Agonistic carotenoid signalling in male red-collared widowbirds: aggression related to the colour signal of both the territory owner and model intruder

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Carotenoid colour displays are widely assumed to be honest indicators of individual health or quality, primarily in mate attraction. Here we show that sexually dimorphic carotenoid ornamentation functions as an agonistic signal in male red-collared widowbirds, *Euplectes ardens*. Mounted male models differing (within natural limits) in the intensity of carotenoid signalling were presented to wild resident males as simulated intruders, perched or made to 'fly' across the territory with the elongated tail folded or keeled. Perched mounts were generally ignored, and stronger aggression towards 'flying' models with a keeled tail (i.e. as in courtship display) than a folded tail suggests the tail display is used to assess the intention of intruding males. Territory owners were less aggressive towards models with intense collar display, suggesting that carotenoid coloration functions as a badge of status in this species. The level of aggressive response was also related to the resident's own badge in that males with larger, redder collars responded more aggressively to the models. In addition, males with a larger collar signal defended larger territories and spent less time in territory defence. Apart from the collar size and 'redness', no other morphological variable predicted the aggressive response of territorial males. Given the previously demonstrated insignificance of the collar in female mate choice, we suggest that the nuptial carotenoid coloration is an honest signal of dominance or fighting ability, sexually selected through male contest competition over territories.

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Carotenoid pigmentation is a major component of conspicuous coloration and sexual dichromatism in birds (Fox & Vevers 1960; Brush 1978; Bortolotti et al. 1996; Badyaev & Hill 2000). Carotenoids are also vital nutrients for all animals and, owing to the presumed costs of foraging, parasite-inhibited uptake, or competing physiological functions (Lozano 1994; Olson & Owens 1998), carotenoid displays have become favourite candidates for honest signalling of resources or 'good genes', primarily in the context of sexual selection (M. Andersson 1994; Hill 1999; Møller et al. 2000). In birds, female mate choice based on male carotenoid display has been tested in several species (see Hill 1999, for a review). However, despite many indications that avian carotenoid ornaments also function in agonistic signalling (e.g. red-winged blackbirds, *Agelaius phoeniceus*: Hansen &

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Rohwer 1986; ring-necked pheasants, *Phasianus colchicus*: Mateos & Carranza 1997; red junglefowl, *Gallus gallus*: Ligon et al. 1990; scarlet-tufted malachite sunbirds, *Nectarinia johnstoni*: Evans & Hatchwell 1992), such results mostly derive from removing or covering the trait rather than showing an effect of natural signal variation. Furthermore, some recent studies that do address carotenoid signal variation, albeit in the non-breeding season, found no or even negative effects on male dominance (Brown & Brown 1988; Belthoff et al. 1994; Wolfenbarger 1999; McGraw & Hill 2000). These somewhat paradoxical results, together with the lack of objective colour quantification in most studies of avian carotenoid displays (i.e. with reflectance spectrometry; see Endler 1990), have left the role of carotenoid honesty in male–male sexual contest competition unresolved.

We investigated the agonistic function of the red 'collar' in male red-collared widowbirds, *Euplectes ardens*, a species well suited for the study of this issue. The male has striking seasonal and sexual dimorphism, and moults from a cryptic sparrow-brown nonbreeding plumage into

a distinct black nuptial plumage with a long tail and a crescent-shaped carotenoid-based chest patch (primarily lutein, zeaxanthin and canthaxanthin; unpublished data). Red-collared widowbirds have a territorial, highly polygynous mating system, where males provide no resources for the females other than nest sites (Craig 1980; Mitchell 1966; Pryke et al., in press). Male competition to acquire and maintain territories is high as judged by a large population of 'floaters' (males in breeding plumage that do not establish a territory) that frequently intrude on to occupied territories to attract females.

Our earlier results suggest that the red collar is selected through male contest competition and not by female choice. First, in territorial males, mating success (number of nests) is strongly determined by tail length, whereas collar size and colour have no or even negative effects on the attraction of nesting females (Pryke et al., in press). Second, floaters have significantly smaller and more yellow collars than the territory owners (S. Andersson, S. R. Pryke, J. Örnborg & M. J. Lawes, unpublished data). However, the above results were purely correlational, which is why experimental tests of the signal function are required.

To demonstrate agonistic signalling, one must determine whether the behaviour of the receiver is affected in a way consistent with the presumed signal content (Dawkins & Krebs 1978). Experimental manipulations of 'status signals' or 'dominance badges' in birds have been successful in a variety of species. However, the majority of these studies have manipulated live birds and introduced them to conspecifics, where the effects on the behaviour of the manipulated bird and the opponent are confounded and difficult to tease apart (Senar 1999). The problems associated with the use of live birds may be solved by the use of models (artificial or mounted specimens; e.g. Studd & Robertson 1985; Jones 1990), by which consistent signal levels can be presented that affect only the behaviour of the target individual. We used mounted red-collared widowbirds manipulated to three different levels of the red collar signal, corresponding to the minimum, average and maximum of the natural variation (including floaters; see above). In addition to the effects of the experimental intruder's signal, the resident male's response level may also vary in relation to his own perceived dominance status based on prior experience (e.g. 'winning begets winning'; Jackson 1991), his own signal expression and other aspects of his morphology. An important aim of the study was therefore to analyse aggressive responses in relation to the natural variation in collar signals and morphology of resident males.

