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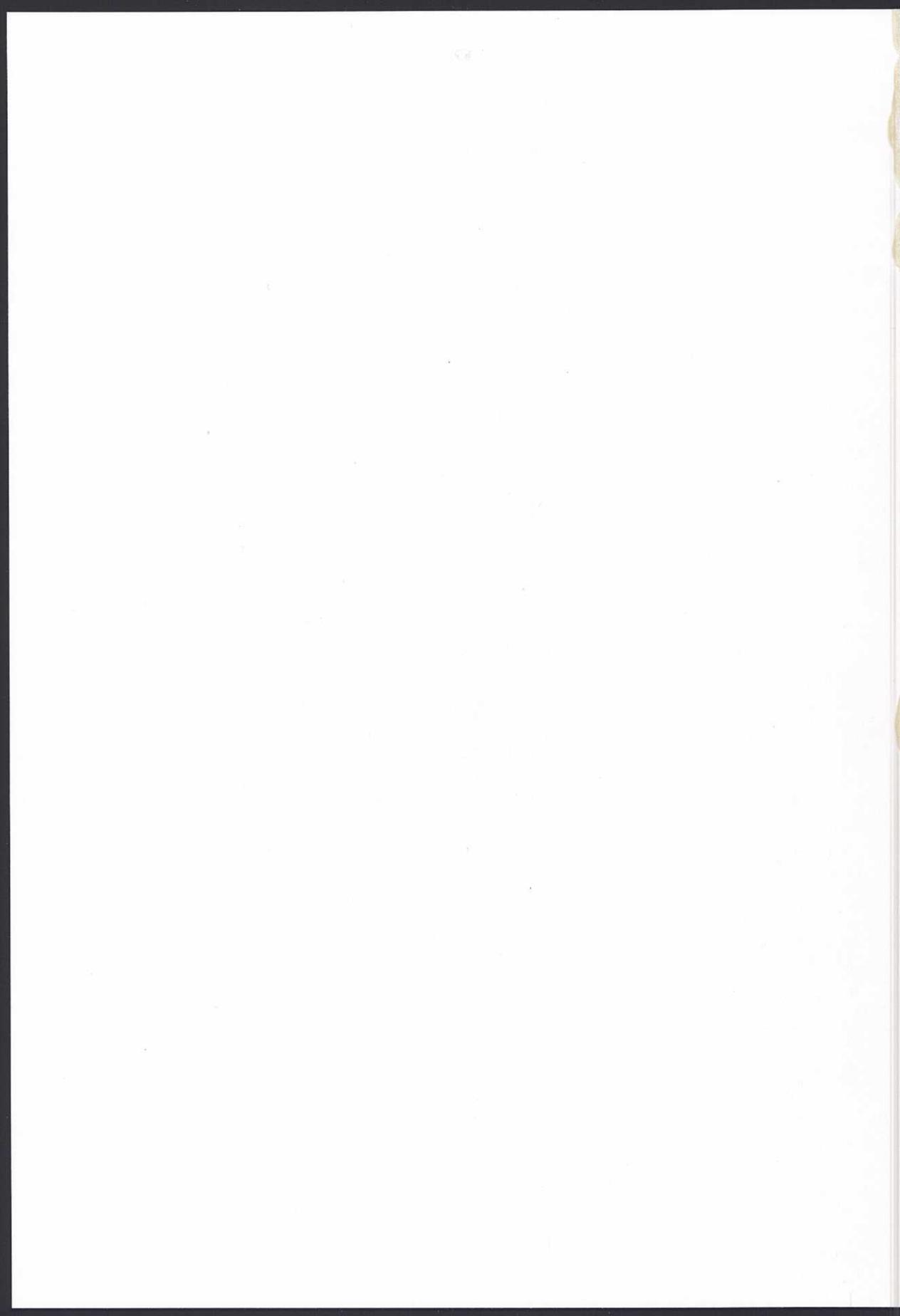
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Ultraviolet coloration and colour
communication in blue tits *Parus caeruleus*

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**Ultraviolet coloration and colour communication
in blue tits *Parus caeruleus***

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Dissertation



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Avhandling för filosofie doktorsexamen i ekologisk zoologi, som enligt Naturvetenskapliga fakultetens beslut kommer att offentlig försvaras fredagen den 22 februari 2002, kl. 09.00 i föreläsningssalen, Zoologiska institutionen, Medicinargatan 18, Göteborg.
Fakultetsopponent är professor Geoffrey Hill, Department of Biological Sciences, Auburn university, Auburn, Alabama, USA.

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ABSTRACT

The mechanisms and signal functions of conspicuous and sexually dimorphic plumage coloration in birds are central issues in the study of sexual selection. By objective quantification of colour (reflectance spectrometry), colour signals can be analysed in relation to receiver and signalling conditions. Coloration in the ultraviolet (UV, 320–400 nm), which birds but not humans perceive, is largely unexplored. Structural plumage colours, regarded as invariant and unlikely to be sexually selected ornaments, are rich in UV.

Reflectance measurements in wild breeding blue tits revealed a strong sexual dimorphism in UV/blue crown plumage, indicating that blue tits are substantially more sexually dichromatic to blue tit than to human eyes. Assortative mating based on crown 'UV-chroma' (relative UV-reflectance) suggests that this is a visual signal of male quality that earlier studies had failed to identify.

In another blue tit population, male UV-reflectance was experimentally manipulated. Among controls, but not among the UV reduced males, females adjusted offspring sex ratio towards sons in response to the natural 'UV-chroma' of their mates. Male 'UV-chroma' also predicted male survival, supporting viability signalling. The relationships between UV colour, male survival, and offspring sex ratio were consistent in two out of three years.

Current theory predicts honesty-enforcing signal costs, but in contrast to dietary and physiological constraints on e.g. carotenoid pigmentation, the mechanisms and costs behind structural colour variation require further study. One source of colour variation in blue tits is seasonal change due to plumage age and abrasion. In a comparison between different seasonal stages, 'UV-chroma' peaked in early spring, whereas the peak location showed a substantial shift from UV shortly after moult (October) towards blue at the end of breeding (June).

External morphometrics of sampled crown feathers suggested mechanisms of sexual and seasonal colour variation. Longer feather barbs (containing the colour structure) in males probably contribute to their stronger 'UV chroma'. Seasonal variation coincided with the amount of abrasion (barb breakage), to which males appear more resistant. Combined with higher rate of barbule loss in males, increased exposure of coloured barbs in early spring might be an adaptation to maximise signal intensity at this time. Honesty-mediating constraints might thus include 'signal maintenance' as well as 'signal production'.

In addition to crown plumage, other structural and pigment-based colour patches were analysed, showing age effects and sex dimorphism consistent with stronger sexual selection on males. Structural UV/blue coloration was correlated between patches, suggesting indirect or direct sexual selection. Furthermore, yellow ventral 'carotenoid chroma' reflects carotenoid concentration in feathers (and thus possibly nutrition and health), and it was stronger in males. The overall UV/blue and yellow plumage thus appears to be a multicomponent signal of vigour in blue tits.

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LIST OF PAPERS

This thesis is a summary based on the following papers, referred to in the text by their roman numerals:

- I. Andersson, S., Örnborg, J. & Andersson, M. 1998. Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265**, 445-450.
- II. Sheldon, B. C., Andersson, S., Griffith, S. C., Örnborg, J. & Sendecka, J. 1999. Ultraviolet colour variation influences blue tit sex ratios. *Nature* **402**, 874-877.
- III. Griffith, S.C., Örnborg, J., Russell, A.F., Andersson, S., & Sheldon, B.C. 2002. Male blue tit attractiveness and offspring sex ratios. Manuscript
- IV. Örnborg, J., Andersson, S., Griffiths, R. & Sheldon, B. C. 2002. Seasonal changes in a sexually selected structural colour signal in blue tits, *Parus caeruleus*. Accepted, subject to acceptable revision in *Biological Journal of the Linnean Society*.
- V. Örnborg, J. & Andersson, S. 2002. Revealing plumage colour: Sexual and seasonal differences in blue tit UV signalling are related to feather morphology and abrasion. Submitted manuscript to *Functional Ecology*
- VI. Örnborg, J. Structural and carotenoid based colour variation in blue tits. Manuscript

A doctoral thesis at a Swedish university is often presented as a collection of papers with a summarising introductory part, which constitute the formal thesis. The papers are published or manuscripts at various stages.

