



Plumage Colouration, Age, Testosterone and Dominance in Male Red Bishops (*Euplectes orix*): A Laboratory Experiment

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Abstract

Visual signals such as plumage characteristics in birds often play a key role in the establishment of dominance in contests 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 whether 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 interspecific and intraspecific communication in many species (e.g. Bradbury & Vehrencamp 1998; Epsmark 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 2003a,b). Because carotenoids cannot be synthesized by animals, they must be ingested and metabolized (Goodwin 1986; Lozano 1994). Carotenoids are exceptional among colour pigments, because they are thought not only to be used in signalling individual quality to prospective mates or rivals (von Schantz et al. 1999; Senar

& Escobar 2002; Ferns & Hinsley 2008), but also to have vital functions as antioxidants and immune system enhancers (Krinsky 1994).

Expression of carotenoid-based plumage colouration in male birds has been linked to various aspects of a male's quality, ranging from immunocompetence and parasitization (Folstad & Karter 1992; Figuerola et al. 1999; Hõrak et al. 2001; Saks et al. 2003; Maney et al. 2008) to testosterone levels (Blas et al. 2006; McGraw et al. 2006; Peters 2007), indicating that carotenoids can play a vital role in signalling. In many bird species, females prefer to mate with males that display the most ornamented colouration (reviewed in Hill 2006a). However, considerably less attention has been paid to the potential role of plumage ornamentation in male–male interactions and the question whether males use carotenoid-based signals to assess one another's fighting ability.

The so-called 'badges of status' were first described by Rohwer's status signalling hypothesis (SSH; Rohwer 1975) for melanin-based ornaments. According to this hypothesis, status signals reduce the amount of aggressive interactions, as birds with unequal fighting ability could assess the rival's dominance status or fighting ability, without having to actually fight over the resource and so risk injury or energy depletion. Social mediation functions as a control system, making cheating an unstable strategy, as potential cheaters are not able to counteract the high level of aggression when actively challenged by dominant males (Senar 1999; Parker & Ligon 2002).

As pointed out by Senar (2006), there seems to be a widespread and generalized relationship of melanin with dominance (reviewed by Senar 1999), while the status signalling function of carotenoids is still poorly studied (but see McGraw & Hill 2000a; Pryke et al. 2002; Pryke & Andersson 2003a,b). Carotenoid-based signalling is different from melanin-based signalling, as the expression of red or orange plumage colouration is more closely linked to individual condition. Many studies have found that the expression of carotenoid-based plumage traits in birds is costly (Olson & Owens 1998) and condition-dependent (reviewed by Hill 2006b; for an exception, see Peters et al. 2008). Therefore, males with the highest level of ornamentation should be in better (nutritional) condition and could be expected to be dominant over less colourful males, leading to the hypothesis that carotenoid-based plumage colouration might be especially suited to be used as a status signal.

However, more recent work has shown that plumage colouration can also be related to 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 plumage 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 using ornamentation that allows competing males to assess one another's competitive ability clearly would be advantageous, because 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, because such a mating pattern is linked to frequent and strong male–male competition for limited resources such as territories and mates. Two bird species with such a mating pattern where the signalling function of carotenoid-based male plumage traits has been studied in detail are the red-collared widowbird (*Euplectes ardens*) and the red-shouldered widowbird (*Euplectes axillaries*). In both species, males have a black breeding plumage with small but conspicuous orange-to-red carotenoid-based plumage patches, and in both species, it has been shown that males with larger and redder patches outcompete opponents with smaller and less colourful patches in staged dyadic contests over food (Pryke et al. 2002; Pryke & Andersson 2003a). Additional experiments in the field revealed that in both species, territorial males with larger and redder patches were better able to establish and defend breeding territories than males with less conspicuous plumage ornaments (Pryke et al. 2002; Pryke & Andersson 2003b), suggesting that the carotenoid-based male plumage traits in these species indicate male dominance status and resource-holding potential.

In this study, we want to determine whether carotenoid-based plumage colouration functions as a status signal indicating dominance and resource-holding potential in another colonial breeding, highly polygynous weaverbird of the same genus, the red bishop (*E. orix*). In this species, males in a brilliant red and black breeding plumage compete

against each other for limited territory space in reed beds or bulrush stands around water, and aggressive male–male interactions in form of territorial disputes are frequently observed (Friedl 2004; Metz et al. 2007). To study the effect of ornamental traits in the context of male–male competition, we used an approach similar to the one used in the studies on red-shouldered and red-collared widowbirds cited earlier, by staging dyadic contests between unfamiliar red bishop 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 inter-correlated 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 into the analysis of experimentally staged male–male contests despite its potential importance in determining dominance among males. It was not the aim of our study to investigate aggressiveness *per se*, i.e. the tendency of an individual to escalate a contest independent of resource-holding potential and resource value (see Barlow et al. 1986; Maynard Smith & Harper 1988), or to differentiate between aggressiveness, dominance and resource-holding potential.

Methods

Study Species

Our study was conducted on red bishop (*E. orix*) males. The red bishop is a polygynous and sexually dimorphic small weaverbird (body weight ranging from 14 to 26 g for females and from 16 to 31 g for males) occurring in sub-Saharan Africa. Males in breeding plumage have a black bill, forehead and face, while the rest of the head, throat, breast and rump are brilliant orange-scarlet. The belly is black, the mantle orange-brown and wings and tail are brown. Red bishop males are characterized by delayed plumage maturation, with full breeding plumage being displayed only at 2 yr of age and older, while one-yr-old males show the same inconspicuous brownish plumage as females and juveniles (Friedl 2004). 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 and actively defend 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 determines male reproductive success (Friedl & Klump 1999; Lawes et al. 2002). Females inspect and accept built nests, and once females have chosen a mate, they copulate several times with the territory holder and then begin lining nests. Eggs (normally 2–4 per clutch) are laid soon after copulation, and females are solely responsible for incubation and raising of young (Friedl 2004).

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 2004). In these situations, dominance is often unclear, and territorial disputes are 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, although 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 2004).

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). In both years, all available males ($n = 14$ in 2007 and $n = 12$ in 2008, because two males died in the course of the year) were used for the experiments. 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 4 and 6

compartments each (width \times length \times height: 1.0 \times 2.0 \times 2.0 m), which were connected via holes (0.2 \times 0.2 m). The aviaries were both located in the middle part of a glass greenhouse and separated by a lane of approx. 2 m in width, resulting in social but not visual isolation of the two groups of birds. We never saw any sign of social interaction between males of the two aviaries. Each compartment contained three 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. Males in the aviary competed vigorously for compartment space to set up territories and build nests that could be offered to breeding females, similar to territorial behaviour under natural conditions in the wild (R. Edler, W. Goymann, I. Schwabl & T. W. P. Friedl, submitted manuscript). 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 1.0 g before every contest. The body condition index was then calculated as the residuals from the linear regression of body weight on tarsus length.

