

Sarah R. Pryke · Staffan Andersson

Carotenoid-based status signalling in red-shouldered widowbirds (*Euplectes axillaris*): epaulet size and redness affect captive and territorial competition

Received: 9 September 2001 / Revised: 20 January 2003 / Accepted: 22 January 2003 / Published online: 15 February 2003
© Springer-Verlag 2003

Abstract In breeding plumage, the African male red-shouldered widowbirds (*Euplectes axillaris*) are black except for red carotenoid-based epaulets ('shoulder patches'), similar to the well-studied American red-winged blackbirds (*Agelaius phoeniceus*). To experimentally test the signal function of the red epaulets in male red-shouldered widowbirds, we manipulated epaulet colour and size (within natural variation), and observed the effects in two competitive contexts. First, in captivity, unfamiliar males with epaulets of different size or redness were staged in dyadic contests over food. Only epaulet manipulations significantly affected contest outcome; red epaulet males out-competed orange and control males, which in turn dominated blackened (no epaulet) males. Epaulet size manipulations had particularly strong effects; enlarged epaulet males vigorously defeated smaller epaulet males within colour treatments, whereas the independent effect of 'redness' (i.e., within size treatments) was much weaker. Second, in a field experiment, epaulets were manipulated prior to territory establishment. Males with enlarged epaulets acquired territories, whereas most males with blackened or reduced signals failed to obtain territories. Furthermore, among the territorial males, birds with enlarged epaulets defended larger territories and spent less time in boundary signalling, yet they received fewer intrusions from conspecifics. In contrast, epaulet manipulations had no effect on either female visitation rates or subsequent female settlement. These results concur with recent studies and suggest that the carotenoid-based epaulets of red-shouldered widowbirds are status signals, sexually selected through male contest competition for territories.

Keywords Carotenoid coloration · Red-shouldered widowbird · Sexual selection · Status signalling

Introduction

Several studies have demonstrated female preferences for larger or more intensely coloured male ornaments or signals, yet the function of such traits in male contest competition has received less attention (Andersson 1994; Grether 1996; Hill 1999). In territorial systems, females often mate exclusively with the resident male (e.g. Searcy 1979a; Smith and Arcese 1989; Friedl and Klump 1999), leading to strong male competition over territories, which is therefore likely to be a primary component of sexual selection. As in other types of conflict, assessment signals or 'badges of status' are likely to evolve as a means to settle conflicts without costly fighting (Rohwer 1982; Senar 1999; Bradbury and Vehrencamp 1998). A number of studies have demonstrated that avian plumage badges can settle conflicts over both food and breeding resources (Järvi and Bakken 1984; Studd and Robertson 1985; Eckert and Weatherhead 1987; Røskift and Rohwer 1987; Maynard Smith and Harper 1988; Evans and Hatchwell 1992). In particular, the red epaulets ('shoulder patches') of male red-winged blackbirds (*Agelaius phoeniceus*) are a classic example of agonistic signalling in birds (reviewed in Searcy and Yasukawa 1995; Orians and Beletsky 1997). In a series of experiments, it has been shown that males with artificially blackened epaulets are often evicted from their territories (Peek 1972; Smith 1972), and that males with larger epaulets are more effective in repelling floaters (Peek 1972; Røskift and Rohwer 1987) and tend to dominate males with smaller epaulets in aviary contests (Searcy 1979b; Eckert and Weatherhead 1987; but see Dufour and Weatherhead 1998). Males may, however, benefit from concealing their badges and hence invoke less aggression when intruding onto the territories of other males (Hansen and Rohwer 1986; Metz and Weatherhead 1992). The epaulets of male red-winged blackbirds are thus modifiable in size and

Communicated by M. Elgar

S. R. Pryke (✉) · S. Andersson
Animal Ecology, Department of Zoology,
Göteborg University, Box 463, 405 30 Göteborg, Sweden
e-mail: sarah.pryke@zool.gu.se
Tel.: +46-31-7733656
Fax: +46-31-416729

represent a dynamic 'coverable badge' (Hansen and Rohwer 1986), allowing individuals to switch between aggressive and submissive behaviour.

In this paper, we investigate the function of the carotenoid-based red epaulets displayed by male red-shouldered widowbirds (*Euplectes axillaris*) during the breeding season. Like red-winged blackbirds, red-shouldered widowbird males in nuptial plumage are black with red plumage patches on the proximal part of their wings (epaulet), which are fully exposed when the wing is not folded. The epaulet ranges in colour from orange to red (lesser wing coverts), and is bordered by a narrow cinnamon brown band (median wing coverts). In perch displays the male epaulets are visible as the males slightly spread their wings, while during flight displays the red epaulets are emphasised by the exaggerated spreading of their wings and the retarded wing beat as they fly slowly over their territories.

Red-shouldered widowbirds inhabit wet grasslands and marsh fringes in equatorial and southern Africa. Sexual dimorphism is pronounced, as males in nuptial plumage are about 35% larger (based on tarsus length) than the cryptic brown females which the males resemble during the non-breeding season. Red-shouldered widowbirds have a polygynous mating system, based on the defence of breeding territories by males, who provide no parental care. Since females nest on male territories (and utilise the male-built nest frames, i.e., 'cock's nests'), a male's mating success critically depends on his success in obtaining and defending a territory, a competition that leads to several males being excluded from occupying territories altogether. These non-territorial male floaters remain in the area and rapidly fill arising vacant territories (Pryke and Andersson 2003).

We manipulated the epaulets of both captive and wild males to determine whether ornament alteration affects the outcome of paired contests in captivity, and territory establishment and defence in the field. Furthermore, the alternative (Hill 1999) or additional (Berglund et al. 1996) epaulet function in female mate choice was tested by exploring the effects on female visitation rates and the number of actively nesting females that males attracted to their territories.

Methods

Paired contests

To assess male dominance in captivity, we utilised a study design similar to that used by Pryke et al. (2002) in which males were forced to compete for access to a limited food resource. Contestants were captured in the Balgowan ($n=24$) and Hilton ($n=21$) regions, KwaZulu-Natal, South Africa, between 16 and 22 December 2001. All birds were fitted with an aluminium band and three colour bands (excluding orange and red bands to minimise potential effects on colour communication; Burley et al. 1988; Metz and Weatherhead 1991). Captive males were held at the University of Natal, Pietermaritzburg, in four outdoor aviaries (1.5 m wide \times 2.7 m long \times 2.2 m high), visually isolated from unfamiliar males caught at a different site. Birds were fed commercial birdseeds,

dehusked sunflower seeds, and vitamins. Numerous dishes were placed in each cage to minimise monopolisation by aggressive males and allow all males unlimited access to food and water.

