Plumage colouration, testosterone and reproductive behaviour in the red bishop (*Euplectes orix*)

Von der Fakultät für Mathematik und Naturwissenschaften
der Carl von Ossietzky Universität Oldenburg
zur Erlangung des Grades und Titels eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

angenommene Dissertation

von Frau Alice Edler

geboren am 05. Mai 1977 in Nürnberg.

Gutachter: PD Dr. Thomas W. P. Friedl

Zweitgutachter: Prof. Dr. Peter H. Becker

Tag der Disputation: 15. September 2009

Dedicated to my parents.

For believing.

Ulrike Aidnik (1952 – 2005)

Werner Aidnik (1945 – 2006)

Table of contents

Appendix A – Wild red bishop males

Appendix B – Aviary red bishop males

GENERAL INTRODUCTION

Introduction

Over the last years, carotenoid-based plumage colouration has been the focus of many studies. Insights have been given into what factors – such as immunological and environmental ones – influence the expression of this plumage, thereby causing an immense variation in ornaments. As red, orange or yellow plumage traits also underlie intense inter- and intrasexual selection, their influence on such things as reproductive effort and success or the outcome of male-male competition have also been under intense scrutiny.

Form and functions of carotenoids

Carotenoids are a special group of organic pigments with unique properties, functions and actions. There are over 700 known carotenoids, divided into two groups – xanthophylls and carotenes. In order to understand their biological importance, it is necessary to understand the relationship between structure, properties and function. Carotenoids belong in the category of tetraterpenoids, meaning they consist of 40 carbon atoms. These are in the form of a polyene chain, which is sometimes terminated by rings. Carotenoids containing oxygen are classified as xanthophylls, whilst those without typically contain only hydrogen and carbon and are grouped under the term “carotenes”, of which the most famous one is probably β-carotene. The observed colour of carotenoids – ranging from pale yellow through bright orange to a deep red – is directly linked to their structure. The double-bonded carbon atoms interact with one another via conjugation, a process in which electrons in the molecule are able to move freely across the molecule. With an increasing number of double bonds between the carbon atoms, electrons have more space to move. This leads to a decrease in the absorbed ranges of energies of light and more frequencies of light are absorbed from the short end of the spectrum of visible light, resulting in a redder appearance (Britton et al. 2008).

Originally, carotenoids evolved in archaebacteria to provide support in lipid membranes and later in photosynthetic organisms as light-harvesting pigments for photosynthesis (Vershinin 1999) by extending the wave length range of light that could be harvested and thereby improving photosynthesis (Britton et al. 2008).

Further, as they are highly reactive to oxidizing agents and free radicals, carotenoids serve an important function as free-radical scavengers. Through this, they protect sensitive systems against oxidative damage, forming the basis of anti-oxidant action (Burton & Ingold 1984, McGraw 2006, Britton et al. 2008), as well as protecting the immune system during immune cell activation and proliferation (Chew 1993, Hughes 2001).

Also, and most importantly for this thesis, carotenoids play a vital role in the world of colour. The yellow, orange and red colouration caused by these pigments is a wide-spread phenomenon in the animal world and can be found in insects, fish and birds, the classic examples being canaries, greater flamingos and gold fish. Animals, however, cannot synthesize carotenoids and need to consume them as an important part of their diet. These ingested pigments can then be metabolized accordingly, meaning that the carotenoids found in tissues are not necessarily the ones ingested (Brush 1990). A classic example for this is the modification of lutein and zeaxanthin into the “canary xanthophylls” as the yellow pigments found in the yellow feathers of canaries, which was first described by Brockman and Völker (1934). The range of carotenoid colouration in the animal kingdom serves several purposes, such as warning and advertising (Summers & Clough 2001, Blount et al. 2003, Casagrande et al. 2006, Bezzerides et al. 2007, Pike et al. 2007, Blount et al. 2009).

After melanins, carotenoids are the second most frequent pigments in bird colouration and were first ascribed as colourants to egg yolk (Willstatter & Escher 1912) and only later to feathers (Brockman & Völker 1934), legs and beaks (Lonnberg 1938) and eyes (Hollander & Owen 1939). Different carotenoids lead to different colours, with astaxanthin, canthaxanthin and adonirubin being responsible for red colouration, while lutein, zeaxanthin and the canary xanthophylls result in yellow colouration. While all birds naturally circulate carotenoids as a result of inevitable dietary uptake, only a few of them actually incorporate these pigments into various integumentary tissues. Also, for the most part, if pigments are deposited, they are only deposited in certain body regions. In the house finch (*Carpodacus mexicanus*) for example, carotenoids only give colour to the crown, breast and rump, while the remaining feathers are melanised. Further, certain carotenoids can be targeted, meaning that only specific pigments are actually used for colouration, even if several others are also consumed (McGraw et al. 2003). The use of carotenoids in ornamental traits has been proposed to be due to their physiological and chemical properties, as they can be used to signal certain aspects of an individual, such as health.

Animal signals and signalling

According to Maynard Smith and Harper (2003), a signal is defined as "...an act or structure which alters the behaviour of other organisms, which evolved because of that effect, and which is effective because the receiver's response has also evolved." Basically, this means that a signal is a morphological, behavioural or chemical feature that has come into existence because it alters or affects the behaviour of its recipient. It also needs to convey information which is of relevance to the receiver, be it about the current state of the signaller or the future behaviour. These signals, however, need to be honest in order for the receiver to want to respond to it. This leads to the question as to what mediates signal honesty. I want to mention two mechanisms, which are proposed by Maynard Smith and Harper (2003). Firstly, Zahavi's Handicap Principle (1975), according to which a signal must be costly to produce in order to be honest. Second, if a signal is an index of some quality, it is honest in itself as the signaller cannot lie about certain aspects such as health or condition (Maynard Smith & Parker 1976). There are a wide variety of signals – those that are made during contests over limited resources such as food or territories or signals occurring during courtship. They can take on several forms, ranging from song over pheromones to body size, all of which pass relevant information from the signaller to the receiver.

According to the Handicap Principle, certain resources need to be subject to an allocation trade-off. This is only the case if the specific resource – in this case, carotenoids – is needed for more than one body function, as well as being in limited supply. As stated above, carotenoids fulfil both criteria. Further, they are not only used for intraspecific, but also for interspecific signalling, such as warning colouration and crypsis. In our case, however, we will concentrate on intraspecific signals.

Influencing factors on colour expression and variation

The expression of carotenoid-based plumage is influenced by several factors, ranging from immunocompetence over parasite load to testosterone levels. The variation resulting from this influence is often used as a visual cue by conspecifics to assess the condition of their counterpart – be it as a prospective mate (female choice) or potential rival (male-male competition).

Immunocompetence and parasites

The impact of immunocompetence and levels of parasitisation on carotenoid-based plumage colouration has been the focus of several studies in the past years, showing a strong link between immune function and ornament intensity and size. Most of these studies focused on the relationship between naturally occurring or experimentally elevated levels of parasitisation and the expression of the ornament. Although the results are often inconsistent, there seems to be a general trend towards a decrease in size or intensity of the colour trait when parasite levels are high or immune functions depressed. The Hamilton-Zuk Hypothesis (1982) proposed a link between colour, immunocompetence and genes, with bright males not only having a lower degree of parasitisation in comparison to duller conspecifics, but also passing this resistance on to their offspring. In terms of immunocompetence, this connection has been shown in several passerine species, for example in greenfinches (*Carduelis chloris*) and zebra finches (*Taeniopygia guttata*). In male greenfinches, Saks et al. (2003) determined that not only did individuals with brighter yellow feathers show a stronger humoral immune response when challenged with a novel antigen, but they were also in better general health, which was indicated by a negative relationship between brightness and the concentration of circulating heterophils. By supplementing male zebra finches with carotenoids, McGraw and Ardia (2003) were able to find out, that supplemented males not only had a redder beak colouration, but that they also mounted stronger immune responses than control males. Both studies give evidence that plumage colouration can be used as a positive indicator for immunocompetence, but there are of course studies showing the exact opposite – brighter or more colourful males suffering from immune suppressive conditions, which was shown in several passerine species. In a different study on male greenfinches, Aguilera and Amat (2007) found contradictory results to those found in the above mentioned study by Saks et al. – the ventral plumage colour of immune challenged males was negatively correlated with the immune response, suggesting instead, that carotenoids in greenfinches are part of an allocation trade-off and invested in colouration instead of immune defence. Similarly, in European blackbirds (*Turdus merula*), Faivre et al. (2003) found that bill colour was negatively correlated with immune response, also suggesting an allocation trade-off in this species. As a last example, Maney et al. (2008) conducted a study on northern cardinals (*Cardinalis cardinalis*) and found that in both females and males, plumage colour was positively related to the heterophil/lymphocyte ratio. In comparison to afore mentioned studies, they proposed that because plumage colour is positively associated with competitiveness, more colourful individuals suffer from more stress due to a higher engagement in competitive behaviours, resulting in the observed positive relationship.

When looking at the link between parasite load and colouration, most studies focused on the relationship between naturally occurring or experimentally elevated parasitisation levels and ornament expression. Although results have been found to be as inconsistent as they are in terms of immunocompetence, the general trend seems to be towards a decrease in size or intensity of the ornament when parasite levels are high. Such negative relationships between colour and parasite prevalence have been found in a variety of studies. In yellowhammers (*Emberiza citrinella*), Sundberg (1995) found a negative relationship between male colour and blood parasite prevalence, showing that more colourful males had a lower degree of parasitisation. This was also determined in greenfinches (Merilä et al. 1999), where bright males had a lower prevalence of *Haematoproteus* parasites.The results of both studies coincide with those found in studies on American goldfinches (*Carduelis tristis*; McGraw & Hill 2000), blackbirds (Baeta et al. 2008) and house finches (*Carpodacus mexicanus*; Hill & Farmer 2005). These studies give evidence to the Hamilton-Zuk hypothesis, allowing plumage colouration to be used as a reliable visual cue for male quality, with brighter and more saturated plumages being an indicator for good health. In comparison, plumage redness in redpoll finches (*Carduelis f. flammea*; Seutin 1994) did not reflect hemoparasitic infection, providing no support for the Hamilton-Zuk hypothesis. A study on great tits (*Parus major*; Fitze & Richner 2002) involving ectoparasites also showed no effect of infestation on carotenoid-based plumage colouration. The positive relationship found by Hõrak et al. (2001) in great tits, with a simultaneous increase in yellow colouration and parasite prevalence in old males, also offers no support for the theory.

Testosterone and "badges of status"

According to the immunocompetence handicap hypothesis (ICHH; Folstad & Karter 1992), testosterone functions as a double-edged sword, enhancing ornamental traits while simultaneously depressing immune functions. Testosterone is also known to regulate not only aggression (Wingfield et al. 1990), but also song (Foerster et al. 2002) and a variety of sexual ornaments and displays. Increased levels are also responsible for a decrease in parental behaviours (de Ridder et al. 2000). Further, because elaborate ornaments are related to high testosterone levels, it was proposed that these dominance signals are costly as their development is controlled by testosterone, which – as stated above – is immunosuppressive (Buchanan et al. 2003). Several studies have shown that large or intense ornamentation is associated with dominance status. In Gambel's quail (*Callipepla gambelii*; Hagelin 2002), for example, the size of head plumes had a significant effect on contest outcome, with large plumed males dominating smaller plumed individuals. In red jungle fowl (*Gallus gallus*), Ligon et al. (1990) found a similar result in respect to not only the comb size of males, but also comb chroma. Size and colour of the comb in this species are strongly affected by testosterone levels and males with very large and very red combs dominated males with smaller and paler combs. When turning to passerine species, the size of the black bib in several species such as house sparrows (*Passer domesticus*; Evans et al. 2000, Gonzalez et al. 2001), Eurasian siskins (*Carduelis spinus*; Senar et al. 1993) and black-capped chickadees (*Poecile atricapillus*; Mennill et al. 2003) was also found to be used as a "badge of status", with individuals with large black bibs being dominant over conspecifics displaying smaller bibs. A study on superb fairy-wrens (*Malurus cyaneus*; Peters et al. 2000) showed that testosterone is needed to moult into nuptial plumage in the first place. When taken to the level of carotenoid-based plumage colouration, it was determined that carotenoids used for colouration cannot be used by the immune system. In a study concerning the role of both testosterone and carotenoids, Blas et al. (2006) proposed that testosterone increases the bioavailability of carotenoids, thereby increasing the level of plasma carotenoids, which can then be allocated accordingly. In a study on house finches, Duckworth et al. (2004) were able to determine that in wild males, redder males had higher testosterone levels than duller conspecifics. In captive males, however, duller males dominated redder ones and were found to have higher testosterone levels, showing that under natural conditions, testosterone was dissociated from dominance. McGraw et al. (2006) were able to experimentally show that beak redness in male zebra finches is positively correlated with testosterone levels, as well as plasma carotenoids increasing with increasing testosterone levels.

Other studies concentrating on the role of carotenoid-based plumage ornaments as status badges in both red-collared (*Euplectes ardens*; Pryke et al. 2002) and red-shouldered widowbirds (*Euplectes axillaris*; Pryke & Andersson 2003), found that males with redder plumage patches dominated duller conspecifics. The dominance signalling function of carotenoid-based plumage patches is further corroborated in a study on Gouldian finches (*Erythrura gouldiae*), where it was also determined that both redder males and females dominated paler individuals (Pryke & Griffith 2006, Pryke 2007).

Condition-dependent signalling

The concept of condition-dependent sexual selection was first proposed by Fisher (1930, also Andersson 1986) and allows females to always select those males as potential mates that will maximize their thereby gained benefits. It can be seen as an all-encompassing term, which unifies all factors influencing the expression of ornamental traits: these are often mediated by condition, which in turn is influenced by several other factors, ranging from nutrition over parasite load to genetic make-up. Nutrition is one of the few factors that directly influences carotenoid-based ornament expression, as carotenoid intake via diet has an effect on the intensity of the expressed colour (see Figure 1).

Although nearly all studies conducted on plumage colouration have focused on a single aspect such as the influence of immunocompetence or dominance, it doesn’t make much biological sense for a female to choose a male on the basis of, for example, his ability to resist parasites *per se*. Instead, as all aspects of an individual seem to be merged under the umbrella term “condition”, a female will always benefit by simply mating with a male in good condition, regardless of the further benefits this encompasses or of the reasons why he is in good condition (Griffith & Pryke 2006).

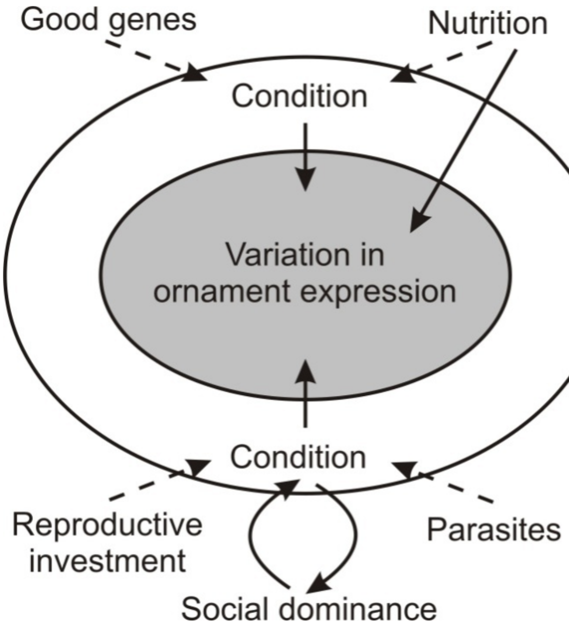

Figure 1: The variation of ornament expression is directly (solid arrows) and indirectly (dashed arrows) influenced by several factors.
After Griffith & Pryke (2006), Bird Coloration – Volume II.

Other studies have shown that carotenoid-based plumage is condition-dependent, such as in greenfinches (Peters et al. 2008), red fodies (*Foudia madagascariensis;* Estep et al. 2006), house finches (Hill 2000) and American goldfinches (McGraw et al. 2005). In all four studies, it was determined that individuals grew duller and less colourful plumage when under food stress before or during moult, therefore allowing colourful plumage to be used as a reliable signal of condition.

Objectives of the thesis

My thesis was divided into two major parts: first the investigation of which factors influence the expression of plumage colouration in male red bishops (*Eupectes orix*) and secondly how these plumage characteristics influence reproductive effort and success, as well as the outcomes of male-male competition. Three hypotheses were laid as the foundation stones of this thesis – Hamilton and Zuk's proposal that bright colouration evolved as an honest signal of parasite resistance (1982), Folstad and Karter's Immunocompetence Handicap Hypothesis (1992) and Rohwer's Status Signalling Hypothesis (SSH; 1975).

The red bishop (*Euplectes orix*) is a sexually dimorph, polygynous weaverbird species, which occurs over large parts of Sub-Saharan Africa. Once they have reached the age of two, males moult into a conspicuous red and black breeding plumage during the breeding season (Figure 2a). Females and juvenile males alike, display a buffy brown plumage with a whitish eye-stripe (Figure 2b). Red bishops are colonial breeders, occurring in reedbeds and bulrush stands along or near water bodies and rivers. Here, breeding males establish small territories, which they actively defend against conspecifics. In these territories, males build multiple nests which are in turn inspected and potentially accepted by females.

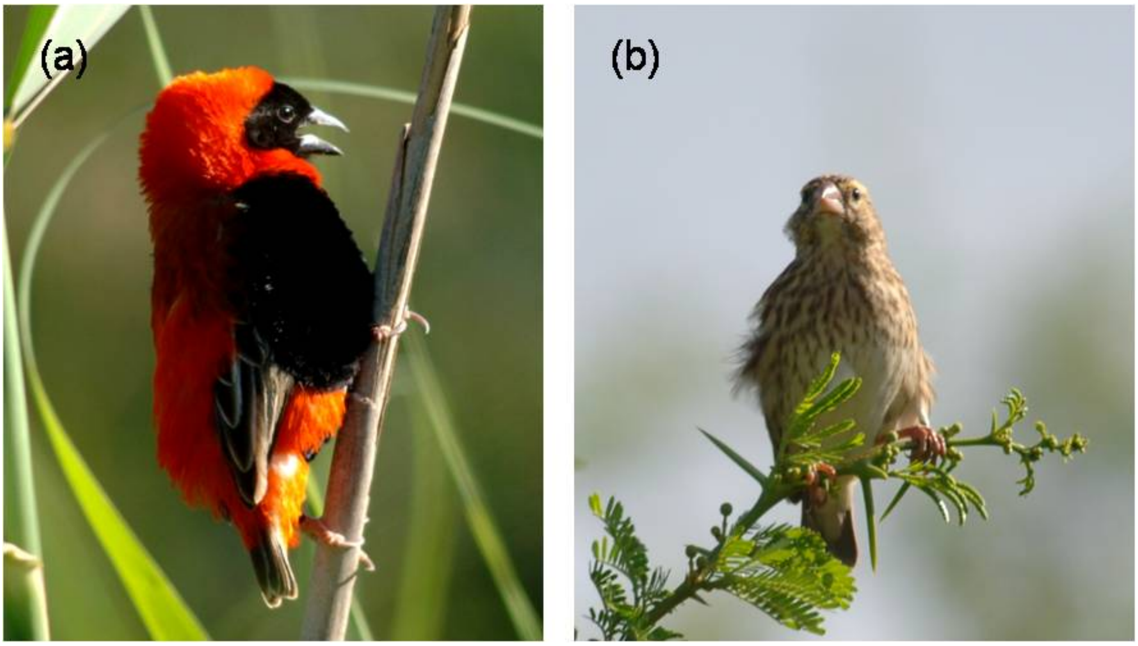

Figure 2: Red bishop (a) male in breeding plumage and (b) female or juvenile.

The strong competition between individual males for territories and mates coupled with a breeding system based on female choice makes the red bishop an ideal species for testing the predictions of the hypotheses named above. While chapters 1 and 2 use data collected on wild males, chapter 3 is an experimental study conducted at the university aviary.

Chapter 1 of the thesis analyses the influence of several immunological and health parameters, as well as age on the expression of plumage colouration in male red bishops and enables us to conclude if certain plumage characteristics in this species are reliable indicators of parasite load, immunological health status (represented by the heterophil/lymphocyte ratio), testosterone levels and body condition. The questions addressed in this study are if (1) the expression of plumage colouration in the red bishop lends further support to the hypotheses postulated by Hamilton and Zuk (1982) and Folstad and Karter (1992) and (2) if the allocation trade-off for carotenoids between the immune system and plumage colouration occurs. Using reflectance and physiological data, as well as blood samples providing data on immunological condition, parasite and testosterone levels, a link was established between plumage colouration and health status.

Chapter 2 examines the influence of the two plumage characteristics (brightness and colouration), testosterone levels and parasitisation on male reproductive effort and success, as well as on territoriality and territory tenure. Plumage colouration often acts as a quality signal, enabling conspecifics to use this as a visual cue for assessing individual condition. This assessment could ultimately determine not only territoriality and tenure (male-male competition via potential dominance signals), but also reproductive success (female choice), which is irrevocably linked to the former. However, the other three factors have been shown to have an effect on individual behaviour, with testosterone for example increasing courtship display. Therefore, using spectrometric and behavioural data in combination with blood and serum samples, the influence of these factors alone, as well as in interaction with one another was tested to determine if and which are key to individual fitness in the long run. The questions behind this study are related to the Hamilton-Zuk hypothesis and the ICHH, in terms of (a) females actively choosing a mate via plumage colouration, as this honestly signals health and parasite resistance and (b) males with a more intense plumage colouration potentially having higher testosterone levels and being in better condition, therefore settling territorial conflicts via dominance signals and having longer tenures.

Chapter 3 describes an experimental study which was conducted in the aviary at the University of Oldenburg. It involved conducting male-male competitions between individual males in two separate seasons. In the first season, males remained unmanipulated, while in the second, plumage colouration of 6 of the 12 males was manipulated, which resulted in these individuals having a more intense plumage colour than their conspecifics. Ultimately, the goal of this study was to determine if carotenoid-based plumage colouration is used a status signal in the red bishop, with redder individuals dominating duller ones. Testosterone levels, as well as body condition and age were also incorporated in the study and the results are discussed in view of Rohwer's Status Signalling Hypothesis and age-related competitive experience.

References

Aguilera E, Amat JA. (2007) Carotenoids, immune response and the expression of sexual ornaments in male greenfinches (*Carduelis chloris*). *Naturwissenschaften* **94:** 895 – 902.

Andersson M. (1986) Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution* **40:** 804 – 816.

Baeta R, Faivre B, Motreuil S, Gaillard M, Moreau J. (2008) Carotenoid trade-off between parasitic resistance and sexual display: an experimental study in the blackbird (*Turdus merula*). *Proceedings of the Royal Society of London B* **275:** 427 – 434.

Bezzerides AL, McGraw KJ, Parker RS, Husseini J. (2007) Elytra color as a signal of chemical defense in the Asian ladybird beetle *Harmonia axyridis*. *Behavioural Ecology and Sociobiology* **61:** 1401 – 1408.

Blas J, Pérez-Rodriguez L, Bortolotti GR, Viñuela J, Marchant TA. (2006) Testosterone increases bioavailability of carotenoids: insights into the honesty of sexual signalling. *PNAS* **103:** 18633 – 18637.

Blount JD, Metcalfe NB, Birkhead TR, Surai PF. (2003) Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* **300:** 125 – 127.

Blount JD, Speed MP, Ruxton GD, Stephens PA. (2009) Warning displays may function as honest signals of toxicity. *Proceedings of the Royal Society of London B* **276:** 871 – 877.

Britton GE, Liaaen-Jensen S, Pfander H. (2008) *Carotenoids. Volume 4: Natural functions*. Boston: Birkhäuser Verlag.

Brockman H, Völker O. (1934) Der gelbe Federfarbstoff des Kanarienvogels (*Serinus canaria canaria*) und das Vorkommen von Carotinoiden bei Vögeln. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* **224:** 193 – 215.

Brush AH. (1990) Metabolism of carotenoids in birds. *FASEB Journal* **4:** 2969 – 2977.

Buchanan KL, Evans MR, Goldsmith AR. (2003) Testosterone, dominance signalling and immunosuppression in the house sparrow. *Behavioural Ecology and Sociobiology* **55:** 50 – 59.

Burton GW, Ingold KU. (1984) Beta-carotene – An unusual type of lipid antioxidant. *Science* **224:** 569 – 573.

Casagrande S, Csermely D, Pini E, Bertacche V, Tagliavini J. (2006) Skin carotenoid concentration correlates with male hunting skill and territory quality in the kestrel *Falco tinnunculus*. *Journal of Avian Biology* **37:** 190 – 196.

Chew B. (1993) Role of carotenoids in the immune response. *Journal of Dairy Science* **76:** 2804 – 2811.

de Ridder E, Pinxten R, Eens M. (2000) Experimental evidence of a testosterone-induced shift from paternal to mating behavior in a facultatively polygynous songbird. *Behavioural Ecology and Sociobiology* **49:** 24 – 30.

Duckworth RA, Mendonça MT, Hill GE. (2004) Condition-dependent sexual traits and social dominance in the house finch. *Behavioral Ecology* **15:** 779 – 784.

Estep LK, Shawkey MD, Hill GE. (2006) Carotenoid-based breast plumage colour, body condition and clutch size in red fodies (*Foudia madagascariensis*). *Ostrich* **77:** 164 – 169.

Evans MR, Goldsmith AR, Norris SRA. (2000) The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). *Behavioural Ecology and Sociobiology* **47:** 156 – 163.

Faivre B, Préault M, Salvadori F, Théry M, Gaillard M, Cézilly F. (2003) Bill colour and immunocompetence in the European blackbird. *Animal Behaviour* **65:** 1125 – 1131.

Fisher RA. (1930) *The genetical theory of natural selection*. Oxford: Claredon Press.

Fitze PS, Richner H. (2002) Differential effects of a parasite on ornamental structures based on melanins and carotenoids. *Behavioral Ecology* **13:** 401 – 407.

Foerster K, Poesel A, Kunc H, Kempenaers B. (2002) The natural plasma testosterone profile of male blue tits during the breeding season and its relation to song output. *Journal of Avian Biology* **33:** 269 – 275.

Folstad I, Karter AJ. (1992) Parasites, bright males and the immunocompetence handicap. *The American Naturalist* **139:** 603 – 622.

Gonzalez G, Sorci G, Smith LC, de Lope F. (2001) Testosterone and sexual signalling male house sparrows (*Passer domesticus*). *Behavioural Ecology and Sociobiology* **50:** 557 – 562.

Griffith SC, Pryke SR. (2006) Benefits to females of assessing color displays. In: Hill GE, McGraw KJ, eds. *Bird Coloration. Volume II: Function and Evolution*. Cambridge: Harvard University Press, 233 – 280.

Hagelin JC. (2002) The kinds of traits involved in male–male competition: a comparison of plumage, behavior, and body size in quail. *Behavioral Ecology* **13:** 32 – 41.

Hamilton WD, Zuk M. (1982) Heritable true fitness and bright birds: a role for parasites. *Science* **218:** 384 – 387.

Hill GE. (2000) Energetic constraints on expression of carotenoid-based plumage coloration. *Journal of Avian Biology* **31:** 559 – 566.

Hill GE, Farmer KL. (2005) Carotenoid-based plumage coloration predicts resistance to a novel parasite in the house finch. *Naturwissenschaften* **92:** 30 – 34.

Hollander WF, Owen RD. (1939) The carotenoid nature of yellow pigment in the chicken iris. *Poultry Science* **18:** 385 – 387.

Hõrak P, Ots I, Vellau H, Spottiswoode C, Møller AP. (2001) Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. *Oecologia* **126:** 166 – 173.

Hughes DA. (2001) Dietary carotenoids and human immune function. *Nutrition* **17:** 823 – 827.

Ligon JD, Thornhill R, Zuk M, Johnson K. (1990) Male-male competition, ornamentation and the role of testosterone in sexual selection in red jungle fowl. *Animal Behaviour* **40:** 367 – 373.

Lonnberg E. (1938) The occurrence and importance of carotenoid substances in birds. *Proceedings of the International Ornithological Congress* **8:** 410 – 424.

Maney DL, Davis AK, Goode CT, Reid A, Showalter C. (2008) Carotenoid-based plumage coloration predicts leukocyte parameters during the breeding season in northern cardinals (*Cardinalis cardinalis*). *Ethology* **114:** 369 – 380.

Maynard Smith J, Parker G. (1976) The logic of asymmetric contests. *Animal Behaviour* **24:** 159 – 175.

Maynard Smith J, Harper D. (2003) *Animal Signals*. Oxford Series in Ecology and Evolution. New York: Oxford University Press.

McGraw KJ. (2006) Mechanics of carotenoid-based coloration. In: Hill GE, McGraw KJ, eds. *Bird Coloration. Volume I: Mechanisms and Measurements*. Cambridge: Harvard University Press, 177 – 243.

McGraw KJ, Hill GE. (2000) Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. *Proceedings of the Royal Society of London B* **267:** 1525 – 1531.

McGraw KJ, Ardia DR. (2003) Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. *The American Naturalist* **162:** 704 – 712.

McGraw KJ, Beebee MD, Hill GE, Parker RS. (2003) Lutein-based plumage coloration in songbirds is a consequence of selective pigment incorporation into feathers. *Comparative Biochemistry and Physiology B* **135:** 689 – 696.

McGraw KJ, Hill GE, Parker RS. (2005) The physiological costs of being colourful: nutritional control of carotenoid utilization in the American goldfinch (*Carduelis tristis*). *Animal Behaviour* **69:** 653 – 660.

McGraw KJ, Correa SM, Adkins-Regan E. (2006) Testosterone upregulates lipoprotein status to control sexual attractiveness in a colorful songbird. *Behavioural Ecology and Sociobiology* **60:** 117 – 122.

Mennill DJ, Doucet SM, Montgomerie R, Ratcliffe LM. (2003) Achromatic signals of color variation in black-capped chickadees, *Poecile atricapilla*: black and white signals of sex and rank. *Behavioural Ecology and Sociobiology* **53:** 350 – 357.

Merilä J, Sheldon BC, Lindström KM. (1999) Plumage brightness in relation to haematozoan infections in the greenfinch *Carduelis chloris*: bright males are a good bet. *Ecoscience* **6:** 12 – 18.

Peters A, Astheimer LB, Boland CRJ, Cockburn A. (2000) Testosterone is involved in acquisition and maintenance of sexually selected male plumage in superb fairy-wrens, *Malurus cyaneus*. *Behavioural Ecology and Sociobiology* **47:** 438 – 445.

Peters A, Delhey K, Andersson S, van Noordwijk H, Förschler MI. (2008) Condition-dependence of multiple carotenoid-based plumage traits: an experimental study. *Functional Ecology* **22:** 831 – 839.

Pike TW, Blount JD, Lindström J, Metcalfe NB. (2007) Availability of non-carotenoid antioxidants affects the expression of a carotenoid-based sexual ornament. *Biology Letters* **3:** 353 – 356.

Pryke SR. (2007) Fiery red heads: female dominance among head color morphs in the Gouldian finch. *Behavioral Ecology* **18:** 621 – 627.

Pryke SR, Andersson S. (2003) Carotenoid-based status signalling in red-shouldered widowbirds (*Euplectes axillaris*): epaulet size and redness affect captive and territorial competition. *Behavioural Ecology and Sociobiology* **53:** 393 – 401.

Pryke SR, Griffith SC. (2006) Red dominates black: agonistic signalling among head morphs in the colour polymorphic Gouldian finch. *Proceedings of the Royal Society of London B* **273:** 949 – 957.

Pryke SR, Andersson S, Lawes MJ, Piper SE. (2002) Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color. *Behavioral Ecology* **13:** 622 – 631.

Saks L, Ots I, Hõrak P. (2003) Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia* **134:** 301 – 307.

Senar JC, Camerino M, Copete JL, Metcalfe NB. (1993) Variation in black bib of the Eurasian siskin (*Carduelis spinus*) and its role as a reliable badge of dominance. *The Auk* **110:** 924 – 927.

Seutin G. (1994) Plumage redness in redpoll finches does not reflect hemoparasitic infection. *OIKOS* **70:** 280 – 286.

Summers K, Clough ME. (2001) The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). *Proceedings of the National Academy of Science USA* **98:** 6227 – 6232.

Sundberg J. (1995) Parasites, plumage coloration and reproductive success in the yellowhammer, *Emberiza citrinella*. *OIKOS* **74:** 331 – 339.

Vershinin A. (1999) Biological functions of carotenoids – Diversity and evolution. *Biofactors* **10:** 99 – 104.

Willstatter R, Escher HH. (1912) Lutein obtained from egg yolks. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* **76:** 214 – 225.

Wingfield JC, Hegner RE, Dufty AM, Ball GF. (1990) The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *The American Naturalist* **136:** 829 – 846.

Zahavi A. (1975) Mate selection – a selection for a handicap. *Journal of Theoretical Biology* **53:** 205 – 214.

CHAPTER 1

Individual quality and carotenoid-based plumage ornaments in male red bishops (*Euplectes orix*): plumage is not all that counts.

Submitted as:
Edler AU, Friedl TWP. “Individual quality and carotenoid-based plumage ornaments in male red bishops (Euplectes orix): plumage is not all that counts”

Abstract

Males in many bird species develop elaborate carotenoid-based plumage ornaments which play an important role as signals of individual quality in intra- or intersexual selection. Here we investigated which factors affect brightness and colouration of the carotenoid-based orange-red breeding plumage in males of the red bishop (*Euplectes orix*), a polygynous and sexually dimorphic weaverbird species. Our study revealed a very complex pattern with the relationships between plumage traits and both heterophil-to-lymphocyte ratio and blood parasite load varying considerably among seasons, suggesting a strong influence of environmental conditions. Furthermore, overall condition of males strongly affected the association pattern between plumage traits and other factors, with males in bad condition being forced to allocate resources away from plumage elaboration to body maintenance or enhancement of immune functions, while males in good condition can afford to invest in plumage ornamentation without obvious detrimental effects on health. Thus, females cannot rely on plumage characteristics alone to gather information on male quality, but have to assess additional traits that advertise general male health status. Finally, we found that plumage brightness decreased linearly with age, while plumage colouration showed a quadratic relationship with age, with males in their prime having a more colourful plumage than both very young and very old males.

Introduction

Communication via visual signals has been shown to be important for conveying information not only intra- but also intersexually. Carotenoid-based ornamentation, expressed in brilliant yellows, oranges and reds, has received much attention, especially in bird species. The colouration of diverse ornaments and appendages, such as wattles (Ohlsson et al. 2003, Smith et al. 2007), combs (Mougeot 2008), eye-rings (Kristiansen et al. 2006, Pérez-Rodríguez & Viñuela 2008) and feathers (Karu et al. 2007, Peters et al. 2008) is achieved by the deposition of carotenoid pigments into the respective appendages. These pigments cannot be synthesized by organisms and therefore need to be ingested as part of the diet and metabolised accordingly. Due to their chemical and physiological properties, carotenoids are not only used in the context of visual signalling, but also function as free-radical scavengers and act as enhancers for the immune system (Krinsky 1994, Møller et al. 2000, McGraw 2005).

Carotenoid-based plumage is a common trait in males of a variety of bird species and several studies have shown that the expression of this trait is influenced by a variety of factors, such as testosterone levels (Gonzalez et al. 2001, Duckworth et al. 2004), degree of parasitisation (Hõrak et al. 2001, Hill & Farmer 2005), health (Lozano 1994, Faivre et al. 2003) and age (Ferns & Hinsley 2008). For this reason, carotenoid-based plumage plays a vital role in communication, not only on an intersexual level, where females can use visual cues to assess different aspects of quality of a potential mate (e.g. von Schantz et al. 1999, Senar & Escobar 2002, Ferns & Hinsley 2008, for review see Hill 2002), but also on an intrasexual level, enabling males to assess the individual competitive ability of prospective rivals (Pryke et al. 2002, Pryke & Griffith 2006, Griggio et al. 2007).

Several hypotheses have linked different aspects of individual quality (i.e., body condition, testosterone levels, parasite load, immunocompetence) to the expression of colouration. With the proposal of the Hamilton-Zuk Hypothesis (Hamilton & Zuk 1982), plumage colouration was suggested to be an honest indicator of parasite resistance, with bright males having fewer parasites than less colourful individuals (Hamilton & Poulin 1997, Lindström & Lundström 2000). This would allow plumage colouration to be used as a reliable visual signal for females when choosing a mate, as they would indirectly benefit from mating with such an elaborately coloured male due to the increased survival probability of their young through enhanced parasite resistance.

The realization that testosterone might mediate the mechanism for the distribution of energy between immune defence and ornamentation lead to the formulation of the Immunocompetence Handicap Hypothesis (ICHH; Folstad & Karter 1992, Wedekind & Folstad 1994) which proposes a link between plumage colouration and different aspects of individual quality. The hypothesis is based on the well-established positive effect of testosterone on both behavioural components of male reproductive effort, such as vocalizations and duration of territory tenure, and morphological aspects, such as brightness and colour of breeding plumage and the size of appendages, for example wattles and combs. Given that ornamentation and immunocompetence compete for the same limited resources such as energy and carotenoids, more elaborately ornamented males show that they can divert valuable immune enhancing carotenoids away from the immune system and invest in ornamentation without serious risks to their general health (Westneat & Birkhead 1998, Aguilera & Amat 2007). As a consequence, only males with good immunocompetence will have high testosterone levels while simultaneously displaying elaborate ornamentation. Although a link between immunocompetence and plumage colouration has been shown in some experimental studies in zebra finches (*Taeniopygia guttata*; Blount et al. 2003, McGraw & Ardia 2003), the precise mechanism behind this potential trade-off in the allocation of carotenoids between elaborate ornaments and the immune system is still under debate (Hõrak et al. 2007, Alonso-Alvarez et al. 2008). In addition, the assumption of the ICHH that testosterone acts as an immunosuppressive agent still remains equivocal (reviewed in Roberts et al. 2004).

The intensity of colouration can further be affected by body condition, as was shown by Estep et al. (2006) in red fodies (*Foudia madagascariensis*), where males in better condition displayed brighter feathers. Similar results were found in male cardinals (*Cardinalis cardinalis*; Jawor & Breitwisch 2004), with red bill colour being a positive predictor of current body condition.

Finally, age has been found to affect ornamentation. Several bird species, red bishop males being among them, display delayed plumage maturation (DPM), with individuals reaching full adult plumage colouration with a delay of one year or more. Furthermore, even when full breeding plumage is attained, males in their first breeding season are often duller than older conspecifics (Komdeur et al. 2005, Delhey & Kempenaers 2006, Bitton & Dawson 2008). However, while these studies compared first-year breeders with older males, there is a lack of studies investigating the exact relationship of plumage characteristics and age in passerine birds, since it is often very difficult to determine the exact age of individual males once they have moulted into full breeding plumage.

The aim of this study was to investigate how much information is conveyed by and which of the above mentioned factors (parasite infections, testosterone levels, immunocompetence, body condition, age) affect the expression of the red plumage colouration in free-living male red bishops (*Euplectes orix*), a colonial breeding and highly polygynous weaverbird species of Sub-Saharan Africa. We captured male red bishops over three consecutive breeding seasons, measured individual plumage reflectance and took blood samples for later analysis of testosterone levels, immunocompetence and parasitemia (assessed by quantitative real-time PCR). In contrast to most other studies on plumage colouration in birds, that often only analysed the relationship of plumage characteristics with one or two of the factors mentioned above, or investigated variation of plumage characteristics over one study season only, we here present an analysis in which we assess the effects of several factors together, including interactions over three study seasons. Where possible, we also determined the exact age of males in order to determine if plumage colouration in male red bishops is influenced by age. Thereby we want to obtain a more complete picture of factors affecting carotenoid-based plumage colouration, in order to evaluate when and under what circumstances plumage characteristics indeed might reflect individual quality.

