

The role of pigment based plumage traits in resolving conflicts

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Abstract

The role of melanin ‘badges of status’, in male-male competition has been well-studied, in contrast, carotenoid based plumage has largely been examined in the context of female mate choice. Recent work has shown that carotenoid signals can also function in male-male competition, although the functions of the two types of signals is currently unclear. Here, we examine the relationships between colouration, dominance and aggression in the crimson finch (*Neochmia phaeton*), a species where males have both conspicuous red carotenoid plumage and a black melanin patch. We examined the importance of carotenoid and melanin based signals in three contexts: 1) among free-living birds interacting at a feeding station: we found that neither colour signal influenced the outcome of interactions; 2) in staged dyadic contest in captivity: we found that coloration from carotenoid pigments was positively related to the probability of winning a contest, while the size of the melanin plumage patch was not related to winning; and 3) in staged dyadic contests where male plumage colour had been masked: we found that the number of interactions required to determine dominance increased. While the underlying natural plumage colour was still important in these contests, birds with more intense carotenoid colouration were now more likely to lose. These results confirm carotenoid-based signalling in male-male contests. However this signal is used in conjunction with other factors such as self-assessment and body condition. Contrary to additional expectations, the black melanin patch was not found to be important in this context.

Key Words: Crimson finch, aggression, colour signal, dominance, melanin, carotenoids

Introduction

Conspicuous colouration is often a sexually selected trait used by females to assess male health, condition, parasite resistance, or resource holding potential (Hamilton and Zuk 1982, Hill 1990). The status-signalling hypothesis, on the other hand, suggests that colourful plumage ornamentation evolved to signal dominance status and fighting ability (Rohwer 1975, Rohwer 1982). According to this hypothesis, conspicuous plumage signals allow individuals to gather information about their opponents' fighting ability or motivation (e.g. Bossema and Burgler 1980, Enquist and Leimar 1983, 1994, Senar 1990, Chaine and Lyon 2008), and may give an opponent the opportunity to withdraw before the conflict escalates to high-cost physical combat (Evans and Hatchwell 1992, Jakobsson et al. 1995). There is increasing evidence that plumage colouration is used in direct male-male competition, both within and outside of the breeding season (reviewed in Santos et al 2011). However the role of colour in settling male-male conflicts remains contentious (Senar 2006), and the relative importance of different colour signals are currently unclear.

The two pigments commonly associated with status-signalling plumage are carotenoids and melanins. Traditionally, research on male-male competition has focused on melanin patches. Melanin is responsible for most brown or black plumage, and patches of melanin plumage are often referred to as badges of status, with the size of the melanin patch signalling dominance or status (Dawkins and Krebs 1978). However, because melanin can be synthesised internally at supposedly little cost to the individual, the honesty of the signal is questionable and its reliability as a social signal remains contentious (Veiga 1995, Jawor and Breitwisch 2003, Roulin 2015). On the other hand, a number of studies point to mechanisms that may keep melanin signals honest (reviewed in Roulin 2015). For example, the development of extensive melanin patches has been associated with high levels of testosterone and melanocortins (McGraw 2008, Ducrest et al. 2008). Both hormones can have pleiotropic

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effects; e.g. testosterone is often positively associated with aggression and negatively associated with immune function (Folstad and Karter 1992, Buchanan et al. 2003), therefore it may play a role in maintaining the honesty of the signal (Anderson 1994, Buchanan et al. 2010). Melanin signals may also be kept honest through social mediation. For instance, if poor quality individuals develop large melanin badges, they may be repeatedly tested by more dominant individuals, and encounter more potentially harmful conflicts (Maynard Smith and Harper 1988, 2003).

Carotenoid pigments are responsible for many of the bright red, orange and yellow plumage displays traditionally linked to female mate choice (Brush 1981, Hill 1991, Møller et al. 2000). However, an increasing number of studies report that carotenoid pigments may also play a role in male-male competition (e.g. Pryke et al. 2002, Pryke and Andersson 2003, Hamilton et al. 2013). Unlike melanins, carotenoids can only be extracted from food, suggesting that carotenoid displays are an intrinsically honest signal of quality (Hill 2006). Furthermore, carotenoids are vital to many physiological processes and their uptake, as well as subsequent deposition in plumage, may be inhibited by parasites (Lozano 1994, Owens and Olson 1998, Møller et al. 2000, Svensson and Wong 2011). The use of carotenoids in status signalling is primarily linked to the hue, rather than patch size (but see Pryke and Andersson 2003b). Recent research has shown that experimentally intensifying red and orange colour patches can increase social dominance (e.g. Evans and Norris 1996, Pryke and Griffith 2006). Other studies have shown that the colour red may be an innate signal of aggression, rather than one learned through social reinforcement (Pryke 2009). Despite this, the role of carotenoids in agonistic encounters is still poorly understood (Senar 2006), and the relative importance of melanin patches vs carotenoid signals is currently unclear. One complication is that many species exhibit either carotenoid or melanin signals, not both,

making it difficult to disentangle the functions of the two pigments based plumage traits (but see McGraw and Hill 2000, Dongen and Mulder 2007).

In this study we examine the relative role of co-occurring melanin and carotenoid plumage characteristics in agonistic encounters between male crimson finches (*Neochmia phaeton phaeton*). Crimson finches are a sexually dimorphic grass finch found across northern Australia. Females are largely brown with red plumage on their faces, rumps and to a small extent on their backs. Males display conspicuous red plumage on their face, chest, back and rump as well as a black patch extending from the undertail coverts to part way up the chest, making them a valuable species with which to explore melanin and carotenoid signalling simultaneously. Crimson finches breed in socially monogamous pairs, within loose colonies of up to 20 pairs (Higgins et al. 2006). While many group living birds develop stable dominance hierarchies with their neighbours, crimson finches are well known for their intensely aggressive behaviour and relentless nature (Forshaw et al. 2012). Although the majority of conflicts are settled without physical fighting, physical contact is not uncommon and the potential for injury or even death is high (Forshaw et al. 2012). Since crimson finches live in stable year-round groups, it is predicted that the males will display one or more plumage characteristic that signals dominance and/or fighting ability (status-signalling hypothesis, Rohwer 1975). To examine the role of plumage coloration in aggressive interactions we: a) documented natural colour variation and interactions in free living/wild populations of crimson finches and b) performed two captive contest experiments (un-manipulated and manipulated plumage colour).

