreasing significance until only variables with $p < 0.10$ remained in the model. The excluded variables were entered one-by-one in the final model to confirm their lack of significance. Data analysis was carried out using the programs Genstat 7 and SPSS 12.0.1.

General pattern of colour change

Only individuals that had been measured at least twice in a given year were included in this analysis. Since not all individuals were measured the same number of times, "repeated-measures ANOVA" could not be used. Instead, we used restricted maximum likelihood models (REML) including bird identity as a random term. Colour variables (hue, UV chroma, chroma and brightness) were the dependent variables in these analyses, and a separate model was constructed for each of them. The full model included year, period (early winter, mid winter, nest building or chick feeding for males and early winter, mid winter, chick feeding for females) and their interaction. When the interaction was significant (see Results), we repeated the analysis for each year separately (to test for period effects), and for each period separately (to test for year effects). These analyses were based on colour measurements of 123 individual males of which 50 were measured twice, 28 three times, 19 four times, 7 five times, 12 six times, 4 seven times and 3 eight times over both study years. For females the analysis was based on 40 individuals, 21 measured twice, 10 three times, 5 four times and 4 five times.

Individual variation in colour change

In a second set of models, we assessed whether individual phenotypic traits or age were associated with the magnitude of colour change (Δcolour), which was used as the dependent variable. While we could have computed Δcolour over several possible intervals of time, we chose to derive only one estimate of colour change over the year, encompassing the longest possible time frame. Individual colour changes were calculated as the difference in colour between chick feeding and early winter ($\Delta\text{colour} = \text{colour}_{\text{chick feeding}} - \text{colour}_{\text{early winter}}$). We restricted our analysis to the change in hue and UV chroma, because (1) these are the only two variables that showed a consistent change during the year (see Results, Figs 1 and 2) and (2) these variables have been shown to correlate with male breeding success and attractiveness (Sheldon et al. 1999; Delhey et al. 2003; Griffith et al. 2003). Hence, changes in hue and UV chroma are probably most biologically relevant. The full models included study year (2002 or 2003) and age (only in the model for male colour change, because only two juvenile females were measured in both periods) as factors, and tarsus length, change in body mass (Δbody mass = body mass_{CF} – body mass_{EW}) and the number of days between measurements as covariates. While testing for the effect of Δbody mass we kept tarsus length as a forced covariate in the model to statistically control for differences in body size (García-Berthou 2001), although excluding it from the model did not change the results. Since we had multiple measurements of tarsus length, a trait that is invariant over a blue tit lifetime, we used average tarsus length in the analyses. Sample sizes for the analyses of Δcolour were 58 males and 20 females, but we only had data on Δbody mass for 57 males.

Finally, we assessed the effect of colour change (Δhue and ΔUV chroma) on male within-pair success (clutch size, likelihood of being cuckolded and proportion of sired offspring) and extra-pair success (likelihood of siring extra-pair offspring and number of sired extra-pair offspring) using binary or ordinal logistic regressions (Tabachnick and Fidell 2000). Full models included male age and study year as factors and Δcolour as covariate, and a different model was built for each colour variable. As correlations between colour and paternity seem to differ between juvenile and adult males (Delhey et al. 2003, Chapter 4), we also included the interaction term age x Δcolour. Sample size for these analyses was 43 males (21 juveniles and 22 adults). Paternity analysis of offspring was performed using eight polymorphic microsatellite markers (*Pca3*, *7*, *8* and *9* (Dawson et al. 2000), *Pocc1* and *6* (Bensch et al. 1996), *Phtr3* (Fridolfsson et al. 1997) and *PK11* (Tanner SM, Richner H, Schuenperli D, unpublished; EMBL accession no: AF041465)). The combined probability of exclusion (Jamieson 1994) for the marker set was >0.999. Paternity was excluded if two or more loci showed mismatches between putative fathers and offspring. For more details on paternity analysis see Delhey et al. (2003, Chapter 4).

As part of another study, in early spring 2003, after measuring crown reflectance, the colour of 47 males was manipulated with marker pens and silicone paste to enhance or reduce the UV reflectance of the crown feathers (see Chapter 4 and 5 for a description of the manipulation). This manipulation had significant short-term effects on crown coloration, but no long-term effects since the same manipulated males measured during chick-feeding did not differ in their crown coloration (all colour variables $p > 0.18$; Delhey, K., A. Peters, A. Johnsen & B. Kempenaers, unpubl.data). Given that some of these males were included in our analyses, we assessed whether this colour manipulation had an effect on colour change. For this we compared the change in crown colour between early winter and chick feeding in three groups of males, non-treated (average Δhue = 23.43, SE = 3.18; average ΔUV chroma = -0.741, SE = 0.510; n = 9), UV enhanced (average Δhue = 21.19, SE = 2.68; average ΔUV chroma = -1. 418, SE = 0.298; n = 9) and UV reduced (average Δhue = 26.01, SE = 2.18;

average ΔUV chroma = -1.599, SE = 0.559; n = 6) birds. The three groups did not differ in the magnitude of their colour change (One-way Anova; Δhue, $F_{2,21} = 0.633$, $p = 0.54$; ΔUV chroma, $F_{2,21} = 0.995$, $p = 0.38$). Moreover, including the factor treatment in the models did not explain a significant part of the variance (in both Δhue and ΔUV chroma, $p > 0.8$) or change any result. We therefore included treated birds in the analyses of colour change and male characteristics. Excluding them did not qualitatively affect the results although it decreased power (data not shown). Colour treatment however, affected male paternity (Chapter 4), hence the relationship between male paternity and colour change was tested excluding treated individuals.

RESULTS

General pattern of colour change

In both study years, male crown coloration changed substantially over the season (Fig. 1). The interaction between year and period was significant for hue ($\chi^2_3 = 24.4$, $p < 0.001$), UV chroma ($\chi^2_3 = 9.5$, $p = 0.023$) and chroma ($\chi^2_3 = 31.8$, $p < 0.001$), but not for brightness ($\chi^2_3 = 5.7$, $p = 0.12$). We therefore analysed within and between year colour changes separately (Table 1). For hue and UV chroma, the significant interaction term reflects the increased rate of change in 2002 compared to 2003 (Table 1), since the pattern of change was similar in both years. As the season progressed, crown hue increased, UV chroma (relative UV reflectance) decreased, and the rate of change in both variables increased (Fig. 1a, b). Chroma, on the other hand, did not show consistent changes in both study years. In 2002 chroma increased uniformly over the season, while in 2003 it initially increased to decrease later on (Fig. 1c). Brightness seemed to peak in mid winter and decreased towards late spring (Fig. 1d). Year differences in crown coloration were evident in most colour variables at all four periods during the year. Overall, male crown coloration was more UV reflectant (higher UV chroma and lower hue) and more chromatic in 2003 than in 2002 (Fig. 1, Table 1). There was a trend for increased brightness in 2003 compared to 2002 but it was only significant in mid winter (Fig. 1d, Table 1). The random term "male identity" was highly significant for all variables (all $p < 0.001$).

Female crown colour showed a similar pattern of seasonal change as male colour (Fig. 2). For all colour variables except chroma, seasonal effects were highly significant (hue, $\chi^2_2 = 191.9$, $p < 0.001$; UV chroma, $\chi^2_2 = 84.1$, $p < 0.001$; chroma, $\chi^2_2 = 3.85$, $p = 0.089$;

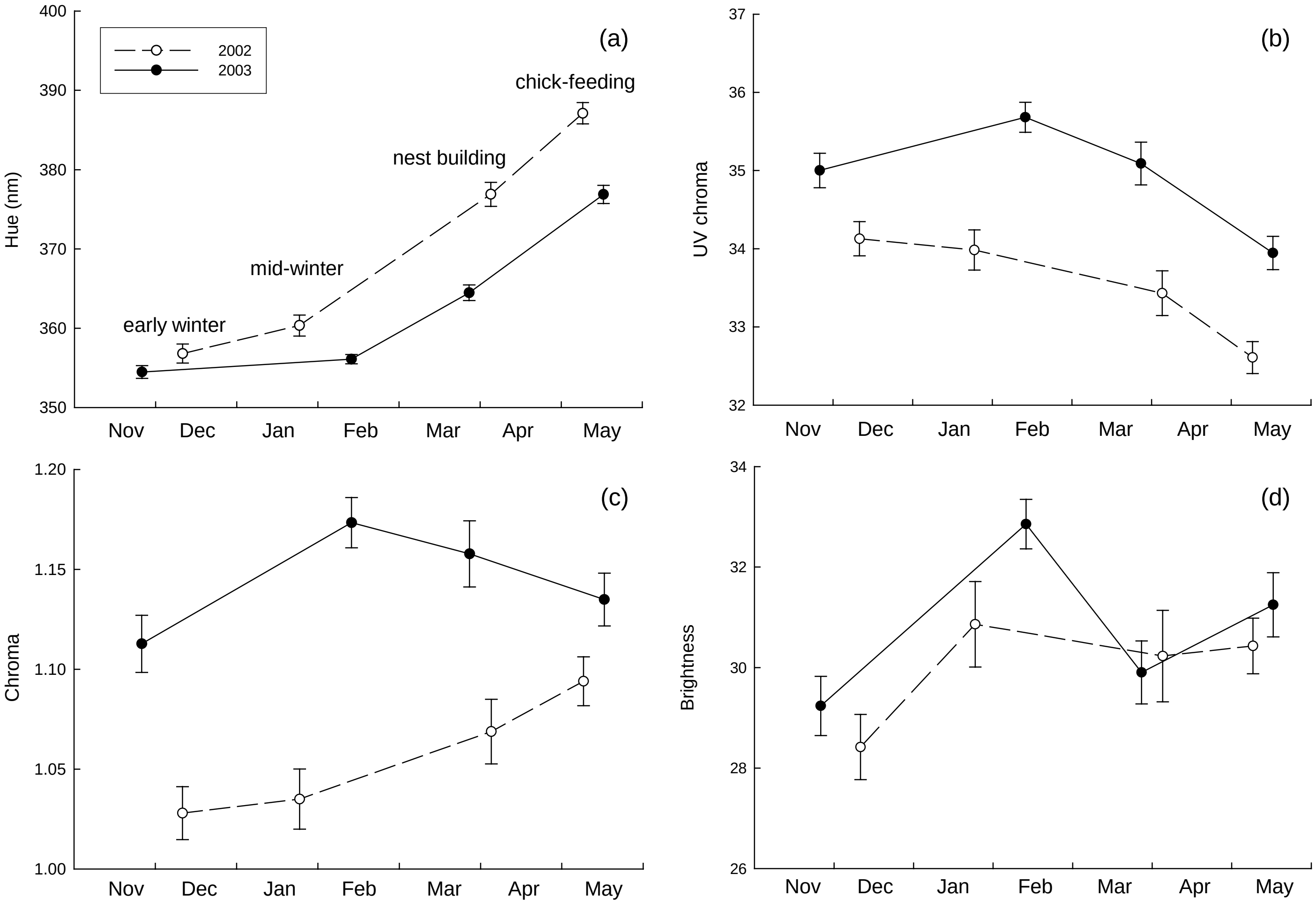

Figure 1. Seasonal pattern of change in male blue tit crown hue (a), UV chroma (b), chroma (c) and brightness (d). Bars depict ± 1 standard error.

Table 1. Within- and between year variation in male blue tit crown colour variables. Means and standard errors (SE) are predicted by restricted maximum likelihood models (REML) including period (EW, MW, NB, CF), year and their interaction as fixed effects and male identity as a random term. Probability values correspond to between-year differences for each period separately (p_{year}), or within-year differences in colour for each year separately (p_{period}); see Methods for more details. EW = Early Winter, MW = Mid Winter, NB = Nest Building and CF = Chick Feeding.

	Hue			**UV chroma**			**Chroma**			**Brightness**[3]		
	mean±SE		p_{year}[1]	mean±SE		p_{year}[1]	mean±SE		p_{year}[1]	mean±SE		p_{year}[1]
	2002	2003		2002	2003		2002	2003		2002	2003	
EW	357±1	354±1	0.069	34.1±1.9	35.2±1.9	<0.001	1.03±0.01	1.12±0.01	<0.001	27.9±1.0	28.8±1.0	0.421
MW	361±1	355±1	0.002	34.0±2.0	35.8±1.9	<0.001	1.04±0.01	1.18±0.01	<0.001	30.5±1.0	32.4±1.0	0.004
NB	376±1	364±1	<0.001	33.5±2.2	35.2±2.0	<0.001	1.08±0.01	1.16±0.01	<0.001	29.8±1.0	29.2±1.0	0.581
CF	387±1	376±1	<0.001	32.7±1.9	33.9±2.0	<0.001	1.10±0.01	1.13±0.01	<0.001	29.8±1.0	30.7±1.0	0.100
p_{period}[2]	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001		<0.001	<0.001	

[1] corresponding to a χ^2 statistic with 1 d.f.; [2] corresponding to a χ^2 statistic with 3 d.f.; [3] log transformed to normalise residuals, predicted means and SE have been back transformed

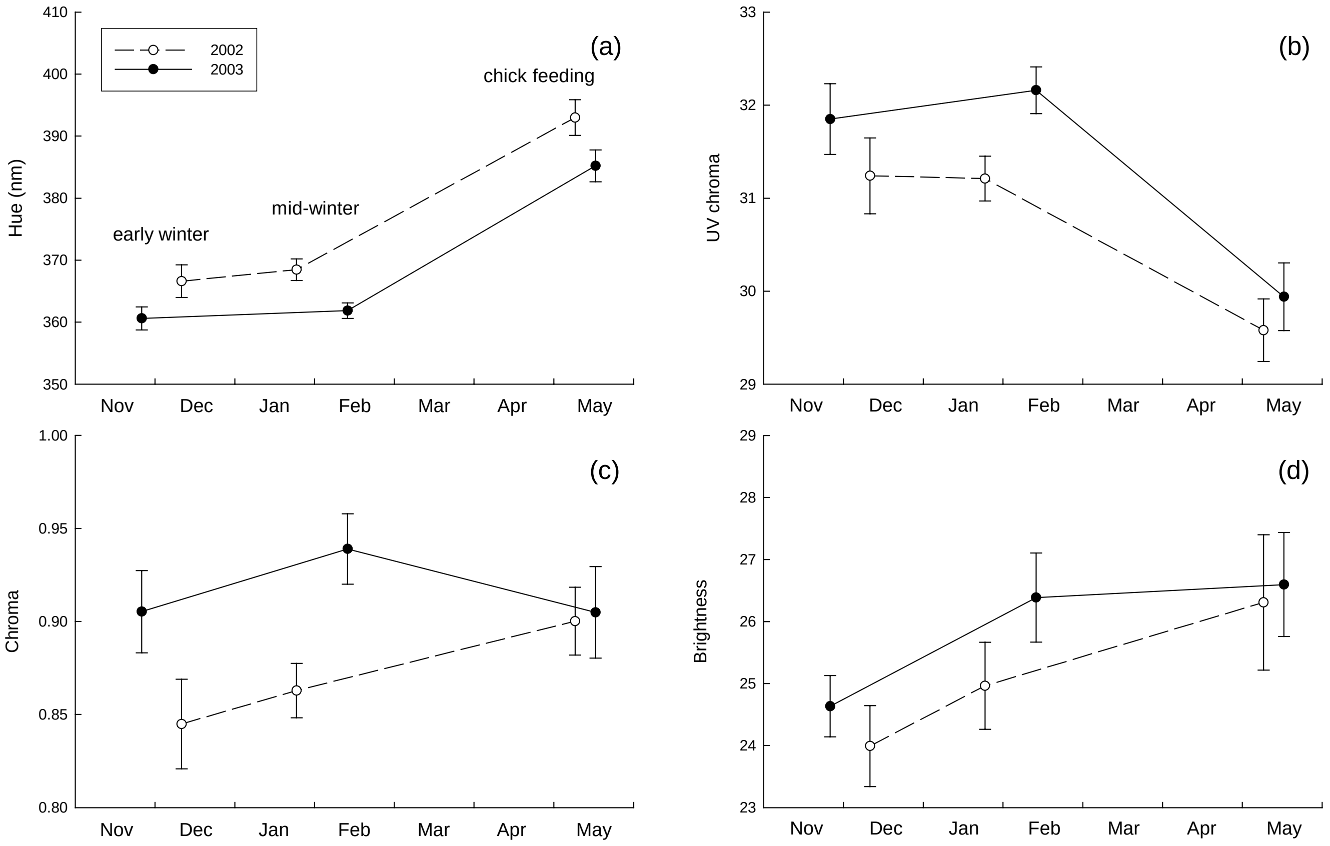

Figure 2. Seasonal pattern of change in female blue tit crown hue (a), UV chroma (b), chroma (c) and brightness (d). Bars depict ± 1 standard error.

brightness, $\chi^2_2 = 13.18$, $p = 0.001$). Year effects were significant for all colour variables although less so for brightness (hue, $\chi^2_1 = 10.62$, $p = 0.001$; UV chroma, $\chi^2_1 = 9.48$, $p = 0.002$; chroma, $\chi^2_1 = 13.37$, $p < 0.001$; brightness, $\chi^2_1 = 3.62$, $p = 0.057$). The interaction between year and period was never significant (hue, $\chi^2_2 = 0.13$, $p = 0.94$; UV chroma, $\chi^2_2 = 3.37$, $p = 0.17$; chroma, $\chi^2_2 = 3.78$, $p = 0.15$; brightness, $\chi^2_2 = 0.11$, $p = 0.95$). The random term "female identity" was highly significant for all colour variables (all $p < 0.001$).

Individual variation in colour change

The individual decline in UV coloration of male and female blue tits from winter to late spring revealed substantial between-individual variation (Fig. 3). On average, males experienced a change in crown hue of 27.5 nm (SE = 1.6, range = 5.6-57.2, n = 58) and of –1.43 in UV chroma (SE = 0.20, range = -5.08-1.31). Changes in female colour were of a similar magnitude (Δhue, average = 24.4 nm, SE = 2.9, range = 5.5-52.7; ΔUV chroma, average = -1.62, SE = 0.33, range = -5.66-1.52; n = 20), and the difference between sexes was not significant (Δhue, t = 0.987, p = 0.33; ΔUV chroma, t = 0.50, p = 0.62). Despite the large seasonal decline in coloration and the great between-individual variability, male winter UV coloration correlated positively

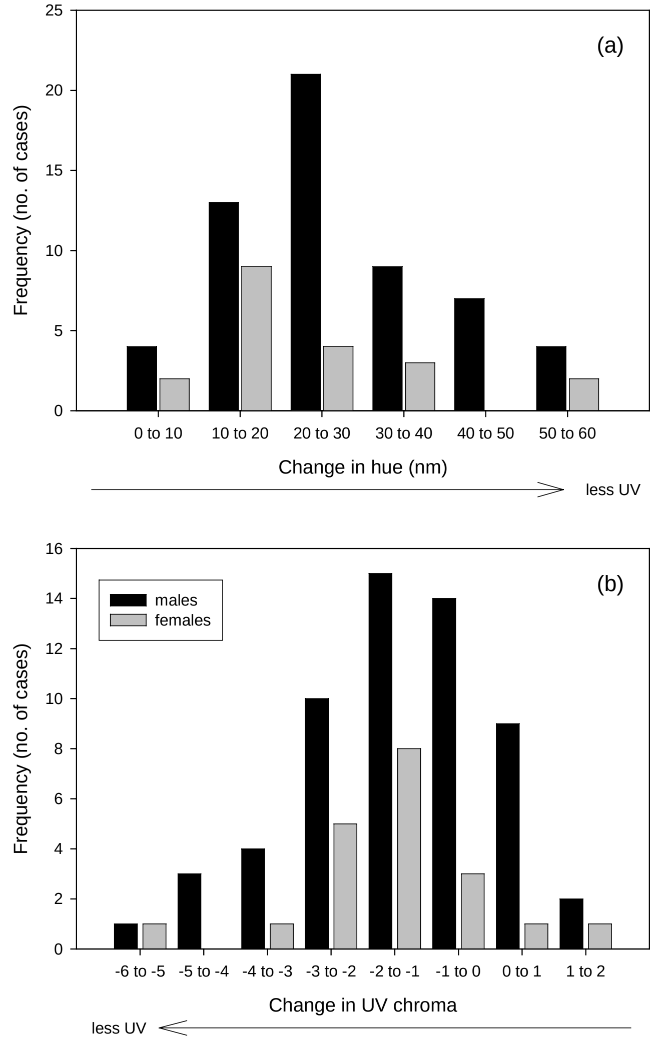

Figure 3. Frequency distribution of seasonal decline in crown hue (a) and crown UV chroma (b) of male and female blue tits.

with late spring colour (hue, r = 0.31, p = 0.02; UV chroma, r = 0.63, p < 0.001, n = 58). In females, the correlation coefficients were of similar magnitude, but only significant for UV chroma (hue, r = 0.29, p = 0.22; UV chroma, r = 0.64, p = 0.002, n = 20). This suggests that the ranking of individuals is not significantly affected by colour change, and indeed non-parametric rank correlations show the same pattern (Kendall tau, males: hue, τ = 0.16, p = 0.080; UV chroma, τ = 0.45, p < 0.001; females: hue, τ = 0.24, p = 0.136; UV chroma, τ = 0.63, p = 0.003).