Red bishops are relatively long-lived small passerines. On average, breeding males captured in a study colony in the Addo Elephant National Park, South Africa, over a period of 3 yr, for which we knew the exact ages were 4.45 ± 1.7 yr old ($n = 23$), with the oldest individual being 7 yr old (T. W. P. Friedl & A. U. Edler unpublished data). However, most males in the field were caught for the first time when already breeding, and for those males, we could only determine a minimum age of 2 yr at the time of first capture. In our study colony in the Addo Elephant National Park, we observed some territorial males that were at least 9 yr of age, indicating that ages of 10 and more yr for breeding male red bishops are no exception in the wild.

For our contest experiments in captivity, we divided all males into two age categories – young and old. This is because we only know the exact age of those males that hatched in the aviary, while we can only give a minimum known age for those

males caught as adults in the field. In 2007, all males of 6 yr of age and younger (range 4–6 yr) were classified as ‘young’ ($n = 7$), while the remaining 7 males that were at least 9 yr of age (minimum age ranged from 9 to 15 yr) were classified as ‘old’. Accordingly, in 2008, those males of 7 yr of age and younger ($n = 6$; range 5–7 yr) were classified as ‘young’ and those at least 10 yr old ($n = 6$; minimum age ranged from 10 to 16 yr) were classified as ‘old’. Thus, in both contest years, males categorized as young were at least 3 yr younger than males categorized as old.

Reflectance Measurements

Reflectance was measured using an Ocean Optics USB2000 spectrometer (Ocean Optics Inc., Dunedin, FL, 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, to standardize 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 approx. 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 (<1 nm resolution; Ocean Optics Inc.) and in relation to a white (WS-2 white reflective tile, 98% reflectance; getspec) 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) and determined that red bishops do not reflect in the range of 300–400 nm, we calculated the three different colourimetrics, average brightness, chroma and hue, based on the raw reflectance data obtained for the range between 400 and 700 nm. 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 & Prager 2006). Chroma (spectral purity) is the relative amount of light from a region of interest and, 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 & Boag (1987). The estimates were high and significant for all plumage variables of both the head (average brightness: $r = 0.96$, $F_{13,27} = 72.55$, $p < 0.001$; chroma: $r = 0.92$, $F_{13,27} = 33.40$, $p < 0.001$; and hue: $r = 0.82$, $F_{13,27} = 14.66$, $p < 0.001$) and the throat (average brightness: $r = 0.96$, $F_{13,27} = 72.59$, $p < 0.001$; chroma: $r = 0.83$, $F_{13,27} = 15.13$, $p < 0.001$; and hue: $r = 0.78$, $F_{13,27} = 11.54$, $p < 0.001$).

Testosterone Levels

Blood samples were obtained by puncturing the brachial vein with a sterile needle. Per male, we collected approx. 20–60 μl of blood. The samples were then centrifuged for the extraction of serum, which was stored in a freezer until testosterone levels were analysed by enzyme immunoassays (see below). In both years, samples for analysis of testosterone levels were taken from males in full breeding plumage shortly before the start of the contests, which was in May for the year 2007 and in late Jun. for the year 2008 (for logistic reasons, male–male contests were conducted later in this year).

Testosterone was measured at the Leibniz Institute for Zoo and Wildlife Research in Berlin by a specifically developed enzyme immunoassay with a double-antibody technique according to Roelants et al. (2002). For testosterone estimation in blood, 0.01–0.05 ml of serum was extracted with 2 ml of butyl t-methyl ether:petroleum ether (30:70, v/v) for 30 min. The samples were frozen, and the fluid petroleum ether phase was removed and evaporated at 55°C. The steroids were dissolved in 0.1 ml of

40% (v/v) methanol, and duplicates of 20 μl each were analysed.

The assay used a polyclonal antibody raised in rabbits against testosterone-11-hemisuccinate-bovine serum albumin, and the label was testosterone-3-carboxymethyl-oxime-horse radish peroxidase. The testosterone standard curve ranged from 0.4 pg per 20 μl to 50 pg per 20 μl , and the cross-reactivity with testosterone was 100%, with 5 α -dihydrotestosterone 10%, androstenedione 2%, estradiol 0.1% and 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-assay and interassay coefficients of variation (CVs) were 8.9% and 12.3%, respectively. Results of the testosterone measurements are presented as nanograms of testosterone per millilitre of serum. Testosterone levels were distributed normally in both 2007 (Kolmogorov–Smirnov test; $Z = 0.843$, $p = 0.476$) and 2008 ($Z = 0.950$, $p = 0.327$). Thus, no data transformation was necessary for statistical analysis.

Male–Male Contests

To test whether 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; and 2008: 36 in total) took place between 7 and 11 a.m. every other day between May 25th and Jun. 7th in 2007 and from Jul. 8th to Jul. 18th in 2008. Males were removed from the main aviaries approx. 15 h before the contests began and placed in individual double cages (82 \times 40 \times 30 cm), 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. To standardize motivation, males were deprived of food but provided with water *ad libitum*. This deprivation time only led to moderate starvation, given that the mean body weight of males at the start of each contest trial (27.2 g; averaged over all contests in both years) was still higher compared to average body weight of males caught in the wild (26.9 g; data collected over three breeding seasons at a red bishop colony in the Addo Elephant National Park, South Africa). 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 min, 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 2 wk (measured and calculated shortly before trial onset), as plumage values do not change over periods as short as 2 wk (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, Tokyo, Japan; see Pryke et al. 2002), a marker that 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 colouration, the colours of experimentally manipulated plumage are more likely to fade within 2 wk. To determine whether manipulations were

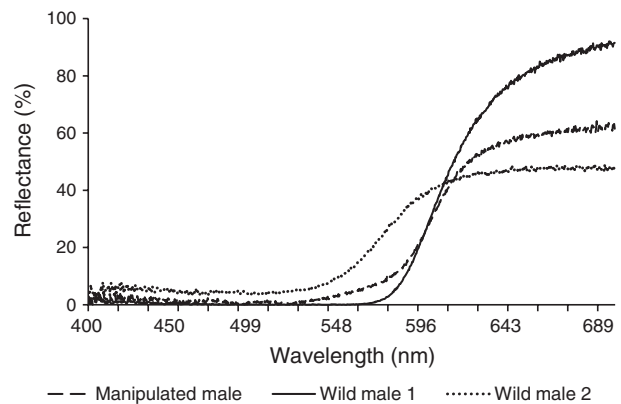


Fig. 1: Reflectance spectra of two red bishop males found in the wild (solid line shows upper extreme of plumage colouration in the field; dotted line shows an example of the lower end of plumage variation) in comparison to a manipulated male (dashed line) in the aviary. The manipulation was applied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

fading, reflectance was measured before each contest and manipulations were reapplied every 6 d, 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 five to six measurements performed for the 12 males over the 12-d trial period (using mean values of head and throat combined) was again high and significant (average brightness: $r = 0.77$, $F_{11,59} = 21.01$, $p < 0.001$; chroma: $r = 0.72$, $F_{11,59} = 16.11$, $p < 0.001$; hue: $r = 0.97$, $F_{11,59} = 182.60$, $p < 0.001$).