Material and methods

Study species and area

The red bishop (*Euplectes orix*) belongs to the family of weaverbirds (*Ploceidae*) and occurs in Sub-Saharan Africa. It is a sexually dimorph species, with all individuals displaying a buffy brown plumage with whitish eye-stripes during the non-breeding season. During the breeding season, mature males (two years and older) moult into a brilliant red and black breeding plumage, with red plumage areas varying in colour intensity between individuals. Red bishops are fairly abundant colonial breeders, occurring along rivers and dams. Males vigorously defend small territories in reed beds consisting mainly of bulrush (*Typha capensis*) and the common reed (*Phragmites spec.*). In their territory, males build as many nests as possible during their tenure, with the number of nests built being an important determinant of male mating success (Friedl & Klump 1999, 2000, Metz et al. 2009). When a female enters the territory, the male carries out various courtship displays until the female either leaves the territory or accepts a nest and allows copulation, which is signalled by the typical copulation solicitation display by female passerines. A female will copulate several times with a male and egg-laying begins shortly after nest acceptance. Once eggs have been laid, the female is usually ignored by the territory owner. Clutch size encompasses two to five eggs, which are incubated for about two weeks by the female alone. After hatching, nestlings are fed by the female and fledge after a period of 11 – 15 days. The main cause of breeding failure (>70% in each season) is predation by water mongoose, *Atilax paludinosis*, cape cobra, *Naja nivea*, and boomslang, *Dispholidus typus*, (Friedl 2004a, AU Edler, unpublished data). More details on red bishop breeding behaviour are given in Craig (1974) and Friedl (2004b).

The study took place during three consecutive breeding seasons between 2005 and 2008, with each breeding season lasting from approximately October to March. The study site was a small dam surrounded by bulrush and common reeds, situated in the Addo Elephant National Park, in the Eastern Cape Province, South Africa (33°26’S, 25°45’E).

Field methods

Behavioural observations and the identification of birds via individual colour ring combinations took place every day. Adult birds were caught with mist nets and walk-in traps, which were situated along the edge of the colony. Upon capture, tarsus and wing length were measured and the weight recorded. The body condition index was calculated using the residuals from the regression of body weight on tarsus length and pooled over all three seasons. We used a total of 79 males, with 13 males having been caught more than once during the course of the study. Blood samples were obtained by puncturing the brachial wing vein with a sterile needle, collecting the blood with a heparinisized microcapillary tube (approximately 20 – 60µl), and transferring the sample into a 1.5ml reaction tube. For blood smears, a drop of blood was placed on a microscope slide and smeared, air-dried and stored in boxes for later analysis. Serum samples were obtained by centrifuging the reaction tubes containing the blood samples with a micro centrifuge (Capsule HF-120, Tomy Kogyo Co., Ltd., Fukushima, Japan), removing the upper layer with the serum using glass pipettes and transferring it into 0.5ml PCR tubes, which were then stored in a freezer. The blood cells remaining in the 1.5ml reaction tubes were topped with a PBS buffer and refrigerated. In total, we collected blood smears and serum samples from 64 individual males, with ten males being sampled at least twice over the course of this study.

Furthermore, we determined a standardised “date of capture during season” for all three seasons. This was necessary, as there were heavy rains during the winter preceding the second field season, which resulted in breeding starting approximately a month earlier than normal. It was therefore not possible to determine an exact start of the breeding season for that year. We defined “day 0” for each season by determining the hatching date of the first nestling in the colony and subtracting 30 days. Individual “date of capture” was then calculated from that date. Finally, we z-transformed “day of capture” to account for differences in length among the three breeding seasons.

We were able to determine the exact age for 18 males, as these individuals had either been captured and ringed as nestlings in the colony or were caught as yearling males along the edge of the colony. Since red bishop males undergo delayed plumage maturation, yearling males are similar to females in appearance during their first year. At the age of two, males moult into the conspicuous breeding plumage for the first time and actively take part in the breeding season. Given that red bishops have a fairly long life-span, with the oldest males ever caught being a minimum of eight years of age (unpublished data), it is not possible to determine the exact age of males if they are in full breeding plumage when captured for the first time.

Plumage reflectance

Spectral reflectance was measured using an Ocean Optics USB2000 spectrometer (Ocean Optics, Inc., Dunedin, Florida, USA), a GetLight-Hal-S tungsten halogen light source (getspec.com, Germany) and a fibre optic reflectance probe, which was fitted with a self-made distance piece, in order to standardise the distance between probe and sample. The probe was held perpendicular to the sample and three consecutive scans were taken from approximately the centre of each measured area (throat, head and lower back, these being the areas in male red bishops showing carotenoid-based plumage colouration), between which the probe was removed and replaced. Reflectance was measured using OOIBase32 (<1nm resolution; Ocean Optics, Inc., Dunedin, Florida, USA) and in relation to a white (WS-2 white reflective tile, 98% reflectance; getspec, Germany), as well as a dark standard (self-made “dark-box”). As red bishops do not reflect in the UV range of 320nm to 400nm (AU Edler & TWP Friedl, unpublished data), we used the data obtained in the range from 400nm to 700nm for further analysis. Overall, we collected reflectance data from 57 individual males, with four males having been recaptured at least once over the three study seasons.

In order to verify that the orange-red breeding plumage in red bishop males is based on carotenoid pigments, we performed the simple two-step chemical extraction method of pigments described in McGraw et al. (2005). After extraction of feather pigments with heated acidified pyridine and adding a 1:1 solution of hexane and tert-butyl methyl ether (TBME), the upper organic phase had an orange colour, confirming that the orange-to-red feather colouration of red bishops is indeed caused by carotenoid pigmentation.

Spectral analysis

The orange-red breeding plumage of red bishop males shows a reflectance spectrum typical for carotenoid-based plumage traits, with no reflectance at the shorter wavelengths and high reflectance at the higher wavelengths corresponding to the orange-to-red part of the spectrum (Fig. 1a). The collected reflectance data was analysed using a Principal Component Analysis (PCA; Cuthill et al. 1999, Grill & Rush 2000, Maney et al. 2008), as this allows a significant reduction of data into principal components (PCs). In comparison to the calculation of colour variables (brightness, chroma and hue), these principal components are statistically independent from one another and are able to accurately describe complex variation across the examined spectrum (Montgomerie 2006).

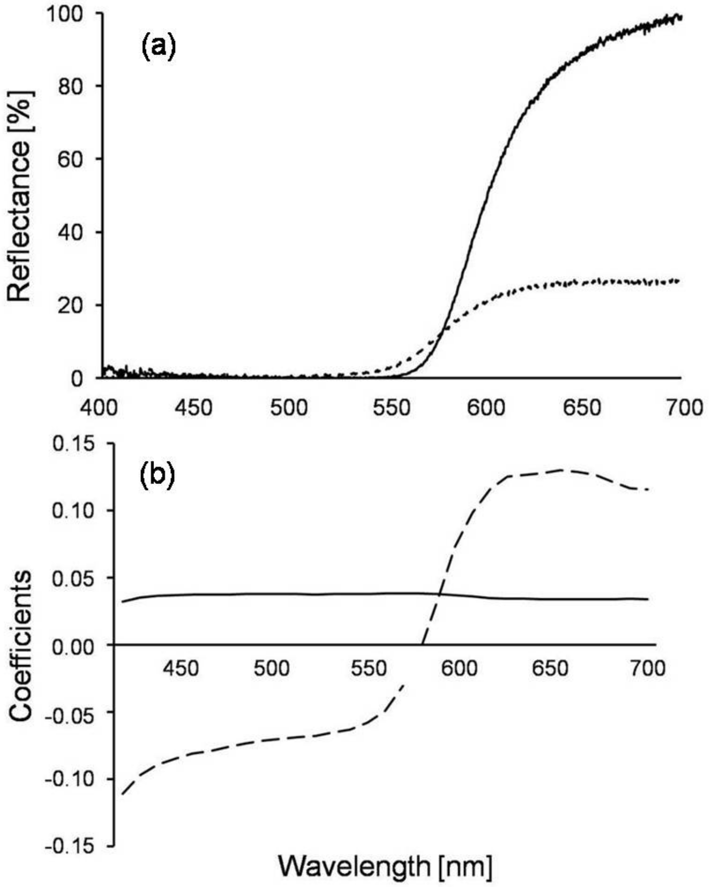

Figure 1: (a) Exemplary reflectance spectra for males with bright (solid line) and dull (dashed line) plumage and (b) principal component coefficients in relation to wavelength [nm] for PC1 and PC2. The first principal component (solid line) accounts for 83.8% of all variation, the second (dashed line) for 13.3%.

As we have three measurements from each of the three body regions measured per male, we first calculated an average of the raw data over the three reflectance spectra, divided these into 30 bins spanning 10nm each (e.g. 400 – 409nm, 410 – 419nm, etc.) using the median values from each segment and performed a PCA. We obtained two principal components for each of the three body regions measured, with at least 91% variation being explained by two PCs in all three cases. As both the first principal components and the second principal components obtained for the three body regions correlated highly significantly with each other (Pearson correlation; all $r > 0.4$, all $P < 0.005$) we further collapsed our data by averaging the three sets of 30 bins per individual and performed another PCA. This resulted in the final two PCs explaining 97% of the variation (PC1: 83.8%; PC2: 13.3%).

Based on the obtained loadings, PC1 is interpreted as brightness of the measured sample, as these loadings are strong and consistently positive over the entire examined range (Cuthill et al. 1999, Grill & Rush 2000). PC2 describes the variation in the relationship between reflectances at high and low wavelengths and therefore depicts chroma and hue (see Fig. 1b).

Leukocyte profiles

For analysis, the obtained blood smears were stained with Wright's stain and examined under a microscope (Zeiss Axioskop 2 *mot plus*, Zeiss, Germany) using a 630x magnification. We counted the amount of erythrocytes and leukocytes, the latter being divided into lymphocytes and heterophilic, eosinophilic and basophilic granulocytes. Consecutive neighbouring microscopic fields were examined, until a total of 100 leukocytes was reached.

The white blood cell count of any given individual can be influenced by several stressors, such as malnutrition, infections and parasites. Lymphocytes are usually the most common white blood cells in avian haemograms and have a tendency to decrease with rising stress levels. Heterophils, on the other hand, being the primary phagocytic white blood cell, increase their circulation in response to infections and stress (Campbell 1995, Feldman et al. 2000, Davis et al. 2008). The interplay of lymphocytes and heterophils with rising stress and inflammation levels allows the calculation of the heterophil/lymphocyte ratio (HLR), which has been shown to be positively associated with heat-shock protein and glucocorticoid levels (Moreno et al. 2002, Davis et al. 2008) and can thus be considered as a reliable indicator of physiological stress induced, for example, through chronic stressors or changes in the environment or social rank (Gross & Siegel 1983, Ots & Hõrak 1996, Davis et al. 2008, Ilmonen et al. 2003, López et al. 2005). Since stress is known to suppress immune functions (Sapolsky 1992, Apanius 1998, Morales et al. 2006), a high HLR, which is indicative of high physiological stress levels will be associated with a concomitant suppression of immunological condition and an increase in susceptibility to infections (Siegel 1995, Ots et al. 1998) or parasitisation (Edler et al. 2004). For further details, see Friedl and Edler (2005).

To assess repeatability of the counts, ten smears were chosen at random and recounted without knowledge of the previous counts. Using the method described in Lessels & Boag (1987), repeatability measures were high for all counts (lymphocytes: $R = 0.8$, $F_{19} = 42.152$, $P < 0.001$; heterophils: $R = 0.9$, $F_{19} = 89.763$, $P < 0.001$, HLR: $R = 0.9$, $F_{19} = 58.458$, $P < 0.001$).

Testosterone levels

Testosterone concentrations of samples collected in the breeding season 2005/06 were determined at the Max-Planck-Institute for Ornithology with a radioimmunoassay following Goymann et al. (2006). Plasma samples (mean ± SD volume: 41.5 ± 20.5µl) were extracted twice with dichloromethane, resulting in an average extraction recovery of 86.0 ± 3.0% (mean ± SD) for 3H-testosterone added prior to extraction. The lower detection limit of the assay was 73pg/tube, resulting in a range of lower detection limits between 39 – 169pg/ml (depending on the amount of plasma available and the respective extraction recovery). To minimize variation, all samples were analyzed in one assay with an intra-assay variation coefficient of 4.5%. Since the testosterone antibody (Esoterix Endocrinology, Calabasas Hill, CA, USA) showed significant cross reactions with 5a-dihydrotestosterone (44%), our testosterone measurements may include a proportion of this other androgen.

Testosterone concentrations of samples collected in the breeding seasons 2006/07 and 2007/08 were determined at the Leibniz Institute for Zoo and Wildlife Research, Berlin, by an enzyme immuno-assay with a double-antibody technique. First, 0.01 – 0.05ml of serum was extracted with 2ml of butyl t-methyl ether:petroleum ether (30:70, v/v) for 30 minutes. The samples were then frozen and the fluid petroleum ether phase was removed and evaporated at 55°C. The steroids were dissolved in 0.1ml of 40% (v/v) methanol, and duplicates of 20µl each were analyzed. The assay used a polyclonal antibody raised in rabbits against testosterone-11-hemisuccinate-BSA, and the label was testosterone-3-carboxymethyl-oxime-horse radish peroxidase. The testosterone standard curve ranged from 0.4pg per 20µl to 50pg per 20µl, and the cross-reactivity with testosterone was 100%, with 5a-dihydrotestosterone 10%, with androstenedione 2%, with estradiol 0.1%, and with progesterone 0.1%. Serial dilutions of a plasma pool from red bishops gave parallelism to the standard testosterone with no differences in slopes ($P > 0.05$). The intra- and inter-assay coefficients of variation (CVs) were 8.9% and 12.3%, respectively.

All results are given in nanograms of testosterone per milliliter of serum.

Parasites

We determined the intensity of infections with blood parasites of the genus *Plasmodium* and *Haemoproteus* by quantitative real-time PCR. In short, parasitemia (i.e. percentage of blood cells infected) was assessed by amplification of a specific 85bp fragment within the plastid-like large subunit ribosomal-RNA (LSU-rRNA) gene (Tan et al. 1997), which is conservative across a range of *Plasmodium* and *Haemoproteus* species. By measuring the accumulation of the product during the PCR (in real-time) using a fluorescent labelled oligonucleotide probe, a threshold was determined at which the fluorescence of the product was raised above background level. The starting quantity of blood parasites in the investigated blood samples of male red bishops was then calculated in comparison to thresholds determined for standards of known quantity (clones of a 594bp fragment within the LSU-rRNA gene from *Plasmodium falciparum* including the target sequence) in the same PCR reaction. For more details see Friedl and Groscurth (submitted manuscript).

We defined males as being parasitized, if parasitemia was above 0.1%, since in passerine birds very low levels of parasitemia with *Plasmodium* or *Haemoproteus* are generally regarded as being harmless to the hosts (e.g. Campbell 1995).

Statistical analysis

We used Generalised Linear Models (GLM) to test the influence of several factors (season, day of capture, testosterone, HLR, level of parasitisation and body condition) on the expression of plumage colouration (PC1 and PC2) in male red bishops. Four males were caught more than once over the course of the study period, and we randomly selected one data set for each of these males to assure statistical independency, resulting in a sample size of *N* = 53. We first included all main effects and two-way interactions in the model, and then removed in a stepwise procedure all non-significant two-way interactions until only significant two-way interactions and the main effects remained in the final model.

The relationship between age and both PC1 and PC2 was investigated for the subset of 18 males for which exact age was known using regression analysis and curve estimation (linear and quadratic models).

All statistical tests were performed using SPSS 15.0 and all given significances are two-tailed.

Results

Influences on plumage colouration

Significant main effects on PC1 were found for season as well as for body condition, and HLR (see Table 1). Male plumage brightness (indicated by high PC1 scores) was highest in the season 2005/06 (mean ± SE: 0.29 ± 0.46), marginally lower in the season 2006/07 (0.15 ± 0.13), and by far lowest in the season 2007/08 (-0.46 ± 0.11). Overall, brighter males (having high PC1 scores) were in better condition and had lower HLR values in comparison to duller individuals.

Table 1: Results of GLM analyses with PC1 and PC2 as dependent variables. The final models shown here include all main effects and all significant two-way interactions (see text). Adjusted R^2 was 0.644 for the analysis of PC1 and 0.486 for the analysis of PC2.

	PC1				PC2			
	Estimate	*F*	*P*	*partial* η^2	*Estimate*	*F*	*P*	*partial* η^2
Season	1.155	5.239	0.011	0.253	1.218	1.794	0.182	0.098
	0.592				0.668			
	0.000				0.000			
Day	-0.033	0.257	0.615	0.008	-0.216	3.358	0.076	0.092
Testosterone	0.010	0.005	0.945	0.000	-0.240	0.903	0.349	0.027
BCI	0.287	7.337	0.011	0.191	0.189	2.425	0.129	0.068
HLR	-1.963	9.718	0.004	0.239	-3.353	8.228	0.007	0.200
Parasites	0.673	3.813	0.060	0.110	1.340	8.371	0.007	0.202
Season*HLR	-0.901	7.873	0.002	0.337	-0.307	3.400	0.045	0.171
	2.030				2.789			
	0.000				0.000			
Season*Parasites	-0.446	7.793	0.002	0.335	-1.082	10.017	<0.001	0.378
	-1.256				-2.099			
	0.000				0.000			
Day*BCI	-0.180	6.100	0.019	0.164				
Testosterone*HLR	0.714	4.279	0.047	0.121	1.255	4.475	0.042	0.119
BCI*HLR	-0.979	19.547	<0.001	0.387				
BCI*Parasites	-0.378	7.826	0.009	0.202	-0.489	4.188	0.049	0.113

The only significant main effects on PC2 were found for HLR and the intensity of infections with blood parasites, with redder males having lower HLR values but higher parasite loads than males less saturated in colour. No other significant main effects of the variables investigated on PC1 or PC2 were found (see Table 1).

There was a significant effect of the interaction between season and HLR on both PC1 and PC2 (Table 1). Both principal components showed a positive relationship with HLR in the season 2006/07, while the relationships were negative in the seasons 2005/06 and 2007/08 (see Fig. 2).

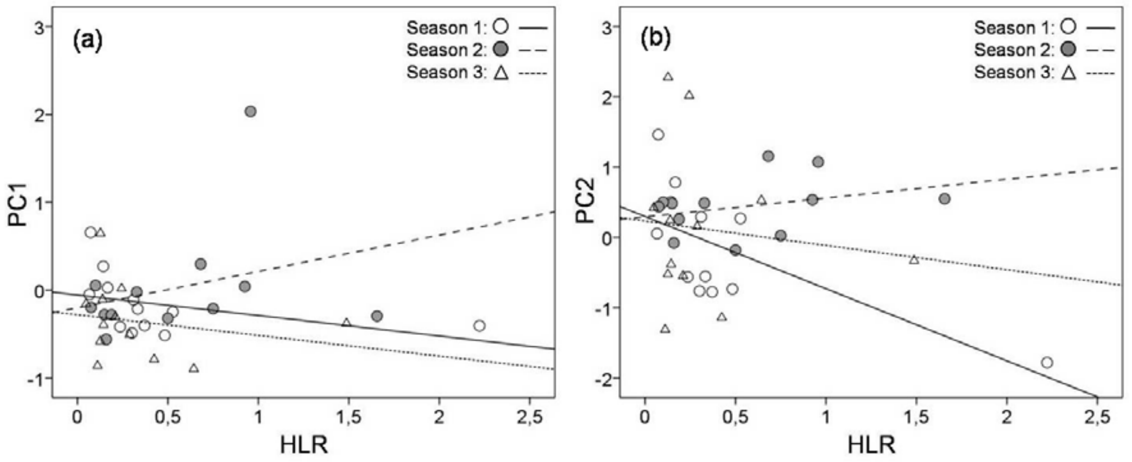

Figure 2: Relationship between HLR and (a) PC1 and (b) PC2 for each of the three study seasons.

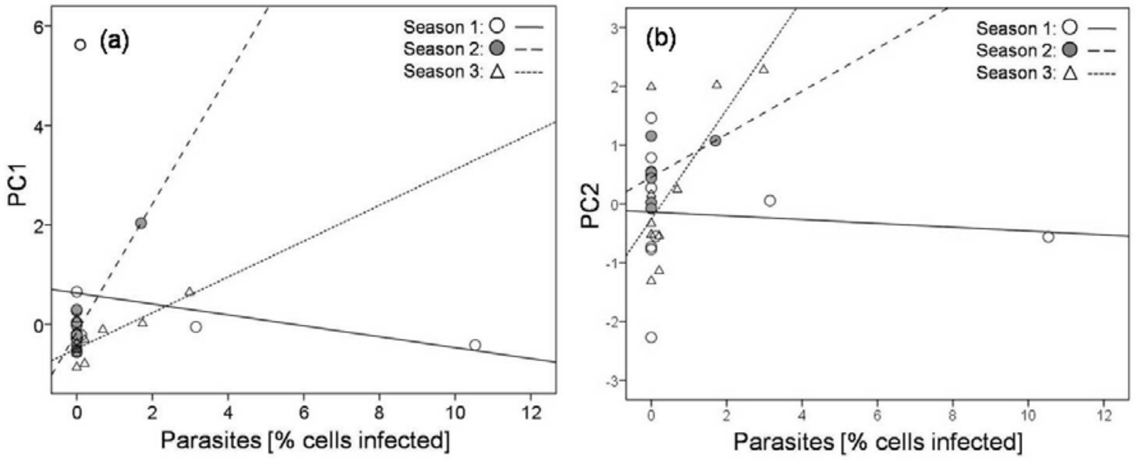

Figure 3: Relationship between parasite load (% cells infected) and (a) PC1 and (b) PC2 for each of the three study seasons.

Furthermore, there was also a significant effect of the interaction between season and parasite load on both PC1 and PC2 (Table 1), with clearly positive relationships between the two PCs and the intensity of infections with blood parasites in the seasons 2006/07 and 2007/08, and slightly negative relationships between the two

PCs and the intensity of infections with blood parasites in the season 2005/06 (see Fig. 3).

The interaction between body condition index and parasite load was significant for both PC1 and PC2, as well as the interaction between testosterone levels and HLR. For males with less than 0.1 % of blood cells infected with parasites (set to zero, see methods) there was a positive relationship between body condition index and both PC1 and PC2, while for males with a parasite load higher than 0.1 % infected blood cells there was a strong negative relationship between body condition index and both PC1 and PC2 (see Fig. 4).

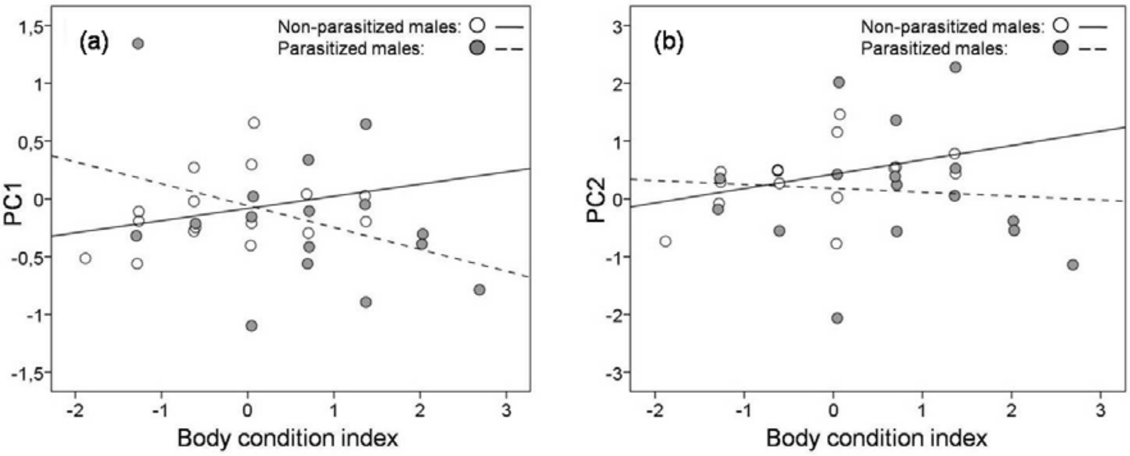

Figure 4: Relationship between body condition and (a) PC1 and (b) PC2, for non-parasitized males (less than 0.1% of blood cells infected; empty dots and solid line) and parasitized males (more than 0.1% cells infected; filled dots and dashed line).

For males that suffered from high stress levels (indicated by high HLR values) there were negative relationships between testosterone levels and both PC1 and PC2, while for males that did not suffer from high stress levels (indicated by low HLR values) there were positive relationships between testosterone levels and both PC1 and PC2 (see Fig. 5).

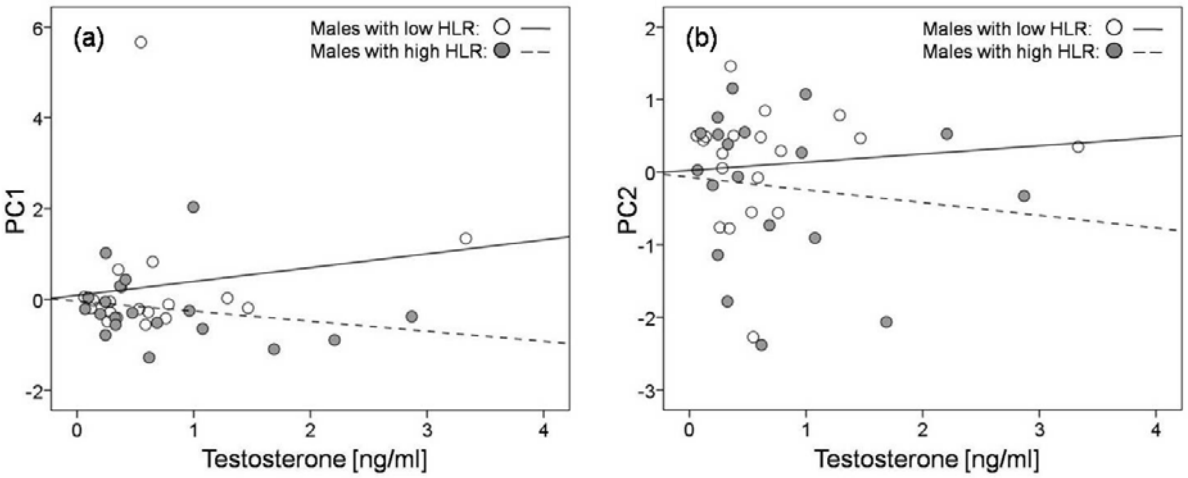

Figure 5: Relationship between testosterone and (a) PC1 and (b) PC2 for males with low (below median; empty dots and solid line) and high (above median; filled dots and dashed line) HLR values.

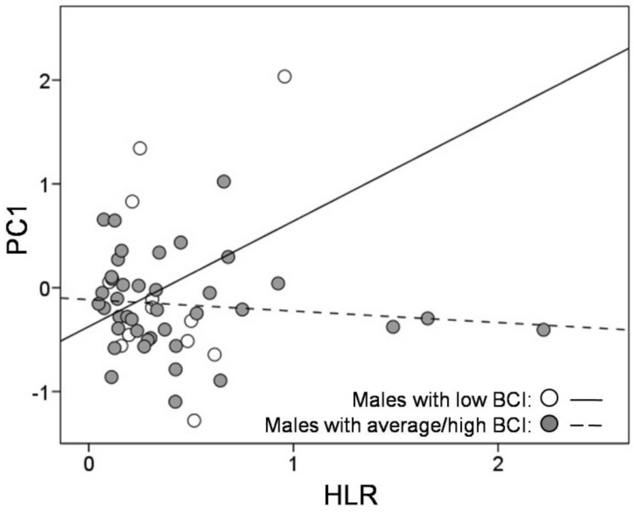

Figure 6: Relationship between PC1 and HLR for males with a low body condition index (lowest quartile of the distribution; empty dots and solid line) and an average to high body condition index (upper three quartiles of the distribution; filled dots and dashed line).

PC1 was further influenced by the interactions between day of capture and body condition, as well as by the interaction between HLR and body condition. For males in bad body condition PC1 decreased over the course of the season, while there was no seasonal effect on PC1 for males in good body condition. Finally, for males in very bad body condition there a positive relationship between PC1 and HLR (i.e. stress level), while for males in good body condition PC1 decreased with increasing HLR (see Fig. 6).

Age and colouration

For a subsample of 18 males for which the exact age was known (see methods) we investigated the relationship between age and both PC1 and PC2 using regression analyses combined with curve estimation to test for linear and quadratic relationships. There was a significant negative linear relationship between age and

PC1 (R^2 = 0.235, F = 4.905, P = 0.042), indicating that plumage brightness decreased linearly with increasing age (see Fig. 7a). In contrast, the relationship between age and PC2 was quadratic rather than linear, with middle-aged males in their prime having a redder and more saturated plumage (indicated by high PC2 scores) compared to both young and very old males (R^2 = 0.364, F = 4.287, P = 0.034; see Fig. 7b).

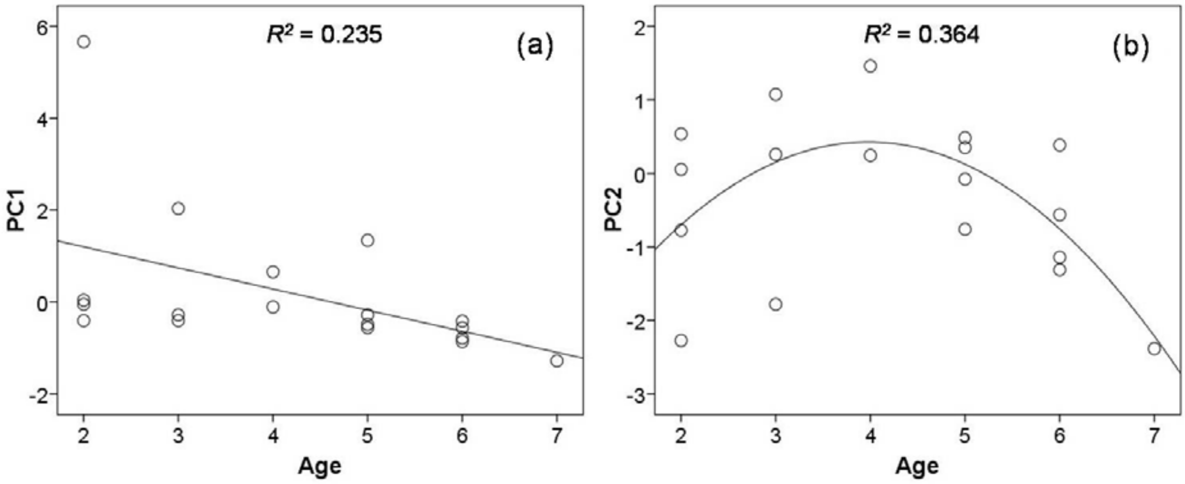

Figure 7: Relationship between male age and (a) PC1 and (b) PC2.

Discussion

As stated before, the aim of our study was to investigate if and how the carotenoid-based orange-to-red breeding plumage of male red bishops is influenced by factors such as infections with blood parasites, testosterone levels, immunocompetence, stress levels, body condition, and age. Based on the associations found, we want to assess whether plumage brightness (reflected by PC1) and/or plumage colour (chroma and hue – referred to as “colour” from now on – reflected by PC2) are suitable to be used as reliable indicators of male quality.

We found significant main effects of HLR (as indicator of physiological stress levels), parasitemia and body condition on PC1 and/or PC2, while there was no significant main effect of testosterone. In addition, our analyses revealed several significant interaction effects which lead to a rather complex and complicated overall picture. In the following, we will discuss our results in detail with special attention to the question of whether plumage characteristics are reliable indicators of male quality as proposed by several hypotheses including the Hamilton-Zuk hypothesis and the ICHH, and whether plumage characteristics are related to male age.

Given that the majority of studies on carotenoid-based plumage in birds focus on colour (i.e. chroma and/or hue) rather than brightness, and given that brightness and colouration may signal different aspects of male quality, we will discuss plumage colouration (reflected by PC2) and plumage brightness (reflected by PC1) separately in the following.

Plumage colouration

Plumage colouration in red bishop males was negatively affected by high physiological stress levels (high HLR values) and the concomitant decrease in immunological condition. Our results fit to the proposed trade-off between immune function and carotenoid-based plumage colouration, since only males that do not suffer from stress and are therefore in good immunological condition should be able to invest carotenoids in elaboration of plumage colouration rather than in enhancing the status of the immune system. Our results are also in accordance with several other studies, which have found that more colourful birds are more immunocompetent (Figuerola et al. 1999, Brawner et al. 2000, Navara & Hill 2003, Saks et al. 2003), indicating that carotenoid-based plumage colouration can be used as a signal of quality.

However, the effect of HLR on plumage colouration differed among the three study seasons as shown by the significant interaction term between HLR and season (Table 1). While in both the season 2005/06 and the season 2007/08 the relationship between stress levels and plumage colouration was negative as expected, males in the season 2006/07 showed the opposite pattern with more colourful plumage (high PC2 scores) at higher stress levels (see Fig. 2b). This result could be explained by the fact that the breeding season 2006/07 differed significantly in timing from the other two study seasons. Due to unusual and extremely heavy winter rains, the 2006/07 season began approximately two months earlier than normal. As rainfall is known to affect the breeding behaviour of red bishops (Friedl 2002), these differences in rainfall pattern might explain the reverse relationship between colouration and HLR for that season. Another study on red bishops has already revealed that the relationship between HLR and reproductive performance in male red bishops varied considerably among seasons (Friedl & Edler 2005), indicating that seasonal variations can indeed have pronounced effects on reproductive parameters.

A surprising and unexpected result was that plumage colouration was positively related to parasitemia, with redder males having more parasites than duller conspecifics. According to both the Hamilton-Zuk hypothesis and the ICHH, this relationship should be the opposite, as parasites are supposed to depress the expression of carotenoid-based plumage colouration through a trade-off in carotenoid allocation between immune function and its purpose as a colourant. Indeed, such a negative relationship between parasite load and plumage colour has been shown in a variety of bird species, such as house finches (*Carpodacus mexicanus*; Hill & Farmer 2005), blackbirds (*Turdus merula*; Baeta et al. 2008) and cirl buntings (*Emberiza cirlus*; Figuerola et al. 1999). Since we could show a negative association between plumage colouration and stress levels (see above), and assuming that higher parasite loads should cause higher stress levels, we would have expected to find also a negative relationship between parasite load and plumage colouration. However, we found an overall positive rather than negative relationship between parasite load and plumage colouration in the red bishop. Furthermore, there was a significant interaction between parasite load and season, with a positive relationship in the seasons 2006/07 and 2007/08, but a negative one in the season 2006/07 (Fig. 3b). Given the inconsistencies of the relationship between parasitemia and plumage colouration across seasons, and the lack of a clear negative effect of blood parasite infections on reproductive performance in male red bishops reported in other studies (Edler et al. 2004, AU Edler & TWP Friedl, unpublished data), we suggest that infections with *Plasmodium* and/or *Haemoproteus* have no strong detrimental effects on male health in red bishops, at least at the low to moderate infection levels found in this study. This seems to be in contrast to the results of several other studies that have shown a negative effect of blood parasites on host condition (e.g., Ots & Hõrak 1998, Hatchwell et al. 2000, Dyrcz et al. 2005). However, the significant interaction between parasite load and body condition on plumage colouration indicates that also in red bishops blood parasites have at least some detrimental effect. While for non-parasitized males (i.e. males with less than 0.1 % of blood cells infected) more intensely coloured individuals are in better condition than less colourful ones, plumage colouration decreases with increasing body condition in parasitized males (see Fig. 4b). This indicates that there is no trade-off between body condition and plumage colouration for males that do not suffer from detrimental effects caused by blood parasites. In contrast, when there is an additional challenge to the immune system caused by blood parasites, males obviously have to trade off the allocation of dietary carotenoids to either immune defence or plumage elaboration. In a study on blackbirds, using experimentally induced parasite infections, Baeta et al. (2008) were able to show that males supplemented with carotenoids were able to maintain bill colour while simultaneously fighting parasites. In comparison, non-supplemented males displayed reduced bill colouration. This result led to the conclusion that only those individuals with a carotenoid-rich diet are able to handle costly parasite infections and confirms that carotenoids are subjected to an allocation trade-off between immunological responses to parasites and ornament elaboration.

According to the ICHH, testosterone is supposed to enhance male behavioural as well as morphological traits involved in mate acquisition. For example, in bird species where the males have carotenoid-based ornamental traits, males with higher levels of testosterone have been shown to display redder ornamentation (e.g. Duckworth et al. 2004, McGraw et al. 2006). In our study, however, we found no significant main effect of testosterone on plumage colouration, though there was a significant effect of the interaction between testosterone and HLR (see Table 1). For males that did not suffer from high stress levels (i.e. males that had HLR values below the median of 0.41), there was no clear relationship between testosterone levels and plumage colouration, while for males with high stress levels (i.e. males that had HLR values above the median of 0.41) high testosterone levels were related to reduced plumage colouration (see Fig. 5b). This effect might be a consequence of the immunosuppressive character of testosterone (directly or indirectly), with high hormone levels forcing the individuals to allocate carotenoids away from plumage elaboration to support the suppressed immune system when they suffered from high stress levels and the concomitant increase of susceptibility to infections and parasites.

Plumage brightness

As stated above, there are surprisingly few studies that have investigated the signalling value of plumage brightness, with brightness here referring to spectral intensity, i.e. the total amount of reflectance over the entire wavelength spectrum measured. Unfortunately the term “brightness” is often misused in the animal colouration literature, with birds having a more saturated plumage (i.e. higher chroma and/or hue values) described as being “brighter” than individuals with less saturated plumage colouration (see Montgomerie 2006). Therefore caution is required when interpreting the results of studies reporting signalling values of plumage characteristics as to what component of plumage measurements the authors are referring to.

Overall, many of the results obtained in red bishop males for plumage brightness were similar to the results shown for plumage colouration above. Similar to plumage colouration, there was an overall negative effect of high stress levels (i.e. high HLR values) on plumage brightness (as reflected by the PC1 scores). In addition, the relationship between HLR and plumage colouration differed among seasons the same way than the relationship between HLR and plumage colouration, with the expected negative relationships in the seasons 2005/06 and 2007/08 and a positive association in the season 2006/07 (see Fig. 2a).

While the positive main effect of parasitemia on plumage brightness just failed to be significant, the interaction between parasite load and season was again highly significant, with an expected but only slightly negative relationship between parasite load and plumage brightness in the season 2005/06 and surprisingly positive associations in the seasons 2006/07 and 2007/08 (see Fig. 3a). Likewise, the significant effect of the interaction between parasite load and body condition on plumage brightness was very similar to the one found for plumage colouration. For non-parasitized males, brighter individuals were in better body condition than duller ones, while for parasitized males the relationship between body condition and plumage brightness was reversed (see Fig. 4a). As stated above, such a pattern might indicate that the detrimental effect of blood parasites forces males to trade the allocation of carotenoids between immune defence against the parasites and plumage elaboration.