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INTRODUCTION

Extravagant plumage colours and other ornamental traits in nature have fascinated humans for a long time. Such traits, which hardly contribute to survival, led Darwin (1871) to put forward the idea of sexual selection, a special case of his theory of natural selection (Darwin 1859). He suggested that female choice has generally been responsible for the evolution of conspicuous male colours and other ornaments. While today there is much evidence that female mate choice favours conspicuous male traits (Andersson 1994), it is less clear what females gain by being choosy and hence what maintains a female preference over evolutionary time. Studies of sexual selection during recent years have often been concerned with some form of honest advertisement models, according to which honest signals of mate quality convey information about possible indirect and/or direct benefits gained during mate acquisition (Andersson 1994; Johnstone 1995; Bradbury & Vehrencamp 1998).

Among the variety of gaudy and elaborate bird ornaments, bright conspicuous colours are together with bird-song the most common display. Colours are often associated with other types of display and thus seem to be an important signal component. Of the two major mechanisms responsible for plumage colours, pigments and structural colours, the latter is the least understood in terms of signalling and has generally been considered invariant and cheap to produce (Borgia 1985). However, this view has recently changed due to the consideration of avian colour-vision in ultraviolet, a common spectral component of structural colours, and other important differences between human and avian colour vision (reviewed in Cuthill et al. 2000b).

Objective analyses of intraspecific variation in plumage colours in general and structural colours in particular are still scarce. Besides providing indications of sexual dimorphism and selection (Andersson 1994), colour variation in relation to e.g. sex, age and condition can also suggest costs that are associated with the colour signal. The development of ultraviolet/visible (UV/VIS) reflectance spectrometry has allowed measurements of plumage reflectance, including also ultraviolet, and objective colour quantification (Endler 1990) is a valuable tool for future studies of avian colour signalling. In this thesis I use UV/VIS reflectance spectrometry to explore plumage reflectance variation and possible signalling functions and costs in the blue tit *Parus c. caeruleus*. This common passerine has previously been subjected to numerous studies of sexual selection (Kempnaers et al. 1992; Kempnaers et al. 1995; Svensson & Nilsson 1996), but not with respect to its most striking ornamental feature, the bright blue and yellow plumage coloration.

Sexual selection

Wallace (1889) explained, probably correctly, plumage variation in terms of natural selection favouring female crypsis during breeding (Götmark et al. 1997; Post 2002). However, he did not satisfactorily explain why males in so many species display conspicuous ornaments but rather regarded these ornaments as "the natural product and the direct outcome of superabundant health and vigour in males" (cf. Andersson 1994). Based on pattern of occurrence of sex dimorphic colours, colours that often are related to reproduction, Darwin (Darwin 1871) concluded that they are favoured by female choice, a suggestion that is now supported by several experimental studies (reviewed in Andersson 1994). One of the most thoroughly investigated species as regards female preferences for conspicuous colours is the Mexican house finch *Carpodacus mexicanus*. In this socially monogamous and sexually dimorphic species, males vary in the extent and hue of carotenoid plumage on head and chest, as a result of differences in foraging success (Hill 1992). In both controlled laboratory experiments and in the wild, female house finches prefer the most coloured males as social

mates (Hill 1990; Hill 1993). Female preferences for bright and conspicuous plumage colours have also been convincingly demonstrated in other bird species (reviewed in Hill 1999), supporting the view of female choice as an important selection pressure behind bright coloration in birds.

Although of less concern in this thesis, it should be pointed out that sexual selection of plumage coloration also can operate via contest competition between males (Andersson 1994); a recent example is the status signalling function of carotenoid 'redness' in a widowbird (Pryke et al. 2001; Pryke et al. 2002).

Signal content

While female preferences for male colour is not a very controversial issue anymore, it is less clear what benefits a female gains and hence what maintains the female preference. The recent attention to receiver psychology (Guilford & Dawkins 1991) calls for a distinction between two components of colour signal design; signal content and signal efficacy (Andersson 2000). First, as regards signal content, females under strong selective pressure to acquire superior mates (Trivers 1972) should favour signals that convey true information (content) about male quality. The 'handicap principle' (Zahavi 1975) predicts that for a signal to convey true (honest) information about sender quality, it should carry a cost directly relevant to this quality. Signals would thereby be expressed in relation to quality since only superior individual would gain a net benefit by extreme expression of the signal (Fisher 1915; Williams 1966; Hamilton & Zuk 1982; Andersson 1986; Pomiankowski 1988; Grafen 1990; Johnstone 1995).

The type of benefits females gain by choosing high quality mates could either be of the kind that increase survival and/or reproductive success of the female (direct benefits) or they could be of the kind that leads to higher reproductive success of offspring (indirect benefits) (Johnstone 1995).

Empirical evidence that plumage colours are expressed in relation to quality is rather scarce and limited to pigment based colours such as the carotenoid displays mentioned above (reviewed in Hill 1999). For structural UV and blue colour, mechanisms of honest signal content remain speculative (Andersson 1999) (but see Keyser & Hill 1999; Keyser & Hill 2000).

Signal efficacy

In studies of colours as sexually selected signals, the division of signal design into the components of signal content (above) and signal efficacy (detectability, discriminability and memorability) (Guilford & Dawkins 1991; Andersson 2000) highlights the importance of objective colour quantification. Estimation of signal efficacy in colour signals requires, among several factors, an understanding of the receiver's ability to detect and discriminate the signal. Research on avian colour perception has revealed profound differences between bird and human colour vision. The most important differences are; (1) the broader spectral range of bird vision (320-700 nm compared to 400-700 nm in humans), (2) four different cone (or receptor) types (compared to three in humans) and (3) filtering of light by coloured oil droplets before reaching the light sensitive part of the cones (reviewed in Bennett & Cuthill 1994; Cuthill et al. 2000b). As colour is derived from comparison of receptor outputs, birds will not only see the UV (320-400 nm) that humans cannot detect but are also likely to see human visible colours differently (Bennett et al. 1994; Burkhardt 1996; Cuthill et al. 2000b). Studies of mating preferences involving colour will thus benefit from closer connections with

sensory and brain physiology, especially for colours with a large part of the reflectance spectrum outside the human visual range (<400 nm) (Bennett et al. 1994).

Bird plumage colour signals

Four colour mechanisms produce the majority of avian plumage colours (Brush 1978). Three of these are pigments: porphyrins, melanins and carotenoids, which absorb incident light differentially in relation to wavelength and thus modify the reflectance of light from the feather. The fourth mechanism is structural coloration (iridescent and non-iridescent) where physical structures inside feathers produce colour by interference phenomena among reflected wavelengths at interfaces between keratin structures and air or melanin (Dyck 1978; Prum et al. 1998; Andersson 1999). Striking (saturated) colours are primarily carotenoid based or structural (or a combination of the two), so these mechanisms will be presented in more detail.

Carotenoid colours

Carotenoid pigments are responsible for many of the bright yellow, orange and red hues in feathers due to their steep absorption peak in the middle of the human visible spectrum (420-500 nm). Carotenoids in vertebrates are all of dietary origin although metabolic conversions can modify their molecular structure and hence colour (Fox & Vevers 1960; Goodwin 1984). Carotenoid colours are thus likely to be influenced by quantity as well as quality of ingested and deposited carotenoids (Brush 1978; Slagsvold & Lifjeld 1985; Hill 1992). Besides being used as plumage colorants, carotenoids are important in a variety of physiological processes such as growth, immune system and colour vision (Lozano 1994; Olson & Owens 1998). Based on these potential foraging and physiological trade offs, they have become classic examples of costly and thereby honest signals of individual quality (reviewed in Olson & Owens 1998; Hill 1999).

Structural colours

Two major types of structural colours exist, iridescent and non-iridescent colours; only the latter one will be dealt with here. Non-iridescent colours (hereafter referred to as structural colours) are produced by light scattering structures inside the barbs of feathers (whereas classical iridescence usually resides in barbules). They produce UV, violet, blue and in combination with carotenoids green plumage colours and occur in a wide range of species (Auber 1957a).

All different structural plumage colours are similarly caused (UV, violet, blue etc), but the exact optical mechanism is debated. Recent advances suggest that a model of coherent scattering from regularly ordered light reflecting surfaces ('scatterers'), first proposed by Dyck (1976), best explains the production of non-iridescent structural colours (Prum 1994; Andersson 1999). Feather interiors of non-iridescent coloured feathers can be seen as a matrix of keratin rods and air channels ('spongy structure') ('the hollow cylinder model' Dyck 1976; Andersson 1999) (figure 1). Melanin granules typically underlie the spongy structure to eliminate incoherent backscattering from tissues below (Auber 1957b).

