Evidence for multiple functions in a sexually selected ornament

Ana V. Leitão [a, b, *], André C. Ferreira [a], Caterina Funghi [a], Sandra Trigo [a, b], Paulo G. Mota [a, b]

[a] CIBIO — Research Centre in Biodiversity and Genetic Resources, University of Porto, Campus Agrário de Vairão, Vairão, Portugal
[b] Department of Life Sciences, University of Coimbra, Coimbra, Portugal

In gregarious animals, social interactions frequently take the form of dominance hierarchies that maintain stable relationships between individuals, and settle disputes without extra costs. Traits that function as signals of status can play an important role in mediating interactions among individuals, both in social and in sexual contexts. Carotenoid ornaments are more generally assumed to be sexually selected and not so relevant to general social contexts. However, it is possible for them to function in social contexts if they signal socially relevant aspects. Here we experimentally analysed social dominance and resource control in male groups of a gregarious species, the European serin, *Serinus serinus*, in relation to a sexual ornament. We tested whether yellow carotenoid-based plumage coloration, age, body size and testosterone were predictors of social dominance over a nonsexual resource (i.e. feeding context). We showed that dominance hierarchies were steep and were related to testosterone levels and ornamental coloration, particularly the male yellow carotenoid-based crown patch. Our results suggest that carotenoid-based colour and testosterone levels can be reliable predictors of social status in agonistic encounters in groups of male serins. Moreover, together with previous work on the sexual function of male coloration, this study provides evidence that male serin yellow coloration has a dual function in both sexual and nonsexual contexts. These results raise the possibility that this ornament may have evolved and be maintained via social selection over social competition/cooperation for reproductive opportunities and ecological resources.
© 2015 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Social relationships in group-living species are an important mechanism for maintaining the balance between gregariousness and conflict between group members. While individuals might increase their fitness by living in groups, they still have to compete over a wide range of limited resources (e.g. food or mating partners), which can lead to increased agonistic interactions between individuals (Alexander, 1974). As aggression can be costly, animals have evolved several strategies to avoid excessive energy expenditure and physical injuries (Parker, 1974), such as the establishment of dominance hierarchies and status signalling. The latter can be an indicator of the opponent's competitive ability, providing decisive information to predict the outcome of a confrontation (Rohwer, 1975) or affect the various stages of agonistic escalation (Chaine & Lyon, 2008; Enquist & Leimar, 1990), which can be particularly relevant in gregarious species, since it can help mediate situations of conflict between members of a group.

The evolution of ornamental traits has commonly been attributed to sexual selection (Andersson, 1994), through either courtship and fighting or mate choice. In contrast, the armament — ornament model predicts that an interaction between contexts can exist, that is, a signal evolved through sexual competition (e.g. weapons) can be co-opted in intersexual choice, since it is a reliable proxy of quality, and occasionally losing its original function (Berglund, Bisazza, & Pilastro, 1996). The concept of social selection considers all socioecological interactions (in both sexual and nonsexual contexts) as selective forces that collectively shape the phenotype (Lyon & Montgomerie, 2012; Tanaka, 1996; West-Eberhard, 1983; but see Clutton-Brock & Huchard, 2013). Thus, the social selection perspective encourages researchers to investigate whether nonsexual contexts are involved in signal evolution (Lyon & Montgomerie, 2012; West-Eberhard, 2014) and, therefore, tests revealing interactions between sexual and nonsexual selection are much needed.

Ornamental traits for social signalling are widespread among vertebrates (Clutton-Brock & Huchard, 2013; Searcy & Nowicki, 2005). Certain traits such as pigment-based coloration can reliably

---

* Correspondence and present address: A. V. Leitão, School of BioSciences (Building 147), University of Melbourne, Parkville, 3010 VIC, Australia.
   E-mail address: anamvleitao@gmail.com (A. V. Leitão).

signal an individual's fighting ability and, as such, predict the outcome of conflicts or the position of individuals within dominance hierarchies. In contrast to melanin pigmentation, there has been much less research on the function of carotenoid-based traits in status-signalling contexts, but studies suggest they are widespread among taxa such as reptiles (Hamilton, Whiting, & Pryke, 2013), fish (Evans & Norris, 1996) and birds (Griggio, Serra, Licheri, Monti, & Pilastro, 2007). Whether or not they have a sexual function, carotenoid-based signals have the potential to convey information in competitive contexts. Variation in carotenoid coloration is associated with an individual's ability to forage, assimilate and process carotenoids (reviewed in Blount, 2004; Olson & Owens, 1998). Also, since these pigments are vital nutrients, important as immunoenhancers and antioxidants (reviewed in Lozano, 1994; Olson & Owens, 1998; see also Hill & Johnson, 2012), it is often the case that these signals are indicators of individual health condition. In such cases, high-quality individuals should exhibit increased ornamentation and thus advertise their superior competitive ability, irrespective of whether carotenoid coloration is dependent on trade-offs for different functions (Lozano, 1994) or is a direct indicator of both physiological and metabolic capacity, as well as immune condition (Hill & Johnson, 2012).