Prior to experiments we took standard measurements of flattened wing chord, culmen, tarsus and tail length (measured to the nearest 0.1 mm). From wing, tarsus and culmen measures, we used a principal components analysis (PCA) to calculate a single body size index. The first principal component (PC1) accounted for 72.7% of the variation in body size measurements, with the loadings ranging from 0.76 to 0.92, indicating positive correlations among the three body size measurements. We measured body mass (to the nearest 0.5 g) just prior to each experiment. Natural and experimental epaulet sizes were estimated by multiplying the length (to the nearest 0.1 mm) of the red shoulder patch (measured from the bend of the wing to the furthest extent of red plumage) by the average of three width measurements (perpendicular to the length). Repeatabilities of the body and epaulet measurements were significant ($r=0.93-0.97$, $F_{50,256}=48.3-89.8$, $P<0.001$; Lessells and Boag 1987).

Epaulets were experimentally manipulated to determine whether there was any causal relationship between the epaulet signals and success in dyadic contests. We manipulated the epaulets in each treatment by bleaching both the black feathers surrounding the epaulets and the epaulet feathers, and then repainting them a different size or colour. The feather pigments were removed by massaging 85% hydrogen peroxide into the feathers. The feathers were then rinsed with warm water and dried. To colour the epaulet signal, we applied yellow, red and black Copic art markers (Too Marker Products, Tokyo, Japan) to all feathers, starting at the base and working to the surface so that each feather on the bleached and original epaulet areas were completely stained. For colour manipulations we selected the two markers that provided the best reflectance match to the extremes of the natural variation in epaulet pigmentation ('red' = cadmium red (R27); 'orange' = cadmium yellow (Y15); Fig. 1). As we were unable to remove all carotenoid pigments from the epaulet feathers (which created a light orange colouring after bleaching), the yellow markers gave the males a dull orange appearance, whereas the red markers successfully concealed the orange plumage of males (Fig. 1).

We created eight different experimental treatments to determine the effect of epaulet size and colour in male contests (Table 1). The blackened epaulet group had their epaulets completely removed by blackening them (Copic Black 100). Red and orange epaulets were

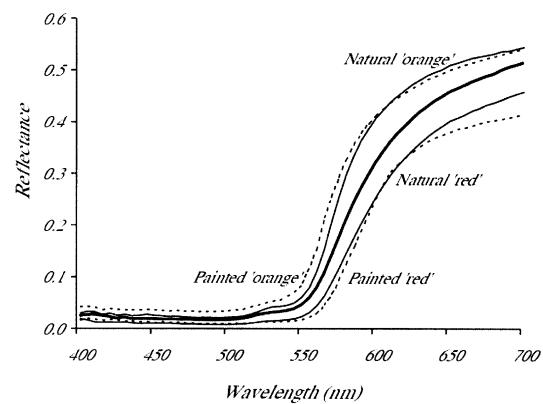


Fig. 1 Reflectance spectra for the natural and manipulated epaulets of male red-shouldered widowbirds (*Euplectes axillaris*). Reflectance for the natural epaulets (solid lines) shows the most intensely coloured 'red' male, the least intensely 'orange' coloured male and the average epaulet colour (thick solid line) for males in the population ($n=310$). The manipulated groups (dashed lines) had their epaulets manipulated with Copic pens to produce reduced or enlarged red ($n=182$) or orange ($n=182$) treatments (see text), which were selected to correspond closely to the natural extremes of colour in the birds

Table 1 Average (\pm SD) experimental epaulet size and hue ('redness') in red-shouldered widowbirds (*Euplectes axillaris*) used in both captive and field experiments. Initial epaulet measurements (prior to manipulation) are provided in parentheses. Measurements were taken once for every male used within each of the seven captivity experimental treatments (including reversals of the manipulations), but not for the multiple manipulations between experiments (e.g. control measurements were taken for only control versus control and not control versus orange males; see Fig. 2)

Treatment	Captiveity experiments			Territory experiments		
	Size (mm ²)	Hue (nm)	n	Size (mm ²)	Hue (nm)	n
Blackened	0 (228.4 \pm 22.1)	0 (582.1 \pm 4.2)	40	0 (235.1 \pm 32.1)	0 (588.4 \pm 5.5)	31
Control	231.2 \pm 38.2 (231.2 \pm 38.2)	590.6 \pm 0.3 (590.6 \pm 5.0)	40	233.2 \pm 40.8 (233.2 \pm 40.8)	590.8 \pm 5.1 (590.8 \pm 5.1)	31
Average red	234.2 \pm 1.8 (238.3 \pm 41.2)	602.7 \pm 0.2 (587.3 \pm 4.8)	40			
Average orange	233.2 \pm 2.1 (230.8 \pm 46.2)	562.9 \pm 0.6 (586.2 \pm 5.3)	40			
Reduced red	155.3 \pm 1.5 (235.6 \pm 21.8)	601.8 \pm 0.1 (581.7 \pm 5.1)	40	151.9 \pm 1.1 (227.1 \pm 30.1)	604.2 \pm 0.4 (585.3 \pm 4.9)	31
Enlarged red	361.7 \pm 2.8 (229.9 \pm 27.4)	603.2 \pm 0.5 (585.2 \pm 5.8)	40	361.8 \pm 2.1 (232.5 \pm 39.2)	603.8 \pm 0.2 (588.2 \pm 4.8)	31
Reduced orange	151.8 \pm 2.3 (237.2 \pm 17.2)	560.3 \pm 0.8 (591.2 \pm 5.8)	40	152.9 \pm 2.4 (230.2 \pm 29.7)	560.1 \pm 0.5 (594.1 \pm 5.3)	31
Enlarged orange	366.3 \pm 3.1 (221.4 \pm 32.1)	561.2 \pm 0.4 (586.8 \pm 7.1)	40	365.2 \pm 3.3 (229.6 \pm 28.6)	559.7 \pm 0.3 (590.0 \pm 5.3)	31

created by bleaching both the feathers around the epaulet and the epaulet area and painting them with the red and yellow pens, respectively, corresponding to the average epaulet size (233.04 \pm 51.1 mm², n=310). The control group had the feathers around the coloured epaulet bleached and then blackened to the original size, while the epaulet was painted similar to the other groups but with a transparent pen (Code 0 colourless). This procedure did not produce any visible change in the appearance of the epaulets (i.e., they remained the original size and colour).