Methods

Morphology measurements

For each bird we measured tarsus ($\pm 0.01\text{mm}$), and mass ($\pm 0.5\text{g}$) which we used to create an indicator of body condition, by regressing the residuals of $\log(\text{mass})$ on $3\log(\text{tarsus length})$ (Andersson 1994). All measurements on all birds were taken by the same person.

Colourimetrics

Crimson finches display two main types of plumage, a black melanin based patch and areas of red carotenoid plumage. In males, red carotenoid plumage is present on the face, chest, back and rump, while melanin based plumage is present on the birds belly, extending up the chest (Figure 1). We estimated the area of the melanin patch as patch length (measured from the top of the patch to the cloaca) multiplied by patch width (all measured with digital callipers to the nearest 0.01mm). To confirm the presence of carotenoid pigments in the red plumage we tested feather samples from six birds, as described in McGraw et al. (2005).

The red colour patches (Figure 1: face, chest, rump and back) were quantified using an Ocean Optics Jaz spectrometer (Ocean Optics, Dunedin USA) with illumination from a xenon Ocean Optics PX-2 light source (Ocean Optics, Dunedin USA) and a fibreoptic probe. Three consecutive scans were taken from the centre of each colour patch, and then averaged for each patch. The probe was fitted with a tip to standardise the distance between the probe and the feathers. The tip was rested on, but not pushed against the feathers. The probe was lifted and moved between scans and recalibrated to the white standard at minimum every hour. Only values between 320 and 700nm were used in the analysis as this corresponds to the visual range of birds (Bennett and Théry 2007). To assess the colour through an avian visual system we analysed spectra in the package ‘*Pavo*’ (Maia et al. 2013) in R 3.2.1 (R

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Development Core Team 2014). Coefficients of variance for melanin and carotenoid measurements are shown in Table S1.

The visual system of crimson finches has not been analysed, however other closely related finches fall into the ultra-violet sensitive (UVS) classification (Bowmaker et al. 1996, Hart et al. 2000). Therefore we used a generalised model of a bird UVS visual system (blue tit) for analysis. To quantify the contrasts between plumage areas and a neutral background (30% grey) we applied the Vorobyev-Osorio model (Vorobyev and Osorio 1998, Siddiqi et al. 2004). This model estimates differences between two areas in units of discrimination threshold or “Just Noticeable Differences” (JND’s, Vorobyev et al. 1998) and constructs a measure of chromatic and achromatic contrasts, while also taking into account photoreceptor noise as a limiting factor in visual discrimination (Vorobyev and Osorio 1998, Osorio and Vorobyev 2008). In this way we are able to quantify colours as they would be seen by other birds. For further details on colour analysis see supplementary material. We used a principle components analysis (PCA), to condense the correlated spectral scores of the red plumage areas; face, back, rump and chest.

Behavioural observations of free-living populations

Fieldwork was conducted between January and September 2013 in areas near Wyndham in the East Kimberley region of Western Australia (15_340S, 128_090E). Crimson finch habitat in this region is characterised by areas of pandanus (*Pandanus spiralis*) close to permanent water sources. Birds were banded with a numbered metal band from the Australian Bird and Bat Banding Scheme and a unique combination of three colour bands for individual recognition. In each of five known feeding areas, two to three feed trays were erected, approximately 100m apart. The trays (20x40cm) were positioned 1 to 1.5m off the ground and were refilled with seed (standard finch mix) every two to three days until the birds

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became familiar with the food source. Behavioural interactions were recorded in August and September 2013 between 5am and 11am, either by direct observations or from video recordings. This time of year corresponds to the non-breeding season and birds generally move in feeding flocks (Higgins et al. 2006, Milenkaya et al. 2011). The video camera or observer was positioned within 5-10 metres of the feeding tray. For each observation an adult banded male was selected as the focal individual and all interactions he was involved in were recorded until he left the feeding tray. Subsequently, another focal male was selected from the individuals still on the feeding tray. An interaction was logged when there was a passive displacement (e.g. supplanting another individual) or an active attack (often resulting in physical contact). The outcome of each interaction was denoted as won or lost by the focal male: individuals were said to have lost if they were displaced from their position or forced to leave the tray as a result of the interaction. Each individual was observed over multiple sessions for a minimum total of 7 minutes (mean=12.54mins \pm 3.7), the date of the observation was also recorded.

Experiments on captive birds

All birds were sourced from a wild-type aviculturist in Eastern Australia, the birds are of known pedigree and are free from plumage mutations that are present in some captive populations. Each bird was banded with an individually numbered band, and measured for condition and colourimetrics using the methods described above. Captive male crimson finches (n=17) were housed individually in wire cages (90x90x70cm), visually isolated from other birds, in climate-controlled aviaries at the Australian National University, Australia.