A positive relation between carotenoid-based plumage and fighting ability has been demonstrated for several bird species, for both red (e.g. widowbirds, *Euplectes ardens* and *Euplectes axillaris*, Pryke, Lawes, & Andersson, 2001; Pryke & Andersson, 2003) and yellow feathers (e.g. rock sparrow, *Petronia petronia*, Griggio et al., 2007; yellow warbler, *Setophaga petechia*, Studd & Robertson, 1985). A recent experimental study with golden-crowned sparrows, *Zonotrichia atricapilla*, has shown that multiple signals of carotenoid and melanin were independently related with social dominance in mixed-sex dominance trials (Chaine, Roth, Shizuka, & Lyon, 2013). In the Gouldian finch, *Erythrura gouldiae*, a genetically based colour-polymorphic species, both red-headed males and females are more aggressive and dominant towards black and yellow colour morphs (Pryke, 2007; Pryke & Griffith, 2006). While all these studies were directed to carotenoid-based status signals, in most cases dominance was only assessed in a sexual competitive context, without considering a social selection perspective.

Our study species, the European serin, *Serinus serinus*, is a gregarious, nonterritorial, socially monogamous finch that exhibits male mate-guarding behaviour (Mota & Hoi-Leitner, 2003). Male European serins display a sexually dichromatic yellow carotenoid-based breast and crown, resulting from a single annual moult 6–8 months before breeding. Male coloration has been shown to be a sexually selected trait preferred by females (Leitão, Monteiro, & Mota, 2014). In this study, we investigated whether male serin yellow carotenoid-based coloration plays a role in male social dominance over nonmating resources. Since testosterone is known to modulate a set of behaviours such as courtship, parental and aggressive behaviours (Adkins-Regan, 2005), we also measured testosterone levels in order to determine its relation to male dominance, and to assess whether it was related to male ornamentation.

We established male groups under laboratory conditions to determine whether male yellow ornamentation was related to aggressive interactions and social dominance, testing the possibility that social competition over nonreproductive resources also influences the evolution of this trait.

METHODS

Housing and Morphological Measurements

We captured 28 male European serins, using mist nets, in the surroundings of Coimbra (40°11′25′ N 8°33′35′ W, Portugal), in the winter (February) of 2012, after moult and before breeding. Individuals were housed in an indoor aviary in the Laboratory of Ethology at the University of Coimbra (40°12′26′N 8°25′21′W, Portugal), where we divided them into seven groups of four males, and housed them in cages (118 × 50 cm and 50 cm high) with ambient temperature of 20 ± 2 °C and natural lighting. Birds had ad libitum access to a commercial seed mixture (canary seed 46%, rapeseed 22%, niger seed 7%, linseed 7%, peeled oats 6%, hempseed 5%, wild seeds 5%, radish seed 1% and spinach seed 1%; European Finches Prestige, Versele-Laga, Deinze, Belgium), tap water and mixed grit with crushed oyster shell. We minimized the opportunity for dominant males to monopolize food resources in the groups by placing multiple food and water receptacles in each cage.

We ringed birds with numbered black plastic rings (A. C. Hughes, Hampton Hill, U.K.), which were replaced by plastic colour rings just before the experiments to allow visual identification during video analysis. Ring colour was not related to any variables analysed (Pearson correlations: $P > 0.10$ for all tests). We also aged birds (first year or adult birds) using plumage marks according to Svensson (1992). We measured morphological parameters including wing (±1 mm), tarsus (±0.01 mm) and mass (±0.5 g) immediately before the experiments. Body size was estimated from the PC1 of a principal component analysis using these variables, which explained 50.8% of variance, had high loadings for tarsus (0.80) and mass (0.88) and a medium loading for wing (0.31). The crown and breast yellow patches were measured by the same person (S. T.) by overlaying a transparent grid on these areas and estimating the number of squares they covered, as described by Hill (1992).

Spectral Analysis

During the month of capture, we performed spectral analysis of males' plumage reflectance, taking into account the passerine bird vision perception range (320–700 nm), using an Ocean Optics USB4000 spectrophotometer, a MikropackMini-DT-2-GS light source (Ocean Optics, Dunedin, FL, U.S.A.) and an optical fibre reflectance probe (Ocean Optics R400-7 UV/VIS), held vertically, attached to a rigid black holder to standardize the distance between probe and sample (distance of 3 mm, providing a sampling area of 28 mm^2). All measurements were standardized using white (Ocean Optics, WS-1-SS) and dark standards. We measured the crown, throat, breast and belly of each male by taking three readings from each area, which were then averaged for each region. We then quantified coloration as perceived by birds, by applying avian visual models (Vorobyev & Osorio, 1998; Vorobyev, Osorio, Bennett, Marshall, & Cuthill, 1998). We calculated cone quantum catches for each of the five cone types present in the avian retina (Bowmaker, Heath, Wilkie, & Hunt, 1997), four single cones and the double cone (using equation 1 in Vorobyev et al., 1998), integrating cone sensitivity, irradiance light and reflectance spectrum of the plumage. Since cone sensitivity data are unavailable for our species, we considered the cone sensitivities of another ultraviolet-sensitive (UVS) representative species, the blue tit, *Cyanistes caeruleus*, for single and double cones (Hart, Partridge, Cuthill, & Bennett, 2000). As a measure of irradiance we used D65, the spectrum of standard daylight. Plumage coloration was quantified using the short-wavelength-sensitive (SWS) ratio and the double cone, representing chromatic and achromatic indices of plumage reflectance (Evans, Hinks, Wilkin, & Sheldon, 2010). We assumed double cones are responsible for achromatic discrimination, since experimental data suggest that they are responsible for luminance vision (Hart, 2001; Jones & Osorio, 2004; Vorobyev & Osorio, 1998). As carotenoid-based plumage coloration results from the pigment absorption of wavelengths otherwise typical of white feathers

(Shawkey, Hill, McGraw, Hood, & Huggins, 2006), we used the same measure as Evans et al. (2010) to quantify yellow pigment concentration, which we perceive as saturation. The SWS ratio compares the SWS cone to the mean quantum cone catch of the other three single cones (UVS, MWS and LWS) so that SWS ratio $= 3^{-1}$ (UVS+MWS+LWS)/SWS. We first used an average across all body parts, and in a later analysis, we split the measurement to determine which body regions were more socially relevant. Visual models were applied using the pavo package (Maia, Eliason, Bitton, Doucet, & Shawkey, 2013), running in R (R Development Core Team, 2015).