Video Analysis

All dyadic contests were recorded on video (Sony HandyCam DCR HC23E, Tokyo, 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 min). 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 minus 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 categorized as a draw and excluded from the analysis.

Statistical Analysis

Both contest years were analysed using General Linear Mixed Models (GLMM) with average brightness, chroma and hue, age, testosterone and body condition index as independent variables and each of the four dominance measures (number of threats, 'wins minus losses', time at feeder and number of worms eaten) as dependent variable in four separate GLMMs. 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 all of these contest analyses. To analyse the binary win/lose response, we used a generalised linear model with a binomial distribution and logit link function, which also allowed us to incorporate male ID as random factor in both years. While our plumage and testosterone data did not differ significantly from a normal distribution, our behavioural values did. Therefore, we used parametric or non-parametric tests respectively for comparisons between groups of males. Descriptive data are given as mean \pm SD unless stated otherwise. All statistical tests were performed with SPSS 17.0, 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 (data obtained from males at a breeding colony of red bishops in the Addo Elephant National Park, South Africa; $n = 56$, with multiple measurements from several males, recaptured over a total of three seasons) showed that in the aviary, males were on average brighter (aviary: 42.69 ± 17.62 ; wild: 28.56 ± 9.06 ; Mann-Whitney U -test, $Z = -3.946$, $p < 0.001$) but had lower chroma (aviary: 0.696 ± 0.076 ; wild: 0.787 ± 0.063 ; $Z = -4.783$, $p < 0.001$) and hue (aviary: 557.07 ± 14.71 ; wild: 581.41 ± 6.58 ; $Z = -6.706$, $p < 0.001$) values than male red

bishops in the wild. However, there was a large overlap between plumage colouration of males in the aviary and in the wild, and the highest values for chroma and hue obtained in the aviary were also above the mean values found in the wild.

Young and old males did not differ with respect to plumage characteristics in either 2007 (average brightness: $t = -0.717$, $p = 0.487$; chroma: $t = 0.209$, $p = 0.838$; hue: $t = -1.488$, $p = 0.163$), before manipulation in 2008 (average brightness: $t = -0.695$, $p = 0.503$; chroma: $t = -0.895$, $p = 0.392$; hue: $t = -1.209$, $p = 0.254$) or after manipulation in 2008 (average brightness: $t = -0.527$, $p = 0.609$; chroma: $t = -0.006$, $p = 0.995$; hue: $t = 0.616$, $p = 0.551$).

The manipulation of plumage in 2008 significantly altered the appearance of males ($n = 6$ males), increasing chroma (paired t -test; $t = -7.534$, $p = 0.001$) and hue ($t = -11.192$, $p < 0.001$; see Fig. 2) while decreasing average brightness ($t = 6.845$,

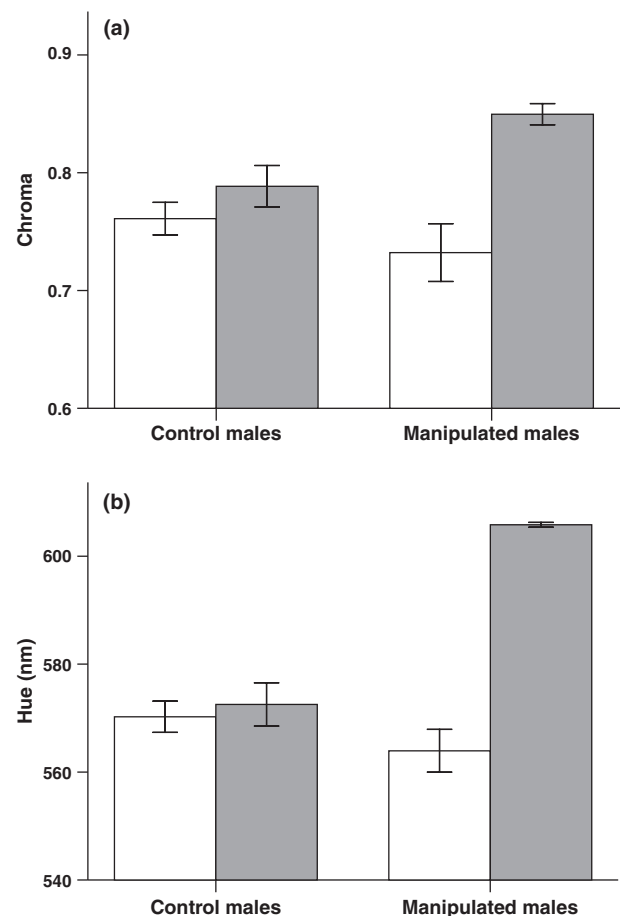


Fig. 2: Mean (\pm SE) values of (a) chroma and (b) hue in control and manipulated red bishop males before (white bars) and after (grey bars) plumage manipulation in 2008.

$p = 0.001$). In control males treated with the colourless blender, plumage remained unaltered ($n = 6$ males; paired t -test; average brightness: $t = 0.70$, $p = 0.515$; chroma: $t = -1.525$, $p = 0.188$; hue: $t = -1.013$, $p = 0.358$; see Fig. 2).

Testosterone Levels

Overall, testosterone levels were significantly higher in 2007 (0.983 ± 0.589) compared to 2008 (0.435 ± 0.481). 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 ($t = 2.541$, $p = 0.018$) or whether we performed a pair-wise t -test with the 12 males that were present in both years ($t = 2.578$, $p = 0.027$). There was no significant correlation between testosterone levels of individual males measured in 2007 and 2008 ($r = 0.240$, $p = 0.447$). Thus, males with high testosterone levels during the breeding season in 1 yr do not necessarily have a high testosterone level in other years (as would be indicated by a positive correlation); nor is there any evidence for a trade-off of testosterone levels between different years (as would be indicated by a negative correlation).

A comparison of testosterone levels between the two age categories (see Fig. 3) revealed that in 2007, young males had significantly higher testosterone levels than older males ($n = 7$ per age category; t -test; $t = 2.873$, $p = 0.015$), while the difference in testosterone levels between males of the two age categories was not significant in 2008 ($t = 0.824$, $p = 0.429$).