The basic design of experimental model studies has been to observe the responses of territorial birds to stationary dummies, but this might be an insufficient stimulus in species where movement is important for signal detection or signal content (Hailman 1977; Fleishman 1992). This seems particularly likely in the red-collared widowbird, where the elaborate flight display (with keeled tail) is a prominent feature of the male's breeding behaviour, and also seems to elicit the strongest aggression from other males (personal observations). We therefore investigated the responses to both stationary and 'flying' models. In addition to improving our results on the function of the carotenoid collar display, this also provided other useful insights into the visual communication system in red-collared widowbirds.

METHODS

Morphometric Measurements

We studied a population of red-collared widowbirds at Mondi Mountain Home Estate in eastern KwaZulu-Natal, South Africa (29°43′S, 30°17′E; elevation ca. 1140 m) from December 1999 to April 2000. The birds inhabited a grassland clearing interspersed with shrubs and marshlands (dams) within afforested plantation.

We captured the birds in mist nets on their territories, at communal feeding sites, or night roosts (dense reed beds situated within marshlands or dams). All males were ringed with an aluminium ring and three unique coloured bands. We measured the lengths of the tarsus (between the bending points at the toes and heel), culmen (tip to the base of the bill) and tail (base to the tip of the feather) to the nearest 0.1 mm and the wing, using the flattened chord method, to the nearest 0.5 mm. As the body size variables (tarsus, culmen and wing length) were intercorrelated, we used the first components of a principal components analysis (PCA), explaining 69% of the variation, as an independent measure of body size. For body mass, we weighed the birds to the nearest 0.5 g with a Pesola spring balance. Since body mass was positively correlated with body size ($r_S=0.34$, $N=185$, $P<0.001$), we calculated an index of body condition based on tarsus length, which is a good indicator of skeletal size (Freeman & Jackson 1990). Body condition was expressed as the residuals from log(body mass) linearly regressed on 3log(tarsus length) (S. Andersson 1994). The ectoparasite load (e.g. lice, ticks and mites) of each male was scored from 0 to 3 (none to heavy load) from an examination of the number of parasites visible on the wing and tail feathers.

Collar Measurement and Colorimetrics

Collar area was calculated to the nearest 0.1 mm as the product of the maximum length (horizontal to the head) and the average of three breadth measures (vertical to the head) of the carotenoid throat patch when holding the head in a normal position.

We measured spectral reflectance (at ± 2 nm resolution) from the red collar with a PS1000 spectrometer (Ocean Optics Inc., Dunedin, U.S.A.), using a HL2000 halogen light source, and a fibreoptic reflectance probe with a 4-mm measuring diameter. The probe was held perpendicularly against the plumage and, using the C-spec software (Ancal Inc., Las Vegas, U.S.A.), we took five consecutive scans (removing the probe between each scan) from the centre of the collar patch and averaged them for each individual. Reflectance spectra were

derived in relation to a WS-2 white standard (>98% reflectance across the measuring range) that was scanned before each individual was measured.

Objective indices of the three main dimensions of colour signals, spectral intensity, location and purity (Hailman 1977), were computed from the raw spectral reflectance data, and then averaged for each individual. The colour variables derived were: (1) 'brightness' (spectral intensity) estimated by $R_{350-700}$, the sum of reflectance from 350 to 700 nm; (2) 'hue' (spectral location) estimated as (λR_{50}), the wavelength at which reflectance is halfway between its minimum (R_{min}) and its maximum (R_{max}); and (3) 'chroma' (spectral purity) which depends on several reflectance shape aspects, such as both slope height and steepness. To avoid a fixed arbitrary spectral segment, we used $\lambda(R_{50})$ as the segment divider, and computed $C[R_{50}]$ as $R[\lambda_{350} - \lambda(R_{50})] - R[\lambda(R_{50}) - \lambda_{700}]/R_{320-700}$. Further details on the methods used to collect and analyse reflectance are described in Pryke et al. (in press).

Territorial Behaviour

We mapped the territories of 43 colour-banded males with the minimum convex polygon method (Odum & Kuenzler 1955). The males defended territories ($\bar{X} \pm SD = 0.41 \pm 0.27$ ha, $N=43$) that were either isolated from one another or contiguous with other territories within the grasslands. Nonterritorial males were caught at communal night roosts in nearby reed beds and were defined as floaters, as they roamed the breeding grounds but did not hold a territory within the study area. Judging from the numerous observations of unknown males in full nuptial plumage intruding on to the territories, and from the large number of adult males caught in night roosts or feeding sites, there was a relatively large population of 'floating' males.