Table 2. Correlates of individual male crown colour (hue and UV chroma) change between winter and spring. Terms included in the final model are depicted in bold.

	ΔHue				ΔUV chroma			
	β (SE)	F	df	p	β (SE)	F	df	p
intercept	150.6 (45.3)				-6.9 (6.4)			
Year (2002-2003)	**8.4 (2.9)[1]**	**8.44**	**1, 55**	**0.005**	-	0.95	1, 53	0.334
age	-	0.52	1, 54	0.475	-	1.41	1, 53	0.241
Δdays	-	0.43	1, 54	0.514	-	0.58	1, 53	0.448
tarsus length	**-7.5 (2.7)**	**7.94**	**1, 55**	**0.007**	**0.28 (0.37)**	**0.55**	**1, 54**	**0.459**
Δbody mass[2]	-	2.69	1, 53	0.107	**-1.18 (0.51)[3]**	**5.43**	**1, 54**	**0.024**
Final model		**7.25**	**2, 55**	**0.002**		**3.18**	**2, 54**	**0.049**

[1]computed relative to year 2003; [2]sample size = 57, [3]if tarsus length is excluded form the model $F_{1,55}$ = 5.86, p = 0.019.

The within-individual decline in male crown hue was more pronounced in 2002 than in 2003 (Table 2, Fig. 1a). Individual changes in hue did not depend on age, nor did they correlate with the change in body mass between winter and late spring (Table 2). However, males with smaller tarsi experienced a larger decline in hue (Table 2, Fig. 4a). Changes in crown UV chroma were not affected by year, age, or morphometrics, but correlated negatively with the change in body mass (Table 2). Males that lost more weight between

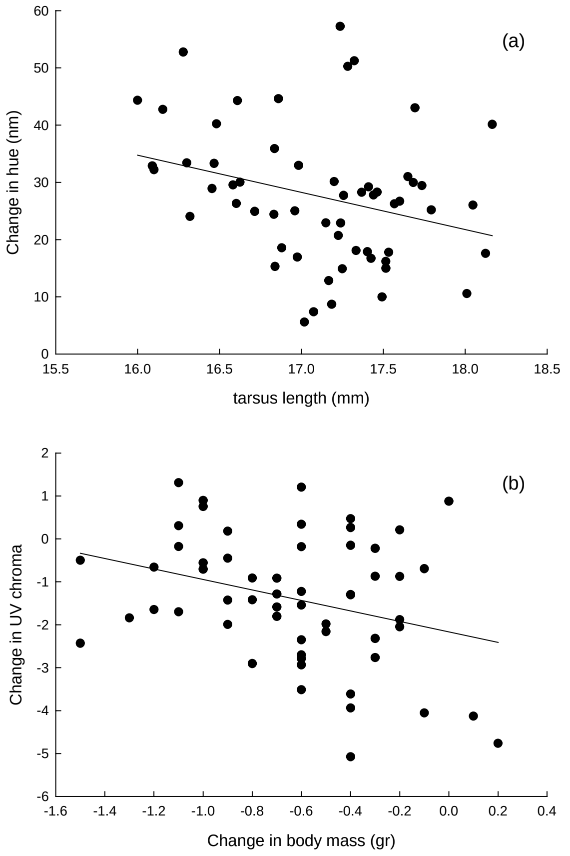

Figure 4. Within-individual changes in male crown hue from winter to spring correlate with tarsus length (a), while changes in male crown UV chroma correlate with changes in body mass during the same period (b). Trend lines based on least squares regression are for visual purposes only, for details on the statistical analyses see Table 2.

winter and spring declined less in UV chroma (Fig. 4b). These patterns are also reflected by the way the overall relationship between male hue and tarsus length changes over the year in the population, from no correlation in winter ($r = 0.02$, $p = 0.84$, $n = 125$) to a weak negative trend in late spring ($r = -0.12$, $p = 0.12$, $n = 156$). Similarly, UV chroma did not correlate with body mass in winter ($r = 0.08$, $p = 0.37$, $n = 124$) but correlated negatively in spring ($r = -0.18$, $p = 0.024$).

Changes in female colour between winter and late spring were not correlated with female size or changes in body mass (both Δhue and ΔUV chroma: tarsus, $p > 0.54$; Δbody mass, $p > 0.22$) but the decline was more pronounced in 2002 than 2003 (year: Δhue, $F_{1,17} = 5.04$, $p = 0.038$; ΔUV chroma, $F_{1,17} = 3.48$, $p = 0.08$) and correlated with the number of days between measurements as well (Δhue, $F_{1,17} = 8.23$, $p = 0.011$; ΔUV chroma, $F_{1,17} = 5.29$, $p = 0.034$).

Colour change and male fertilisation success

Colour change did not explain variation in male within-pair success (Table 3). Similarly, after controlling for the effects of year (in this sample, males in 2002 sired more extra-pair offspring than in 2003) and age (adult males sire more extra-pair offspring in the blue tit (Kempenaers et al. 1997; Delhey et al. 2003)), colour change did not explain variation in male extra-pair success (Table 3).

Table 3. Effects of colour change on male within- and extra-pair reproductive success. Separate models were built for each colour variable (Δhue and ΔUV chroma). Terms included in the final model are depicted in bold.

	Within-pair paternity (WPP)						Extra-pair paternity (EPP)			
	Clutch size[1]		Likelihood of losing WPP[2]		Proportion of WPP[3]		Likelihood of siring EPP[2]		Number of extra-pair offspring[4]	
	χ^2	p	χ^2	p	χ^2	P	χ^2	p	χ^2	p
Year	1.59	0.20	0.41	0.522	0.73	0.393	**6.25**	**0.012**	**4.47**	**0.034**
Age	0.86	0.35	0.59	0.443	0.76	0.381	**13.62**	**< 0.001**	**11.29**	**< 0.001**
Δhue	0.48	0.487	0.001	0.972	0.76	0.383	0.58	0.443	0.43	0.511
Δhue * age	0.25	0.618	0.69	0.404	0.18	0.672	0.56	0.455	0.43	0.511
ΔUV chroma	1.65	0.198	0.86	0.352	0.23	0.627	0.08	0.778	0.01	0.912
ΔUV chroma * age	0.70	0.401	0.33	0.563	0.68	0.409	0.17	0.677	0.26	0.611

[1]ordinal logistic regression controlling for decreasing clutch size with date, $\chi^2_1 = 6.10$, $p = 0.013$; [2]binary logistic regression, [3]ordinal logistic regression with number of within-pair offspring as dependent variable while controlling for the number of typed offspring ($\chi^2_1 = 37.81$, $p < 0.001$), [4]ordinal logistic regression

DISCUSSION

This study shows that the crown colour of individual blue tits changed dramatically between early winter and late spring in both study years (Figs 1 and 2). The most obvious change was a general decline in the UV reflectance of the crown, illustrated here by a decline in UV chroma and an increase in hue (i.e. becoming more long- wave). The average decline in UV coloration was as large as one standard deviation of the original (winter) UV chroma and up to 4 standard deviations of the original hue.

Seasonal changes in crown coloration of male and female blue tits were qualitatively similar to those reported by Örnborg et al. (2002) in a cross-sectional analysis for two Swedish populations. Hue and UV chroma followed virtually the same pattern while changes in chroma and brightness were somewhat different. The seasonal change in chroma was similar to the one reported for the Swedish populations in 2003, but different in 2002 (Fig. 1c). Brightness on the other hand increased towards spring in Sweden, while in our study population it peaked in mid winter for males and in spring for females (Figs 1d and 2d). Different patterns of change in crown brightness were also evident between Swedish populations (Örnborg et al. 2002). We do not have satisfactory explanations for these differences. Nonetheless, the remarkably similar pattern of change between years and populations in hue and UV chroma, suggests that seasonal declines in UV reflectance of the crown are a general phenomenon in this species, probably caused by the same underlying mechanisms.

Mechanisms of colour change and individual quality

Changes in blue tit crown colour are most likely due to the combined action of soiling and feather wear (Örnborg et al. 2002). Wear has been hypothesised to have especially strong effects on structurally coloured feathers (Fitzpatrick 1998), while soiling could constitute a mechanism specifically affecting the colour of highly UV reflectant plumage. The progressive accumulation of dirt and fat on the feathers might be responsible for the decline in UV reflectance, since these substances often absorb UV light (Örnborg et al. 2002; Zampiga et al. 2004). Thus, the degree of UV reflectance of the plumage might be a good indicator of investment in feather maintenance, which would prevent or diminish wear and soiling (Zampiga et al. 2004). Birds devote a substantial part of their daily time budget to feather maintenance activities (Cotgreave and Clayton 1994; Walther and Clayton 2005). It is likely that preening involves costs in terms of time, energy expenditure (Goldstein 1988) or production of substances used in feather maintenance, such as uropygial gland secretions

(Jacob and Ziswiler 1982). If maintaining plumage UV reflectance is costly, only high quality individuals would be able to maintain their plumage intact, resulting in a positive association between individual quality and UV colour change. If large differences in individual quality exist in the population, this should translate into large between-individual variability in the degree of colour change. Indeed, individual change in crown UV coloration over the year was highly variable (Fig. 3), with some individuals declining much more than others.

While the degree of individual decline in UV coloration was unrelated to sex or age, changes in male, but not female crown colour, correlated with phenotypic traits. Male tarsus length correlated negatively with changes in crown hue, that is, smaller males increased more in crown hue (i.e. they became less UV) than larger males (Fig. 4a). Tarsus length is a highly heritable trait (Dhondt 1982), which is also sensitive to rearing conditions, since nestlings grow shorter tarsi if raised in a food-stressed environment (Kunz and Ekman 2000; Limbourg et al. 2004). Larger fledglings show higher survival in the closely related great tit (*Parus major*, Garnett 1981), and this suggests that males with longer tarsi may be of higher than average quality. Our results suggest that larger, high quality blue tit males, produce feathers of better quality or devote more time to plumage maintenance.

Changes in UV chroma, while unrelated to male size, correlated negatively with changes in condition (estimated as change in body mass, Table 2). Males that lost more mass between winter and spring declined less in crown UV reflectance (Fig. 4b). This suggests that keeping crown UV reflectance more intact may entail physiological costs, or that investment in other costly activities like chick provisioning or territory defence may co-vary with the ability to maintain crown UV coloration. Experimental manipulation of male condition or investment in reproduction, coupled with repeated measurements of crown coloration, will be necessary to disentangle these possibilities.

Implications for sexual selection

Previous studies in the blue tit suggest that more UV ornamented (higher UV chroma, lower hue) males enjoy fitness advantages since their females are more faithful, produce more male offspring and invest more in chick provisioning (Sheldon et al. 1999; Delhey et al. 2003; Limbourg et al. 2004; Johnsen et al. in press). On the other hand, older, less UV ornamented males are more successful at cuckolding other males (Delhey et al. 2003). Hence, changes in UV chroma and hue have the potential to influence male fitness. This becomes more evident if we consider the magnitude of these changes. The average decline in crown hue between winter and spring is larger than sexual dichromatism (compare Figs 1 and 2), while changes in UV chroma exceed age related differences in coloration (Chapter 2). Moreover, differences due to seasonal variation in colour are larger than differences in hue between cuckolded and non-cuckolded males (ca. 12 nm, Delhey et al. 2003) or differences in UV chroma between males that did and did not survive the winter (ca. 0.1, Sheldon et al. 1999).

Whether and how blue tits use the information from this remarkable degree of change in ornament expression is not clear. Our data suggest however, that colour changes have little impact on male within- or extra-pair success, since neither component of male fertilisation success was related to male colour change (Table 3). Hence, although we cannot exclude that females are sensitive to male colour changes, evidence so far indicates that they do not affect female faithfulness or male attractiveness as an extra-pair partner. Alternatively, females may only assess male coloration at certain key time points during the breeding cycle, for example when birds pair up for the first time or in early spring when copulations take place. Colour manipulation experiments suggest that females react to manipulations of male colour as early as the nest building period (Sheldon et al. 1999), and as late as the chick feeding stage of the breeding cycle (Limbourg et al. 2004; Johnsen et al. in press). Male-male agonistic interactions, on the other hand, could be affected by colour expression even earlier in the year during territory acquisition in autumn or winter (Alonso-Alvarez et al. 2004). Addressing the question on when colour signalling is most important will be necessary to understand how sexual selection acts on blue tit crown coloration and colour maintenance (Sullivan 1990).

To conclude, in the blue tit seasonal declines in UV reflectance of the crown feathers are probably the rule, and this could also apply to other bird species with highly UV reflective plumage (see for example Bridge and Eaton 2005). Thus, maintaining the UV reflectance of the feathers may constitute an additional cost enforcing the honesty of this type of coloration (Zampiga et al. 2004). Future studies in other species and on different types of colours should address whether and how the degree of colour change could reflect individual quality. Meanwhile, the time has come to put aside the traditional view of feather colours as static ornaments and start thinking of bird plumage as a more plastic trait.

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Chapter 3

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Chapter 4 - Higher fertilization success of less UV ornamented blue tits: correlational and experimental evidence

Kaspar Delhey, Anne Peters, Arild Johnsen and Bart Kempenaers
Max Planck Institute for Ornithology, Seewiesen

ABSTRACT

Cases where less ornamented males are favoured through sexual selection are rare among birds. Here we show, based on data from three consecutive breeding seasons, that male blue tits with less UV-ornamented crown feathers sire more offspring. This pattern was mainly driven by the higher success of older, less UV-ornamented males at siring extra-pair offspring. The reason behind this relationship is unclear although we hypothesize that being less UV-ornamented may enable adult males to intrude nearby territories by mimicking juveniles. To test the causality of these relationships we experimentally enhanced (UV(+) treatment) or reduced (UV(-) treatment) male crown UV-reflectance within the natural range of variation. Contrary to our expectations UV(-) males were less likely to sire extra-pair offspring than UV(+) males. Treatment had no effect on the likelihood of losing paternity in a male's own nest. Since the experimental evidence does not support the observational data a direct effect of male crown colour on extra-pair success cannot be confirmed. However potential pitfalls of the experiment, like fading of treatment with time and mismatches between behaviour and coloration, call for new improved manipulation techniques and detailed behavioural observations to conclusively test for the effect of blue tit crown coloration on male extra-pair success.

INTRODUCTION

In sexually dimorphic birds more ornamented males are usually more successful in mate attraction. Generally, those males that express the most extreme form of a particular ornament, such as extravagant plumage or bright coloration, obtain more matings and/or mates of higher quality by either out-competing other males (male-male competition) or being directly preferred by females (female choice) (Darwin 1871; Ryan and Keddy-Hector 1992; Andersson 1994). All other things being equal, pairing with many or high-quality females leads to increased breeding success, and sexual selection is thereby mostly directional, towards increased ornamentation.

Conversely, only relatively few examples exist where males with a less exaggerated sexual trait experience higher breeding success through sexual selection. Most of these cases refer to smaller males being more successful by virtue of their increased agility, aerobatic abilities or reduced energetic requirements (Blomqvist et al. 1997; Balmford et al. 2000; Voigt et al. 2005). In most of these species sexual selection for smaller males leads also to reversed size dimorphism (Szekely et al. 2004). Examples where males are the larger or more ornamented sex, but nonetheless smaller or less ornamented males seem to be favoured by sexual selection are rare. In some populations of house sparrows (*Passer domesticus*) and house finches (*Carpodacus mexicanus*) for instance, females prefer to mate with less ornamented males since these are willing to invest more in brood rearing (Griffith et al. 1999; Badyaev and Hill 2002). In these cases male ornamentation is maintained by opposing positive selection on ornament elaboration through alternative mechanisms: more ornamented male house finches are preferred by young inexperienced females and pair early in the year (Badyaev and Hill 2002), while more ornamented house sparrows show higher over-winter survival (Griffith et al. 1999). However, in the absence of these opposing selective forces male ornamentation might become reduced (Saetre et al. 1997) or even completely lost in the course of evolutionary history (Wiens 2001).

Recently we documented higher annual reproductive success for less ornamented blue tit males (Delhey et al. 2003). In this species both sexes display brilliant ultraviolet (UV)/blue crown feathers, but males are more UV ornamented than females (Andersson et al. 1998; Hunt et al. 1998). Several lines of evidence suggest that male UV ornamentation is under direct sexual selection through female choice. In the wild, blue tits have been found to mate assortatively by crown UV coloration (Andersson et al. 1998), while choice chamber experiments revealed a preference for “UV-intact” against “UV- blocked” mates (Hunt et al. 1999). Moreover, experimental and correlational evidence suggest that females perceive highly UV-reflectant males as attractive since they bias brood sex ratios towards sons (Sheldon et al. 1999; Griffith et al. 2003) and invest relatively more in brood rearing and nest defense if paired to more UV-reflectant males (Limbourg et al. 2004; Johnsen et al. in press). While in these studies, more UV-ornamented males were preferred, in our population we found that, older, less UV ornamented, males were more successful at siring extra-pair offspring (Delhey et al. 2003). This selection pressure through extra-pair success was opposed by selection through within-pair success since more UV males were cuckolded less. However, since the former was stronger, there was an overall weak negative selection on male UV ornamentation through annual fertilization success.

The aim of this study is twofold. First, we re-evaluate the correlational patterns reported by Delhey et al. (2003). This study was based on data from a single breeding season and selection pressures may vary among years. Furthermore, one cannot exclude that the reported correlations, even though significant, are due to type I statistical errors. We therefore analyse the relationship between crown colour and paternity using a larger data set collected during three consecutive breeding seasons (2001-2003). Second, we experimentally test the relationship between ornament expression and within- and extra-pair paternity, by manipulating male crown coloration. Experimental studies testing the causal link between male ornamentation and paternity are surprisingly scarce (Griffith et al. 2002), and often male ornaments are manipulated far beyond the natural degree of variation (Smith et al. 1991; Johnsen et al. 1998) making the interpretation of results difficult. In an attempt to avoid these problems we use a new method to manipulate male crown colour within the natural range of variation.