In the Results, we report analyses based on the UVS visual system. We assumed that *S. serinus* has the same visual system as its close relative *Serinus canaria*, which is known to have the UVS visual system (Das, Wilkie, Hunt, & Bowmaker, 1999). However, since the actual visual system is not known for *S. serinus*, we also conducted similar analyses based on the violet-sensitive (VS) visual system (see Appendix Tables A1–A3). Results were similar to those obtained using the UVS visual system.

Testosterone Assays

Blood samples from each bird were taken early in the breeding season (April), to obtain individual testosterone levels. We could not take another sample immediately before the experiments, owing to constraints on the amount of blood we could take from a bird of this size, as well as the fact that these birds were later used in another experiment (Trigo & Mota, 2014). Therefore, testosterone levels, as measured in this study, must be taken as baseline for breeding conditions and are not directly related to the social groups, since they were measured before the groups were formed. The present study was conducted before Trigo and Mota (2014) experiment, with several months separating them.

Samples were taken in the morning with individuals being handled for 2–4 min. Blood was collected from the brachial vein (approximately 100 μl), using a heparinized capillary tube. After centrifugation, the plasma samples (5–50 μl) were stored at $-20\,^{\circ}$C until hormonal analysis.

Testosterone concentrations were measured by radioimmunoassay in ISPA (Lisbon, Portugal), following a previously described method (Canário & Scott, 1989). Samples were assayed in duplicate for testosterone, using an antibody for testosterone (Fitzgerald, Acton, MA, U.S.A.; ref.: WLI-T3003; new ref.: 20R-TR018W) and a specific label ($[1,2,6,7-^{3}H]$-Testosterone, Amersham Biosciences, Little Chalfont, U.K.; ref. TRK402-250μCi). The lower detection limit of the assay was 0.039 ng/ml. The intra-assay coefficients of variation were 2.4% and 4.5%; the inter-assay variation was 11.5%. Sufficient plasma for assays was obtained for 18 of the 28 males.

Social Competition Experiment

We conducted social competition experiments in June, at the end of the breeding season, and before moulting. To promote new social interactions and the establishment of new dominance hierarchies, we formed seven new male groups of four randomly chosen unfamiliar males 2 days before the trials. Each group was housed in an indoor cage similar to the housing cages, with four perches, four plastic feeders and four plastic water containers. We created a competition context for access to a limited food resource adapted from other protocols (Boogert, Reader, & Laland, 2006; Pryke & Griffith, 2006). To standardize motivation, in each trial all the individuals were deprived of food for 2 h (starting between 1200 and 1230 hours) before testing. Tests were run between 1400 and 1430 hours and started after we placed a feeder in the middle of the cage, which allowed only one individual to feed at a time. The food provided during the experiments was the same mixture provided during normal housing. Over 5 consecutive days, the agonistic interactions in each cage were filmed for 30 min.

We analysed the tests with the software Observer XT 10.0 (Noldus Information Technology, Wageningen, The Netherlands). We opted to analyse the first 15 min of video of the trials as we confirmed that, after that time, all the birds had already eaten and no substantial agonistic interactions were observed between individuals. We recorded behaviours already described for typical cardueline finches and other small birds. Social competition was assessed by the time spent monopolizing the feeder and aggressiveness (displays and attacks). We defined threat displays as behavioural postures during which the bird extends the neck and directs the head down, towards an opponent, with beak open, and with or without rapid wing movements. We defined attack as any instance in which the actor attempted to peck the other individual, with or without chasing, jumping or flying towards it. The actor and the recipient of the display or attack were identified for all confrontations. We calculated 'aggressiveness' as the sum of attacks and displays made by each individual. Moreover, we also recorded the total time spent in the feeder, as a measure of resource monopolization. We did not consider latency to feed, since faster individuals were not always those that controlled the feeder (A. V. Leitão, A. C. Ferreira, C. Funghi, S. Trigo & P. G. Mota, personal observation). We defined 'activity' for each individual as the total number of hops between perches and the feeder, and checked for differences in general activity at group level. We calculated a dominance index for each individual using David's score ranking method (David, 1988; Gammell, de Vries, Jennings, Carlin, & Hayden, 2003). David's score provides a measure of overall individual success, by considering individual agonistic interactions (wins and losses) in a group and the relative strengths of the opponents, where individuals that have higher scores are dominant over the others. The David's scores were corrected for different interaction frequencies between group members using de Vries, Stevens, & Vervaecke (2006) correction. This score was calculated based on the sum of aggressive interactions across the 5 observation days, after checking for consistency in these behaviours between the days (see Results). After calculating the dominance index, we assessed the degree to which individuals differed from each other in dominance status, by calculating the steepness of the dominance hierarchy from the absolute slope fitted to the normalized David's score on rank order for each experimental group (de Vries et al., 2006).