We observed each territory owner daily between dawn and dusk (for 15 min) when males defended territories aggressively from conspecifics. We used a random number table to determine the order in which males were observed. All activities and their durations were recorded continuously on to a dictophone with the time given to the nearest 5 s. For analyses, we extracted data on the time spent active on a territory, the time spent in territory defence, the number of aggressive interactions, encounter length and fights.

Model Experiments

We made nine models from skins of adult male red-collared widowbirds. The nine males were captured from the study population (ca. 900 widowbirds) and killed by affixation (i.e. euthanasia within ca. 10 s with an increasing concentration of carbon dioxide gas) at the University of Natal, South Africa. The procedure was carried out in accordance with the guidelines of the Ethics Committee at the University of Natal and with permission from the KwaZulu-Natal Conservation Services, South Africa. The first set of experiments was
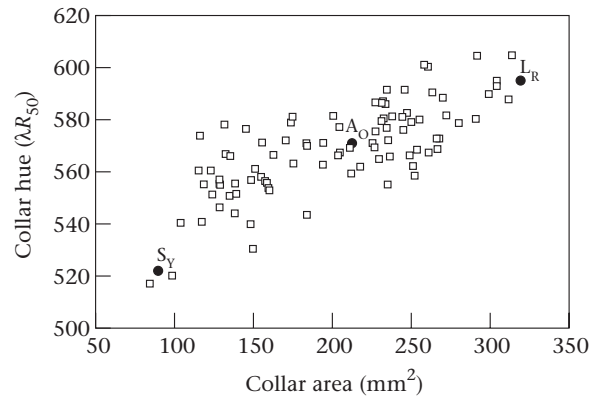
Figure 1. Correlation between collar area (mm²) and colour (hue (λR_{50})) for the population ($r_S = 0.76$, $N=93$, $R^2 = 57.2\%$, $P < 0.001$), showing the three collar categories (S_Y: small yellow; A_O: average orange; L_R: large red) for the models.

designed to test whether the collar, and not some correlated trait, was responsible for the differing levels of aggression between males. The size and the hue (colour) of the collar were highly correlated (Fig. 1), with the product of these two dimensions contributing to the greatest difference between floating and territory owners (S. Andersson, S. R. Pryke, J. Örnborg & M. J. Lawes, unpublished data). Therefore, we selected the model collars from the natural variation in this combined signal measure. Separating the independent effects of colour versus size was not our goal in this study; rather, we wanted to mimic the natural variation in carotenoid display intensity. Thus, one model category displayed a small yellow collar (S_Y), another an average-sized orange collar (A_O) and the last a large, red collar (L_R). We made S_Y collars by blackening the yellow feathers with a marker pen (Sanford Sharpie permanent marker, Mesa, Arizona, U.S.A.) to reduce the collar area to the lower limit of natural variation in collar size (Fig. 1). A_O collars were chosen from the population to represent the average collar area and colour (Fig. 1). We constructed the L_R model by gluing supplemental feathers taken from other live birds with similar red-coloured collars on to the mount. The large collar was constructed such that the collar area was increased evenly in all dimensions (i.e. length and breadth).

In addition, we positioned each of these three categories of collar models in different postures such that one model type was perched, and the other two 'flying' (with the tail either folded or keeled), to investigate whether motion patterns affected a male's agonistic reaction. The perched models were constructed in a vigilant posture displayed by conspicuously perched resident males, with the culmen directed upward and the tail flared behind him. We fastened these models on to wooden dowels that could be inserted on to a wooden perch for positioning in the field. The 'flying' models were stuffed red-collared widowbird males constructed with the wings spread as in flight and with the tail either folded (as in directed flight) or keeled to form an inverted sickle-shape (as in courtship displays). We made keeled-tail models by setting and gluing the tail feathers in

a deep vertical keeled position during the taxidermic procedure, and folded-tail models by positioning the tail feathers directly behind the body. We shortened the tail (by cutting the tail feathers) such that tail lengths for all the perched and flying models represented a below-average tail length of 200 mm.

We randomly presented the nine models (i.e. three perched models, three folded-tail models and three keeled-tail models each with a different collar display: S_Y, A_O and L_R) to the 43 marked males holding territories within the study area. We presented these nine models three times in the breeding season. The first coincided with the prenesting period about 2 weeks before we located the first nest (20 December 1999 to 11 January 2000), the second was during the most intensive nesting period when the majority of nests had their first eggs (12 February to 4 March 2000), and the last was at the end of the breeding season, when the majority of the nest-lings had fledged (2–19 April 2000). Each male was therefore presented with the nine models three times during the breeding season (i.e. a total of 27 models). All observations were made during the 3–4 h after dawn, as this is the time of highest activity. The perched mounts were situated conspicuously in the centre of each territory so that they were visible in all directions. The models were positioned while the male was occupied off territory so that the observer was away from the model by the time it was detected. We recorded 10-min focal samples of male activity beginning once the resident had sighted the model. We assumed that the male had seen the mount after he had turned to face the model.