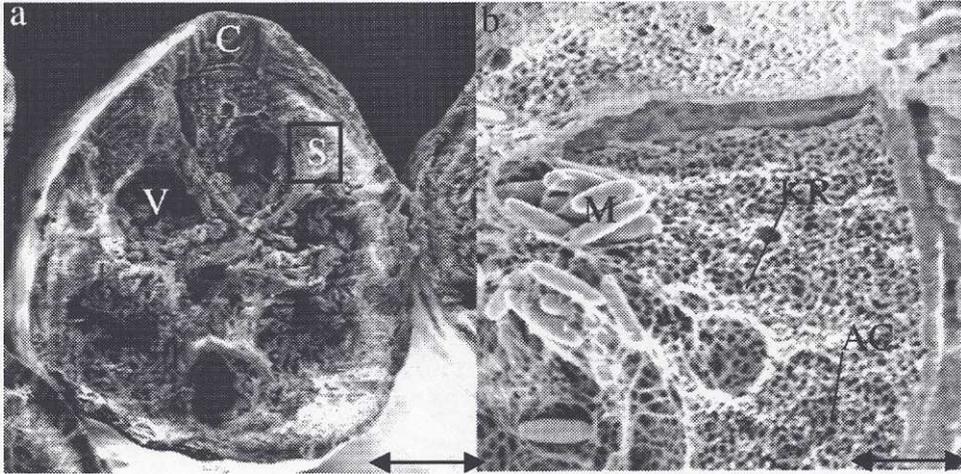


Figure 1. EM cross-section of blue tit crown barb in 1800x magnification (a) and 10.000x magnification (b). C: cortex, S: spongy layer, V: vacuole, M: melanin granule, KR: keratin rod, AC: air-channel. Scale bar in (a) 10 μ m and (b) 2 μ m. The black square in (a) indicate structures enlarged in (b).

'Scatters' consist of keratin/air interfaces in nanoscale organization, with approximately fixed distance between 'scatters' (Prum 1994), determining the location of the peak of maximum reflectance ('hue'), the wavelengths of strongest constructive interference (Prum 1994). Important determinants of the resulting colour are thus the dimensions, regularity and thickness of the 'spongy structure' (Andersson 1999). At a larger scale and important in this thesis (paper V), the total amount of colour producing structure exposed in a plumage patch should affect properties such as 'chroma' (relative intensity of the dominant wavelengths) and 'brightness' (Andersson 1999).

Occurrence of structural plumage colours

Besides the studies of sexually selected UV reflection in blue tits *Parus caeruleus* presented in this thesis, there are several other examples of avian UV ornamentation (reviewed in Bennett et al. 1994; Finger & Burkhardt 1994; Andersson & Amundsen 1997; Bennett et al. 1997; Hunt et al. 1998; Johnsen et al. 1998; Keyser & Hill 1999). However, simply demonstrating UV reflectance is not strong evidence of UV signalling since the UV-component can be a nonadaptive secondary effect of colour signalling in longer wavelengths (Andersson 1996). 'Pure' UV reflectance's (i.e. main reflectance below 400 nm) is scarce, well documented only in the black lory *Chalopsitta spp.* (Burkhardt & Finger 1991) and the Asian whistling thrushes *Myiophonus spp.* (Andersson 1996). The reasons for this apparent scarcity is unknown but ecological and/or physical constraints, such as the composition of the light habitat and lower signal efficiency of UV signals at long distance signalling have been suggested (Andersson 1996).

Structural colours and sexual selection

Until recently, structural coloration has typically been regarded as lacking the variation and quality-indicating mechanisms presumed by sexual selection (Borgia 1985; Gray 1996). However, the use of UV/VIS reflectance spectrometry has changed this view and there are now several examples of UV colours as variant and important in mate choice. In addition to sexual communication, UV-vision has also been found to function in foraging (Viitala et al. 1995; Church et al. 1998) and possibly also in orientation (Vos Hzn et al. 1994; reviewed in Cuthill et al. 2000a). The first study to show evidence of UV reflecting plumages affecting behaviour was Maier (1993) who showed that Pekin robins *Leiothrix lutea* discriminated between mates viewed under normal UV and UV-deprived conditions (using filters). This implied that UV-cues were important in social signalling but did not exclude the possibility of discrimination between birds of different brightness (i.e. not colour perception). By using the same technique as Maier (1993) but controlling for brightness with the use of filters with different opacity, Bennett et al. (1996) showed that female zebra finches have strong preferences for males viewed under normal UV-including light over males seen in UV-deprived light conditions. Even stronger evidence for ultraviolet colour vision and communication was achieved by manipulating a colour signal directly (rather than the entire light environment). Andersson & Amundsen (1997) used a UV-blocking 'paint' to reduce the UV-component of the brilliant UV/violet chest of bluethroats *Luscinia svecica*. In outdoor aviaries on the breeding ground, females discriminated between these males and controls with equally but spectrally uniformly reduced 'brightness', thus demonstrating ultraviolet colour vision. Applying the same technique on free ranging bluethroats, Johnsen et al (1998) found that females avoided UV-reduced males both as social males and extra pair mates. The above experiments show that UV cues in plumage do affect mate choice, at least in the sense that completely removing it affects female preferences.

Mate choice based on natural UV reflectance variation was first demonstrated in starlings *Sturnus vulgaris* by Bennett et al. (1997). Female preference ranks of males viewed in either UV deprived light or UV-including light were highly correlated within but not between treatments, showing that natural variation in UV reflectance consistently affect mating preferences.

Honest structural colour signals?

As mentioned, the predicted costs of signal production or signal emission (Johnstone 1995; Johnstone 1997) have not been identified for structural colour properties. Speculations include potential costs in terms of energy and time for producing the colour structure as well as effects of developmental stability on the thickness and regularity of the nanostructure (Fitzpatrick 1998; Andersson 1999). Maintenance of the plumage signal might also incur costs that only high quality individuals can afford. For example, abrasion of the parts (barbs) containing the colour producing structure is likely to affect the resulting colours. Hence, costs of keeping the plumage in good condition (e.g. preening, finding shelter and food etc.) may affect the colour and mediate honest signalling of phenotypic quality.

Apart from blue tits (this study), the only indication of structural colours being a signal of phenotypic quality comes from the blue grosbeak *Guiraca caerulea*, in which males in better nutritional condition during moult grew UV/blue plumage patches of both larger size and a more shortwaved hue (Keyser & Hill 1999). Furthermore, these males were larger and held territories of both larger size and higher quality (Keyser & Hill 2000). With these few indications of honest signalling mechanisms, there are few clues to the benefits of female choice based on structural colours. It is interesting, however, that in a comparative study by

Owens and Hartley (1998), it was found that extra pair copulation (EPC) frequency covaried with sexual dichromatism based on structural, but not with melanin or carotenoid coloration. This seems to suggest genetic mate choice based on structural colours, which is interesting considering the likely genetic aspects of the developmental stability affecting structural colour properties proposed by Fitzpatrick (1998) and Andersson (1999).

MATERIAL AND METHODS

The blue tit

The nominate race of blue tit *Parus caeruleus caeruleus* is a small (9-13 g) passerine commonly found in deciduous forests of Fenno-scandia and the Baltic region. It is generally considered as a resident in its distribution area but some years it makes eruptive movements, mainly to the west and south (Cramp & Perrins 1993). Blue tits feed mainly on insects and spiders but readily accept seeds and fruits during times of food scarcity.

Blue tits are described in the literature as being only slightly dimorphic, both in morphology and coloration (Cramp & Perrins 1993). Both sexes have colourful plumage, containing both carotenoid pigment colours and structural colours. Adults undergo a complete moult after the breeding season while juveniles undergo a partial moult. Males often start to moult before females and during times when still caring for young (Cramp & Perrins 1993).