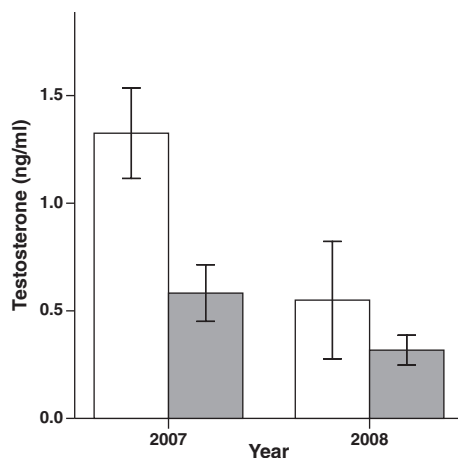


Fig. 3: Mean (\pm SE) testosterone levels [ng/ml] of young (white bars) and old (grey bars) red bishop males in 2007 and 2008.

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 coefficient $r_s = 0.440$, $p < 0.001$), the amount of worms eaten ($r_s = 0.304$, $p = 0.002$) and the time spent at the feeder ($r_s = 0.228$, $p = 0.024$). In addition, there was a significant positive correlation between the amount of time spent at the feeder and the amount of worms eaten ($r_s = 0.744$, $p < 0.001$). For the 2008 contests, there was a very similar pattern, with all correlations listed above also being highly significant (all $r_s > 0.35$, all $p < 0.01$). However, in contrast to the contests 2007, the correlations between the amount of threats and both the amount of time spent at the feeder and the amount of worms eaten were also significant ($r_s = 0.457$, $p < 0.001$ and $r_s = 0.368$, $p = 0.001$).

Contests 2007

The GLMMs for the 2007 contests showed significant effects of age (Parameter estimate = -3.25 ± 0.90 , $F_{1,96} = 13.0$, $p = 0.001$; see Fig. 4), hue (Parameter estimate = -0.20 ± 0.08 , $F_{1,96} = 6.52$, $p = 0.013$) and chroma (Parameter estimate = 47.78 ± 21.30 , $F_{1,96} = 5.03$, $p = 0.029$) on the amount of 'wins minus losses'. Overall, age had the strongest effect, with older males winning more interactions. Both the positive effect of chroma and the negative effect of hue were weaker and contradicted each other, given that chroma and hue were positively correlated with each other ($n = 14$, $r_s = 0.851$, $p < 0.001$). There were also significant positive effects of age (Parameter estimate = -2.91 ± 1.08 , $F_{1,96} = 7.29$, $p = 0.033$; see Fig. 4) and chroma (Parameter estimate = 71.75 ± 25.40 , $F_{1,96} = 7.98$, $p = 0.027$) and a significant negative effect of hue (Parameter estimate = -0.25 ± 0.09 , $F_{1,96} = 7.58$, $p = 0.027$) on the number of threats. No significant effects were found for time at the feeder or the number of worms eaten as dependent variable, but on average, older males spent more time at the feeder and ate more worms than younger males (see Fig. 4). None of the GLMM analyses revealed a significant effect of testosterone or body condition index.

Using a generalised linear model with 'win/lose' as a binary response, we found a strong age effect and a somewhat weaker but still significant effect of hue on the dependent variable (Table 1). Older males were more likely to win a contest compared

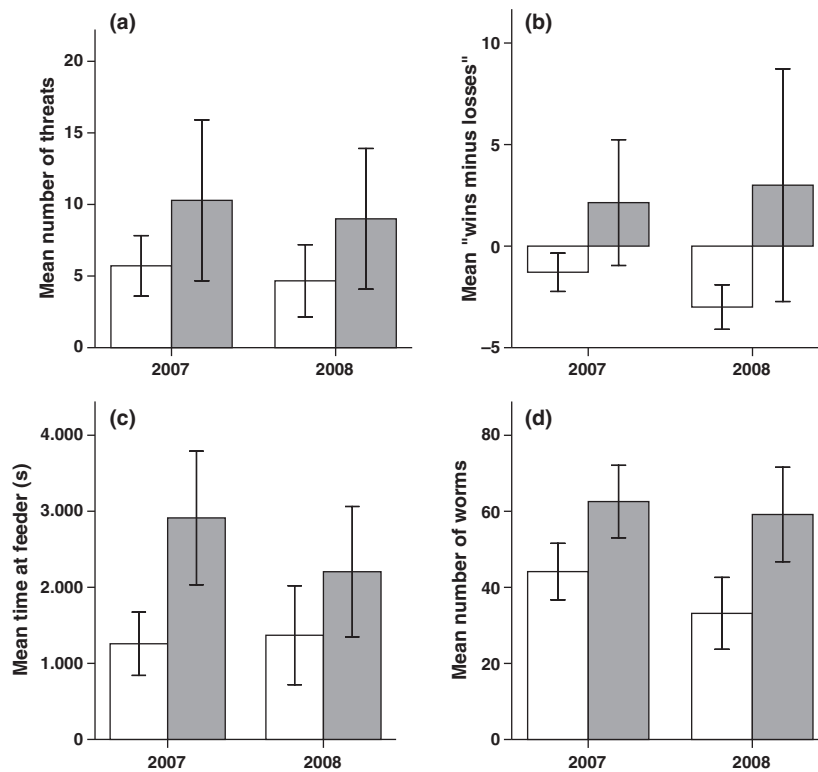


Fig. 4: Comparison of males of the age categories 'young' (white bars) and 'old' (grey bars) during both trial seasons (2007 and 2008) in regard to (a) number of threats, (b) 'wins minus losses', (c) time spent at the feeder and (d) the amount of worms eaten (mean \pm SE).

Table 1: 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 (\pm SE)	Wald X^2	p
2007			
Age	-8.976 \pm 2.874	9.753	0.002
Average brightness	0.075 \pm 0.066	1.276	0.259
Chroma	60.762 \pm 36.867	2.716	0.099
Hue	-0.294 \pm 0.129	5.199	0.023
Testosterone [ng/ml]	0.451 \pm 0.301	2.256	0.133
Body condition index	-0.320 \pm 0.391	0.671	0.413
2008			
Age	-3.665 \pm 1.699	4.655	0.031
Average brightness	0.287 \pm 0.172	2.795	0.095
Chroma	16.237 \pm 13.259	1.50	0.221
Hue	0.138 \pm 0.060	5.203	0.023
Testosterone [ng/ml]	0.303 \pm 0.167	3.302	0.069
Body condition index	-0.604 \pm 0.294	4.237	0.040