The flying models were designed to 'fly' through the centre of the resident's territory. We positioned two aluminium poles on either side but outside the resident's territory boundaries and connected by thick transparent fishing line (Siglon monofilament fishing line, Sunline Ltd, Kaisha, Japan). The line was positioned on the pole such that the models would 'fly' from a high point just inside the neighbouring territory down into the experimental territory about 50 cm above the grass height (typical for intruding males) and then exit the territory on the other side, landing well outside the experimental territory. Once the poles and line were set up between the territories they were left for at least 1 h before the experiment began, to minimize the effect of the presence of the observer on the territory. When returning, the observer would not enter the territory but set up the model outside it. A harness of transparent fishing line was made for the flying models, which was attached to the experimental line by a small steel hook. Only when the male was vigilant was the model released and gently pushed so that it 'flew' through the experimental territory.

The above experimental approach was based on the assumption that increased aggressive displays and a closer approach by territorial males towards an intruding male were indicative of male competition induced by the model male's phenotype. For each male red-collared widowbird that responded to the models, we measured the distance of the closest approach and the response time (the time from when the model was displayed until the territory owner approached the model). Songs were not played during any model presentations because intruding red-collared widowbirds do not sing when entering occupied territories (Craig 1980; personal observations).

Presentations to the individual males during the three different breeding stages were separated by 1–2 days to minimize the possibility of habituation. Randomizing the order of presentation for both the posture and collar parameter model categories also experimentally controlled the variation in response from habituation. However, habituation is unlikely to be a serious problem in these data sets because males rarely hit the models and responses at the end of the nesting season were similar to those on first encounters (see below). To control for the novelty of 'flying' models through the territory, we randomly presented each resident male with a 'flying' male red-shouldered widowbird, *Euplectes axillaris*, model during each breeding stage (at the prenesting, nesting and postnesting phase). Although red-collared widowbird males do occasionally chase red-shouldered widowbirds (and vice versa), these two species defend overlapping territories with an apparent mutual tolerance for each other (Craig 1980; personal observations).

Data Analysis

We used partial correlations to isolate the effect of male (morphometric) characteristics on the interrelated territorial behaviour variables. A sequential Bonferroni adjustment was used to adjust significance levels for the multiple partial correlations. A multiple analysis of variance (MANOVA) was used to determine whether the model collar categories (collar area, brightness, chroma and hue) differed within each of the three model collar types (S_Y, A_O and L_R). Male response (closest approach distance and reaction time) to the models was examined with a repeated measures analysis of variance (RM-ANOVA; Statistica 1996). We compared means within and between males for the different models (folded tail and keeled tail) and collar types (S_Y, A_O and L_R) over the three breeding stages (prenesting, nesting and postnesting). The interactions between all these effects were analysed to determine whether the change in one effect was independent of the variation in the other. When F tests indicated significance, we used Scheffé's multiple comparison tests to test where specific variables differed from one another (i.e. which combination of variables contributed to the significance).

Statistical tests are two tailed.

RESULTS

Territorial Behaviour

The size of the territory defended was related to the size ($F_{1,42}=32.19$, $R^2=45.9\%$, $P<0.0001$) and colour (hue; $F_{1,32}=21.52$, $R^2=41.1\%$, $P<0.0001$) of the resident male's collar. Resident males signalled territory boundaries by sitting prominently on the boundary with their culmen

Table 1. Partial correlation coefficients between behaviour variables and the territorial male traits

	Territorial behaviour variables				
Male trait	Active time on territory	Time spent conspicuous	Time in territory defence	Number of aggressive interactions	Encounter length
Territory area	−0.32	−0.25	−0.59**†	−0.37	−0.33
Tail length	0.04	0.07	0.13	0.17	0.08
Body size (PC1)	−0.28	−0.11	−0.22	−0.31	−0.36
Collar area	−0.73***†	−0.52**†	−0.44**†	−0.65***†	−0.46**†
Collar chroma	−0.17	−0.21	−0.11	−0.14	−0.41*
Collar hue	−0.51**†	−0.41*	−0.38*	−0.47**†	−0.48*
Collar brightness	0.08	−0.02	0.09	−0.23	0.06
Body condition	−0.22	−0.17	−0.21	−0.43*	−0.34
Ectoparasite load	−0.13	−0.19	−0.24	−0.29	−0.28

N=33 for collar hue, chroma and brightness, 43 for the other male traits.
*P<0.05; **P<0.01; ***P<0.001; †Significant after Bonferroni adjustment at α=0.05.

directed forward and upwards, body feathers slightly ruffled (especially on the chest) and tail partially spread. Intrusions by other males on to occupied territories were common. Territory owners tended to focus all their attention on intruders and commonly performed boundary displays, with their chest (and collar) ruffled and culmen projected forward and upwards. If the intruder did not flee, the resident male would displace intruding males by flying directly towards them. Owners initiated chase flights towards intruders within 28.5 ± 79 s ($\bar{X}$ ± SE, N=486) of the intruder landing. After the attack the resident male sometimes pursued the intruder over many neighbouring territories (73% of all chases), especially if the male entered the territory displaying to females. Fights were defined as conflicts between males where physical contact was made: either pecking or biting the opponent (N=14 for all fights observed), or, more rarely, grasping and grappling with claws and beak (N=5). In each observed fight (N=19) the winner of the conflict was the resident, which retained the territory.