METHODS

Study site and general methods

This study was carried out between 2001 and 2003 at Kolbeterberg (48º 13’ N, 16º 20’ E), in the outskirts of Vienna, Austria. The study site consists of a ca. 35 ha plot within a large deciduous mixed forest. The plot contained 220 nestboxes in 2001 and 250 in 2002 and 2003. Each year, from the end of March onwards, we regularly checked each nestbox for signs of occupancy. Occupied boxes with complete nests (i.e. lined cups) where checked every day to determine laying date and clutch size. After incubation initiation we left the nests undisturbed until the expected hatching date, when boxes were checked daily again until hatching finished. All unhatched eggs and dead nestlings found in the nest were collected and stored in

70% ethanol. As part of another project, 20 clutches were collected under license (10 in 2001 and 10 in 2003) after one day of incubation, eggs were opened and embryos stored in ethanol. Broods were inspected at least three more times before fledging (which happens around day 19-20 post hatch). A small blood sample (5-25 µl) was drawn from the brachial vein of nestlings at day 9-14 post-hatch, and each chick was banded with a numbered metal ring. Adults were captured at the nestbox while feeding 8-14 day old chicks. If unbanded they were fitted with a numbered metal ring and a unique combination of plastic colour bands, and a blood sample (10-50 µl) was taken from the brachial vein. Adult birds were sexed by presence or absence of the brood patch and aged as juveniles or adults following Svensson (1992). After banding and blood sampling we measured the colour of the crown (see below).

Colour measurements

Reflectance of the crown feathers was measured with a S-2000 spectrometer with a DH-2000-FHS deuterium-halogen light source (Ocean Optics). Spectrometer and lamp were connected through a bifurcated fiber optic probe, fitted at the end with a plastic cylinder to standardise measuring distance and exclude ambient light. The probe was held perpendicular to the surface of the feathers, and we took readings of five standardized spots (11.3 mm^2 each). Reflectance (R) was calculated relative to a WS-2 white standard using the program Spectra-Win. Raw spectra were imported into a spreadsheet program and smoothed using a running average over a 10 nm interval. Previous studies on blue tit crown coloration (Sheldon et al. 1999; Delhey et al. 2003; Griffith et al. 2003; Limbourg et al. 2004; Johnsen et al. in press) indicate that patterns of variability in male crown coloration are adequately and meaningfully summarised by the following two variables: (1) hue or spectral location, determined as the wavelength of peak reflectance (λR_{max}), and (2) the relative amount of UV reflectance or "UV chroma", calculated as reflectance in the UV range (300-400nm) divided by total reflectance (($R_{300-400}/R_{300-700}$) x 100) and expressed as a percentage. A more exaggerated signal is one that shows a reflectance peak shifted further towards or into the UV (i.e. a smaller value of hue) and a higher relative UV reflectance (higher UV chroma) (Andersson 1999; Keyser and Hill 1999; Keyser and Hill 2000).

Colour manipulation experiment

The colour treatment was designed to modify crown reflectance within the natural range of variation found in our population. In previous studies UV-reflectance was almost completely blocked, resulting in an unnaturally truncated reflectance spectrum (Andersson and

Amundsen, 1997; Johnsen et al., 1998; Sheldon et al., 1999). We captured 47 males in March-April 2003, on average 18 days before their mates laid the first egg (range 1-25 days), measured pre-manipulation colour as described above (b) and manipulated male crown colour. Males of both age classes were sequentially allocated to either UV(+) treatment (n = 22), where relative reflectance in the UV was enhanced, or UV(-) treatment (n = 25), where UV reflectance was reduced. To manipulate crown coloration we used Edding 4500 "T-Shirt Marker" pens (Ahrensburg, Germany). UV(+) treated males were painted on the crown feathers with a light blue pen (Edding col.10), while UV(-) males were first painted with a dark blue pen (Edding col.03) and then with the light blue pen (Edding col.10) on top. To enhance water-resistance of both treatments, we applied a coat of silicone-based fly-dressing (Balzer Silicone-Fett, Germany) after painting the feathers. After completing the treatment, crown colour was measured again. Treated males behaved normally and their ability to raise the crown feathers was not impaired.

Before manipulation males from both treatment groups did not differ in crown colour (all $p > 0.35$). After treatment, UV(+) males had a more UV-shifted hue (UV(+) = 353 nm, SE = 1.4, range: 342-371; UV(-) = 401.5 nm, SE = 2.6, range: 367-434; $t = 16.2$, $p < 0.001$) and higher UV chroma (UV(+) = 33%, SE = 0.2, range: 31-35; UV(-) = 31%, SE = 0.3, range: 28-34; $t = -6.58$, $p < 0.001$) than UV(-) birds. After manipulation, the colour variables were largely within the natural range of variation of this population (see Chapter 5 for a graph of the reflectance spectra).

To assess the durability of the treatment we recaptured 16 males on average 15 days post-manipulation (range 7-25 days). UV(-) males (n = 9) were still significantly less UV chromatic (paired t-test, $t_8 = 4.20$, $p = 0.003$) and had a less UV-shifted hue (paired t-test, $t_8 = -3.179$ $p = 0.013$) than before treatment. Crown reflectance of the UV(+) males (n = 8) was no longer significantly different from pre-treatment values ($p > 0.30$). Nonetheless, both treatment groups still differed significantly in crown hue ($t_{14} = 2.178$, $p = 0.047$), with UV(-) males having a less UV-shifted hue than UV(+) males. To account for this fading of the treatment we included the time (in days) between treatment and laying date as a covariate in the analysis (see below).

Paternity analysis

We used eight polymorphic microsatellite markers (*Pca3*, *7*, *8* and *9* (Dawson et al. 2000), *Pocc1* and *6* (Bensch et al. 1996), *Phtr3* (Fridolfsson et al. 1997) and *PK11* (Tanner SM, Richner H, Schuenperli D, unpublished; EMBL accession no: AF041465)) to determine the paternity of 2430 offspring (including dead nestlings and embryos from unhatched eggs) from 227 broods. We followed a standard PCR protocol using about 20 ng genomic DNA, 0.25 U of taq DNA polymerase (Promega) and 1.5 mM $MgCl_2$. The PCR profiles had the following annealing temperatures: 53˚C (*Pca8*, *Phtr3*) 55 ˚C (*Pca3*), 56˚C (*Pocc1*), 57˚C (*Pca9*, *Pocc6*) and 60˚C (*Pca7*, *PK11*). Amplified fragments were resolved on an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

The combined probability of exclusion (Jamieson 1994) for the marker set was >0.999. Paternity was excluded if two or more loci showed mismatches between putative fathers and offspring. In 2051 cases, there was no (*n* = 1987) or one mismatch (*n* = 73) with the social father. For these offspring, the average probability of false inclusion (Jeffreys et al. 1992) was $2.15 \times 10^{-4} \pm 8.19 \times 10^{-4}$s.d. (range $4.77 \times 10^{-3} – 9.44 \times 10^{-10}$). We therefore conclude that they were sired by the social male and that the single mismatches were due to mutations or typing errors. The remaining offspring showed two or more mismatches with the putative father and were thus sired by extra-pair males. For 215 of the extra-pair offspring, an alternative male matched the paternal genotype completely. Fifteen offspring showed a single mismatch with the putative extra-pair father, and in 12 of these cases the male was the unequivocal sire of another offspring in that brood. For these 15 offspring the average probability of false inclusion was $2.90 \times 10^{-5} \pm 9.71 \times 10^{-5}$ s.d. (range $3.79 \times 10^{-4} – 1.61 \times 10^{-8}$) and we therefore assigned them to their putative extra-pair fathers. Overall the probability of false inclusion for the 230 assigned extra-pair offspring was $2.79 \times 10^{-5} \pm 2.09 \times 10^{-4}$ (range $3.07 \times 10^{-3} – 7.59 \times 10^{-10}$).

Statistical analysis

We constructed Generalized Linear Models (GLM) using SPSS 12. Full models were simplified by excluding variables in order of decreasing significance until only terms with $p < 0.1$ remained in the model. Excluded variables were included in the final model one by one to confirm their lack of significance. Throughout the paper parametric tests were used when variables were normally distributed, otherwise we employed their non-parametric equivalents. All tests are two-tailed.

Correlational data: the aim of this part of the study was to verify the relationships between within- and extra-pair success and male crown colour observed in 2001 (Delhey et al. 2003). Since both used colour variables, hue and UV chroma are highly correlated ($r = -0.76$, $p < 0.001$, $n = 127$) we analyzed their effects in separate models. Data from the three years were pooled and for males that bred in more than one year we randomly included only one breeding attempt in the analyses to avoid pseudoreplication. In case of polygyny only the primary brood of a male was used (Johnsen et al. 2001). Polygyny is rare in our study population (less than 10% of the males, Chapter 6), and including the offspring from secondary females as part of within-pair success does not change the results (not shown). Polygynous and monogamous males did not differ in crown colour (Mann-Whitney test: hue, $Z = -0.61$, $p = 0.54$; UV chroma, $Z = -0.22$, $p = 0.82$; $n_{monogamous} = 116$, $n_{polygynous} = 11$). As male colour differed between years (Chapter 2, 3, see also Fig. 1), we standardized (mean = 0, standard deviation = 1) hue and UV chroma for each year separately. This procedure has the desirable side effect that effect sizes can be directly compared between both colour variables. We used ordinal and binary logistic regressions (Thomson et al. 1998) to analyse the effect of colour on the following dependent variables: clutch size, likelihood of being cuckolded, likelihood of being a cuckolder, proportion of offspring sired in the own brood, number of sired extra-pair offspring and total fertilization success. Full models included study year and male age (juvenile or adult) as factors and male crown colour as a covariate. Additionally we tested for the interaction between male age and colour. The between-year consistency of the significant relationships between colour and fertilization success was tested by including *a posteriori* the corresponding colour*year interactions in the final model.

Experimental data: first, we used univariate tests to compare breeding success (date of first egg and clutch size) and fertilization success (proportion of within-pair offspring, number of extra-pair young and total fertilization success) between UV(-) and UV(+) males. However, the effect of UV reflectance manipulations may depend on confounding variables such as pre-manipulation colour (Sheldon et al. 1999), see Results), male age, or the delay between treatment and the start of laying (due to fading of the treatment, see above). Therefore, we included these terms as explanatory variables in binary or ordinal logistic regressions with breeding and fertilisation success as dependent variates and treatment ((UV(+) and UV(-)) as a factor. We also tested the interactions age*treatment, age*pre-manipulation colour and treatment*pre-manipulation colour (see Predictions in Results for rationale).

RESULTS

Correlational Results

We genotyped a total of 1877 offspring from 172 unmanipulated broods. The proportion of broods containing at least one extra-pair offspring was higher in 2001 (65%; 33/51) than in 2002 (56%, 47/84) or 2003 (56%, 21/37), but these differences were not significant (χ^2_2 = 1.07, p = 0.583). On average males sired 85.2% (SE = 1.4; median = 91.28%, range 0-100%) of the offspring in their own nest and there was little variation between study years (2001, mean = 84.6±2.4%, median = 90.0% (27-100); 2002, 85.1±2.2%, median = 91.6% (0-100); 2003, 86.1±3.0%, median = 91.6% (15-100)).

Clutch size, after controlling for the effects of year and laying date, correlated weakly with male crown colour (Table 1). Females paired to less UV males (higher hue, lower UV chroma) tended to lay larger clutches. This effect was independent of laying date (in this population early laid clutches are usually larger), since excluding this variable from the model did not change the effect of male colour (hue, p = 0.047; UV chroma, p = 0.073) and crown colour is unrelated to laying date (both colour variables, p > 0.6). The trend that less UV-ornamented males have larger clutches, albeit weak, was consistent over the three study years (not shown).

There was no consistent relationship between male crown colour and within-pair paternity in the three study years (Table 1, Fig. 1a). There was a trend indicating that cuckolded males, irrespective of age, had a less UV-shifted hue than males with full paternity in their broods, but the difference was small (Table 1). Although annual differences in this relationship were not sufficiently large to support a significant hue*year interaction (χ^2_2 = 2.60, p = 0.27), the trend for higher within-pair success for more UV males was clearly absent in 2002 and very weak in 2003 (Fig.1a). Additionally, there was no similar relationship between the likelihood of being cuckolded and UV chroma (Table 1). The proportion of within-pair paternity did not correlate significantly with male crown colour (Table 1).

Extra-pair success was mainly affected by male age, older males being more successful at siring extra-pair offspring (Table 2). On average, adult males sired 1.4 (SE = 0.3, n = 45) extra-pair offspring against 0.4 (SE = 0.1, n = 80) sired by juveniles (Mann-Whitney test, Z = -3.14, p = 0.002), and 40% (18/45) of adult males sired at least one extra-pair offspring against 16% (13/80) of juveniles (χ^2_1 = 8.71, p = 0.003). Crown hue seemed to have only a weak effect on extra-pair success, while there was no relationship between crown UV chroma

Table 1. Statistical effects of crown colour on variation in within-pair success of male blue tits (n = 125 males). Colour variables (hue and UV chroma) were tested in separate models (see Methods for details). Effect size (B) and the corresponding standard error (SE) are given for significant terms (except for year).

	Within-pair success								
	Clutch size[1]			Likelihood of losing within-pair paternity[2]			Proportion of within-pair paternity[3]		
	B (SE)	χ^2	p	B (SE)	χ^2	P	B (SE)	χ^2	p
Year	--	**9.78**	**0.007**	--	0.31	0.86	--	0.40	0.82
Age	--	0.09	0.76	--	0.36	0.54	--	0.63	0.43
Hue	**0.32 (0.16)**	**3.76**	**0.052**	**0.48 (0.18)**	**3.24**	**0.072**	--	0.86	0.35
Hue*Age	--	0.005	0.94	--	0.96	0.33	--	0.81	0.37
	--			--			--		
UV chroma	**-0.30 (0.16)**	**3.37**	**0.066**	--	0.02	0.88	--	0.06	0.81
UV chroma*Age	--	0.003	0.96	--	0.19	0.66	--	0.00	0.99

[1] ordinal logistic regression controlling for the effects of laying date, $\chi^2_1 = 7.11$, p = 0.007. [2] binary logistic regression. [3] ordinal logistic regression controlling for the effect of number of typed eggs, $\chi^2_1 = 81.28$, $p < 0.001$.

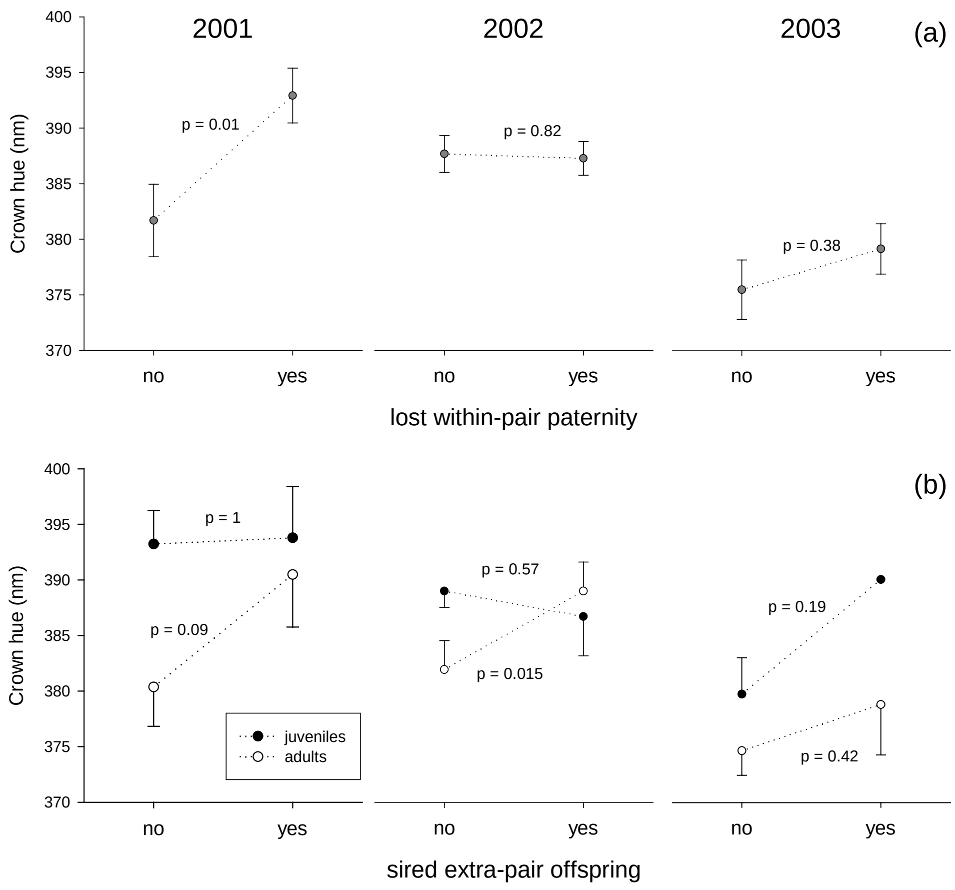

Fig. 1. Differences in crown hue between blue tit males that (a) did and did not loose within-pair paternity and (b) did and did not sire extra-pair offspring (shown separately for juveniles and adults). P-values are based on Mann-Whitney U-tests.

Table 2. Statistical effects of crown colour on variation in extra-pair success of male blue tits (n = 125 males). Colour variables (hue and UV chroma) were tested in separate models (see Methods for details). Effect size (B) and the corresponding standard error (SE) are given for significant terms (except for year).

	Extra-pair success					
	Likelihood of siring extra-pair offspring[1]			Number of extra-pair offspring[2]		
	B (SE)	χ^2	p	B (SE)	χ^2	p
Year	--	0.87	0.65	--	0.01	0.99
Age	**-1.23 (0.43)[3]**	**8.45[3]**	**0.004**	**-1.56 (0.46)[3]**	**9.74[3]**	**0.002**
Hue	**0.30 (0.24)**	**1.64[3]**	**0.20**	**0.37 (0.24)**	**2.49[3]**	**0.11**
Hue * Age	**-0.93 (0.51)[3]**	**3.41**	**0.064**	**-1.06 (0.49)[3]**	**4.56**	**0.032**
UV chroma	--	0.16	0.69	--	0.30	0.58
UV chroma *Age	--	0.08	0.77	--	0.47	0.49

[1]binary logistic regression. [2]ordinal logistic regression. [3]computed after excluding the interaction hue*age from the model.[3]computed relative to adult birds.

and extra-pair success (Table 2). However, the significant interaction hue*age (Table 2) and visual inspection of the data (Fig. 2b), suggest that the relationship between hue and extra-pair success may be different in both age classes. After re-analysing the data for both age classes separately, there was a clear relationship between extra-pair success and hue among adults, but not among juvenile males. Adult males that sired extra-pair offspring had a less UV-shifted hue than adult males that did not sire extra-pair young (t-test, $t_{53} = -2.41$, $p = 0.019$; Fig. 2b) but this was not the case among juvenile males (t-test, $t_{89} = 0.10$, $p = 0.92$; Fig. 2b). Moreover, the number of extra-pair offspring sired correlated positively with hue among adult ($r_s = 0.33$, $p = 0.012$, $n = 55$) but not juvenile males ($r_s = 0.01$, $p = 0.93$, $n = 91$). These differences between juvenile and adult males were relatively consistent over the three study years as indicated by the non-significant year*hue*age interaction (likelihood of siring extra-pair offspring: $\chi^2_2 = 1.51$, $p = 0.47$, Fig. 2b; number of extra-pair offspring: $\chi^2_2 = 3.32$, $p = 0.19$).