To determine the effect of epaulet size, independent of coloration, epaulets were manipulated to the lower (154 mm², n=310) and upper limits (362 mm², n=310) of the population and painted either red or yellow, producing reduced and enlarged versions of each of the two colour treatments (see Table 1). To prevent familiarisation with the manipulations, the colour treatment on the epaulets was removed from the birds using 70% ethanol (i.e., only the uncoloured bleached patches remained, except in the unbleached control birds) before returning them to their housing aviaries. Seven different experiments were performed using 20 different dyads (i.e., 40 males) in each of these experiments (Fig. 2). Initially, all the experiments with control dyads were performed (i.e., unbleached epaulets) before using the birds in the manipulated epaulet size and colour experiments (which required irreversible bleached epaulets; see above). The sequence of all other experiments, as well as the trials within experiments, were varied and randomised. To eliminate the possibility that contestants had recent prior acquaintance with one another, paired individuals were randomly selected from each of the two different localities (Hilton or Balgowan). Most males were used in each of the seven experiments, but never more than once and every dyad was unique. To further remove confounding individual differences (e.g. previous winning experience; Jackson 1988), dyadic trials were repeated 7–11 days later with the epaulet treatments reversed between the pairs (except for the control dyads which were repeated 2–3 days after their initial experiments).

Birds were allowed 6 days to habituate to captivity before the dominance experiments. Contests were staged in a neutral cage, visually isolated from all other birds. The cage contained equal numbers of perches on either side. To ensure that neither contestant was satiated at the onset of a trial, we removed all food from the aviary compartment at 1800 hours the evening before and ran all trials in the first 5 h of morning daylight. During the 15-min trials we recorded the nature and outcome of all aggressive interactions at a central, easily monopolised feeder. Interactions (displacements) at the feeder were recorded as either 'active suppliants' (involving aggressive displays and/or physical attacks) or 'passive suppliants' where a bird simply fled when approached by its opponent. The individual that retained control of the food dish following an interaction was considered the winner of that interaction, and the

individual that won the majority of the interactions (>75%) in a given trial was taken as the winner of that trial. No ties were recorded and only two dyads were excluded from subsequent analyses because dominance could not be easily assigned. As alternative measures of competitive success, we also recorded the first bird to feed and the total amount of time (to the nearest second) that each individual spent at the feeder. All birds were released at their capture site on the last day of the trials.

To avoid type I errors involved with multiple pairwise comparisons and to evaluate the contribution of other potential interacting effects (e.g. body size, body condition, premanipulation epaulet size and colorimetrics, individual ability, capture locality, housing cage, trial reversals) we used generalised linear models (GLM) as they are able to determine variable design effects on binary response data. For each response variable, the win/loss outcomes from each dyad were modelled as Bernoulli dependent variables with a logit link function (Genstat version 5 VSN 2000). Models were generated incorporating all possible combinations of the measured effects and their interactions. The model with the lowest Akaike's information criterion (AIC), which balances the fit of the model against the number of parameters used (Anderson and Burnham 2001) was accepted as the model best fitting the data.

Territorial contests

The study population inhabited a grass and marshland area at Balgowan, situated in the KwaZulu-Natal Midlands of South Africa. Males were captured in mist nets at their night roosts and ringed between 24 November and 16 December 2001, before they established breeding territories. At capture, we measured aspects of body and epaulet size (as above). The first principal component for body size (calculated as above) accounted for 75.3% of the variation in the three body size measurements among wild birds.

Males were sequentially assigned to one of six epaulet treatments as they were captured (as detailed above but excluding the average colour treatments; see Table 1). As a result, none of the male morphological measurements (including their previous epaulet size and hue) differed among the six treatment groups (see Table 2 and Results).

Territory boundaries were mapped by plotting the perches of displaying owners and the locations of aggressive interactions between males, and defined using the convex polygon method (Odum and Kuenzler 1955). The territory maps were digitised and analysed in ArcView (version 3.1 ESRI 1996) to estimate territory area. To ensure that no male replacements or changes in territory size had taken place, the presence of a male on a territory and territory size were updated every three weeks. Once established, no males lost their territories and differences in territory size between

Table 2 Average (\pm SD) differences among epaulet treatment groups in male morphological ($n=31$ males per treatment) and territory characteristics (see Fig. 4 for sample sizes of males establishing territories). Significance tests are provided in the text

	Large red	Large orange	Control	Reduced red	Reduced orange	Blackened
Morphological characteristics						
Tail length	74.9 \pm 4.1	72.5 \pm 4.7	75.1 \pm 3.9	74.4 \pm 3.5	4.7 \pm 4.4	75.7 \pm 4.9
Body size (PC1)	1.3 \pm 1.1	1.2 \pm 1.4	1.3 \pm 0.9	1.1 \pm 1.3	1.3 \pm 1.0	1.4 \pm 1.3
Body mass	29.5 \pm 2.3	30.5 \pm 3.4	29.8 \pm 4.2	30.2 \pm 1.9	32.1 \pm 3.2	31.5 \pm 2.9
Initial epaulet size	233 \pm 60	249 \pm 71	231 \pm 58	259 \pm 45	271 \pm 68	221 \pm 73
Initial epaulet brightness	197 \pm 36	182 \pm 45	172 \pm 37	182 \pm 41	198 \pm 31	201 \pm 39
Initial epaulet chroma	0.69 \pm 0.51	0.63 \pm 0.32	0.58 \pm 0.48	0.72 \pm 0.58	0.63 \pm 0.41	0.78 \pm 0.55
Initial epaulet hue	589 \pm 6	593 \pm 8	583 \pm 6	581 \pm 9	588 \pm 7	589 \pm 8
Territorial characteristics						
Vegetation type	4.5 \pm 1.1	4.8 \pm 1.7	4.2 \pm 1.3	4.2 \pm 1.1	4.5 \pm 1.6	4.4 \pm 1.1
Vegetation density	65.8 \pm 11.3	69.3 \pm 13.2	57 \pm 12.1	59 \pm 11.1	63.2 \pm 12.5	70.1 \pm 12.7
Number of cock's nests	9.6 \pm 3.9	8.2 \pm 2.4	8.7 \pm 3.1	10.1 \pm 3.8	9.4 \pm 12.1	9.8 \pm 13.1
Number of active nests	2.8 \pm 1.4	3.1 \pm 1.2	2.5 \pm 1.3	2.2 \pm 1.2	2.7 \pm 1.7	2.1 \pm 1.3
Female visitation rate	0.09 \pm 0.17	0.16 \pm 0.12	0.23 \pm 0.09	0.28 \pm 0.11	0.27 \pm 0.13	0.26 \pm 0.15

updates were so slight that area parameters were unaltered (change in territory size: 0.02 ± 0.01 ha; Wilcoxon's signed-rank test: $z=1.02$, $n=79$, $P=0.48$).