Another result for plumage brightness which is very similar to our findings for plumage colouration is the lack of a significant main effect of testosterone and the significant interaction between testosterone and HLR (see Table 1). For males that did not suffer from high stress levels (i.e. males that had HLR values below the median of 0.41), there was the expected positive relationship between testosterone levels and plumage brightness, while for males that suffered from high stress levels (i.e. males that had HLR values above the median of 0.41) high testosterone levels were related to reduced plumage brightness (see Fig. 5a). As discussed above for plumage colouration, this effect might be a consequence of an immunosuppressive effect of testosterone, where males with both high hormone levels and high stress levels have to allocate carotenoids away from plumage elaboration to support the suppressed immune system.

The most obvious difference regarding the factors affecting plumage brightness and plumage colouration is that body condition seems to have a stronger effect on plumage brightness than on colouration. This is reflected by the significant positive main effect of body condition on plumage brightness together with two significant interaction effects involving body condition that were not found for plumage colouration (see Table 1). The positive association between plumage brightness and body condition indicates that brightness can be used by females as a visual cue for individual condition when assessing potential mates. While most correlational and experimental studies have found significant relationships between feather colour (i.e. chroma and/or hue) and body condition rather than between brightness and body condition (see Hill 2002), there are some studies showing that brightness is a better indicator of male quality than plumage colouration. In a study on golden-collared manakins, *Manacus vitellinus*, Stein and Uy (2006) found that collar brightness was correlated with body size and mating success, while principal components reflecting chroma and hue did not show such correlations. Likewise, in red fodies only brightness but not chroma of male breast plumage was significantly related to body condition (Estep et al. 2006), and a study on greenfinches (*Carduelis chloris*) showed that males with brighter yellow feathers were able to mount a stronger immune response to a novel antigen than duller conspecifics (Saks et al. 2003).

The fact that both HLR and body condition were positively linked to plumage brightness in red bishop males suggests that a female choosing a bright male over a duller counterpart would benefit considerably, as the potential mate is not only in better condition, but also has a higher immunocompetence. Shawkey and Hill (2005) demonstrated that structural components of feather barbs which are responsible for the underlying white structural colour of pigmented feathers can strongly influence brightness of carotenoid-containing tissues. While the production mechanisms and potential metabolic costs of such white structural colouration are virtually unknown, it seems possible that plumage brightness of carotenoid-based plumage ornaments is an important but over-looked indicator of quality in birds. Clearly, more studies are required that investigate the signalling value of plumage brightness (in contrast to plumage colouration) and the underlying mechanism responsible for inter-individual variation in brightness of such carotenoid-based plumage ornaments.

The interaction between day of capture and body condition was also found to have a significant effect on brightness. When in good condition, male plumage brightness did not change over the course of the breeding season, while for males in bad body condition brightness declined over the season. One possible explanation for this pattern would be that plumage brightness is affected by preening behaviour and males in good body condition are likely to spend more time preening; these males might thus better be able to maintain their plumage brightness throughout the breeding season as compared to males in worse body condition.

Finally, the significant interaction between HLR and body condition confirms the patterns described before (Fig. 5). For males in very bad body condition (i.e. body condition in the lowest quartile of the distribution) there is a positive relationship between plumage brightness (PC1 scores) and HLR, again indicating a trade off with individuals being able to allocate resources either in plumage brightness or in health maintenance, but not both (Fig. 6). In contrast, for males in average or good body condition (i.e. body condition in the upper three quartiles of the distribution) the relationship between HLR and plumage brightness is negative, suggesting that males in good body condition have more available resources which can be invested in the immune system, allowing them to simultaneously produce bright feathers while keeping their stress level and immune system on a suitably functioning level.

Plumage characteristics and their relation to age

The investigation of the relationship between age and the two principal components revealed a linear negative relationship between age and PC1 with older males having lower PC1 scores (i.e. having less bright plumage) than younger ones, and a quadratic relationship between age and PC2, with very young and very old males showing less plumage colouration than males in their prime.

There are some studies that have looked at the relationship between age categories (mainly classifying individuals as yearlings or adults) and plumage brightness of plumage patches reflecting in the UV range. In male bluebirds (*Sialia sialis*), tree swallows (*Tachycineta bicolor*) and pied flycatchers (*Ficedula hypoleuca*) it was found that older males had brighter plumage than younger conspecifics (Siitari & Huhta 2002, Siefferman et al. 2005, Bitton et al. 2007). Another study on tree swallows (Bitton & Dawson 2008) revealed that older males (recaptured adults) had both brighter and more intensely coloured bluish-green plumage than younger ones (newly captured as adults). In a study on blue tits (*Parus caeruleus*), Delhey and Kempenaers (2006) classified individuals into three age categories – yearlings, second year individuals and individuals older than two years of age. They showed that males and females not only became brighter (across the entire spectrum), but also that both UV and carotenoid chroma increased with age. One of the very few studies using the exact age of males conducted by Budden and Dickinson (2009) on bluebirds, found that head plumage brightness increased in older individuals in a sample of males ranging from one to five years of age. Interestingly, the pattern for the association between age and brightness of the carotenoid-based breeding plumage in red bishops with a decrease of brightness in older birds is the opposite to that most often found for brightness of structural colours in the UV and blue range of the spectrum. Currently we do not know why age and brightness of the carotenoid-based plumage traits of red bishop males are negatively rather than positively related. However, regardless of whether brightness increases or decreases with age, it can still be used by conspecifics as visual cue for assessing age and perhaps age-related aspects of individual quality.

We are not aware of any study investigating the relationship between exact age in years and colouration (i.e. chroma and/or hue) of carotenoid-based plumage traits in birds. Probst et al. (2007) reported a quadratic relationship between age and plumage characteristics in Kirtland's warblers (*Dendroica kirtlandii*); however, plumage was assessed only by composite plumage scores based on a combination of size and subjective colour intensity of a mixture of black and yellow plumage patches. The fact that many small passerines have a rather short life-span and exact age is very difficult to assess in the field for the species with longer life spans might explain the lack of data on the relationship between age and colouration of carotenoid-based plumage in passerines. Red bishops have a fairly long life-span (oldest captured male was a minimum of eight years) compared to similar sparrow-sized passerines, and it may be advantageous for females to be able to identify males that are in their prime and distinguish them from first year breeders or old males. Age-related changes in reproductive success are well known for some bird species (e.g. Evans 1997, Espie et al. 2000, Penteriani et al. 2003), but a linear relation where reproductive effort and mating success steadily increase with age is unlikely in long-lived birds (Forslund & Pärt 1995), since very old males often perform worse than younger males because of decreasing body condition and other senescence effects. Rather, a quadratic relationship with an initial increase of reproductive performance followed by a decline later in life can be expected, as has been found, for example, in red-billed choughs, *Pyrrhocorax pyrrhocorax* (Reid et al. 2003), and in stitchbirds, *Notiomystis cincta* (Low et al. 2007). Similar quadratic relationships have also been found between age and survival in some passerines such as serins (*Serinus serinus*; Figuerola & Senar 2007) and European blackbirds (Grégoire et al. 2004). The age-related changes of plumage colouration in male red bishops followed the same pattern, with males in their prime having a more saturated plumage (higher PC2 scores) compared to both younger and older males. It remains to be investigated whether reproductive success and survival in male red bishops show a similar age-related pattern, which might then be partly attributable to the age-related pattern of plumage colouration found in this study.

Conclusion

Our study revealed a complex pattern of factors determining carotenoid-based plumage ornamentation in red bishop males. In general, plumage brightness and plumage colouration seem to be affected by the same variables and factors, with plumage brightness probably being a better indicator of male quality than plumage colouration. However, the relationships between plumage traits and factors such as HLR and blood parasite load varied considerably among seasons, indicating a strong effect of environmental conditions. Furthermore, several significant interactions suggest that the overall condition of males (body condition, stress levels, blood parasite load) can strongly affect the association pattern between plumage traits and other factors, with high stress levels or parasite loads as well as bad body condition forcing males to allocate resources away from plumage elaboration to maintenance or enhancement of immune functions. That is, males which are in generally good condition can afford to invest in plumage ornamentation without obvious detrimental effects on health, while males in bad overall condition face a trade-off: they can invest either in plumage ornamentation or in maintenance of body condition, health and immune function, but not simultaneously in both.

There are two conclusions that we can draw from these results. First, females cannot use plumage traits alone to gather information on male quality, but have to assess additional factors that advertise general male health status (possible through observations of male behaviour). Otherwise it would be difficult for females to distinguish between males in bad condition that invest most of the available energy in plumage ornamentation at the cost of body maintenance, and males in general good health that can afford to invest in plumage ornamentation without detrimental effects on health. Second, studies that investigate the signalling value of plumage traits should include several indices of health and should cover more than one season to be able to evaluate the complex and variable pattern of associations between plumage ornamentation and other male traits.

Acknowledgements

We would like to thank the National Parks Board of South Africa and the Chief Directorate Environmental Affairs at the Department of Economic Affairs, Environment and Tourism in the Eastern Cape Province for the permission to carry out this study in the Addo Elephant National Park. We are also very grateful to the entire staff at the Addo Elephant National Park for their continuous support. Many thanks go out to Dr. Wolfgang Goymann and Ingrid Schwabl at the Max-Planck Institute for Ornithology and to Dr. Martin Dehnhard and Marlies Rohleder at the Leibniz Institute for Zoo and Wildlife Research for determination of testosterone levels, and to Elisabeth Groscurth for carrying out the real time PCR for determination of parasitisation. Alice Edler was supported by a grant from the DAAD (Deutscher Akademischer Austauschdienst). This study was funded by the Deutsche Forschungsgemeinschaft (DFG FR 2096/2-2).

References

Aguilera E, Amat JA. (2007) Carotenoids, immune response and the expression of sexual ornaments in male greenfinches (*Carduelis chloris*). *Naturwissenschaften* **94:** 895 – 902.

Alonso-Alvarez C, Pérez-Rodríguez L, Mateo R, Chastel O, Viñuela J. (2008) The oxidation handicap hypothesis and the carotenoid allocation trade-off. *Journal of Evolutionary Biology* **21:** 1789 – 1797.

Apanius V. (1998) Stress and immune defense. In: Møller AP, Milinski M, Slater PJB, eds. *Stress and Behavior. Advances in the Study of Behavior, 27*. London: Academic Press, 133 – 153.

Baeta R, Faivre B, Motreuil S, Gaillard M, Moreau J. (2008) Carotenoid trade-off between parasitic resistance and sexual display: an experimental study in the blackbird (*Turdus merula*). *Proceedings of the Royal Society of London B* **275:** 427 – 434.

Bitton P-P, Dawson RD. (2008) Age-related differences in plumage characteristics of male tree swallows *Tachycineta bicolor*: hue and brightness signal different aspects of individual quality. *Journal of Avian Biology* **39:** 446 – 452.

Bitton P-P, O'Brien EL, Dawson RD. (2007) Plumage brightness and age predict extrapair fertilization success of male tree swallows, *Tachycineta bicolor*. *Animal Behaviour* **74:** 1777 – 1784.

Blount JD, Metcalfe NB, Birkhead TR, Surai PF. (2003) Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* **300:** 125 – 127.

Brawner WR, Hill GE, Sundermann CA. (2000) Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. *The Auk* **117:** 952 – 963.

Budden AE, Dickinson JL. (2009) Signals of quality and age: the information content of multiple plumage ornaments in male western bluebirds *Sialia mexicana*. *Journal of Avian Biology* **40:** 18 – 27.

Campbell TW. (1995) *Avian Hematology and Cytology*. Ames: Iowa State University Press.

Craig AJFK. (1974) Reproductive behaviour of the male red bishop bird. *Ostrich* **45:** 149 – 160.

Cuthill IC, Bennett ATD, Patridge JC, Maier EJ. (1999) Plumage reflectance and the objective assessment of avian sexual dichromatism. *The American Naturalist* **153:** 183 – 200.

Davis AK, Maney DL, Maerz JC. (2008) The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology* **22:** 760 – 772.

Delhey K, Kempenaers B. (2006) Age differences in blue tit *Parus caeruleus* plumage colour: within-individual changes or colour-biased survival? *Journal of Avian Biology* **37:** 339 – 348.

Dyrcz A, Wink M, Kruszewicz A, Leisler B. (2005) Male reproductive success is correlated with blood parasite levels and body condition in the promiscuous aquatic warbler (*Acrocephalus paludicola*). *The Auk* **122:** 558 – 565.

Duckworth RA, Mendonça MT, Hill GE. (2004) Condition-dependent sexual traits and social dominance in the house finch. *Behavioral Ecology* **15:** 779 – 784.

Edler R, Klump GM, Friedl TWP. (2004) Do blood parasites affect reproductive performance in male red bishops (*Euplectes orix*)? A test of the Hamilton-Zuk hypothesis. *Ethology Ecology & Evolution* **16:** 315 – 328.

Espie RHM, Oliphant LW, James PC, Warkentin IG, Lieske DJ. (2000) Age-dependent breeding performance in merlins (*Falco columbarius*). *Ecology* **81:** 3404 – 3415.

Estep LK, Shawkey MD, Hill GE. (2006) Carotenoid-based breast plumage colour, body condition and clutch size in red fodies (*Foudia madagascariensis*). *Ostrich* **77:** 164 – 169.

Evans MR. (1997) Nest-building signals male condition rather than age in wrens. *Animal Behaviour* **53:** 749 – 755.

Faivre B, Préault M, Salvadori F, Théry M, Gaillard M, Cézilly F. (2003) Bill colour and immunocompetence in the European blackbird. *Animal Behaviour* **65:** 1125 – 1131.

Feldman BF, Zinkl JG, Jain NC. (2000) *Schalm's veterinary hematology*. 5th edition. New York: Lippincott, Williams and Wilkins.

Ferns PN, Hinsley SA. (2008) Carotenoid plumage hue and chroma signal different aspects of individual and habitat quality in tits. *Ibis* **150:** 152 – 159.

Figuerola J, Senar JC. (2007) Serins with intermediate brightness have a higher survival in the wild. *Oikos* **116:** 636 – 641.

Figuerola J, Muñoz E, Gutiérrez R, Ferrer D. (1999) Blood parasites, leucocytes and plumage brightness in the cirl bunting, *Emberiza cirlus*. *Functional Ecology* **13:** 594 – 601.

Folstad I, Karter AJ. (1992) Parasites, bright males, and the immunocompetence handicap. *The American Naturalist* **139:** 603 – 622.

Forslund P, Pärt T. (1995) Age and reproduction in birds – hypotheses and tests. *Trends in Ecology and Evolution* **10:** 374 – 378.

Friedl TWP. (2002) The effect of rainfall on the breeding behaviour of the red bishop, *Euplectes orix. Ostrich* **73:** 181 – 184.

Friedl TWP. (2004a) Breeding success in a red bishop (*Euplectes orix*) colony in the Eastern Cape, South Africa, and its relation to nest site characteristics. *Ostrich* **75**: 95 – 105.

Friedl TWP. (2004b) Breeding behaviour of the red bishop (*Euplectes orix*): a synthesis and new observations. *Vogelwarte* **42:** 178 – 190.

Friedl TWP, Edler R. (2005) Stress-dependent trade-off between immunological condition and reproductive performance in the polygynous red bishop (*Euplectes orix*). *Evolutionary Ecology* **19:** 221 – 239.

Friedl TWP, Klump GM. (1999) Determinants of male mating success in the red bishop (*Euplectes orix*). *Behavioural Ecology and Sociobiology* **46:** 387 – 399.

Friedl TWP, Klump GM. (2000) Nest and mate choice in the red bishop (*Euplectes orix*): female settlement rules. *Behavioral Ecology* **11:** 378 – 386.

Gonzalez G, Sorci G, Smith LC, de Lope F. (2001) Testosterone and sexual signalling in male house sparrows (*Passer domesticus*). *Behavioural Ecology and Sociobiology* **50:** 557 – 562.

Goymann W, Trappschuh M, Jensen W, Schwabl I. (2006) Low ambient temperature increases food intake and dropping production, leading to incorrect estimates of hormone metabolite concentrations in European stonechats. *Hormones and Behavior* **49:** 644 – 653.

Grégoire A, Préault M, Cézilly F, Wood MJ, Pradel R, Faivre B. (2004) Stabilizing natural selection on the early expression of a secondary sexual trait in a passerine bird. *Journal of Evolutionary Biology* **17:** 1152 – 1156.

Griggio M, Serra L, Licheri D, Monti A, Pilastro A. (2007) Armaments and ornaments in the rock sparrow: a possible dual utility of a carotenoid-based feather signal. *Behavioural Ecology and Sociobiology* **61:** 423 – 433.

Grill CP, Rush VN. (2000) Analysing spectral data: comparison and application of two techniques. *Biological Journal of the Linnean Society* **69:** 121 – 138.

Gross WB, Siegel HS. (1983) Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Diseases* **27:** 972 – 979.

Hamilton WJ, Poulin R. (1997) The Hamilton and Zuk hypothesis revisted: a meta-analytical approach. *Behaviour* **134:** 299 – 320.

Hamilton WD, Zuk M. (1982) Heritable true fitness and bright birds: a role for parasites? *Science* **218:** 384 – 386.

Hatchwell BJ, Wood MJ, Anwar M, Perrins CM. (2000) The prevalence and ecology of the haematozoan parasites of European blackbirds, *Turdus merula*. *Canadian Journal of Zoology* **78:** 684 – 687.

Hill GE. (2002) *A red bird in a brown bag: the function and evolution of colourful plumage in the house finch*. New York: Oxford University Press.

Hill GE, Farmer KL. (2005) Carotenoid-based plumage coloration predicts resistance to a novel parasite in the house finch. *Naturwissenschaften* **92:** 30 – 34.

Hõrak P, Ots I, Vellau H, Spottiswoode C, Møller AP. (2001) Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. *Oecologia* **126:** 166 – 173.

Hõrak P, Saks L, Zilmer M, Karu U, Zilmer K. (2007) Do dietary carotenoids alleviate the cost of immune activation? An experiment with greenfinches. *The American Naturalist* **170:** 625 – 635.

Ilmonen P, Hasselquist D, Langefors Å, Wiehn J. (2003) Stress, immunocompetence and leukocyte profiles of pied flycatchers in relation to brood size manipulation. *Oecologia* **136:** 148 – 154.

Jawor JM, Breitwisch R. (2004) Multiple ornaments in male northern cardinals, *Cardinalis cardinalis*, as indicators of condition. *Ethology* **110:** 113 – 126.

Karu U, Saks L, Hõrak P. (2007) Carotenoid coloration in greenfinches is individually consistent irrespective of foraging ability. *Physiological and Biochemical Zoology* **80:** 663 – 670.

Komdeur J, Oorebeek M, van Overveld T, Cuthill IC. (2005) Mutual ornamentation, age, and reproductive performance in the European starling. *Behavioral Ecology* **16:** 805 – 817.

Krinsky NI. (1994) The biological properties of carotenoids. *Pure and Applied Chemistry* **66:** 1003 – 1010.

Kristiansen KO, Bustnes JO, Folstad I, Helberg M. (2006) Carotenoid coloration in great black-backed gull *Larus marinus* reflects individual quality. *Journal of Avian Biology* **37:** 6 – 12.

Lessels CM, Boag PT. (1987) Unrepeatable repeatabilities: a common mistake. *The Auk* **104:** 116 – 121.

Lindström K, Lundström J. (2000) Male greenfinches (*Carduelis chloris*) with brighter ornaments have higher virus infection clearance rate. *Behavioural Ecology and Sociobiology* **48:** 44 – 51.

López G, Figuerola J, Varo N, Soriguer R. (2005) White wagtails *Motacilla alba* showing extensive post-juvenile moult are more stressed. *Ardea* **93:** 237 – 244.

Low M, Pärt T, Forslund P. (2007) Age-specific variation in reproduction is largely explained by the timing of territory establishment in the New Zealand stitchbird *Notiomystis cincta*. *Journal of Animal Ecology* **76:** 459 – 470.

Lozano GA. (1994) Carotenoids, parasites, and sexual selection. *Oikos* **70:** 309 – 311.

Maney DL, Davis AK, Goode CT, Reid A, Showalter C. (2008) Carotenoid-based plumage coloration predicts leukocyte parameters during the breeding season in northern cardinals (*Cardinalis cardinalis*). *Ethology* **114:** 369 – 380.

McGraw KJ. (2005) The antioxidant function of many animal pigments: are there consistent health benefits of sexually selected colourants? *Animal Behaviour* **69:** 757 – 764.

McGraw KJ, Ardia DR. (2003) Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. *The American Naturalist* **162:** 704 – 712.

McGraw KJ, Hudon J, Hill GE, Parker RS. (2005) A simple and inexpensive chemical test for behavioral ecologists to determine the presence of carotenoid pigments in animal tissues. *Behavioural Ecology and Sociobiology* **57:** 391 – 397.

McGraw KJ, Correa SM, Adkins-Regan E. (2006) Testosterone upregulates lipoprotein status to control sexual attractiveness in a colourful songbird. *Behavioural Ecology and Sociobiology* **60:** 117 – 122.

Metz M, Klump GM, Friedl TWP. (2009) Male nest-building behaviour and mating success in the red bishop (*Euplectes orix*). *Behaviour* **146**: 771 – 794.

Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, Surai PF. (2000) Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence, or detoxification ability? *Avian and Poultry Biology Reviews* **11:** 137 – 159.

Montgomerie R. (2006) Analyzing colors. In: Hill GE, McGraw KJ, eds. *Bird Coloration. Volume I: Mechanisms and Measurements.* Cambridge: Harvard University Press, 90 – 147.

Morales J, Moreno J, Lobato E, Merino S, Tomas G, Martinez de la Puente J, Martinez J. (2006) Higher stress protein levels are associated with lower humoral and cell-mediated immune responses in pied flycatcher females. *Functional Ecology* **20:** 647 – 655.

Moreno J, Merino S, Martinez J, Sanz, JJ, Arriero E. (2002) Heterophil/lymphocyte ratios and heat-shock protein levels are related to growth in nestling birds. *Ecoscience* **9:** 434 – 439.

Mougeot F. (2008) Ornamental comb colour predicts T-cell-mediated immunity in male red grouse *Lagopus lagopus scoticus*. *Naturwissenschaften* **95:** 125 – 132.

Navara KJ, Hill GE. (2003) Dietary carotenoid pigments and immune function in a songbird with extensive carotenoid-based plumage coloration. *Behavioral Ecology* **14:** 909 – 916.

Ohlsson T, Smith HG, Råberg L, Hasselquist D. (2003) Effects of nutrition on sexual ornaments and humoral immune responsiveness in adult male pheasants. *Ethology Ecology and Evolution* **15:** 31 – 42.

Ots I, Hõrak P. (1996) Great tits *Parus major* trade health for reproduction. *Proceedings of the Royal Society of London B* **263:** 1443 – 1447.

Ots I, Hõrak P. (1998) Health impact of blood parasites in breeding great tits. *Oecologia* **116:** 441 – 448.

Ots I, Murumägi A, Hõrak P. (1998) Haematological health state indices of reproducing great tits: methodology and sources of natural variation. *Functional Ecology* **12:** 700 – 707.

Penteriani V, Balbontin J, Ferrer M. (2003) Simultaneous effects of age and territory quality on fecundity in Bonelli's eagle *Hieraaetus fasciatus*. *Ibis* **145:** E77 – E82.

Pérez-Rodríguez L, Viñuela J. (2008) Carotenoid-based bill and eye ring coloration as honest signals of condition: an experimental test in the red-legged partridge (*Alectoris rufa*). *Naturwissenschaften* **95:** 821 – 830.

Peters A, Delhey K, Andersson S, van Noordwijk H, Förschler MI. (2008) Condition-dependence of multiple carotenoid-based plumage traits: an experimental study. *Functional Ecology* **22:** 831 – 839.

Probst JR, Donner DM, Bozek MA. (2007) Continuous, age-related plumage variation in male Kirtland's warblers. *Journal of Field Ornithology* **78:** 100 – 108.

Pryke SR, Griffith SC. (2006) Red dominates black: agonistic signalling among head morphs in the colour polymorphic Gouldian finch. *Proceedings of the Royal Society of London B* **273:** 949 – 957.

Pryke SR, Andersson S, Lawes MJ, Piper SE. (2002) Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color. *Behavioral Ecology* **13:** 622 – 631.

Reid JM, Bignal EM, Bignal S, McCracken DI, Monaghan P. (2003) Environmental variability, life-history covariation and cohort effects in the red-billed chough *Pyrrhocorax pyrrhocorax*. *Journal of Animal Ecology* **72:** 36 – 46.

Roberts ML, Buchanan KL, Evans MR. (2004) Testing the immunocompetence handicap hypothesis: a review of the evidence. *Animal Behaviour* **68:** 227 – 239.

Saks L, Ots I, Hõrak P. (2003) Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia* **134:** 301 – 307.

Sapolsky RM. (1992) Neuroendocrinology of the stress response. In: Becker JB, Breedlove SM, Crews D, eds. *Behavioral Endocrinology.* Cambridge: MIT Press, Cambridge, 287 – 324.

Senar JC, Escobar D. (2002) Carotenoid derived plumage coloration in the siskin *Carduelis spinus* is related to foraging ability. *Avian Science* **2:** 19 – 24.

Shawkey MD, Hill GE. (2005) Carotenoids need structural colours to shine. *Biology Letters* **1:** 121 – 124.

Siefferman LM, Hill GE, Dobson FS. (2005) Ornamental plumage coloration and condition are dependent on age in eastern bluebirds *Sialia sialis*. *Journal of Avian Biology* **36:** 428 – 435.

Siegel HS. (1995) Stress, strains and resistance. *British Poultry Science* **36:** 3 – 22.

Siitari H, Huhta E. (2002) Individual color variation and male quality in pied flycatchers (*Ficedula hypoleuca*): a role of ultraviolet reflectance. *Behavioral Ecology* **13:** 737 – 741.

Smith HG, Råberg L, Ohlsson T, Granbom M, Hasselquist D. (2007) Carotenoid and protein supplementation have differential effects on pheasant ornamentation and immunity. *Journal of Evolutionary Biology* **20:** 310 – 319.

Stein AC, Uy JAC. (2006) Plumage brightness predicts male mating success in the lekking golden-collared manakin, *Manacus vitellinus*. *Behavioral Ecology* **17:** 41 – 47.

Tan TMC, Nelson JS, Ng HC, Ting RCY, Kara UAK. (1997) Direct PCR amplification and sequence analysis of extrachromosomal *Plasmodium* DNA from dried blood spots. *Acta Tropica* **68:** 105 – 114.

von Schantz T, Bensch S, Grahn M, Hasselquist D, Wittzell H. (1999) Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society of London B* **266:** 1 – 12.

Wedekind C, Folstad I. (1994) Adaptive or nonadaptive immunosuppression by sex-hormones. *The American Naturalist* **143:** 936 – 938.

Westneat DF, Birkhead TR. (1998) Alternative hypotheses linking the immune system and mate choice for good genes. *Proceedings of the Royal Society of London B* **265:** 1065 – 1073.

CHAPTER 2

Reproductive behaviour in male red bishops (*Euplectes orix*): how important is plumage colouration?

Submitted as:
Edler AU, Friedl TWP. "Reproductive behaviour in male red bishops (Euplectes orix): how important is plumage colouration?"

Abstract

The role of bright plumage colouration for female choice has been the focus of several studies, with females often preferring the brightest and most elaborately ornamented males, which are often also the healthiest and highest quality individuals. In our study, we tested the influence of plumage, testosterone levels and blood parasite load (determined by quantitative real-time PCR) on reproductive performance in the red bishop (*Euplectes orix*), a sexually dimorphic and polygynous weaverbird species, where males in a carotenoid-based orange-to-red breeding plumage defend small territories in colonies to which they try to attract females. We found no difference between territorial males and floaters with regard to plumage traits, testosterone levels or parasite load. For territorial males, reproductive effort measured in terms of the number of nests built was positively related to territory tenure (which per se was largely influenced by breeding season duration), plumage brightness and blood parasite load, while testosterone levels had no effects. Male reproductive success in terms of number of nests accepted was mainly determined by the number of nests built (confirming previous findings), but was also negatively related to blood parasite load, while we found no clear influence of plumage characteristics or testosterone levels. Male attractiveness independently of the number of nests built was also positively related to the number of accepted nests, possibly indicating some form of positive feedback loop, as expected, for example, when female mate choice copying occurs. Based on our results we can conclude that in the red bishop high infection levels with blood parasites negatively influence male mating success, while we have to reject the idea that plumage characteristics affect male reproductive success. However, plumage colouration might play a role as a signal in male-male competition for territories.

Introduction

Even though the exact ways in which female preferences evolve are unknown, the mechanism behind many conspicuous male traits is intersexual selection via female choice, leading to a system consisting of showy males and choosy females (Andersson 1994, Bennett & Owens 2002). The displayed male ornamentation shown in many species is often characterised by a high variability across the individuals of a population, and this observed variation allows females to choose a male which will maximise potentially gained benefits, be they direct (i.e. fertility assurance, paternal care, or high quality territories) or indirect (e.g. genes for parasite resistance and/or immunocompetence then obtained by offspring).

Visual signals and colour-based ornamentation in particular, have received a lot of attention in the field of sexual selection (e.g. Maynard Smith & Harper 2003, Searcy & Nowicki 2005). Colour-based ornaments can vary not only in size, but also in colour quality, with individuals with the most intense or biggest ornaments usually being the most attractive (e.g. Andersson 1994). Specifically carotenoid-based signals have been the focus of many studies concerning reproductive effort and success, and there is a great number of avian studies showing that males displaying larger or more colourful carotenoid-based ornaments were preferred as mates and obtained higher reproductive success (for review see Hill 2006a).

Given that there is overwhelming evidence that females prefer males with the most elaborated colour ornaments, the question remains as to what carotenoids signal from which females could benefit? A topic that received considerable attention in this context is the relationship between carotenoid-based colouration and parasite load. Following the seminal paper by Hamilton and Zuk (1982) in which they presented the idea of parasite-mediated sexual selection, several studies reported a negative relationship between male colouration and levels of parasitisation at the intraspecific level, in accordance with the Hamilton-Zuk hypothesis (Milinski & Bakker 1990, Houde & Torio 1992, Shykoff & Widmer 1996, Brawner et al. 2000). The usual interpretation of such a negative relationship was that high-quality males with inherently good parasite resistance or immunocompetence signal their superior quality via elaborated colour ornaments. In contrast, individuals with inferior quality are less colourful ornamented and more susceptible to parasite infections. There should, of course, also be costs associated with this type of signalling, in order to maintain certain honesty to male quality signalling. One of the main costs associated with carotenoid-based signalling, is that carotenoids play a vital part as immune system enhancers and antioxidants (Lozano 1994, Olson & Owens 1998, von Schantz et al. 1999). Carotenoids which have been allocated to the colouration of diverse ornaments are lost to the immune system. If an individual is able to allocate pigments away from immune defence and into display, colour signals are then a reliable indicator of good health. A further cost is the transport and metabolism of ingested pigments needed for display, processes which can also limit colouration if they cannot be carried out sufficiently (Hill 2000, 2002). The various costs are not mutually exclusive, but can be additive. This would lead to the conclusion that an individual in poor condition may have more difficulty in producing colourful displays, if carotenoids are not only difficult to obtain, but also helpful in fighting infections and diseases, as well as costly to transport and modify accordingly. Therefore, signal honesty is maintained and females can base their choice of a prospective mate on a reliable indicator of overall condition, in the form of ornament colouration.

More recently, however, it has been recognized that there are complications to this aspect of carotenoid signalling. Since carotenoids cannot be synthesized, they have to be ingested as part of the food and metabolized accordingly. Several studies have shown that carotenoid pigmentation is directly affected by diet, thereby demonstrating an important link between nutrition and carotenoid levels (Hill 1992, 2006b, Grether 2000). Assuming that the amount of carotenoids obtained in the diet is proportional to how much food was actually ingested and therefore proportional to the amount of energy and nutrients obtained, then carotenoid-based signals are a reliable indicator for individual nutritional condition. Alternatively, some individuals could be more successful than other in searching for carotenoid-rich food items, in which case carotenoid-based signals would reflect foraging ability rather than nutritional condition. Under the assumption that all individuals in a population have equal access to food resources containing the desired pigments, then those individuals investing more in colouration will have a lower immune defence and a higher level of parasitisation, resulting in a positive relationship between colouration and parasite load (Folstad et al. 1994, Shykoff & Widmer 1996). On the other hand, if access varies, then those individuals with good access can afford both high levels of ornamentation and immune defence, showing a negative correlation between colouration and parasite load (Hill 2006b). The relationship between carotenoid-based colouration and level of parasite infections can be further modified by variation in individual immunological condition, parasite resistance, physiological stress levels or health.

In this study, we wanted to determine if the carotenoid-based orange to red plumage areas in male red bishops (*Euplectes orix*) affect male reproductive performance (i.e. reproductive effort and success). The red bishop is a sexually dimorphic, colonial breeding and polygynous weaver bird species of southern Africa, where males compete for limited territories to which they try to attract females. In an accompanying study we have found that both plumage colouration and brightness were negatively influenced by physiological stress levels (represented by the heterophil/lymphocyte ratio), and that plumage brightness, but not colouration, was negatively associated to body condition (Edler & Friedl, submitted), leading to the conclusion that brightness might be a better indicator of individual quality than colouration. Here we investigate whether male reproductive effort and success is influenced by plumage colouration, including blood parasite load (determined by quantitative real-time PCR) and testosterone levels as explanatory variables.

Material & methods

Study species and area

The red bishop (*Euplectes orix*) is a sexually dimorph weaverbird species occurring in Sub-Saharan Africa. During the non-breeding season, all individuals display a buffy brown plumage with a whitish eye-stripe. At the start of the breeding season, males two years of age and older moult into a bright red and black nuptial plumage, with colour intensity of the red areas varying between individuals. Red bishops are colonial breeders, with colonies often situated in reed beds or bulrush stands along or near water. Males defend small territories in these colonies and build as many nests as possible within the territory during their tenure, with both tenure and the number of nests built being positively related to individual reproductive success (Friedl & Klump 1999, 2000, Lawes et al. 2002, Metz et al. 2009). Males have a variety of displays, both for territory defence when confronted with territory intruders and for mate attraction when females enter the territory (Craig 1974, Friedl 2004). In the latter case, the male carries out courtship behaviour until the female either leaves the territory or accepts a nest and allows copulation, which is signalled by the typical copulation solicitation display characteristic to female passerines. A female will copulate several times with the territory holder and egg-laying (clutch size: 2 – 5 eggs) commences soon after nest acceptance. Once the eggs have been laid, the female is usually ignored by the territorial male. Incubation of the eggs and feeding of the young, which hatch approximately two weeks after egg-laying, is the sole responsibility of the female. The nestlings fledge about 11 to 15 days after hatching. More details on red bishop breeding behaviour can be found in Friedl (2004).

The study took place during three consecutive breeding seasons (approximately October to March) from 2005 to 2008. The studied colony was situated in a small dam surrounded by bulrush (*Typha capensis*) and common reeds (*Phragmites spec.*) located in the Addo Elephant National Park in the Eastern Cape Province, South Africa (33°26'S, 25°45'E). The main cause of breeding failure (>70% per season, unpublished data) in our study colony during the course of this study was predation by water mongoose (*Atilax paludinosis*), boomslang (*Dispholidus typus*) and cape cobra (*Naja nivea*). Not all males in full breeding plumage observed in the study colony were territorial, and we defined all mature males which were seen in the colony but never defended a breeding territory during their attendance as non-territorial floaters.

Field Methods

Behavioural observations and the identification of birds via individual colour ring combinations took place every day. Adult birds were caught with the help of mist nets and walk-in traps, which were situated along the edge of the colony. Upon capture, tarsus and wing length were measured and the weight recorded. We captured a total of 79 males, with 13 males having been caught more than once during the course of the study. Blood samples were obtained by puncturing the brachial wing vein with a sterile needle, collecting the blood with a heparinisized microcapillary tube (approximately 20 – 60µl), and transferring the sample into a 1.5ml reaction tube. Serum samples were obtained by centrifuging the reaction tubes containing the blood samples with a micro centrifuge (Capsule HF-120, Tomy Kogyo Co., Ltd., Fukushima, Japan), removing the upper layer with the serum using glass pipettes and transferring it into 0.5ml PCR tubes, which were then stored in a freezer. The blood cells remaining in the 1.5ml reaction tubes were topped with a PBS buffer and refrigerated. In total, we collected blood smears and serum samples from 64 individual males, with ten males being sampled at least twice over the course of this study.

We randomly selected one data set for each male caught more than once over the course of the three seasons. Further, we only used males with complete data sets for plumage reflectance and blood samples, reducing our sample size for this study to a total of 53 males, with 39 of them being territorial and 14 non-territorial.

We established a standardised method to determine both the length of all three seasons as well as tenure for individual males during these seasons. The beginning of the season in seasons one and three was defined as the date the first completed nest was found in the colony. However, due to unusually heavy rainfall in the winter 2006, the second season started extremely early and there were already hatchlings in the colony upon arrival and start of observations. Therefore, we defined the start of the second breeding season as the calculated hatching date of the first-hatched nestlings minus 30 days. The end of all three seasons was determined by the date the colony's last nest was completed plus 30 days (approximately the time period between nest acceptance and fledging).

In regard to territory tenure of individual males, "day 1" of tenure was defined as the date on which an individual male completed his first nest. In the second season, the start of territory tenure for those males already in the colony upon arrival was set to the beginning of the breeding season, as we cannot be sure exactly when these males established their territories. All other males that established territories after the beginning of October had their tenure defined as mentioned above for the other two seasons. As males often occupy their territories without building a nest for extended time periods, tenure end was determined based on daily behavioural observations – we defined territory tenure as terminated when a male was not observed defending his territory for three consecutive days.

We further determined the number of nests built (as a measure for reproductive effort) and the number of nests accepted (as a measure for reproductive success) for each territorial male. This was accomplished by nest checks which were carried out every other day. During these nest checks, new nests in territories were tagged and the date and number of eggs laid in each nest was recorded. Due to the high predation rate, the number of nests accepted is used as a measure for reproductive success, as the amount of nests with hatchlings and fledglings is rather small. The number of accepted nests has previously been shown to be significantly correlated to the number of eggs laid, the number of hatchlings and the number of fledglings in the territory (Friedl & Klump 1999, Metz et al. 2009). Given that the number of nests built has been shown to be an important determinant of mating success in red bishops (Friedl & Klump 1999, Lawes et al. 2002, Metz et al. 2009), we calculated the residuals of the regression of nests accepted against nests built to obtain an additional measure of reproductive success. This variable will be termed "male attractiveness" from here on, since it reflects the ability of a male to attract females to its territory independent from the number of nests built. These residuals were calculated on a seasonal basis with data from all territorial males in the breeding colony included (not only the data for the limited set of territorial males with a complete dataset regarding plumage characteristics and parasitemia).