Pairwise comparisons between cuckolding males and the males they cuckolded confirmed that cuckolders are on average older than the males they cuckold (Wilcoxon paired test, $Z = -3.4$, $p = 0.001$, $n = 49$). Overall there were no differences in colour between cuckolder and cuckolded males (paired t-test: hue, $t = 0.78$, $p = 0.44$; UV chroma, $t = -1.17$, $p = 0.24$, $n = 48$). However, in those cases were the cuckolder was an adult male ($n = 33$) it had a less UV-shifted hue (paired t-test, $t = 2.19$, $p = 0.036$) and lower UV chroma (paired t-test, $t = -2.26$, $p = 0.030$) than the male it cuckolded. This was not the case when the cuckolder was a juvenile male (paired t-test, hue, $t = -1.25$, $p = 0.23$; UV chroma, $t = 1.13$, $p = 0.28$; $n = 15$).

Total fertilization success, the sum of within-pair and extra-pair offspring, was mainly influenced by male age (B(SE) = -1.23 (0.35); $\chi^2_1 = 12.49$, $p < 0.001$). Adult males sired on average 10.8 (SE = 0.6) offspring against 9.4 (SE = 0.3) sired by juveniles (Mann-Whitney, $Z = -2.58$, $p = 0.010$). This was mainly due to adult male's higher extra-pair success (see above), since the number of within-pair offspring did not differ significantly between age classes (adults: 9.5 (SE = 0.4); juveniles: 9.0 (SE = 0.3); Mann-Whitney, $Z = -1.37$, $p = 0.17$). After controlling for age differences, total fertilization success was negatively related to the expression of UV coloration (hue, B(SE) = 0.55 (0.17), $\chi^2_1 = 10.53$, $p = 0.001$; B(SE) = UV chroma, -0.35 (0.16), $\chi^2_1 = 4.27$, $p = 0.038$). The interaction between age*colour was not significant indicating that this pattern was similar for both age classes ($p > 0.17$). Similarly, the colour*year interaction was non-significant for both colour variables ($p > 0.45$).

Predictions for the experiment

Based on the results form the correlations, somewhat different from the findings of Delhey et al. (2003), we could make the following predictions regarding the experimental results. (1) If the pattern found in 2001 and the weak trend in the overall data set (Fig. 1a) reflect a female preference for more UV-ornamented social mates (since they are less cuckolded), males in the UV(+) treatment should experience (slightly) higher within-pair breeding success than UV(-) males. (2) If being less UV ornamented directly improves the chances of adult males to sire extra-pair offspring we would expect adult UV(-) males to have higher extra-pair success. These predictions would be supported by significant treatment effects or a significant treatment*age interaction. However, these relatively simple predictions are complicated in that the response to treatment may depend on the colour prior to treatment. Indeed, Sheldon et al. (1999) showed that a reduction in UV reflectance has a much stronger negative effect on males that were highly UV reflective before the treatment than on naturally UV dull males. Hence, we would expect a more marked decrease in within-pair paternity among UV(-) males which were highly UV-reflectant prior to treatment. Following this line of thought we would also expect a similar negative correlation in the UV(+) treated males, since naturally less UV-ornamented males may benefit more from an experimental boost in presumed attractiveness than naturally highly UV-reflectant males. A significant negative effect of pre-manipulation colour on within-pair success would lend support to these predictions.

Experimental Results

We obtained paternity estimates for 23 UV(-), 19 UV(+) and 37 unmanipulated males in 2003. In total we typed 836 offspring in these broods. Extra-pair offspring accounted for 15% (126/836) of the typed offspring and overall extra-pair offspring were found in 60% of the broods (48/79). Three males (one UV(-) and two UV(+) males) sired none of the offspring in their broods and the proportion of sired offspring ranged from 0 to 100% with a mean of 83% (SE = 3).

Females mated to UV(+) males laid their first egg earlier than females mated to UV(-) or unmanipulated males (Table 3). Surprisingly, given that in this population earlier clutches are usually larger (Table 1), UV(+) males tended to have smaller clutches than UV(-) males (Table 2). Overall, there were no differences in the proportion of sired offspring between UV(-) and UV(+) males, although UV(+) males had lower within-pair success (Table 3).

Table 3. Differences in reproductive success between UV(-) and UV(+) treated male blue tits. Values for unmanipulated males in 2003 are given for comparison. Shown are means and their standard errors (in brackets).

	UV(-) N = 23	UV(+) N = 19	Mann-Whitney U-test	Unmanipulated N = 37
Date of first egg[1]	105.7 (0.7)	103.3 (1.1)	Z = -2.24, p = 0.025	104.4 (0.6)
Clutch size	11.4 (0.24)	10.7 (0.4)	Z = -1.66, p = 0.096	11.5 (0.2)
% within-pair offspring	83.6 (4.6)	77.3 (7.2)	Z = -0.17, p = 0.87	86.1 (3.0)
No. extra-pair offspring	0.4 (0.4)	1.4 (0.7)	Z = -1.89, p = 0.059	0.8 (0.3)
Total fertilization success	9.2 (0.7)	9.7 (1.3)	Z = -0.37, p = 0.71	10.2 (0.4)

[1] 1 January = day 1

Table 4. Effect of the colour manipulation treatment, and potentially confounding variables, on male blue tit within-pair and extra-pair success, n = 23 UV(-) and 19 UV(+) males, df = 1 for all variables. Significant terms are depicted in bold, see text for details. All tested pre-treatment colour*treatment and pre-treatment colour*age interactions were non significant (p > 0.4).

	Within-pair paternity (WPP)				Extra-pair paternity (EPP)			
	Likelihood of losing WPP[1]		Proportion of WPP[2]		Likelihood of siring EPP[1]		Number of extra-pair offspring[3]	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Treatment	0.61	0.43	0.32	0.57	**4.32**	**0.037**	**3.89**	**0.048**
Age	0.10	0.75	0.004	0.95	2.65	0.10	2.52	0.11
Pre-treatment hue	0.07	0.78	1.08	0.30	0.63	0.43	0.48	0.49
Pre-treatment UV chroma	0.52	0.47	0.69	0.40	0.21	0.64	0.31	0.57
Treatment-to-1stegg[4]	1.70	0.19	1.79	0.18	0.10	0.74	0.17	0.68
Treatment*age	0.07	0.78	0.88	0.35	0.52	0.47	0.60	0.44

[1]binary logistic regression, [2]ordinal logistic regression with number of within-pair offspring as dependent variable while controlling for the number of typed offspring (χ^2_1 = 34.69, $p < 0.001$), [3]ordinal logistic regression, [4]number of days between treatment and the start of laying,

Seven out of 23 males (30%) were cuckolded in the UV(-) treatment and 8 out of 19 (42%) in the UV(+) treatment ($\chi^2_1 = 0.61$, $p = 0.43$).

Extra-pair success tended to be higher for UV(+) males, since they sired more extra-pair offspring (Table 3). Indeed, only one out of 23 UV(-) males sired extra-pair offspring, against 5 out of 19 UV(+) males ($\chi^2_1 = 4.10$, $p = 0.043$). Total fertilization success did not differ between treatments (Table 3).

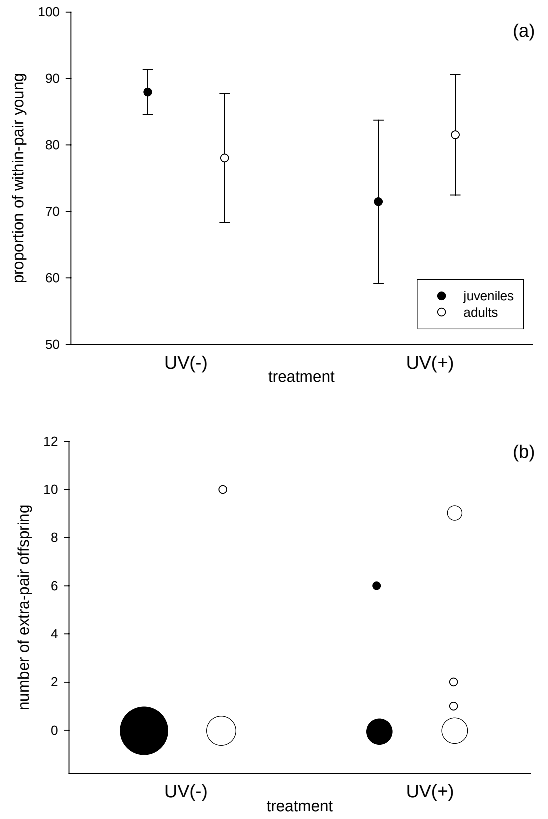

Fig. 2. Effects of plumage colour treatment. UV(+) or UV(-) on (a) within-pair paternity (proportion of young sired) and (b) extra-pair success (number of extra-pair young sired). Shown are means and standard errors in (a). In (b) increasing dot size represents 2, 6, 7, 9 and 13 data points, respectively.

Multivariate models containing potentially confounding variables largely confirmed the results from the previous analyses (Table 4). Neither age, colour pre-treatment or the time interval between treatment and the date of first egg had an effect on the proportion of within-pair offspring (Fig. 4a) or on the likelihood of being cuckolded. The number of extra-pair offspring, and the likelihood of siring extra-pair offspring were weakly influenced by treatment and by male age, older and UV(+) males having higher extra-pair success (Fig 4b). Neither the time interval between treatment and the date of first egg or the colour pre-treatment contributed significantly to these models (Table 4). Since only one UV(-) and only one juvenile male sired extra-pair offspring this precluded the inclusion of the interactions pre-treatment colour*treatment and pre-treatment colour*age in the models. However, there were no obvious trends between colour pre-treatment and number of extra-pair offspring within the subgroup of UV(+) adult males (hue, $r_s = 0.28$, $p = 0.41$; UV chroma, $r_s = -0.18$, $p = 0.60$; $n = 11$).

DISCUSSION

Correlations

Based on a large sample collected over three breeding seasons we found largely no (or a very weak) relationship between crown colour and within-pair fertilization success. Extra-pair fertilization success was correlated with male crown colour but only among adults, with less UV-ornamented males being more successful at siring extra-pair offspring (Table 1, Fig. 1b). This negative correlation between UV ornamentation and extra-pair success plus a weak negative correlation with clutch size, resulted in an overall negative relationship between total fertilization success and the degree of male ornamentation in both age classes (Table 1): less UV-ornamented blue tit males sired more offspring, confirming the result from our previous study (Delhey et al. 2003).

Why do older, less UV, males sire more extra-pair young?

In many species of passerine birds adult males are much more successful at siring extra-pair offspring than juveniles (Griffith et al. 2002). This pattern has been interpreted as female preference for high quality males, which have proven their viability (Brooks and Kemp 2001), but could also reflect a change in behavioural strategies as males age (Johnsen et al. 2003). Hence higher extra-pair success of adult males could be due to a combination of male tactics and female preference (Westneat and Stewart 2003). Indeed, in the blue tit both male- and female-driven pursuit of extra-pair copulation seem to exist since females have been observed to undertake around-dawn extra-pair forays (Kempenaers et al. 1992; own obs.) while males often intrude other male's territories (Foerster and Kempenaers 2005). If adult males invest more time in extra-pair behaviours and/or are preferred by females, being less UV may be beneficial for older males either (1) because females directly prefer less UV ornamented males as extra-pair partners or (2) because being less UV facilitates intrusions into territories of other males. Alternatively, (3) crown coloration may not be causally involved in extra-pair success but correlate with an unknown trait that enhances extra-pair success. If this were the case we expect to see no effect of colour manipulation on male extra-pair success (see discussion of experimental results below). We now focus on the first two possibilities.

The hypothesis of direct female preference for less UV adult males in this population finds some support in the pattern of brood sex allocation (Chapter 5). Females paired to adult males produce more male biased broods with decreasing UV ornamentation of their mate, while this pattern is reversed in females paired to juvenile males (Chapter 5). According to the theory of sex allocation based on mate attractiveness, females should produce more male offspring if paired to a sexually attractive male, since sons would benefit by inheriting their father's attractiveness (West and Sheldon 2002). If this applies to the blue tit it would mean that females find less UV-ornamented older males more attractive. However, it remains puzzling then why females are not more faithful to less UV ornamented adult males or even to adult males in general (Table 1, Fig. 1b).

Alternatively, adult blue tits might benefit from being less UV ornamented through increased success at intruding into neighbouring territories. Male blue tits usually respond extremely aggressively towards taxidermic mounts simulating an intruder. However, aggressiveness is considerably reduced if mounts are manipulated to reduce crown UV reflectance (Alonso-Alvarez et al. 2004). Hence reduced UV-ornamentation might be a desirable attribute among older males seeking extra-pair copulations in foreign territories. In other species, dull juveniles are more likely to be tolerated by older males since they pose little danger to their paternity (Greene et al. 2000), and likewise juvenile blue tits are far less proficient at obtaining extra-pair paternity (Table 1). Interestingly, those adult males that sire extra-pair offspring resemble UV-dull juveniles (Fig. 2b): age-differences in crown colour are only evident among males that did not sire extra-pair offspring (hue juveniles: 387.9 nm, SE = 1.4, n = 67; hue adults: 378.3 nm, SE = 1.8, n = 27; t-test for unequal variances, t = 3.99, $p < 0.001$) and not among males that did sire extra-pair offspring (hue juveniles: 388.5 nm, SE = 3.0, n = 13; hue adults: 385.4 nm, SE = 3.5, n = 18; t-test, t = 0.54, p = 0.59). This lends support to the idea that UV-dull adult males could be mimicking juveniles to deceive territorial males about their true age when roaming for extra-pair copulations.

What do more UV ornamented males gain?

Our results imply that there is little to be gained from being a highly UV ornamented blue tit male in our study population. Although the likelihood of being cuckolded was smaller for more UV males in one of three years, the effect is small compared to the increase in extra-pair success associated with decreased UV ornamentation. The obvious question then becomes: what selects for UV coloration in this species? While the present study addresses selection through the number of sired offspring, there are other important selective forces operating in the life of a male blue tit, such as surviving and obtaining and keeping a territory and a (high quality) mate.

A survival advantage appears an unlikely candidate for a selective force maintaining crown UV ornamentation, since annual survival of juvenile and adult male (and female) blue tits is unrelated to their crown colour (Chapter 2). On the other hand, an advantage of highly UV-ornamented males in obtaining a territory or a mate could result in a strong selection pressure for ornament exaggeration (Siefferman and Hill 2005), potentially surpassing the more subtle effects of extra-pair success. Although there is a suggestion that crown coloration is involved in male-male interactions (Alonso-Alvarez et al. 2004), what attributes of a male determine the outcome of territorial interactions in the blue tit is unknown. Similarly, we lack information on which males were unsuccessful at finding a mate, since they are not part of the breeding population, and remain undetected. Theoretically, more ornamented males could also benefit by obtaining a higher quality mate. In another blue tit population assortative mating by crown coloration was observed (Andersson et al. 1998) implying mutual mate choice based on crown ornamentation as an indicator of quality (Hunt et al. 1999). However, in the three years of our study we found no evidence of assortative mating by crown coloration (Chapter 6), and female coloration did not reflect female quality in terms of reproductive output (Chapter 6). Since it is therefore unlikely that crown colour is related to the quality of the mate obtained or to survival, future studies should address the role of male crown coloration in acquiring a territory and a mate.

Experimental Results

Within-pair paternity was not significantly affected by the experiment. Neither treatment nor any other variable included in our models had an effect on the proportion of within-pair paternity or the likelihood of being cuckolded (Table 3). The lack of treatment effect coupled with the weak, inconsistent relationship between crown UV reflectance and within-pair paternity in the correlational data set (Table 1) undermines the hypothesis that females are more faithful to more UV-ornamented males or that these males are more successful at defending their paternity (Delhey et al. 2003). However, potential pitfalls of the experiment (see below), the significant albeit weak selection pressure in one year (Delhey et al. 2003) and the fact that selection pressures often vary considerably between years and populations (this study, Griffith et al. 2003) makes dismissal of selection of male blue tit crown colour through within-pair paternity premature. Nonetheless, it seems that selection on male colour through this component of fertilization success is weak at best.

Extra-pair success was affected by treatment, with UV(+) males being more likely to cuckold other males and hence sire more extra-pair offspring than UV(-) males (Fig. 2b).

However, this pattern is the opposite of what we had expected based on the observed relationships (Table 1). If crown colour has a direct effect on extra-pair success, adult males from the UV(-) treatment should have been more successful at siring extra-pair offspring. Alternatively, we hypothesized that crown colour may be negatively correlated to a, yet unidentified, variable which increases a male's chances of siring extra-pair offspring (Griffith et al. 2003). Under this scenario we would expect no treatment effect on extra-pair paternity. The results from the experiment, however, do not fit with any of these predictions. It could be argued that treatment effects were weak and hence the result could be a statistical artifact (type I error). However, note that the treatment effect on extra-pair success was stronger than the effect of age (Table 3), a male attribute known to be very important for extra-pair success in this and other species (Griffith et al. 2002).

Conflicting results: confounded correlations or flawed experiment?

The results from the experiment highlight the fact that previous knowledge about correlational patterns is necessary for a meaningful interpretation of the outcome of phenotypic manipulations. Had we performed the experiment without previous information on the relationship between extra-pair paternity and crown colour, we would have concluded that less UV reflectant males are avoided and/or more UV males preferred by females as extra-pair mates. The logical conclusion would then have been that blue tit male crown UV reflectance is under direct sexual selection through extra-pair mating success. While it remains a possibility that, everything else being equal, females prefer more UV-ornamented males as extra-pair partners, this potential preference is not enough to counter the overriding advantage at siring extra-pair young that older, less UV males have in real life.

Experimental manipulations are often regarded as the most appropriate way of teasing apart causative effects from correlated responses (Kempenaers and Sheldon 1997; Milinski 1997). However, manipulating a bird's phenotype may have unexpected consequences that could confound the results. For example, when birds have multiple ornaments that show some degree of intercorrelation (Møller and Pomiankowski 1993; Andersson et al. 2002), manipulating only one ornament may cause mismatches between otherwise correlated traits (Sheldon et al. 1999). In blue tits, besides the conspicuous crown feathers, males also show UV/blue coloured wing coverts and tail feathers, and colour expression is correlated among these patches (Sheldon et al. 1999, Delhey et al unpubl. data). These correlations were potentially disrupted by the manipulation of the crown plumage, an inherent problem of ornament colour manipulation. Additionally, mismatches between manipulated appearance and behaviour can cause unexpected experimental results. For example, enlarging the badge of status in Harris sparrows (*Zonotrichia querula*) led to increased dominance rank only if coupled with simultaneous testosterone implants, to also increase dominance behaviour (Rohwer and Rohwer 1978). In our blue tit population male crown coloration correlates with natural testosterone levels in an age-dependent manner, with testosterone levels increasing with increasing UV ornamentation in juveniles, and decreasing with increasing UV colour in adults (Peters et al. submitted). Hence, changing male colour without changing male testosterone could have confounded our experimental results. Other problems with current experimental approaches are manipulations that go beyond the natural range of variation, for example using sunblock to manipulate UV reflectance (Johnsen et al. 1998; Sheldon et al. 1999), or the fading of treatment with time as in the present study. Therefore, being aware of the potential weaknesses of the experimental approach used is a crucial step towards the meaningful interpretation of its results.