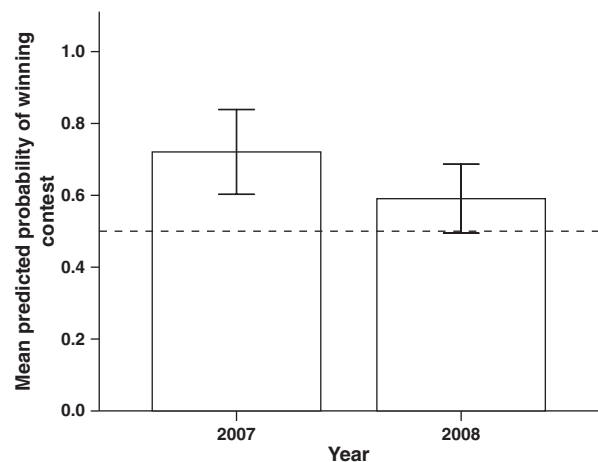


Fig. 5: Mean winning probabilities for males of the age category 'old' in contests conducted in 2007 and 2008, as predicted by the generalized linear models with 'win/lose' as binary response variable reported in Table 1. The error bars represent the 95% confidence intervals. The dashed line indicates a winning probability of 50%.

to younger males (Fig. 5), while hue had a negative effect on winning probability, with less red and more orange-coloured males (i.e. males with a shorter wavelength hue) being more likely to win a contest

than redder counterparts (i.e. males with a longer wavelength hue) when the influence of all the other variables was controlled for (see Table 1). These

results are in accordance with the positive effect of age and the negative effect of hue on both the amount of 'wins minus losses' and the number of threats described earlier. There were no significant effects of average brightness, chroma, testosterone levels or body condition index on contest outcome (see Table 1).

Contests 2008

The 2008 GLMMs revealed that no male traits were significantly related to 'wins minus losses' and time at feeder. However, there was a significant positive effect of hue on both the number of threats (Parameter estimate = 0.12 ± 0.41 , $F_{1,70} = 8.192$, $p = 0.008$) and the number of worms eaten (Parameter estimate = 0.22 ± 0.11 , $F_{1,70} = 4.315$, $p = 0.047$), as well as a tendency for age to positively affect the number of worms eaten (Parameter estimate = -5.40 ± 2.44 , $F_{1,70} = 4.882$, $p = 0.058$). No other significant effects or tendencies were found. However, it should be noted that similar to the contests in 2007, older males had on average higher values than younger males for all four dominance measures (see Fig. 4).

The generalised linear model for the 2008 contests showed significant effects of age, hue and body condition index on the binary response 'win/lose' (see Table 1). The only result consistent with 2007 is the positive effect of age, with older males having a higher probability of winning a contest (Fig. 5). The negative effect of body condition index on the probability of winning shows that males that had a low body weight in relation to their size (i.e. thin and probably hungry males) were more likely to win a contest in 2008. The positive effect of hue on the binary response variable 'win/lose' with redder males having a higher probability of winning in 2008 contradicts the negative effect of hue found in 2007 (see above). Contest outcome in 2008 was not significantly affected by average brightness, chroma or testosterone levels (see Table 1).

Effects of Plumage Manipulations on Behaviour and Contest Outcome

We compared behaviour and contest outcome between both contest seasons for those competing male dyads in which one male was manipulated during the 2008 trials. Males with their plumage hue and chroma experimentally increased in 2008 did not threaten their unmanipulated opponents more often in 2008 compared to 2007 when both

opponents were unmanipulated (paired *t*-test, $t = 1.382$, $p = 0.189$). In addition, the manipulated males did not spend more time at the feeder (paired *t*-test, $t = 1.787$, $p = 0.096$) and did not eat more mealworms (paired *t*-test, $t = 0.503$, $p = 0.623$) in the 2008 contests compared to the 2007 contests against the same unmanipulated male. Likewise, in the 2008 contests, the unmanipulated control males did not threaten their manipulated opponents with increased hue and chroma less often (paired *t*-test, $t = 1.284$, $p = 0.220$), did not spend less time at the feeder (paired *t*-test, $t = -1.746$, $p = 0.103$) and did not eat a lower number of mealworms (paired *t*-test, $t = -0.468$, $p = 0.647$) compared to 2007 when both opponents were unmanipulated. Finally, manipulated males with increased chroma and hue were not more likely to win a contest against an unmanipulated opponent in 2008 compared to 2007 when both opponents were unmanipulated (McNemar test, $p = 0.375$).

Discussion

Signals that convey honest information on status and fighting ability in contests are widespread among animals (Bradbury & Vehrencamp 1998; Epsmark et al. 2000; Maynard Smith & Harper 2003; Searcy & Nowicki 2005), and avian plumage traits seem to be especially suited to be used as such badges of status (Maynard Smith & Harper 2003). However, most work on status signalling in birds has been performed on melanin-based ornaments, while the possible function of carotenoid-based plumage traits as signals in contests is less well understood (Senar 2006). Here, we performed captive dominance trials in male red bishops that prominently display their carotenoid-based bright orange-red and black breeding plumage in threat displays and contests. In staged dyadic contests over food, we found no significant effect of average brightness on contest outcome, while the barely significant effects of chroma and hue on contest outcome were inconsistent and – in case of hue – in opposite directions in both years. We found, however, a strong and consistent effect of age on the outcome of contests over food, with older males having a higher probability of winning. Thus, our results indicate a strong age effect on contest outcome in male red bishops, while plumage characteristics seem to 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 & Sarrazin (2007) show that in Griffon vultures

(*Gyps fulvus*), older males were more likely to be aggressive and dominant than younger individuals, and similar findings have been reported for Arctic barnacle geese (*Branta leucopsis*, Stahl et al. 2001), yellowhammers (*Emberiza citronella*, Sundberg 1995), urban pigeons (*Columba livia*, Sol et al. 1998), black kites (*Milvus migrans*, Sergio et al. 2009), song sparrows (*Melospiza melodia*, Smith et al. 1980) and 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. Alternatively, older males might be dominant over younger ones because their residual reproductive value is lower and they therefore are expected to take higher risks in fighting contests (Grafen 1987; Sergio et al. 2009).

In contrast to the strong age effect on contest outcome in male red bishops, the effects of the different plumage variables were weak, inconsistent and somewhat contradictory (see Results). 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 less colourful (i.e. lower chroma and/or hue values) counterparts (Pryke et al. 2002; Pryke & Andersson 2003a,b). Similar results have been obtained in studies on yellow warblers (*Dendroica petechia*, Studd & Robertson 1985) and Gouldian finches (*Erythrura gouldiae*, Pryke & Griffith 2006). However, in some of these studies, both patch colour and patch size of carotenoid-based plumage ornaments were simultaneously manipulated and analysed, and it is not entirely clear whether the strong effects on contest outcome observed were attributable to patch size, patch colour or a combination of both (Pryke et al. 2002; Pryke & Andersson 2003b). Other studies found a strong effect of the size of carotenoid-based plumage patches on contest outcome but did not analyse plumage colour (Griggio et al. 2007; Karubian et al. 2008). In contrast, a negative relationship between plumage colouration and dominance with duller males dominating brighter males was found in house finches (McGraw & Hill 2000a,b), and experiments in red-winged blackbirds revealed that males with reddened epaulettes experienced more territorial challenges and lost their territories more often compared to control males (Yasukawa et al. 2009). Finally, Wolfenbarger (1999) showed that carotenoid-based colouration had no influence at all on dominance status in

northern cardinals. Overall, the published studies (including this study) investigating the role of carotenoid-based plumage colouration for status signalling have revealed different and contradictory results, indicating that more intense carotenoid-based plumage traits in birds are not always linked to dominance signalling.