Reflectance and spectral analysis

We measured the spectral reflectance of the orange-red plumage areas of red bishop males using an Ocean Optics USB2000 spectrometer (Ocean Optics, Inc., Dunedin, Florida, USA), connected to a GetLight-Hal-S tungsten halogen light source (getspec.com, Germany) and a fibre optic reflectance probe fitted with a self-made distance piece to standardise the distance between probe and sample. We took three consecutive scans from approximately the centre of each measured area (head, throat and lower back), between which the probe was removed and replaced. Reflectance data (resolution (<1nm) were generated in relation to a white (WS-2 white reflective tile, 98% reflectance; getspec.com, Germany) and dark standard (self-made “dark box”) using OOIBase32 spectral software (Ocean Optics, Inc., Dunedin, Florida, USA). The analysis of plumage colour was restricted to the range from 400nm to 700nm, as red bishops do not reflect in the UV region between 320 – 400nm (AU Edler & TWP Friedl, unpublished data). Complete sets of reflectance data were collected for 53 males.

The three sets of raw reflectance data obtained for each of the three body areas measured were first averaged and then divided into 30 bins spanning 10nm each (e.g. 400 – 409nm, 410 – 419nm, etc.) using the median values from each 10nm segment. We then performed a Principal Component Analysis on these averaged raw data (Cuthill et al. 1999, Grill & Rush 2000, Maney et al. 2008), as this allows a significant reduction of a number of correlated variables into a few principal components (PCs) that summarize most of the variation (Montgomerie 2006). We obtained two principal components for each of the three body regions, which explained at least 91% of the variation in all three cases. Both the first principal components and the second principal components obtained for the three body regions correlated highly significantly with each other (Pearson correlation; all $r > 0.4$, all $P < 0.005$); therefore we averaged the three sets of 30 bins per individual and performed another PCA resulting in the final two principal components explaining 97% of the variation in the reflectance spectra. The first principal component (PC1; 83.8% of the variance explained) is flat and positive throughout the whole spectrum and thus reflects colour brightness, while the second principal component (PC2; 13.3% of the variance explained) represents variation in spectral shape and consequently reflects chroma and hue, which we will refer to as saturation from now on (see Edler & Friedl, submitted).

Parasites

We assessed parasitemia (i.e. percentage of blood cells infected with blood parasites of the genus *Plasmodium* and *Haemoproteus*) using a quantitative real-time PCR approach based on the so-called TaqMan assay probe strategy (for more details regarding quantitative real-time PCR and probe strategies see for example Dorak 2006). In short, the intensity of infections with blood parasites was determined by amplification of a specific 85bp fragment within the plastid-like large subunit ribosomal-RNA (LSU-rRNA) gene, which is conservative across a range of *Plasmodium* and *Haemoproteus* species (Tan et al. 1997). Real-time PCR was performed with the iCycler IQ Real-Time detection System (Bio-Rad). We measured the accumulation of the product during the PCR using a dual-labeled oligonucleotide probe (labelled with 6-FAM as a reporter molecule at the 5'-end and with a Black Hole Quencher at the 3'-end), and determined the threshold cycle at which the fluorescence of the product raised above background level. Based on the fact that there is a linear relationship between the logarithm of the starting amount of a template and its threshold cycle, we could then calculate the starting amount of blood parasites in the investigated blood samples of male red bishops using a standard curve determined for templates of known quantity (clones of a 594bp fragment within the LSU-rRNA gene from *Plasmodium falciparum* including the target sequence) in the same PCR reaction. We then converted the obtained values of blood parasite DNA per sample into a more meaningful measure for the level of infection, i.e. percentage of blood cells infected with blood parasites. For more details of the molecular methods and the calculation of parasitemia see Friedl and Groscurth (submitted manuscript). Given that in passerine birds very low levels of parasitemia with *Plasmodium* or *Haemoproteus* are generally regarded as being harmless to the hosts (e.g. Campbell 1995), we defined males as being parasitized only if the percentage of blood cells infected was higher than 0.1%.

Testosterone

The determination of testosterone levels for the serum samples collected in the breeding season 2005/06 was performed at the Max-Planck-Institute for Ornithology using a radioimmuno- assay following Goymann et al. (2006). Plasma samples (mean ± SD volume: 41.5 ± 20.5µl) were extracted with dichloromethane two times, resulting in an average extraction recovery of 86.0 ± 3.0% (mean ± SD) for 3H-testosterone added prior to extraction. The lower detection limit of the assay was 73pg/tube, equivalent to a range of lower detection limits between 39pg/ml and 169pg/ml (depending on the amount of plasma available and the respective extraction recovery). All samples were analyzed in one assay to reduce variation, and the intra-assay coefficient of variation was 4.5%. Given that the testosterone antibody used (Esoterix Endocrinology, Calabasas Hill, CA, USA) showed significant cross reactions with 5a-dihydrotestosterone (44%), our testosterone measurements may include a proportion of this other androgen.

The samples collected in the breeding seasons 2006/07 and 2007/08 were analysed at the Leibniz Institute for Zoo and Wildlife Research, Berlin using an enzyme immuno-assay with a double-antibody technique. Following extraction of serum (0.01 – 0.05 ml) with 2ml of butyl t-methyl ether:petroleum ether (30:70, v/v) for 30 minutes, the samples were frozen and the fluid petroleum ether phase was removed and evaporated at 55°C. After dissolving the steroids in 0.1ml of 40% (v/v) methanol, duplicates of 20µl each were analyzed. The assay used a polyclonal antibody raised in rabbits against testosterone-11-hemisuccinate-BSA, and testosterone-3-carboxymethyl-oxime-horse radish peroxidase served as label. The testosterone standard curve ranged from 0.4pg to 50pg per 20µl, and the cross-reactivity with testosterone was 100%, with 5a-dihydrotestosterone 10%, with androstenedione 2%, with estradiol 0.1%, and with progesterone 0.1%. Serial dilutions of a plasma pool from red bishops gave parallelism to the standard testosterone with no differences in slopes (P > 0.05). The intra- and inter-assay coefficients of variation (CVs) were 8.9% and 12.3%, respectively. All results are presented as nanograms of testosterone per milliliter of serum.

Statistical analysis

We performed a binary logistic regression model to determine whether male territoriality during the three study seasons was predicted by season, testosterone levels, parasitemia or plumage characteristics (PC1 and PC2). General Linear Models (GLM) were used to analyse how male reproductive effort (territory tenure, number of nests built) and success (number of nests accepted, male attractiveness) were influenced by the above mentioned factors. Based on previous findings showing that the number of nests built was influenced by territory tenure and the number of nests accepted was influenced by the number of nests built (Friedl & Klump 1999, Lawes et al. 2002, Metz et al. 2009), we included territory tenure and number of nests builts as independent variables in the respective analyses. In all GLMs, we first included all main effects and two-way interactions in the models and then removed all non-significant interactions in a stepwise process, until only significant interactions and the main effects remained in the final models.

Results

Territoriality

A binary logistic regression revealed that the probability of a male to become territorial was not affected by its testosterone level, blood parasite load, or plumage characteristics (Table 1).

Table 1: Results of a binary logistic regression with territoriality (yes/no) as dependent variable, season as factor and testosterone level, PC1, PC2 and parasite load as covariates.

	P	*Wald*	*B*
Season	0.211	3.113	
Season(1)	0.735	0.115	0.447
Season(2)	0.186	1.752	-1.322
Testosterone	0.451	0.569	-0.395
PC1	0.167	1.912	1.246
PC2	0.146	2.115	-0.751
Parasites	0.479	0.501	0.310

Territory tenure

Territory tenure was strongly affected by season, with considerably longer tenures in the season 2006/07 (mean ± SD, 115.2 ± 35.3 days) compared to both season 2005/06 (71.0 ± 31.6 days) and 2007/08 (74.2 ± 28.6 days). In addition, there was a marginally significant main effect of plumage brightness (PC1) and a tendency for parasite load to affect tenure (Table 2).

Furthermore, the interactions between season and testosterone, season and PC1, season and parasites, testosterone and PC1, and finally PC1 and parasites had significant effects on territory tenure. The three significant interaction terms involving season showed a similar pattern, with negative relationships between testosterone, PC1 and parasite load with tenure in the second season, and positive or no relationship between testosterone, PC1 and parasite load with tenure in the first and third study season (see Fig. 1).

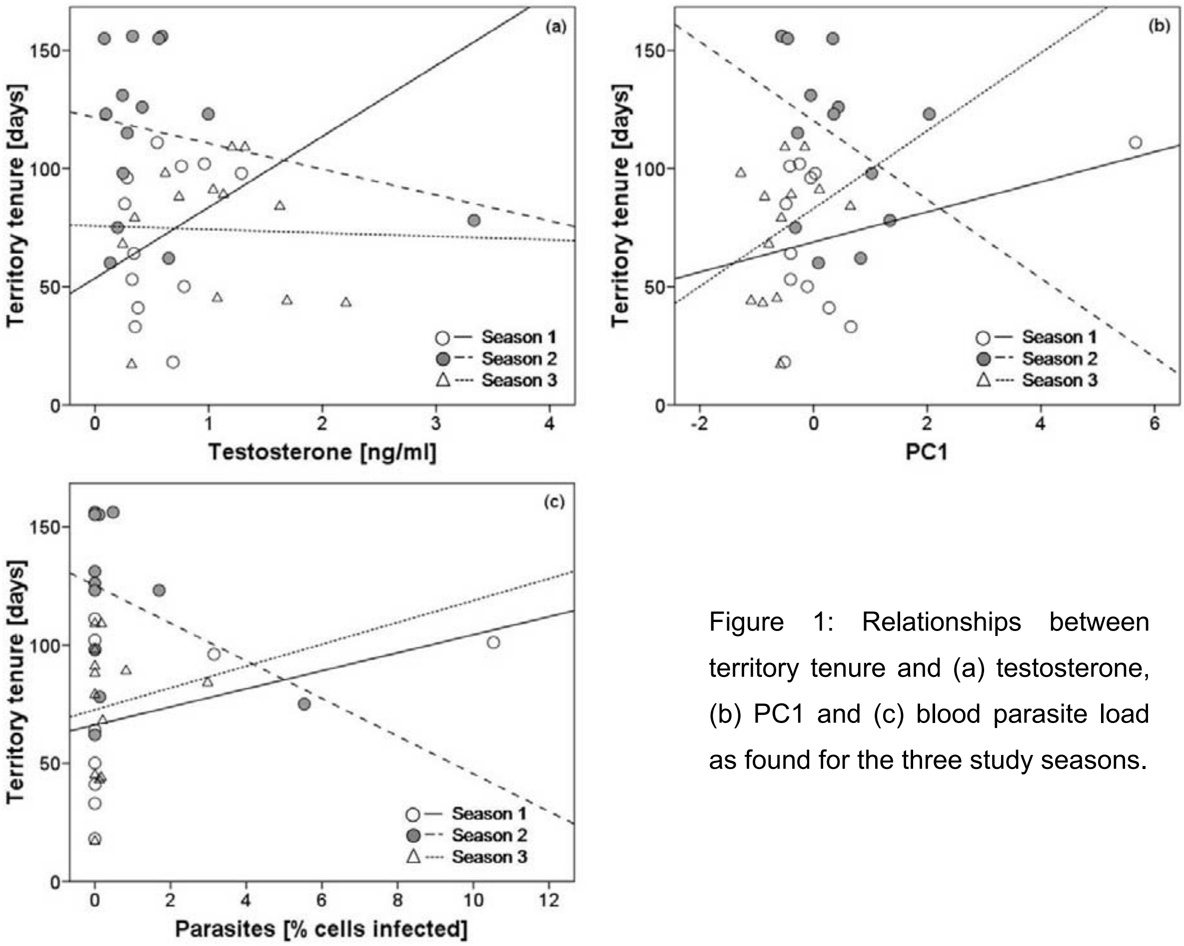

Figure 1: Relationships between territory tenure and (a) testosterone, (b) PC1 and (c) blood parasite load as found for the three study seasons.

Likewise, the interactions between PC1 (reflecting plumage brightness) and testosterone, and between PC1 and parasite load revealed a very similar pattern, with a clear positive relationship between PC1 and territory tenure for males having high testosterone levels or high parasite loads, and only very weak positive relationships between PC1 and territory tenure for males with low testosterone levels or non-parasitized males (Fig. 2).

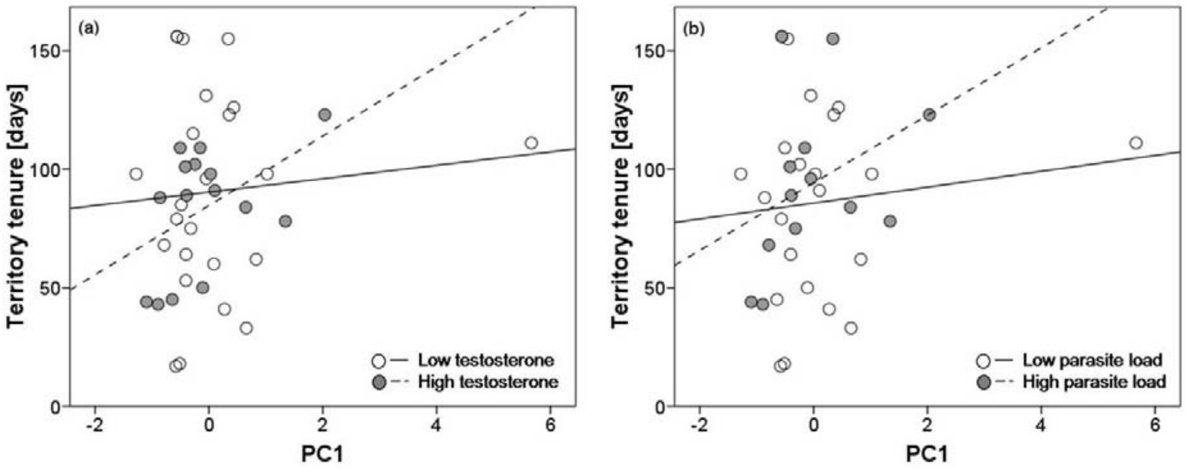

Figure 2: Relationship between territory tenure and PC1 for (a) males with high or low testosterone levels, and (b) non-parasitized and parasitized males.

Table 2: Results of a GLM with territory tenure as dependent variable, season as factor, and testosterone level, PC1, PC2 and parasite load as covariates. Shown here is the final model with all main effects and significant two-way interactions (adjusted R^2 of the model: 0.571).

	Tenure			
	F	*P*	*partial* η^2	*B*
Season	15.501	0.000	0.608	-5.570
				144.978
Testosterone	0.402	0.533	0.020	59.748
PC1	4.355	0.050	0.179	-51.922
PC2	0.135	0.717	0.007	-3.016
Parasites	3.646	0.071	0.154	-37.963
Season * Testosterone	4.644	0.022	0.317	-4.132
				152.897
Season * PC1	5.508	0.012	0.355	21.068
				-30.753
Season * Parasites	9.273	0.001	0.481	52.039
				26.650
Testosterone * PC1	4.829	0.040	0.195	72.279
Parasites * PC1	6.901	0.016	0.257	23.338

Number of nests built

There were several significant main effects (but no significant 2-way interaction effects) in the GLM model with the number of nests built as dependent variable, together explaining almost 75 % of the observed variation (Table 3).

Table 3: Results of a GLM with the number of nests built as dependent variable, season as factor, and testosterone level, PC1, PC2, parasite load, and territory tenure as covariates. Shown here is the final model with all main effects; all two-way interactions were non-significant and therefore omitted from the final model (adjusted R^2 of the model: 0.741).

	Number of nests built			
	F	*P*	*partial* η^2	*B*
Season	3.613	0.41	0.211	-2.322
				0.387
Testosterone	0.299	0.589	0.011	-0.332
PC1	7.801	0.009	0.224	0.960
PC2	5.081	0.033	0.158	-0.831
Parasites	16.051	<0.001	0.373	0.740
Tenure	31.872	<0.001	0.541	0.068

Territory tenure had the strongest effect, with males with long territory tenures building more nests than individuals with short tenures (Fig. 3a). Independent of territory tenure, there was also a strong positive relationship between parasite load and the number of nests built (Fig. 3b). Finally, the number of nests built was also related to both measures of plumage colouration. The number of nests built increased with increasing PC1 scores (i.e. there was a positive relationship between number of nests built and plumage brightness) and decreased with increasing PC2 scores (i.e. there was a negative relationship between number of nests built and plumage saturation).

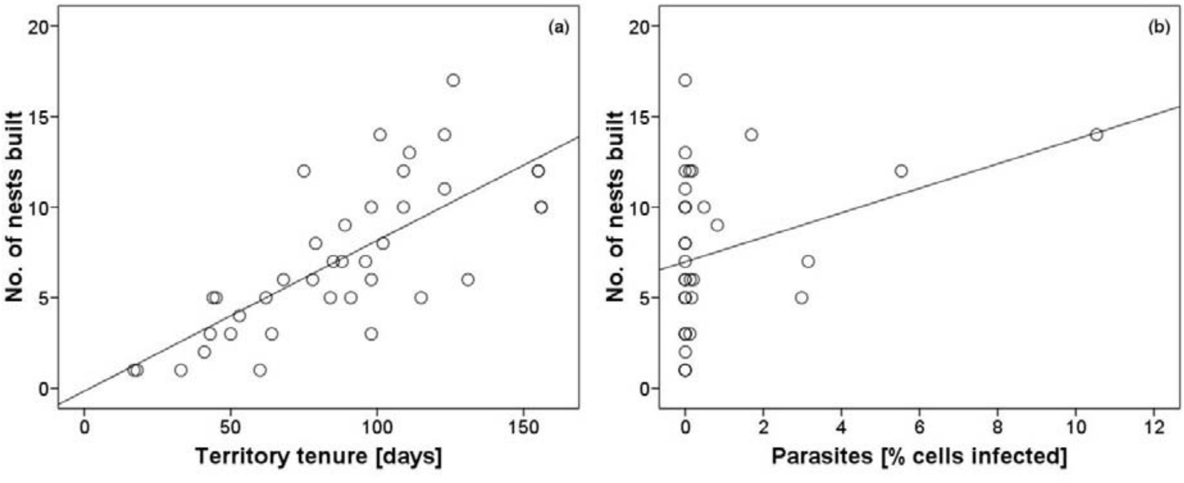

Figure 3: Relationship between the number of nests built and (a) territory tenure in days and (b) blood parasite load, pooled over the three study seasons.

Number of nests accepted

The number of nests built had the strongest influence on the number of nests accepted, alone explaining 72% of the observed variation (see Table 4). Males that built more nests than conspecifics also had more nests accepted. There was also a significant negative main effect of parasitemia, as well as significant effects of the 2-way interactions between parasitemia and PC2, between parasitemia and the number of nests built, and between parasitemia and testosterone levels (see Table 4).

Table 4: Results of a GLM with the number of nests accepted as dependent variable, and testosterone level, PC1, PC2, parasite load, and the number of nests built as covariates. Shown here is the final model with all main effects and significant two-way interactions (adjusted R^2 of the model: 0.787).

	Number of nests accepted			
	F	*p*	partial η^2	*B*
Testosterone	1.417	0.245	0.052	-0.410
PC1	0.821	0.373	0.031	-0.210
PC2	2.165	0.153	0.077	0.391
Parasites	4.405	0.046	0.145	-2.138
Nests built	67.490	<0.001	0.722	0.585
Parasites * PC2	5.684	0.025	0.179	1.020
Parasites * NBuilt	7.722	0.010	0.229	0.269
Parasites * Testosterone	7.605	0.011	0.226	-1.350

Male attractiveness

Attractiveness (i.e. the residuals of the regression of number of nests accepted against number of nests built as a measure of male reproductive success independent of the strong effect of number of nests built) was significantly affected by the number of nests accepted, parasitemia, and PC1. Males with a high value for attractiveness had a larger total number of nests accepted, fewer blood parasites and lower PC1 scores (less plumage brightness) than less attractive males (Table 5).

Table 5: Results of a GLM with male attractiveness (residuals of the regression of the number of nests accepted against the number of nests built) as dependent variable, and testosterone level, PC1, PC2, parasite load, and the number of nests accepted as covariates. Shown here is the final model with all main effects and significant two-way interactions (adjusted R^2 of the model: 0.502).

	Male attractiveness			
	F	*P*	*partial* η^2	*B*
Testosterone	0.319	0.576	0.011	-0.159
PC1	4.700	0.039	0.144	-0.330
PC2	0.699	0.410	0.024	0.144
Parasites	6.069	0.020	0.187	-0.626
Nests accepted	8.939	0.006	0.242	0.213
Parasites * NAccept	6.858	0.014	0.197	0.096

The significant effect of the interaction between parasites and number of nests accepted on male attractiveness is illustrated in Figure 4. For males with a number of nests accepted below average there was a negative relationship between parasitemia and male attractiveness, while the relationship was positive for males with a number of nests accepted above average.

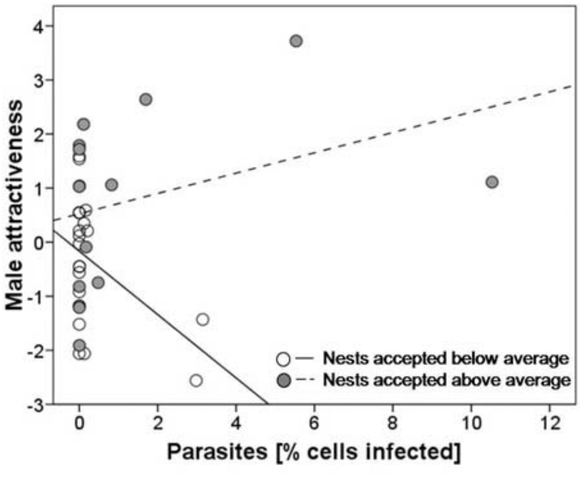

Figure 4: Relationship between male attractiveness and blood parasite load for males with a number of nests accepted below or above average.

Discussion

Territoriality

Especially in a polygynous breeding system, several factors can influence the competition between individuals in gaining a territory and therefore obtaining the potential to breed. Our results show – rather surprisingly - no difference between territorial males and floaters with regard to plumage characteristics, testosterone levels and parasite load. In contrast, other studies on closely related polygynous weaverbird species displaying carotenoid-based plumage ornaments found differences between floaters and territorial males. Andersson et al. (2002), for example, showed that territorial red-collared widowbird (*Euplectes ardens*) males not only had larger and redder collars in comparison to floaters, but they were also in worse nutritional condition due to the constant physical challenges of holding a territory. Pryke et al. (2002) provided additional experimental evidence that territory acquisition is determined by size and redness of plumage ornaments in red-collared widowbirds. Similar results have been reported in red-shouldered widowbirds (*Euplectes axillaris*; Pryke & Andersson 2003a, b). Studies on other bird species examining the difference between floaters and territorial males have shown that there are factors other than plumage characteristics that determine the status of an individual. In Zenaida doves (*Zenaida aurita*), Sol et al. (2005) found that floaters were smaller and had shorter wings when compared to territory owners. A study on black kites (*Milvus migrans*) revealed that age was the most likely predictor of territoriality (Sergio et al. 2009). However, in red-winged blackbirds (*Agelaius phoenicius*; Shutler & Weatherhead 1991) or tree swallows (*Tachycineta bicolor*; Peer et al. 2000), no morphological or behavioural differences between territory owners and floaters could be detected. There are several possible explanations for the lack of differences between floater and territorial males as found in our study. First, this result might reflect trade-offs between individual health state, development of plumage ornamentation, and investment in reproduction. Males in inherently good condition could afford to invest in the energetically demanding and stressful activities of establishing and defending territories without compromising both parasite defence and their ability to develop bright and saturated plumage, ending up at about the same level of plumage ornamentation and blood parasite load as compared to floater males which do not suffer these costs of territorial activities. Second, there is the possibility that males defined as floaters in our study due to not being observed as territory holders in our colony might in fact be no real floaters but instead only short term visitors that hold territories in one of the two other red bishop colonies in the vicinity of our study site.

Territory tenure

In regard to tenure, season had the largest effect, with mean territory tenure being highest in the second study season, followed by seasons three and one. The strong effect of season can be explained by the unusually heavy rainfalls in the winter preceding the second study season, which resulted in the breeding season both starting approximately two months earlier and lasting about one month longer than the other two seasons. Strong effects of rainfall on breeding behaviour in red bishops have been reported before (Friedl 2002), and it has been shown that not only timing and duration of the breeding season were related to rainfall patterns, but also that breeding activity in terms of number of nests built and accepted was influenced by rainfall (Friedl 2002).

As already mentioned above, the three significant interaction terms involving season showed a similar pattern, with negative relationships between testosterone, PC1 and parasite load with territory tenure in the second season, and positive or no relationship between testosterone, PC1 and parasite load with tenure in the first and third study season. These interaction effects can probably be attributed to the different environmental conditions prevailing during the three study seasons, and clearly show that the influence of parasite load and testosterone level on territory tenure is not always the same. Based on their finding that average stress levels in red bishop males are positively related to overall breeding activity in terms of the total number of nests built and accepted in the whole breeding colony per season, Friedl and Edler (2005) have concluded that trade-offs between health and male reproductive performance might be important only in breeding seasons with high levels of environmentally or socially induced physiological stress. Such a mechanism could explain the fact that we found negative relationships between territory tenure and both parasite load and testosterone level in the second field season, which was characterised by considerably longer duration and higher levels of breeding activity as compared to the other two seasons investigated. Assuming that males in this season already suffered from high stress levels, the additional negative effect of both blood parasites and high testosterone levels (immunosuppression) on host fitness potentially decreased their ability to fend off competitors for territories. In contrast, in the first and third study season the males did not suffer from such high physiological stress levels due to lower levels of breeding activity and negative impacts of blood parasites and high testosterone levels might not be strong enough to impair their ability for territory defence.

Plumage brightness (reflected by PC1) has a significant main effect on territory tenure, and tenure was also significantly affected by the interaction of PC1 with both blood parasite load and testosterone. Territory tenure clearly increased with increasing PC1 scores for males with high parasite loads and high testosterone levels, while there were only weak positive or no clear relationships between PC1 and territory tenure for males with low testosterone levels or non-parasitized males. As shown in a previous study on red bishops (Edler & Friedl, submitted), brightness is a good indicator of male quality, with brighter males being in better body condition, as well as having lower HLR values – both factors signal good overall condition. Therefore, we suggest that brighter males are capable of coping with higher parasite loads, as well as higher testosterone levels – both of which have been found to have a negative impact on (host) condition (Folstad & Karter 1992, Evans et al. 2000, Garvin et al. 2006) – as they are in overall better condition and are therefore able to defend their territories for longer periods of time. But why did we find no such positive relationship between territory tenure and PC1 for males with low testosterone levels or non-parasitized males? We suggest that for males that do not suffer from the negative impacts of high parasite loads or high testosterone levels the link between territory tenure and PC1 (itself linked to condition) is not as strong as for males with the additional burden of blood parasite infections or the immunosuppressive effect of high testosterone levels.

There is hardly any data on the relationship between brightness of carotenoid-based plumage ornaments (in contrast to saturation, i.e. chroma and hue) and territory tenure or territory acquisition in the literature. An exception is the study on American redstarts (*Setophaga ruticilla*) by Reudink et al. (2009), who showed that plumage brightness plays a significant part in acquiring high quality territories, with males displaying brighter yellow-orange plumage holding higher quality territories than duller conspecifics.

Reproductive effort (number of nests built)

In an earlier study on red bishops, Friedl and Klump (1999) were able to show that territory tenure significantly influences the number of nests built by an individual male, a result we were able to corroborate in our study. Further, parasitemia had a significant effect, with males with a higher parasite load building more nests than others. This could be explained by males investing more energy into building nests and therefore having lower reserves for parasite defence (Sheldon & Verhulst 1996). A previous study on red bishops (Edler et al. 2004) found no significant effect of blood parasite intensity on reproductive effort (also measured by the number of nests built); however, parasite load in this study was determined by counting parasites in blood smears, which is less reliable and accurate compared to quantitative real time PCR as used in our study. Several other studies have reported a positive relationship between parasite prevalence and reproductive effort, and a recent meta-analysis investigating the relationship between experimentally altered reproductive effort and blood parasite infection levels in birds found a significant positive though relatively weak overall effect of reproductive effort on parasitemia (Knowles et al. 2009).

Both principal components reflecting plumage brightness (PC1) and colour (PC2) were also significantly related to the number of nests built, with brighter and less saturated males building more nests than other conspecifics. Given that we were able to show that plumage brightness seems to be a better quality indicator than plumage colour (Edler & Friedl, submitted), this leads us to conclude that indeed, brighter but less red males are in better condition than duller and redder males, enabling them to invest more time and energy into nest building. Lastly, the significant main effect of season independently of territory tenure suggests that the three study seasons differed not only with regard to duration (affecting the number of nests built through its strong influence on territory tenure), but also with regard to some other additional factor related to nest-building activity.

Other studies linking carotenoid-based plumage colouration and male reproductive effort have for example been conducted on northern cardinals (*Cardinalis cardinalis*; Linville et al. 1998), and blackbirds (*Turdus merula*; Préault et al. 2005), with both showing that reproductive effort increases with increasing colouration. In cardinals, the male proportion of feeding increased with increasing plumage colour, while in blackbirds, males with more orange bills were better fathers as they displayed higher nest visiting rates than duller conspecifics, resulting in a higher number of fledglings. Similar results have been obtained with regard to the carotenoid-based tarsus colouration in kestrels (*Falco tinnunculus*), where males with more intensely coloured tarsi (in terms of hue) had higher female provisioning rates and better quality territories (Casagrande et al. 2006). We are not aware of any study linking brightness of male carotenoid-based plumage ornaments with reproductive effort or paternal care.

Reproductive success (number of nests accepted)

The strongest influence on the number of nests accepted we found was the positive effect of the number of nests built, confirming results of previous studies on red bishops (Friedl & Klump 1999, Lawes et al. 2002, Metz et al. 2009). We also found a negative relationship between parasite levels and the number of nests accepted. This result is accordance with other studies showing that individuals displaying higher degrees of parasitisation often have a lower reproductive success. For example, in an experimental study on house martins (*Delichon urbica*), Marzal et al. (2005) determined that males treated against blood parasites had a significantly higher reproductive success than untreated males. In aquatic warblers (*Acrocephalus paludicola*), males with lower *Trypanosoma* infections had higher reproductive success than males with higher infection levels (Dyrzc et al. 2005). A similar negative relationship between intensities of parasite infections and male reproductive success has been reported for many other species (e.g. Ots & Hõrak 1998, Merino et al. 2000, MacDougall-Shackleton et al. 2002). The fact that the three significant two-way interactions all involved parasitemia also suggest that blood parasite infections influence male reproductive success and/or female mate choice decisions in red bishops.

Male attractiveness (residuals of nests accepted against nests built)

Male attractiveness (defined as the residuals of nests accepted against nests built calculated for the enlarged data set; see methods) was positively affected by the number of nests accepted. This result might indicate that males that were already successful in attracting mates to their territories were able to attract even more females via some form of positive feedback loop. One possible scenario that could result in such a pattern is female mate-choice copying. Other data indicating that mate choice copying in female red bishops might occur were reported by Friedl and Klump (2000), who investigated female settlement pattern in detail. They found that in two out of four study seasons females were more likely to accept nests in territories with at least one other breeding female as compared to territories with the same number of empty available nests but without another breeding female. However, female mate-choice copying in red bishops seems to be counteracted by an increased predation risk (at least in some years) for nests in territories containing other nests with nestlings (Friedl & Klump 2000). In addition, the linear relationship between number of nests built and number of nests accepted reported for red bishops by Friedl and Klump (1999) indicates that males with many nests do not have a disproportional higher reproductive success, which also argues against female mate-choice copying being an important component of the red bishop mating system. Clearly, more studies are needed to investigate whether and under what circumstances female mate choice copying is present in red bishops.

There was a significant and negative main effect of parasitism on male attractiveness, with attractiveness decreasing with increasing parasite loads, which might be explained similar to the negative main effect of parasitemia on the number of nest accepted discussed above.

Finally, attractiveness was influenced by the interaction between parasite load and the number of nests accepted. The attractiveness of males with less than the average number of nests accepted decreased with increasing parasite load, while the attractiveness of males with more than the average number of accepted nests increased with increasing parasite load. As argued before, we suggest that more successful males (i.e. males with a number of nests accepted above average) are also the qualitatively better males which can afford to invest more time in nest building and courtship display, because their immune system is strong enough to cope with an increased blood parasite burden. In contrast, less successful males with a number of nests accepted below average are probably also less fit males that have to trade off investment in reproduction against body maintenance and parasite defence.

Conclusion

Contrary to our expectations, we were not able to find a clear influence of plumage characteristics on male reproductive success in the red bishop. This contrasts with many other avian studies that investigated the relationship between the expression of carotenoid-based ornaments and male reproductive success, since most of these studies found that males displaying larger or more colourful carotenoid-based ornaments were preferred as mates and obtained higher reproductive success (for review see Hill 2006a). However, plumage brightness, but not plumage saturation, was positively related to reproductive effort, corroborating results from another study showing that plumage brightness seems to be a better indicator of male quality than plumage hue or chroma (Edler & Friedl, submitted). Our study also revealed no effect of testosterone levels on either reproductive effort or success. Finally we found strong effects of blood parasite loads, with the level of parasite infections being positively related to reproductive effort (reflecting allocation trade-offs) and negatively related to reproductive success. Overall, our results clearly do not lend support to the idea that the orange-red plumage areas of male red bishops play a role for female mate choice decisions. However, plumage colouration might have an intrasexual signalling function in the context of male-male competition for territories, but this idea has yet to be experimentally tested.

Acknowledgments

We would like to thank the entire staff at the Addo Elephant National Park for their continuous support during the course of the long-term project on red bishop breeding behaviour. We are grateful to the National Parks Board of South Africa and the Chief Directorate Environmental Affairs at the Department of Economic Affairs, Environment and Tourism in the Eastern Cape Province for the permission to carry out this study in the Addo Elephant National Park. Dr. Wolfgang Goymann and Ingrid Schwabl at the Max-Planck Institute for Ornithology and Dr. Martin Dehnhard and Marlies Rohleder at the Leibniz Institute for Zoo and Wildlife Research kindly determined testosterone levels, and Elisabeth Groscurth performed the real time PCR for the quantitative assessment of parasitemia as fast and reliably as always. Alice Edler was funded by a grant from the DAAD (Deutscher Akademischer Austauschdienst), and this study was further supported by the Deutsche Forschungsgemeinschaft (DFG FR 2096/2-2).

References

Andersson M. (1994) *Sexual selection*. Princeton: Princeton University Press.

Andersson S, Pryke SR, Örnborg J, Lawes MJ, Andersson M. (2002) Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. *The American Naturalist* **160:** 683 – 691.

Bennett PM, Owens IPF. (2002) *Evolutionary Ecology of Birds*. Oxford: Oxford University Press.

Brawner WR, Hill GE, Sundermann CA. (2000) Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. *The Auk* **117:** 952 – 963.

Campbell TW. (1995) *Avian Hematology and Cytology*. 2nd edition. Ames: Iowa State University Press.

Casagrande S, Csermely D, Pini E, Bertacce V, Tagliavini J. (2006) Skin carotenoid concentration correlates with male hunting skill and territory quality in the kestrel *Falco tinnunculus*. *Journal of Avian Biology* **37:** 190 – 196.

Cuthill IC, Bennett ATD, Partridge JC, Maier EJ. (1999) Plumage reflectance and the objective assessment of avian sexual dichromatism. *The American Naturalist* **160:** 183 – 200.

Craig AJFK. (1974) Reproductive behaviour of the male red bishop bird. *Ostrich* **45:** 149 – 160.

Dorak MT. (2006) *Real-time PCR*. New York: Taylor and Francis.

Dyrzc A, Wink M, Kruszewicz, Leisler B. (2005) Male reproductive success is correlated with blood parasite levels and body condition in the promiscuous aquatic warbler (*Acrocephalus paludicola*). *The Auk* **122:** 558 – 565.

Edler R, Klump GM, Friedl TWP. (2004) Do blood parasites affect reproductive performance in male red bishops (*Euplectes orix*)? A test of the Hamilton-Zuk hypothesis. *Ethology Ecology and Evolution* **16:** 315 – 328.

Evans MR, Goldsmith AR, Norris SRA. (2000) The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). *Behavioural Ecology and Sociobiology* **47:** 156 – 163.

Folstad I, Karter AJ. (1992) Parasites, bright males, and the immunocompetence handicap. *The American Naturalist* **139:** 603 – 622.

Folstad I, Hope AM, Karter A, Skorping A. (1994) Sexually selected color in male sticklebacks – a signal of both parasite exposure and parasite resistance. *Oikos* **69:** 511 – 515.

Friedl TWP. (2002) The effect of rainfall on the breeding behaviour of the red bishop, *Euplectes orix*. *Ostrich* **73:** 181 – 184.

Friedl TWP. (2004) Breeding behaviour of the red bishop (*Euplectes orix*): a synthesis and new observations. *Vogelwarte* **42:** 178 – 190.

Friedl TWP, Edler R. (2005) Stress-dependent trade-off between immunological condition and reproductive performance in the polygynous red bishop (*Euplectes orix*). *Evolutionary Ecology* **19:** 221 – 239.

Friedl TWP, Klump GM. (1999) Determinants of male mating success in the red bishop (*Euplectes orix*). *Behavioural Ecology and Sociobiology* **46:** 387 – 399.

Friedl TWP, Klump GM. (2000) Nest and mate choice in the red bishop (*Euplectes orix*): female settlement rules. *Behavioral Ecology* **11:** 378 – 386.

Garvin MC, Szell CC, Moore FR. (2006) Blood parasites of nearctic-neotropical migrant passerine birds during spring trans-gulf migration: impact on host body condition. *Journal of Parasitology* **92:** 990 – 996.

Goymann W, Geue D, Schwabl I, Flinks H, Schmidl D, Schwabl H, Gwinner E. (2006) Testosterone and corticosterone during the breeding cycle of equatorial and European stonechats (*Saxicola torquata axillaris* and *S. t. rubicola*). *Hormones & Behaviour* **50:** 779 – 785.

Grether G. (2000) Carotenoid limitation and mate preference in evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution* **54:** 1712 – 1724.

Grill CP, Rush VN. (2000) Analysing spectral data: comparison and application of two techniques. *Biological Journal of the Linnean Society* **69:** 121 – 138.

Hamilton WD, Zuk M. (1982) Heritable true fitness and bright birds: a role for parasites? *Science* **218:** 384 – 387.

Hill GE. (1992) The proximate basis of variation in carotenoid pigmentation in male house finches. *The Auk* **109:** 1 – 12.

Hill GE. (2000) Energetic constraints on expression of carotenoid-based plumage coloration. *Journal of Avian Biology* **31:** 559 – 566.

Hill GE. (2002) *A red bird in a brown bag: the function and evolution of colourful plumage in the house finch*. New York: Oxford University Press.

Hill GE. (2006a) Female mate choice for ornamental coloration. In: Hill GE, McGraw KJ, eds. *Bird Coloration. Volume II: Function and Evolution.* Cambridge: Harvard University Press, 137 – 200.

Hill GE. (2006b) Environmental regulation of ornamental coloration. In: Hill GE, McGraw KJ, eds. *Bird Coloration. Volume I: Mechanisms and Measurements.* Cambridge: Harvard University Press, 507 – 560.

Houde AE, Torio AJ. (1992) Effect of parasitic infection on male colour pattern and female choice in guppies. *Behavioral Ecology* **3:** 346 – 351.