Surprisingly few studies have experimentally tested the link between male ornamentation and paternity, possibly due to difficulties in designing methods to realistically manipulate male phenotype. Numerous studies have described correlations between the expression of male ornaments and within- and/or extra-pair success (see review in Griffith et al. 2002). However, experimental manipulation of the male ornament to test for a direct causal link with fertilization success was attempted for only two species: barn swallows (*Hirundo rustica*), where tail length was manipulated (Smith et al. 1991; Saino et al. 1997) and bluethroats (*Luscinia svecica*) where throat patch UV reflectance was reduced with sunscreen (Johnsen et al. 1998). Moreover, in two studies the experimental results did not fit the correlational evidence, most likely due to the fact that manipulations were beyond the natural range of variation (Smith et al. 1991; Johnsen et al. 1998; Johnsen et al. 2001). Hence experimental evidence supporting a relationship between paternity and ornamentation in birds is still badly needed.

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Chapter 5 - Brood sex ratio and male UV ornamentation in blue tits: conflicting correlational and experimental evidence?

Kaspar Delhey, Anne Peters, Arild Johnsen, and Bart Kempenaers

Max Planck Institute for Ornithology, Seewiesen.

ABSTRACT

Sex-allocation theory predicts that females paired to attractive males should bias the brood sex ratio towards male offspring since these would inherit the attractiveness of their father. We studied sex allocation based on male ornamentation, using three years of data from an Austrian population of blue tits. Brood sex ratios varied with male UV coloration in an age-dependent manner. For juvenile males, the proportion of sons increased with increasing UV ornamentation, in agreement with previous findings that blue tit females seem to consider highly UV-reflectant males more attractive. However, the relationship between UV ornamentation and brood sex ratio was reversed for adult males, with females paired to less UV-ornamented adult males producing more sons. This pattern fits with the observation that, in our population, less UV ornamented adult males sire the majority of the extra-pair young and therefore more young in total. To test the causality of the association between brood sex ratio and coloration, we experimentally increased, UV(+), or decreased, UV(-), male coloration within the natural range. Contrary to our expectations there was no significant effect of the treatment, male age, or their interaction on brood sex ratio. However, in UV(-), but not UV(+) males, the proportion of sons was negatively correlated with male coloration before manipulation. This suggests that the treatment caused more UV ornamented males to decline more in attractiveness than less ornamented males and highlights that effects of experimental treatment may vary unexpectedly with the phenotype of the subject.

INTRODUCTION

Sex-allocation theory predicts that when parents derive higher relative fitness benefits from producing offspring of one sex instead of the other, they should adjust the sex ratio of their young accordingly (Trivers and Willard 1973). While there is growing empirical evidence consistent with such adaptive sex-allocation by female birds (see meta-analysis in West and Sheldon 2002), negative or inconsistent results are also widespread (Komdeur and Pen 2002; Krackow 2002; Pike and Petrie 2003; Rosivall et al. 2004). Moreover, in many cases it is difficult to predict what patterns to expect if adaptive brood sex ratio adjustment would take place (West and Sheldon 2002). These issues have cast doubt on the generality of adaptive sex allocation in birds, suggesting that positive evidence is over-represented among published studies (Andersson 1994; Palmer 1999; Krackow 2002).

Strong evidence for adaptive sex allocation in birds comes from female sex ratio manipulation based on male attractiveness, because a clear prediction can be made under an adaptive sex allocation scenario: females mated to more attractive males should bias brood sex ratio towards sons (West and Sheldon 2002; but also see Ewen et al. 2004). This would be predicted given that males usually have higher variance in reproductive success than females, and the most successful males are usually the most ornamented (Andersson 1994). Therefore sons would benefit more than daughters by inheriting their fathers' attractiveness.

Even if correlational evidence supports adaptive female sex-allocation based on male attractiveness, a causal relationship between male phenotype and brood sex ratio can only be demonstrated by experimental manipulation of male traits. The blue tit (*Parus caeruleus*) is one of the few species for which correlational studies of sex-allocation and male ornaments have been combined with experimental manipulation of male attractiveness. In this species, males and females have a bright blue crest that is used in intra-specific displays (Cramp and Perrins 1993). Although the sexes look superficially similar to the human eye, they show strong sexual dimorphism in the near ultraviolet range (UV, 300-400 nm), with males being relatively more UV-reflectant than females(Andersson et al. 1998; Hunt et al. 1998). Blue tit females paired to males with high survival prospects have been shown to bias brood sex ratio towards sons in a Swedish (Svensson and Nilsson 1996) but not in a British population (Leech et al. 2001). Furthermore, in another Swedish population, more UV ornamented blue tit males showed higher over-winter survival (Sheldon et al. 1999; Griffith et al. 2003). These results suggest that females could use the degree of UV ornamentation to assess their mate's quality. Indeed, correlational evidence showed that, in two out of three study years, females biased brood sex ratio towards male offspring if paired to a highly UV ornamented male

(Griffith et al. 2003). Surprisingly, experimental reduction of male attractiveness, by applying sunblock chemicals on the crown feathers (which largely eliminated UV reflectance) did not result in female-biased brood sex ratio (Sheldon et al. 1999). However, this treatment reversed the positive relationship between crown coloration and sex ratio: those males that had been more UV ornamented before manipulation subsequently showed a more female biased brood sex ratio, presumably since these males suffered a greater reduction in UV reflectance due to the treatment (Sheldon et al. 1999).

To improve our understanding of adaptive sex ratio manipulation, replication of studies across different years and populations are necessary (Hasselquist and Kempenaers 2002; Komdeur and Pen 2002), since consistent results would provide support for the generality of sex-allocation strategies (Griffith et al. 2003). Here we report on a three-year correlational study to assess whether (seemingly adaptive) patterns of sex-allocation related to male UV ornamentation, as found in the Swedish blue tit population, also occur in our study population in Vienna, Austria. However, recent evidence (Delhey et al. 2003) suggests that patterns of male attractiveness may differ between these populations, leading to different predictions for adaptive sex allocation. In our population paternity analysis revealed that less UV ornamented adult males have higher extra-pair and total reproductive success than more ornamented males (Delhey et al. 2003; Chapter 4), while overwinter survival is unrelated to male crown colour (Chapter 2). Since less UV ornamented adult males seem to have higher fitness in our population, we predict that females paired to such males should bias brood sex ratio towards sons. In addition to the observational study, we performed a colour manipulation experiment to assess the causality of the observed pattern. Unlike Sheldon et al. (1999), however, we did not eliminate UV reflectance but increased and decreased crown UV coloration within the natural range.

METHODS

General Methods

Fieldwork was carried out in Vienna, Austria (48º 13’ N, 16º 20’ E) between 2001 and 2003. The study area, of approximately 35 ha is part of a large continuous tract of deciduous mixed woodland, and contained 220 nestboxes in 2001 and 250 in 2002 and 2003.

We collected data on basic reproductive biology by regular nestbox checks from the end of March until June. Adult birds were caught in nestboxes either in winter while roosting or while feeding 8-10 day old chicks in spring. All captured birds were banded with a numbered aluminium ring and a unique combination of three plastic colour bands. A small blood sample (5-50 µl) was taken from the brachial vein and morphometric and colour (only in spring, see (b)) measurements were performed. We measured tarsus (with callipers, to the nearest 0.05 mm), and body mass (with an electronic balance, to the nearest 0.1 g). Birds were sexed by presence or absence of a brood patch (in spring) and aged according to Svensson (1992) as juveniles or adults.

A small blood sample (5-25 µl) was taken from the nestlings between day 9 and 14 after hatching for molecular sex determination. Additionally, all unhatched eggs and dead chicks found before blood sampling were collected and preserved in ethanol.

Colour measurement

Reflectance of the crown feathers was measured during the chick-feeding phase of the breeding cycle in late spring (late April to late May). We used a S-2000 spectroradiometer and DH-2000-FHS Deuterium-Halogen light source (Ocean Optics, Eerbek, Netherlands) connected through a bifurcated fibre optic probe. The probe, mounted with a cylindrical plastic tube to exclude ambient light and standardise measuring distance, was held perpendicular to the feathers. Measurements were taken of five standardised spots on the crown. Reflectance was calculated relative to a white standard (WS-2) with the software Spectrawin (Top Sensor Systems). Raw spectra were smoothed by a running average calculated on a 10 nm interval. Colour coefficients describing the variation in spectral shape were calculated from the smoothed spectra and averaged for each individual. 'Brightness' (spectral intensity) was calculated as the average reflectance (R_{av}) in the 300 to 700 nm interval, which encompasses the entire visual sensitivity range of passerine birds. 'Hue' (spectral location) was estimated as the wavelength of peak reflectance ($\lambda(R_{max})$), and 'chroma' (spectral purity) was calculated as $(R_{max}- R_{min})/R_{av}$, that is, the difference between peak and trough divided by brightness. Additionally, we calculated UV chroma, ($R_{300-400}/R_{300-700}$), representing the relative amount of reflected UV light. This variable has been found to correlate with brood sex ratios in other studies of blue tits (Sheldon et al. 1999; Griffith et al. 2003). Some of the variables used to describe colour variation are strongly correlated (hue vs. chroma, $r = -0.51$; hue vs. UV chroma, $r = -0.76$; chroma vs. UV chroma, $r = 0.90$ and chroma vs. brightness, $r = 0.23$, all $p < 0.01$; $n = 124$ males) while others are not (hue vs. brightness, $r = 0.05$ and UV chroma vs. brightness $r = 0.07$, both $p > 0.4$; $n = 124$ males).

Colour manipulation experiment

The aim of the treatment was to manipulate the crown colour signal within the natural range of variation found in our population, as opposed to previous studies where the UV band was almost completely blocked (Andersson and Amundsen 1997; Johnsen et al. 1998; Sheldon et al. 1999). Males were captured in early spring 2003 (March-April), while they were roosting in nestboxes at night (n = 30) or by employing playback and mist nets during the day (n = 17). On average males were captured and manipulated 17.9 (SE = 0.9) days before their mates laid their first egg (range 1-25 days). Pre-manipulation colour was measured and males of both age classes were sequentially allocated to one of two treatments: UV(+), where relative reflectance in the UV was enhanced, and UV(-), where UV reflectance was reduced. To manipulate crown coloration, Edding 4500 "T-Shirt Marker" pens (Ahrensburg, Germany) were used. Similar marker pens have been used successfully to manipulate carotenoid-based and structural coloration in birds (Pryke et al. 2002; Ballentine and Hill 2003). In the UV(+) treatment, crown feathers were painted with a light blue pen (Edding col.10), while in the UV(-) treatment, the crown was first painted with a dark blue pen (Edding col.03) and then with the light blue pen (Edding col.10) on top. To enhance water-resistance of both treatments, we applied a coat of silicone-based fly-dressing (Balzer Silicone-Fett, Germany) after painting the feathers. After completing the treatment, crown colour was measured again. Treated males behaved normally and their ability to raise the crown feathers was not impaired.

Molecular sexing

We determined offspring sex using either the Z-linked microsatellite marker *Phtr3* (Fridolfsson et al. 1997), or the sexing primers *P2* and *P8* (Griffiths et al. 1998). In the samples from 2001 and 2002, the majority of offspring were sexed using *Phtr3* (83% and 53%, respectively) while the remaining were sexed with *P2*/*P8*. In 2003, all offspring were sexed with the *P2*/*P8* primers. Fluorescently labelled PCR products were run on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, U.S.A.) and fragment sizes were scored using Genotyper 1.1 (Applied Biosystems). Due to early embryo mortality or possibly unfertilised eggs we were unable to sex all samples. On average, we sexed 92.7% of all offspring (range: 46-100%, N = 115 broods) in the correlational data set. From the experimental broods we sexed on average 92% of the offspring, but there where treatment differences. We sexed only 87.5% (range: 30-100%) of the offspring in the UV(-) treatment against 97.3% (range: 75-100%) in the UV(+). This difference was mainly due to three broods in the UV (-) treatment where we sexed less than 70% of the offspring due to partial brood mortality. This is unlikely to have biased our results since in the analysis we weighed each data point by the proportion of sexed offspring (see (e)), and there was no relationship between the proportion of offspring sexed and the sex ratio of the experimental broods (Spearman rank correlation, $r_s = 0.00$, $p = 0.99$, $N = 44$), or non-experimental broods ($r_s = 0.097$, $p = 0.30$, $N = 115$). Moreover, excluding the three broods with less than 70% sexed offspring resulted in qualitatively similar results, albeit with lower power (not shown).

Statistical analysis

Brood sex-ratios were analysed with Generalised Linear Models (GLM) using number of male offspring as the dependent variable and brood size (number of sexed offspring) as the binomial denominator. We used a logit link and corrected for over- or under-dispersion (Wilson and Hardy 2002). In all models, each data point was weighted by the proportion of the brood that was sexed. Repeating the analyses without this weighting does not affect the patterns (not shown). When individuals were recorded breeding in more than one year, we randomly included only one breeding attempt and each bird (male or female) entered the analysis only once. Only first broods were included in the analyses, and in case of polygynous males only their primary broods were used, defined as the earliest of all broods of a particular male.

Given that most colour variables were highly correlated (see (b)), we constructed independent models for each of them. All male traits (colour variable, morphometrics, age and the interaction between age and male colour) as well as date of first egg, clutch size and year (2001, 2002 or 2003) were entered in the initial model. Explanatory variables were sequentially excluded in order of decreasing significance until only terms with $p < 0.1$ remained in the model. Excluded terms were then re-entered one-by-one in the final model to confirm that they did not explain a significant part of the variation. The same procedure was used to analyse the effects of colour treatment on brood sex ratios. In this case the full model contained treatment, male age, colour pre-treatment (see Sheldon et al. 1999 and Discussion for rationale), delay in days between treatment and first egg in the brood, and the interactions age x treatment, colour pre-treatment x treatment, colour pre-treatment x age and age x treatment x colour pre-treatment. When there were significant age or treatment interactions (see Results), we additionally performed the analyses separately for the two age classes or treatments. Residuals of all models were examined to assess departure from normality (Wilson and Hardy 2002).

A potential confounding factor when correlating brood sex ratio with male attractiveness, is sex allocation based on paternity (Bensch 1999). If female infidelity relates to male coloration (Delhey et al. 2003) and if extra-pair young are sired by high quality males, then females are expected to bias the sex ratio of broods containing many extra-pair young towards males (Bensch 1999). There is little evidence that this is the case in the blue tit: extra-pair young were more likely to be male in a Belgian population (Kempenaers et al. 1997), although in this study offspring sex was determined based on plumage colour and not molecular sexing, while no sex-bias was found in a British population (Leech et al. 2001). Likewise, in our study population the proportion of male offspring was unrelated to the proportion of extra-pair offspring in the brood (Spearman rank correlation, $r_s = 0.11$, $p = 0.23$, $n = 118$). Nevertheless, we repeated the correlational analyses excluding broods with mixed paternity. Because all the patterns of sex allocation based on male colour remained unchanged we only report the results from the full data set here.

RESULTS

Correlational data

In total 1228 offspring from 115 clutches were sexed, 641 of which were male (52%, SE = 1.4%). This was not significantly different from 50% (One-sample t-test, $t = 1.44$, $df = 114$, $p = 0.15$), and the sex ratio distribution did not differ significantly from a random, binomial distribution (randomisation test with 1000 iterations, $n = 115$, $p = 0.52$).

Overall, male UV ornamentation was not correlated with brood sex ratio (Table 1). However, there was a highly significant interaction between male colour and age (Table 1): among juvenile males, the proportion of male offspring in the brood was positively related to UV chroma (Fig. 1a), whereas the reverse was true for older males (Fig. 1b). The interaction between male age, UV chroma and year was not significant ($F_{2,100} = 0.22$, $p = 0.80$) and the trend was similar in the three study years (Fig. 1). The interaction between male age and UV chroma was confirmed in the separate analyses of the two age classes (adult males only; $F_{1,38} = 8.17$, $p = 0.007$, effect = -13.78, SE = 4.87; juvenile males only; $F_{1,68} = 4.60$, $p = 0.036$, effect = 8.43, SE = 3.95)). Similar interactions with age were found for chroma ($F_{1,106} = 9.42$, $p = 0.003$) and hue ($F_{1,106} = 7.81$, $p = 0.006$), but there was no effect of male brightness on brood sex ratio either in interaction with age ($F_{1,106} = 0.31$, $p = 0.58$) or as a main effect ($F_{1,108}$

= 0.37, p = 0.55). Brood sex ratio did not vary with year, clutch size, lay date or male size or condition (Table 1).

The pattern was qualitatively similar when we restricted the analysis to broods where 100% of the offspring were sexed (UV chroma x age, $F_{1,51}$ = 7.80, p = 0.007; chroma x age, $F_{1,51}$ = 4.37, p = 0.042; hue x age, $F_{1,51}$ = 2.27, p = 0.14; brightness x age, $F_{1,51}$ = 0.83, p = 0.37), suggesting that the bias in sex ratio was present at ovulation and not due to sex-biased offspring mortality.

Table 1. Generalised linear model on brood sex ratio variation in the correlational data set (2001-2003). Terms in bold are included in the final model. Terms in the final model that were part of a significant interaction could not be dropped from the model and thus show no F statistic or p value.

	Estimate (SE)	F(df1, df2)	p
Constant	**-2.57 (1.33)**	**-**	**-**
Age	**7.11 (1.99)**	**-**	**-**
UV chroma	**8.43 (4.15)**	**-**	**-**
Age * UV chroma	**-22.21 (6.12)**	**13.35 (1, 106)**	**< 0.001**
Body mass[1]		0.60 (1, 102)	0.442
Tarsus length		0.31 (1, 105)	0.581
Year		0.78 (2, 105)	0.462
Date of first egg		0.75 (1, 105)	0.390
Clutch size		0.01 (1, 105)	0.941

[1] sample size is smaller since three males were released before being weighed.