Blue tits are socially monogamous but with regularly occurring polygyny, up to 20% of the males in some populations (Kempnaers et al. 1995). Pair formation occurs at the end of winter with social pairs persisting into the next season if both partners survive. Breeding starts already from the age of 1 year and breeding pairs defend territories of varying sizes, depending on habitat quality and suitable nest cavities. Blue tits readily accept nest-boxes for breeding, making them a convenient study-species. Nest-building, incubation and brooding of young is done almost exclusively by the female, but males feed the female during incubation and also assists in feeding of the young. Average clutch size is 10-12 eggs and incubation 12-15 days. Chicks are fed for 16-20 days before fledging. Blue tits also show high levels of extra-pair fertilizations (Kempnaers et al. 1992; Kempnaers 1995; Krokene et al. 1998), and females appear to actively seek copulations with neighbour males with high survival probability to the subsequent season (Kempnaers et al. 1992; Kempnaers et al. 1997). Furthermore, in a Swedish blue tit population, females paired with males of high viability (survival prospects) adjusted offspring sex ratio towards sons (Svensson & Nilsson 1996; but see Leech et al. 2001). Together, the evidence suggests viability based female mate choice in blue tits, but how this information is communicated is unclear.

Fieldwork was done in two populations of nest box breeding blue tits in two deciduous woodlands in southern Sweden, one outside Göteborg on the Swedish west coast and the second on the island of Gotland in the Baltic Sea. A total of 413 blue tits were caught and measured over the years 1995, 1998-2001 at four different life stages, post-moult (October), winter (January), nest-building (April and May) and chick-feeding (June). Although some birds were recaptured between years, no bird was included more than once in analyses, e.g. reflectance or breeding parameters.

Birds were captured using mistnets at a feeder-station during post-moult and winter, while breeding territorial blue tits were caught by mistnets, song playback, decoys and in some occasions nestbox traps. Birds were sexed and aged according to Svensson (1992) and ringed with aluminum rings and in the Göteborg population with additional colour rings for field identification. In addition, breeding birds caught during chick-feeding were sexed on the basis of the presence (female) or absence (male) of an incubation patch. Small blood samples (5-50 µl) from adults and nestlings (1-10 days old) were obtained from the carpal vein for

molecular sex determination and future parentage analysis. Standard biometry measurements were taken (wing length, weight, tarsus length) with the length of the erectable crown feathers also measured in 1995 and October 2001. Spectrometric measurements of plumage colours were also done (method below). From several adults, five to ten feathers from the central part of the crown were collected with soft-edged tweezers. Care was taken to sample feathers from approximately the same spot between birds. In addition, many adults birds in the Gotland population were also recaptured during chick feeding to confirm the initially assigned parents for each brood. After being ringed, measured and blood sampled birds were released back to their capture place and when applicable, monitored at regular intervals to determine laying date, clutch size, brood size and number of fledglings.

Molecular sex-identification

Avian molecular sexing is achieved by using the size differences of the amplified products from the two homologous chromo-helicase-DNA binding (CHD) genes situated on the sex chromosomes (ZZ in males and ZW in females). Two slightly different protocols were used between study years. In 1995, following DNA extraction using the Qiagen blood amp kit (Qiagen Inc., Hilden, Germany), the PCR protocols of Ellegren (1996) was followed. Three primers (2945F, cfR and 3224R) which together amplify a 630 bp fragment on the Z-linked avian CHD gene (both sexes) and a 210 bp fragment from the W-linked fragment in females only were used. Amplified products were separated electrophoretically on agarose gels and then visualised with ethidium bromide. All individuals show one strong band (630 bp fragment) while females had one additional weaker band (210 bp fragment). In 1998, following chelex DNA extraction, PCR primers P2 and P8 (Griffiths et al. 1998) were used to amplify introns of sex specific length from the CHD gene. Products were resolved on 6% denaturing polyacrylamide gels and visualised by silver staining. In males, primers P2 and P8 amplify a single fragment (CHD-Z) while in females they amplify two differentially sized fragments (CHD-W and CHD-Z). Molecular sex identification confirmed the sexing based on biometry and plumage of all birds from 1995 (except one pair) and 1998 (I, II).

Reflectance spectrometry

Objective measures of plumage reflectance from six patches on the blue tit (figure 2) were obtained with a PS1000 or S2000 reflectance spectrometer (Ocean Optics Inc., Dunedin, USA).

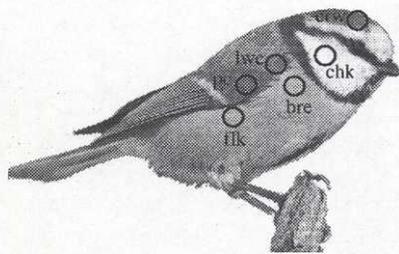


Figure 2. Reflectance spectrometric measured patches on the blue tit; crown (*crw*), cheek (*chk*), lesser wing coverts (*lwc*), primary coverts (*pc*), breast (*bre*) and flank (*flk*).

Briefly described, a fiber-optic cable with six illuminating fibers surrounding a measuring fiber transmits light from a UV/VIS light source (DH2000 deuterium-halogen lamp), encompassing the entire spectrum from 320-700 nm. The reflected light is then transmitted back through the measuring fiber to the spectrometer in which it is refracted onto an array of light sensitive diodes. A cylindrical sheath was attached to the fiber-probe to standardise the measuring distance and exclude ambient light. The probe was held perpendicular to the plumage and 3-5 scans were taken, removing the probe between each scan. Each obtained reflectance measure is then divided with the corresponding reflectance from a white standard with approximately 100% reflectance over the relevant spectra. The computer software (CSPEC, Ancal Inc. San Diego, USA) plots these relative measurements according to wavelength to give a 'continuous' function of the wavelength composition of measured colours.

Reflectance data was imported to a spreadsheet program where objective indices of the three main perceptual dimensions of colour vision ('hue', 'chroma' and 'brightness') (Hailman 1977) are computed as described below. The understanding of bird colour signals requires detailed knowledge about several aspects of a bird's colour perception, including cone spectral sensitivities, neural wiring and neural processing, data that are available for very few animals. A logical approach to objective analysis of colour was therefore to extract parameters from reflectance spectra with relevance to colour perception. Certain features of reflectance spectra, such as position and steepness of peaks and cutoffs, strongly predict the perception of 'hue' and 'chroma' in human colour vision systems and it seems reasonable to assume similar colour sensations also in other colour vision systems (I; Endler 1990). Another commonly used method of objective analysis of colour is Principal Component Analysis (PCA) (Cuthill et al. 1999). This method transforms a large number of correlated variables (wavelengths in the case of reflectance analysis) into a few orthogonal variables (the principal components (PC)). Each PC then summarises the variation in each plane and in the case of reflectance analysis, PC1-3 captures most of the variation (>98%). However, the major drawbacks of PCA are that the resulting PCs are not always easily understood in terms of colour perception ('hue', 'chroma' and 'brightness') and that they are unique for the dataset that generated them and thus not easily comparable between studies (Grill & Rush 2000). Furthermore, in capturing the variation in human colour space of blue tit reflectance measurements, PCA had less explanatory power than the simple, objective estimates (peak heights, steepness, etc.) (S. Andersson, unpublished data) used throughout this thesis.

'Hue' is likely to be strongly correlated with the wavelength of peak reflectance ($\lambda(R_{\max})$), especially in 'simple' spectra (unimodal spectra) since it roughly measures which cones are stimulated the most. Hence, in a human colour vision system, a reflectance spectra with $\lambda(R_{\max})$ of 420 nm will be sensationed as a blue colour while a $\lambda(R_{\max})$ of 600 nm will be seen as red (Endler 1990). $\lambda(R_{\max})$ has been used as hue measure in several studies analyzing blue plumage colours. Variation in $\lambda(R_{\max})$ of blue tit blue plumage colours is a surprisingly good predictor ($r^2 > 0.4$) of human perceived 'hue' considering that $\lambda(R_{\max})$ of this reflectance is situated outside the human visible range.

There are several ways to objectively estimate 'chroma' (spectral purity). Generally, spectra that exhibit steeper slopes and greater intensity differences between spectral segments will appear more 'chromatic' than spectra with smaller changes (Endler 1990). Therefore 'chroma' was calculated as the product of relative height difference ($(R_{\max} - R_{\min}) / R_{\text{average } 320-700}$) and the value of maximum negative slope (maxfall) of the reflection peak to give our 'peaky chroma' measure ($((R_{\max} - R_{\min}) / R_{\text{average } 320-700}) * [\text{maxfall}]$). Our calculated 'peaky chroma' measure shows a strong correlation with human colour vision based 'chroma' ($r^2 > 0.7$). We also computed UV-chroma as the proportion of UV reflectance to total reflectance ($R_{320-400} / R_{320-700}$). This measures specifically address the contribution of UV to the signal, and

captures variation in both 'chroma' (relative peak height) and 'hue' (peak position in relation to the 400 nm border of human vision).