Knowles SCL, Nakagawa S, Sheldon BC. (2009) Elevated reproductive effort increases blood parasitaemia and decreases immune function in birds: a meta-regression approach. *Functional Ecology* **23:** 405 – 415.

Lawes MJ, Slotow R, Andersson S. (2002) Male nest building but not display behaviour directly influences mating success in the polygynous red bishop (*Euplectes orix*). *Ostrich* **73:** 87 – 97.

Linville SU, Breitwisch R, Schilling AJ. (1998) Plumage brightness as an indicator of parental care in northern cardinals. *Animal Behaviour* **55:** 119 – 127.

Lozano GA. (1994) Carotenoids, parasites, and sexual selection. *Oikos* **70:** 309 – 311.

MacDougall-Shackleton EA, Derryberry EP, Hahn TP. (2002) Nonlocal male mountain white-crowned sparrows have lower paternity and higher parasite loads than males singing local dialect. *Behavioral Ecology* **13:** 682 – 689.

Maney DL, Davis AK, Goode CT, Reid A, Showalter C. (2008) Carotenoid-based plumage coloration predicts leukocyte parameters during the breeding season in northern cardinals (*Cardinalis cardinalis*). *Ethology* **114:** 369 – 380.

Marzal A, de Lope F, Navarro C, Møller AP. (2005) Malarial parasites decrease reproductive success: an experimental study in a passerine bird. *Oecologia* **142:** 541 – 545.

Maynard Smith J, Harper D. (2003) *Animal Signals*. New York: Oxford University Press.

Merino S, Moreno J, Sanz JJ, Arriero E. (2000) Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). *Proceedings of the Royal Society of London B* **267:** 2507 – 2510.

Metz M, Klump GM, Friedl TWP. (2009) Male nest-building behaviour and mating success in the red bishop (*Euplectes orix*). *Behaviour* **146:** 771 – 794.

Milinski M, Bakker TCM. (1990) Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* **344:** 330 – 333.

Montgomerie R. (2006) Analyzing colors. In: Hill GE, McGraw KJ, eds. *Bird Coloration. Volume I: Mechanisms and Measurements.* Cambridge: Harvard University Press: 90 – 147.

Olson VA, Owens IPF. (1998) Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology and Evolution* **13:** 510 – 514.

Ots I, Hõrak P. (1998) Health impact of blood parasites in breeding great tits. *Oecologia* **116:** 441 – 448.

Peer K, Robertson RJ, Kempenaers B. (2000) Reproductive anatomy and indices of quality in male tree swallows: the potential reproductive role of floaters. *The Auk* **117:** 74 – 81.

Préault M, Chastel O, Cézilly F, Faivre B. (2005) Male bill colour and age are associated with parental abilities and breeding performance in blackbirds. *Behavioural Ecology and Sociobiology* **58:** 497 – 505.

Pryke SR, Andersson S, Lawes MJ, Piper SE. (2002) Carotenoid status signalling in captive and wild red-collared widowbirds: independent effects of badge size and color. *Behavioral Ecology* **13:** 622 – 631.

Pryke SR, Andersson S. (2003a) Carotenoid-based status signalling in red-shouldered widowbirds (*Euplectes axillaris*): epaulet size and redness affect captive and territorial competition. *Behavioural Ecology and Sociobiology* **53:** 393 – 401.

Pryke SR, Andersson S. (2003b) Carotenoid-based epaulettes reveal male competitive ability: experiments with resident and floater red-shouldered widowbirds. *Animal Behaviour* **66:** 217 – 224.

Reudink MW, Studds CE, Marra PP, Kyser TK, Ratcliffe LM (2009) Plumage brightness predicts non-breeding season territory quality in a long-distance migratory songbird, the American redstart *Setophaga ruticilla*. *Journal of Avian Biology* **40:** 34 – 41.

Searcy WA, Nowicki S. (2005) *The Evolution of Animal Communication - Reliability and Deception in Signaling Systems*. Princeton: Princeton University Press.

Sergio F, Blas J, Hiraldo F. (2009) Predictors of floater status in a long-lived bird: a cross-sectional and longitudinal test of hypotheses. *Journal of Animal Ecology* **78:** 109 – 118.

Sheldon BC, Verhulst S. (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology and Evolution* **11:** 317 – 321.

Shutler D, Weatherhead PJ. (1991) Owner and floater red-winged blackbirds: determinants of status. *Behavioural Ecology and Sociobiology* **28:** 235 – 241.

Shykoff JA, Widmer A. (1996) Parasites and carotenoid based signal intensity: how general should the relationship be? *Naturwissenschaften* **83:** 113 – 121.

Sol D, Elie M, Marcoux M, Christovsky E, Porcher C, Lefebvre L. (2005) Ecological mechanisms of a resource polymorphism in Zenaida doves of Barbados. *Ecology* **86:** 2397 – 2407.

Tan TMC, Nelson JS, Ng HC, Ting RCY, Kara UAK. (1997) Direct PCR amplification and sequence analysis of extrachromosomal *Plasmodium* DNA from dried blood spots. *Acta Tropica* **68:** 105 – 114.

von Schantz T, Bensch S, Grahn M, Hasselquist D, Wittzell H. (1999) Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society of London B* **266:** 1 – 12.

CHAPTER 3

Plumage colouration, age, testosterone and dominance in male red bishops (*Euplectes orix*): a laboratory experiment

Submitted as:
Edler AU, Friedl TWP. “Plumage colouration, age, testosterone and dominance in male red bishops (Euplectes orix): a laboratory experiment”

Abstract

Visual signals such as plumage characteristics in birds often play a key role in the establishment of dominance hierarchies by acting as a badge of status that can be used to assess individual fighting ability. We studied the role of plumage colouration in males of the red bishop (*Euplectes orix*), a sexually dimorphic and polygynous weaverbird species occurring in Sub-Saharan Africa and breeding in dense colonies around water. Males are highly territorial and often engage in competition over limited resources such as breeding sites and potential mates. By experimentally staging male-male contests over a limited food source, we wanted to determine if the orange-red breeding plumage in this species serves as a dominance signal between individuals, with males with redder plumage being dominant over those with duller plumage. In the first set of experiments, we staged contests between unfamiliar and unmanipulated males. The setup of the second set of experiments was identical to the first, with the exception that those males with the lowest chroma and hue values had their plumage experimentally reddened within the range of the natural variation. In addition to plumage colouration, we incorporated testosterone levels, body condition and age into the analysis of factors contributing to contest outcome. Our results show a consistent and strong age effect in both sets of experiments, which seems to be independent of plumage colouration, testosterone and body condition. This suggests that in the red bishop the outcome of male-male competitions over limited resources is determined by age-related acquired experience rather than by status signalling through plumage colouration.

Introduction

Visual signals in terms of colourful body parts play a vital role in both inter- and intraspecific communication in many species (e.g. Bradbury & Vehrencamp 1998, Espmark et al. 2000, Searcy & Nowicki 2005). Special attention has been paid to the striking yellow, orange and bright red colouration of diverse ornamental traits which is achieved by depositing carotenoid pigments in the respective appendages, such as skin (cichlids: Evans & Norris 1996; sticklebacks: Bakker & Sevenster 1983), combs and wattles (red jungle fowl: Zuk et al. 1990; black grouse: Siitari et al. 2007) or feathers (malachite sunbirds: Evans & Hatchwell 1992; red-shouldered widowbirds: Pryke & Andersson 2003). Since carotenoids cannot be synthesized by organisms, they must be ingested as an essential part of an individual's diet (Goodwin 1986, Lozano 1994) and metabolised accordingly, as they are not only used in signalling individual quality to prospective mates or rivals (von Schantz et al*.* 1999, Senar & Escobar 2002, Ferns & Hinsley 2008), but also have vital functions as antioxidants and immune system enhancers (Krinsky 1994).

Carotenoid-based plumage colouration is a very conspicuous trait in males of many bird species. Many studies have shown that the expression of these colours is linked to various aspects of a male's quality, ranging from immunocompetence and parasitisation (Folstad & Karter 1992, Figuerola et al. 1999, Hõrak et al. 2001, Saks et al. 2003, Maney et al. 2008) to testosterone levels (Evans et al. 2000, Blas et al. 2006, McGraw et al. 2006, Peters 2007), indicating that carotenoids can play a vital role in signalling. Indeed, it has been demonstrated in a large number of birds where males display carotenoid-based plumage colouration, that females use this male ornamentation as a visual cue for mate assessment (review by Hill 1999). However, considerably less attention has been given to the potential role of plumage ornamentation in male-male interactions, and the question if males use carotenoid-based signals to assess one another's fighting ability.

These so-called "badges of status" were first ascribed by Rohwer's Status Signalling Hypothesis (SSH; Rohwer 1975) to melanin-based ornaments and most likely evolved as a means to settle conflicts over limited resources without costly fighting with the help of easily assessable colour markers. According to this hypothesis, these status signals reduce the amount of aggressive interactions, as birds with unequal fighting ability could assess the rival's dominance status, without having to actually fight over the resource and so risk injury or energy depletion. A potential problem arising in this system is the strategy of cheaters, where birds of low status manage to signal high dominance. However, these strategies do not last long, as cheaters are not able to counteract the high level of aggression when actively challenged by dominant males, as these melanin markers may have been cheap to produce but are costly to maintain - the signalling system is socially mediated (Andersson 1994, Senar 1999, Parker & Ligon 2002).

However, carotenoid-based signalling is different to melanin-based signalling, as the expression of red or orange plumage colouration is more or less irrevocably linked to individual condition (Honest Advertisement Hypothesis; Zahavi 1975, 1977, Kodrick-Brown & Brown 1984). This means that cheating here is not possible, as carotenoid-based colouration is costly to produce in the first place (Olson & Owens 1998, Pryke & Andersson 2003, McGraw et al. 2005), allowing only those individuals to display brilliant plumage that are in the best condition. Therefore, those males with the highest level of ornamentation should be dominant over duller males as they are in better nutritional condition, leading to the conclusion that carotenoid-based plumage colouration is especially suitable to be used as a status signal. But more recent work has shown that plumage colouration *per se* is not only determined by condition and not only signals individual quality, but can also be influenced by other factors such as age and testosterone levels. These factors in turn can deliver cues on competitive ability to potential opponents – older males could potentially be more brilliant in colour (Delhey & Kempenaers 2006, Bitton & Dawson 2008, Ferns & Hinsley 2008), which would signal age and therefore experience in dominance behaviours (e.g., Sundberg 1995), while testosterone not only mediates the level of expressed aggression (Wingfield et al. 2001, Soma 2006), but also enhances ornamental traits (Gonzalez et al. 2001, Duckworth et al. 2004). In any case, regardless of whether dominance in agonistic interactions is achieved by older age, superior body condition, better fighting abilities, higher testosterone levels, or a combination of these factors, a signalling mechanism that allows competing males to assess one another's competitive ability clearly would be advantageous, since it could potentially reduce engagement in costly fighting (Rohwer 1982, Senar 1999).

Such a signalling mechanism involving badges of status is expected to be particularly important in birds with a polygynous mating system and small territories, since such a mating pattern is linked to frequent and strong male-male competition for limited resources such as territories and mates. In this study, we want to determine if carotenoid-based plumage colouration functions as a status signal in the red bishop (*Euplectes orix*), a colonial breeding highly polygynous weaverbird, where colourful males in a brilliant red and black breeding plumage compete against each other for limited territory space in reedbeds or bulrush stands around water. To study the effect of ornamental traits in the context of male-male competition, we staged dyadic contests between unfamiliar males over a limited food resource in a controlled laboratory setup. In addition to investigating the role of plumage colouration on the outcome of these contests, we tested for potential effects of age, testosterone levels and individual body condition to elucidate how these factors might be intercorrelated and what factor is the most important one in determining the outcome of male-male interactions in red bishops. Age, in particular, has rarely been incorporated in the analysis of experimentally staged male-male contests despite its potential importance in determining rank and dominance hierarchies among males.

Material and methods

Study species

Our study was conducted on red bishop (*Euplectes orix*) males. The red bishop is a polygynous and sexually dimorph weaverbird occurring in Sub-Saharan Africa, with males displaying a brilliant red-orange and black breeding plumage, while females and juveniles are a buffy brown. Red bishops are fairly abundant colonial breeders and can be found in reed beds consisting mainly of bulrush (*Typha capensis*) and the common reed (*Phragmites spec.*) around water. Males are highly territorial, actively defending their territories against intruders while simultaneously trying to attract as many females as possible. During their territory tenure, territorial males try to build as many nests as possible, as the number of nests built not only determines male reproductive success (Friedl & Klump 1999, Lawes et al. 2002), but is also a reliable indicator of individual quality (Friedl and Klump 1999). Females inspect and accept built nests, as well as initiating copulations. Once females have chosen a mate, they copulate several times with the territory holder and then begin lining nests. Eggs (normally two to four per clutch) are laid soon after copulation and females are solely responsible for incubation and raising of young (Friedl 2004a). Females display aggressive behaviour towards other females inspecting occupied nests as a prevention of intraspecific brood parasitism (Friedl 2004a, 2004b). During aggressive encounters with other individuals, males display a variety of behaviours. So-called “supplant chases” are directed at intruders into the territory and are the most frequent display of aggression. At territory boundaries, neighbouring males are often seen threatening one another, which involves the flicking of the tail as well as stretching the neck, raising the feathers around the head and pointing with the bill towards the opponent, thereby prominently displaying the orange-red colouration of both head and throat (Craig 1974, Friedl 2004a). In this situation, the dominance hierarchy is often unclear and is settled via threats and even occasional fighting. Threats can also be observed at feeding grounds, where males will rush at one another with raised head feathers, though sometimes an undirected fluffing of head feathers can be enough to drive subordinate individuals away from the immediate vicinity of the feeding male. Between individuals, subordinate status is signalled with sleeked plumage (Craig 1974, Friedl 2004a).

Housing conditions

The birds were kept in two aviaries at the University of Oldenburg, Germany (53° 9’ N, 8° 13’ E). In the summer 2007, when the first part of the experiment was conducted (see below), the two aviaries housed a total of 46 birds (aviary 1: 7 males and 18 females; aviary 2: 7 males and 14 females), while in 2008, the aviaries contained a total of 42 birds (aviary 1: 6 males and 16 females; aviary 2: 6 males and 13 females). Some of the birds were caught in South Africa in 1994 or 2000 as adults of unknown age, while all the other individuals hatched in the aviary and, consequently, their exact age was known. All birds were individually marked with a numbered metal ring and a unique colour combination of four plastic rings. The two aviaries consisted of four and six compartments each (width x length x height: 1.0m x 2.0m x 2.0m), which were connected via holes (0.2m x 0.2m). The aviaries were both located in the middle part of a glass greenhouse and separated by a lane of approximately 2m in width, resulting in social but not visual isolation of the two groups of birds. Each compartment contained 3 horizontal wooden perches, which were positioned in the front, middle and back, as well as four sets of vertical bamboo scaffolding (each consisting of a set of three poles) located in each of the four corners of a compartment, which could be used for nest building during the breeding season. Food and water dispensers were provided in each compartment, giving all individuals *ad libitum* access to both. The birds were kept under natural light conditions at all times.

Body condition index and age

For both contest years, tarsus length was measured once before contests began, while male body weight was measured on a laboratory precision balance (Pesola precision scale, Pesola AG, Switzerland) to the nearest 1.0g before every contest. The body condition index was then calculated as the residuals from the linear regression of body weight on tarsus length.

As we only know the exact age of those males that hatched in the aviary and can only give a minimum known age for those males caught in the field, we divided all males into two age categories – young and old. In 2007, all males six years of age and younger (range 4 to 6 years) were classified as “young” (*N* = 7), while the remaining seven males which were at least nine years of age (minimum age ranged from 9 to 15 years) were classified as “old”. Accordingly, in 2008 those males seven years of age and younger (*N* = 6; range 5 to 7 years) were classified as “young” and those at least ten years old (*N* = 6; minimum age ranged from 10 to 16 years) were classified as “old”. Thus, in both contest years males categorized as young were at least three years younger than males categorized as old.

Reflectance measurements

Reflectance was measured using an Ocean Optics USB2000 spectrometer (Ocean Optics Inc., Dunedin, Florida, USA), a GetLight-Hal-S tungsten halogen light source (getSpec.com, Germany) and a fibre optic reflectance probe, which was fitted with a self-made distance holder, in order to standardise the distance between probe and sample and to reduce noise from surrounding light. The probe was held perpendicular to the sample and three consecutive scans were taken from approximately the centre of each measured area, between which the probe was removed and replaced. The measured areas were the throat and head of each individual, as these areas are displayed during aggressive interactions between males (see “Study species”). Reflectance was measured using OOIBase32 software (<1nm resolution; Ocean Optics Inc., Dunedin, Florida, USA) and in relation to a white (WS-2 white reflective tile, 98% reflectance; getspec, Germany), as well as a dark standard (self-made “dark-box”).

After we tested for UV reflectance using a halogen-deuterium light source (AvaLight-DHS, getSpec.com, Germany) and determined that red bishops do not reflect in the range of 300nm to 400nm, we calculated the three different colourimetrics average brightness, chroma and hue based on the raw reflectance data obtained for the range between 400nm and 700nm. Brightness (spectral intensity) is a measure of the total reflectance coming from the sample and is the sum of all reflectances over the whole spectrum of wavelengths measured (R_{400} – R_{700}). As spectrometers do not always have the same resolution, we then calculated average brightness as the total reflectance over all wavelengths, divided by the number of measured data points (Delhey et al. 2005, Siefferman & Hill 2005) to enable comparisons of our plumage brightness data with those of other studies. Hue (spectral location), which is defined as “colour”, indicates which wavelengths contribute most to the total reflectance (sum of reflectance over all wavelengths) and is calculated as λR_{50}, which is the wavelength at which reflectance is halfway between its minimum (R_{min}) and maximum (R_{max}) (Pryke et al. 2001, Smiseth et al. 2001, Andersson et al. 2002). Chroma (spectral purity) measures how much of the reflectance actually comes from the region of interest and so, in our case, is calculated as the sum of reflectance in the orange-to-red part of the light spectrum ($R_{590} – R_{700}$) divided by total reflectance ($R_{400} – R_{700}$) (Shawkey et al. 2003, Peters et al. 2004, Siefferman & Hill 2005). Based on the three consecutive measurements per area, we calculated an average value for average brightness, chroma and hue for each of the two areas (head and throat), which again was averaged into a mean value over these two areas for each individual, resulting in "total body" values for each individual. Repeatability of the three consecutive measurements of the head and throat in 2007 was calculated according to Lessels and Boag (1987). Although the estimates were moderate, they were highly significant for all plumage variables of both the head (average brightness: $R = 0.9$, $F_{41} = 104.102$, $P < 0.001$; chroma: $R = 0.6$, $F_{41} = 25.806$, $P < 0.001$; hue: $R = 0.4$, $F_{41} = 11.505$, $P < 0.001$) and the throat (average brightness: $R = 0.8$, $F_{41} = 72.187$, $P < 0.001$; chroma: $R = 0.5$, $F_{41} = 16.942$, $P < 0.001$; hue: $R = 0.5$, $F_{41} = 13.706$, $P < 0.001$). The fact that we did not measure the same spot three times but different areas of the head and throat, explains the moderate repeatability values, therefore combining measurement errors and spatial variability in colouration of individual plumage patches (see Delhey & Kempenaers 2006 for similar results).

Testosterone levels

Blood samples were obtained by puncturing the brachial vein with a sterile needle. Per male, we collected approximately 20µl to 60µl of blood. The samples were then centrifuged for the extraction of serum which was stored in a freezer until testosterone levels were analysed by radio-immuno-assays (see below). Samples for analysis of testosterone levels were taken twice in the first contest year (2007), once before males started moulting into breeding plumage (February 2007) and again, once moult was completed by all males (May 2007). In 2008, serum samples were taken three times – once again before moult into nuptial plumage (January 2008), after completion of moult (May 2008) and a third time approximately a week before contests began (June 2008).

Testosterone was measured by an enzyme immuno-assay with a double-antibody technique. For testosterone estimation in blood, 0.01ml to 0.05ml of serum was extracted with 2ml of butyl t-methyl ether:petroleum ether (30:70, v/v) for 30 minutes. The samples were frozen, and the fluid petroleum ether phase was removed and evaporated at 55°C. The steroids were dissolved in 0.1ml of 40% (v/v) methanol, and duplicates of 20µl each were analyzed.

The assay used a polyclonal antibody raised in rabbits against testosterone-11-hemisuccinate-BSA, and the label was testosterone-3-carboxymethyl-oxime-horse radish peroxidase. The testosterone standard curve ranged from 0.4pg per 20µl to 50pg per 20µl, and the cross-reactivity with testosterone was 100%, with 5a-dihydrotestosterone 10%, with androstenedione 2%, with estradiol 0.1%, and with progesterone 0.1%.

The results are given in nanograms of testosterone per milliliter of serum. Serial dilutions of a plasma pool from red bishops gave parallelism to the standard testosterone with no differences in slopes ($P > 0.05$).The intra- and inter-assay coefficients of variation (CVs) were 8.9% and 12.3%, respectively.

Male-male contests

In order to test if plumage colouration influences the outcome of male-male competitions, we staged two separate experimental blocks of dyadic contests, in which every male from one aviary was paired with every male from the other aviary. The contests (2007: 49 in total; 2008: 36 in total) took place between 7 and 11 a.m. every other day between May 25th and June 7th in 2007 and from July 8th to July 18th in 2008. Males were removed from the main aviaries approximately 15 hours before the contests began and placed in individual double cages (82cm x 40cm x 30cm), with competing males on either side of a removable sliding door. The cages consisted of wood and had a wire-mesh floor. Each double cage contained four perches, one in each corner and one on each side of the feeder. In order to standardize motivation, males were deprived of food but provided with water *ad libitum*. When contests began, the partition was removed and a feeder containing 20 mealworms was placed in the middle of the cage. If mealworms were dropped, they fell through the wire mesh floor and became unavailable for both males for the remainder of the dyadic contest. All trials lasted 25 minutes and the males were returned to their respective aviaries afterwards. In the first experimental block (season 2007, *N* = 14 males), dyadic trials were conducted with unmanipulated males only. For this block we used only one set of values for average brightness, chroma and hue per bird for the entire contest period of two weeks (measured and calculated shortly before trial begin), as plumage values do not change over periods as short as two weeks (unpublished data). The second experimental block was conducted in the following year (2008, *N* = 12 males), and trials were carried out with one half of the males having had their plumage manipulated (see below). By conducting the two experimental blocks in two separate breeding seasons, we ensured that males competing against one another in the second experimental block had no memory of outcomes of previous male-male contests against the same opponents in the first experimental block, which could potentially affect contest outcome (Wiley et al. 1999, Hsu et al. 2006). All parts of the experiment were approved by the appropriate authorities (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit: 33.9-42502-04-07/1312).

Manipulations

In the second experimental block conducted in 2008, males (*N* = 12 males; two males had died in the course of the year) were divided into two groups according to chroma values. Those six males with the lowest chroma values had those orange-red plumage areas manipulated which are displayed in male-male interactions (head and throat), while the other six were used as control males. To increase chroma and hue, males subjected to manipulation were treated with R27 (Copic marker, Too Marker Products, Japan), a marker which corresponds closely to the natural colour variation found in the field (see Fig. 1). Control males were treated with a transparent pen (colourless blender, code "0"). While we used one value of average brightness, chroma and hue for each bird in the first experimental block in which all males were unmanipulated (see above), we had separate plumage values for each contest for each individual in the second experimental block, as, in contrast to natural plumage coloration, the colours of experimentally manipulated plumage are more likely to fade within two weeks.

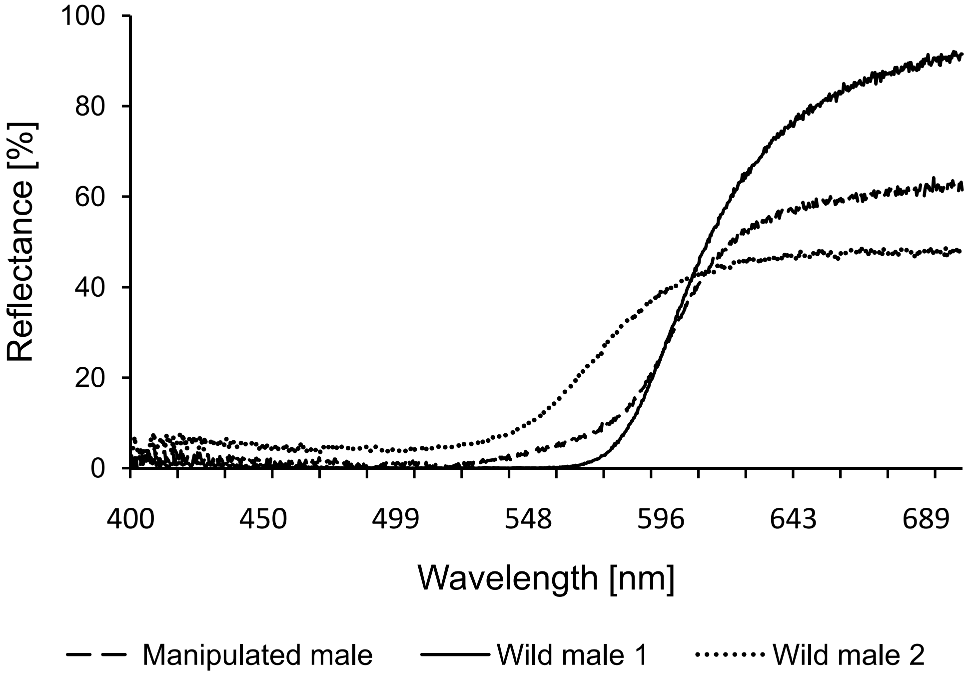

Figure 1: Reflectance spectra of two red bishop males found in the wild (solid line shows upper extreme of plumage colouration in the field; dotted line shows an example of the lower end of plumage variation) in comparison to a manipulated male (dashed line) in the aviary. The manipulation was applied with a red Copic marker selected to correspond to the colour variation found in the field.

In order to determine if manipulations were fading, reflectance was measured before each contest and manipulations were reapplied every six days, when the average value for chroma decreased by 0.1 or mean average brightness increased by a minimum of 2.0. Repeatability (Lessels & Boag 1987) for the six measurements performed for each male over the 12 day trial period (using mean values of head and throat combined) was again moderate but highly significant (average brightness: R = 0.6, F_{71} = 24.491, $P < 0.001$; chroma: R = 0.4, F_{71} = 11.712, $P < 0.001$; hue: R = 0.9, F_{71} = 123.186, $P < 0.001$).

Video analysis

All dyadic contests were recorded on video (Sony HandyCam DCR HC23E, Japan), to ensure no disturbance through observer presence. Each video was analysed twice, once for each competing male in the dyad. The analysis was conducted with the help of an Excel macro (Dr. Ralf Edler), which was programmed to run for the entire duration of the contest (25 minutes). During this time, all behaviours displayed by the competing individuals were recorded. As measures of dominance we used (1) the number of times an individual threatened his opponent; (2) the number of interactions an individual won in comparison to his opponent (“wins – losses”); (3) the time in seconds each male spent at the feeder (defined as the time in seconds that an individual spent on one of the two perches to the left and right of the feeder, thereby denying access to food for the opponent); and (4) the number of worms eaten. Furthermore, in all competitions containing threats, all males were classified as either “winner” or “loser” of each trial depending on which male had won the majority of interactions regardless of the amount of interactions that took place during the dyadic contest (binary response variable “win/lose”). In cases where both males had won an equal number of interactions, the trial was categorised as a draw and excluded from the analysis.

Statistical analysis

Both contest years were analysed using General Linear Mixed Models (GLMM) with the plumage variables, age, testosterone and body condition index as main effects. As every male from one aviary competed against every male from the second aviary, but never more than once against the same male in the same year, we included male ID as a random factor in both contest analyses. To analyse the binary win/lose response, we used a Generalised Linear Model (GLM) with a binomial distribution and logit link function, which also allowed us to incorporate male ID as random factor in both years. Our plumage data did not differ significantly from a normal distribution, but our behavioural values did. Therefore, we used parametric or non-parametric tests respectively for comparisons between groups of males. All statistical tests were performed with SPSS 15.0, and all given significances are two-tailed.

Results

Plumage reflectance

A comparison of plumage colouration between aviary (*N* = 26, plumage data pooled over both contest seasons with 14 males in 2007 and 12 in 2008) and wild males (*N* = 58, with multiple measurements from several males, recaptured over a total of 3 seasons) showed that in the aviary, males were on average brighter (aviary: 40.161 ± 18.291; wild: 24.462 ± 7.629; Mann-Whitney-U test; *Z* = -4.967, *P* < 0.001), but had significantly lower chroma (aviary: 0.661 ± 0.09; wild: 0.873 ± 0.113; *Z* = -6.668, *P* < 0.001) and hue (aviary: 547.47 ± 17.394; wild: 583.01 ± 7.186; *Z* = -7.229, *P* < 0.001) values than male red bishops in the wild.

When analysing male plumage data obtained in 2007 (*N* = 14 males), we found that chroma and hue correlated significantly positively with one another (*r* = 0.823, *P* < 0.001), while both correlated negatively with average brightness (chroma: *r* = -0.887, *P* < 0.001; hue: *r* = -0.600, *P* = 0.023). Plumage values in 2008 measured before the manipulation (*N* = 12 males) showed a similar pattern, though average brightness did not correlate significantly with hue (*r* = -0.356, *P* = 0.283). However, there was a significant negative correlation between average brightness and chroma (*r* = -0.659, *P* = 0.027) and a highly significant positive correlation between chroma and hue (*r* = 0.883, *P* < 0.001). After we manipulated plumage, there was a significant positive correlation between chroma and hue (*r* = 0.933, *P* < 0.001) and significantly negative correlations between average brightness and both chroma (*r* = -0.606, *P* = 0.037) and hue (*r* = -0.766, *P* = 0.004) when including all males (*N* = 12) in the analysis. A correlation of mean plumage values for males manipulated with the R27 marker only (*N* = 6) showed a significant relationship between chroma and hue (*r* = 0.905, *P* = 0.013), but not between average brightness and chroma or hue.

A pair-wise comparison of plumage variables between 2007 and 2008 (measurements taken before manipulation) showed no significant difference between years for individual males (*N* = 12 males) with respect to chroma and hue (Table 1). There was, however, a difference in average brightness (paired t-test, *t* = 4.485, *P* = 0.001), with individual males being significantly brighter in 2007 than in 2008 (Table 1).

Table 1: Descriptive statistics for average brightness, chroma, and hue (mean ± SD) for 12 red bishop males in breeding plumage measured in 2007 and 2008 (before plumage manipulations). *P*-values refer to paired t-tests for differences between years.

	2007	2008	Comparison	
	Mean ± SD	*Mean ± SD*	*t*	*p*
Average Brightness	51.565 ± 18.091	26.944 ± 3.868	4.485	0.001
Chroma	0.656 ± 0.925	0.668 ± 0.962	-0.341	0.740
Hue [nm]	548.04 ± 16.825	547.71 ± 19.976	0.049	0.962

Young and old males did not differ with respect to plumage characteristics in either 2007 (average brightness: *t* = -0.937, *P* = 0.367; chroma: *t* = 0.018, *P* = 0.986; hue: *t* = -1.595, *P* = 0.137), before manipulation in 2008 (average brightness: *t* = 1.910, *P* = 0.085; chroma: *t* = -0.006, *P* = 0.996; hue: *t* = 0.111, *P* = 0.914) or after manipulation in 2008 (average brightness: *t* = -0.344, *P* = 0.738; chroma: *t* = 0.038, *P* = 0.970; hue: *t* = 0.663, *P* = 0.523).

The manipulation of plumage in 2008 significantly altered the appearance of males (*N* = 6 males), increasing chroma (paired t-test; *t* = -9.701, *P* < 0.001) and hue (*t* = -7.918, *P* = 0.001; see Figure 2), while decreasing average brightness (*t* = 9.215, *P* < 0.001). In control males treated with the colourless blender, plumage remained unaltered (*N* = 6 males; paired t-test; average brightness: *t* = 1.429, *P* = 0.212; chroma: *t* = -1.888, *P* = 0.118; hue: *t* = -1.050, *P* = 0.342; see Fig. 2).

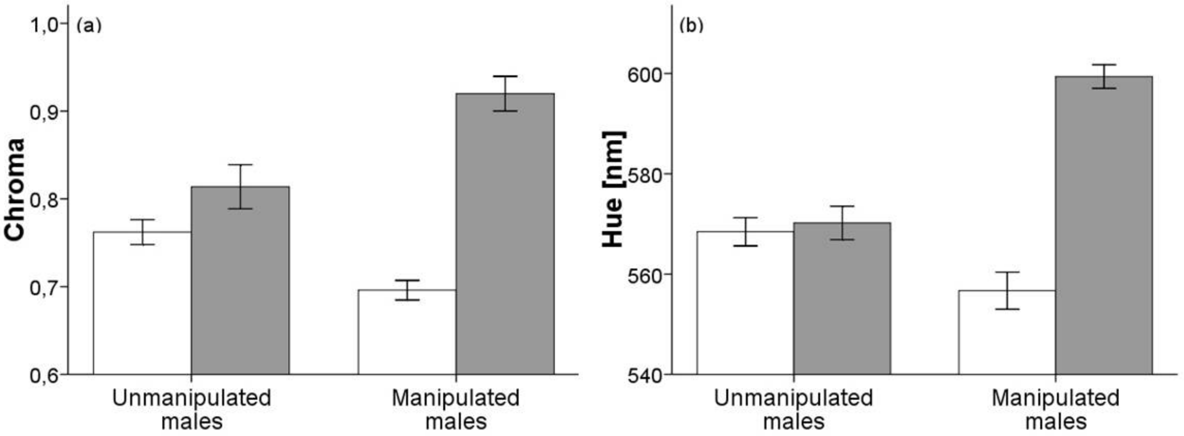

Figure 2: Mean (± SE) values of (a) chroma and (b) hue in control and manipulated red bishop males before (white bars) and after (grey bars) plumage manipulation in 2008.

Testosterone levels

Overall, testosterone levels before moult tended to be higher in 2008 compared to 2007, and testosterone levels after moult were significantly or almost significantly higher in 2007 compared to 2008. The results were essentially the same regardless of whether we compared all males (14 males in 2007 and 12 males in 2008) with an independent sample t-test (before moult: *t* = -1.444, *P* = 0.179; after moult: *t* = 2.038, *P* = 0.054) or whether we performed a pair-wise t-test with the 12 males that were present in both years (before moult: *t* = -1.496, *P* = 0.165; after moult: *t* = 3.335, *P* = 0.009; see also Table 2).

Table 2: Testosterone levels [ng/ml] of red bishop males at different times during the breeding cycle for 2007 (*N* = 14) and 2008 (*N* = 12).

	2007 *Mean ± SD*	2008 *Mean ± SD*
Before moult	0.079 ± 0.049	0.292 ± 0.488
After moult	0.983 ± 0.589	0.576 ± 0.250
Before contests	-	0.435 ± 0.481

In 2007 there was no significant correlation of testosterone levels of individual males measured before (February) and after (May) moult (*N* = 14; *r* = 0.084, *P* = 0.785). Likewise, in 2008, there were no significant relationships between testosterone levels before moult (January), shortly after moult (May), and just before contests (June) (*N* = 12 for all correlations; before moult and after moult: *r* = 0.027, *P* = 0.946; before moult and before contests: *r* = 0.038, *P* = 0.912; after moult and before contests: *r* = 0.571, *P* = 0.085). Thus, males with high testosterone levels at a certain time in the breeding cycle do not necessarily have a high testosterone level at other times within the same breeding season (as would be indicated by positive correlations); nor is there any evidence for a trade-off of testosterone levels between different times within a season (as would be indicated by negative correlations).

A comparison between individual levels before and after moult showed that in 2007, the testosterone levels were significantly higher after moult than before moult (paired t-test; *t* = -5.581, *P* < 0.001), while in 2008 individual testosterone levels did not differ significantly among the three sampling times (before moult, shortly after moult, and before start of the contests (one-way repeated measures ANOVA; *F* = 0.635, *P* = 0.543).

A comparison of testosterone levels between the two age categories (see Fig. 3) revealed that in 2007 young males had significantly higher testosterone levels after moult than older males (*N* = 7 per age category; t-test; *t* = 2.873, *P* = 0.015), while there were no significant differences between the two age groups before moult (*t* = -0.846, *P* = 0.414). In 2008 (*N* = 6 per age category), there were no significant differences in testosterone levels between young and old males after moult (*t* = 0.993, *P* = 0.350) and before contests (*t* = 0.827, *P* = 0.427), but there was a tendency for older males to have higher testosterone levels than younger males before moult (*t* = -1.594, *P* = 0.172).

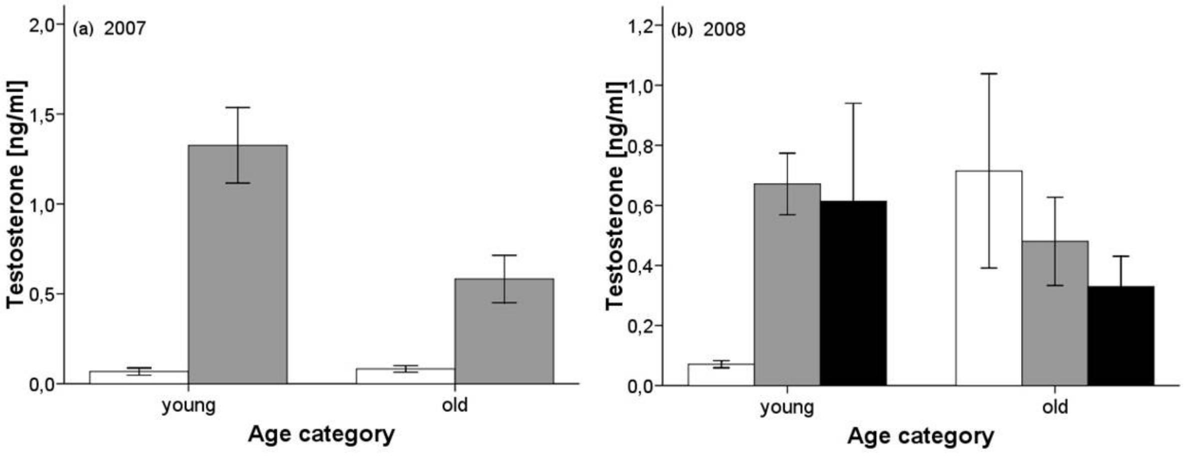

Figure 3: Mean (± SE) testosterone levels [ng/ml] of young and old red bishop males before moult (white bars), after moult (grey bars) and just before contests (black bars) for (a) 2007 and (b) 2008.

Relationships between measures of dominance

For the contests conducted in 2007, there were significant positive correlations between the dominance measure "wins minus losses" and the amount of threats (Spearman correlation; r_s = 0.636, *P* < 0.001), the amount of worms eaten (r_s = 0.304, *P* = 0.002), and the time spent at the feeder (r_s = 0.228, *P* = 0.024). The amount of times an individual threatened his counterpart was positively correlated to the amount of times the interaction was subsequently won (r_s = 0.440, *P* < 0.001), which by itself was positively correlated to the time spent at the feeder (r_s = 0.360, *P* = 0.029) and the amount of worms eaten (r_s = 0.522, *P* = 0.001). Finally, the amount

of time spent at the feeder and the amount of worms eaten were also significantly correlated with each other ($r_s = 0.744$, $P < 0.001$).
For the 2008 contests, there was essentially the same pattern, with all correlations listed above also being highly significant (all $r_s > 0.35$, all $P < 0.01$).