The four measures of territorial behaviour indicate the relative amount of time residents spent in territorial defence and maintenance (Table 1). First, redder- and larger-collared males spent significantly less time on their territory and larger-collared males spent less time conspicuous on their territory (Table 1). These results correspond to less time spent in sight by redder- and larger-collared males on the territory. Second, males defending larger territories (with larger and redder collars) also spent significantly less time in territory defence (i.e. signalling territory boundaries and aggressive displays; Table 1). Third, if badge size is important in agonistic interactions, we would expect the conflicts to be settled mainly by low-intensity behaviour (i.e. settled primarily by badge display). The time spent in agonistic interactions is a product of two variables, the number of interactions and the duration of each interaction. When the number of separate agonistic encounters is considered, larger- and redder-collared males in slightly better condition received fewer intruders and hence interactions from floaters (Table 1). There was also a significant difference in the duration of agonistic encounters, with larger- and redder-collared males spending less time in aggressive interactions. Therefore, these males spent less time in aggressive encounters and received fewer intrusions from conspecifics.

Response to Models

### Effect of breeding stage and model type

The aggressive response of resident males to all models was related to the stage of breeding during presentation (Table 2). For all model types, males were significantly more aggressive during the nesting period than the pre-nesting (Scheffé test: P<0.001) and postnesting (Scheffé test: P<0.001) stages. Resident males also tended to react faster and approach closer to the models with displayed tails and small yellow collars during the nesting stage than during the other presentation times, although not significantly so (see interactions of presentation time∗model and collar∗presentation time in Table 2).

Males responded differently to the type of model displayed (Table 2). There were very few responses to the perched models (only 12% of males responded), and these were excluded from subsequent analyses. Similarly, the 'flying' red-shouldered widowbird model was generally ignored and although four males did react (9%), none of them approached the model closer than 3 m. The keeled-tail models were approached significantly quicker than the folded-tail models (Fig. 2a) and this effect was independent of collar signal since there was no interaction between model type and collar type (Table 2). There was also a significant difference in the approach distance of resident males to the flying and displaying models (Fig. 2b), and keeled-tail models were approached closer (Scheffé test: P<0.001) and attacked more than the flying ones. In addition, males tended to approach keeled-tail models with smaller yellow collars closer than other collar types, although this result was not quite significant (collar∗model interaction; Table 2). Several residents (14%) struck the keeled-tail models without hesitation, pecking violently and often removing tails and wings. However, model type did not further affect the variation in aggressiveness, as there were no other

Table 2. Repeated measures ANOVA (see text) for the effects of presentation time, model type and the model's collar on the reaction time and proximal distance approached by territorial males to the models

	Reaction time			Approach distance		
Source of variation	F	df	P	F	df	P
Between males						
Presentation time (prenesting, nesting and postnesting)	740.91	2,82	<0.001	27.42	2,82	<0.001
Model (folded tail and keeled tail)	4792.13	1,41	<0.001	107.53	1,41	<0.05
Presentation time*Model	14.29	2,82	0.085	8.73	2,82	0.089
Collar (small yellow (S_Y), average orange (A_O) and large red (L_R))	1.61	2,82	0.132	135.92	2,82	<0.001
Collar*Presentation time	4.94	4,164	0.074	4.99	4,164	0.095
Collar*Model	0.43	4,164	0.696	10.89	4,164	0.084
Within males						
Male (43 territorial males)	16.92	41,1	<0.01	49.89	41,1	<0.01
Male*Model	0.59	1,41	0.799	7.34	1,41	0.096
Male*Collar	19.98	2,82	0.042	119.36	2,82	0.001

Only significant interactions are reported.
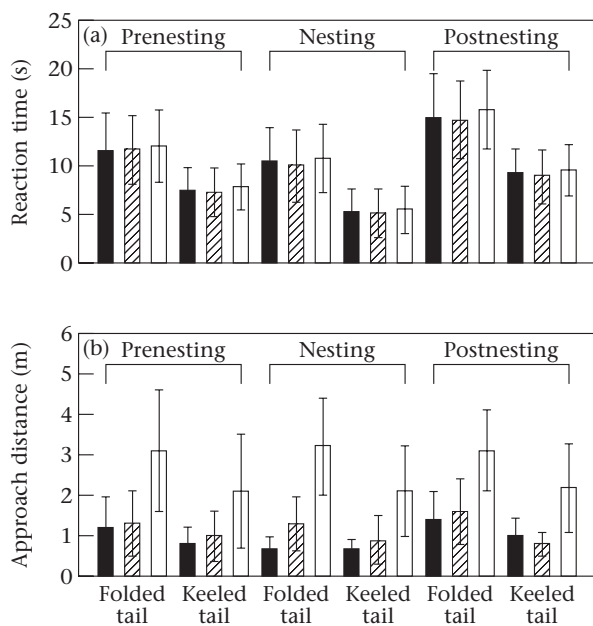
Figure 2. (a) Residents' reaction time and (b) distance approached by residents to the different model postures (folded tail and keeled tail) and model collars (■: S_Y=small yellow; ▨: A_O=average orange; □: L_R=large red) during the three presentation times (prenesting, nesting and postnesting). Means are given±SD.