'Brightness' (spectral intensity) was estimated by $R_{320-700}$, the sum of reflectance from 320-700 nm, corresponding to the typical visual range of birds, which show a close association with human perceived 'brightness' ($r^2 > 0.9$).

UV-manipulation method

In paper II, manipulation of male crown UV reflectance was done to study the effect on male attractiveness. A UV reduction of plumage reflectance was obtained by smearing a UV-absorbing coating directly onto the plumage patch with cotton swabs. The coating consisted of a mix of fly dressing based on preen gland fat (CDC) and two UVA absorbing sun block chemicals (Parsol 1789 and MCX, Roche) which was the experimental treatment (UVR). The control treatment (C) consisted of CDC only (for more detailed information about manipulation technique, see Andersson & Amundsen 1997). The effect of this UVR treatment is a strong reduction of reflected UV light (although invisible to human eyes) while the controls are almost identical as untreated males (figure 3).

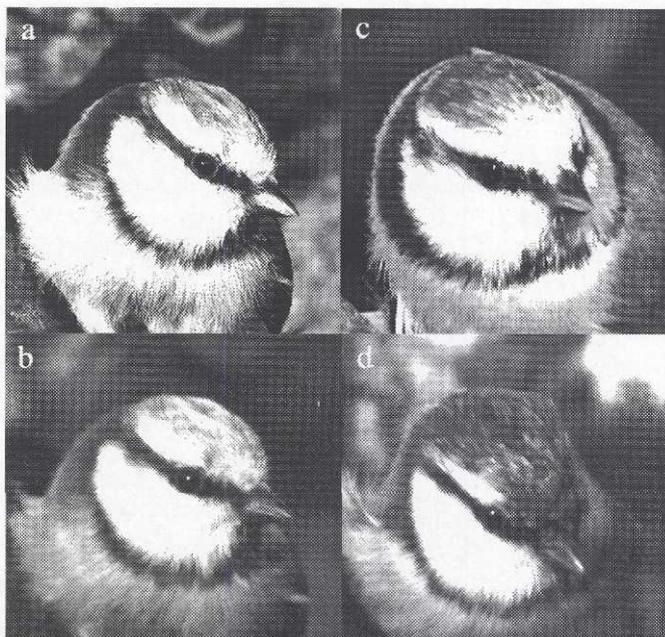


Figure 3. Pictures of crown from one control male (a, b) and one UVR male (c, d) taken under full daylight. Picture (a) and (c) taken with a lens that transmit all human visible wavelengths while picture (b) and (d) taken with a lens that only transmit UV. Note the large contrast between crown and cheek in male with UVR treatment (d) seen through the UV transmitting lens compared with control male (b).

Feather morphology measurements

In paper IV we study the external morphology and abrasion of single feathers sampled from different individuals during different times of the year. Total feather length, length of rachis, individual barb length, barb width and barbule density were measured from digital images taken through a stereo-microscope. We quantified feather wear as average barbule density and number of broken barbs at different times of the year. Number of broken barbs is the number of barbs at least one standard deviation shorter than the average for that particular barb class, as estimated from the newly grown, and presumably minimally abraded, feathers collected from newly moulted birds. Barbule density, known to decrease with plumage age (Ginn & Melville 1983), was calculated as the average number of barbules per mm barb, corrected for broken barbs.

RESULTS AND DISCUSSION

'Hidden' sexual dichromatism in crown UV-coloration (I)

Sexual dichromatism is in many cases believed to be the outcome of sexual selection through female choice (Andersson 1994). Despite strong evidence for viability-based sexual selection in blue tits, based on high levels of extra pair fertilizations (Kempnaers et al. 1992), blue tit plumage is very similar between the sexes (Cramp & Perrins 1993) and believed unimportant in intra-specific signalling (Stokes 1960). However, these statements are based on visual inspection of plumage reflectance by the UV-blind and yellow-biased colour vision of humans.

Avian colour vision is known to differ considerably from that of humans (reviewed in Cuthill et al. 2000b), the most obvious difference being the sensitivity to UV in birds. The evidence of UV reflection from structural plumage colours in other species and the fact that these colours are important in mate choice (Bennett et al. 1996; Andersson & Amundsen 1997; Bennett et al. 1997) suggest that the structural colours of blue tits also have the potential to reflect UV and function in intraspecific signalling. Motivated by the fact that the crown plumage is known to be used in both agonistic and sexual displays (Stokes 1960), the aim of paper I was to explore the structural blue crown colour in blue tits by the use of UV/VIS spectrometry. As a first test of a signal function, we also explore how this colour influences mate-choice in this species.

Forty-one blue tits (14 subadult and 9 adult females, 12 subadult and 6 adult males) measured in April/June in Göteborg, during the period of chick feeding, showed a marked sexual dichromatism when including the UV-waveband. Male crown reflectance has maximum peak reflectance $\lambda(R_{\max})$ at shorter wavelengths (figure 4), and larger differences between maximum and minimum reflectance than females, resulting in colour with a more shortwave 'hue' (-13 nm) and higher 'chroma' in males. Analysing the contrast of blue tit plumage coloration in relation to the prevailing background typical at the time of display, shows that contrast against the background is maximised in the UV-range (I). The seasonal change in $\lambda(R_{\max})$ (paper IV) towards longer wavelengths as the season progress is likely to underestimate the contrast analysis. A more shortwaved 'hue' at the time of intraspecific signalling in late winter/early spring should thus further increase conspicuousness in the UV range.

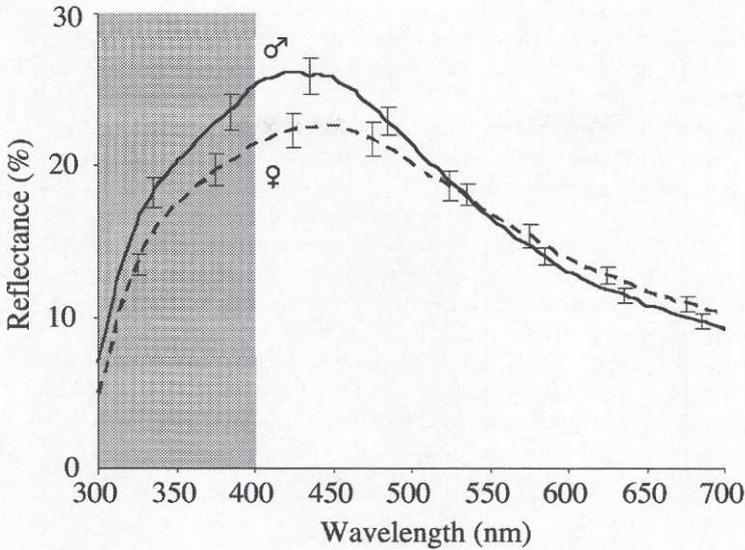


Figure 4. The blue tit crown signal spectra ($\pm s.e.$) in males ($n=18$, solid line) and females ($n=23$, dashed line).

A strong sexual UV-dimorphism in the crown and other parts of blue tit plumage was reported simultaneously by Hunt et al. (1998). This demonstrated that the UV sexual dichromatism is robust and spans across other blue tit subspecies (Sweden: *Parus caeruleus caeruleus*, Britain: *Parus caeruleus obscurus*). Among the 18 breeding pairs studied, there was significant assortative mating with respect to 'UV-chroma', which was not confounded by age. This implies that the crown UV-ornament (or correlated aspects of other not measured plumage coloration) functions as an intraspecific signal. The conspicuous display of the crown patch in the male courtship dance (Stokes 1960) suggests that it plays a role in female choice. Furthermore, the erectable crown plumage also showed a sexual size dimorphism (controlling for body size) with 8% longer male feathers, further supporting the idea that crown plumage serves as a sexual signal during mate choice.

However, the Swedish study (paper I) and the British study (Hunt et al. 1998) showed quantitative differences in spectral peak position ($\lambda(R_{max})$), which are discussed further in paper IV.

To conclude, in their own cognition and seen in typical light habitat, blue tit plumages are likely to appear strikingly sex dimorphic, with males being the more conspicuous sex as predicted by sexual selection theory. The influence of crown coloration on mating pattern (assortative mating) together with the use of this ornament during courtship displays (Stokes 1960) suggest that females use UV-coloration as a cue to discriminate between males and perhaps also vice versa.