Two types of captive contests were staged in January 2014: dyads with un-manipulated plumage colours (n = 37); and dyads with chest plumage colour experimentally manipulated

(n = 44). All males competed in both types of contests, but always against unfamiliar opponents. Trials lasted 20 minutes and were conducted in wire cages (90x90x70cm) that represented neutral territory and were visually isolated from other birds. Opponents competed over a single food source in the form of a familiar feeder setup so only one bird could feed at a time. To standardise motivation all food was removed from the males' cages the evening before the contests as detailed in similar studies (Lemel and Wallin 1993, McGraw and Hill 2000, Pryke et al. 2002). Prior to release into the test cage, males were temporarily assigned a random colour band (green, blue, white or yellow) for identification on the video recording. Competing males were then released simultaneously into the testing cage. All contests were conducted within 2.5 hours of sunrise (approximately between 6 and 8:30am) after which time birds were returned to their original cages and their food replaced. Each trial was filmed (Sony HDR-CX220 Handycam) and all interactions were scored from the video. The winner was said to be the individual who won the majority of interactions (>70%). Trials that resulted in no interactions were considered draws (n = 17 of 81) and were excluded from analysis (Pryke et al. 2001, Pryke and Anderson 2003a).

In the second contest type, both individuals in the dyad had their chest plumage experimentally manipulated. For each contest trial, we darkened the red chest colour of one male using a non-toxic marker, effectively blocking the carotenoid colouration (Black100 Copic Too Marker Products, Toyko). This treatment had the added effect of extending the melanin patch. To control for the effect of marking, the other male in the dyad had his chest coloured with a red marker (R2 Copic Too Marker Products, Toyko) that closely matched the natural red plumage colour (see figure 1). This control also standardised the red chest colouration of the males. Chest colour was chosen for manipulation because of its prominence on the bird there was no chance of the manipulation not being seen by the other

contestants. Additionally, chest colouration has been used to investigate signal in other species (e.g. Wolfenbarger 1999) and was chosen in preference to the face to avoid the sensitive area around the eyes. Each bird competed as both darkened and a control, but always against an unfamiliar opponent. Plumage colour was measured using a spectrometer and analysed using the bird visual model as described above.

Analyses and statistics

To evaluate factors influencing contest success, a generalized linear mixed model (GLMM) with binomial (win/loss) link function was used. Principle components analysis was conducted in JMP 11.0 while all other analysis was run in R 3.1.2 (R Development Core Team 2014) using the packages ‘*lme4*’ (Bates et al. 2014), ‘*arm*’ (Gelman and Su 2014), ‘*MuMIn*’ (Barton 2014) and ‘*AICcmodavg*’ (Mazerolle 2013). To determine which variables were important in shaping contest outcome, initial models included all combinations of condition, colourimetrics and melanin patch size. In free-living populations each observed interaction was treated as a separate data point and analysed using measurements from the focal bird only, as the ID of the opponent was not always known. The duration of observations (minutes) was included as a fixed effect and individual ID fitted as random factor. We used the same approach in captive dyads except instead of individual interactions, we used overall trial outcome and fitted trial order as a factor. For each contest, we calculated the differences in morphological and colour measurements between the two competitors as each winner cannot be considered independent of the loser. Prior to analysis we standardised variables using the package ‘*arm*’ (Gelman and Su 2014).

We used model averaging techniques (Burnham and Anderson 2002) to evaluate the relative importance of colour variables and condition on contest success. When models were too large

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to converge we followed the suggestion of Bolker et al. (2009) and removed variables that had the least impact based on Akaike information criterion, corrected for small sample size (AICc; Grueber et al. 2011, Symonds and Moussalli 2011). Using the model averaging approach a set of all possible combinations of models is created from a global model. These models are then ranked according to goodness-of-fit to the data, based on AICc scores. We calculated the differences in AICc ($\Delta AICc$) between the best model (i.e. with the lowest AICc) and every other model and considered only models with a $\Delta AICc < 3$. Using these top models, we then estimated the relative importance of each variable by summing the weights of all models containing that variable. The relative importance reflects how each variable improves the model fit and estimates the probability that it is part of the best model (Symonds and Moussalli 2011). All models were also run as regressions however the top models were found to be the same.

To evaluate the effect of treatment (control or darkened) on the number of interactions per contest we used a GLM with Poisson link function, with the number of interactions as the dependant variable. A second GLM with binomial link was used to test the effect of treatment on contest outcome. In this case we used treatment to predict contest outcome.

RESULTS

Principle Components

We conducted PCA's on spectral data after putting it through the avian visual system, to create an indicator of overall colour or 'redness'. To do this we used both chromatic and achromatic measures of red plumage areas, face, back, rump and chest. All principle components (PC's) with an eigenvalue of greater than one were retained for further analysis.

In free living populations PC1 and PC2 explained a total of 65% variance (table 1). The

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coefficients in PC1 were all positively weighed and of similar magnitude. In the captive population the first three PC's accounted for 80% of the variation in the colour (table 1). PC1 explained 42% with all the top coefficients loading positively (chest achromatic, back achromatic, back chromatic). Additionally, the presence of carotenoid pigments was confirmed for all feather samples tested.

Free-living populations

In total 731 interactions were recorded for the 17 focal males observed on the feeding trays. Of the 14 models with $\Delta AICc < 3$, the null hypothesis was the best model (AICc 397.6), while the second best model included just condition (AICc 397.9; for a full list of models see supplementary S2). The length of each observation was fitted as a fixed effect and so has a relative importance score of 1 (i.e. it appears in all the models), while condition shows a lower score of 0.37 and a negative estimate (table 2); i.e. birds in worse condition were more likely to win an interaction. There were no significant correlations between the variables in all models ('redness', melanin patch size, condition) and interaction terms were not included in the models.

Captive population

Dyads with un-manipulated plumage colour.

In captive dyads with un-manipulated plumage colour ($n = 37$), the only predictor of contest outcome was redness PC1 (table 3). Birds with 'redness' score higher than their opponent were more likely to win contests. The top model contained redness PC1 and PC2 while the second contained just PC1, the difference in AICc values was 0.3 (full list of models in supplementary S3). Patch length appeared in just one and condition in two of the top five models with $\Delta AICc < 3$. The PCA1 score for redness had a relative importance of 1.0 as it

appeared in all the top models (table S3). The second highest relative importance was PCA2 (0.42). Approximately 20% of contests resulted in a draw, either because the birds did not interact or because there was no clear winner.