To estimate territory quality and the suitability of the territory for nesting, we assessed the mean vegetation type and density (rank scale from 0 to 10) at sampling points situated 20 m apart across the study area. Vegetation measurements were taken every 2 weeks and averaged across the breeding season.

Nest locations were marked in the territories and plotted onto the territory maps. As males build numerous cock's nests (which the females line and use), we recorded the number of cock's nests on each territory as they may influence female settlement (e.g. Savalli 1994; Friedl and Klump 1999). The number of active nests (those containing eggs or nestlings) on a territory was used to measure female mating preferences and hence male reproductive success (see Pryke and Andersson 2002).

We observed epaulet-treated males for 15 min weekly (for a period of 12 weeks) during the first 4 h of daylight to quantify the frequency and duration of aggressive interactions. The order of males observed was randomised and all activities and their durations continuously recorded. For analyses, we extracted data on display rate, the time present on a territory, the time in territorial signalling, male intrusion rates, aggressive interactions, encounter durations and female visitation rates.

To avoid type I errors involved in repeatedly testing the same hypothesis, we used three separate multivariate analysis of variance (MANOVA) models to test for differences in morphological, behavioural and nesting variables among the treatment groups. Where F -tests were significant, Scheffé's multiple comparison tests were used to test which treatments contributed to the significance. No transformations were performed as the residuals in all of the MANOVA models were normally distributed (Shapiro-Wilk W -tests, all $P>0.1$) and had equal variances (equality-of-variances F -tests, all $P>0.1$). All statistical tests are two-tailed and means are presented \pm SD unless indicated otherwise.

Epaulet colorimetrics

The red epaulet colour is based on carotenoid pigments in the feathers (unpublished data). Spectral reflectance (at ± 2 nm resolution) of the natural and manipulated epaulets were measured using a S2000 spectrometer (Ocean Optics, Dunedin, Fla., USA), HL2000 halogen light source, WS-2 white reference, a fibre-optic reflectance probe (Avantes, Eerbeek, Netherlands), and C-spec software (Ancal, Las Vegas, Nev., USA). For each individual, following a reference scan (WS-2 white standard), the probe was positioned perpendicular to the feather surface and five consecutive scans (removing the probe between each) were taken from the

centre of the epaulet. Although bird vision typically extends down towards 320 nm (Burkhardt 1989), UV/VIS measurements of the epaulets and pens have confirmed that the weak reflectance below 400 nm indicates no or minimal contribution of ultraviolet to the carotenoid-based signal.

Objective indices of the three main dimensions of colour signals (spectral intensity, location and purity; Hailman 1977) were computed from the scans and averaged for each individual. Measurements of the multiple scans for each of the three colour indices were highly repeatable ($r=0.89\text{--}0.93$, $F_{240,1180}=47.2\text{--}75.8$, $P<0.001$). Spectral intensity ('brightness') was estimated by $R_{350\text{--}700}$, the sum of reflectance from 350–700 nm. Spectral location ('hue', here referred to as 'redness') was estimated as $\lambda(R50)$, the wavelength at which reflectance is halfway between its minimum (R_{\min}) and its maximum (R_{\max}). Spectral purity ('chroma') has a more complex dependence on several aspects of reflectance shape, such as slope height and steepness. To incorporate both these slope aspects, we used individual $\lambda(R50)$ as segment dividers, and computed chroma (C_{R50}) as $R_{350\text{--}\lambda(R50)}-R_{\lambda(R50)\text{--}700}/R_{350\text{--}700}$. Details on the methods used to measure and analyse reflectance are provided in Pryke et al. (2001a).

Results

Effects of epaulet manipulation on dyadic contests

The best fitting GLM explaining the win/loss outcome of the seven experiments (140 trials) identified the same model for all three response variables; the overall trial winner (AIC=228.7, $\chi^2=61.93$, $df=259$, $P<0.001$), feeding order (AIC=213.5, $\chi^2=53.21$, $df=259$, $P<0.001$), and the highest proportion of time spent feeding (AIC=175.4, $\chi^2=39.86$, $df=259$, $P<0.001$). This model identified the effects of the epaulet manipulations as the only significant predictor of contest outcome; redder males were more likely to defeat controls and, to a lesser extent, orange males during contests (probabilities for the outcome of overall trials are provided in Fig. 2). Epaulet size had a particularly strong effect since within colour treatments (i.e., red and orange), enlarged red males won nearly all contests over smaller red males (93%) and larger orange males dominated smaller ones (82%; Fig. 2). Control males had similar and equal probabilities of dominating

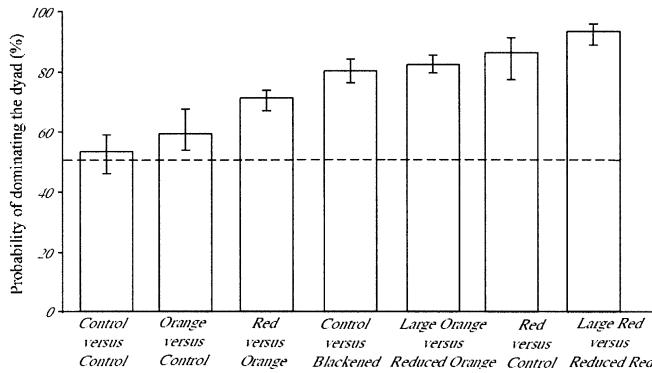


Fig. 2 Within a dyad, the probability (%) of the male with a given epaulet treatment (listed first in the bottom axis labels) dominating his treated opponent (listed second). Probabilities are generated from the coefficients of the best-fit generalised linear model [GLM; Probability = $e^{(\text{coefficient})}/(1+e^{(\text{coefficient})})$] for the win/loss outcome of the contests. The error bars represent the 95% confidence intervals, calculated from the standard error of the coefficients. The dashed line indicates where two males have an equal likelihood (i.e., 50%) of dominating the dyad

other control and orange males, but control birds strongly dominated blackened males (without epaulets). Furthermore, within the control dyads (i.e., with natural epaulets), males with the largest epaulet signals defeated their smaller epaulet opponents ($\chi^2=53.2-80.6$, $df=39$, $P<0.001$ for all three contest measures). Apart from the control males, there was no effect on contest outcome in relation to the initial epaulet characteristics, indicating that all treated birds, irrespective of their own epaulet size, avoided larger and redder males. Likewise, none of the other potential determinants, such as body size (PC1), body condition, capture date, housing cage, individual status or trial sequence (i.e., the initial or reversed epaulet treatment within the pair), had any detectable influence on the outcome of contests.