Sex ratio adjustment in relation to male UV-attractiveness (II and III)

When the relative fitness returns of sons and daughters differ, sex allocation theory predicts biased investment in the more profitable sex (Charnov 1982), for example by adjustment of the offspring sex ratio. One potential reason for different indirect fitness returns from sons and daughters is variation in paternally inherited attractiveness, which influences the future

reproductive success of sons more than daughters. Females mated to attractive males should thereby bias her offspring sex ratio towards sons. After the pioneering study by Burley (1986), molecular sex determination methods have recently made it possible to detect adaptive sex ratio adjustments in the wild.

With the newly identified epigamic UV-coloration and assortative mating (I) and a method to manipulate UV-reflectance (Andersson & Amundsen 1997), the blue tit presented an ideal system for testing the prediction of sex allocation in response to male attractiveness. Moreover, a previous blue tit study had indeed found a correlation between brood sex ratio and male viability (winter survival) (Svensson & Nilsson 1996). Yet another study had found that males with high subsequent survival were preferred by females as extra pair copulation mates (Kempnaers et al. 1997). However, neither of the studies identified any likely cues by which females discriminated between males of different survival qualities. Could this be the function and evolutionary background of the sexually dimorphic UV-coloration?

To test this hypothesis, we carried out an experimental study on a blue tit population on Gotland in collaboration with Ben Sheldon and Simon Griffith at Uppsala University. Prior to egg laying in 1998, male crown UV reflectance was either reduced (UVR in figure 5) or control treated (same fat coating, but without the UV-blocking chemicals) (C in figure 5).

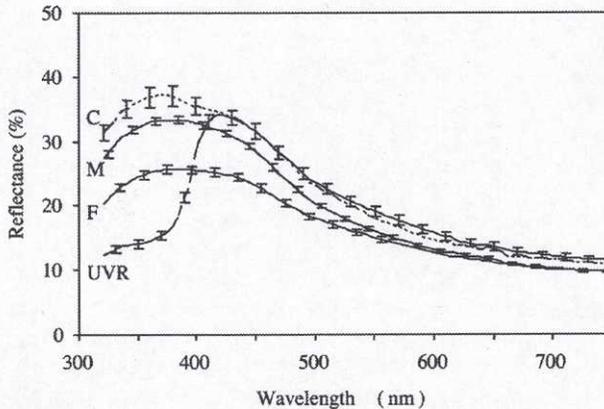


Figure 5. Average spectral reflectance (320-750 nm) from blue tit crown plumage (percentage reflected radiance compared to that of a white standard). M=males, F=females, UVR=UV-reduced males and C=control treated males. Where spectra converge, standard error bars are excluded from female (F) and control male (C).

Masking the male ultraviolet reflectance reversed a positive correlation between reflectance ('UV-chroma') and brood sex ratio observed in control pairs (figure 6a,b; continuous line). Thus females mated to males with a naturally high UV chroma and shortwave hue adjust their offspring sex ratio toward sons as predicted if the UV-colour functions as a heritable signal of phenotypic quality.

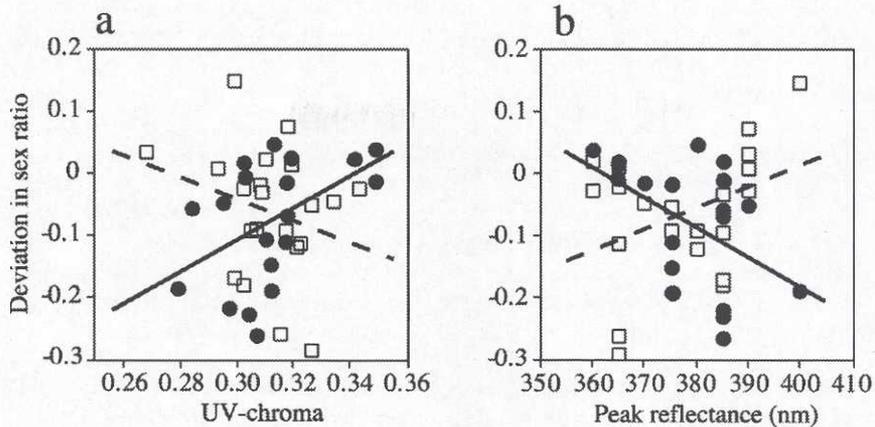


Figure 6. Relationships between spectral reflectance parameters and deviations from expected sex ratio (positive deviations in the sex ratio indicate an excess of males). Data are shown for broods of blue tits from UV-reduced (dashed lines, open symbols) and control (continuous lines, filled symbols). **a**, 'UV-chroma', likelihood ratio control group: $\chi^2_1=4.04$, $p=0.044$, UVR group: $\chi^2_1=4.28$, $p=0.039$ **b**, peak reflectance, likelihood ratio control group: $\chi^2_1=5.94$, $p=0.015$, UVR group: $\chi^2_1=8.77$, $p=0.003$.

Somewhat surprising was the finding of the opposite pattern amongst UVR males when their pre-manipulation values were used. Females mated to males, which initially (before manipulation) had high 'UV-chroma' and shortwave 'hue', adjusted offspring sex ratio towards daughters (figure 6a,b; dashed line). However, the correlation between colour patches within individuals (VI) could result in a higher treatment effect in naturally UV-bright males due to a higher contrast between the manipulated crown and the surrounding unmanipulated plumage patches.

The analysis of sex ratio adjustment showed influences from variables other than male treatment (attractiveness), namely age of male and female, laying date and habitat, all of which are theoretically predicted to influence sex ratio by the sex allocation theory (Charnov 1982). The proportion of variance in sex ratios explained after entering all the relevant variables are high (51-55%), suggesting a high degree of female control over primary sex ratio. However, the mechanism by which females adjust offspring sex ratios remains unknown (but see Krackow 1995, for further discussion).

As discussed above, previous studies have found a higher survival in males that females preferred as EPC partners (Kempnaers et al. 1997). Recapture of 1998 experimental birds the following year revealed a positive significant relationship between survival (assuming that non-recaptured males died) and 'UV-chroma', suggesting that it serves as a viability indicator. Surviving males also showed a significantly higher proportion of sons in their brood the previous year, a relationship also reported by (Svensson & Nilsson 1996).

Skepticism regarding the ability and evidence for adaptive non-random allocation of sex to offspring in birds have been put forward (Radford & Blakey 2000) because of the non-congruent patterns between studies of this subject. However, in a re-analysis of a larger sample size, comprising three years of brood sex ratio data from the same population, confirmed in two out of three years the positive relationship between proportion of sons in male broods and male crown UV-coloration and future prospects to survive (III).

To conclude, variation in blue tit crown structural coloration seems partly to be sexually selected by female adaptive sex ratio adjustments in relation to this character. The positive relation between male survival and crown coloration suggest this character as a signal of phenotypic quality.

Seasonal changes in crown UV-reflectance (IV)

Assortative mating and sex ratio adjustment in the field (I, II) as well as aviary mate choice trials (Hunt et al. 1999) have strongly suggested that the structural UV coloration of blue tits constitutes a target for female choice. The UV-signal intensity ('UV-chroma') also predicted male survival until the next breeding season (II, III), suggesting that UV-colour might function as an honest viability indicator, potentially benefiting females through more viable offspring. However, the mechanism by which the blue tit UV signal honestly advertises male quality is unknown, largely because the proximate sources of variation in structural colours in general, and UV-colours in particular, rarely have been investigated. Clarification of phenotypic and environmental sources of signal variation is thus important when trying to understand signal function and potentially honesty enforcing costs. Furthermore, a substantial discrepancy in $\lambda(R_{\max})$ between the previous British (Hunt et al. 1998) and the Swedish (I) studies of the reflectance dimorphism, prompted us to investigate the source of this variation.

One potential explanation (paper I) is a temporal change in plumage colour, since the two studies were carried out at different times of the year, i.e. at different plumage ages (British study: February-March, Swedish study: April-June).

In this study we therefore focus on seasonal colour variation in blue tits. We report colour measurements from 400 blue tits, sampled during four times of the year to clarify signal variation and resolve the discrepancy in $\lambda(R_{\max})$ reported in previous studies (paper I; Hunt et al. 1998).

The average reflectance spectra in figure 7 show a significant change in $\lambda(R_{\max})$ as the season progress from moult to chick-feeding. The most UV-shifted reflectance peak is found in newly moulted birds (males: 359 nm, females: 373 nm). As the season progress, $\lambda(R_{\max})$ shifts towards longer wavelengths and is located above 400 nm for both sexes by the time of chick-feeding, a shift of 46 nm in males and 40 nm in females.