Statistical Analysis

We first tested for possible group differences in activity, aggressiveness (number of individual attacks and displays) and time spent in the feeder with a one-way analysis of variance (ANOVA). Within-individual repeatability was calculated for aggressiveness and time spent in the feeder across the 5 days from the variance analysis ANOVA (Lessells & Boag, 1987). When we found repeatability, we averaged variables across the 5 days of tests, in order to have a single measurement for each individual. The relationship between dominance index (David's score), average of aggressiveness, and average of time spent in the feeder across all individuals was examined using Pearson correlations.

We tested which factors were predictors of social dominance using generalized linear mixed models (GLMM), with dominance index as the dependent variable and group as a random factor,

assuming normal distribution of error terms. The models incorporated all possible combinations of variables that could be related to dominance, namely plumage chromaticity (SWS ratio) and plumage achromaticity (double cone), as well as age, body size, and crown and breast patch size. We report the results (F and P values) for the fixed effects. The model did not include variables that were correlated to avoid problems of collinearity (Pearson correlations: $P > 0.10$ for all variables included).

Having found a significant effect of SWS ratio (plumage chromaticity) over dominance index (see Results), we performed a GLMM focusing on plumage chromaticity of each individual body part as predictors (crown, throat, breast and belly SWS ratio; variables were not correlated with Pearson correlations: all $P > 0.05$) in order to partition the trait signal and assess its components.

We performed a third model analysis, with the same specifications as the other models, to test for testosterone as a predictor variable of dominance index. This model was made separately due to the smaller sample size, since we only had a subsample of birds with measured testosterone levels ($N = 18$). The correlation between testosterone and other variables that could explain social dominance (total SWS ratio, crown SWS ratio and body size) was analysed using Pearson correlations.

All variables fulfilled the statistical assumptions. Statistical analysis were performed with R using packages nlme (Pinheiro, Bates, DebRoy, & Sarkar, 2011), MuMIn (Barton, 2009) and Hmisc (Harrell, 2012).

Ethical Note

This work was performed in accordance with the Portuguese National Authority for Animal Health (DGAV). Permits for animal capture, transport, maintenance, handling and experiments were carried out under I.C.N.B. licences (49/2012/CAPT) to P.G.M. according to Portuguese legislation.

Birds were captured and transported to the aviary in small groups in cages (70×35 cm and 50 cm high), and upon arrival moved to their housing cages. In the aviary, the birds' status was checked daily, and they were maintained with care to ensure their health and wellbeing. The veterinary surgeon also checked the birds' general state and housing conditions. During experiments, agonistic physical interactions (pecking) were rarely observed, and when they occurred never caused physical injury. After experiments and before release, birds were transferred to a large indoor aviary for a 5-day period of flight training, to improve flight performance and reach normal activity. Individuals were then released in their capture locations where they joined groups of conspecifics.

RESULTS

Male groups did not differ in aggressiveness ($F_{6,21} = 1.697$, $P = 0.171$), time spent in the feeder ($F_{6,21} = 0.508$, $P = 0.796$) or general activity ($F_{6,21} = 1.680$, $P = 0.175$).

Within groups, the traits defining dominance were consistent across days (time spent in the feeder: $r_S = 0.356$, $F_{27,108} = 3.775$, $P < 0.001$; aggressiveness: $r_S = 0.268$, $F_{27,108} = 2.839$, $P < 0.001$). Also, these behavioural variables were correlated with the dominance index: individuals that retained control of the feeder for longer were also more aggressive ($r_S = 0.569$, $N = 28$, $P = 0.002$) and had a higher dominance index (David score and aggressiveness: $r = 0.681$, $N = 28$, $P < 0.001$). The dominance relationship between members of each group was found to be steep (mean $\pm$ SE $= 0.70 \pm 0.063$; Fig. 1), which is indicative of strong hierarchies.

Only plumage chromaticity was a significant predictor of dominance score (Fig. 2), while plumage achromaticity, age, body
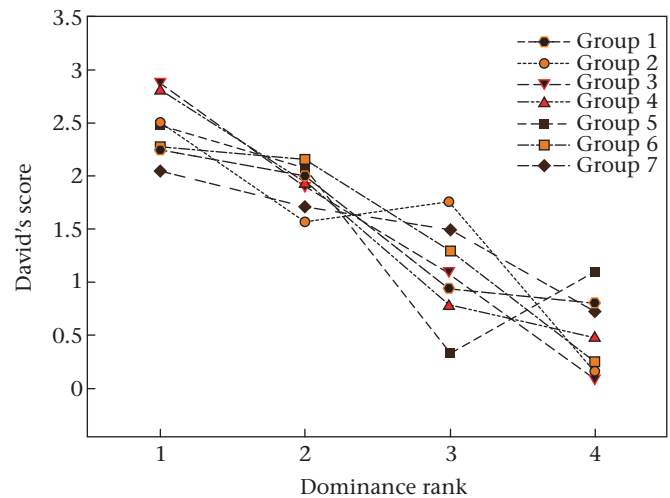

Figure 1. Dominance hierarchy steepness. Relation between David's score (normalized) and the rank of each individual within each group (seven groups with different symbols). The absolute value of the slope for each group measures the steepness of the hierarchy.

size, and crown and breast patch size were not significantly related to social dominance (Table 1).