Dyads with manipulated plumage colour

In colour manipulated dyads (n=44), model averaging showed the PC1 value of natural 'redness' and condition to be the best predictors of contest outcome, both with high relative importance values (table 3). In this case 'redness' had a negative effect, while condition had a positive effect on contest outcome. The top model (AICc 38.92) contained PC1, and condition and was 2.58 AICc's above the second top model (AICc 41.5), which contained PC1, PC2 and condition (table S4). Again, roughly 20% of contests were considered a draw, i.e. the birds did not interact or there was no clear winner.

Effects of manipulated color

There was no effect of treatment/manipulation; control individuals won 56% of contests (n=36, GLM p=0.51, figure 3a). However there were significantly more interactions in colour manipulated dyads than in un-manipulated dyads (n = 44, GLM p=<0.001, mean interactions per contest; un-manipulated plumage 9.05, manipulated plumage 17.11, figure 3b). in total 59% of contests were won by the bird that initiated it.

Discussion

We examined the role of carotenoid and melanin based plumage in male-male contests by testing the relationships between contest outcomes, colour and condition. In the group living crimson finch, we found that morphology and colour were not good predictors of contest outcome when birds competed for a food resource in the wild. However, in captive dyads

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between unfamiliar individuals, carotenoid plumage was the most important predictor of contest outcome. Hence, it may act as a signal of fighting ability and aggressive disposition between unfamiliar birds. In contrast, melanin patch size was unrelated to contest outcome. Manipulation of the most prominent carotenoid patch, the chest, did not predict contest outcome, although it significantly increased the number of interactions per contest. Despite the manipulation, the individuals underlying natural colour was still important, but in the opposite direction. Together these results suggest that carotenoid, and not melanin plumage, is an important social signal in crimson finches.

Aggression in free-living populations

In free-living birds, we found no relationship between contest outcome and any of the potential predictors of fighting ability including redness, melanin patch size, and body condition. In fact, condition was shown to be the most important variable with individuals in poorer condition having a slightly better chance of winning an interaction. This scenario is often called the ‘desperado effect’ or ‘divisive asymmetry’ (Grafen 1987, Morrell et. al. 2005) and has previously been shown in many bird species (e.g. yellowhammers, *Emberiza citronella*, Sundberg 1995, Griffon vultures, *Gyps fulvus*, Bose and Sarrazin 2007, red bishops, *Euplectes orix*, Edler and Friedl 2010). Such patterns are expected when the reward is greater than the cost of fighting, when motivational factors such as hunger differ between individuals, or the level of competition is low (Senar 2006). However, because the relative importance of condition was low (0.50, table 3), its effect on interaction outcome should be considered weak at best, and should be interpreted with caution.

Why did we not detect effective predictors of contest outcome? One reason may be that our study focused on adult males. In red bishops (Edler and Friedl 2010), the age based

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dominance hierarchy was so high that it masked any other signals of dominance. Such strong age related dominance structures, in which older individuals dominate, appear common in birds (e.g. Balph et al. 1979, Rohwer et al. 1981, Enoksson 1988). Additionally, our observations did not include individuals that did not come to the feed tray. If there was a non-random bias regarding which birds did and did not visit the tray (e.g. low vs. high ranking individuals) this may have biased the interactions we were able to observe. Furthermore, we could not account for more subtle displays, e.g. exhibition flights, which may discourage lower quality individuals from engaging in a contest (Alonso-Alvarez et al. 2004). This is likely to be especially important as crimson finches are year-round residents and form relatively stable groups. Finally, familiar birds are likely to have already established dominance hierarchies that do not require physical interactions. Plumage signals may therefore be more important when interacting with unfamiliar birds, which would most likely occur during dispersal.

Captive dyads: un-manipulated plumage

In staged trials where birds were unfamiliar with each other, we found that the redness score was overwhelmingly the best predictor of contest outcome. This further supports the possibility that familiarity of birds in natural flocks may have masked the effect of carotenoids in predicting contest outcome in the wild. Under sexual selection both carotenoid and melanin based signals are important for social signalling, however they are generally thought of as functioning in disparate contexts (Senar 2006). Here we add to the growing number of studies demonstrating that carotenoids can also function as signals in male-male competition (e.g. Evans and Hatchwell 1992, Pryke et al. 2001, Pryke and Andersson 2003a). Furthermore, the traditional dominance signal, the size of the melanin patch, was not important in contest outcome.

Captive dyads: manipulated plumage

When we manipulated the prominent chest signal, outwards/visible plumage colour no longer predicted contest outcome: a similar number of darkened and control birds won contests (figure 3a). However, the underlying natural plumage colour was still an effective predictor of contest outcome, although in this case redness had a negative effect on contest outcome. Other studies have also found that after colour manipulation, naturally redder birds remained dominant (e.g. Pryke and Griffith 2006), while others found that the strength of the signal changed or disappeared (Wolfenbarger 1999, Edler and Field 2010). Wolfenbarger (1999) suggested this indicates that while carotenoid colour is an important signal in male-male dominance, it is not the only factor influencing contest outcome. In support of this we found that in manipulated colour contests, condition had an important influence on contest outcome, birds in better condition were more likely to win contests. These results also add support for the hypothesis that melanin is not an important signal in crimson finches. In addition to blocking the red plumage on the chest, our experimental darkening also greatly enlarged the melanin patch. Despite this increase, treatment (darkened or control) was unrelated to contest outcome in manipulated plumage contests. Additionally melanin patch size was unrelated to contest outcome in both manipulated and un-manipulated contests. However, the melanin patch may still play a role in signalling: Endler (2012) suggests that conspicuousness is dependent on contrasts between adjacent colour patches. In the crimson finch, the melanin patch contrasts strongly with the red chest, and may strengthen the red signal.