Epaulet treatments also affected the type of displacement (i.e., active or passive); using interaction type as the response variable showed a strong effect of only epaulet manipulation in the best fitting GLM model ($AIC=58.6$, $\chi^2=80.7$, $df=259$, $P<0.001$). For example, interactions between control males tended to be active (87.3%; Fig. 3) and more frequent than interactions among males in the other groups (Mann-Whitney U -tests: $z=1.93-4.52$, $n=40$, $P=0.05-0.001$ for all experiments except orange versus control: $z=1.12$, $P=0.26$). In contrast, most suppliants in the larger (98.3% large red; 89.2% large orange) and red (versus control 91.9%; versus orange 83.7%) epaulet treatments were passive since the losing bird would move away from the feeder at the approach of the opponent (Fig. 3). Thus, contestants paired to males with larger experimental epaulet signals also showed lower overt aggression.

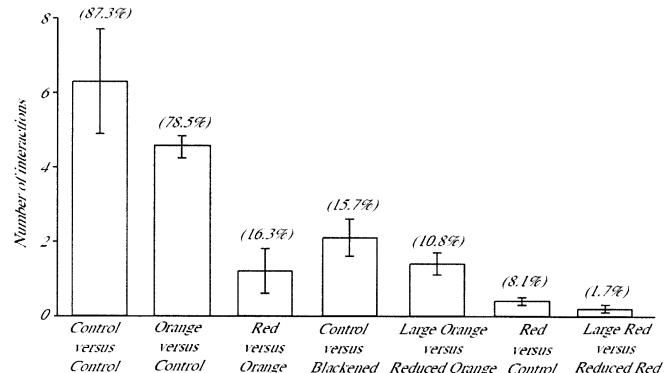


Fig. 3 The average ($\pm SD$) number of aggressive interactions in dyadic contests between epaulet treated males over limited food resources. The percentage of active suppliants (i.e., aggressive displays and/or physical displacements) in these interactions are provided above the bars. The remaining interactions involved passive suppliants (i.e., the feeding bird retreats when approached by an opponent)

Effects of epaulet treatments on territorial contests

The males actively defended well-defined territories and did not allow conspecific males to trespass the boundaries. Males defend their territories using threatening perch displays; with neck feathers raised to a ruff, the tail partially fanned and wings slightly spread, revealing the red epaulets in striking contrast to the black body plumage. When necessary, these displays are backed up by residents aggressively chasing and fighting the intruding males.

Of the 186 epaulet-manipulated males (31 per treatment), 79 males subsequently acquired territories: 19 controls established territories, which was significantly fewer than among enlarged red males ($n=30$; Fisher's exact test: $P<0.001$), significantly more than among blackened ($n=2$; $P<0.001$), reduced orange ($n=4$; $P<0.001$) and reduced red males ($n=9$; $P=0.004$), but not different from among enlarged orange males ($n=16$; $P=0.45$). Treated males unable to establish territories seemed to remain in the area as floaters since five blackened, three reduced orange and two reduced red males were subsequently recaptured (2 days to 5 weeks later) at communal night roosts. Due to the low numbers of blackened and reduced orange males establishing territories (two and four, respectively), these were excluded from the subsequent analyses.

Morphological characters and epaulet colouration may affect territory establishment. However, there was no difference in body size (PC1), body condition, tail length and initial epaulet size and colorimetrics (hue, brightness and chroma) among the treatment groups (MANOVA: Wilks' Lambda =0.83, $F_{7,65}=1.06$, $P=0.39$; Table 2) or between successful and unsuccessful territorial males (multivariate logistic regression: $\chi^2=1.28$, $df=7$, $n=186$, $P=0.57$).

The difference in territory establishment is likely due to indirect effects of the treatments on behaviour as there

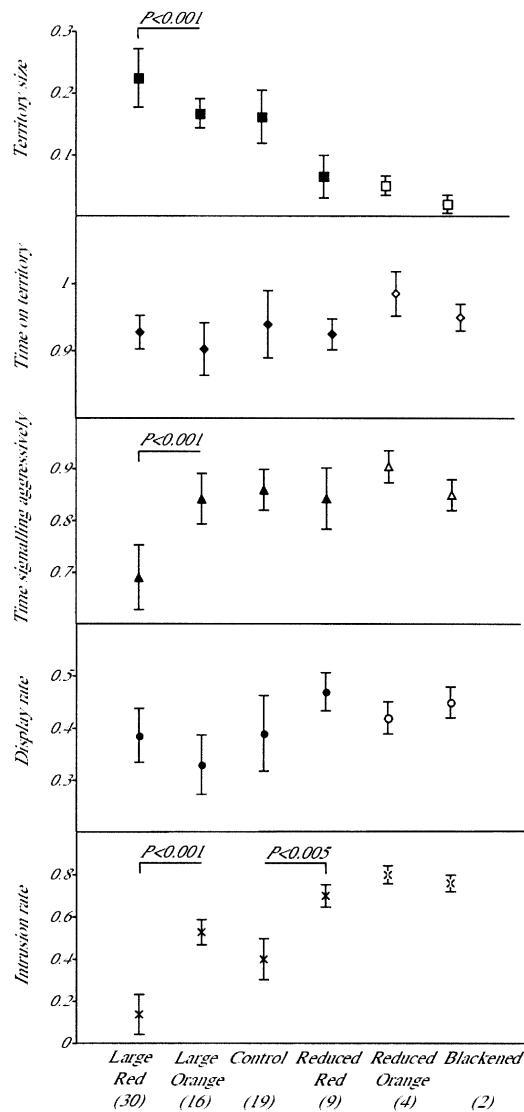


Fig. 4 The effects (mean±SD) of the epaulet manipulations on territorial behaviours. The blackened and reduced orange treatment groups (open symbols) were omitted from the MANOVA due to the low number of males establishing territories. There were significant differences among the treatment groups in territory size (ha), time spent in aggressive signalling (min^{-1}), and intrusion rates (min^{-1}) but not in the time present on territories (min^{-1}) or in display rates (min^{-1}). P -values indicate significant differences (Scheffé's test) between the highlighted group and all other treatment groups (see Results for statistical tests). Samples sizes of males acquiring territories are provided in parentheses below the epaulet treatment groups. Treatment groups are ordered in relation to their decreasing effect on dominance as judged by territory size as well as experiments in captivity