When we separately analysed the effects of plumage chromaticity in each of the males' yellow body parts, only the crown SWS ratio showed a significant effect over dominance ($F_{1,17} = 9.977$, $P = 0.005$; Fig. 3), whereas throat ($F_{1,17} = 0.793$, $P = 0.385$), breast ($F_{1,17} = 0.820$, $P = 0.379$) and belly SWS ratio ($F_{1,17} = 0.001$, $P = 0.990$) did not.

Testosterone levels positively affected social dominance, with males with higher levels of testosterone also showing higher dominance rank ($F_{1,10} = 7.620$, $P = 0.020$; Fig. 4). However, the difference between individuals in testosterone was not correlated with male ornamentation, either SWS ratio of all body parts ($r_S = 0.190$, $N = 18$, $P = 0.443$) or crown SWS ratio ($r_S = 0.100$, $N = 18$, $P = 0.705$). It was also not correlated with body size ($r = 0.040$, $N = 18$, $P = 0.868$).

Similar results were found when we used the VS visual system in visual models (see Appendix Tables A1, A2 and A3).
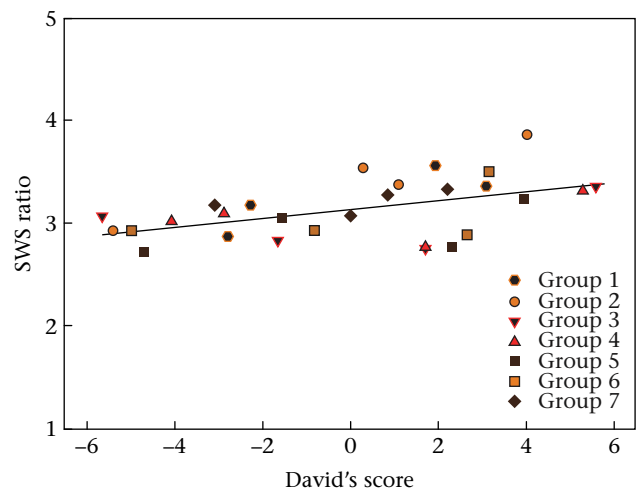

Figure 2. Relationship between plumage SWS ratio (chromaticity) and David's score (social dominance). Each symbol corresponds to a different group of males.

Table 1
Summary of generalized linear mixed models (GLMM) of the David's score (social dominance)

Variables	Estimate	SE	F	P
Plumage chromaticity (SWS ratio)	5.475	2.432	5.069	0.039
Plumage achromaticity (double cone)	−3.429	23.270	0.022	0.885
Breast patch size	0.028	0.056	0.246	0.627
Crown patch size	0.153	0.440	0.120	0.734
Body size	−0.088	0.658	0.018	0.895
Age	0.592	1.610	0.135	0.718

Predictors were plumage chromaticity (SWS ratio), plumage achromaticity (double cone), breast patch size, crown patch size, body size, age. 'Group' was also included as a random factor, to control for differences between experimental groups.
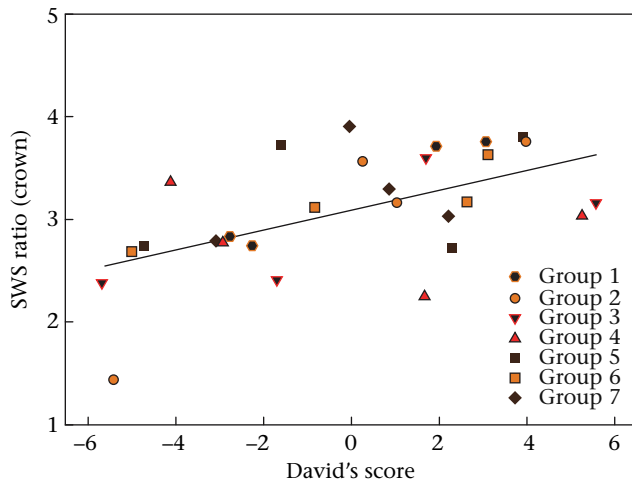

Figure 3. Relationship between crown SWS ratio (chromaticity) and David's score (social dominance). Each symbol corresponds to a different group of males.

DISCUSSION

Our results show that male serins in captive groups form steep dominance hierarchies, which is indicative of strong consistent differences between individuals in their agonistic interactions. We also demonstrated that plumage coloration and testosterone levels can independently predict social dominance in a feeding context.
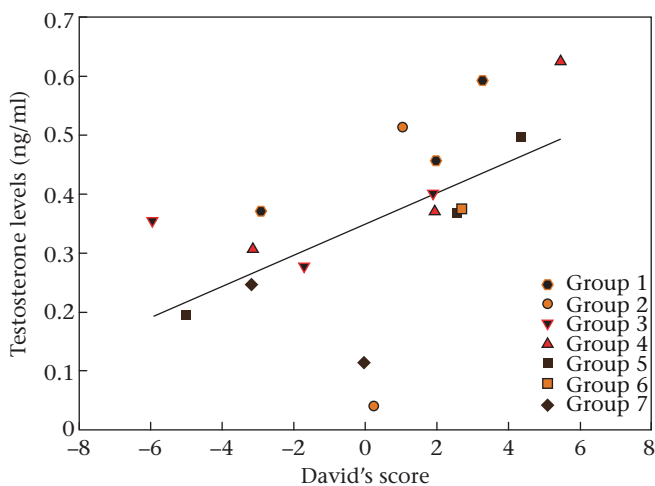

Figure 4. Relationship between testosterone and David's score (social dominance). Each symbol corresponds to a different group of males.