The clearest result of this study is that masking the natural red plumage colour led to an increase in the number of male-male interactions per contest. This suggests that when colour signals were altered, individuals found it more difficult to make a visual assessment of the

unfamiliar opponent's status or fighting ability. As a consequence, competing males spent more time and energy establishing dominance. More time spent in aggressive interactions not only increases the chance of injury, but also reduces the time available to invest in other activities such as foraging, feeding chicks and/or mate guarding (Senar 2006), as well as increasing predation risk (Jakobsson et al. 1995). Given that crimson finches live in small stable groups, and their propensity towards aggressive behaviour, a plumage signal which reduces the time spent engaging in potentially dangerous interactions may be very valuable.

Conclusion

Our results suggest that, in crimson finches, red carotenoid based plumage, rather than the black melanin based plumage, is used as a signal of aggression and competitive ability in agonistic contests. When plumage colour is manipulated (darkened), it becomes an unreliable signal and males spend more time determining dominance. Individual condition also becomes important for determining contest outcome/dominance in the manipulated contests. This study supports previous findings that red signals are important in dominance interactions (e.g Bakker and Sevenster 1983, Evans and Norris 1996, Pryke et al. 2001, Crowley and Magrath 2004, Pryke et al. 2009), and shows that carotenoids are important in a broader context than previously predicted. The use of red plumage signals in male-male competition does not rule out the possibility of the red plumage also being used in mate choice. Future studies should consider the interaction between mate choice and male-male competition and how it can affect the evolution of plumage signals.

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References

- Alonso-Alvarez, C., Doutrelant, C. and Sorci, G. 2004. Ultraviolet reflectance affects male-male interactions in the blue tit (*Parus caeruleus ultramarinus*). - *Behav. Ecol*, 15: 805-809.
- Andersson, S. 1994. Costs of sexual advertising in the lekking Jackson's widowbird *Euplectes jacksoni*. - *Auk*, 96: 1-10.
- Bakker, T. C. M. and Sevenster, P. 1983. Determinants of dominance in male sticklebacks (*Gasterosteus aculeatus L.*). - *Behaviour*. 86: 55-57.
- Balph, M. H., Balph, D. E. and Romesburg, H. C. 1979. Social status signalling in winter flocking birds: an examination of a current hypothesis. - *Auk*. 96: 78-93.
- Barlow, G. W. 1983. Do gold midas cichlid fish win fights because of their color, or because they lack normal coloration? A logistic solution. - *Behav. Ecol. Sociobiol.* 13: 197-204.
- Barton, K. 2014. MuMIn: Multi-model inference. R package version 1.10.5
- Bates, D., Maechler, M., Bolker, B. and Walker, S. 2014. *_lme4: Linear mixed-effects models using Eigen and S4_*. R package version 1.1-7 -<http://CRAN.R-project.org/package=lme4>>.
- Bennett, A. T. D. and Théry, M. 2007. Avian color vision and coloration: Multidisciplinary evolutionary biology. - *Am. Nat.* 169: 1-6.
- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. and White, J. S. S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. - *Trend. Ecol. Evol.* 24: 127-135.
- Bossema, I. and Burgler, R. R. 1980. Communication during monocular and binocular looking in European Jays (*Garrulus G Glandarius*). - *Behaviour*. 77: 274-283.

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

Bowmaker, J. K., Heath, L. A., Wilkie, S. E. and Hunt, D. M. 1997. Visual Pigments and Oil Droplets from Six Classes of Photoreceptor in the Retinas of Birds. - *Vision. Res.* 37: 2183-2194.

Buchanan, K. L., Evans, M. R. and Goldsmith, A. R. 2003. Testosterone, dominance signalling and immunosuppression in the House Sparrow, *Passer domesticus*. - *Behav. Ecol. Sociobiol.* 55: 50-59.

Buchanan, K., Evans, M., Roberts, M., Rowe, L. and Goldsmith, A. 2010. Does testosterone determine dominance in the house sparrow *Passer domesticus*? An experimental test. - *J. Avian Biol.* 41: 445-451.

Burnham, K. P. and Anderson, D. R. 2002. Model selection and multi-model inference - a practical information-theoretic approach. 2nd ed. - Springer-Verlag.

Brush, A. 1981. Carotenoids in wild and captive birds. - In Bauernfeind, J. C., Adams, C. R. and Marusch, W.L. (eds), Carotenoids as colorants and vitamin A precursors. Academic Press New York pp. 539-562.

Chaine, A. S. and Lyon, B. E. 2008. Intra-sexual selection on multiple plumage ornaments in the lark bunting. *Anim. Behav.* 76: 657-667.

Crowley, C. E. and Magrath, R. D. 2004. Shields of offence: signalling competitive ability in the dusky moorhen, *Gallinula tenebrosa*. *Aust. J. Zool.* 52: 463-474.

Dawkins, R. and Krebs, J. 1978. Animal signals: information or manipulation? In: Krebs, J.R. and Davies, N. B. (eds), *Behavioural Ecology: an Evolutionary Approach*. Blackwell. pp. 282-309.

Dongen, W.D.F. and Mulder, R.A. 2007. Relative importance of multiple plumage ornaments as status signals in golden whistlers (*Pachycephala pectoralis*). - Behav. Ecol. Sociobiol. 62: 77-86.

Ducrest, A.-L., Keller, L. & Roulin, A. 2008. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. - Trends Ecol. Evol. 23: 502–510.

Edler, A. U. and Friedl, T. W. P. 2010. Plumage Colouration, Age, Testosterone and Dominance in Male Red Bishops (*Euplectes orix*): A Laboratory Experiment. - Ethology. 116: 806-820.