However, it has to be noted that male age was not included in the analyses in any of the studies mentioned earlier that found positive effects of carotenoid-based plumage characteristics on dominance and contest outcome. Various studies have shown that there is age-related plumage variation, with older males being more colourful than younger counterparts (Wiebe & Bortolotti 2002; Delhey & Kempenaers 2006; Probst et al. 2007; Bitton & Dawson 2008). In cases where age and plumage colouration are positively correlated, it seems likely that status signalling via plumage ornaments that indicate age occurs. In our aviary population of red bishops, age was not consistently related with plumage colouration, and there were no significant differences between males categorized as young or old with regard to average brightness, chroma or hue (see Results). 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 or being prone to more risk-taking and behaving accordingly. This interpretation is confirmed by the fact that older red bishop males in the 2007 contests threatened their counterparts significantly more often than younger males and, consequently, won significantly more interactions. Overall, our results suggest that in red bishops, age and its matching dominance behaviours might override potential signalling effects of plumage colouration. However, given this strong age effect on the one hand and our rather small sample size on the other hand, it may be possible that plumage colouration serves as a badge of status within age classes when differences in age-related dominance behaviours are weak or absent but that our sample size was too small to detect such a weaker plumage effect on the outcome of contests between males of similar age.

All males used in our contest experiments were 4 yr of age and older (at the time the experiments were conducted, younger males were not available). Thus, the youngest age class of adult males that moult into the colourful breeding plumage and show full reproductive activity in the wild (i.e. males 2

and 3 yr of age) was not included in this experiment. While there were no significant differences with regard to plumage characteristics between males categorized as young or old in our experiments, there seems to be a quadratic relationship between male plumage colouration and age in the wild, with an initial increase in plumage colouration (chroma and hue) up to three or 4 yr of age being followed by a decrease at higher age (T. W. P. Friedl & A. U. Edler unpublished data). Hence, based on our experiments, we cannot exclude the possibility that plumage colouration functions as a signal of status in contests where 2-yr-old or 3-yr-old males (which were not included in our experiment) are involved. Recently published data obtained on a red bishop colony in the wild showed that over the course of three breeding seasons, there was considerable variation among years with regard to the relationship between male plumage traits and factors indicating male condition and health (Edler & Friedl 2010). Furthermore, the significant effects of several interactions between individual stress levels, body condition and blood parasite load on male plumage colouration add up to a rather complex pattern (see Edler & Friedl 2010). In our opinion, these results indicate that under natural conditions, plumage colouration in red bishop males might not be linked consistently and reliably enough to male quality to be used as a status signal in male–male contests.

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 2008 contests, with those birds that had a worse body condition than their counterparts having a higher probability of winning. Similar results have been shown in studies on great tits (*Parus major*, Lemel & Wallin 1993), house sparrows (*Passer domesticus*, Andersson & Åhlund 1991) and American goldfinches (*Carduelis tristis*, Popp 1987), where 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).

Perhaps surprisingly, testosterone showed no significant effect on contest outcome or aggressive behaviour (number of threats) in both the 2007 and the 2008 contests. 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 shown to be positively correlated with the level of aggressiveness displayed by an individual. However, in our study, younger males had higher testosterone titres than older individuals (with the differences being significant in 2007 but non-significant in 2008), suggesting that younger rather than older males should win more contests. As mentioned earlier 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 testosterone plays a more important role when individuals of the same age are staged against one another.

It has been shown across multiple taxa 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, given that the time delay between interactions is not too long. 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 the effects of previous wins or losses on the behaviour in future interactions persist (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.

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 seems to override the potential 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 that have been thought to be more important.

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Literature Cited

- Andersson, S. & Åhlund, M. 1991: Hunger affects dominance among strangers in house sparrows. *Anim. Behav.* **41**, 895–897.
- Andersson, S. & Prager, M. 2006: Quantification of coloration. In: *Bird Coloration, Vol 1. Mechanisms and measurements* (Hill, G. E. & McGraw, K. J., eds). Harvard Univ. Press, Cambridge, pp. 41–89.
- Bakker, C. M. & Sevenster, P. 1983: Determinants of dominance in male sticklebacks (*Gasterosteus aculeatus* L.). *Behaviour* **86**, 55–71.
- Barlow, G. W., Rogers, W. & Fraley, N. 1986: Do Midas cichlids win through prowess or daring? *Behav. Ecol. Sociobiol.* **19**, 1–8.
- Beaugrand, J. P., Goulet, C. & Pavette, D. 1991: Outcome of dyadic conflict in male green swordtail fish, *Xiphophorus helleri*: effect of body size and prior dominance. *Anim. Behav.* **41**, 417–424.
- Bitton, P. P. & Dawson, R. D. 2008: Age-related differences in plumage characteristics of male tree swallows *Tachycineta bicolor*: hue and brightness signal different aspects of individual quality. *J. Avian Biol.* **39**, 446–452.
- Blas, J., Pérez-Rodríguez, L., Bortolotti, G. R., Viñuela, J. & Marchant, T. A. 2006: Testosterone increases bioavailability of carotenoids: insights into the honesty of sexual signalling. *Proc. Natl. Acad. Sci. USA* **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, J. W. & Vehrencamp, S. 1998: *Principles of Animal Communication*. Sinauer Associates Inc., Sunderland.
- Craig, A. J. F. K. 1974: Reproductive behaviour of the male red bishop bird. *Ostrich* **45**, 149–160.
- Delhey, K. & Kempenaers, B. 2006: Age differences in blue tit *Parus caeruleus* plumage colour: within-individual changes or colour-biased survival? *J. Avian Biol.* **37**, 339–348.
- 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*). *Proc. R. Soc. Lond. B* **270**, 2057–2063.
- Drummond, H. & Osório, J. L. 1992: Training siblings to be submissive losers: dominance between booby nestlings. *Anim. Behav.* **44**, 881–893.
- Duckworth, R. A., Mendonça, M. T. & Hill, G. E. 2004: Condition-dependent sexual traits and social dominance in the house finch. *Behav. Ecol.* **15**, 779–784.
- Edler, A. U. & Friedl, T. W. P. 2010: Individual quality and carotenoid-based plumage ornaments in male red bishops (*Euplectes orix*): plumage is not all that counts. *Biol. J. Linn. Soc.* **99**, 384–397.
- Epsmark, Y., Amundsen, T. & Rosenqvist, G. 2000: *Animal Signals: Signalling and Signal Design in Animal Communication*. Tapir Academic Press, Trondheim.
- Evans, M. R. & Hatchwell, B. J. 1992: An experimental study of male adornment in the scarlet-tufted malachite sunbird: I. The role of pectoral tufts in territorial defence. *Behav. Ecol. Sociobiol.* **29**, 413–419.
- Evans, M. R. & Norris, K. 1996: The importance of carotenoids in signaling during aggressive interactions between male firemouth cichlids (*Cichlasoma meeki*). *Behav. Ecol.* **7**, 1–6.
- Ferns, P. N. & Hinsley, S. A. 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*. *Funct. Ecol.* **13**, 594–601.
- Folstad, I. & Karter, A. K. 1992: Parasites, bright males and the immunocompetence handicap. *Am. Nat.* **139**, 603–622.
- Friedl, T. W. P. 2004: Breeding behaviour of the red bishop (*Euplectes orix*): a synthesis and new observations. *Vogelwarte* **42**, 178–190.
- Friedl, T. W. P. & Klump, G. M. 1999: Determinants of male mating success in the red bishop (*Euplectes orix*). *Behav. Ecol. Sociobiol.* **46**, 387–399.
- Gonzalez, G., Sorci, G., Smith, L. C. & de Lope, F. 2001: Testosterone and sexual signalling in male house sparrows (*Passer domesticus*). *Behav. Ecol. Sociobiol.* **50**, 557–562.
- Goodwin, T. W. 1986: Metabolism, nutrition, and function of carotenoids. *Annu. Rev. Nutr.* **6**, 274–297.
- Grafen, A. 1987: The logic of divisively asymmetric contests: respect for ownership and the desperado effect. *Anim. Behav.* **35**, 462–467.
- 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. *Behav. Ecol. Sociobiol.* **61**, 423–433.