were significant differences in territorial activities among the treatment groups (MANOVA: Wilks' Lambda =0.18, $F_{5,67}=13.11$, $P<0.001$; Fig. 4). There were differences in the amount of time each group spent in aggressive interactions (Fig. 4). The reduced red group received significantly more intrusions from other males (Scheffé's test: $P<0.005$; Fig. 4), whereas the intrusion rate was lowest in the enlarged red group (Scheffé's test: $P<0.001$;

Fig. 4). Since intrusion rate was unrelated to territory size ($r_s=0.11$, $n=79$, $P=0.57$), territory area alone could not explain this significant pattern among treated males. No significant effects were found for the time spent on a territory ($F_{5,67}=3.42$, $P=0.32$) or display rate ($F_{5,67}=2.76$, $P=0.48$), although enlarged red males signalled their territory boundaries less than the other groups ($F_{5,67}=14.22$, $P<0.005$; Scheffé's test: $P<0.001$; Fig. 4). As no males in the enlarged red epaulet group were involved in aggressive encounters with intruders, measures of encounter rate and duration were excluded from the MANOVA model. However, both the reduced red and enlarged orange groups received more aggressive interactions (ANOVA: $F_{2,34}=5.62$, $P=0.003$) and spent longer in encounters (ANOVA: $F_{2,34}=6.12$, $P=0.002$) than the control males.

Territory size was also highly variable (0.17 ± 0.08 , range 0.02–0.38 ha, $n=79$) and differed among the treatment groups ($F_{5,67}=17.43$, $P<0.001$). Males with enlarged red epaulets defended larger territories than the other treatment groups (Scheffé test: $P<0.001$ for all; Fig. 4). Territory quality (vegetation type and density), however, did not differ among treatments (vegetation type: $F_{5,67}=0.15$, $P=0.84$; vegetation density: $F_{5,67}=2.06$, $P=0.61$) and was unrelated to territory size (vegetation type: $r_s=0.04$, $n=79$, $P=0.93$; vegetation density: $r_s=0.09$, $n=79$, $P=0.68$).

Therefore, compared to the enlarged group, males with control and reduced epaulets acquired smaller territories, had more and longer aggressive interactions, and consequently spent more time in territorial defence and conflicts.

Effects of epaulet treatments on male mating success

The number of active nests was evenly distributed among the four treatment groups of males that acquired territories ($F_{5,67}=0.80$, $P=0.79$), although the numbers of females visiting these territories differed ($F_{5,67}=6.72$, $P=0.005$; Table 2). However, after controlling for differences in territorial behaviours among the groups (i.e., residuals derived from the territorial behaviour MANOVA model; see above), there were no differences in female visitation rates ($F_{1,78}=0.07$, $P=0.80$) or the number of nesting females settling on the male's territory ($F_{1,78}=0.89$, $P=0.35$) among the groups. In contrast, although there was no difference in the number of male-built cock's nests among the treatment groups ($F_{5,67}=0.29$, $P=0.65$; Table 2), within territories, the number of active nests was positively correlated to the number of cock's nests ($r_s=0.79$, $n=79$, $P=0.03$). Furthermore, the number of cock's nests was unrelated to territory size ($r_s=0.05$, $n=79$, $P=0.91$) and habitat quality (vegetation type: $r_s=0.07$, $n=79$, $P=0.83$; vegetation density: $r_s=0.12$, $n=79$, $P=0.61$), suggesting that male building activities, rather than territory quality, are the main constraint on the production of these nests. No other morphological (including the original as well as manip-

ulated epaulets) or behavioural traits were related to the number of cock's nests built so that the cause of this variation remains unknown.

Considering the importance of tail length in widowbird mate choice (e.g. Andersson 1982, 1992; Pryke et al. 2001a), which was experimentally preferred even in this short-tailed species (Pryke and Andersson 2002), we also investigated the relationship between tail length and female preferences. However, both female visits ($F_{1,78}=0.13$, $P=0.72$) and nesting decisions ($F_{1,78}=0.85$, $P=0.33$) were unaffected by the natural variation in tail length in this study.

Discussion

The carotenoid-based red epaulets of nuptial male red-shouldered widowbirds are prominently exposed during male threat displays and contests, and our experiments corroborate an agonistic signal function in contests over both food and territories. In captive dominance trials, males with redder and larger treated epaulets strongly dominated males with smaller and more yellow epaulets. Similarly, in field experiments, only males with enlarged and reddened epaulets acquired and maintained territories. The results are consistent with other studies, such as red-winged blackbirds (Peek 1972; Smith 1972; Hansen and Rohwer 1986) and scarlet-tufted malachite sunbirds (Evans and Hatchwell 1992) that demonstrate the importance of carotenoid-based male plumage coloration in settling territorial conflicts. Bright red coloration in red-shouldered widowbirds and most other birds is achieved primarily by the deposition of carotenoids into the feathers (Fox and Vevers 1960; Hudon 1991). Carotenoid pigments are acquired exclusively through the diet and are physiologically essential as vitamin A precursors, antioxidants, and immune-system enhancers (Kodric-Brown 1989; Lozano 1994). As a consequence of these multiple acquisition and usage demands, carotenoid ornaments are thought to be particularly sensitive to environmental constraints (e.g. pigment limitation, parasite infection; Olson and Owens 1998; Hill 1999; Grether et al. 1999; Møller et al. 2000). Carotenoid-based coloration may thus function as an indicator to females of male condition, foraging ability, parental care, genetic quality, age or some other male characteristic (Andersson 1994; Gray 1996; Hill 1999). In male widowbirds, however, female choice seems to be strongly focused on tail elongation (Andersson 1982, 1992; Pryke et al. 2001a; Pryke and Andersson 2002), whereas the red colouration appears to signal fighting ability to male rivals (Pryke et al. 2001b, 2002; Pryke and Andersson 2003).