significant two- or three-way interaction terms involving model type.

Effect of colour signal

There was no significant difference within the three model types (perched, folded tail and keeled tail) used for each of the three model collar categories (S_Y, A_O and L_R) for any of the collar variables (collar area, brightness, chroma and hue; MANOVA: Wilks's λ=0.71–0.93, $F_{6,8}$=1.3–2.9, NS for all combinations).

Although there was no effect of collar signal on reaction time (Fig. 2a), there was a clear effect on the approach distance (Fig. 2b): males approached the S_Y and

A_O collars closer than the maximum colour signal (Scheffé test: $P<0.001$ for both). The trend for the S_Y versus the A_O colour signal was consistent with this, but not significant (Fig. 2b). This suggests that residents reacted initially to the display type of the model (i.e. keeled tail), but thereafter escalated or reduced their aggression depending on the collar signal.

Individual Variation in Response

Response in relation to own collar signal

We also included the responses of each male as an effect in the model to determine if all males reacted similarly to the models. There was significant individual variation in both reaction time and closest approach, but the effect of the model collar remained since there was a significant interaction between the individual and the model's collar (Table 2). The variation in the resident's aggressive response to the models was related to his own collar (see below; Table 3). For all the models presented (after controlling for the effects of presentation time, model type and model collar in the RM-ANOVA model), the time taken to react (Fig. 3) and the distance approached (Fig. 4) were highly significantly related to the collar area of the territorial male and less so to the colour of his collar (see below). The most aggressive responses involved the largest collared male who often physically attacked the models, in one case completely decapitating the wire-reinforced flying model.

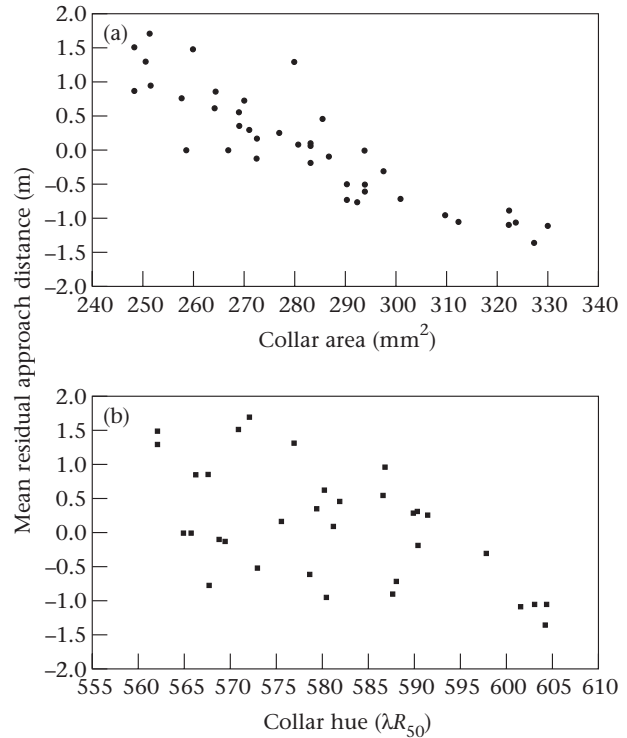*Response in relation to morphometrics and aggression*