Individuals with higher plumage chromaticity and higher levels of testosterone were more aggressive and dominant. Other factors might affect the outcome of contests and the achievement of dominance, such as body size or age. However, our analysis did not reveal such correlation: neither different body size nor age variation seem to have an effect on social dominance, which could indicate that age differences and variation in size do not actually give an advantage in social dominance in serins.

Our results support the growing evidence that carotenoid coloration is involved in status signalling (Evans & Norris, 1996; Griggio et al., 2007; Hamilton et al., 2013; Young, Cain, Svedin, Backwell & Pryke, 2015), and can be important in social competition. Moreover, we demonstrated that the same sexually selected signal (Leitão et al., 2014) can similarly be used as a status signal in the context of social competition over nonreproductive resources (food). These results are indicative of a dual function of the yellow coloration, and suggest that this ornamentation may have evolved and be maintained not only via competition for reproductive opportunities (sexual selection), but also via competition for access to other resources in nonsexual contexts, that is, social selection.

Status signalling can be particularly relevant in gregarious birds (West-Eberhard, 1983) such as the serin, since they can reveal information regarding an individual's competitive ability to conspecifics and mediate conflict situations. At the group level, individual differences in coloration might arise as a result of trade-off mechanisms that maintain this variation in coloration. Living in groups conveys several advantages for more subordinate (less colourful) males, as they can benefit from the presence of other group members through protection and social information use (e.g. follow better foragers, Tóth & Griggio, 2011). Although this can increase competition for food, more colourful males can also benefit from attracting individuals to the group by decreasing the risk of predation (group size effect on vigilance and dilution effect; Alexander, 1974). At the individual level, if more dominant individuals achieve more control over food resources (from which the pigments are derived), they could be more efficient at expressing coloration, which can directly signal their superior competitive ability to others.

Our experiment also demonstrates that social dominance is positively associated with the difference between individuals in testosterone level, indicating that dominance status is hormonally mediated in male serins. It is generally recognized that testosterone is correlated with aggressive displays, at least during periods of male − male competition (high testosterone levels are associated with high levels of aggression; Wingfield, Ball, Dufty, Hegner, & Ramenofsky, 1987). Our results show that the difference between individuals in testosterone level attained 2 months before we established the experimental groups predicted future social dominance, which is an indication that it relates in a stable way to dominance. Basal testosterone levels could be indicative of high testosterone production during aggressive interactions, as individuals can vary consistently in their androgen production (reviewed in Kempenaers, Peters, & Foerster, 2008). Still, our results show no correlation between testosterone levels and the males' colour traits. Sex-steroid hormones such as testosterone can modulate the expression of sexual traits by favouring the allocation of pigments to ornaments, as in melanin-based coloration (Andersson, 1994; Mougeot, Perez-Rodriguez, Martinez-Padilla, Leckie, & Redpath, 2007). Regarding carotenoids, the majority of studies suggest that testosterone can stimulate carotenoid transport and increase carotenoid availability (Blas, Perez-Rodriguez, Bortolotti, Viñuela, & Marchant, 2006; Peters, 2007), while others did not find a direct link between testosterone and colour expression (Day, McBroom, & Schlinger, 2006; Peters, Roberts, Kurvers, & Delhey, 2012). In a recent experiment with serins, Trigo and Mota

(2014) reported a moderate negative effect of implanted testosterone on the saturation and size of yellow coloration of males. Still, the relationship between testosterone and carotenoid-based plumage coloration is unclear, and needs further research.

Although coloration and testosterone were not correlated, they both predicted male dominance. This could result from reduced power of the test due to a small sample size in relation to effect sizes or derive from complex relationships between testosterone and coloration with dominance. Actually, while testosterone may cause the dominant behaviour (Adkins-Regan, 2005) coloration is, in this case, signalling it. In spite of these possible differences, both relate significantly to dominance, which underlies the robustness of colour signalling. More tests are needed to understand the different physiological mechanisms underlying carotenoid coloration and testosterone and their possible difference in functional pathways in relation to dominance status.

Overall, our experiment reveals that yellow carotenoid-based coloration and testosterone levels in the European serin predict social dominance in a nonsexual, feeding context. These results, together with previous research, show that yellow coloration is sexually preferred by females (Leitão et al., 2014), indicating that a dual function of a carotenoid ornament is possible. These results also highlight the importance of considering contexts other than sexual contexts when studying armament − ornament signal processes, which can be more appropriately framed within social selection.

Acknowledgments

References

Adkins-Regan, E. (2005). *Hormones and animal social behavior*. Princeton, NJ: Princeton: University Press.

Alexander, R. D. (1974). The evolution of social behavior. *Annual Review of Ecology and Systematics, 5*, 325–383.

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

Barton, K. (2009). *MuMIn: Multi-model inference. R package, version 0.12.2*. Available at: http://r-forge.r-project.org/projects/mumin/.

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

Blas, J., Perez-Rodriguez, L., Bortolotti, G. R., Viñuela, J., & Marchant, T. A. (2006). Testosterone increases bioavailability of carotenoids: insights into the honesty of sexual signaling. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 18633–18637.

Blount, J. D. (2004). Carotenoids and life-history evolution in animals. *Archives of Biochemistry and Biophysics, 430*, 10–15.