Endler, J. A. 2012. A framework for analysing colour pattern geometry: adjacent colours. - Biol. J. Linnean Soc.107: 233-253.

Enoksson, B. 1988. Age- and sex-related differences in dominance and foraging behaviour of nuthatches *Sitta europaea*. - Anim. Behav. 36: 231-238.

Enquist, M. and Leimar, O. 1983. Evolution of Fighting Behaviour: Decision Rules and Assessment of Relative Strength. - J. Theor. Biol. 102: 387- 410.

Evans, M. R. and Norris, K. 1996. The importance of carotenoids in signalling during aggressive interactions between male firemouth cichlids (*Cichlasoma meeki*). - Behav. Ecol. 7: 1-6.

Evans, M. R. and 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.

Folstad, I. and Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. - Am. Nat. 139: 603-622.

Accepted Article
Forshaw, J. M., Shephard, M. and Pridham, A. 2012. Grassfinches of Australia. - CSIRO Publishing.

Gelman, A. and Su, Y. 2014. arm: Data Analysis Using Regression and Multilevel/Hierarchical Models. R Version 1.7-07. - <<http://CRAN.R-project.org/package=arm>>.

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. and 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.

Grueber, C. E., Nakagawa, S., Laws, R. J. and Jamieson, I. G. 2011. Multimodel inference in ecology and evolution: challenges and solutions. - *J Evol Biol.* 24: 699-711.

Hamilton, W. D. and Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? - *Science.* 218: 384-387.

Hamilton, D. G., Whiting, M. J. and Pryke, S. R. 2013. Fiery frills: carotenoid based colouration predicts contest success in frillneck lizards. - *Behav. Ecol.* 24:1138-1149.

Hart, N. S., Partridge, J. C. and Bennett, A. T. D. 2000. Visual pigments, cone oil droplets and ocular media in four species of estrildid finch. - *J. Comp. Physiol. A.* 186: 681-694.

Higgins, P. J., Peter, J. M. and Cowling, S. J. 2006. 'Handbook of Australian, New Zealand and Antarctic Birds. Vol. 7: Boatbill to Starlings.' - Oxford Uni Press.

Hill, G. E. 1990. Female House Finches prefer colourful males: sexual selection for a condition-dependent trait. - *Anim. Behav.* 40: 563-572.

Hill, G. E. 1991. Plumage colouration is a sexually selected indicator of male quality. - *Nature.* 350: 337-339.

Hill, G. E. 2006. Environmental regulation of ornamental coloration. In: Hill, G. E. and McGraw, K. J. (eds), Bird Coloration Volume 1: Mechanisms and Measurements. - Harvard Uni Press. pp. 507-560.

Jakobsson, S., Brick, O. and Kullberg, C. 1995. Escalated fighting behaviour incurs increased predation risk. - Anim. Behav. 49: 235-239.

Jawor, J. M. and Breitwisch, R. 2003. Melanin ornaments, honesty, and sexual selection. - Auk. 120: 249-265

JMP[®], Version 11.1.1. SAS Institute Inc., Cary, NC, 1989-2007.

Lemel, J. and Wallin, K. 1993. Status signalling, motivational condition and dominance: an experimental study in the great tit, *Parus major L.* - Anim. Behav. 45: 549-558.

Maia, R., Eliason, C. M., Bitton, P. P., Doucet, S. M. and Shawkey, M. D. 2013. Pavo: an R Package for the analysis, visualization and organization of spectral data. - Methods Ecol. Evol. 4: 906–913.

Maynard Smith, J and Harper, D. 1988. The evolution of aggression: can selection generate variability? - Philos. Trans. R. Soc. B. 319: 557-570.

Maynard Smith, J and Harper, D. 2003. Animal Signals. - Oxford Uni Press

Mazerolle, M. J. 2013. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 1.35.- <<http://CRAN.R-project.org/package=AICcmodavg>>.

McGraw, K. and Hill, G. 2000. Plumage brightness and breeding-season dominance in the house finch: a negatively correlated handicap? - Condor. 102: 456-461.

McGraw, K. J., Hudon, J., Hill, G. E. and Parker, R. S. 2005. A simple and inexpensive chemical test for behavioral ecologists to determine the presence of carotenoid pigments in animal tissues. - Behav. Ecol. Sociobiol. 57: 391-397.

McGraw, K. J. 2008. An update on the honesty of melanin-based color signals in birds. - Pigment Cell Melanoma Res. 21: 133-138.

Milenkaya, O., Legge, S. and Walters, J. R. 2011. Breeding biology and life-history traits of an Australasian tropical granivore, the Crimson Finch (*Neochmia phaeton*). - Emu 111: 312-320.

Møller, A. P., Biard, C., Blount, J. D., Houston, D. C., Ninni, P., Saino, N. and Surai, P. F. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immune-competence or detoxification ability? - Poult. Avian. Biol. Rev. 11: 137-159.

Morrell, L. J., Lindström, J. and Ruxton, G. D. 2005. Why are small males aggressive? - Proc. R. Soc. B. 272: 1235-1241.

Osorio, D. and Vorobyev, M. 2008. A review of the evolution of animal colour vision and visual communication signals. - Vision Res. 48: 2042-2051.

Olson, V. A. and Owens, I. P. F. 1998. Costly sexual signals: are carotenoids rare, risky or required? - Trend. Evol. Ecol. 13: 510-514.

Pryke, S., Lawes, M., Andersson, S. 2001. Agonistic carotenoid signalling in male red-collared widowbirds: aggression related to the colour signal of both the territory owner and model intruder. - Anim. Behav. 62: 695-704.

Pryke, S. R., Andersson, S., Lawes, M. J. and Piper, S. E. 2002. Carotenoid status signalling in captive and wild red-collared widowbirds: independent effects of badge size and color. - Behav. Ecol. 13: 622-631.