- Hau, M., Wikelski, M., Soma, K. K. & Wingfield, J. C. 2000: Testosterone and year-round territorial aggression in a tropical bird. *Gen. Comp. Endocrinol.* **117**, 20–33.
- Hill, G. E. 2006a: Female mate choice for ornamental coloration. In: *Bird Coloration, Vol 2. Function and Evolution* (Hill, G. E. & McGraw, K. J., eds). Harvard Univ. Press, Cambridge, pp. 137–200.
- Hill, G. E. 2006b: Environmental regulation of ornamental coloration. In: *Bird Coloration, Vol 1. Mechanisms and Measurements* (Hill, G. E. & McGraw, K. J., eds). Harvard Univ. Press, Cambridge, pp. 507–560.
- Hörak, P., Ots, I., Vellau, H., Spottiswoode, C. & Møller, A. P. 2001: Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. *Oecologia* **126**, 166–173.
- Hsu, Y. & Wolf, L. L. 2001: The winner and loser effect: what fighting behaviours are influenced? *Anim. Behav.* **61**, 777–786.
- Hsu, Y., Earley, R. L. & Wolf, L. L. 2006: Modulation of aggressive behaviour by fighting experience: mechanisms and contest outcomes. *Biol. Rev.* **81**, 33–74.
- Johnsen, T. S. 1998: Behavioural correlates of testosterone and seasonal changes of steroids in red-winged blackbirds. *Anim. Behav.* **55**, 957–965.
- Karubian, J., Sillett, T. S. & Webster, M. S. 2008: The effects of delayed plumage maturation on aggression and survival in male red-backed fairy-wrens. *Behav. Ecol.* **19**, 508–516.
- Krinsky, N. I. 1994: The biological properties of carotenoids. *Pure Appl. Chem.* **66**, 1003–1010.
- Lawes, M. J., 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. *Anim. Behav.* **45**, 549–558.
- Lessels, C. M. & Boag, P. T. 1987: Unrepeatable repeatabilities: a common mistake. *Auk* **104**, 116–121.
- Lozano, G. A. 1994: Carotenoids, parasites, and sexual selection. *Oikos* **70**, 309–311.
- Maney, D. L., Davis, A. K., Goode, C. T., 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. & Harper, D. G. C. 1988: The evolution of aggression: can selection generate variability? *Philos. Trans. Roy. Soc. London B* **319**, 557–570.
- Maynard Smith, J. & Harper, D. 2003: *Animal signals*. Oxford Series in Ecology and Evolution. Oxford Univ. Press, Oxford.
- McGraw, K. J. & Hill, G. E. 2000a: Carotenoid-based ornamentation and status signalling in the House Finch. *Behav. Ecol.* **11**, 520–527.
- McGraw, K. J. & Hill, G. E. 2000b: Plumage brightness and breeding-season dominance in the house finch: a negatively correlated handicap? *Condor* **32**, 90–94.
- McGraw, K. J., Correa, S. M. & Adkins-Regan, E. 2006: Testosterone upregulates lipoprotein status to control sexual attractiveness in a colorful songbird. *Behav. Ecol. Sociobiol.* **60**, 117–122.
- Metz, M., Geberzahn, N., Hansen, L. H., Klump, G. M. & Friedl, T. W. P. 2007: Effects of behavioural time budgets and nest-building efficiency on male reproductive performance in red bishops (*Euplectes orix*). *J. Ornithol.* **148**, 145–155.
- Olson, V. A. & Owens, I. P. F. 1998: Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol. Evol.* **13**, 510–514.
- Parker, T. H. & Ligon, J. D. 2002: Dominant male red jungle fowl (*Gallus gallus*) test the dominance status of other males. *Behav. Ecol. Sociobiol.* **53**, 20–24.
- Peters, A. 2007: Testosterone and carotenoids: an integral view of trade-offs between immunity and sexual signalling. *Bioessays* **29**, 427–430.
- Peters, A., Denk, A. G., Delhey, K. & Kempenaers, B. 2004: Carotenoid-based bill colour as an indicator of immunocompetence and sperm performance in male mallards. *J. Evol. Biol.* **17**, 1111–1120.
- Peters, A., Delhey, K., Andersson, S., van Noordwijk, H. & Förstler, M. I. 2008: Condition-dependence of multiple carotenoid-based plumage traits: an experimental study. *Func. Ecol.* **22**, 831–839.
- Piper, W. H. & Wiley, H. 1989: Correlates of dominance in wintering white-throated sparrows: age, sex and location. *Anim. Behav.* **37**, 298–310.
- Popp, J. W. 1987: Resource value and dominance among American goldfinches. *Bird Behav.* **7**, 73–77.
- Probst, J. R., Donner, D. M. & Bozek, M. A. 2007: Continuous, age-related plumage variation in male Kirtland's warblers. *J. Field Ornithol.* **78**, 100–108.
- Pryke, S. R. & Andersson, S. 2003a: Carotenoid-based status signalling in red-shouldered widowbirds (*Euplectes axillaris*): epaulet size and redness affect captive and territorial competition. *Behav. Ecol. Sociobiol.* **53**, 393–401.
- Pryke, S. R. & Andersson, S. 2003b: Carotenoid-based epaulettes reveal male competitive ability: experiments with resident and floater red-shouldered widowbirds. *Anim. Behav.* **66**, 217–224.
- Pryke, S. R. & Griffith, S. C. 2006: Red dominates black: agonistic signalling among head morphs in the colour polymorphic Gouldian finch. *Proc. R. Soc. Lond. B* **273**, 949–957.
- Pryke, S. R., Andersson, S. & Lawes, M. J. 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, S. R., Andersson, S., Lawes, M. J. & Piper, S. E. 2002: Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color. *Behav. Ecol.* **13**, 622–631.