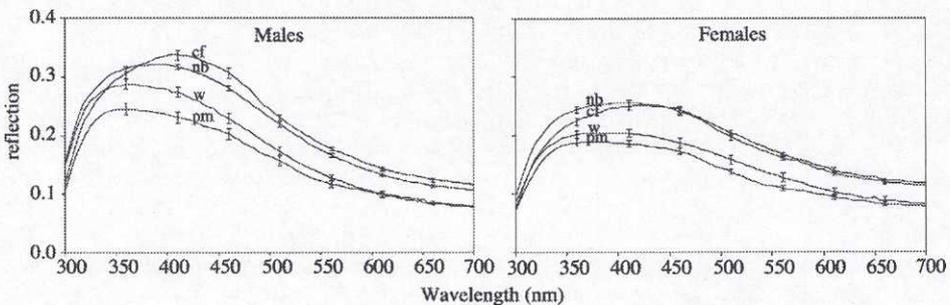


Figure 7. Male and female blue tit (ages pooled) UV crown plumage measured (mean \pm s.e) over the four stages, post-moult (pm), winter (w), nest-building (nb) and chick-feeding (cf).

Other colour perception variables also change with plumage age. As shown in figure 7, 'brightness' increases significantly as the season progress. 'UV-chroma' (relative UV-reflectance), also changes as the season progress, partly because of changes in relative peak height, but primarily because $\lambda(R_{\max})$ drifts out of the UV, thereby decreasing 'UV-chroma'.

To conclude, blue tit UV coloration change over the season, perhaps as a consequence of increased feather wear. Furthermore, the seasonal variation in structural coloration reported here largely explains the discrepancy in position of maximum reflectance $\lambda(R_{\max})$ between the British and the Swedish studies.

Plumage wear cause seasonal changes in structural UV-coloration (V)

Traditionally, structural colours have been viewed as invariant and cheap to produce (Borgia & Collias 1990; Gray 1996). This view has been challenged by several recent studies, showing extensive variation in structural colours and function in mate choice (I, II; Andersson & Amundsen 1997; Bennett et al. 1997; Hunt et al. 1997; Hunt et al. 1998; Johnsen et al. 1998; Hunt et al. 1999; Keyser & Hill 1999; Keyser & Hill 2000). However, the mechanisms and costs of producing structural colours remain a speculative issue. Production-costs of the colour producing nanostructure have been suggested to ensure honesty in structural colour signals (Andersson 1999; Fitzpatrick, 1998). On a larger scale, the relative amount of colour structure exposed in a patch, both vertically along the light path (I) and in the plumage plane (i.e. coloured barbs as opposed to other components), should also affect the reflectance and then mainly spectral purity ('chroma') and 'hue'. External morphology and size of signalling feathers are then likely to have a significant impact on plumage reflectance. This suggests that simpler, more general costs of feather growth (e.g. Lindström et al. 1993) and/or maintenance of plumage feathers might also constitute critical constraints on the colour signal. In this paper, we analyse the role of external feather morphology as one possible proximate basis of structural UV signal variation in blue tits. In addition to exploring the physical basis of the previously described sexual dichromatism (paper I; Hunt et al. 1998) and the individual reflectance variation, this study was also prompted by a recently documented seasonal colour change (IV).

Despite a rather crude analysis based on a small sample of single plucked feathers, several features of external feather morphology and abrasion are potentially important proximate mechanisms causing the observed structural colour variation in blue tits. Firstly, males have longer crown feathers compared to females, as a result of both longer rachis and barbs (figure 8). Most of this sexual length dimorphism stems from males having on average 8.7% longer barbs (in which the colour structure resides) compared to only 2.0% longer male rachis.

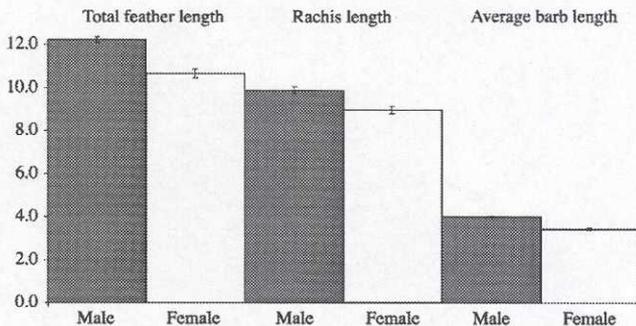


Figure 8. Average (mean \pm s.e.) total feather length, average rachis length and average barb length for male and female crown feathers (ages pooled) (all measurements on y-axis in mm).

Secondly, abrasion pattern in the two measured feather wear variables (barbule density and number of broken barbs) showed a striking sexual dimorphism. Loss of black barbules showed an initially much higher rate in males compared to females (figure 9a). Already in winter, males had lost 50% of their black barbules while females only lost less than 20%. The other morphological mechanism in relation to sexual dichromatism is that male barbs seemed more resistant (or less subjected) to abrasion (figure 9b), as judged by the several times higher frequency of broken barbs in female feathers sampled in chick feeding compared to male feathers.

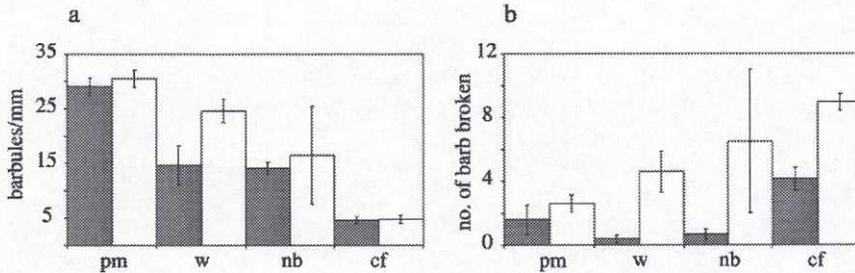


Figure 9. Seasonal change in barbule density (a) and number of barbs broken (b) for males (filled bars) and females (open bars). Abbreviations: pm=post-moult, w=winter, nb=nest-building, and cf=chick-feeding.

Taken together, the longer and more abrasion resistant male barbs and maybe also the more rapid shedding of barbules are probably important proximate mechanisms behind the sexual dimorphism in 'UV-chroma' of the blue tit crown plumage reported earlier (I, II). As a result of these effects, male crown feathers seem to have maximised reflectance surface from barbs (and thus intensity of the colour signal) since barbule density is more then halved and barbs remain largely intact. Speculatively, these effects of male higher abrasion rate of barbules and higher resistance against barb breakage might represent a sexually dimorphic adaptation to maximise the colour signal in late winter when supposedly signal selection on male is strongest. In support for this is the suggestive signalling peak reached in late winter, with respect to 'chroma' of crown coloration (IV; figure 10), when pair formation is occurring in blue tits (Cramp & Perrins 1993).

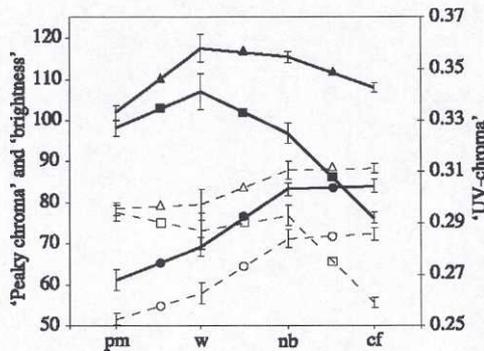


Figure 10. Seasonal changes in 'UV-chroma' (squares), 'peak-chroma' (triangles) and 'brightness' (circles) for males (thick solid line and filled symbols) and females (thin dashed line and open symbols). (Data from paper IV).

The pattern of changes in external feather morphology seems consistent with the earlier described seasonal changes in crown plumage reflectance (IV) but the effects were not strong enough to predict individual reflectance variation within stages (likely as a result of small sample size), with one exception; the negative correlation between barb breakage and 'UV-chroma' in the chick feeding period. Based on the rather crude abrasion estimate (number of broken barbs in one feather) the explanatory value (19% in males and 23% in females) must be considered quite strong and suggest feather wear, at least partly, as the mechanism behind the seasonal change in crown coloration.

According to current signalling theory, signal honesty is obtained when aspects of signal design make it impossible and/or unprofitable for low quality individuals to employ displays that are not representative of their state (Johnstone 1997). The pattern of sexual dimorphism in feather wear, covariation between external feather morphology and colour variation due to season and sex, fits well in this frame of honest signalling. Firstly, barbule density may therefore affect thermoregulatory capacity (Middleton 1986; Redfern & Alker 1996) as well as colour properties of the plumage. The higher male rate of barbule loss, when insulation properties of plumage are likely to be important (winter), to maximise signal intensity, is thus likely to represent a handicap signal (Zahavi 1975). Secondly, the suggested relationship between external feather morphology and colour implied in this study suggests feather wear as the factor causing the colour change. Feather wear may be greater in birds that must forage more or that must forage in sub-optimal areas, travel further while foraging, roosting at bad sites or flee potential threats more frequently. A low quality individual can simply not avoid the cost of increased feather wear and this is then indicated by the strength of the UV-colour signal.