Contests 2007

The GLMM for the 2007 contests showed significant effects of age (Parameter estimate = -2.723 ± 0.890, $F_{1,58} = 9.366$, $P = 0.003$), hue (Parameter estimate = -0.131 ± 0.056, $F_{1,58} = 5.544$, $P = 0.022$) and chroma (Parameter estimate = 27.461 ± 13.150, $F_{1,58} = 4.361$, $P = 0.041$) on the amount of “wins minus losses”. Overall, age had the strongest effect, with older males winning more interactions, while the positive effect of chroma and the somewhat contradictory negative effect of hue were weak and barely significant. In addition, there was a tendency for body condition index to influence the amount of “wins minus losses” (Parameter estimate = -0.217 ± 0.120, $F_{1,58} = 3.288$, $P = 0.075$), with males in worse condition winning more interactions. Age also had a tendency to influence the time spent at the feeder (Parameter estimate = -456.443 ± 226.805, $F_{1,7} = 4.050$, $P = 0.086$) and the amount of worms eaten (Parameter estimate = -5.872 ± 2.756, $F_{1,7} = 4.540$, $P = 0.072$), with older males spending more time at the feeder and eating more worms than younger ones (Figure 4). No other significant effects or tendencies were found in this analysis.

Using a Generalised Linear Model with “win/lose” as a binary response, we found that age, hue and testosterone had a significant effect on the dependent variable, with older males, as well as those with higher testosterone levels being more likely to win. Hue had a negative effect on winning probability, showing that duller males were more likely to win than more colourful counterparts when the influence of all the other variables was controlled for (see Table 3).

Contests 2008

The 2008 GLMMs showed a trend for older males eating more worms than younger males (Parameter estimate = -5.669 ± 2.570, $F_{1,8} = 4.868$, $P = 0.057$), while there was no age effect on “wins minus losses” ($F_{1,8} = 1.313$, $P = 0.285$), the amount of threats ($F_{1,7} = 0.321$, $P = 0.590$) or the time spent at the central feeding bowl ($F_{1,8} = 0.726$, $P = 0.419$). There was also no evidence of any of the plumage variables (average brightness, chroma and hue), testosterone or body condition index having a significant effect on measures of dominance.

The Generalised Linear Model for the 2008 contests showed significant effects of age, body condition index, average brightness and hue on the binary response "win/lose" (see Table 3 and Figure 4).

Table 3: Results of a Generalized linear mixed model with the winning probability (binary response "win/lose") as dependent variable for both the 2007 and 2008 contests. Represented are the corresponding B values with their standard errors, as well as Wald X^2 and the significance of the effect.

		B (± SE)	*Wald X^2*	*P*
2007	Intercept	87.927 ± 22.504	15.266	< 0.001
	Age	-11.002 ± 3.236	11.560	0.001
	Average brightness	-0.091 ± 0.092	0.979	0.322
	Chroma	0.893 ± 15.556	0.003	0.954
	Hue	-0.152 ± 0.045	11.589	0.001
	Testosterone [ng/ml]	0.620 ± 0.266	5.453	0.020
	Body Condition Index	-0.493 ± 0.341	2.095	0.148
2008	Intercept	-108.253 ± 35.079	9.523	0.002
	Age	-3.671 ± 1.711	4.604	0.032
	Average brightness	0.325 ± 0.133	6.010	0.014
	Chroma	13.046 ± 12.151	1.153	0.283
	Hue	0.151 ± 0.064	5.535	0.019
	Testosterone [ng/ml]	0.293 ± 0.188	2.427	0.119
	Body Condition Index	-0.613 ± 0.296	4.290	0.038

The only result consistent with 2007 is the positive effect of age, with older males having a higher probability of winning a contest. The negative effect of body condition index on the probability of winning is in line with the negative effect of body condition on "wins minus losses" as found in 2007 (see above). However, the positive effect of hue on the binary response variable “win/lose” with redder males having a higher probability of winning contradicts the negative effect of hue found in 2007 (see above), while the positive effect of brightness found in 2008 was absent in 2007.

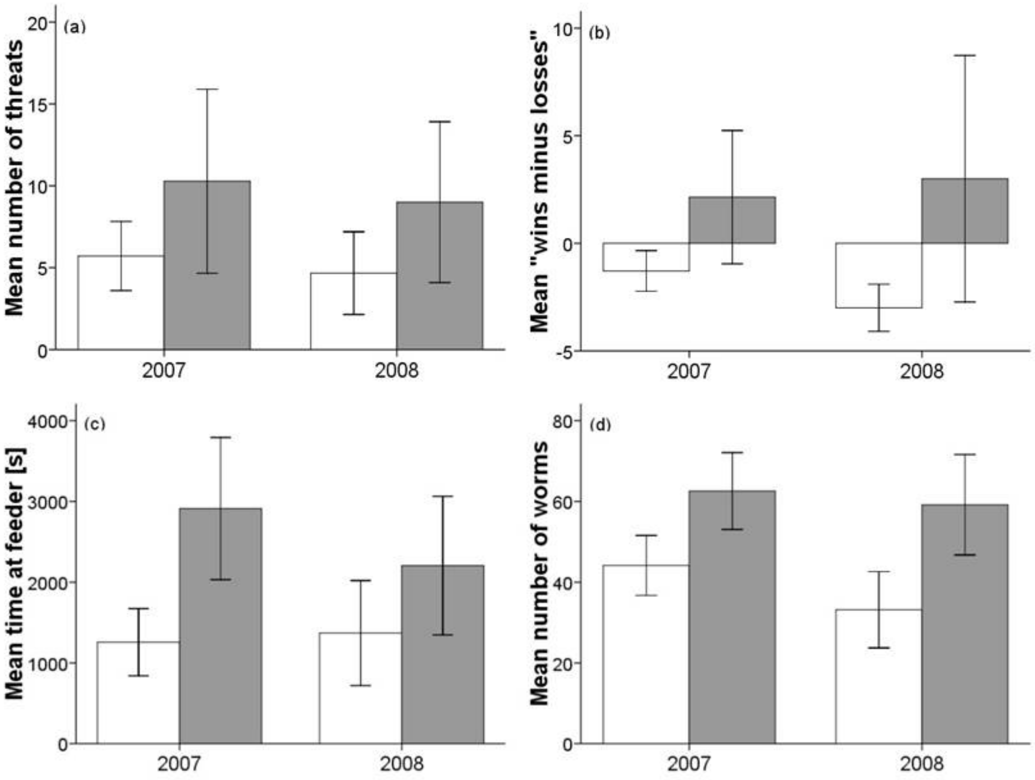

Figure 4: Comparison of the age categories “young” (white bars) and “old” (grey bars) during both trial seasons (2007 and 2008) in regard to (a) number of threats, (b) “wins minus losses”, (c) time spent at the feeder, and (d) the amount of worms eaten (mean ± SE).

Effects of plumage manipulations on behaviour and contest outcome

We compared the difference in plumage variables (average brightness, chroma and hue) between both contest seasons for those competing male dyads in which one male was manipulated during the 2008 trials. The manipulation of males resulted in a decrease (albeit not significant) of plumage difference in hue (paired t-test; t = 1.747, P = 0.103) and chroma (t = 1.373, P = 0.191) between competing males, meaning that these male pairs became more similar in hue and chroma than they were in the previous year. The differences in average brightness between competing males for these male dyads did not change significantly (average brightness: t = -0.428, P = 0.675).

If hue and chroma indeed act as status signals in male-male interactions, a smaller difference between two competing males would be expected to lead to more intense and aggressive interactions and to increase the winning probability of the male whose plumage colouration was experimentally increased. However, a comparison of the total number of interactions taking place between these pairs of males in both years showed that the number of interactions did not increase significantly after having decreased differences in plumage hue (paired t-test; $t = -1.526$, $P = 0.149$). In addition, males with their plumage hue and chroma experimentally increased did not threat their unmanipulated opponents more often in 2008 compared to 2007 where both opponents were unmanipulated (paired t-test; $t = -1.382$, $P = 0.189$). Finally, manipulated males were not more likely to win a contest against an unmanipulated opponent in 2008 compared to 2007 where both opponents were unmanipulated (McNemar test; $P = 0.375$).

Discussion

To our knowledge, our study is one of the few that has incorporated not only plumage colouration into the analysis of which traits influence contests over a limited food source under laboratory conditions in birds, but also includes testosterone levels, body condition and, most importantly, age. Our results show that age has both the most important and the only consistent effect on the outcome of contests over food between brightly coloured red bishop males. In contrast, we found no clear and consistent effect of average brightness, chroma and hue, indicating that plumage characteristics play only a minor role in settling male-male contests in this species.

A strong effect of age on dominance structures has been shown in several other studies. For example, Bose and Sarrazin (2007) show that in Griffon vultures (*Gyps fulvus*), older males were more likely to be aggressive and dominant than younger individuals, a finding that has also been shown for Arctic barnacle geese (*Branta leucopsis*; Stahl et al. 2001), yellowhammers (*Emberiza citronella;* Sundberg 1995), urban pigeons (*Columba livia*; Sol et al. 1998) or white-throated sparrows (*Zonotrichia albicollis*; Piper & Wiley 1989). All these studies state that age predicts dominance, as birds could potentially learn to dominate opponents as they grow older and more experienced.

When looking at the effects of the different plumage variables average brightness, chroma and hue in both contest years, our results are somewhat contradictory, with both chroma and hue influencing winning probability in 2007 but in opposing directions (chroma positive and hue negative), while in 2008, chroma has no visible effect and hue as well as average brightness show positive influences. There are several studies that have shown positive relationships between carotenoid-based plumage colouration and dominance status in birds. For example, in both red-shouldered widowbirds and red-collared widowbirds, males with redder ornamental traits were dominant over duller counterparts (Pryke et al. 2002, Pryke & Andersson 2003). Similar results have been obtained in studies on yellow warblers (*Dendroica petechia;* Studd & Robertson 1985), Gouldian finches (*Erythrura gouldiae;* Pryke & Griffith 2006) rock sparrows (*Petronia petronia;* Griggio et al. 2007) and red-backed fairy-wrens (*Malurus melanocephalus*; Karubian et al. 2008). In comparison, there are also some studies showing negative or no relationships. Hill (2002) found that in house finches duller males were more likely to dominate brighter males (see also McGraw & Hill 2000a, b), and Wolfenbarger (1999) showed that carotenoid-based colouration had no influence at all on dominance status in northern cardinals. Overall, the published studies dealing with the effect of carotenoid-based plumage colouration on social status have revealed different and contradictory results.

However, it has to be noted that male age was not included in the analyses in any of the studies on the effect of plumage characteristics on dominance mentioned above. Various studies have shown that there is age-related plumage variation, with older males being more colourful than younger counterparts (Wiebe & Bortolotti 2002, Probst et al. 2007). In these cases where age and plumage colouration are positively linked together, it seems likely that status signalling via plumage ornaments occurs. In the red bishop (or at least in our aviary population of red bishops), age was not consistently correlated with plumage colouration. Thus, our results indicate that at least in trials between males of different age categories, plumage colouration does not play a vital role in establishing dominance hierarchies. Rather, dominance seems to be established by age-related behaviour alone with older males having more competition experience and behaving accordingly. This interpretation is confirmed by the fact that older red bishop males in our experiments tended to threaten counterparts more often than younger males, which resulted in their winning more interactions. Overall, our results suggest that in red bishops, age and its matching dominance behaviours might override the potential signalling effects of plumage colouration.

As well as testing for plumage colouration and age effects, we also included body condition index and testosterone levels in our analysis. Body condition index influenced trial outcome during the 2007 contests, with those birds winning more interactions that had a worse body condition than their counterparts. This is a result that has also been shown in other studies involving great tits (*Parus major;* Lemel & Wallin 1993), house sparrows (*Passer domesticus;* Andersson & Åhlund 1991) and American goldfinches (*Carduelis tristis;* Popp 1987), in which hungrier subordinates were able to beat dominant opponents. In these cases it has been suggested that if the value of the limited resource is higher than the cost of actually fighting, then contests should not be settled by a badge of status such as plumage colouration, but based on other motivational factors such as hunger or a low body condition index (Senar 1999).

Testosterone showed a positive influence on contest outcome during 2007, with those males with higher testosterone levels having a higher winning probability. In several studies, such as those on spotted antbirds (*Hylophylax naevioides*; Hau et al. 2000), dark-eyed juncos (*Junco hyemalis carolinensis*; Zysling et al. 2006), red-winged blackbirds (*Agelaius phoeniceus;* Johnsen 1998) and sedentary song sparrows (*Melospiza melodia morphna*; Wingfield 1994), testosterone titres have been to shown to be positively correlated with the level of aggressiveness displayed by an individual. Surprisingly, younger males had higher testosterone titres than older individuals after moult in 2007, suggesting that younger rather than older males should win more contests. As mentioned above and similar to the effect of plumage colouration, we suggest that the somewhat weaker testosterone effect was overridden by the strong and obviously testosterone-independent age effect in our experiments. It remains to be shown whether the positive testosterone effect plays a more important role when individuals of the same age are staged against one another.

It has also been shown for several species, such as fish (Beaugrand et al. 1991, Hsu & Wolf 2001), invertebrates (Whitehouse 1997) and birds (Drummond & Osório 1992), that past experience with competitors can affect future interactions between these individuals. By conducting our experiments in two separate breeding seasons, we wanted to ensure that the time period between dyadic competitions between familiar males was long enough for competing males to “forget” their experience with certain opponents. While it is not exactly known for how long this “temporal decay” actually works (Hsu et al. 2006), we are confident that we prevented such a memory effect by the experimental design with the two contests between each pair of males being conducted with a pause of a whole year. However, there is another factor which might have influenced the outcome of our dominance trials – the observer effect. The two separate aviaries in which our birds were held were not visually isolated from one another. Hence, it is possible that males, even though they do not interact socially, eavesdrop on the interactions between males in the opposite aviary and then adjust their perception of the competitive ability of these other competitors according to the outcome of the observed interactions (Hsu et al. 2006). This phenomenon of eavesdropping has been demonstrated in many fish (Oliveira et al. 1998, McGregor & Peake 2000, Brown & Laland 2003) and bird species (Naguib et al. 1999, Peake et al. 2001, Amy & Leboucher 2007) and evidence has been given that the information an individual has gained from observing aggressive interactions between others influences the decision as to how to interact with this previously observed conspecific, thereby avoiding potential costs of actually fighting (Oliveira 2005).

The carotenoid-based plumage of male red-bishops might not only serve as a status signal, but also as an ornament for mate attraction ("armament-ornament model", Berglund et al. 1996), meaning that different receivers are receptive to different aspects of the same signal. In respect to yellow, orange or red plumage this has been shown in rock sparrows (Griggio et al. 2007), where the size of the yellow throat patch is not only used in male-male competition, but also in female choice as a signal of male quality. Other studies have only been able to show one function of the studied carotenoid-based ornamental trait – either as a status signal (red-collared widow-birds, Pryke et al. 2001) or as a cue for female mate choice (siskins, Senar et al. 2005; house finches, Hill et al. 1999). In both situations, larger and/or more colourful ornaments are more imposing or attractive. The possibility of a dual function of male red bishop plumage still needs to be studied.

To conclude, we were able to determine a significant and consistent age effect on the outcome of male-male contests over food in red bishops. This age effect potentially overrode the signalling effects of plumage colouration, as well as potential effects of testosterone levels or body condition on contest outcome. Further experiments are needed in which the age effect is detangled from those of plumage colouration, testosterone, and body condition to clarify under which circumstances the age-related dominance hierarchies in red bishop males are modified by these other factors. In any case, our results show that age plays a more important role in settling male-male contests than previously acknowledged, potentially overriding other factors which have been thought to be more important.

Acknowledgements

We would like to thank Martin Dehnhard and Marlies Rohleder from the Institute for Zoo and Wildlife Research in Berlin for conducting the testosterone analyses. This study was funded by the Deutsche Forschungsgemeinschaft (DFG FR 2096/2-2).

References

Amy M, Leboucher G. (2007) Male canaries can visually eavesdrop on conspecific food interactions. *Animal Behaviour* **74:** 57 – 62.

Andersson M. (1994) *Sexual Selection*. Princeton: Princeton University Press.

Andersson S, Åhlund M. (1991) Hunger affects dominance among strangers in house sparrows. *Animal Behaviour* **41:** 895 – 897.

Andersson S, Pryke SR, Örnborg J, Lawes MJ, Andersson M. (2002) Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. *The American Naturalist* **160:** 683 – 691.

Bakker CM, Sevenster P. (1983) Determinants of dominance in male sticklebacks (*Gasterosteus aculeatus L.*). *Behaviour* **86:** 55 – 71.

Beaugrand JP, Goulet C, Pavette D. (1991) Outcome of dyadic conflict in male green swordtail fish, *Xiphophorus helleri*: effect of body size and prior dominance. *Animal Behaviour* **41:** 417 – 424.

Berglund A, Bisazza A, Pilastro A. (1996) Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biological Journal of the Linnaen Society* **58:** 385 – 399.

Bitton P-P, Dawson RD. (2008) Age-related differences in plumage characteristics of male tree swallows *Tachycineta bicolor*: hue and brightness signal different aspects of individual quality. *Journal of Avian Biology* **39:** 446 – 452.

Blas J, Pérez-Rodríguez L, Bortolotti GR, Viñuela J, Marchant TA. (2006) Testosterone increases bioavailability of carotenoids: Insights into the honesty of sexual signalling. *PNAS* **103:** 18633 – 18637.

Bose M, Sarrazin F. (2007) Competitive behaviour and feeding rate in a reintroduced population of griffon vultures *Gyps fulvus*. *Ibis* **149:** 490 – 501.

Bradbury JW, Vehrencamp S. (1998) *Principles of Animal Communication*. Sunderland: Sinauer Associates Inc.

Brown C, Laland KN. (2003) Social learning in fishes: a review. *Fish and Fisheries* **4:** 280 – 288.

Craig AJFK. (1974) Reproductive behaviour of the male red bishop bird. *Ostrich* **45:** 149 – 160.

Dale J. (2006) Intraspecific variation in coloration. In: Hill GE, McGraw KJ, eds. *Bird Coloration. Volume II: Function and Evolution*. Cambridge: Harvard University Press, 36 – 86.

Delhey K, Johnsen A, Peters A, Andersson S, Kempenaers B. (2005) Paternity analysis reveals opposing selection pressures on crown coloration in the blue tit (*Parus caeruleus*). *Proceedings of the Royal Society of London B* **270:** 2057 – 2063.

Delhey K, Kempenaers B. (2006) Age differences in blue tit *Parus caeruleus* plumage colour: within-individual changes or colour-biased survival? *Journal of Avian Biology* **37:** 339 – 348.

Drummond H, Osório JL. (1992) Training siblings to be submissive losers: dominance between booby nestlings. *Animal Behaviour* **44:** 881 – 893.

Duckworth RA, Mendonça MT, Hill GE. (2004) Condition-dependent sexual traits and social dominance in the house finch. *Behavioral Ecology* **15:** 779 – 784.

Espmark Y, Amundsen T, Rosenqvist G. (2000) *Animal Signals: Signalling and Signal Design in Animal Communication*. Trondheim: Tapir Academic Press.

Evans MR, Hatchwell BJ. (1992) An experimental study of male adornment in the scarlet-tufted malachite sunbird: I. The role of pectoral tufts in territorial defence. *Behavioural Ecology and Sociobiology* **29:** 413 – 419.

Evans MR, Norris K. (1996) The importance of carotenoids in signaling during aggressive interactions between male firemouth cichlids (*Cichlasoma meeki*). *Behavioral Ecology* **7:** 1 – 6.

Evans MR, Goldsmith AR, Norris SRA. (2000) The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). *Behavioural Ecology and Sociobiology* **47:** 156 – 163.

Ferns PN, Hinsley SA. (2008) Carotenoid plumage hue and chroma signal different aspects of individual and habitat quality in tits. *Ibis* **150:** 152 – 159.

Figuerola J, Muñoz E, Gutiérrez R, Ferrer D. (1999) Blood parasites, leucocytes and plumage brightness in the cirl bunting, *Emberiza cirlus*. *Functional Ecology* **13:** 594 – 601.

Folstad I, Karter AK. (1992) Parasites, bright males and the immunocompetence handicap. *The American Naturalist* **139:** 603 – 622.

Friedl TWP. (2004a) Breeding behaviour of the red bishop (*Euplectes orix*): a synthesis and new observations. *Vogelwarte* **42:** 178 – 190.

Friedl TWP. (2004b) Breeding success in a red bishop (*Euplectes orix*) colony in the Eastern Cape, South Africa, and its relation to nest site characteristics. *Ostrich* **75:** 95 – 105.

Friedl TWP, Klump GM. (1999) Determinants of male mating success in the red bishop (*Euplectes orix*). *Behavioural Ecology and Sociobiology* **46:** 387 – 399.

Gonzalez G, Sorci G, Smith LC, de Lope F. (2001) Testosterone and sexual signalling in male house sparrows (*Passer domesticus*). *Behavioural Ecology and Sociobiology* **50:** 557 – 562.

Goodwin TW. (1986) Metabolism, nutrition, and function of carotenoids. *Annual Review of Nutrition* **6:** 274 – 297.

Griggio M, Serra L, Licheri D, Monti A, Pilastro A. (2007) Armaments and ornaments in the rock sparrow: a possible dual utility of a carotenoid-based feather signal. *Behavioural Ecology and Sociobiology* **61:** 423 – 433.

Hau M, Wikelski M, Soma KK, Wingfield JC. (2000) Testosterone and year-round territorial aggression in a tropical bird. *General and Comparative Endocrinology* **117:** 20 – 33.

Hill GE. (2002) A *red bird in a brown bag: the function and evolution of colorful plumage in the House Finch*. New York: Oxford University Press.

Hill GE, Nolan PM, Stoehr AM. (1999) Pairing success relative to male plumage redness and pigment symmetry in the house finch: temporal and geographic constancy. *Behavioral Ecology* **10:** 48 – 53.

Hõrak P, Ots I, Vellau H, Spottiswoode C, Møller AP. (2001) Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. *Oecologia* **126:** 166 – 173.

Hsu Y, Wolf LL. (2001) The winner and loser effect: what fighting behaviours are influenced? *Animal Behaviour* **61:** 777 – 786.

Hsu Y, Earley RL, Wolf LL. (2006). Modulation of aggressive behaviour by fighting experience: mechanisms and contest outcomes. *Biological Reviews* **81:** 33 – 74.

Johnsen TS. (1998) Behavioural correlates of testosterone and seasonal changes of steroids in red-winged blackbirds. *Animal Behaviour* **55:** 957 – 965.

Kodrick-Brown A, Brown JH. (1984) Truth in advertising: the kind of traits favored by sexual selection. *The American Naturalist* **124:** 309 – 323.

Krinsky NI. (1994) The biological properties of carotenoids. *Pure and Applied Chemistry* **66:** 1003 – 1010.

Lawes MJ, Slotow R, Andersson S. (2002) Male nest building but not display behaviour directly influences mating success in the polygynous red bishop (*Euplectes orix*). *Ostrich* **73:** 87 – 91.

Lemel J, Wallin K. (1993) Status signalling, motivational condition and dominance: an experimental study in the great tit, *Parus major L*. *Animal Behaviour* **45:** 549 – 558.

Lessels CM, Boag PT. (1987) Unrepeatable repeatabilities: a common mistake. *The Auk* **104:** 116 – 121.

Lozano GA. (1994) Carotenoids, parasites, and sexual selection. *Oikos* **70:** 309 – 311.

Maney DL, Davis AK, Goode CT, Reid A, Showalter C. (2008) Carotenoid-based plumage coloration predicts leukocyte parameters during the breeding season in northern cardinals (*Cardinalis cardinalis*). *Ethology* **114:** 369 – 380.

McGraw KJ, Hill GE. (2000a) Carotenoid-based ornamentation and status signalling in the house finch. *Behavioral Ecology* **11:** 520 – 527.

McGraw KJ, Hill GE. (2000b) Plumage brightness and breeding-season dominance in the house finch: a negatively correlated handicap? *Condor* **32:** 90 – 94.

McGraw KJ, Correa SM, Adkins-Regan E. (2006) Testosterone upregulates lipoprotein status to control sexual attractiveness in a colorful songbird. *Behavioural Ecology and Sociobiology* **60:** 117 – 122.

McGregor PK, Peake T. (2000) Communication networks: social environments for receiving and signalling behaviour. *Acta Ethologica* **2:** 71 – 81.

Naguib M, Fichtel C, Todt D. (1999) Nightingales respond more strongly to vocal leaders of simulated dyadic interactions. *Proceedings of the Royal Society of London B* **266:** 537 – 542.

Oliveira RF. (2005) Hormones, social context and animal communication. – In: McGregor PK, ed. *Animal Communication Networks*. Cambridge: Cambridge University Press, 481 – 520.

Oliveira RF, McGregor PK, Latruffe C. (1998) Know thine enemy: fighting fish gather information from observing conspecifics interactions. *Proceedings of the Royal Society of London B* **265:** 1045 – 1049.

Olson VA, Owens IPF. (1998) Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology and Evolution* **13:** 510 – 514.

Parker TH, Ligon JD. (2002) Dominant male red jungle fowl (*Gallus gallus*) test the dominance status of other males. *Behavioural Ecology and Sociobiology* **53:** 20 – 24.

Peake TM, Terry AMR, McGregor PK, Dabelsteen T. (2001) Male great tits eavesdrop on simulated male-to-male vocal interactions. *Proceedings of the Royal Society of London B* **268:** 1183 – 1187.

Peters A, Denk AG, Delhey K, Kempenaers B. (2004) Carotenoid-based bill colour as an indicator of immunocompetence and sperm performance in male mallards. *Journal of Evolutionary Biology* **17:** 1111 – 1120.

Peters A. (2007) Testosterone and carotenoids: an integral view of trade-offs between immunity and sexual signalling. *BioEssays* **29:** 427 – 430.

Piper WH, Wiley H. (1989) Correlates of dominance in wintering white-throated sparrows: age, sex and location. *Animal Behaviour* **37:** 298 – 310.

Popp JW. (1987) Resource value and dominance among American goldfinches. *Bird Behaviour* **7:** 73 – 77.

Probst JR, Donner DM, Bozek MA. (2007) Continuous, age-related plumage variation in male Kirtland's warblers. *Journal of Field Ornithology* **78:** 100 – 108.

Pryke SR, Andersson S, Lawes MJ. (2001) Sexual selection of multiple handicaps in the red-collared widowbird: female choice of tail length but not carotenoid display. *Evolution* **55:** 1452 – 1463.

Pryke SR, Andersson S, Lawes MJ, Piper SE. (2002) Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color. *Behavioral Ecology* **13:** 622 – 631.

Pryke SR, Andersson S. (2003) Carotenoid-based status signalling in red-shouldered widowbirds (*Euplectes axillaris*): epaulet size and redness affect captive and territorial competition. *Behavioural Ecology and Sociobiology* **53:** 393 – 401.

Pryke SR, Griffith SC. (2006) Red dominates black: agonistic signalling among head morphs in the colour polymorphic Gouldian finch. *Proceedings of the Royal Society of London B* **273:** 949 – 957.

Rohwer SA. (1975) The social significance of avian winter plumage variability. *Evolution* **29:** 593 – 610.

Rohwer SA. (1982) The evolution of reliable and unreliable badges of fighting ability. *American Zoologist* **22:** 531 – 546.

Saks L, Ots I, Hõrak P. (2003) Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia* **134:** 301 – 307.

Searcy WA, Nowicki S. (2005) *The Evolution of Animal Communication: Reliability and Deception in Signaling Systems*. Princeton: Princeton University Press.

Senar JC. (1999) Plumage colouration as a signal of social status. In: Adams NJ, Slotow RH, eds. *Proceedings of the 22nd International Ornithological Congress*. Durban: Birdlife South Africa, 1669 – 1686.

Senar JC, Escobar D. (2002) Carotenoid derived plumage coloration in the siskin *Carduelis spinus* is related to foraging ability. *Avian Science* **2:** 19 – 24.

Senar JC, Domenech J, Camerino M. (2005) Female siskins choose mates by the size of the yellow wing stripe. *Behavioural Ecology and Sociobiology* **57:** 465 – 469.

Shawkey MD, Estes AM, Siefferman LM, Hill GE. (2003) Nanostructure predicts intraspecific variation in ultraviolet-blue plumage colour. *Proceedings of the Royal Society of London B* **270:** 1455 – 1460.

Siefferman LM, Hill GE. (2005) UV-blue structural plumage coloration and competition for nestboxes in male eastern bluebirds. *Animal Behaviour* **69:** 67 – 72.

Siitari H, Alatalo RV, Halme P, Buchanan KL, Kilpimaa J. (2007) Color signals in the black grouse (*Tetrao tetrix*): Signal properties and their condition dependency. *The American Naturalist* **169:** S81 – S92.

Smiseth PT, Örnborg J, Andersson S, Amundsen T. (2001) Is male plumage reflectance correlated with paternal care in bluethroats? *Behavioral Ecology* **12:** 164 – 170.

Sol D, Santos DM, Garcia J, Cuadrado M. (1998) Competition for food in urban pigeons: the cost of being juvenile. *Condor* **100:** 298 – 304.

Soma KK. (2006) Testosterone and aggression: Berthold, birds and beyond. *Journal of Neuroendocrinology* **18:** 543 – 551.

Stahl J, Tolsma PH, Maarten JJE, Drent RH. (2001) Subordinates explore but dominants profit: resource competition in high Arctic barnacle goose flocks. *Animal Behaviour* **61:** 257 – 264.

Studd MV, Robertson RJ. (1985) Evidence for reliable badges of status in territorial yellow warblers (*Dendroica petechia*). *Animal Behaviour* **33:** 1102 – 1113.

Sundberg J. (1995) Female yellowhammers *(Emberiza citrinella)* prefer yellower males: a laboratory experiment. *Behavioural Ecology and Sociobiology* **37:** 275 – 282.

von Schantz T, Bensch S, Grahn M, Hasselquist D, Wittzell H. (1999) Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society of London B* **266:** 1 – 12.

Whitehouse MJ. (1997) Experience influences male–male contests in the spider *Argyrodes antipodiana* (Theridiidae: Aranea). *Animal Behaviour* **53:** 913 – 923.

Wiebe KL, Bortolotti GR. (2002) Variation in carotenoid-based color in northern flickers in a hybrid zone. *Wilson Bulletin* **114:** 393 – 400.

Wiley RH, Steadman L, Chadwick L, Wollerman L. (1999) Social inertia in white-throated sparrows results from recognition of opponents. *Animal Behaviour* **57:** 453 – 463.

Wingfield JC. (1994) The regulation of territorial behavior in the sedentary song sparrow, *Melospiza melodia morphna*. *Hormones and Behavior* **28:** 1 – 15.

Wingfield JC, Lynn SE, Soma KK. (2001) Avoiding the "costs" of testosterone: ecological basis of hormone-behavior interactions. *Brain Behavior and Evolution* **57:** 239 – 251.

Zahavi A. (1975) Mate selection – a selection for a handicap. *Journal of Theoretical Biology* **53:** 205 – 214.

Zahavi A. (1977) The cost of honesty (further remarks on the handicap principle). *Journal of Theoretical Biology* **67:** 603 – 605.

Zuk M, Thornhill R, Ligon JD, Johnson K, Austad S, Ligon SH, Thornhill NW, Costin C. (1990) The role of male ornaments and courtship behavior in female mate choice of red jungle fowl. *The American Naturalist* **136:** 459 – 473.

Zysling DA, Greives TJ, Breuner CW, Casto JM, Demas GE, Ketterson ED. (2006) Behavioral and physiological responses to experimentally elevated testosterone in female dark-eyed juncos (*Junco hyemalis carolinensis*). *Hormones and Behaviour* **50:** 200 – 207.

APPENDIX

Appendix A1 – Plumage characteristics of wild males

Plumage characteristics (average brightness, chroma, hue) of wild red bishop males (ring number and date of capture are given) for each season. Shown are measurements taken from the head (H), lower back (LB) and throat (T), as well as the average (AV) over the three areas. Further, principal components (PC1 and PC2) calculated from the average values of the three colour values are given.

Ring number	**Season**	**Date**	**Average Brightness**				**Chroma**				**Hue**				**Principal components**	
			H	**LB**	**T**	**AV**	**H**	**LB**	**T**	**AV**	**H**	**LB**	**T**	**AV**	**PC1**	**PC2**
BC92716	2005/06	09.12.2005	15.944				0.774				589.21				-0.745	-1.717
BC92773	2005/06	09.12.2005	105.874	94.515			0.444	0.454			524.67	535.43			5.665	-2.273
BC92882	2005/06	07.02.2006	33.568	28.620	23.091	28.426	0.842	0.856	0.812	0.837	588.61	590.69	583.56	587.62	0.027	0.783
BH03882	2005/06	19.12.2005	30.171	30.593	19.888	26.884	0.828	0.781	0.778	0.796	594.41	584.36	602.85	593.87	-0.049	0.054
BH03981	2005/06	27.01.2006	38.765				0.736				581.53				0.793	1.038
BH04140	2005/06	30.01.2006	20.580	26.533	17.013	21.376	0.840	0.831	0.761	0.811	594.06	586.74	577.21	586.00	-0.416	-0.562
BH09171	2005/06	10.02.2006	24.309	23.621	25.200	24.377	0.874	0.847	0.856	0.859	593.51	586.39	588.99	589.63	-0.247	0.269
BH09191	2005/06	19.12.2005	26.896	27.846	17.183	23.975	0.778	0.794	0.689	0.754	578.93	581.64	567.43	576.00	-0.213	-0.554
BH09217	2005/06	09.12.2005	77.428	77.457			0.482	0.457			505.61	571.68			3.070	-2.516
BH09227	2005/06	07.12.2005	39.784	34.926			0.790	0.786			583.45	589.78			0.657	1.461
BH09296	2005/06	13.12.2005	19.946				0.826				590.80				-0.514	-0.733
BH09347	2005/06	10.02.2006	23.551	24.357	20.354	22.754	0.797	0.800	0.643	0.747	577.34	579.38	551.57	569.43	-0.403	-0.775
BH36321	2005/06	09.12.2005	22.063	18.640			0.707	0.634			583.23	571.41			-0.405	-1.781
BH36324	2005/06	13.12.2005	27.440	24.909			0.854	0.793			592.04	578.02			-0.109	0.292
BH36339	2005/06	19.12.2005	19.192	24.613	17.077	20.294	0.831	0.820	0.791	0.814	592.04	587.75	589.11	589.63	-0.486	-0.761
BH36381	2005/06	27.01.2006	29.684	34.465	30.348	31.499	0.781	0.779	0.764	0.774	594.19	585.83	591.14	590.39	0.272	0.504

BC92666	2006/07	30.10.2006	39.953	19.075	39.953	32.994	0.786	1.029	0.786	0.867	569.26	584.58	569.26	574.37	0.338	1.361
BC92827	2006/07	15.11.2006	25.193	21.523	30.845	25.854	0.811	0.860	0.757	0.809	576.32	583.79	572.91	577.67	-0.115	0.124
BC92888	2006/07	27.10.2006	18.696	23.656	26.325	22.892	0.838	0.864	0.817	0.840	577.79	583.91	581.64	581.11	-0.320	-0.181
BH03933	2006/07	09.11.2006	13.594	13.847	33.410	20.284	1.108	1.095	0.825	1.009	582.66	583.00	570.97	578.88	-0.561	0.386
BH03981	2006/07	30.10.2006	18.514	20.969	25.933	21.805	0.987	0.937	0.881	0.935	588.20	582.78	579.38	583.45	-0.455	0.500
BH04242	2006/07	24.11.2006	13.926	20.484	25.266	19.892	0.973	0.934	0.861	0.923	588.88	589.44	583.23	587.18	-0.560	-0.078
BH09214	2006/07	27.10.2006	24.381	16.227	20.973	20.527	1.010	1.189	1.111	1.103	565.74	570.40	580.62	572.25	2.035	1.074
BH09227	2006/07	09.11.2006	19.188	17.686	28.880	21.918	1.018	1.033	0.845	0.965	576.43	577.45	570.75	574.88	1.023	0.517
BH09239	2006/07	27.10.2006	21.727	22.592	37.452	27.257	0.937	0.923	0.766	0.875	583.79	582.32	574.15	580.09	-0.050	0.755
BH09260	2006/07	22.11.2006	18.355	19.956	38.858	25.723	1.107	1.040	0.716	0.954	581.76	573.14	554.55	569.82	1.343	0.351
BH09418	2006/07	03.11.2006	23.579	25.256	23.397	24.077	0.882	0.876	0.899	0.886	584.70	585.83	587.07	585.87	-0.279	0.484
BH09459	2006/07	24.11.2006	13.331	19.405	40.557	24.431	1.168	1.007	0.620	0.931	597.11	588.09	556.27	580.49	0.356	-0.034
BH09465	2006/07	06.11.2006	24.539	23.708	27.421	25.222	0.846	0.889	0.841	0.859	582.78	588.09	583.12	584.66	-0.191	0.469
BH09491	2006/07	09.11.2006	20.248	18.511	20.651	19.803	1.002	1.053	1.052	1.035	574.38	580.97	585.83	580.39	0.997	0.673
BH36379	2006/07	27.10.2006	17.215				1.103				597.57				-0.818	0.552
BH36384	2006/07	03.11.2006	21.333	22.398	27.802	23.844	0.882	0.879	0.833	0.865	584.47	587.87	583.34	585.23	-0.281	0.257
BH36471	2006/07	30.10.2006	10.734	16.855	22.610	16.733	1.104	1.024	0.987	1.038	574.61	580.29	584.02	579.64	0.436	-0.062
BH36477	2006/07	30.10.2006	17.144	20.466	30.580	22.730	1.072	0.990	0.873	0.978	584.58	578.02	584.35	582.32	0.829	0.847
BH36495	2006/07	03.11.2006	18.114	27.166	26.641	23.974	0.899	0.887	0.898	0.895	584.81	588.54	587.75	587.03	-0.295	0.549
BH36496	2006/07	03.11.2006	25.054	22.837	34.398	27.430	0.862	0.842	0.778	0.827	583.00	580.85	579.15	581.00	-0.021	0.488
BH36547	2006/07	23.11.2006	19.529	16.835	29.496	21.953	1.084	1.149	0.944	1.059	581.18	588.43	581.98	583.86	0.297	1.155
BH36559	2006/07	15.11.2006	21.697	19.022	28.612	23.111	0.941	0.940	0.883	0.921	588.66	587.64	584.70	587.00	0.041	0.536
BH36590	2006/07	01.12.2006	25.072	20.820	40.823	28.905	0.892	0.912	0.697	0.834	581.76	588.43	568.25	579.48	0.085	0.537
BH36596	2006/07	07.12.2006	27.138	19.748	25.595	24.161	0.750	1.019	0.928	0.899	570.41	590.12	587.75	582.76	0.054	0.500
BH36598	2006/07	07.12.2006	23.021	20.735	31.697	25.151	0.910	0.917	0.781	0.869	587.97	589.22	573.82	583.67	-0.196	0.437
BH36600	2006/07	13.12.2006	18.738	19.187	35.634	24.520	0.856	0.957	0.758	0.857	577.66	587.75	574.60	580.00	-0.210	0.025
BC92827	2007/08	23.01.2008	20.257	17.325	27.585	21.723	0.813	0.856	0.818	0.829	575.29	583.91	578.92	579.37	-0.392	-0.383

BH04054	2007/08	10.01.2008	26.202	22.716	28.048	25.655	0.854	0.872	0.830	0.852	584.70	586.96	583.79	585.15	-0.155	0.423
BH09197	2007/08	29.01.2008	15.737	15.725	9.442	13.635	0.854	0.841	0.858	0.851	584.64	584.02	579.89	582.85	-1.098	-2.065
	2007/08	20.02.2008	20.632	21.180	27.894	23.235	0.899	0.874	0.835	0.869	591.25	585.60	580.62	585.82	-0.319	0.167
BH09214	2007/08	11.01.2008	19.805	20.747	34.003	24.852	0.901	0.847	0.687	0.812	586.51	582.44	568.13	579.03	-0.169	-0.210
BH09227	2007/08	10.01.2008	17.109	20.250	29.241	22.200	0.875	0.858	0.805	0.846	587.97	583.34	581.42	584.24	-0.372	-0.234
	2007/08	22.02.2008	13.181	19.412	20.602	17.731	1.004	0.908	0.924	0.945	591.02	583.57	587.06	587.22	-0.702	-0.368
BH09265	2007/08	29.01.2008	6.802	7.771	10.216	8.263	0.834	0.911	0.882	0.876	574.03	584.81	583.46	580.77	-1.280	-2.383
	2007/08	20.02.2008	22.043	17.342	17.200	18.862	0.851	0.802	0.807	0.820	586.51	581.41	580.97	582.96	-0.578	-0.899
BH09491	2007/08	19.12.2007	15.529	17.956	28.522	20.669	1.038	1.009	0.861	0.969	579.49	583.57	575.05	579.37	-0.505	0.158
BH36445	2007/08	19.12.2007	12.630	14.020	20.723	15.791	1.265	1.186	1.088	1.179	576.31	579.15	576.77	577.41	-0.894	0.527
BH36605	2007/08	27.11.2007	37.888	33.716	40.887	37.497	0.829	0.766	0.874	0.823	578.11	567.28	580.51	575.30	0.647	2.277
	2007/08	19.02.2008	20.553	14.991	26.047	20.530	0.818	0.836	0.776	0.810	578.46	579.26	575.75	577.82	-0.458	-0.734
BH36608	2007/08	30.11.2007	32.624	26.399	33.024	30.683	0.879	0.924	0.960	0.921	582.65	595.76	600.15	592.85	0.104	1.989
BH36611	2007/08	05.12.2007	18.321	22.241	27.892	22.818	0.902	0.754	0.760	0.805	593.85	575.97	575.96	581.93	-0.305	-0.546
BH36613	2007/08	14.12.2007	14.463	16.658	16.496	15.872	0.877	0.839	0.849	0.855	584.35	580.74	575.06	580.05	-0.787	-1.141
BH36615	2007/08	20.12.2007	16.593	11.645	16.085	14.774	0.909	0.970	0.881	0.920	585.60	586.17	584.22	585.33	-0.860	-1.311
BH36617	2007/08	07.01.2008	18.685	17.689	21.226	19.200	0.868	0.844	0.880	0.864	586.05	581.76	589.10	585.64	-0.582	-0.523
BH36624	2007/08	11.01.2008	20.507	23.697	34.035	26.080	0.825	0.845	0.797	0.822	580.51	582.21	581.08	581.27	-0.106	0.244
BH36628	2007/08	16.01.2008	23.429	24.144	18.310	21.961	0.822	0.801	0.862	0.828	579.15	574.16	584.59	579.30	-0.378	-0.330
BH36631	2007/08	29.01.2008	19.683	16.073	18.206	17.987	0.847	0.872	0.823	0.847	582.32	586.06	579.61	582.66	-0.644	-0.908
BH36661	2007/08	25.02.2008	20.230	20.174	17.125	19.177	0.853	0.839	0.863	0.852	583.34	587.98	583.33	584.88	-0.566	-0.716

Appendix A2 – Testosterone, parasites, blood picture and body condition of wild males

Blood sample and catch number of all captured wild males from 2005/06 to 2007/08, as well as testosterone levels [ng/ml], degree of parasitisation [% cells infected with blood parasites], number of lymphocytes, heterophils and eosinophils per 100 leukocytes, heterpophil/lymphocyte ratio (HLR), total leukocyte count (TLC) and body condition index (BCI) on date of capture.