Boogert, N. J., Reader, S. M., & Laland, K. N. (2006). The relation between social rank, neophobia and individual learning in starlings. *Animal Behaviour, 72*, 1229–1239.

Bowmaker, J. K., Heath, L. A., Wilkie, S. E., & Hunt, D. M. (1997). Visual pigments and oil droplets from six classes of photoreceptor in the retina of birds. *Vision Research, 37*, 2183–2194.

Canário, A. V. M., & Scott, A. P. (1989). Synthesis of 20α-hydroxylated steroids by ovaries of the dab (*Limanda limanda*). *General and Comparative Endocrinology, 76*, 147–158.

Chaine, A. S., & Lyon, B. E. (2008). Intrasexual selection on multiple plumage ornaments in the lark bunting. *Animal Behaviour, 76*, 657–667.

Chaine, A. S., Roth, A. M., Shizuka, D., & Lyon, B. E. (2013). Experimental confirmation that avian plumage traits function as multiple status signals in winter contests. *Animal Behaviour, 86*, 409–415.

Clutton-Brock, T. H., & Huchard, E. (2013). Social competition and selection in males and females. *Philosophical Transactions of the Royal Society B: Biological Sciences, 368*, 20130074.

Das, D., Wilkie, S. E., Hunt, D. M., & Bowmaker, J. K. (1999). Visual pigments and oil droplets in the retina of a passerine bird, the canary *Serinus canaria*: microspectrophotometry and opsin sequences. *Vision Research, 39*, 2801–2815.

David, H. A. (1988). *The method of paired comparisons*. London: Charles Griffin.

Day, L. B., McBroom, J. T., & Schlinger, B. A. (2006). Testosterone increases display behaviors but does not stimulate growth of adult plumage in male golden-collared manakins (Manacus vitellinus). *Hormones and Behavior, 49*, 223–232.

Enquist, M., & Leimar, O. (1990). The evolution of fatal fighting. *Animal Behaviour, 39*, 1–9.

Evans, S. R., Hinks, A. E., Wilkin, T. A., & Sheldon, B. C. (2010). Age, sex and beauty: methodological dependence of age- and sex dichromatism in the great tit *Parus major*. *Biological Journal of the Linnean Society, 101*, 777–796.

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

Gammell, M. P., de Vries, H., Jennings, D. J., Carlin, C. M., & Hayden, T. J. (2003). David's score: a more appropriate dominance ranking method than Clutton-Brock et al.'s index. *Animal Behaviour, 66*, 601–605.

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. *Behavioral Ecology and Sociobiology, 61*, 423–433.

Hamilton, D. G., Whiting, M. J., & Pryke, S. R. (2013). Fiery frills: carotenoid-based coloration predicts contest success in frillneck lizards. *Behavioral Ecology, 24*, 1138–1149.

Harrell, F. E. (2012). *And with contributions from many other users Hmisc (Harrell Miscellaneous)*. R package version 3.9-3. Retrieved from: http://biostat.mc.vanderbilt.edu/trac/Hmisc.

Hart, N. S. (2001). The visual ecology of avian photoreceptors. *Progress in Retinal and Eye Research, 20*, 675–703.

Hart, N. S., Partridge, J., Cuthill, I., & Bennett, A. (2000). Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: the blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). *Journal of Comparative Physiology A, 186*, 375–387.

Hill, G. E. (1992). Proximate basis of variation in carotenoid pigmentation in male house finches. *Auk, 109*, 1–12.

Hill, G. E., & Johnson, J. D. (2012). The vitamin A-Redox hypothesis: a Biochemical basis for honest signaling via carotenoid pigmentation. *American Naturalist, 180*, 127–150.

Jones, C. D., & Osorio, D. (2004). Discrimination of oriented visual textures by poultry chicks. *Vision Research, 44*, 83–89.

Kempenaers, B., Peters, A., & Foerster, K. (2008). Sources of individual variation in plasma testosterone levels. *Philosophical Transactions of the Royal Society B, 363*, 1711–1723.

Leitão, A. V., Monteiro, A. H., & Mota, P. G. (2014). Ultraviolet reflectance influences female preference for colourful males in the European serin. *Behavioral Ecology and Sociobiology, 68*, 63–72.

Lessells, 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.

Lyon, B. E., & Montgomerie, R. (2012). Sexual selection is a form of social selection. *Philosophical Transactions of the Royal Society B: Biological Sciences, 367*, 2266–2273.

Maia, R., Eliason, C. M., Bitton, P.-P., Doucet, S. M., & Shawkey, M. D. (2013). pavo: an R package for the analysis, visualization and organization of spectral data. *Methods in Ecology and Evolution, 4*, 906–913.

Mota, P. G., & Hoi-Leitner, M. (2003). Intense extrapair behaviour in a semicolonial passerine does not result in extrapair fertilizations. *Animal Behaviour, 66*, 1019–1026.

Mougeot, F., Perez-Rodriguez, L., Martinez-Padilla, J., Leckie, F., & Redpath, S. M. (2007). Parasites, testosterone and honest carotenoid-based signalling of health. *Functional Ecology, 21*, 886–898.

Olson, V. A., & Owens, I. P. F. (1998). Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology & Evolution, 13*, 510–514.

Parker, G. A. (1974). Assessment strategy and the evolution of fighting behaviour. *Journal of Theoretical Biology, 47*, 223–243.