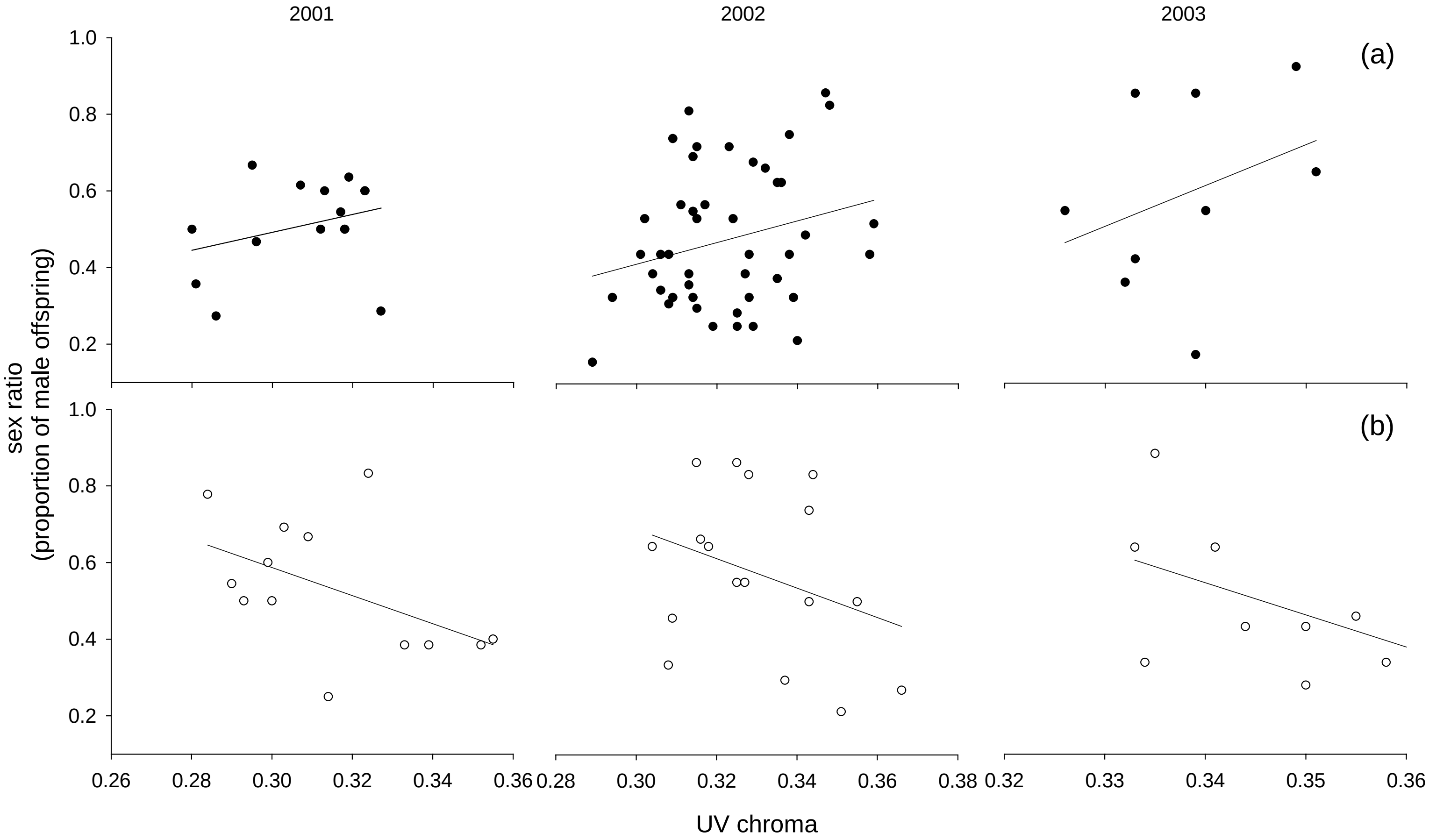

Fig. 1. Correlations between brood sex ratio (proportion of male offspring) and crown UV chroma in juvenile (a) and adult (b) blue tit males. Least squares trend lines are given for visual purposes only, for details on the statistical analysis see Methods and Table 1.

Colour Manipulation Experiment

Effects of treatment on male colour

Neither crown colour prior to treatment nor morphometrics differed between the two treatment groups (all $p > 0.35$). However, after the treatment, crown colour was significantly different between the groups (Table 2, Fig. 2). As intended, UV(+) birds reflected more in the UV range than UV(-) birds. Colour coefficients were within the range found in untreated birds (Table 2), except for two UV(-) males which had a more long-wave hue (418 and 434 nm) than the upper limit (410 nm) of the natural range.

Table 2. Crown colour variables after manipulation in the two treatment groups, presented as means +/- SE. The natural range of variation is based on crown colour measurements of 124 individual males between winter and spring in 2002 and 2003 (K. Delhey, A. Peters, A. Johnsen and B. Kempenaers, unpubl. data).

	UV(-) N = 25	UV(+) N = 22	t	p	Natural range N = 124
Brightness	38.85 (0.86)	42.10 (1.04)	-2.41	0.02	18.1-52.7
Chroma	0.969 (0.018)	0.987 (0.016)	-0.706	0.484	0.81-1.39
Hue (nm)	401.5 (2.63)	353 (1.43)	16.2	<0.0001	331-410
UV chroma	0.31 (0.003)	0.33 (0.002)	-6.58	<0.0001	0.28-0.39

To assess the durability of the treatment, we recaptured 16 birds (9 UV(-) and 7 UV(+)) on average 15.4 days after manipulation (range 7-25 days). The treatment had faded considerably but was still detectable in the UV reduced birds. UV(-) males had a significantly lower UV chroma (paired t-test, $t_8 = 4.20$, $p = 0.003$) and chroma (paired t-test, $t_8 = 2.52$, $p = 0.036$), and a more long-wave hue (paired t-test, $t_8 = -3.18$ $p = 0.013$) than before treatment. The colour of UV(+) males was no longer significantly different from pre-manipulation values (paired t-test, all $p < 0.30$). When we compared between treatments, UV(-) birds still showed a significantly more long-wave hue than UV(+) birds ($t_{14} = 2.18$, $p = 0.047$), but no significant differences were evident in any of the other colour variables (all $p > 0.80$). When crown colour was re-measured during chick feeding, on average 49 (range 30-57) days after treatment, there were no longer any detectable differences in colour between treatments (all colour variables, $p > 0.18$, $n = 36$).

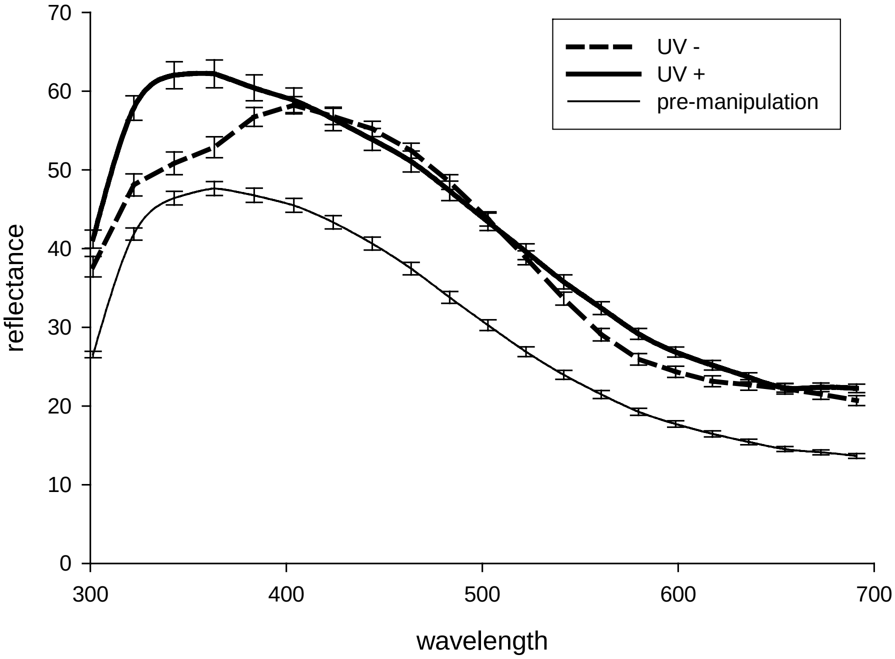

Fig. 2. Average reflectance spectra corresponding to pre-manipulation crown colour and UV(-) and UV(+) treatments (sample size 25 and 22 males respectively). Vertical bars represent standard errors (every 20nm).

Effects of treatment on sex ratios

From the 47 colour-manipulated males, we obtained data on sex ratio for 24 UV(-) and 20 UV (+) males. Based on the correlational results we predicted that in the UV(+) treatment juveniles would have a more male biased brood sex ratio than adults, and that the opposite would be true in the UV(-) treatment, yielding a significant age by treatment interaction. However, this is not what we found (Table 3, Fig. 3). Rather, the interaction between pre-manipulation UV chroma and treatment had a significant effect on brood sex ratio (Table 3). In the UV(-) treatment, males with higher pre-manipulation UV chroma had fewer sons in their broods while there was no trend in the UV(+) treatment (Figure 4). This pattern was confirmed in the separate analyses for the two treatment groups (UV(-) only, $F_{1,22} = 8.82$, $p = 0.007$, effect = -18.55, SE = 6.35; UV(+) only, $F_{1,18} = 0.00$, $p = 0.98$, effect = -0.18, SE =

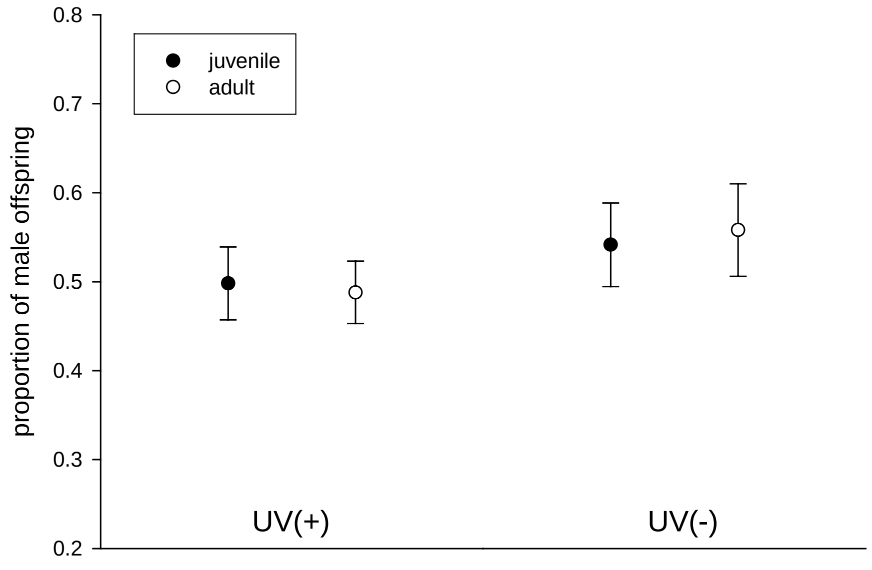

Fig. 3. Average sex ratio of broods (proportion of male offspring) of juvenile and adult males subjected to UV(+) and UV(-) treatments. Vertical bars represent standard errors.

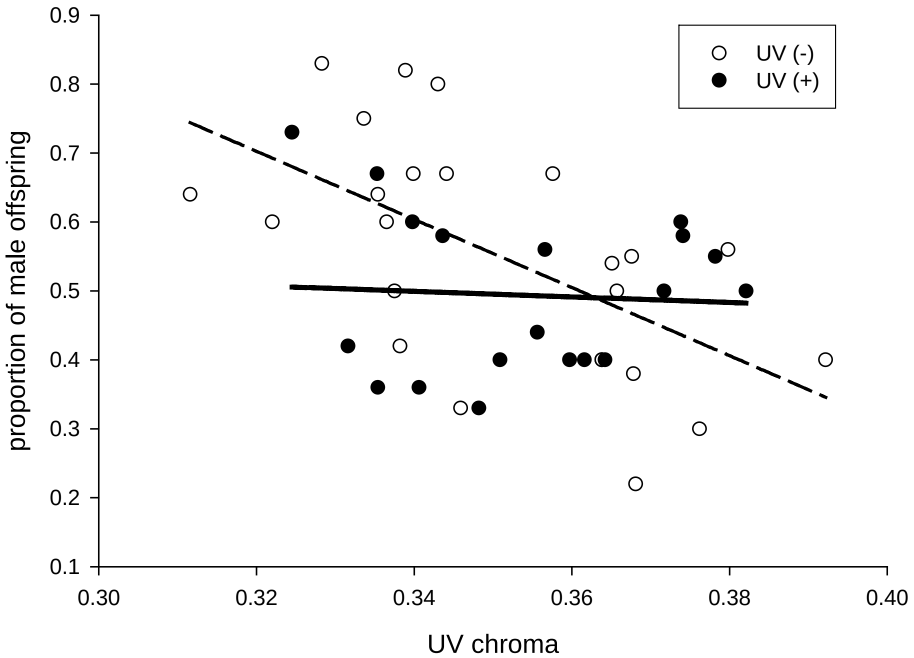

Fig. 4. Correlations between male pre-manipulation UV chroma and brood sex ratio (proportion of male offspring) for UV(+) and UV(-) treatment groups. Closed symbols and solid line correspond to UV(+) treated males, while open symbols and dashed line to UV(-) treated males.

6.22). The same interaction was present when using pre-manipulation chroma as the explanatory colour variable (chroma x treatment, $F_{1,40} = 4.67$, $p = 0.037$), but there were no effects of pre-manipulation hue and brightness either in interaction with treatment (hue x treatment, $F_{1,40} = 0.11$, $p = 0.75$; brightness x treatment, $F_{1,40} = 2.24$, $p = 0.14$) or alone (hue, $F_{1,42} = 0.93$, $p = 0.34$; brightness, $F_{1,42} = 0.03$, $p = 0.86$). In the UV(-) treatment group, males that had higher UV chroma and chroma before manipulation, suffered a greater reduction in coloration (pre-treatment UV chroma vs. change in UV chroma, $r = -0.65$, $p = 0.001$; pre-treatment chroma vs. change in chroma, $r = -0.71$, $p = 0.001$), while males with more UV-shifted pre-manipulation hue were not more affected by the UV(-) treatment than less UV-shifted males (pre-treatment hue vs. change in hue, $r = -0.24$, $p = 0.25$; $n = 25$). The time in days between colour treatment of the male and the first egg laid by his mate had no effect on brood sex ratios either alone (Table 3) or in interaction with pre-treatment colour ($p > 0.3$).

Table 3. Generalised linear model on brood sex ratio variation in the colour manipulation experiment (2003). Terms in bold are included in the final model. Terms in the final model that were part of a significant interaction could not be dropped from the model and thus show no F statistic or p value.

	Estimate (SE)	F(df1,df2)	p
constant	**6.69 (2.07)**	-	-
days to first egg[1]		0.28 (1, 39)	0.602
Age		0.32 (1, 39)	0.575
UV chroma	**-18.55 (5.89)**	-	-
Treatment	**-6.66 (3.21)**	-	-
Age*UV chroma		0.01 (1, 38)	0.938
Age*Treatment		0.05 (1, 38)	0.818
Treatment*UV chroma	**18.36 (9.12)**	**4.08 (1, 40)**	**0.05**
Age*Treat*UV		0.08 (1, 36)	0.777

[1] difference in days between date of treatment and date of first egg laid by its female

DISCUSSION

Correlational data gathered over three years suggest that, in our study population, the pattern of sex-allocation by females based on male UV ornamentation is more complex than previously reported for a Swedish blue tit population (Sheldon et al. 1999; Griffith et al. 2003). Male UV ornamentation was still the most important predictor of brood sex ratio in our data set, but the relationship between male UV ornamentation and brood sex ratio had opposite signs in the two age classes (Fig. 1). Females paired to more ornamented juvenile males biased their brood sex ratio towards sons, while those paired to highly ornamented adult males overproduced daughters. This pattern had strong statistical support and was similar in the three study years (Fig. 1).

Age-dependent male attractiveness?

The opposite relationship between male UV ornamentation and brood sex ratio in the two age classes fits, at least partially, with previous results which suggest that the correlation between male UV ornamentation and breeding success changes with age (Delhey et al. 2003). Data on paternity collected in parallel to this study indicate that less UV ornamented adult males are consistently more successful at siring extra-pair offspring (Chapter 4) and have higher overall annual fertilisation success. It is not clear whether this pattern reflects direct female preference, the outcome of male-male interactions or whether it is due to a yet unknown correlated variable (see Chapter 4). However, the fact that females produce more sons when mated to less UV ornamented adult males suggests that females may recognise such males as successful. Their sons would then benefit by inheriting their father’s phenotypic traits (crown colour or others) which may in turn increase their future breeding success.

In contrast to the pattern in adult males, sex allocation suggests that females prefer more UV males when paired to juveniles. While this agrees with previous correlations between UV ornamentation and sex ratios in this species (Sheldon et al. 1999; Griffith et al. 2003), it is not clear why more UV juvenile males should be considered attractive in our study population. Although more UV ornamented males, irrespective of age, seem to suffer less from cuckoldry (Delhey et al. 2003), this advantage is small and the pattern is not consistent between years (Chapter 4). Another possibility is that UV crown coloration signals male competitive ability (Alonso-Alvarez et al. 2004) and being UV-ornamented enables juveniles to obtain and defend a territory (Siefferman and Hill 2005). This in agreement with the observation that, in our study population, more UV ornamented juveniles have higher levels of circulating testosterone in spring (Peters et al submitted), a hormone linked to the expression of agonistic behaviour in birds and other animals (Wingfield 1990). Finally, more UV ornamented males could be signalling their viability, and hence their superior genetic quality as in a Swedish blue tit population (Sheldon et al. 1999; Griffith et al. 2003). However, in our study population crown coloration is unrelated to male survival among both juveniles and adults (Chapter 2), and there is no direct link between male survival prospects and sex ratio (brood sex ratio: survivors = 0.48, SE = 0.02; non-survivors = 0.51, SE = 0.02; Mann Whitney U-test, Z = -1.13, p = 0.26).

If females are indeed biasing brood sex ratios towards male offspring when paired to high quality males of either age class, we could expect a correlation between male “fitness” and female sex allocation. Moreover, if females use more traits other than male colour to assess mate quality we could expect an even stronger correlation between male “fitness” and brood sex ratio. However, male annual fertilization success (the sum of all within-pair and extra-pair offspring a male sired, standardized by year and age class (data from Chapter 4), was not correlated with brood sex ratios (Spearman rank correlation: juveniles, $r_s = 0.11$, $p = 0.35$, $n = 74$; adults, $r_s = 0.01$, $p = 0.97$, $n = 40$). This result remains unchanged if we use unstandardized values instead, or if we analyse age classes together. Hence, females paired to males that were the most succesful for a given year and age class did not produce more male offspring. While the lack of a relationship may suggest that sex allocation based on male coloration in this population does not confer a fitness advantage to the females, the above test should not be considered conclusive given that (1) the fitness estimate we used may not accurately reflect male quality since the key measure of success in this case would be the breeding success of sons, and (2) it is likely that females can assess male ornamentation more easily than his total fertilization success. Hence, a much larger sample would be required to find a correlation between two variables (male fitness and female sex allocation) that are not directly related but instead covary with a third variable (male colour). Moreover, all three variables are measured or assesed with a certain degree of error by both researchers and/or females.

Why no simple treatment effect on brood sex ratios?

Correlational data, especially if based on large sample sizes, are useful in suggesting possible causal relationships between ornamental traits and female preference. However, only experimental manipulation of the particular trait can provide evidence for the proposed causal link. In our case, the colour manipulation experiment seems to have failed to corroborate the correlational pattern: based on the observed opposite relationship between male UV ornamentation and brood sex ratio in the two age classes we had expected to find an age by treatment interaction. However, we did not find that the treatment had different effects on the age classes nor a treatment effect *per se* (Fig. 3). Unlike a previous colour manipulation experiment in blue tits (Sheldon et al. 1999) we did not use UV-blocking chemicals to eliminate reflectance in the UV range. Instead, we applied two different marker pens that produced more subtle changes in crown UV colour which remained largely within the natural range of variation. However, despite our more natural colour manipulation, our experimental results are similar to the previous experiment (Sheldon et al. 1999) that also failed to demonstrate a simple effect of treatment on sex allocation.