Ring number	Season	Date	Blood sample	Catch	Testosterone [ng/ml]	Parasites	Lympho	Hetero	Eosino	HLR	TLC	Weight	Tarsus	BCI
BC78299	2005/06	10.02.2006	1519	E	0.396	0.89	56	27	15	0.48	39.08	25	25	-1.273
BC92716	2005/06	09.12.2005										23	25	-1.254
BC92773	2005/06	09.12.2005	2081	D	0.547	0						22	26	-0.585
BC92882	2005/06	07.02.2006	2190	B	1.288	0	78	13	7	0.17	32.38	25	29	1.361
BH03882	2005/06	19.12.2005	2382	C	0.282	3.15	76	5	12	0.07	18.70	25	29	1.361
BH03981	2005/06	27.01.2006	2478	B	1.691	0	57	14	25	0.25	14.01	24	27	0.054
BH04140	2005/06	30.01.2006	2632	E	0.76	10.53	72	17	10	0.24	37.40	24	28	0.712
BH09171	2005/06	10.02.2006	2961	B	0.962	0	55	29	16	0.53	15.67	24	26	-0.605
BH09191	2005/06	19.12.2005	2979	B	0.532	0.12	57	19	18	0.33	13.02	24	26	-0.605
BH09217	2005/06	09.12.2005										21	29	1.400
BH09227	2005/06	07.12.2005	3015	B	0.353	0	82	6	11	0.07	20.23	22	27	0.073
BH09296	2005/06	13.12.2005	3083	C	0.686	0	58	28	13	0.48	13.64	20	24	-1.883
BH09347	2005/06	10.02.2006	3134	B	0.342	0	62	23	14	0.37	24.04	26	27	0.034
BH36321	2005/06	09.12.2005	3309	A	0.326		27	60	13	2.22	65.04	22	26	-0.585
BH36322	2005/06	13.12.2005	3310	A	0.466		42	30	21	0.71	38.20	21	27	0.083
BH36324	2005/06	13.12.2005	3312	A	0.784	0	58	18	19	0.31	11.21	24	25	-1.264
BH36339	2005/06	19.12.2005	3327	A	0.261		73	22	5	0.30	39.08	25	28	0.703
BH36379	2005/06	16.01.2006	3367	A	0.415	0	42	33	18	0.79	18.90	26	28	0.693
BH36381	2005/06	27.01.2006	3369	A	0.378	0	70	10	17	0.14	16.45	26	26	-0.624
BH36396	2005/06	10.02.2006	3384	A	0.245	0	70	7	18	0.10	8.47	25	27	0.044
BC92666	2006/07	30.10.2006	1976	B	0.082	0.11	61	21	15	0.34	32.94	25	28	0.703
BC92827	2006/07	15.11.2006	2135	D	0.158	0.22	50	22	23	0.44	15.66	26	27	0.034
BC92888	2006/07	27.10.2006	2196	C	0.199	5.53	50	25	22	0.50	13.38	27	25	-1.293

BH03933	2006/07	09.11.2006	2430	C	0.33	0.48	54	23	18	0.43	8.35	26	28	0.693
BH03981	2006/07	30.10.2006	2478	C	0.561	0	51	10	36	0.20	7.21	26	25	-1.283
BH04242	2006/07	24.11.2006	2734	C	0.587	0	69	11	14	0.16	11.66	26	25	-1.283
BH09214	2006/07	27.10.2006	3002	B	0.996	1.7	46	44	9	0.96	16.02	26	24	-1.942
BH09227	2006/07	09.11.2006	3015	C	0.246	0	53	35	11	0.66	19.02	26	26	-0.624
BH09239	2006/07	27.10.2006	3027	E	0.242	0	44	26	29	0.59	7.27	25	26	-0.615
BH09260	2006/07	22.11.2006	3047	C	3.333	0.13	52	13	32	0.25	7.90	25	25	-1.273
BH09418	2006/07	03.11.2006	3205	B	0.611	0	74	11	10	0.15	41.77	26	26	-0.624
BH09459	2006/07	24.11.2006	3246	B	0.096	0	81	13	6	0.16	23.73	25	27	0.044
BH09465	2006/07	06.11.2006	3252	B	1.466	0	55	17	24	0.31	21.14	24	25	-1.264
BH09491	2006/07	09.11.2006	3278	B	0.171	0	62	18	18	0.29	21.17	24	25	-1.264
BH36379	2006/07	27.10.2006										26	27	0.034
BH36384	2006/07	03.11.2006	3372	B	0.283		69	13	15	0.19	18.63	26	27	0.034
BH36471	2006/07	30.10.2006	3459	A	0.415	0	49	22	19	0.45	9.27	23	28	0.722
BH36477	2006/07	30.10.2006	3465	A	0.648	0	66	14	16	0.21	15.95	25	25	-1.273
BH36495	2006/07	03.11.2006	3483	A	0.474	0	32	53	10	1.66	19.78	25	28	0.703
BH36496	2006/07	03.11.2006	3484	A	0.142	0	67	22	8	0.33	24.16	26	26	-0.624
BH36547	2006/07	23.11.2006	3562	A	0.37	0	47	32	14	0.68	9.84	25	27	0.044
BH36559	2006/07	15.11.2006	3547	A	0.096	0	40	37	17	0.93	19.48	27	28	0.683
BH36590	2006/07	01.12.2006	3578	A	0.134		70	8	18	0.11	18.72	25	27	0.044
BH36596	2006/07	07.12.2006	3584	A	0.061		80	8	11	0.10	30.86	24	25	-1.264
BH36598	2006/07	07.12.2006	3586	A	0.119	0	80	6	11	0.08	17.16	24	29	1.371
BH36600	2006/07	13.12.2006	3588	A	0.069	0	44	33	17	0.75	16.66	25	27	0.044
BC92666	2007/08	29.02.2008	1976	C	0.131	0.1	81	9	6	0.11	35.06	25	30	2.020
BC92827	2007/08	23.01.2008	2135	E	1.129	0.82	81	12	3	0.14	24.12	25	30	2.020
BC92921	2007/08	26.02.2008	2227	E	0.515	0	68	14	16	0.21	8.15	24	25	-1.264
BH03963	2007/08	30.11.2007	2460	C	0.212	1.74	78	19	2	0.24	31.77	23	27	0.063
BH04054	2007/08	10.01.2008	2551	D	1.319	0.17	84	4	8	0.05	7.96	25	27	0.044
BH04236	2007/08	28.12.2007	2728	C	1.786	0	66	28	3	0.42	30.27	25	27	0.044
BH09029	2007/08	29.02.2008	2820	C	0.253	0	77	12	5	0.16	11.55	25	29	1.361
BH09197	2007/08	29.01.2008	2985	C	1.689	0.17	52	22	16	0.42	9.52	25	27	0.044
	2007/08	20.02.2008	2985	D	0.688	0.32	70	16	12	0.23	6.05	25	27	0.044
BH09214	2007/08	11.01.2008	3002	C	0.476	3.06	72	20	3	0.28	15.20	24	25	-1.264

BH09227	2007/08	10.01.2008	3015	D	0.866	0	77	13	5	0.17	14.78	24	27	0.054
	2007/08	22.02.2008	3015	E	0.162	0	66	10	20	0.15	7.72	24	27	0.054
BH09265	2007/08	29.01.2008	3052	D	0.618	0	55	28	10	0.52	12.17	23	25	-1.254
	2007/08	20.02.2008	3052	E	0.891	0	60	24	12	0.40	5.08	23	26	-0.595
BH09491	2007/08	19.12.2007	3278	C	1.204	0	59	17	21	0.29	15.09	22	28	0.732
	2007/08	29.02.2008	3278	D	0.063	0						22	27	0.073
BH36397	2007/08	29.02.2008	3385	B	0	0	74	13	10	0.17	7.47	25	28	0.703
BH36445	2007/08	19.12.2007	3433	A	2.207	0.12	56	36	8	0.64	12.01	24	29	1.371
BH36605	2007/08	27.11.2007	3593	A	1.626	2.98	79	10	7	0.13	24.57	24	29	1.371
	2007/08	19.02.2008	3593	B	1.172	1.25	55	33	3	0.60	15.65	24	28	0.712
BH36608	2007/08	30.11.2007	3596	A	1.039	0	81	9	6	0.11	19.63	24	29	1.371
	2007/08	26.02.2008	3596	B	0.54	0	78	11	7	0.14	14.11	25	26	-0.615
BH36611	2007/08	05.12.2007	3599	A	2.594	0.21	81	17	1	0.21	13.64	24	30	2.030
BH36613	2007/08	14.12.2007	3601	A	0.243	0.21	66	28	1	0.42	14.38	24	31	2.688
BH36615	2007/08	20.12.2007	3603	A	0.739	0	81	9	7	0.11	14.66	23	27	0.063
BH36616	2007/08	28.12.2007	3604	A	1.249	1.51	84	16	0	0.19	7.61	25	27	0.044
	2007/08	29.02.2008	3604	B	0.11	0.44	70	16	11	0.23	13.94	25	27	0.044
BH36617	2007/08	07.01.2008	3605	A	0.322	0	87	11	0	0.13	7.37	25	28	0.703
BH36624	2007/08	11.01.2008	3612	A	1.003	0.69	72	10	14	0.14	7.20	24	28	0.712
BH36628	2007/08	16.01.2008	3616	A	2.869	0	39	58	2	1.49	22.49	25	27	0.044
BH36631	2007/08	29.01.2008	3619	A	1.076	0	52	32	16	0.62	15.07	24	25	-1.264
BH36661	2007/08	25.02.2008	3649	A	0.348	0	70	19	8	0.27	15.90	24	26	-0.605
BH36667	2007/08	29.02.2008	3655	A	0.051	0	72	5	22	0.07	6.53	21	27	0.083

Appendix A3 – Territoriality, territory tenure and reproductive behaviour of wild males

Age, territoriality (yes/no), date of territory establishment, tenure [number of days], number of nests built, number of nests accepted and the residuals of nests accepted against built (male attractiveness), as well as the number of eggs laid in each nest, the number of young hatched and number of young fledged.

Ring number	Season	Age	Territorial yes/no	Territory	Territory establishment	Tenure (days)	Nests built	Nests accepted	Residuals	No. of eggs	No. of hatchlings	No. of fledglings
BC78299	2005/06		no									
BC92716	2005/06		yes	Z	16.11.2005	93	14	5	-0.89	10	8	4
BC92773	2005/06	2	yes	AJ	16.11.2005	111	13	4	-1.91	10	4	2
BC92882	2005/06		yes	AI	16.11.2005	98	3	0	-0.45	0	0	0
BH03882	2005/06	2	yes	BK	28.11.2005	96	7	1	-1.43	3	0	0
BH03981	2005/06		yes	AV	22.11.2005	87	11	1	-3.41	3	1	0
BH04140	2005/06	6	yes	BA	24.11.2005	101	14	7	1.11	16	7	1
BH09171	2005/06		yes	BG	28.11.2005	102	8	2	-0.92	5	3	0
BH09191	2005/06		no									
BH09217	2005/06		yes	CA	09.01.2006	42	4	1	0.05	2	2	0
BH09227	2005/06	4	yes	S	16.11.2005	33	1	1	1.54	4	0	0
BH09296	2005/06		yes	BU	09.12.2005	18	1	0	0.54	0	0	0
BH09347	2005/06	2	yes	BZ	06.01.2006	64	3	1	0.55	3	0	0
BH36321	2005/06	3	yes	BY	31.12.2005	53	4	1	0.05	2	2	0
BH36322	2005/06		yes	BV	09.12.2005	48			0.04			
BH36324	2005/06		yes	AY	24.11.2005	50	3	0	-0.45	0	0	0
BH36339	2005/06	5	yes	AS	22.11.2005	85	7	5	2.57	12	6	0
BH36379	2005/06	2	yes	BC	24.11.2005	75			-0.92			
BH36381	2005/06		yes	CD	29.01.2006	41	2	1	1.04	3	3	0
BH36396	2005/06	4	yes	AM	19.11.2005	108			0.59			
BC92666	2006/07		yes	J	07.10.2006	155	12	9	2.18	27	16	9
BC92827	2006/07	6	yes	AK	07.10.2006	122	11	7	0.72	16	5	0
BC92888	2006/07		yes	AC	07.10.2006	75	12	10	3.72	22	4	3

BH03933	2006/07	6	yes	M	07.10.2006	156	10	5	-0.75	15	10	6
BH03981	2006/07		yes	O	09.10.2006	155	12	6	-0.82	19	7	0
BH04242	2006/07	5	yes	AF	07.10.2006	156	10	7	1.79	21	6	0
BH09214	2006/07	3	yes	AE	07.10.2006	123	14	10	2.64	28	8	5
BH09227	2006/07		yes	S	07.10.2006	98	10	4	-1.21	12	7	4
BH09239	2006/07		yes	C	07.10.2006	98	6	1	-2.06	2	2	2
BH09260	2006/07	5	yes	BE	05.11.2006	78	6	1	-2.06	3	0	0
BH09418	2006/07	5	no									
BH09459	2006/07		yes	T	07.10.2006	123	11	8	1.72	21	2	2
BH09465	2006/07		no									
BH09491	2006/07		yes	H	07.10.2006	135	12	7	0.72	23	15	15
BH36379	2006/07		yes	AP	11.10.2006	118	10	5	-0.75	13	9	0
BH36384	2006/07	3	yes	AV	16.10.2006	115	5	0	-2.52	0	0	0
BH36471	2006/07		yes	F	07.10.2006	126	17	10	1.03	32	16	6
BH36477	2006/07		yes	B	07.10.2006	62	5	1	-1.52	1	0	0
BH36495	2006/07		no									
BH36496	2006/07		no									
BH36547	2006/07		no									
BH36559	2006/07	2	no									
BH36590	2006/07		yes	BG	23.11.2006	60	1	0	-0.37	0	0	0
BH36596	2006/07		no									
BH36598	2006/07		no									
BH36600	2006/07		no									
BC92666	2007/08		yes	G	09.11.2007	108	11	3	-0.71	8	5	0
BC92827	2007/08		yes	U	28.11.2007	89	9	4	1.82	12	5	5
BC92921	2007/08		yes	H	09.11.2007	109	10	4	1.06	13	5	2
BH03963	2007/08		no									
BH04054	2007/08		yes	E	09.11.2007	109	12	4	-0.09	10	8	0
BH04236	2007/08		yes	V	28.11.2007	89	5	1	-0.41	2	0	0
BH09029	2007/08		no									
BH09197	2007/08		yes	AZ	16.01.2008	44	5	2	0.59	6	6	0
BH09214	2007/08		yes	AJ	18.12.2007	72	2	0	0.12	0	0	0
BH09227	2007/08	7	yes	Q	26.11.2007	88	11	4	0.68	11	6	3
BH09265	2007/08	7	yes	W	28.11.2007	98	6	2	0.21	6	6	0

BH09491	2007/08		yes	F	09.11.2007	109	10	2	-0.56	5	2	0
BH36397	2007/08		yes	N	26.11.2007	100	8	4	1.44	12	6	0
BH36445	2007/08		yes	AE	28.11.2007	43	3	1	0.35	2	0	0
BH36605	2007/08		yes	T	26.11.2007	84	5	0	-2.56	0	0	0
BH36608	2007/08		yes	R	10.12.2007	91	5	3	1.59	9	3	3
BH36611	2007/08		no									
BH36613	2007/08	6	yes	AK	19.12.2007	68	6	2	0.21	7	4	0
BH36615	2007/08	6	yes	M	26.11.2007	88	7	1	-1.18	2	0	0
BH36616	2007/08	4	yes	L	12.11.2007	97	6	3	1.59	9	0	0
BH36617	2007/08		yes	BF	31.01.2008	17	1	0	0.12	0	0	0
BH36624	2007/08	4	no									
BH36628	2007/08		no									
BH36631	2007/08		yes	AT	10.01.2008	45	5	1	-0.03	3	3	3
BH36661	2007/08		yes	AI	18.12.2007	79	8	1	-1.18	1	0	0
BH36667	2007/08		no									

Appendix B1 – Plumage characteristics of aviary males 2007

Date and plumage characteristics (average brightness, chroma and hue) of the head (H) and the throat (T), as well as the average (AV) of the two areas of males participating in the 2007 contests.

Ring	Date	Average Brightness			Chroma			Hue		
		H	T	AV	H	T	AV	H	T	AV
5	25.05.2007	66.841	67.847	67.344	0.607	0.614	0.611	545.51	551.69	548.60
16	25.05.2007	27.289	37.390	32.340	0.760	0.689	0.725	558.99	557.07	558.03
86	25.05.2007	67.860	71.793	69.827	0.591	0.612	0.602	536.31	544.59	540.45
88	25.05.2007	62.693	74.656	68.675	0.590	0.601	0.596	547.23	545.86	546.54
94	25.05.2007	53.651	61.736	57.694	0.638	0.668	0.653	562.66	565.63	564.15
95	25.05.2007	26.222	36.887	31.555	0.831	0.715	0.773	570.29	556.96	563.63
109	25.05.2007	64.871	65.214	65.043	0.620	0.615	0.618	556.62	550.52	553.57
113	25.05.2007	31.839	29.933	30.886	0.740	0.743	0.742	558.90	562.43	560.67
114	25.05.2007	25.771	27.279	26.525	0.815	0.798	0.807	565.85	565.85	565.85
157	25.05.2007	60.179	67.982	64.081	0.535	0.586	0.561	492.90	538.97	515.93
159	25.05.2007	33.949	40.494	37.222	0.732	0.671	0.702	552.50	541.15	546.83
165	25.05.2007	73.582	52.476	63.029	0.592	0.423	0.508	540.23	504.93	522.58
169	25.05.2007	76.623	72.151	74.387	0.583	0.582	0.583	533.33	526.87	530.10
171	25.05.2007	27.910	36.589	32.249	0.697	0.666	0.682	547.34	542.41	544.88

Appendix B2 – Plumage characteristics of aviary males 2008, both manipulated and unmanipulated

Trial number, male ID and information on manipulation status (yes/no), as well as plumage characteristics (average brightness, chroma and hue) of the head (H), throat (T) and the average (AV) of the two areas of males participating in the 2008 contests.

Trial	Ring	Date	Manipulated yes/no	Average Brightness			Chroma			Hue		
				H	T	AV	H	T	AV	H	T	AV
1-1	5	07.07.2008	yes	17.563	16.299	16.931	0.994	1.019	1.007	606.45	607.12	606.79
1-1	88	07.07.2008	no	21.139	24.226	22.683	0.933	0.856	0.895	581.87	575.86	578.87
1-2	16	07.07.2008	no	34.020	17.469	25.745	0.770	0.265	0.518	563.81	507.60	535.71
1-2	94	07.07.2008	no	24.518	24.373	24.446	0.880	0.885	0.883	578.02	579.14	578.58
1-3	113	07.07.2008	yes	14.735	12.865	13.800	0.924	0.772	0.848	595.65	596.83	596.24
1-3	95	07.07.2008	yes	14.118	19.550	16.834	1.062	0.951	1.007	609.47	597.68	603.58
1-4	157	07.07.2008	yes	13.387	16.985	15.186	1.016	0.936	0.976	605.10	601.73	603.42
1-4	109	07.07.2008	no	23.619	33.193	28.406	0.901	0.744	0.823	584.36	570.96	577.66
1-5	165	07.07.2008	no	26.463	35.150	30.807	0.862	0.755	0.809	581.08	566.30	573.69
1-5	114	07.07.2008	yes	13.539	21.626	17.583	0.964	0.807	0.886	606.56	592.72	599.64
1-6	169	07.07.2008	no	32.110	42.243	37.177	0.766	0.679	0.723	564.03	550.09	557.06
1-6	159	07.07.2008	yes	15.528	21.151	18.340	0.948	0.885	0.917	607.23	599.14	603.18
2-1	169	09.07.2008	no	30.710	38.193	34.452	0.771	0.706	0.739	563.46	554.33	558.90
2-1	114	09.07.2008	yes	23.765	22.341	23.053	0.805	0.875	0.840	604.65	597.34	601.00
2-2	5	09.07.2008	yes	19.780	21.032	20.406	0.909	0.894	0.902	607.12	605.10	606.11
2-2	159	09.07.2008	yes	23.268	20.292	21.780	0.857	0.854	0.856	605.21	600.26	602.74
2-3	16	09.07.2008	no	34.077	39.842	36.960	0.762	0.700	0.731	566.99	561.64	564.32
2-3	88	09.07.2008	no	32.468	27.929	30.199	0.796	0.824	0.810	571.07	576.43	573.75
2-4	113	09.07.2008	yes	22.350	24.767	23.559	0.873	0.875	0.874	600.83	603.75	602.29
2-4	94	09.07.2008	no	25.934	31.328	28.631	0.792	0.815	0.804	573.25	578.47	575.86
2-5	157	09.07.2008	yes	26.009	24.175	25.092	0.803	0.843	0.823	601.05	602.40	601.73
2-5	95	09.07.2008	yes	20.186	26.253	23.220	0.850	0.809	0.830	606.67	597.00	601.84
2-6	165	09.07.2008	no	25.569	29.593	27.581	0.857	0.811	0.834	583.34	577.22	580.28
2-6	109	09.07.2008	no	26.701	25.213	25.957	0.835	0.857	0.846	579.27	583.79	581.53

3-1	165	11.07.2008	no	21.972	29.675	25.824	0.879	0.814	0.847	586.06	576.77	581.42
3-1	95	11.07.2008	yes	19.469	25.229	22.349	0.852	0.769	0.811	608.91	605.43	607.17
3-2	169	11.07.2008	no	37.641	35.255	36.448	0.698	0.722	0.710	552.49	557.07	554.78
3-2	109	11.07.2008	no	26.192	24.491	25.342	0.830	0.870	0.850	578.36	586.06	582.21
3-3	5	11.07.2008	yes	19.850	15.325	17.588	0.878	0.920	0.899	601.62	611.16	606.39
3-3	114	11.07.2008	yes	18.168	11.807	14.988	0.826	1.050	0.938	604.88	611.61	608.25
3-4	16	11.07.2008	no	33.289	31.991	32.640	0.761	0.780	0.771	565.86	566.88	566.37
3-4	159	11.07.2008	yes	20.696	14.606	17.651	0.840	0.944	0.892	604.31	611.72	608.02
3-5	113	11.07.2008	yes	19.804	15.084	17.444	0.875	0.945	0.910	602.52	607.57	605.04
3-5	88	11.07.2008	no	28.800	27.691	28.246	0.861	0.850	0.856	580.40	578.47	579.43
3-6	157	11.07.2008	yes	22.169	23.427	22.798	0.845	0.809	0.827	606.89	604.87	605.88
3-6	94	11.07.2008	no	25.283	28.019	26.651	0.816	0.830	0.823	575.97	580.96	578.47
4-1	157	13.07.2008	yes	22.955	23.417	23.186	0.823	0.833	0.828	603.07	603.97	603.52
4-1	88	13.07.2008	no	27.666	28.332	27.999	0.830	0.818	0.824	579.27	579.27	579.27
4-2	165	13.07.2008	no	23.636	42.875	33.256	0.855	0.648	0.752	580.63	555.48	568.05
4-2	94	13.07.2008	no	21.360	19.682	20.521	0.838	0.798	0.818	579.83	575.18	577.51
4-3	169	13.07.2008	no	36.148	39.064	37.606	0.723	0.698	0.711	556.39	554.90	555.65
4-3	95	13.07.2008	yes	15.933	16.820	16.377	0.920	0.876	0.898	612.83	604.54	608.68
4-4	5	13.07.2008	yes	16.273	23.686	19.980	0.951	0.822	0.887	609.47	606.90	608.18
4-4	109	13.07.2008	no	23.429	27.757	25.593	0.868	0.824	0.846	584.92	583.80	584.36
4-5	16	13.07.2008	no	38.114	36.753	37.434	0.733	0.780	0.757	561.97	567.90	564.93
4-5	114	13.07.2008	yes	17.347	20.646	18.997	0.835	0.839	0.837	608.02	604.20	606.11
4-6	113	13.07.2008	yes	22.374	18.700	20.537	0.845	0.891	0.868	603.08	609.36	606.22
4-6	159	13.07.2008	yes	18.053	19.610	18.832	0.915	0.893	0.904	606.33	606.78	606.55
5-1	113	15.07.2008	yes	21.868	14.730	18.299	0.840	0.900	0.870	604.43	611.83	608.13
5-1	114	15.07.2008	yes	19.085	17.520	18.303	0.830	0.833	0.832	606.00	601.84	603.92
5-2	157	15.07.2008	yes	26.244	20.379	23.312	0.800	0.820	0.810	604.31	607.01	605.66
5-2	159	15.07.2008	yes	15.635	18.087	16.861	0.932	0.882	0.907	609.36	606.22	607.79
5-3	165	15.07.2008	no	22.746	24.170	23.458	0.874	0.754	0.814	583.91	571.42	577.67
5-3	88	15.07.2008	no	28.700	20.856	24.778	0.795	0.815	0.805	571.20	573.13	572.17
5-4	169	15.07.2008	no	30.320	28.936	29.628	0.735	0.729	0.732	558.79	557.30	558.04
5-4	94	15.07.2008	no	20.538	22.032	21.285	0.867	0.875	0.871	580.74	584.47	582.61
5-5	5	15.07.2008	yes	19.681	15.650	17.666	0.860	0.896	0.878	604.42	605.55	604.99
5-5	95	15.07.2008	yes	16.868	21.454	19.161	0.891	0.850	0.871	607.57	603.30	605.43

5-6	16	15.07.2008	no	32.605	27.873	30.239	0.732	0.679	0.706	563.58	552.59	558.09
5-6	109	15.07.2008	no	23.862	20.066	21.964	0.850	0.867	0.859	583.23	585.49	584.36
6-1	16	17.07.2008	no	32.613	23.349	27.981	0.748	0.811	0.780	563.01	565.73	564.37
6-1	95	17.07.2008	yes	15.174	15.317	15.246	0.975	0.965	0.970	610.03	608.80	609.42
6-2	113	17.07.2008	yes	20.137	10.467	15.302	0.909	1.071	0.990	607.23	613.84	610.54
6-2	109	17.07.2008	no	23.263	19.380	21.322	0.896	0.920	0.908	584.13	585.26	584.70
6-3	157	17.07.2008	yes	19.648	17.672	18.660	0.764	0.855	0.810	610.04	607.68	608.86
6-3	114	17.07.2008	yes	15.003	11.780	13.392	0.835	0.938	0.887	610.37	610.93	610.65
6-4	165	17.07.2008	no	24.878	28.262	26.570	0.866	0.808	0.837	582.89	579.03	580.96
6-4	159	17.07.2008	yes	13.994	12.344	13.169	0.969	1.004	0.987	614.63	606.56	610.60
6-5	169	17.07.2008	no	24.920	36.586	30.753	0.790	0.674	0.732	566.54	550.78	558.66
6-5	88	17.07.2008	no	25.790	25.944	25.867	0.870	0.873	0.872	576.66	577.79	577.23
6-6	5	17.07.2008	yes	13.091	20.149	16.620	0.919	0.775	0.847	615.75	610.15	612.95
6-6	94	17.07.2008	no	24.200	22.847	23.524	0.862	0.827	0.845	577.10	574.25	575.67

Appendix B3 – Age categories and testosterone levels of all males during the 2007 and 2008 contests

Age category (2007: 1 = younger than 6, 2 = older than 6; 2008: 1 = younger than 7, 2 = older than 7) and testosterone levels [ng/ml] before and after moult in 2007, as well as before moult, after moult and just before contests began in 2008.

Ring	**Age Category**	**2007**				**2008**					
		before moult		**after moult**		**before moult**		**after moult**		**before contests**	
		Date	**T. [ng/ml]**	**Date**	**T. [ng/ml]**	**Date**	**T. [ng/ml]**	**Date**	**T. [ng/ml]**	**Date**	**T. [ng/ml]**
5	2	12.02.2007	0.09	23.05.2007	0.80	29.01.2008	1.55	16.05.2008	0.70	25.06.2008	0.62
16	2	12.02.2007	0.05	23.05.2007	0.90	29.01.2008	0.50	16.05.2008	0.77	25.06.2008	0.29
86	2	12.02.2007	0.13	23.05.2007	0.92						
88	2	12.02.2007	0.14	23.05.2007	0.29	29.01.2008	0.81	16.05.2008	0.25	25.06.2008	0.25
94	2	12.02.2007	0.14		.	29.01.2008	0.00		.	25.06.2008	0.40
95	2	12.02.2007	0.03	23.05.2007	0.36	29.01.2008	0.00	16.05.2008	0.20	25.06.2008	0.16
109	2	12.02.2007	0.06	23.05.2007	0.23	29.01.2008	0.00		.	25.06.2008	0.19
113	1	12.02.2007	0.00	23.05.2007	0.95	29.01.2008	0.03	16.05.2008	0.41	25.06.2008	0.31
114	1	12.02.2007	0.17	23.05.2007	1.82	29.01.2008	0.10	16.05.2008	0.73	25.06.2008	0.53
157	1	12.02.2007	0.05	23.05.2007	1.35	29.01.2008	0.08	16.05.2008	0.91	25.06.2008	1.89
159	1	12.02.2007	0.05	23.05.2007	2.33	29.01.2008	0.05	16.05.2008	0.85	25.06.2008	0.21
165	1	12.02.2007	0.04	23.05.2007	0.87		.	16.05.2008	0.49	25.06.2008	0.24
169	1	12.02.2007	0.07	23.05.2007	1.04	29.01.2008	0.09	16.05.2008	0.46	25.06.2008	0.13
171	1	12.02.2007	0.10	23.05.2007	0.93						

Appendix B4 – Body condition and behaviour of participating males in the 2007 contests

Male ID, trial number, weight [g], tarsus length [mm] and body condition index (BCI) are given for each male in every contest, along with the number of times the observed male threatened his opponent (threat) and the number of times he was threatened (thrtnd), the amount of times he won (win) or lost (lose) an interaction, or the interaction outcome was undecided (draw). Further, the amount of "wins – losses" (WML) are given, along with if a male won (= 1) or lost (= 0) the entire trial (WLC; win/lose contest). Draws are represented by "9". The amount of times the observed male fled from his opponent (flight), the time spent at the feeder (time [s]) and the amount of worms eaten (worms), as well as – in seconds – the time spent flying (fly), approaching the feeder (appr.) and sitting in a corner (corner) are also given.