Pryke, S. R. and Andersson, S. 2003a. Carotenoid-based epaulettes reveal male competitive ability: experiments with resident and floater red-shouldered widowbirds. - Anim. Behav. 66: 217-224.

Pryke, S. R. and Andersson, S. 2003b. 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. and Griffith, S. C. 2006. Red dominates black: agonistic signalling among head morphs in the colour polymorphic Gouldian finch. - Proc. R. Soc. B. 273: 949-957.

Pryke, S. R. and Griffith, S. C. 2009. Socially mediated trade-offs between aggression and parental effort in competing color morphs. - Am. Nat. 174:455-464.

Pryke, S. R. 2009. Is red an innate or learned signal of aggression and intimidation? - Anim. Behav. 78: 393-398.

R Development Core Team. 2014. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. - <<http://www.R-project.org/>>.

Rohwer, S. 1975. The social significance of avian winter plumage variability. - Evolution. 29: 593-610.

Rohwer, S. 1982. The evolution of reliable and unreliable badges of fighting ability. - Am. Zool. 22: 531-546.

Rohwer, S. and Ewald, P. W. 1981. The cost of dominance and advantage of subordination in badge signalling system. Evolution. 35: 441-454.

Rosenberg, R. H. and Enquist, M. 1991. Contest behaviour in Weidemeyer's admiral butterfly, *Limenitis weidemeyerii* (Nymphalidae): the effect of size and residency - Anim. Behav. 42:805-811.

Roulin, A. 2015. Condition-dependence, pleiotropy and the handicap principle of sexual selection in melanin-based colouration. Biol. Rev. doi: 10.1111/brv.12171

Rowland, W. J.,

Bolyard, K. J. and Halpern, A. D. 1995. The dual effect of stickleback nuptial coloration on rivals: manipulation of a graded signal using video playback. - *Anim. Behav.* 50: 267-272.

Senar, J. C. 1990. Agonoistic communication in social species - What is communicated? - *Behaviour.* 112: 270-283.

Senar, J. 2006. Color displays as intrasexual signals of aggression and dominance. - In: Hill, G.E. and McGraw, K. J. (eds), *Bird Coloration. Volume II: Function and Evolution.* Harvard Uni Press. pp. 87-136.

Santos, E. S. A., Scheck, D. and Nakagawa, S. 2011 Dominance and plumage traits: meta-analysis and metaregression analysis. - *Anim. Behav.* 82: 3-19

Siddiqi, A., Cronin, T. W., Loew, E. R., Vorobyev, M. and Summers, K. 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog *dendrobates pumilio*. - *J. Exp. Biol.* 207: 2471-2485.

Sundberg, J. 1995. Female yellowhammers (*Emberiza citrinella*) prefer yellower males: a laboratory experiment. - *Behav. Ecol. Sociobiol.* 37:275-282.

Svensson, P. A. and Wong, B. B. M. 2011. Carotenoid-based signals in behavioural ecology: a review. - *Behaviour.* 148:131-189.

Symonds, M. R. E. and Moussalli, A. 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. - *Behav. Ecol. Sociobiol.* 65: 13-21.

Tinbergen, N. 1955. *The Study of Instinct.* - Oxford Uni. Press.

Veiga, J. P. 1995. Honest signalling and the survival cost of badges in the house sparrow. - *Evolution.* 49: 570-572.

Vorobyev, M. and Osorio, D. 1998. Receptor noise as a determinant of colour thresholds. - Proc. R. Soc. B. 265: 351-358.

Vorobyev, M., Osorio, D., Bennett, A. T. D., Marshall, N. J. and Cuthill, I. C. 1998.

Tetrachromacy, oil droplets and bird plumage colours. - J. Comp. Physiol. A. 183: 621–633.

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.

Table Legends

Table 1: Results from principal components analysis for colour measures, including eigenvalue and percentage of variance each PC explains. Bold numbers indicate the most important variables (>0.4) for each PC.

		Free-living		Captive		
		PC1	PC2	PC1	PC2	PC3
Face	chromatic	0.27	0.55	-0.32	0.06	0.63
	achromatic	0.34	0.52	-0.14	0.59	0.47
Back	chromatic	0.37	-0.21	0.44	-0.23	0.09
	achromatic	0.46	-0.01	0.47	0.09	0.02
Rump	chromatic	0.32	-0.38	-0.14	0.52	-0.51
	achromatic	0.27	0.13	0.35	0.51	-0.03
Chest	chromatic	0.28	-0.47	0.32	-0.14	0.30
	achromatic	0.46	-0.05	0.47	0.22	0.13
Eigenvalue		3.24	1.94	3.37	1.58	1.40
% of variance explained		40.5	24.0	42.0	20.0	18.0

Table 2: Summary of model averaging for the predictors of interaction outcome, in free-living crimson finches. Length of observations was fitted as a fixed factor. High relative importance indicates that the variable is more likely to be in the best model.

Variable	Estimate	SE	Adjusted Confidence Interval	Relative Importance
Intercept	2.92	0.35	2.24, 3.61	-
Length of observations	0.81	0.69	-0.54, 2.17	1.00
Condition	-1.00	0.69	-2.35, 0.35	0.50
Redness PC2	-0.63	0.57	-1.74, 0.48	0.42
Redness PC1	-0.67	0.66	-1.97, 0.63	0.38
Patch area	-0.41	0.62	-1.63, 0.82	0.31

Table 3: Summary of model averaging for potential predictors of contest outcomes in captive dyads. **Bold** variables indicate those with an importance over 0.5 and a confidence interval that does not cross 0. High relative importance indicates that the variable is more likely to be in the best model. Red PC scores relate to the natural or underlying colour of the bird and are independent of colour treatment.