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

Peters, A., Roberts, M. L., Kurvers, R. H. J. M., & Delhey, K. (2012). Testosterone treatment can increase circulating carotenoids but does not affect yellow carotenoid-based plumage colour in blue tits *Cyanistes caeruleus*. *Journal of Avian Biology, 43*, 362–368.

Pinheiro, J., Bates, D., DebRoy, S., & Sarkar, D. (2011). *nlme: Linear and nonlinear mixed effects models R package version 3.1-98*. Retrieved from: http://CRAN.R-project.org/package=nlme.

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

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

Pryke, S. R., & Griffith, S. C. (2006). Red dominates black: agonistic signalling among head morphs in the colour polymorphic Gouldian finch. *Proceedings of the Royal Society B: Biological Sciences, 273*, 949–957.

Pryke, S. R., Lawes, M. J., & 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. *Animal Behaviour, 62*, 695–704.

R Development Core Team. (2013). *R: a Language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. Retrieved from: http://www.R-project.org/.

Rohwer, S. A. (1975). The social significance of Avian Winter plumage variability. *Evolution, 29*, 593.

Searcy, W. A., & Nowicki, S. (2005). *The evolution of animal communication: Reliability and deception in signalling systems.* Princeton, NJ: Princeton University Press.

Shawkey, M. D., Hill, G. E., McGraw, K. J., Hood, W. R., & Huggins, K. (2006). An experimental test of the contributions and condition dependence of microstructure and carotenoids in yellow plumage coloration. *Proceedings of the Royal Society B: Biological Sciences, 273*, 2985–2991.

Studd, M. V., & Robertson, R. J. (1985). Evidence for reliable badges of status in territorial yellow warblers (*Dendroica petechia*). *Animal Behaviour, 33*, 1102–1113.

Svensson, L. (1992). In *Identification guide to the European passerines* (4th ed.). Thetford, U.K.: British Trust for Ornithology.

Tanaka, Y. (1996). Social selection and the evolution of animal signals. *Evolution, 50*, 512–523.

Tóth, Z., & Griggio, M. (2011). Leaders are more attractive: birds with bigger yellow breast patches are followed by more group-mates in foraging groups. *PLoS One, 6*, e26605.

Trigo, S., & Mota, P. G. (2014). A test of the effect of testosterone on a sexually selected carotenoid trait in a cardueline finch. *Ecological Research, 30*, 25–31.

Vorobyev, M., & Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society B: Biological Sciences, 265*, 351–358.

Vorobyev, M., Osorio, D., Bennett, A. T. D., Marshall, N. J., & Cuthill, I. C. (1998). Tetrachromacy, oil droplets and bird plumage colours. *Journal of Comparative Physiology A, 183*, 621–633.

de Vries, H., Stevens, J. M. G., & Vervaecke, H. (2006). Measuring and testing the steepness of dominance hierarchies. *Animal Behaviour, 71*, 585–592.

West-Eberhard, M. J. (1983). Sexual selection, social competition, and speciation. *Quarterly Review of Biology, 58*, 155–183.

West-Eberhard, M. J. (2014). Darwin's forgotten idea: the social essence of sexual selection. *Neuroscience & Biobehavioral Reviews, 46*, 501–508.

Wingfield, J. C., Ball, G. F., Dufty, A. M., Hegner, R. E., & Ramenofsky, M. (1987). Testosterone and aggression. *American Scientist, 75*, 602–608.

Young, C. M., Cain, K. E., Svedin, N., Backwell, P. R. Y., & Pryke, S. R. (2015). The role of pigment based plumage traits in resolving conflicts. *Journal of Avian Biology.* http://dx.doi.org/10.1111/jav.00742.

Appendix

Table A1

Summary of generalized linear mixed models (GLMM) of the David's score (social dominance), using violet-sensitive (VS) spectral sensitivity in the visual model

Variables	Estimate	SE	F	P
Plumage chromaticity (SWS ratio)	10.706	5.229	4.129	0.058
Plumage achromaticity (double cone)	−5.809	23.720	0.048	0.809
Breast patch size	0.022	0.056	0.071	0.701
Crown patch size	0.113	0.446	0.205	0.804
Body size	0.159	0.669	0.056	0.816
Age	0.616	1.658	2.255	0.716

Predictors were plumage chromaticity (SWS ratio), plumage achromaticity (double cone), breast patch size, crown patch size, body size, age. 'Group' was also included as a random factor, to control for differences between experimental groups.

Table A2

Summary of generalized linear mixed models (GLMM) of the David's score (social dominance), for the effects of plumage chromaticity using violet-sensitive (VS) spectral sensitivity in the visual model

Variables	Estimate	SE	F	P
SWS ratio for crown	10.706	5.229	4.129	0.058
SWS ratio for throat	−5.809	23.720	0.048	0.809
SWS ratio for throat	0.022	0.056	0.071	0.701
SWS ratio for breast	0.113	0.446	0.205	0.804
SWS ratio for belly	0.159	0.669	0.056	0.816

Table A3

Correlation between testosterone levels and social dominance, coloration (SWS ratio for all body parts and SWS for crown) and body size

Variables	Statistics
David's score (social dominance)	r_S=0.569, P=0.014
Plumage chromaticity (SWS ratio)	r_S=0.165, P=0.512
SWS ratio for crown	r_S=0.118, P=0.641
Body size	r_S=0.143, P=0.570

$N = 18$ for each variable.