Trial	Ring	Weight	Tarsus	BCI	Threat	Thrtnd	Win	Draw	Lose	WML	WLC	Flight	Time [s]	Worms	Fly	Appr.	Corner
1-1	86	32.0	24.8	1.88	0	5	3	2	0	3	1	2	329	9	0	82	775
1-1	5	39.9	25.1	9.20	5	0	0	2	3	-3	0	12	488	8	19	144	1119
1-3	88	34.1	23.0	7.48	0	0	0	0	0	0	9	2	9	0	77	0	1411
1-2	16	31.1	25.9	-1.15	0	0	0	0	0	0	9	1	764	16	18	27	658
1-2	94	21.9	23.1	-4.92	0	2	0	1	1	-1	0	8	4	1	49	24	1409
1-3	95	27.3	24.3	-1.85	2	0	1	1	0	1	1	0	983	15	0	40	461
1-4	109	26.7	22.6	0.85	0	1	1	0	0	1	1	0	1030	12	0	38	434
1-4	113	33.0	24.9	2.69	1	0	0	0	1	-1	0	2	78	6	0	0	1410
1-5	114	32.9	24.7	2.98	0	1	0	0	1	-1	0	26	29	4	71	24	1346
1-5	157	32.2	23.9	3.83	1	0	1	0	0	1	1	0	832	9	11	45	562
1-6	159	29.8	25.5	-1.68	2	1	0	3	0	0	9	8	9	1	52	38	1350
1-6	165	29.4	23.9	1.03	1	2	0	3	0	0	9	9	4	0	55	0	1437
1-7	169	33.0	23.5	5.41	0	0	0	0	0	0	9	1	245	10	0	3	1248
1-7	171	25.8	24.0	-2.77	0	0	0	0	0	0	9	1	137	6	2	33	1320
2-1	171		24.0		0	0	0	0	0	0	9	17	273	6	6	67	1127
2-1	16		25.9		0	0	0	0	0	0	9	9	958	11	19	32	479

2-2	86		24.8		0	5	0	4	1	-1	0	1	680	9	8	132	482
2-2	95		24.3		5	0	1	4	0	1	1	7	502	9	43	77	835
2-3	88		23.0		1	4	0	1	4	-4	0	0	0	0	34	0	1434
2-3	113		24.9		4	1	4	1	0	4	1	0	179	12	10	71	1211
2-4	94		23.1		0	0	0	0	0	0	9	2	33	3	22	117	1314
2-4	157		23.9		0	0	0	0	0	0	9	0	388	9	4	21	1084
2-5	109		22.6		0	4	0	4	0	0	9	0	1312	14	0	8	177
2-5	165		23.9		4	0	0	4	0	0	9	7	107	3	6	72	1249
2-6	114		24.7		0	0	0	0	0	0	9	2	17	2	70	5	1399
2-6	169		23.5		0	0	0	0	0	0	9	4	27	3	48	10	1406
2-7	159		25.5		0	0	0	0	0	0	9	17	128	5	96	65	1183
2-7	5		25.1		0	0	0	0	0	0	9	4	187	13	20	69	1214
3-1	159		25.5		5	2	2	4	1	1	1	1	29	6	19	45	1383
3-1	95		24.3		2	5	1	4	2	-1	0	2	200	10	11	163	1091
3-2	171		24.0		0	2	0	2	0	0	9	0	143	6	6	49	1294
3-2	113		24.9		2	0	0	2	0	0	9	0	512	12	29	19	923
3-3	86		24.8		17	0	0	17	0	0	9	0	508	8	6	43	907
3-3	157		23.9		0	17	0	17	0	0	9	9	360	10	17	31	1024
3-4	88		23.0		0	8	0	7	1	-1	0	0	1	0	96	2	1356
3-4	165		23.9		8	0	1	7	0	1	1	4	39	3	40	138	1186
3-5	94		23.1		0	0	0	0	0	0	9	0	47	6	0	246	1199
3-5	169		23.5		0	0	0	0	0	0	9	0	0	0	64	0	1434
3-6	109		22.6		0	0	0	0	0	0	9	2	760	16	0	107	627
3-6	5		25.1		0	0	0	0	0	0	9	0	16	3	21	75	1386
3-7	114		24.7		0	0	0	0	0	0	9	1	66	11	27	47	1357
3-7	16		25.9		0	0	0	0	0	0	9	1	975	10	0	125	397
4-1	114	31.5	24.7	1.58	0	1	0	1	0	0	9	1	116	11	0	28	1352
4-1	113	29.4	24.9	-0.91	1	0	0	1	0	0	9	0	367	5	0	29	1099
4-2	159	31.3	25.5	-0.18	3	2	1	4	0	1	1	0	22	6	34	9	1415
4-2	157	30.2	23.9	1.83	2	3	0	4	1	-1	0	0	149	9	19	32	1288
4-3	171	28.0	24.0	-0.57	0	2	0	1	1	-1	0	0	142	8	3	62	1284
4-3	165	26.8	23.9	-1.57	2	0	1	1	0	1	1	0	320	10	0	259	910
4-4	86	28.1	24.8	-2.02	0	0	0	0	0	0	9	0	308	18	0	134	1056
4-4	169	29.1	23.5	1.51	0	0	0	0	0	0	9	0	0	0	111	0	1386

4-5	88	30.3	23.0	3.68	0	0	0	0	0	0	9	14	79	8	18	163	1221
4-5	5	34.5	25.1	3.80	0	0	0	0	0	0	9	12	187	9	0	257	1043
4-6	94	23.7	23.1	-3.12	0	1	0	0	1	-1	0	2	39	7	0	98	1355
4-6	16	33.6	25.9	1.35	1	0	1	0	0	1	1	2	274	12	0	400	816
4-7	109	26.3	22.6	0.45	0	11	4	3	4	0	9	4	449	9	0	200	786
4-7	95	27.2	24.3	-1.95	11	0	4	3	4	0	9	14	130	7	11	97	1129
5-1	109	25.8	22.6	-0.05	0	0	0	0	0	0	9	2	1177	9	0	22	297
5-1	157	30.1	23.9	1.73	0	0	0	0	0	0	9	7	202	9	14	67	1208
5-2	114	31.7	24.7	1.78	1	0	1	0	0	1	1	2	79	12	4	40	1369
5-2	165	24.7	23.9	-3.67	0	0	0	0	1	-1	0	1	90	4	3	161	1243
5-3	159	30.2	25.5	-1.28	0	0	0	0	0	0	9	2	4	0	186	23	1281
5-3	169	29.4	23.5	1.81	0	0	0	0	0	0	9	0	0	0	201	0	1297
5-4	171	27.0	24.0	-1.57	0	0	0	0	0	0	9	1	262	9	0	141	1095
5-4	5	35.1	25.1	4.40	0	0	0	0	0	0	9	0	399	12	2	173	924
5-5	86	29.0	24.8	-1.12	5	0	3	2	0	3	1	0	632	8	0	159	693
5-5	16	33.4	25.9	1.15	0	2	0	2	0	0	9	10	451	11	5	135	887
5-6	88	29.2	23.0	2.58	0	9	0	2	7	-7	0	4	97	4	21	129	1227
5-6	95	27.7	24.3	-1.45	9	0	7	2	0	7	1	0	504	13	1	167	795
5-7	94	22.7	23.1	-4.12	0	0	0	0	0	0	9	0	81	9	0	98	1319
5-7	113	29.1	24.9	-1.21	0	0	0	0	0	0	9	1	134	9	0	1	1362
6-1	94	20.0	23.1	-6.82	2	0	2	0	0	2	1	1	127	12	6	39	1356
6-1	165	24.5	23.9	-3.87	0	2	0	0	2	-2	0	2	236	5	0	173	1076
6-2	109	26.1	22.6	0.25	0	0	0	0	0	0	9	0	727	17	3	4	763
6-2	169	30.2	23.5	2.61	0	0	0	0	0	0	9	9	16	0	117	3	1348
6-3	114	31.9	24.7	1.98	0	2	0	0	2	-2	0	1	45	10	11	14	1423
6-3	5	35.0	25.1	4.30	2	0	2	0	0	2	1	1	145	7	4	8	1335
6-4	159	30.1	25.5	-1.38	0	0	0	0	0	0	9	2	8	1	93	11	1383
6-4	16	33.5	25.9	1.25	0	0	0	0	0	0	9	0	1187	19	35	64	212
6-5	171	26.0	24.0	-2.57	0	2	0	1	1	-1	0	6	368	6	2	117	989
6-5	95	27.7	24.3	-1.45	2	0	1	1	0	1	1	0	376	11	0	138	977
6-6	86	28.1	24.8	-2.02	10	0	9	1	0	9	1	1	1059	12	0	62	348
6-6	113	28.1	24.9	-2.21	0	10	0	1	9	-9	0	10	92	9	2	15	1355
6-7	88	27.7	23.0	1.08	0	0	0	0	0	0	9	6	73	7	42	12	1364
6-7	157	30.2	23.9	1.83	0	0	0	0	0	0	9	0	793	10	5	13	687

7-1	88	27.6	23.0	0.98	0	0	0	0	0	0	9	0	115	8	5	28	1349
7-1	169	29.6	23.5	2.01	0	0	0	0	0	0	9	0	0	0	0	0	1498
7-2	94	22.3	23.1	-4.52	0	0	0	0	0	0	9	3	206	9	6	62	1220
7-2	5	34.3	25.1	3.60	0	0	0	0	0	0	9	1	84	6	0	25	1388
7-3	109	26.2	22.6	0.35	0	2	0	0	2	-2	0	0	640	11	1	25	827
7-3	16	32.3	25.9	0.05	2	0	2	0	0	2	1	26	275	10	7	251	940
7-4	114	31.8	24.7	1.88	0	1	0	1	0	0	9	4	106	7	36	9	1342
7-4	95	27.2	24.3	-1.95	1	0	0	1	0	0	9	0	710	10	0	116	672
7-5	159	29.8	25.5	-1.68	0	0	0	0	0	0	9	0	336	9	2	44	1116
7-5	113	27.0	24.9	-3.31	0	0	0	0	0	0	9	1	196	11	0	9	1292
7-6	171	25.6	24.0	-2.97	1	2	1	1	1	0	9	3	290	10	25	57	1122
7-6	157	30.0	23.9	1.63	2	1	1	1	1	0	9	1	730	7	10	78	673
7-7	86	28.7	24.8	-1.42	0	0	0	0	0	0	9	0	744	11	5	31	718
7-7	165	24.4	23.9	-3.97	0	0	0	0	0	0	9	25	95	8	18	167	1189

Appendix B5 – Body condition and behaviour of participating males in the 2008 contests

Male ID (ring), trial number, weight [g], tarsus length [mm] and body condition index (BCI) are given for each male in every contest, along with the number of times the observed male threatened his opponent (threat) and the number of times he was threatened (thrtnd), the amount of times he won (win) or lost (lose) an interaction, or the interaction outcome was undecided (draw). Further, the amount of "wins – losses" (WML) are given, along with if a male won (= 1) or lost (= 0) the entire trial (WLC; win or lose contest). Draws are represented by "9". The amount of times the observed male fled from his opponent (flight), the time spent at the feeder (time [s]) and the amount of worms eaten (worms), as well as – in seconds – the time spent flying (fly), approaching the feeder (appr.) and sitting in a corner (corner) are also given.

Trial	Ring	Weight	Tarsus	BCI	Threat	Thrtnd	Win	Draw	Lose	WML	WLC	Flight	Time [s]	Worms	Fly	Appr.	Corner
1-1	5	33.0	25.1	5.73	0	1	1	0	0	1	1	1	141	8	1	89	1269
1-1	88	22.5	23.0	0.00	1	0	0	0	1	-1	0	11	167	9	11	4	1318
1-3	16	34.0	25.9	4.91	4	0	0	4	0	0	9	0	972	12	17	18	493
1-2	94	22.0	23.1	-0.73	0	4	0	4	0	0	9	15	0	0	16	18	1466
1-2	113	23.5	24.9	-3.32	3	14	1	3	13	-12	0	5	242	8	1	81	1176
1-3	95	25.5	24.3	0.05	14	3	13	3	1	12	1	0	447	11	45	21	987
1-4	157	33.5	23.9	8.96	0	1	0	0	1	-1	0	4	906	7	4	92	498
1-4	109	20.0	22.6	-1.59	1	0	1	0	0	1	1	0	402	12	0	31	1067
1-5	165	25.5	23.9	0.96	0	0	0	0	0	0	9	3	125	6	4	55	1316
1-5	114	28.5	24.7	2.14	0	0	0	0	0	0	9	0	512	9	3	15	970
1-6	169	32.5	23.5	8.87	0	0	0	0	0	0	9	0	0	0	62	0	1438
1-6	159	28.5	25.5	0.32	0	0	0	0	0	0	9	0	0	0	34	0	1466
2-1	169	30.0	23.5	6.37	0	0	0	0	0	0	9	0	0	0	9	0	1491
2-1	114	28.5	24.7	2.14	0	0	0	0	0	0	9	0	0	0	13	0	1487
2-2	5	31.0	25.1	3.73	1	0	1	0	0	1	1	2	77	15	1	66	1356
2-2	159	26.5	25.5	-1.68	0	1	0	0	1	-1	0	6	14	3	28	138	1320

2-3	16	32.0	25.9	2.91	0	2	0	1	1	-1	0	0	970	5	1	18	511
2-3	88	22.0	23.0	-0.50	2	0	1	1	0	1	1	6	146	15	5	30	1319
2-4	113	23.5	24.9	-3.32	2	0	2	0	0	2	1	0	1191	20	0	33	276
2-4	94	20.5	23.1	-2.23	0	2	0	0	2	-2	0	1	0	0	0	19	1481
2-5	157	31.0	23.9	6.46	0	4	0	0	4	-4	0	0	37	6	0	2	1461
2-5	95	24.5	24.3	-0.95	4	0	4	0	0	4	1	0	156	8	0	4	1340
2-6	165	23.5	23.9	-1.04	0	1	0	0	1	-1	0	6	64	0	49	48	1339
2-6	109	20.0	22.6	-1.59	1	0	1	0	0	1	1	0	1140	18	0	117	243
3-1	165	22.0	23.9	-2.54	0	2	0	0	2	-2	0	9	181	5	25	48	1246
3-1	95	22.5	24.3	-2.95	2	0	2	0	0	2	1	0	796	15	3	16	685
3-2	169	28.5	23.5	4.87	0	0	0	0	0	0	9	0	0	0	18	0	1482
3-2	109	19.5	22.6	-2.09	0	0	0	0	0	0	9	0	1495	20	0	3	2
3-3	5	29.5	25.1	2.23	1	0	1	0	0	1	1	2	130	16	4	67	1299
3-3	114	26.5	24.7	0.14	0	1	0	0	1	-1	0	2	40	2	5	98	1357
3-4	16	30.0	25.9	0.91	0	0	0	0	0	0	9	0	928	13	10	31	531
3-4	159	24.5	25.5	-3.68	0	0	0	0	0	0	9	0	0	0	22	0	1478
3-5	113	23.5	24.9	-3.32	4	0	1	3	0	1	1	1	808	4	0	69	623
3-5	88	22.0	23.0	-0.50	0	4	0	3	1	-1	0	1	195	13	0	4	1301
3-6	157	29.5	23.9	4.96	0	0	0	0	0	0	9	0	510	14	23	11	956
3-6	94	20.5	23.1	-2.23	0	0	0	0	0	0	9	3	29	3	38	92	1341
4-1	157	28.5	23.9	3.96	3	1	4	0	0	4	1	1	400	8	40	14	1046
4-1	88	20.5	23.0	-2.00	1	3	0	0	4	-4	0	1	135	5	60	77	1228
4-2	165	22.0	23.9	-2.54	0	0	0	0	0	0	9	0	176	9	13	24	1287
4-2	94	19.5	23.1	-3.23	0	0	0	0	0	0	9	0	29	5	9	45	1417
4-3	169	27.5	23.5	3.87	0	0	0	0	0	0	9	0	0	0	17	0	1483
4-3	95	22.0	24.3	-3.45	0	0	0	0	0	0	9	0	368	16	1	8	1123
4-4	5	29.0	25.1	1.73	2	5	2	0	5	-3	0	4	28	5	26	40	1406
4-4	109	19.5	22.6	-2.09	5	2	5	0	2	3	1	0	186	11	0	65	1249
4-5	16	28.5	25.9	-0.59	1	0	1	0	0	1	1	0	344	6	7	38	1111
4-5	114	25.5	24.7	-0.86	0	1	0	0	1	-1	0	1	82	10	8	94	1316
4-6	113	23.5	24.9	-3.32	2	8	7	3	0	7	1	0	130	16	0	12	1358
4-6	159	24.0	25.5	-4.18	8	2	0	3	7	-7	0	3	117	0	0	168	1215
5-1	113	24.5	24.9	-2.32	2	0	1	1	0	1	1	0	1493	11	0	0	7
5-1	114	26.5	24.7	0.14	0	2	0	1	1	-1	0	0	13	4	0	33	1454

5-2	157	28.5	23.9	3.96	0	0	0	0	0	0	9	0	456	14	0	4	1040
5-2	159	24.5	25.5	-3.68	0	0	0	0	0	0	9	1	25	5	0	41	1434
5-3	165	22.0	23.9	-2.54	0	0	0	0	0	0	9	1	37	5	23	16	1424
5-3	88	21.5	23.0	-1.00	0	0	0	0	0	0	9	0	0	0	25	0	1475
5-4	169	27.0	23.5	3.37	0	0	0	0	0	0	9	0	0	0	8	0	1492
5-4	94	21.0	23.1	-1.73	0	0	0	0	0	0	9	0	0	0	0	0	1500
5-5	5	29.0	25.1	1.73	0	3	0	1	2	-2	0	5	65	7	18	44	1373
5-5	95	25.0	24.3	-0.45	3	0	2	1	0	2	1	0	495	13	9	34	962
5-6	16	28.5	25.9	-0.59	0	1	0	0	1	-1	0	0	42	1	1	4	1453
5-6	109	20.0	22.6	-1.59	1	0	1	0	0	1	1	0	1098	19	0	0	402
6-1	16	27.5	25.9	-1.59	0	10	0	1	9	-9	0	3	195	4	5	15	1285
6-1	95	22.0	24.3	-3.45	10	0	9	1	0	9	1	0	606	16	1	39	854
6-2	113	23.5	24.9	-3.32	2	0	0	0	2	-2	0	1	64	0	1	54	1381
6-2	109	19.5	22.6	-2.09	0	2	2	0	0	2	1	0	1169	17	0	0	331
6-3	157	27.5	23.9	2.96	1	0	1	0	0	1	1	0	452	10	79	10	959
6-3	114	26.5	24.7	0.14	0	1	0	0	1	-1	0	2	60	10	53	14	1373
6-4	165	21.5	23.9	-3.04	0	1	0	0	1	-1	0	3	22	2	3	120	1355
6-4	159	24.0	25.5	-4.18	1	0	1	0	0	1	1	0	61	11	0	19	1420
6-5	169	26.0	23.5	2.37	0	0	0	0	0	0	9	0	0	0	0	0	1500
6-5	88	21.5	23.0	-1.00	0	0	0	0	0	0	9	0	163	20	0	1	1336
6-6	5	28.0	25.1	0.73	0	0	0	0	0	0	9	0	112	15	0	127	1261
6-6	94	20.5	23.1	-2.23	0	0	0	0	0	0	9	0	5	2	1	57	1437

SUMMARY

Summary

Bright red, orange or yellow plumage colouration is a common ornamental trait in males of many bird species. This plumage is carotenoid-based and directly linked to an individual's diet, as carotenoids cannot be synthesized and need to be ingested. There are various hypotheses linking carotenoid-based plumage colouration to several aspects of an individual, such as paternal care, immunological condition, parasite load, testosterone, or dominance status, but, in my thesis, I concentrated on three of these: (1) The Hamilton-Zuk hypothesis (1982) postulates that brighter males have a lower degree of parasitisation, enabling females to use this bright plumage colouration as a quality signal. (2) Folstad and Karter's Immunocompetence Handicap Hypothesis (ICHH, 1992) tries to explain the mechanism behind the expression of ornaments, with testosterone functioning as a "double-edged sword" – on the one hand it contributes to the enhancement of a trait, on the other, it compromises the immune system. Therefore, not unlike the Hamilton-Zuk hypothesis, it attributes a quality signalling function to ornamental traits, as only males in the best condition are able to simultaneously display bright plumage and have an intact immune system. (3) The "Status Signalling Hypothesis" (Rohwer 1975, 1982) revolves around a signalling mechanism allowing rivals to easily assess one another's fighting ability via colour badges (which can be influenced by testosterone levels, age or body condition), thereby reducing the risk of engaging in costly fighting. In most cases, the individual with the larger or more intense colour signal is dominant.

In my thesis, I presented three studies revolving around the brilliant red plumage displayed by male red bishops (*Euplectes orix*), a highly polygynous and colonial breeding weaver bird species of Sub-Saharan Africa. The aim was to determine (a) how much information on individual quality is conveyed by plumage colouration, (b) how plumage influences reproductive performance and (c) if plumage characteristics are used as a dominance signal in this species.

In the first part of my thesis, I examined which factors (body condition, testosterone, blood parasite load, immunological condition) affect both plumage colouration and brightness in wild red bishop males. I was not able to lend clear support to either the Hamilton-Zuk hypothesis or the ICHH. Rather, a complex pattern between plumage

and the heterophil/lymphocyte ratio (reflecting physiological stress and immunological condition), as well as parasitisation was revealed, which varied considerably between seasons, suggesting a strong influence of environmental conditions. Further, the overall condition of individual males also strongly affected the relationship between plumage traits and the other factors. On the one hand, males in bad condition that allocate their limited resources to plumage elaboration suffer from a decrease in immunological condition and parasite resistance, while, on the other hand, males in good condition are able to simultaneously invest in both ornamental traits and immune functions. This leads to the conclusion that in terms of female choice, potential mates cannot rely on plumage characteristics alone – they also need to assess other factors giving information on male health (i.e., behaviour) in order to obtain a clear impression of actual male quality. Another aspect investigated was the relationship between the two plumage traits and age. While brightness decreases linearly with age, plumage colouration showed a quadratic relationship with age, leading to the conclusion that both characteristics have the potential to be used as reliable age indicators. It remains to be investigated, if reproductive success and survival follow a similar pattern.

In the second part of my thesis, I concentrated on the influence of plumage characteristics, testosterone and parasites on male reproductive behaviour (reproductive effort and success), including the question as to which of these factors determine territoriality. I was able to determine that, contrary to expectations, plumage colouration seemed to have no effect on either reproductive effort or success, while brightness was positively linked to reproductive effort in terms of number of nests built. I was further able to determine a form of allocation trade-off, as males building many nests also suffered from increased parasitemia. However, reproductive success was negatively influenced by blood parasite load. Lastly, I found no differences between territorial males and floaters. To conclude, I was not able to verify the Hamilton-Zuk Hypothesis in this chapter. It remains to be investigated, if the red-orange plumage of wild red bishop males plays a role in status signalling.

The determination if plumage colouration is used as status signal in red bishops was the topic of the last part of my thesis. According to the Status Signalling Hypothesis, males with larger and/or more brilliant ornaments are dominant over individuals with smaller or duller ones. In order to test this hypothesis, I carried out two sets of experiments by staging male-male contests with unfamiliar males over a limited food source in the aviary. In the first block, competing males remained unmanipulated, while in the second block, those males with the lowest chroma values had their plumage manipulated to a more intense red colouration. In both experimental blocks I expected males with more intense plumage colouration to be more dominant. Including age, testosterone levels and body condition in the analysis of contest outcome, the obtained results were somewhat surprising. I found a very strong age effect in both seasons, with older males constantly dominating younger individuals regardless of plumage colouration, testosterone levels or body condition. The obtained results suggest that the outcome of male-male contests is determined by age-related acquired experience and not by the status signalling function of plumage traits. In order to detangle this age effect from the potential signalling effects of plumage colouration, further plumage-manipulation experiments need to be carried out with individuals of similar age.

To summarize, the analysis of carotenoid-based plumage ornaments in male red bishops and its influence on reproductive behaviour and male dominance has proven to be less straight-forward than expected. There were no clear and consistent effects of plumage ornaments on any component of male reproductive performance, and several correlations involving plumage brightness and/or colouration varied considerably among seasons. Furthermore, the relationships between plumage traits and reproductive parameters as well as dominance status were often influenced by male age, health or immunological condition. Thus, I was not able to fully support or deny either the Hamilton-Zuk or the ICHH in terms of plumage colouration and quality, and I could not support Rohwer’s Status Signalling Hypothesis.

Zusammenfassung auf Deutsch

Leuchtend rotes Gefieder ist ein häufig auftretendes sekundäres Geschlechtsmerkmal bei den Männchen vieler Vogelarten. Diese Gefiederfärbung basiert auf Carotinoiden, welche durch die Nahrung aufgenommen werden müssen, da Tiere sie nicht selbst synthetisieren können. Es gibt einige Hypothesen, nach denen carotinoid-basierte Gefiederfärbung mit den unterschiedlichsten Aspekten bzw. Merkmalen eines Männchens wie z.B. väterliche Brutpflege, Gesundheitszustand, Parasitenbefall oder Testosteron in Zusammenhang steht, wobei ich mich in meiner Doktorarbeit auf folgende drei Hypothesen konzentriert habe: (1) Die Hamilton-Zuk Hypothese (1982) postuliert, dass Männchen mit röterem Gefieder einen niedrigeren Parasitenbefall aufweisen, wodurch es Weibchen ermöglicht wird, dieses Merkmal als Qualitätssignal zu verwenden. (2) Die „Immunocompetence Handicap“ Hypothese (ICHH) von Folstad und Karter (1992) versucht den Mechanismus zu erklären, welcher hinter der Ausprägung eines Ornaments steckt, wobei Testosteron als zweischneidiges Schwert fungiert – einerseits trägt es zur Verstärkung des Merkmals bei, andererseits beeinträchtigt es jedoch das Immunsystem. Demzufolge spricht die ICHH – wie die Hypothese von Hamilton und Zuk – diesen Merkmalen eine Signalfunktion zu, da nur Männchen in einem sehr guten Gesundheitszustand leuchtendes Gefieder ausbilden können, während sie gleichzeitig ein intaktes Immunsystem aufweisen. (3) Die „Status Signalling“ Hypothese (Rohwer 1975, 1982) besagt, dass es innerhalb einer Art einen Signalmechanismus gibt, welcher es konkurrierenden Individuen ermöglicht die Wettbewerbsfähigkeit seines Rivalen einzuschätzen ohne tatsächlich miteinander kämpfen zu müssen. Dies geschieht durch Größe und/oder Intensität von Farbsignalen, die meistens von anderen Aspekten, wie z.B. Körperzustand, Testosteron oder Alter beeinflusst werden. In den meisten Fällen ist das Individuum mit dem größeren und/oder intensiveren Signal dominant.

In der vorliegenden Doktorarbeit habe ich drei Studien präsentiert, in denen ich mich mit dem leuchtend roten Gefieder von männlichen Oryxwebern (*Euplectes orix*) beschäftigt habe. Der Oryxweber ist eine polygyne Webervogelart, welche im südlichen Afrika vorkommt und dort in Kolonien brütet. Das Ziel der Arbeit war zu bestimmen (a) wie viel Information über individuelle Qualität durch Gefiederfärbung vermittelt wird, (b) in wie fern Gefiedermerkmale Fortpflanzungsaufwand und –erfolg

beeinflussen und (c) ob Gefiedermerkmale beim Oryxweber als Statussignal im Rahmen von Männchen-Männchen Interaktionen verwendet werden.

In dem ersten Teil meiner Doktorarbeit habe ich untersucht, welche Faktoren (Körperzustand, Testosteron, Blutparasitenbefall, Gesundheitszustand) einen Einfluss auf die Gefiederintensität (Summe der Gesamtreflektanz über den gesamten Wellenlängenbereich) und –farbe bei freilebenden Oryxwebermännchen haben. Die Ergebnisse konnten allerdings weder die Hamilton-Zuk Hypothese noch die ICHH unterstützen. Es hat sich jedoch herauskristallisiert, dass komplexe Zusammenhänge zwischen Gefieder, HLR (Verhältnis zwischen Heterophilen und Lymphozyten im Differentialblutbild; gilt als Indikator für physiologischen Stress und Immunzustand) bestehen, die zwischen den einzelnen Saisons erheblich variieren und somit einen starken Einfluss von Umweltfaktoren andeuten. Des Weiteren konnte gezeigt werden, dass der allgemeine körperliche Zustand von Männchen einen starken Einfluss auf den Zusammenhang zwischen Gefiedermerkmalen und anderen Faktoren hat. Einerseits leiden Männchen in schlechtem körperlichen Zustand, die ihre limitierten Ressourcen in die Gefiederfärbung investieren, unter einem erhöhten Parasitenbefall und verringerter Immunokompetenz. Andererseits können Männchen in gutem Zustand gleichzeitig in die Gefiederausprägung, sowie ins Immunsystem investieren. Daraus kann man schließen, dass sich potentielle Partner nicht nur auf Gefiederfärbung verlassen können – sie müssen auch andere Variablen, die Aufschluss über den Gesundheitszustand eines Männchens geben, beurteilen, um ein vollständiges Bild der Qualität eines Männchens zu erhalten. Zuletzt wurde der Zusammenhang zwischen Alter und den beiden Gefiedermerkmalen untersucht. Hier wurde festgestellt, dass die Intensität mit zunehmendem Alter linear abnimmt, während die Farbe einem quadratischen Verlauf folgt. Somit haben beide Charakteristika das Potential als Alterssignal verwendet zu werden; es muss allerdings noch untersucht werden, ob Fortpflanzungserfolg und Überlebenswahrscheinlichkeiten einem ähnlichen Muster folgen.

Im zweiten Teil meiner Arbeit habe ich untersucht, ob sich Gefiedermerkmale auf Territorialität und Fortpflanzungsverhalten (Fortpflanzungsaufwand und -Erfolg) der Oryxwebermännchen auswirken. Ich konnte feststellen, dass – im Gegensatz zu allen Erwartungen – Gefiederfarbe keinerlei Einfluss auf das Fortpflanzungsverhalten hatte. Gefiederintensität und auch Parasitenbefall hatten einen signifikant positiven Einfluss auf den Fortpflanzungsaufwand. Bezüglich des Aufwands kann man eine Zuweisung von limitieren Ressourcen erkennen, da Männchen die sehr viel in Fortpflanzungsaufwand (Anzahl gebauter Nester) investieren, einem erhöhten Parasitenbefall ausgesetzt sind. Der Fortpflanzungserfolg hingegen korrelierte negativ mit der Intensität des Befalls mit Blutparasiten. Weiterhin habe ich keine Unterschiede zwischen territorialen und nicht-territorialen Männchen gefunden. Zusammenfassend kann ich sagen, dass ich die Hamilton-Zuk Hypothese in diesem Kapitel nicht unterstützen konnte. Es bleibt zu untersuchen, ob das rote Gefieder von Oryxwebermännchen eine Funktion als Statussignal hat.

Die Untersuchung der Frage, ob Gefiederfärbung in dieser Art als Statussignal verwendet wird, war das Thema meines letzten Kapitels. Entsprechend der „Status Signalling Hypothesis“ sind Männchen mit größeren und/oder intensiver gefärbten Gefiedermerkmalen dominant gegenüber anderen Individuen mit kleineren oder matteren Ornamenten. Um diese Hypothese zu testen habe ich zwei Experimente durchgeführt, in denen zwei unbekannte Männchen bei der Auseinandersetzung um eine limitiere Ressource gegeneinander antreten mussten. Im ersten Experiment blieben die konkurrierenden Männchen unmanipuliert. Im zweiten Experiment wurden diejenigen Männchen manipuliert, welche die niedrigsten Chroma-Werte aufwiesen, um ihnen somit eine kräftigere rote Färbung zu geben. In beiden Experimenten habe ich erwartet, dass die Männchen mit röterem Gefieder dominanter sein würden. Die Analyse beinhaltete auch die Faktoren Alter, Testosteron und körperlichem Zustand und lieferte unerwartete Ergebnisse. In beiden Experimenten war ein sehr starker Alterseffekt zu erkennen, wobei ältere Männchen – ungeachtet von Gefiederfärbung, Testosteron oder Körperzustand – konstant dominant waren. Die Ergebnisse deuten an, dass der Ausgang von Männchen-Männchen Interaktionen durch altersbedingte Erfahrung bestimmt wird und nicht durch die Status signalisierende Funktion von Gefiedermerkmalen. Um diesen Alterseffekt von potentiellen Signalfunktionen zu trennen, müssten weitere Gefiedermanipulationsexperimente mit Individuen der gleichen Altersklasse wiederholt werden.

Zusammenfassend kann gesagt werden, dass die Analyse des Einflusses der Carotenoid-basierten Gefiedermerkmale von Männchen des Oryxwebers auf Fortpflanzungsverhalten und Dominanz weniger klare Ergebnisse erbracht hat als erwartet. Es gab keinen eindeutigen konsistenten Einfluss von Gefiedermerkmalen auf das Fortpflanzungsverhalten. Zusätzlich existierte eine erhebliche saisonale Variation bezüglich der Zusammenhänge verschiedener Faktoren mit Gefiederintensität und/oder Gefiederfärbung. Des Weiteren wurden die Zusammenhänge zwischen Gefiedermerkmalen und Fortpflanzungsverhalten sowie Dominanz oft von Alter, Testosteron und dem Gesundheitszustand eines Individuums zusätzlich beeinflusst. Demzufolge können die Ergebnisse meiner Arbeit die Hamilton-Zuk Hypothese, die „Immunocompetence Handicap“ Hypothese und die „Status Signalling“ Hypothese nicht eindeutig unterstützen.

ACKNOWLEDGEMENTS

First of all, I would like to thank my supervisor PD Dr. Thomas Friedl for giving me the opportunity to carry out this thesis and for making it possible to work in Addo for three consecutive seasons – mainly by myself – knowing that I would get the work done without his supervision and being able to gather much needed and appreciated experience. Also for not only always having an ear no matter what the problem may have been, for letting me run with ideas I may have had – and of course reining me back in when things got a little out of perspective – but also for putting up with my moods, for all the laughter and jokes shared. But mainly I would like to thank him for in believing in me and my ability to carry out this project.

Thanks also go out to our head of department, Professor Dr. Georg Klump, for allowing me to become a part of the department in the first place, for useful insights and advice into a multitude of things and for ensuring interesting discussions over lunch.

I'd also like to thank Prof. Dr. Peter Becker for agreeing to become my second supervisor on a fairly short notice.

For all the lab work done during the last years, I'd first like to thank Elisabeth Groscurth for not only the DNA fingerprinting and real-time PCR analyses she did for me, but also patiently trying to teach me the molecular techniques used and always lending a helping hand when I wanted to try out pigment extractions or needed to dispose of dyes (possibly without staining the entire corridor blue on my way to the temporary collection point). Not to forget the Christmas emails and the warm welcomes home. For the analyses of serum samples to determine testosterone levels, I'd like to thank Dr. Wolfgang Goymann and Ingrid Schwabl at the Max Planck Institute for Ornithology, as well as Dr. Martin Dehnhard and Marlies Rohleder at the Leibniz Institute for Zoo and Wildlife Research.

My husband, Dr. Ralf Edler – look at what we've become! Without him, I'm sure I would have given up long ago. For those inspiring and sometimes truly eye-opening discussions at all hours of the day and night, the patient explaining of the mysterious workings of statistics (yes, sometimes it took a while for things to sink in and even then I may still have said "yes, ok, now I get it..." even though I didn't), the invaluable programming of macros which made my life so much easier, the attempt to teach me to "think simple" when faced with problems and that I don't need to understand every last tiny detail of everything to be successful. Not to mention the long-distance problem-solving between continents on the phone! And basically all the love, support and belief in me I needed to get through this thesis somewhat in touch with my sanity. You truly made up for it!

In South Africa, I'd firstly like to thank National Parks board, for enabling me to carry out my field work in the Addo Elephant National Park. Thanks go out to Lucius Moolman (regional park manager) and Norman Johnson (park manager), for their ongoing support during my years in Addo. Further, special thanks go out to John and Melanie Adendorff, for not only being a constant source of advice throughout the years and for the warm welcomes back to Addo, but also for some truly invaluable insights into many things such as game capture, potential water mongoose problem solving and for helping keep the project running.

To four special ladies who became my family away from home: Katie Gough and Jordana Meyer, my partners in fieldwork crime and the best wooden wendy neighbours to be had, as well as Ilse Welgemoed and Robyn Woods, who offered sanctuaries away from wooden hut life (especially during thunderstorms!). To all four of you: thank you for all the discussions about the large and small things in life, for all the laughter we shared, the road trips, the Christmases, the unforgettable trips around the park at all hours of the day and night, the dinners, the drinks, rainy couch days and movie nights, the heaps of other fun and wacky things we did and, not to forget, the support during harder times. There is so much to mention and I know I've forgotten things, but let me just say that coming back to Addo every 6 months was like coming home. I couldn't have done it without you.

Anban Padayachee – Guy, you'll always have a place in my heart. "It's mine to offer and yours to accept." You offered so much: a place to stay whenever and for however long it was needed, no questions asked. Fishing trips, beach trips, boat trips, copious amounts of food and drink, invaluable insights into everyday life and most importantly friendship.

And many other people in South Africa, who made the stays extremely enjoyable and lent a helping hand if necessary: John Allderman and family at the Orange Elephant, Jed Bird, Driekus Buys, Heleen and Dave Druce, Mark Eaton, Gina and Matt Hayward, Maritjie Landmann, Michelle Mathee, Megan and Bruce Taplin, the rest of the Woods family, the rangers, the office staff, uncountable students and volunteers that came and went over the years, and many many other people.

There are of course, also a bunch of people here in Oldenburg that I’d like to thank for not only the good times we’ve shared, but also for various bits of help which made life that little bit easier. Dr. Ulrike Langemann, whose door was always open when advice was needed and who often gave a different perspective on things, opening doors that had until then been overlooked. Folkert Seeba for lending a helping hand whenever needed and for being who he is. Lastly, for making the lab such a pleasant, creative and fun environment to work in, I’d like to thank Rainer Beutelmann, Tobias Bockhorst, Holger Dierker, Lena-Vanessa Dollezal, Ilona Dwehus, Arne Feinkohl, Elise Göckus, Susanne Groß, Sabine Haskamp, Annika Horn, Naoya Itatani, Martina Jäger, Simon Jones, Astrid Klinge, Mirjam Knörnschild, Kilian Köbrich, Julia Maier, Markus Metz, Maike Niebuhr, Nina Pohl, Colette Pokar, Irene van den Heuvel, Anne Vellage and Melanie Zokoll – van der Laan.

This last paragraph is for my family – related, by marriage and substitute. Although my parents are not around anymore to witness this somewhat amazing little feat of mine, I end it with the knowledge that I would have made them proud. Though both very sceptical of my decision back in the day when I first started studying biology, they soon realized it was the right choice for me. Typical for parents, there was of course the constant question as to when I would finally finish and what I would do then, but there was never a moment of doubt for them that I would succeed. And I did. This is for you.

Next in line, I’d like to thank my aunt, Ulrike Hopf. She willingly and unconditionally stepped into my parents’ stead for the remainder of my PhD. Her constant love and support helped me carry on with what I had started. Even though most of the time she had no idea what I was rambling on about, she would listen patiently and gave me advice when she could.

Also to my relatively newly gained family, especially my mother-in-law, Sigrid Edler, for welcoming me so warmly into her family, naturally making me a part of it all and giving me the feeling that I was now an indispensable part of something bigger. Thank you for everything, for never hesitating when something was needed and for treating me like your own. To my sister- and brother-in-law, Britta and Thorsten Streit, for their undying interest in how things were going and always listening patiently when I got stuck in rambling mode about how fantastic Africa was yet again or how tired I was of writing.

The Friedrichs, for all the picking up and dropping off at the airport at all hours of the day and night, waiting for me with open arms, my thick winter jacket and copious amounts of coffee and breakfast when arriving back home at 6 a.m. after 6 months in the field and always being the first to listen to all the stories. Not to forget the sponsoring of an invaluable piece of technology (the FuturePad mini PC) which made life in the field so much easier and without which, we would have used uncountable pencils, notepads and cassette tapes, not to mention the never-ending hours spared us listening to ourselves droning on and on on the latter.
Lastly, the Taylors – the best substitute family I could ever have hoped for – who have accompanied me on my way mostly long-distance for the past nearly 30 years, always worried about my well-being, making sure that I was doing alright wherever I was and, sure as clockwork, sending that first Christmas parcel of the year to Addo.

Thanks to all of you.

CURRICULUM
VITAE

Personal information

Surname, name	Edler, Alice Ulrike (née Aidnik)
Address	Meesjenweg 4 26215 Wiefelstede Germany 04402 / 916 0951
Date of birth	05.05.1977
Place of birth	Nürnberg, Germany
Nationality	German

University education

Since 07/2005	PhD at the Carl von Ossietzky University Oldenburg Title: Plumage colouration, testosterone and reproductive behaviour in the red bishop (*Euplectes orix*)."
11/1999 – 07/2002	Biology, Friedrich-Alexander University Erlangen-Nürnberg
10/2002 – 05/2005	Biology; Carl von Ossietzky University Oldenburg Major in ecology
Degree	Diploma (gut) Title: „Blutbild, Immunsystem und Fortpflanzungsaufwand bei Weibchen des Oryxwebers (*Euplectes orix*)."

Field studies and teaching experience during PhD

05/2005	Supervision of practical “Behaviour and information processing”
10/2005 – 03/2006	Field studies at the Addo Elephant National Park, South Africa
04/2006 – 10/2006	Supervision of Diploma thesis (Vanessa Gwosdz)
10/2006 – 03/2007	Field studies at the Addo Elephant National Park, South Africa
04/2007 – 10/2007	Supervision of Diploma thesis (Silke Bock)
05/2007 – 08/2007	Supervision of Bachelor practical (Melanie Jahreis)
10/2007 – 03/2008	Field studies at the Addo Elephant National Park, South Africa
04/2008 – 04/2009	Supervision of Diploma thesis (Annika Horn)

Grants during PhD

08/2005	Travel grant from the Wolfgang-Schulenberg Programme
09/2005 – 03/2006	Scholarship from the “Deutscher Akademischer Austauschdienst“ (DAAD)

Conferences during PhD

08/2005	International Ethological Conference, Budapest, Hungary Poster: Aidnik AU, Goymann W, Schwabl I, Friedl TWP „Is there a trade-off between reproductive effort and immunocompetence in female red bishops (*Euplectes orix*)?”
08/2006	International Ornithological Conference, Hamburg, Germany Talk: Aidnik AU, Friedl TWP “The effect of plumage colouration on male-male interactions and reproductive success in the red bishop (*Euplectes orix*)”
08/2007	Conference of the European Ornithological Union, Vienna, Austria Talk: Aidnik AU, Friedl TWP “Plumage colouration, health status and testosterone levels in male red bishops (*Euplectes orix*)”
09/2008	Pan-African Ornithological Conference, Capetown, South Africa Talk: Aidnik AU, Friedl TWP “Who’s the boss? Dominance and plumage colouration in male-male competitions in the red bishop (*Euplectes orix*)”

Oldenburg, 6 July 2009

Alice Edler

Oldenburg, 21. September 2009

Erklärung gemäß § 10 Absatz (2) Punkt b) der Promotionsordnung der Fakultät für Mathematik und Naturwissenschaften der Carl von Ossietzky Universität Oldenburg vom 11.12.2003.

Die vorgelegte Arbeit wurde selbstständig verfasst und es wurden nur die angegebenen Hilfsmittel benutzt. Es wurden noch keine Teile der Dissertation veröffentlicht.

Alice Edler