	Variable	Estimate	Adjusted SE	Confidence interval	Relative Importance
Un-manipulated plumage colour	Intercept	-16.90	13.64	-43.64, 9.84	-
	Red PC1	82.51	35.15	13.61, 151.41	1.00
	Red PC2	-17.06	14.96	-46.39, 12.27	0.42
	Condition	20.60	9.83	1.33, 39.86	0.28
	Patch area	-35.13	16.28	-67.04, -3.23	0.10
Manipulated plumage colour	Intercept	-0.36	1.86	-4.00, 3.29	-
	Red PC1	-8.84	9.24	-26.95, 9.26	0.77
	Condition	7.76	6.52	-5.01, 20.54	0.77
	Red PC2	-0.98	2.72	-6.32, 4.35	0.14
	Patch area	-0.22	3.11	-6.32, 5.88	0.13

Figure Legends

Figure 1: Left: Male crimson finch in adult plumage, indicating the colour patches used in analysis. Spectrometry measures taken from carotenoid based red areas: Face (1), Chest, (2), Back (3), Rump (4), as well as the black melanin based patch (5). Top right: Photographs of the treatments a) Un-manipulated (natural) b) Control c) Darkened. Bottom right: Reflectance curves for average un-manipulated and manipulated plumage colourations (control and darkened).

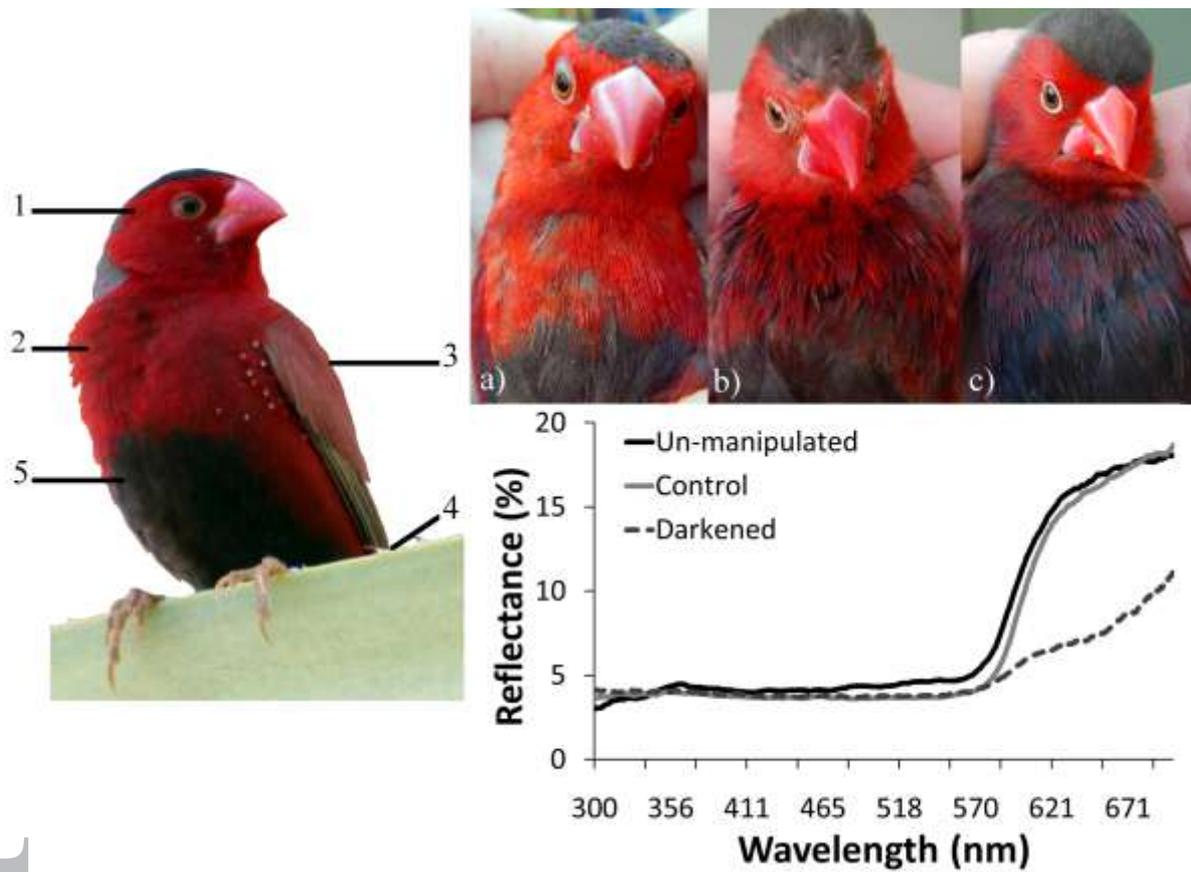


Figure 2: Results for model averaging of captive dyads, illustrating the influence of red carotenoid plumage in manipulated and un-manipulated contests, and the influence of condition in the manipulated contests only. The line indicates the values predicted from the model averaging while the size of the data point indicates how many data points it represents.

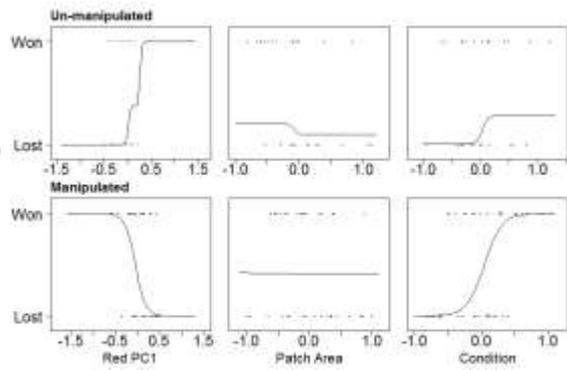


Figure 3: Effect of plumage manipulations on dyadic contests a) the number of contests won by control vs manipulated birds. b) The average number of interactions per contest in manipulated vs un-manipulated trials.