- Roelants, H., Schneider, F., Göritz, F., Streich, J. & Blottnner, S. 2002: Seasonal changes of spermatogonial proliferation in roe deer, demonstrated by flow cytometric analysis of *c-kit* receptor, in relation to follicle-stimulating hormone, luteinizing hormone, and testosterone. *Biol. Reprod.* **66**, 305–312.
- Rohwer, S. A. 1975: The social significance of avian winter plumage variability. *Evolution* **29**, 593–610.
- Rohwer, S. A. 1982: The evolution of reliable and unreliable badges of fighting ability. *Amer. Zool.* **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.
- von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. 1999: Good genes, oxidative stress and condition-dependent sexual signals. *Proc. R. Soc. Lond. B* **266**, 1–12.
- Searcy, W. A. & Nowicki, S. 2005: *The Evolution of Animal Communication: Reliability and Deception in Signaling Systems*. Princeton Univ. Press, Princeton.
- Senar, J. C. 1999: Plumage colouration as a signal of social status. In: *Proceedings of the 22nd International Ornithological Congress* (Adams, N. J. & Slotow, R. H., eds). Birdlife South Africa, Durban, pp. 1669–1686.
- Senar, J. C. 2006: Color displays as intrasexual signals of aggression and dominance. In: *Bird Coloration, Vol 2. Function and Evolution* (Hill, G. E. & McGraw, K. J., eds). Harvard Univ. Press, Cambridge, pp. 87–136.
- Senar, J. C. & Escobar, D. 2002: Carotenoid derived plumage coloration in the siskin *Carduelis spinus* is related to foraging ability. *Avian Sci.* **2**, 19–24.
- Sergio, F., Blas, J. & Hiraldo, F. 2009: Predictors of floater status in a long-lived bird: a cross-sectional and longitudinal test of hypotheses. *J. Anim. Ecol.* **78**, 109–118.
- Shawkey, M. D., Estes, A. M., Siefferman, L. M. & Hill, G. E. 2003: Nanostructure predicts intraspecific variation in ultraviolet-blue plumage colour. *Proc. R. Soc. Lond. B* **270**, 1455–1460.
- Siefferman, L. M. & Hill, G. E. 2005: UV-blue structural plumage coloration and competition for nestboxes in male Eastern bluebirds. *Anim. Behav.* **69**, 67–72.
- Siitari, H., Alatalo, R. V., Halme, P., Buchanan, K. L. & Kilpimaa, J. 2007: Color signals in the black grouse (*Tetrao tetrix*): signal properties and their condition dependency. *Am. Nat.* **169**, S81–S92.
- Smiseth, P. T., Örnborg, J., Andersson, S. & Amundsen, T. 2001: Is male plumage reflectance correlated with paternal care in Bluethroats? *Behav. Ecol.* **12**, 164–170.
- Smith, J. N. M., Montgomerie, R. D., Taitt, M. J. & Yom-Tov, Y. 1980: A winter feeding experiment on an island song sparrow population. *Oecologia* **47**, 164–170.
- Sol, D., Santos, D. M., Garcia, J. & Cuadrado, M. 1998: Competition for food in urban pigeons: the cost of being juvenile. *Condor* **100**, 298–304.
- Soma, K. K. 2006: Testosterone and aggression: Berthold, birds and beyond. *J. Neuroendocrinol.* **18**, 543–551.
- Stahl, J., Tolsma, P. H., Maarten, J. J. E. & Drent, R. H. 2001: Subordinates explore but dominants profit: resource competition in high Arctic barnacle goose flocks. *Anim. Behav.* **61**, 257–264.
- Studd, M. V. & Robertson, R. J. 1985: Evidence for reliable badges of status in territorial yellow warblers (*Dendroica petechia*). *Anim. Behav.* **33**, 1102–1113.
- Sundberg, J. 1995: Female yellowhammers (*Emberiza citrinella*) prefer yellower males: a laboratory experiment. *Behav. Ecol. Sociobiol.* **37**, 275–282.
- Whitehouse, M. J. 1997: Experience influences male-male contests in the spider *Argyrodes antipodiana* (Theridiidae: Aranea). *Anim. Behav.* **53**, 913–923.
- Wiebe, K. L. & Bortolotti, G. R. 2002: Variation in carotenoid-based color in Northern Flickers in a hybrid zone. *Wilson Bull.* **114**, 393–400.
- Wiley, R. H., Steadman, L., Chadwick, L. & Wollerman, L. 1999: Social inertia in white-throated sparrows results from recognition of opponents. *Anim. Behav.* **57**, 453–463.
- Wingfield, J. C. 1994: The regulation of territorial behavior in the sedentary song sparrow, *Melospiza melodia morphna*. *Horm. Behav.* **28**, 1–15.
- Wingfield, J. C., Lynn, S. E. & Soma, K. K. 2001: Avoiding the “Costs” of testosterone: ecological basis of hormone-behavior interactions. *Brain Behav. Evol.* **57**, 239–251.
- Wolfenbarger, L. L. 1999: Is red coloration of male Northern Cardinals beneficial during the nonbreeding season: a test of status signalling. *Condor* **101**, 655–663.
- Yasukawa, K., Enstrom, D. A., Parker, P. G. & Jones, T. C. 2009: Epulet color and sexual selection in the red-winged blackbird: a field experiment. *Condor* **111**, 740–751.
- Zuk, M., Thornhill, R., Ligon, J. D., Johnson, K., Austad, S., Ligon, S. H., Thornhill, N. W. & Costin, C. 1990: The role of male ornaments and courtship behavior in female mate choice of red jungle fowl. *Am. Nat.* **136**, 459–473.
- Zysling, D. A., Greives, T. J., Breuner, C. W., Casto, J. M., Demas, G. E. & Ketterson, E. D. 2006: Behavioral and physiological responses to experimentally elevated testosterone in female dark-eyed juncos (*Junco hyemalis carolinensis*). *Horm. Behav.* **50**, 200–207.