The lack of an overall treatment effect on brood sex ratio is puzzling, and so far there is no satisfactory explanation for it. Based on our correlational data we had expected to find that juvenile UV(+) and adult UV(-) males had male biased brood sex ratios. Likewise, the basic expectation in Sheldon et al's (1999) experiment was that reduction of UV reflectance should result in reduced male attractiveness and female-biased broods. However, in neither case was the predicted effect of the treatment found. This was not a result of females simply ignoring the treatment since we, like Sheldon et al. (1999), found an effect of treatment in interaction with pre-manipulation colour (see below). Moreover, the lack of a simple treatment effect is not necessarily an artefact of this type of colour manipulations. Both methods (UV-blocking sunscreen and marker pens) have been used to manipulate male crown colour later in the season during chick-feeding to test for female differential allocation (Limbourg et al. 2004; Johnsen et al. in press). In both cases simple treatment effects were found with females paired to UV-reduced males investing relatively less in brood provisioning and/or nest defence than controls or UV enhanced males. Thus, why colour manipulations in early spring yield no simple treatment effects on brood sex ratios still remains an unanswered question that should be addressed in future studies. Until this issue is resolved, a causal relationship between brood sex ratio and male crown coloration remains to be unequivocally demonstrated in blue tits.

The effect of pre-manipulation coloration

Instead of a direct effect of the treatment we found that brood sex ratios were negatively related to the pre-manipulation colour, although only in the UV(-) treatment (Fig. 4). Again, this experimental result is similar to the previous colour manipulation experiment using sun block chemicals (Sheldon et al. 1999): the magnitude of brood sex ratio skew was negatively related to male UV-colour prior to manipulation. Assuming that females are sensitive to changes in coloration of their mate, this effect is most likely related to the fact that more UV-reflectant males suffered a greater decline in coloration due the UV(-) treatment. Females mated to initially more attractive males might have perceived the treatment as a greater decline in attractiveness and produced fewer males (as also suggested by Sheldon et al. 1999). Alternatively, if a sudden decrease in crown coloration were indicative of disease or dramatic loss of condition, affecting the amount of expected male help in raising the brood, females should bias the sex ratio towards the less costly offspring (Bensch 1999), in this case females (Johnsen et al. 2003). However, a more effective way to minimise the effect of reduced male investment in brood rearing would have been a reduction in clutch size. Since clutch size was actually slightly larger in UV(-) compared to UV(+) males, this explanation appears unlikely (Chapter 4).

Pre-manipulation colour only predicted brood sex ratio for males in the UV(-) treatment, while there was no obvious relationship between pre-manipulation colour and sex ratio among UV(+) birds (Fig. 4). This might be (a) because the effects of the UV(+) treatment were weaker than those of the UV(-) and vanished faster, (b) because females do not respond to an increase in male attractiveness or (c) because the UV(+) treatment was not perceived as an enhancement of relative UV reflectance. Although our experiment manipulated the colour signal largely within the natural range of variation, the UV(+) treatment appeared less effective than the UV(-) treatment. Waterproofing of the colour treatment with silicon-based fly dressing increased the glossiness, and therefore the brightness of the feathers, and this also affected other colour variables. Since chroma and UV chroma are calculated relative to overall brightness, even birds in the UV(+) treatment were relatively less chromatic than prior to treatment. Thus, although UV(+) birds were more "ultraviolet" than UV(-) birds, they did not have higher UV chroma than untreated birds. This might explain why we did not detect an effect of the pre-manipulation colour on brood sex ratio for males from the UV(+) treatment (Figure 4). It is worth noting that the increase in achromatic brightness due to the use of either silicone-based or natural preen-fat fly-dressing substances is also a problem for the control group in other colour manipulation experiments (e.g. Sheldon et al. 1999; Limbourg et al. 2004).

Conclusions

Our data reveal a more complex relationship between sex ratio and male ornamentation than previously reported for the blue tit (Griffith et al. 2003). Incongruent results between different populations of the same species might be seen as evidence against the occurrence of adaptive sex manipulation by females (Krackow 2002). However, life history-differences between populations can have profound effects on sexual signalling strategies and this will determine which males are more attractive to females (Badyaev and Qvärnstrom 2002; Rosivall et al. 2004).

In contrast to the correlational results, experimental manipulation of crown coloration failed to confirm age-dependent colour signalling but produced similar results to previous colour manipulations in this species (Sheldon et al. 1999). In both studies, the UV-reduction treatment produced a sudden change in male appearance, and the magnitude of the effect on brood sex ratio depended on male colour prior to manipulation. This highlights the importance of considering the different effects that the same treatment may cause in different individuals. In many studies manipulations are assumed to have identical effects on subjects disregarding the fact that not all individuals will respond in the same way to the treatment. A phenotype-dependent response to treatment might be a widespread but often overlooked factor affecting or obscuring experimental results.

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Chapter 6 - Does female crown coloration signal individual quality in the blue tit?

Kaspar Delhey, Anne Peters and Bart Kempenaers
Max Planck Institute for Ornithology, Seewiesen

ABSTRACT

Traditionally, the subdued expression of secondary sexual traits in females was considered a correlated response to sexual selection acting on males. Recently however, studies on several bird species have shown that males prefer to mate with more ornamented females and that the expression of female ornaments may be a good indicator of female quality. Here, we studied female ultraviolet (UV)/blue crown coloration in the blue tit. Since blue tits have been shown to mate assortatively by crown UV-reflectance, and that males prefer UV-reflectant females in captivity, we hypothesised that female coloration could signal female quality and thus be under direct sexual selection. Based on a large sample collected over three years we found little evidence in support of this hypothesis. Female chroma (spectral purity) and perhaps UV chroma, but not hue or brightness, were positively correlated with body condition. However, coloration was not related to breeding performance or mating patterns. In one out of three years clutch initiation was earlier in more chromatic females but there was no such relationship for UV chroma, hue or brightness. Moreover, there was no evidence that clutch size, fledging success or nestling provisioning rate were higher in more ornamented females. To the contrary, there was a weak trend for more UV-ornamented females to fledge fewer young. Moreover, we found no evidence for assortative mating by crown colour, suggesting that male mate choice for more ornamented females is weak or non-existent in our study population. Finally, crown colour did not differ between females mated to monogamous or polygynous males. We conclude that in our study population female colour is unlikely to be under direct (sexual) selection.

INTRODUCTION

In most species of sexually dichromatic birds males are more ornamented than females and these differences are thought to have evolved through sexual selection acting on males (Andersson 1994). In many cases, however, females do not lack ornamentation altogether, but display a subdued version of the male's secondary sexual traits (Amundsen 2000). This has often been interpreted as a correlated response to selection for ornamentation on males resulting from their shared genome (Lande 1980). In this case, the expression of female ornaments is assumed to be non-functional or even maladaptive, and no (or a negative) relationship is expected between female fitness and the degree of ornamentation. This traditional view of female ornamentation has recently been challenged by comparative studies suggesting that evolutionary changes in the degree of female ornamentation are not strongly constrained by genetic correlations (Irwin 1994; Burns 1998). Moreover, experimental and observational studies on a variety of bird species have revealed links between female ornamentation, female quality and male mate choice (reviewed in Amundsen 2000). Thus, at least in some species, the degree of female ornamentation could have evolved through direct sexual selection.

Sexual selection on female ornamentation can be brought about by direct male mate choice and/or through agonistic interactions between females (Amundsen 2000). Male mate choice is more likely to occur if large differences in quality between females exist, especially when investment in reproduction by both sexes is high (Johnstone et al. 1996). Hence, female ornamentation might provide information about her phenotypic quality, allowing males to select mates of high fecundity or good parental abilities. Given that usually only high quality males can afford being selective without risk of remaining unpaired, strong assortative mating of high quality birds is expected when both males and females exert mate choice (Johnstone et al. 1996; MacDougall and Montgomerie 2003). Assortative mating is also possible if females actively compete for males, and female competitive ability is reflected by the degree of ornamentation (MacDougall and Montgomerie 2003). In this case high quality (and ornamented) females end up pairing with the preferred high quality (and ornamented) males by outcompeting other females.

Here we study the function of female crown coloration in the blue tit (*Parus caeruleus*), a short-lived, hole-nesting, passerine bird. In this species both sexes display conspicuous UV/blue colored crest feathers (Andersson et al. 1998; Hunt et al. 1998), which are used in agonistic and courtship signaling (Cramp and Perrins 1993). Males are more UV reflectant than females (Andersson et al. 1998; Hunt et al. 1998), and in males this trait has been shown to be sexually selected through female mate choice and male-male interactions (Sheldon et al. 1999; Delhey et al. 2003; Alonso-Alvarez et al. 2004; Limbourg et al. 2004). In contrast with our level of understanding of the functions of crown coloration in male blue tits we know very little about its role in females. Several lines of evidence however, suggest that female crown coloration could be under sexual selection. First, female crown coloration, although less intense than in males, shows comparable levels of variability and age-related changes (Chapter 2). Second, strong assortative mating by the degree of UV reflectance of the crown was demonstrated in a Swedish population (Andersson et al. 1998), suggesting that mutual mate choice may take place in this species. Indeed, third, in mate choice trials male blue tits displayed strong preference for females viewed behind UV transparent filters compared to females behind UV blocking filters (Hunt et al. 1999). Fourth, female-female competition over territories or mates is common in populations with female-biased sex ratios (Kempenaers 1994). If female colour mediates female agonistic interactions, more colourful females may be more successful at expelling or dominating other females, thereby preventing polygyny (Sandell 1998).

The aim of this study is to assess whether female blue tit crown coloration could be under direct sexual selection in our Austrian study population. We examined a large data set collected over three years for evidence of assortative mating by crown colour and for correlations between female coloration, morphology, reproductive effort and breeding success. Additionally, we compared crown colour of monogamous females and primary and secondary females of polygynous males.

METHODS

Study area and general methods

This study was carried out between 2001 and 2003 in a nest box breeding blue tit population at Kolbeterberg (48˚ 13’ N, 16˚ 20’ E) in the outskirts of Vienna, Austria. The study site consists of a plot of ca. 35 ha., which is part of a large continuous deciduous forest and contained 220 nestboxes in 2001 and 250 in 2002 and 2003. From mid-March onwards we regularly checked all nest boxes to monitor breeding activity. Active nests were checked every few (2-5) days during the nest-building phase, and on a daily basis after the nest was completed until the last egg was laid, to establish the exact laying date and clutch size. After females started incubation, nests were left undisturbed until the expected hatching date (12 days after incubation start), when they were checked again daily until hatching was complete.

Nests were inspected at least three more times before fledging (which occurs around day 19-20 post hatch in our population).

All birds were captured at the nest box while feeding 8-14 days old chicks. Unbanded individuals were then fitted with a numbered aluminium ring and a unique combination of three plastic colour bands, and a small (5-50 μl) blood sample was drawn from the brachial vein. Birds were aged as juvenile or adult by the colour of their primary coverts following Svensson (1992) and sexed by presence-absence of a brood patch. In addition, we measured tarsus length (with caliper to the nearest 0.1 mm), body mass (with electronic balance to the nearest 0.1 g.) and colour of the crown feathers (by reflectance spectrometry, see (b)).

Colour measurement

Reflectance of the crown was measured in Spring between mid-May and early June. We used a S-2000 spectrometer and a DH-2000-FHS deuterium halogen lamp (Ocean Optics, Eerbek, The Netherlands). Spectrometer and lamp were connected through a bifurcated fibre optic probe, fitted at the end with a plastic cylinder to standardise measuring distance and exclude ambient light. The probe was held perpendicular to the surface of the feathers, and we took readings of five standardized spots (11.3 mm^2). Reflectance (R) was calculated relative to a WS-2 white standard using the program Spectra-Win (Ocean Optics). Raw spectra were imported into a spreadsheet program, smoothed using a running average calculated over a 10nm interval, and the following variables were computed. (1) Brightness, an estimate of the total light reflected by the feathers, can be calculated as the sum of reflectances between 300 and 700 nm ($\Sigma\ R_{300-700}$). However, studies and spectrometers usually differ in the number of data points contained in a reflectance spectrum (pers. obs). Hence, to allow direct comparison among studies, we report average reflectance (R_{av}), which is the total reflectance ($\Sigma\ R_{300-700}$) divided by the number of data points. (2) Hue or spectral location was determined as the wavelength of peak reflectance (λR_{max}). (3) Chroma or spectral purity was calculated as the difference between peak and trough divided by total reflectance ($(R_{max}-R_{min})/R_{300-700}$). (4) The relative amount of UV reflectance or "UV chroma" was calculated as reflectance in the UV range (300-400nm) divided by total reflectance and expressed as a percentage ($(R_{300-400}/R_{300-700}) \times 100$).

One potential caveat of using colour measurements taken in late spring is that in the blue tit, crown UV reflectance declines dramatically between winter and spring (Örnborg et al. 2002; Chapter 3). Hence female colour during chick feeding might differ to an unknown extent from the colour earlier in the year, and - more importantly - the degree of decline might be related to female quality (Örnborg et al. 2002). We do not have enough data on female winter colour to repeat the analyses presented in this study using winter colour instead of spring colour. However, female spring and winter colour tend to be positively correlated (UV chroma, $r = 0.64$, $p = 0.002$; hue, $r = 0.29$, $p = 0.216$; chroma, $r = 0.35$, $p = 0.126$; brightness, $r = 0.44$, $p = 0.052$; $n = 20$; Chapter 3) and previous analysis showed that, unlike in males, the degree of decline in female crown UV colour is uncorrelated with other phenotypic traits such as size or condition (Chapter 3). Hence, we assume here that crown colour measured in spring constitutes a meaningful estimate of female ornamentation.

Breeding success and female status

Female breeding success was estimated using the following parameters: (1) laying date, (date of first egg), (2) clutch size, and (3) fledging success (number of offspring fledged). The few nests that were predated (mainly by woodpeckers (*Dendrocopos major*) or snakes (*Elaphe longissima*)) were excluded from the analysis of fledging success.

Females were classified in three categories according to their pairing status. Monogamous females were those females whose male had no other known social mate, while females mated to polygynous males were classified as primary or secondary, whereby primary female is the first one to start laying. Primary females receive most male assistance (Kempenaers 1995), while the secondary females usually receive very little help from the male to raise their chicks (pers. obs). In four cases females raised their brood without any male help, and no male could be identified as the social partner. These females were excluded from the analysis.

Feeding rates

In May 2002 we recorded male and female provisioning rates of 30 pairs that were feeding 13 day old chicks. During one (24 pairs) or two (6 pairs) observation bouts of one hour made from a blind, we determined the total number of feeding trips per hour to the nest box by each parent. Both male and female had previously been captured, banded and measured (see above). In 23 pairs, one member of the pair (determined randomly) was additionally marked with non-permanent purple hair dye on the white forehead patch for ease of individual identification during the observations. In the other pairs male and female were identified solely by their colour bands. The colour mark on the forehead temporarily changed the appearance of the bird (the colour faded after a 4-5 days). If this also altered its attractiveness, this could theoretically have affected the degree of investment by its partner (Limbourg et al. 2004; Johnsen et al. in press). To test for this possibility we compared feeding rates in broods were the male, the female, or neither parent were marked. In no case did the identity of the marked bird significantly affect male, female, or total feeding rates (in all cases $p > 0.3$). Moreover including which parent was colour marked in the statistical models did not change the results (not shown).

Statistical analysis

Since the aim of this study was to determine if there were consistent relationships between female colour and attributes of female quality and breeding success, we initially analysed data from the three study years together (statistically controlling for year differences when necessary). If a relationship with female colour appeared to exist we then included the interaction with year in the model to assess whether the pattern was consistent in the three study years. Some birds were present in more than one year, and to avoid pseudoreplication we randomly included only one measurement per individual when analysing the three years pooled.

Simple correlations were used to test the occurrence of assortative mating by crown coloration in each of the three study years. Partial correlations controlling for year (and a measure of skeletal size, tarsus length) were used to assess the existence of correlations between female colour, size and condition (body mass controlling for tarsus length). To analyse the effect of female colour on breeding success variables and nestling provisioning we used generalized linear models (GLM) that included potentially confounding variables such as year and female age as covariates. A different model was built for each of the four colour variables. Models were reduced by excluding variables in order of decreasing significance until only variables with $p < 0.1$ remained in the model. The lack of significance of excluded terms was confirmed by including them one by one in the final model. In these analyses we included females mated to monogamous males or, in case of polygyny, only the primary female. Laying date, clutch size and number of fledglings were non-normally distributed and the data could not be normalized through transformations. We therefore analysed the effect of female colour on these variables using ordinal logistic regressions (Tabachnick and Fidell 2000). Feeding rates were analysed using GLMs with normal error distribution and identity link. All p values are two tailed. Statistical tests were carried out using SPSS 12.0.1.

The relationship between female colour and breeding success may be obscured by female differential allocation based on male colour (Sheldon 2000). Hence we initially included male colour variables in all models to test for this possibility. In no case did the inclusion of male colour in the model alter the relationships between female colour and the variable of interest (not shown), and in most models male colour had no significant effect ($p > 0.1$). The only exception was a significant relationship between male brightness and laying date: females paired to brighter males laid their first egg earlier ($\chi^2_1 = 7.63$, $p = 0.006$).

RESULTS

Assortative mating

We found no indication that blue tits in our study population mated assortatively by crown colour in any of the three study years (Table 1). Correlations between male and female colour were inconsistent between years and not significant for all four colour variables.

Table 1. No evidence for assortative mating by crown coloration in blue tit pairs.

	2001 n = 47 pairs	2002 n = 59 pairs	2003 n = 29 pairs
Hue	r = 0.17, p = 0.241	r = -0.05, p = 0.663	r = -0.12, p = 0.541
UV chroma	r = 0.18, p = 0.223	r = 0.14, p = 0.284	r = 0.02, p = 0.908
Chroma	r = -0.2, p = 0.885	r = 0.10, p = 0.428	r = -0.13, p = 0.506
Brightness	r = 0.22, p = 0.137	r = 0.05, p = 0.720	r = -0.20, p = 0.294

Body size and condition

Female skeletal size, measured as tarsus length, did not correlate with any colour variable (partial correlation controlling for year; hue, $r = 0.06$; UV chroma, $r = 0.03$; chroma $r = 0.02$; brightness $r = 0.004$; all $p > 0.5$, $n = 117$). Female body condition (body mass controlling for size differences) correlated positively with female chroma (partial correlation controlling for year and tarsus length: $r = 0.23$, $p = 0.009$, Fig. 1) and the same was true for UV chroma albeit non-significantly so ($r = 0.16$, $p = 0.079$). The pattern remained the same without controlling for tarsus length (partial correlation controlling for year: chroma, r = 0.23, p = 0.014; UV chroma, r = 0.16, p = 0.092). Brightness and hue were not significantly correlated with female body condition (partial correlation controlling for year and tarsus length: brightness, r = 0.016, p = 0.89; hue, r = -0.12, p = 0.18).