To sum up, beside the earlier suggested production cost of structural colours (I), other more generally costs associated with external feather morphology are also suggested here. Which, if any, of these costs mechanisms are at work is the scope for future studies of UV/blue colour signalling in blue tits.

General structural and carotenoid based colour variation in blue tits (VI)

In blue tits, the discovery of sexual crown plumage dichromatism, mainly in the ultraviolet (320-400 nm) (I; Hunt et al. 1998), has allowed the identification of a signal by which females select males and adjust their offspring sex ratio. It also appears to be a signal of phenotypic quality as judged by a higher winter survival of males with more UV-chromatic crowns (II). Sexual dimorphism and sexual selection of the blue tit crown patch is thus relatively well established, but how this relates to the remainder of the extravagant plumage coloration is unclear. The combination of extensive dorsal structural colours and carotenoid-based (Partali et al. 1987) yellow chest and belly is quite unique, at least among European birds (Mullarney et al. 1999). Several of these colour patches have been found to be sexually dichromatic, with more UV biased and chromatic colours in males (Hunt et al. 1998). However, these overall blue tit plumage patches has not been extensively analysed in terms of both age and sex effects, morphology and other sources of variation relevant to sexual selection.

Carotenoid plumage colours are known to signal individual quality and influence mate choice in several species (reviewed in Hill 1999). In the closely related great tit *Parus major*, yellow ventral carotenoid coloration show variation in relation to sex, age, habitat and health (Slagsvold & Lifjeld 1985; Eeva et al. 1998; Horak et al. 2001). Carotenoid coloration in blue tits is described as being slightly sexually dimorphic (Cramp & Perrins 1993) but no objective analyses of it have been done yet.

In this paper, I therefore analyse plumage reflectance in relation to sex and age from six different plumage patches and possible correlations between these plumage patches (the

blue crown, lesser wing coverts and primary coverts, the carotenoid yellow breast and flank and the white cheek).

Besides the known age differences in hue of primary coverts (Svensson 1992), confirmed in this study, an age difference of chroma ('peaky' chroma and UV-chroma) in all three measured UV/blue patches were revealed. These age differences might represent delayed plumage maturation, which is an indication of sexual selection (Butcher & Rohwer 1988; Hill 1996; Ligon 1999).

For the blue wing patches (lesser wing coverts and primary coverts), we found that sexual dimorphism is at least as strong as in the crown patch. Furthermore, UV-chroma, which appears to be the most important signal aspect in blue tit sexual signalling, (paper I, II) showed positive correlations between patches within individuals. Thus, UV-chroma show strong sexual dimorphism in all the three dorsal plumage patches.

The white cheek patch was significantly brighter in females than in males, in contrast to the white forehead measured by (Hunt et al. 1998). Furthermore, in terms of colour (spectral shape), and given that blue tits perceive white like humans do, the flatter cheek reflectance in older birds should be a 'purer' white' - also to the blue tits.

Turning to the reflectance spectra for the yellow breast and flank patches, males show a significantly lower reflectance (i.e. stronger absorption) around 450 nm, similar to the results in Hunt et al. (1998). The yellow colour is caused by two carotenoids, lutein and zeaxanthin (Partali et al. 1987, S. Andersson & A. Johansson, unpublished data) with the characteristic two-humped absorption peak at 450 nm inverted in the reflectance spectrum. The reflectance dimorphism corresponds to 6% and 5% higher male 'carotenoid chroma' of the breast and flank respectively. The only age effect on carotenoid coloration was a slight hue difference in the breast patch; older birds had a 2 nm more shortwaved reflectance 'cut-on' ($\lambda(R50)$), likely a result of higher carotenoid absorption.

To conclude, in addition to the earlier documented sexual dimorphism and signal function of crown UV-coloration (I, II; Hunt et al. 1998), other plumage patches of the extravagant blue tit plumage also show sex and age differences consistent with a generally more chromatic UV and yellow plumage, and whiter cheeks in older birds compared to subadults. This suggests that sexual selection is acting (directly or indirectly) on the entire structural and carotenoid colour pattern. Further studies and experiments are needed to clarify how signal selection (in both sexes) and ecological and morphological constraints have shaped the brilliant (blue and yellow) plumage of blue tits.

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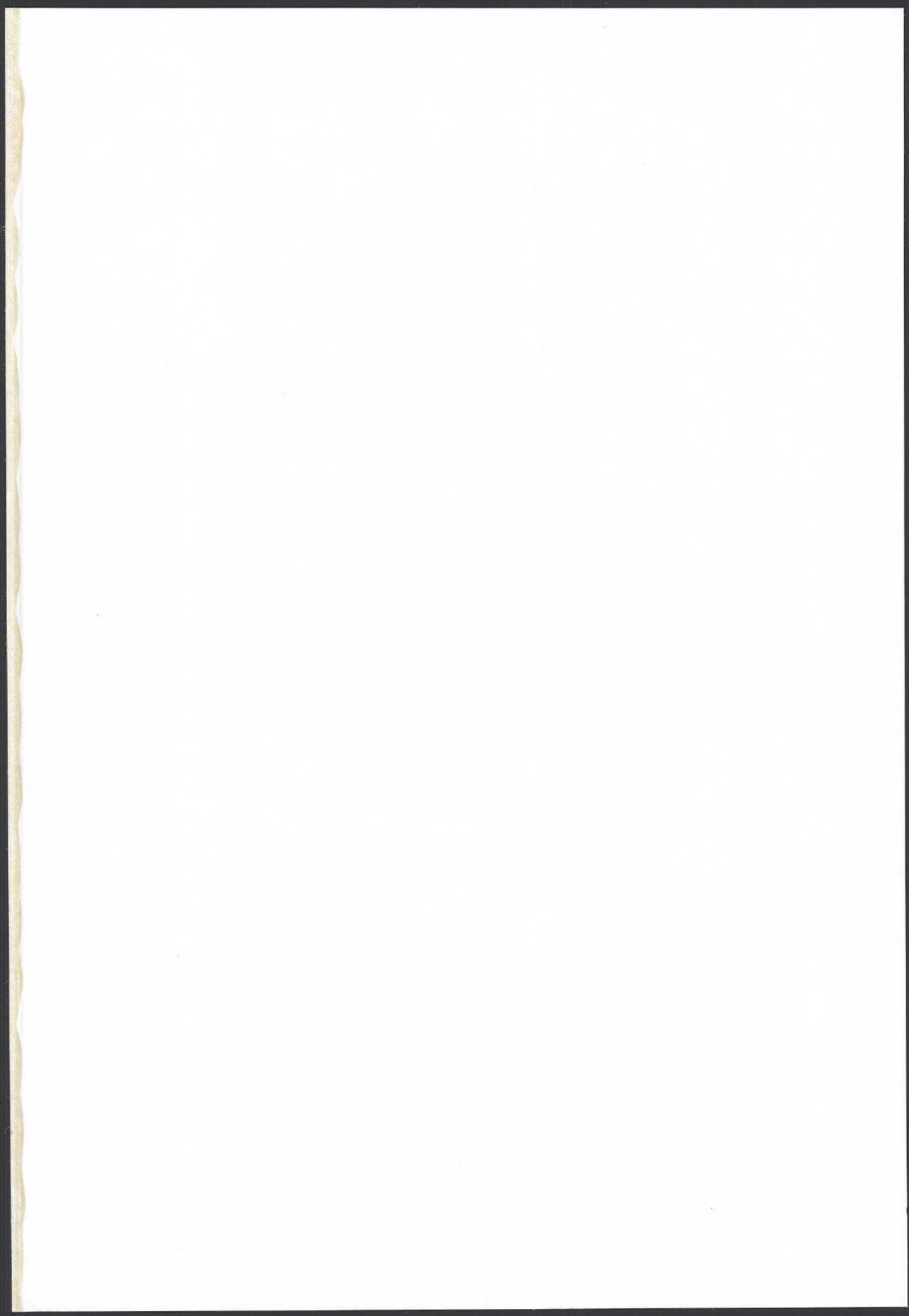
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