Several of the male morphometric characteristics were interrelated and we therefore used a stepwise multivariate regression analysis to determine which morphometric traits were important in aggressive interactions (Table 3). The selection of the variables in the model was based on a backward and forward stepwise regression to determine the amount of explained variance (R^2) that was removed or added, respectively, by each variable. All measurements were standardized (mean 0, variance 1) to obtain comparable parameter estimates (β) and the residuals
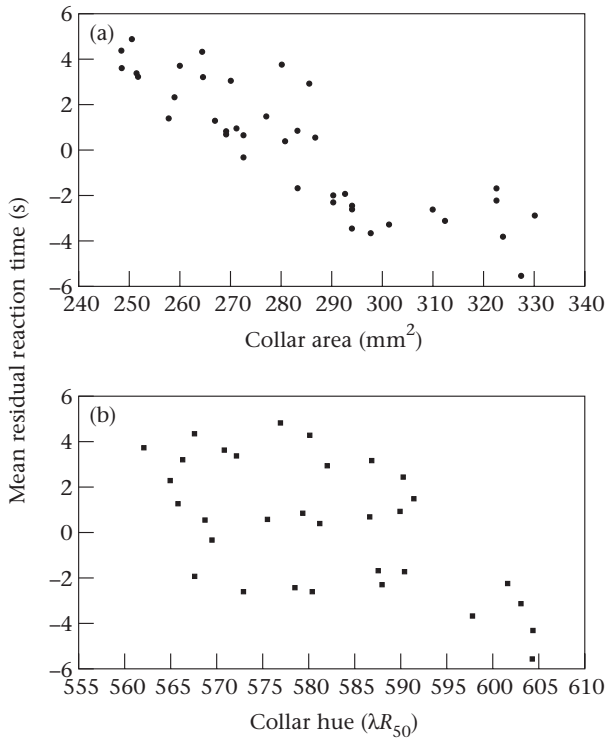
Figure 3. The mean residual reaction time of each male to the models in relation to the (a) size ($F_{1,42}$=102.39, R^2=72.9%, P<0.0001) and (b) colour ($F_{1,32}$=18.84, R^2=38.8%, P<0.0001) of the resident male's collar. The mean residual measures for each male were derived while controlling for the effect of presentation time, model type and model collar (i.e. residuals derived from the RM-ANOVA model; see Table 2). The higher residual values indicate a relatively longer time taken to react to the models.

Figure 4. The mean residual proximal distance of each male to the models in relation to the (a) size ($F_{1,42}$=143.43, R^2=79.1%, P<0.0001) and (b) colour ($F_{1,32}$=15.17, R^2=32.9%, P<0.0005) of the resident male's collar. The mean residual measures for each male were derived while controlling for the effect of presentation time, model type and model collar (i.e. residuals derived from the RM-ANOVA model; see Table 2). The lower residual values indicate a relatively closer approach to the models.

did not depart from a normal distribution in any of the models (Kolmogorov–Smirnov tests: D=0.43–0.97, P=0.3–0.8). Reaction time (as the dependent variable), log transformed, was primarily related to collar area (Table 3), which accounted for more than 36% of the variation. The colour of the collar accounted for less of the variation (R^2=19%). When proximal distance scores were used as the measure of aggressive response, a highly significant relationship with collar area was also obtained (R^2=32%). In addition, the size of the resident's territory (R^2=15%), his body condition (R^2=13%) and ectoparasite load (R^2=8%) also contributed to this aggression measure, making the overall model highly significant (R^2=69.1%, P<0.001).

If the collar indicates the status of the territorial male, it should also covary with male aggression as measured by territory size. The rank order of territory size was log transformed before being entered into the model. The full model explained almost 71% of the variation in aggression, with collar area contributing to most of the variation in territorial position (R^2=39%; Table 3). Except for the size and the colour of the collar, no other single variable (included in the model) was significantly associated with rank (Table 3). The highly significant effect of collar area and colour on different male aggression

indices suggests that, when body size and other traits are held constant, males respond primarily to the investment in carotenoid plumage.

Thus males with a greater carotenoid advertisement expended less effort in territorial maintenance, advertisement of residency and agonistic interactions but showed more aggression to the model presentations.

DISCUSSION

In contrast to the primary mate choice function of carotenoid displays (reviewed in Hill 1999), our study confirms an intrasexually selected, status-signalling function of carotenoid coloration in red-collared widowbirds. In addition to the observed correlations with territory size and success in male–male interactions, our results show that: (1) stronger signals in intruders were avoided (less closely approached) more than average and weaker model signals; (2) larger- and redder-collared residents responded more aggressively to models (i.e. attacked more) and approached them closer than smaller-collared territory owners. Resident males with larger territories were also more aggressive than those with smaller territories. This was not an effect of territory size as such (via intrusion rate) since larger-collared residents received fewer intrusions by floaters and were involved in fewer

Table 3. Multiple stepwise regression model of the best combination of characters for predicting aggression (reaction time (R^2=67.3%, P<0.001) and closest distance approached (R^2=69.1%, P<0.001) to the models) and dominance (R^2=70.8%, P<0.001)

Male trait	Male aggression and dominance variables					
	Reaction time		Proximal distance		Territory dominance	
	β	t_{33}	β	t_{33}	β	t_{33}
Territory area			0.38	1.67**		
Collar area	0.81	5.98***	0.80	5.11***	0.84	6.12***
Hue	0.33	2.86**			0.46	3.91**
Chroma	0.14	1.72	0.13	1.86	0.13	1.76
Body size (PC1)	0.19	1.78				
Ectoparasite load	0.12	1.24	0.21	2.14**	0.14	1.97
Body condition			0.29	2.37**	0.19	1.82

All measurements were standardized to obtain comparable parameter estimates (β).
**P<0.01; ***P<0.001.

conflicts than males with smaller collars. The increase in male aggressiveness is probably caused by the crucial need to establish and defend display territories. An individual may benefit in part from having higher status because it allows conflict to be settled at lower cost (e.g. energy, injury).