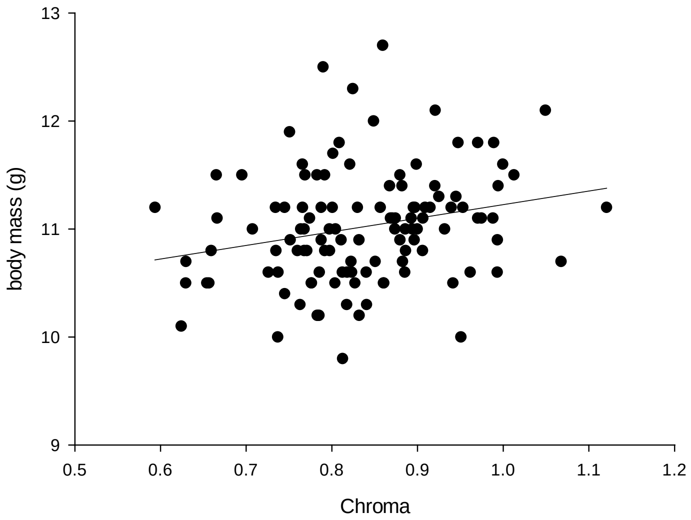

Fig. 1. Females in better body condition (higher body mass) have more chromatic crown feathers. Least-squares regression line given for visual aid only, for statistical details see Results.

Breeding success

Laying date, after controlling for year differences, was negatively correlated with chroma (Table 2). However this relationship was only present in 2002, as indicated by the significant chroma*year interaction ($\chi^2_2 = 8.05$, p = 0.018; Fig. 2). Clutch size, after controlling for year differences, was negatively correlated with laying date but unrelated to female crown colour (Table 2). Even though early laid clutches were larger, and more chromatic females started laying earlier, there was no relationship between chroma and clutch size when we excluded laying date from the model ($\chi^2_1 = 0.05$, p = 0.823). Fledging success (number of fledged offspring) was negatively correlated with the expression of female UV coloration (Table 2). More UV-ornamented females (lower hue, higher UV chroma) fledged fewer young (Fig. 3), and this relationship was weak but consistent over the three study years as indicated by the non-significant colour*year interaction (UV chroma*year, $\chi^2_2 = 1.00$, p = 0.605; hue*year, $\chi^2_2 = 0.59$, p = 0.741). Brightness and chroma were unrelated to fledging success (Table 2).

Table 2. Ordinal logistic regression models analysing the relationship between female colour and breeding success. Each colour variable was tested in a separate model (see Methods for details). Terms in bold are included in the final models. Effect sizes (unstandardized regression coefficients) and their corresponding standard errors (SE) are given for significant terms (except for year) while 95% confidence interval (CI) are presented for non-significant results.

		Laying date[1] (n = 117)			Clutch size[2] (n = 117)			Number of fledglings[3] (n = 100)		
	df	Effect/CI	χ^2	p	Effect/CI	χ^2	P	Effect/CI	χ^2	P
Laying date	1	--	--	--	**-0.09 (0.03)**	**8.54**	**0.003**	(-0.07-0.03)	0.72	0.396
Year	2	**--**	**86.31**	**< 0.001**	**--**	**10.32**	**0.005**	--	1.98	0.371
Female age	1	(-0.36-1.02)	0.91	0.337	(-0.93-0.46)	0.45	0.502	(-0.39-1.03)	0.74	0.389
Hue	1	(-0.04-0.01)	1.82	0.176	(-0.01-0.03)	0.90	0.341	**0.03 (0.01)**	**6.01**	**0.014**
UV chroma	1	(-0.30-0.09)	1.06	0.302	(-0.38-0.03)	2.70	0.100	**-0.22 (0.10)**	**4.76**	**0.029**
Chroma	1	**-4.42 (1.73)**	**6.18**	**0.013**	(-4.80-2.44)	0.42	0.515	(-3.43-3.54)	0.001	0.973
Brightness	1	(-0.05-0.09)	0.36	0.546	(-0.03-0.11)	1.14	0.285	(-0.05-0.11)	0.52	0.469

All age*colour interactions: [1] p > 0.17, [2] p > 0.31, [3] p > 0.28.

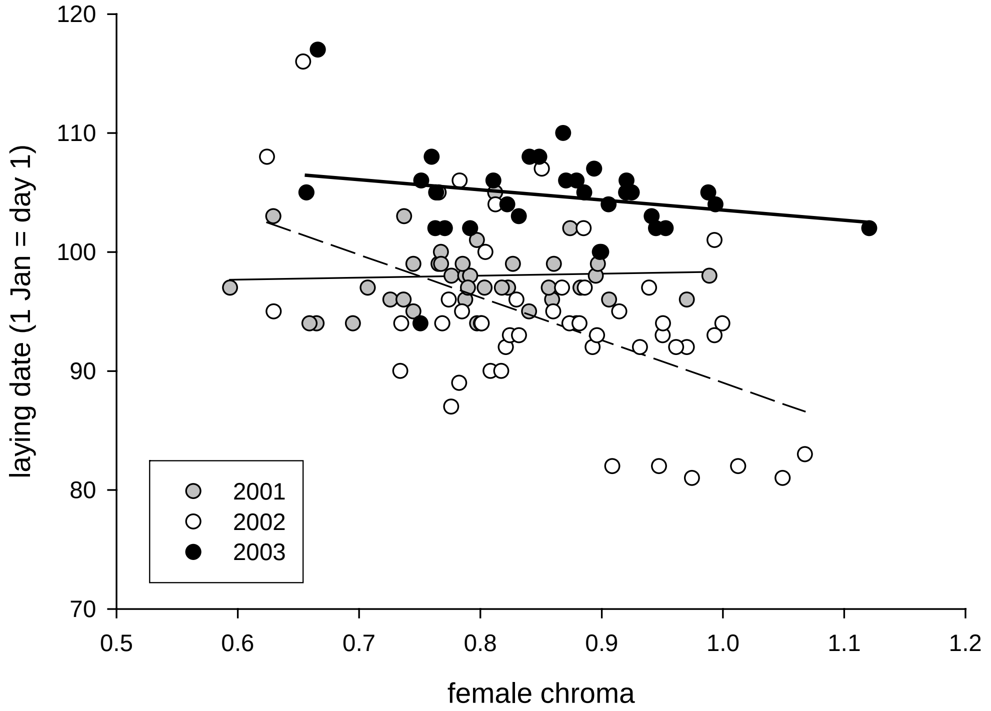

Fig. 2. Female crown chroma and laying date (date of first egg) correlate significantly only in one out of three study years. Least-squares regression lines correspond to each of the three study years, and are shown for visual purposes only. For details on statistical analysis see Table 1.

Nestling provisioning

Female feeding rate was unrelated to her crown colour (Table 3). Brood provisioning rate was only affected by female age, with older females feeding at higher rate than juveniles. There was no indication that male investment in brood provisioning differed if mated to a more colourful or older female. Male feeding rate correlated only with brood size (Table 3). The results are similar if instead of absolute feeding rates we used the proportion of feeds by each pair member (not shown).

Table 3. Generalised linear model analysing the relationship between female and male chick feeding rate (feeds/hour) and female crown colour. Each colour variable was tested in a separate model (see Methods for details). Terms in bold are included in the final models. Effect sizes (unstandardized regression coefficients) and their corresponding standard errors (SE) are given for significant terms while 95% confidence interval (CI) are presented for non-significant ones.

	Female feeding rate[1]				Male feeding rate			
	Effect/CI	F	df	p	Effect/CI	F	df	p
Female age	**- 5.78 (2.61)**[2]	**4.91**	**1, 28**	**0.035**	(-6.46-5.62)	0.02	1, 27	0.887
Brood size	(-0.49-1.63)	1.22	1, 27	0.278	**1.29 (0.53)**	**5.91**	**1, 28**	**0.022**
Female hue	(-0.06-0.37)	2.15	1, 27	0.154	(-0.06-0.38)	2.22	1, 27	0.147
Female UV chroma	(-242.7-96.2)	0.78	1, 27	0.383	(-267.4-72.2)	1.39	1, 27	0.248
Female chroma	(-26.30-14.60)	0.34	1, 27	0.562	(-27.58-15.11)	0.36	1, 27	0.554
Female brightness	(-0.46-0.61)	0.08	1, 27	0.780	(-0.47-0.76)	0.22	1, 27	0.642

[1] All colour*age interactions $p > 0.42$. [2] computed relative to adult females.

Polygyny

Polygyny was relatively rare in our study population, with only 9% of the males (16 out of 178) having two social mates. Hence the sample size for primary and secondary females was low, reducing the power of our comparisons. The colour of monogamous (n = 106), primary (n = 11) and secondary (n = 7) females was not significantly different (Table 4). A pairwise comparison between the colour of primary and secondary females of each polygynous male yielded similar results (paired t-test; hue, t = -0.83, p = 0.449; UV chroma, t = 0.76, p = 0.486; chroma, t = 0.46, p = 0.665; brightness, t = -0.33, p = 0.756; n = 5) but note that sample size was extremely low.

Table 4. Colour differences between females mated to monogamous males, and primary and secondary females of polygynous males. Shown are mean ± s.e.

	Female status			
	Monogamous N = 106	Primary N = 11	Secondary N = 7	
Hue	396.4 (1.5)	393.5 (4.8)	393.1 (6.4)	$F_{2, 119} = 0.03$, $p = 0.97^1$
UV chroma	28.7 (0.2)	29.2 (0.6)	29.9 (1.0)	$F_{2, 119} = 1.25$, $p = 0.29^1$
Chroma	0.83 (0.01)	0.88 (0.04)	0.92 (0.05)	$F_{2, 119} = 2.25$, $p = 0.11^1$
Brightness	25.8 (0.4)	25.5 (1.1)	24.9 (1.6)	$F_{2, 121} = 0.14$, $p = 0.86$

[1] correcting for significant ($p < 0.01$) year differences in colour.

DISCUSSION

Using a large data set collected over three consecutive years, we found little evidence suggesting that the expression of crown colour may act as a sexual signal of quality in female blue tits. In our study population female crown colour was largely unrelated to aspects of female quality like clutch size or parental investment in nestling provisioning and even negatively related to the number of fledglings produced. Moreover, we failed to find any evidence of assortative mating by crown colour, lending no support to the occurrence of mutual mate choice in our population. Finally, we found no indication that crown coloration differs between females paired to monogamous or polygynous males.

Lack of assortative mating and male mate choice

Assortative mating by the expression of ornamental traits, especially if ornamentation is linked to individual quality, has been associated with the occurrence of mutual mate choice in birds (Jawor et al. 2003; MacDougall and Montgomerie 2003). Mutual mate choice was hypothesised to take place in the blue tit based on the observation that there is strong, positive assortative mating by crown UV reflectance reported for a Swedish blue tit population (Andersson et al. 1998) and that there is male preference for UV-intact, against UV-reduced, females in choice chamber experiments (Hunt et al. 1999). However, in the three years of our study we found no evidence that blue tits mated assortatively by crown colour (Table 1). Assortative mating may be prevented by constraints on males and females to pair with their preferred partner. In our population, blue tits seem to form pair bonds throughout the year when vacancies arise, and this may restrict the extent of possible mate choice (Foerster et al. 2003). Differences in pairing behaviour or in the constraints of social mate choice may account for between-population differences in assortative mating by crown coloration, even if underlying patterns of mate preferences are the same.

Alternatively, male preference for ornamented females could be expressed later in the breeding cycle, for example if males invest differentially in brood rearing when paired to highly ornamented females (the differential allocation hypothesis, Sheldon 2000). This would be analogous to female differential allocation based on male UV ornamentation, which has been shown to occur in the blue tit (Limbourg et al. 2004; Johnsen et al. in press). Although we did not experimentally manipulate female crown coloration, and our sample size is relatively low, the correlational results do not support male differential allocation based on female ornaments (Table 3). The only correlate of male feeding rate was brood size and we found no relationship, or even a trend, between male investment and female colour or age. Hence, although we can not rule out that male mate choice based on female crown colour takes place in our study population, the data presented here are not consistent with this hypothesis.

Female colour and female quality

Based on honest signaling theory, ornaments are assumed to function as indicators of individual quality (Johnstone 1995). Although most of the evidence comes from male ornaments, in some species female ornamentation has been shown to correlate with attributes of female phenotypic quality such as body condition (Amundsen et al. 1997; Jawor et al. 2004), laying date (Møller 1993; Jawor et al. 2004; Safran and McGraw 2004), breeding success (Møller 1993; Safran and McGraw 2004) and parental investment (Linville et al. 1998; Jawor et al. 2004). However lack of correlation between ornamentation and female quality has also been reported (Muma and Weatherhead 1989; Hill 1993; Tella et al. 1997; Rhode et al. 1999; Smiseth and Amundsen 2000).

We found evidence that one aspect of colour expression, chroma or saturation, was positively correlated with female condition (Table 2, Fig. 1). Correlations between female condition and the expression of secondary sexual traits might reflect the higher phenotypic quality of ornamented females (Amundsen et al. 1997; Jawor et al. 2004). This is consistent with the finding that more chromatic females started laying earlier (Fig. 2, Table 2). Early laying females are assumed to be of higher quality, as early laid clutches are usually larger (Table 2) and early females are more likely to re-nest in case of breeding failure or may attempt a second clutch in some species (Price et al. 1988). However, the correlation between chroma and laying date was not consistent between years (Fig. 2), and although early laying females produced larger clutches this did not translate into higher breeding success of more chromatic females (Table 2). These results suggest that selection on crown colour through early laying is weak in this population. Moreover, and against the hypothesis that more colorful females are of higher phentoypic quality, less UV coloured females (higher hue and lower UV chroma) tended to fledge more young than colorful females (Table 2). Hence, our analyses did not reveal any consistent selective advantage of being a colorful female in this population.

Even if more ornamented females do not produce more young, males could still benefit by mating with them if the degree of female ornamentation reflects her brood rearing abilities

(Hoelzer 1989). This was however not the case, female investment in brood rearing, (measured as feeding rate of 13 day old chicks) was unrelated to her crown colour (Table 3) and only influenced by female age, whereby older females fed at higher rates. Despite their higher investment in brood rearing however, older females did not fledge more young (Table 2).

Female colour and female-female competition

There is increasing evidence that females often engage in fights with other females while competing for mates or territories (Wolf 1969; Trail 1990; Langmore and Davies 1997) and female aggressiveness can prevent males from becoming polygynous by evicting prospecting female floaters (Kempenaers 1994; Sandell 1998). Polygyny is relatively common in the blue tit (Kempenaers 1994), and while polygynous males clearly benefit from it, their females do not, since male parental effort is divided between the broods (Kempenaers 1995). Thus, it is expected that already mated females aggressively try to prevent other females from settling nearby.

Secondary sexual traits like song or plumage ornamentation may help settling aggressive interactions between females by acting as honest signals of competitive ability or badges of status (Butcher and Rohwer 1989; Amundsen 2000). Among male blue tits crown UV/blue coloration seems to play such a role in male-male agonistic interactions (Alonso-Alvarez et al. 2004). If female coloration fulfils a similar function we predicted that monogamous females, which have successfully prevented their mates from becoming polygynous, would be more colourful than females that failed to do so (i.e. primary females of polygynous males). We found no evidence in support of this idea, but our sample of primary females was low, and thus our conclusions should be regarded as tentative. However, in contrast to our predictions, primary females were (non-significantly) more ornamented than monogamous females (Table 5).

Predictions regarding colour differences between primary and secondary females are less straightforward. If secondary females settle because they could overcome the territorial female’s resistance through aggressive interactions we would expect more ornamented secondary females. On the other hand, less ornamented (less attractive) secondary females may settle because they are tolerated as low quality neighbours in which case the secondary female would be less ornamented (see Greene et al. 2000 for a similar example with males). Our data do not provide support to any of the above hypotheses, although sample size was extremely low. However, future comparisons between females of different mating status might provide further insight on the link between female ornamentation and dominance signalling (Amundsen 2000).

Conclusions

Based on our results we conclude that male blue tits in our population do not benefit by pairing with more UV-ornamented females. Hence, our data do not support the hypothesis that male preference for more ornamented females found in captivity (Hunt et al. 1999) would be adaptive in our study population. Our results resemble the findings of other studies where male preference for more ornamented females in captivity did not translate into higher breeding success of males paired to showy females in the field (Hill 1994; Amundsen et al. 1997; Rhode et al. 1999). However, it remains a possibility that female ornamentation in the blue tit may reflect a yet unmeasured aspect of quality, such as heritable parasite resistance (Potti and Merino 1996). Meanwhile, we cannot reject the hypothesis that the expression of UV/blue crown coloration in female blue tits is but a correlated response to sexual selection on males.

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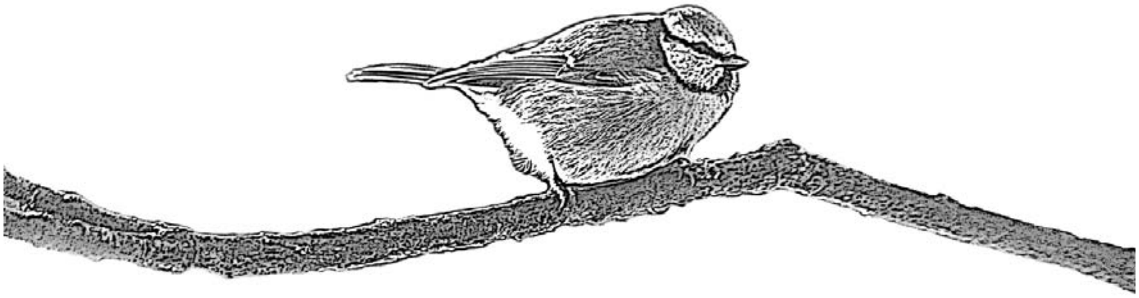

CURRICULUM VITAE

Name	Johann Kaspar Valdemar DELHEY
Present address	Max-Planck Institute for Ornithology, Vogelwarte Radolfzell, Schlossallee 2, D- 78315, Radolfzell, GERMANY Tel: 07732-150151 e-mail: delhey@orn.mpg.de
Date of birth	15 December 1974
Place of birth	Tandil, Argentina.
Secondary Education	
1988-1993	Ciclo Básico U.N.S. and Escuela de Agricultura y Ganadería “Adolfo Joaquín Zabala”
Tertiary Education	
1994-2000	Biology studies at the Universidad Nacional del Sur, Bahía Blanca, Argentina.
2000	Final Thesis: "*Comunidades de aves en áreas de caldenal del sudoeste de la provincia de Buenos Aires*" [Bird communities in caldén woodlands of the SW of Buenos Aires province] 36 pp +iii.
since Dec 2001	MPI Ornithology, Seewiesen, Germany Doctoral thesis: “Sexual Selection and blue tit (*Parus caeruleus*) crown coloration” supervised by Prof. Dr. Bart Kempenaers.

Scholarships

September 1995-October 1996	Beca C.C.Olrog, Asociación Ornitológica del Plata.
April 1998-February 1999	Beca de Introducción a la Investigación para Alumnos Avanzados, Universidad Nacional del Sur, Secretaría de Ciencia y Tecnología; CSU-659/97.

Working experience

Dec 1994 – May 1995	Volunteer Research Assistant. The effect of shorebirds on benthic species. Supervisor: Dr. Oscar Iribarne, Universidad Nacional de Mar del Plata
Dec 1999	Volunteer Research Assistant. Distribution and abundance of Buff-breasted Sandpipers on their wintering grounds in Argentina, Uruguay and Brazil. Supervisor: Dr. Richard Lanctot, US Fish and Wildlife Service.
May-August 2000	Volunteer Research Assistant. Seabird breeding biology study on Middleton Island in the Gulf of Alaska. Supervisor: Ms. Verena Gill, Dr. Scott Hatch, U.S. Geological Survey, Alaska Biological Science Center.
February-November 2001	Visiting Scientist. Max-Planck-Research Centre for Ornithology, Seewiesen, Germany. Sexual selection in blue tits and bluethroats. Supervisor: Dr. Bart Kempenaers.

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