Badge size and, particularly, colour ('redness') of the red collars in male red-collared widowbirds are crucial for territory establishment and dominance in dyadic competition (Pryke et al. 2002). In contrast, this study suggests that, at least when experimentally altered, badge size rather than redness is the important signal component in red-shouldered widowbirds. For example, within the

asymmetratically sized colour groups (i.e., large red/orange versus small red/orange), males with the larger badges strongly dominated opponents with smaller badges. Similarly, males with naturally larger epaulets in control dyads out-competed their contestants with smaller epaulets. Epaulet size is also important in competition for territories (i.e., territorial versus non-territorial males), with resident males possessing larger and slightly redder epaulets than floating males (Pryke and Andersson 2003). However, since epaulet size and colour are positively correlated (Pryke and Andersson 2003), the combined visual effect of large red epaulets may be more important than that of badge size per se.

Apart from the generally invoked, but rarely substantiated, constraints on carotenoid access or allocation (Hill 1999; Møller et al. 2000), we have no direct evidence of an honesty-enforcing mechanism in the agonistic carotenoid signal of red-shouldered widowbirds. A significant difference in carotenoid-based plumage colouration between resident (which are 'redder') and floater ('yellower') males (Pryke and Andersson 2003) suggests that epaulets signal competitive ability, but further studies of pigment acquisition and utilisation are needed to clarify the cost(s) mediating this apparent 'honesty'.

In contrast to the well-studied case of red-winged blackbirds, we find no clear indication that cheating is socially controlled through increased challenging of stronger signals. Male red-winged blackbirds that exaggerate their social status (e.g. by continuously exposing their coverable epaulets) may provoke increased aggression from conspecifics, which tests the reliability of the badge (Hansen and Rohwer 1986; Metz and Weatherhead 1992). Therefore, epaulet-enlarged males struggled to maintain territories since their epaulets were increased beyond the size that males could back up (Metz and Weatherhead 1992). Contrary to the red-winged blackbirds, however, captive male red-shouldered widowbirds rarely challenged birds with redder or larger epaulets (passive suppliants), whereas aggressive interactions were longer and more numerous between control males (active suppliants) displaying similar badges. Similarly, in the field, manipulations also influenced the aggressive interactions among males. Males with enlarged epaulets received fewer intrusions and spent less time in defence and aggressive interactions, indicating that owners displaying larger epaulets are able to defend their territories at relatively lower costs. Therefore, unlike red-winged blackbirds where males are more aggressive to birds with enlarged epaulets (Hansen and Rohwer 1986), epaulet-treated red-shouldered widowbirds with smaller epaulets were subordinate to those with larger epaulets.

The contrasts between these two species probably relates to the coverable nature of the red-winged blackbird epaulet. Covering the epaulet is beneficial to red-winged blackbird males as a signal of subordinance, allowing males to trespass onto territories of other males with less likelihood of being attacked (Hansen and Rohwer 1986). Epaulets in red-winged blackbirds are generally hidden when the male is in a relaxed posture

(i.e., not displaying or in aggressive interactions); the red part of the epaulet is then completely covered by the black over-wing coverts, so that only the narrow, yellow margin of the epaulet is visible (Orians and Beletsky 1997). In contrast, red-shouldered widowbird epaulets are at least partly exposed even in relaxed perching (i.e., perching with wings completely folded). Here the red signal appears to function as an agonistic signal similar to that of red-collared widowbirds, where only males with the largest and reddest collars establish territories (Andersson et al. 2002; Pryke et al. 2002), and receive fewer aggressive intrusions and interactions than smaller and more yellow collared males (Pryke et al. 2001b, 2002).

Ornamental traits used in contest competition may function as honest indicators of male quality in female choice as well (Andersson 1994; Berglund et al. 1996). However, nesting female red-shouldered widowbirds ignore the male epaulets and instead appear to select males building more cock's nests. Although females were not found to respond to any territory characteristics studied here (apart from the number of cock's nests), a territory is a crucial resource for males since it is a prerequisite for attracting females. Furthermore, competition for breeding territories is intense with only the largest epaulet males acquiring territories, to the exclusion of other males that remain in the area as floaters (Pryke and Andersson 2003).

In conclusion, the red epaulets of male red-shouldered widowbirds are examples of carotenoid-based status signals, contrary to the earlier evidence suggesting that avian status 'badges' are usually melanin-based (e.g. house sparrow bibs). Since large epaulets are vital for territory acquisition, which in turn is a prerequisite for mate attraction, this is an example of agonistic sexual selection (*sensu* Butcher and Rohwer 1988). Traditionally, status signal costs and 'honesty' have been attributed to social costs (e.g. social mediation and life-history trade-offs) and more recently, immunocompetence (reviewed in Senar 1999). In species with carotenoid-based plumage, however, the costs involved in producing carotenoid pigmentation may, alternatively or additionally, reinforce the signal honesty of dominance badges.

Acknowledgements Thanks to the owners and managers of Tschonga Farm for allowing us to work on their land, and to Malte Andersson and two anonymous referees who provided helpful comments on the manuscript. Permission to collect, house (permit no. 28562/2001) and work with wild red-shouldered widowbirds (permit no. 28561/2001) was granted by the KwaZulu-Natal Wildlife Services, and all experimental work was approved by the University of Natal's Animal Ethics Committee (reference no. AE/01/10). Funding was provided by the Swedish Science Council (to S.A.) and an academic exchange program from the Swedish STINT foundation (to S.P.)

References

- Anderson DR, Burham KP (2001) Commentary on models in ecology. *Bull Ecol Soc Am* 82:160–161
- Andersson M (1982) Female choice selects for extreme tail length in a widowbird. *Nature* 299:818–820
- Andersson M (1994) Sexual selection. Princeton University Press, Princeton, NJ.
- Andersson S (1992) Female preference for long tails in lekking Jackson's widowbirds: experimental evidence. *Anim Behav* 43:379–388
- Andersson S, Pryke SR, Örnborg J, Lawes MJ, Andersson M (2002) Multiple receivers, multiple ornaments and a trade-off between agonistic and epigamic signaling in a widowbird. *Am Nat* 160:683–691
- Berglund A, Bisazza A, Pilastro A (1996) Armaments and ornaments: An evolutionary explanation of traits of dual utility. *Biol J Linn Soc* 58:385–399
- Bradbury JB, Vehrenamp SL (1998) Principles of animal communication. Sinauer, Sunderland, Mass.
- Butcher GS, Rohwer S (1988) The evolution of conspicuous and distinctive coloration for communication in birds. In: Power DP (ed) Current ornithology. Plenum Press, New York, pp 51–108
- Burkhardt D (1989) UV vision: a bird's eye view of feathers. *J Comp Physiol A* 164:787–796
- Burley N, Krantzberg G, Radman P (1982) Influence of colour-banding on the conspecific preferences of zebra finches. *Anim Behav* 30:444–455
- Dufour KW, Weatherhead PJ (1998) Bilateral symmetry and social dominance in captive male red-winged blackbirds. *Behav Ecol Sociobiol* 42:71–76
- Eckert CG, Weatherhead PJ (1987) Owners, floaters and competitive asymmetries among territorial red-winged blackbirds. *Anim Behav* 35:1317–1323
- Evans MR, Hatchwell BJ (1992) An experimental study of male adornment in the scarlet-tufted malachite sunbird: I. The role of pectoral tufts in territorial defence. *Behav Ecol Sociobiol* 29:413–419
- Fox HM, Vevers G (1960) The nature of animal colours. Macmillan, New York
- Friedl TWP, Klump GM (1999) Determinants of male mating success in the red bishop (*Euplectes orix*). *Behav Ecol Sociobiol* 46:387–399
- Gray DA (1996) Carotenoids and sexual dichromatism in North American passerine birds. *Am Nat* 148:453–580
- Grether GF (1996) Intrasexual competition alone favours a sexually dimorphic ornament in the rubyspot damselfly *Hetaerina americana*. *Evolution* 50:1949–1957
- Grether GF, Hudon J, Millie DF (1999) Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proc R Soc Lond B* 266:1317–1322
- Hailman JP (1977) Optical signals. Indiana University Press, Bloomington
- Hansen AJ, Rohwer S (1986) Coverable badges and resource defence in birds. *Anim Behav* 34:69–76
- Hill GE (1999) Mate choice, male quality, and carotenoid-based plumage colouration. In: Adams NJ, Slotow, RH (eds) *Proc Int Ornithol Congr* 22:1654–1668
- Hudon, J. (1991) Unusual carotenoid use by the Western Tanager (*Piranga ludoviciana*) and its evolutionary implications. *Can J Zool* 69:2311–2320
- Jackson WM (1988) Can individual differences in history of dominance explain the development of linear dominance hierarchies? *Ethology* 79:71–78
- Järvi T, Bakken M (1984) The function of the variation in the breast stripe of the great tit (*Parus major*). *Anim Behav* 32:590–596
- Kodric-Brown A (1989) Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behav Ecol Sociobiol* 25:393–401
- Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. *Auk* 104:116–121
- Lozano GA (1994) Carotenoids, parasites, and sexual selection. *Oikos* 70:309–311
- Maynard Smith J, Harper DGC (1988) The evolution of aggression: can selection generate variability. *Phil Trans R Soc Lond B* 319:557–570

- Metz KJ, Weatherhead PJ (1991) Color bands function as secondary sexual traits in male red-winged blackbirds. *Behav Ecol Sociobiol* 28:23–27
- Metz KJ, Weatherhead PJ (1992) Seeing red: uncovering coverable badges in red-winged blackbirds. *Anim Behav* 43:223–229
- Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, Surai PF (2000) Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian Poult Biol Rev* 11:137–159
- Odum EP, Kuenzler EJ (1955) Measurement of territory and home range size in birds. *Auk* 72:128–137
- Olson VA, Owens IPF (1998) Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol Evol* 13:510–514
- Orians GH, Beletsky LD (1997) Red-winged blackbirds: decision-making and reproductive success. University of Chicago Press, Chicago
- Peek FW (1972) An experimental study of the territorial function of vocal and visual display in the male red-winged blackbird (*Agelaius phoeniceus*). *Anim Behav* 20:112–118
- Pryke SR, Andersson S (2002) A generalized female bias for long tails in a short-tailed widowbird. *Proc R Soc Lond B* 279:2141–2146
- Pryke SR, Andersson S (2003) Carotenoid-based epaulets reveal male competitive ability: experiments with resident and floater red-shouldered widowbirds. *Anim Behav* (in press)
- Pryke SR, Andersson S, Lawes MJ (2001a) Sexual selection of multiple handicaps in the red-collared widowbird: female choice of tail length but not carotenoid display. *Evolution* 55:1452–1463
- Pryke SR, Lawes MJ, Andersson S (2001b) Agonistic carotenoid signalling in male red-collared widowbirds: aggression related to the colour signal of both territory owner and model intruder. *Anim Behav* 62:695–704
- Pryke SR, Andersson S, Lawes MJ, Piper SE (2002) Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color. *Behav Ecol* 13:622–631
- Rohwer S (1982) The evolution of reliable and unreliable badges of fighting ability. *Am Zool* 22:531–546
- Røskræft E, Rohwer S (1987) An experimental study of the function of the red epaulettes and black body colour of male red-winged blackbirds. *Anim Behav* 48:105–111
- Savalli UM (1994) Mate choice in the yellow-shouldered widowbird—correlates of male attractiveness. *Behav Ecol Sociobiol* 35:227–234
- Searcy WA (1979a) Female choice of mates: A general model for birds and its application to red-winged blackbirds. *Am Nat* 114:77–100
- Searcy WA (1979b) Morphological correlates of dominance in captive male red-winged blackbirds. *Condor* 81:417–420
- Searcy WA, Yasukawa, K (1995). Polygyny and sexual selection in redwinged blackbirds. Princeton University Press, Princeton, N.J.
- Senar JC (1999) Plumage colouration as a signal of social status. In: Adams NJ, Slotow, RH (eds) *Proc Int Ornithol Congr* 22:1669–1686
- Smith DG (1972) The role of the epaulettes in the red-winged blackbird (*Agelaius phoeniceus*) social system. *Behaviour* 41:251–268
- Smith JNM, Arcese P (1989) How fit are floaters? Consequences of alternate territorial behaviors in a non-migratory sparrow. *Am Nat* 133:830–845
- Studd MV, Robertson RJ (1985) Evidence for reliable badges of status in territorial yellow warblers (*Dendroica petechia*). *Anim Behav* 33:1102–1112