A number of studies have shown that variation in plumage badges indicates an individual's dominance status in nonbreeding flocks, for example, *Zonotrichia leucophrys* (Parsons & Baptista 1980; Fugle et al. 1984), *Parus major* (Järvi & Bakken 1984) and *Zonotrichia querula* (Rohwer 1985). The patch size and/or hue varies considerably in each of these species. Since much of this variability is sex related, colourfulness is thought to affect an individual's dominance status because it is reliably correlated with differences that can directly affect an individual's ability to defend resources (i.e. 'status signal', Rohwer 1982; Senar 1999). Status signalling is likely to be advantageous between strangers in territorial systems when there are sufficiently high rates of intrusion by strangers, since neighbours supposedly have knowledge of each other (Rohwer 1975, 1982; Evans & Hatchwell 1992). In the red-collared widowbird system, intrusions from unfamiliar birds (floaters) are common and 82% of intrusions were by non-neighbours. The status-signalling hypothesis therefore applies to this territorial breeding system.

As large-collared models were more successful in deterring aggressive interactions from resident males, birds with stronger (larger and redder) carotenoid signals appear to defend their territories more effectively. This suggests that there should be strong directional selection for enlarged badge size. What then confines the collar to a small patch on the throat? The most likely explanation in this case is that the male's carotenoid display represents an equilibrium between agonistic and epigamic signalling. A negative correlation between the carotenoid signal (size and colour) and tail length, which is crucial for mate choice (Pryke et al., in press), suggests a developmental trade-off between the two plumage signals (S. Andersson, S. R. Pryke, J. Örnborg & M. J. Lawes, unpublished data).

The perching models received little aggression (or even response), unlike other indirect studies on widowbirds where mounted skins of adult male widowbirds (Savalli 1994) and dummy models (S. Andersson 1993) were used to enhance capture rates. This suggests that red-collared widowbird males either generally tolerate perching or feeding males, or at least need a preceding flight to provoke aggression. The much stronger response to 'flying' models highlights the importance of mimicking movements in model-presenting experiments, rather than relying on weak responses to stationary models which might give erroneous results as to the experimental effect.

The size of the tail had no effect on aggressiveness and was also negatively related to territory area. Tail size thus seems unlikely to be important in dominance, but the presence and display of the tail, rather than its size, may still be an important cue to the intention of an intruding male as a competitor. Our finding, that territory owners will escalate and respond more aggressively to keeled-tail rather than folded-tail flying models, indicates that the owner increases his level of aggression in relation to the perceived intent of the intruder. In such a territorial system, where territories are limited and intruders are unlikely to supplant owners for territories, it may be necessary for floaters to attempt to attract females.

Much attention has been given to how signal reliability is controlled in a natural population (e.g. Rohwer 1982; Maynard Smith 1988; Grafen 1990; Johnstone & Norris 1993). For example, the 'handicap principle' (Zahavi & Zahavi 1997) suggests that signals must be costly, otherwise 'low-quality' individuals could cheat by producing showy signals and consequently destabilize the signalling system. The honesty of melanin-based status badges may be controlled by the costs of displaying the badge, such as social mediation (Rohwer 1982; Senar 1999) and life history trade-offs (Gustafsson et al. 1995; Griffith 2000). However, the expression of carotenoid-based plumage coloration has potential direct honesty-enforcing production costs. As carotenoid pigments cannot be synthesized and have to be acquired from the diet, carotenoid-based trait expression is related to the male's ability to obtain,

physiologically transport, process and deposit carotenoid pigments into the feathers (Goodwin 1984; Brush 1990). At the time of moult into nuptial plumage, the male's foraging ability, nutritional condition, parasite suscepti-bility and immunocompetence could all influence carotenoid expression (Lozano 1994; Olson & Owens 1998; Hill 1999). Carotenoid coloration might be a good indicator of fighting ability since it contains information about several aspects of male vigour (Dufva & Allander 1995; Endler & Houde 1995; Bortolotti et al. 1996; Evans & Norris 1996; Godin & Dugatkin 1996). If there is interference competition over the food source from which the pigments are derived (e.g. carotenoid-rich invertebrates), carotenoid coloration can be directly related to competitive ability. We do not know whether such feeding competition exists to the extent that it imposes a limitation on access to carotenoid pigments, but during communal flock feeding, birds compete aggressively over suddenly available invertebrates (e.g. termites, flying ants and butterflies). In addition, the variation in carotenoid-based pigmentation is influenced by environmental stresses, such as parasite and pathogen infection (Olson & Owens 1998). Carotenoids are also important in immune system functioning (Lozano 1994), such that carotenoid-based pigmentation may express the allocation of carotenoids between nuptial plumage and immunocompetence. Red carotenoid-based nuptial plumage may thus indicate a male's vigour, such as superior health, ability to forage for scarce carotenoid-rich resources and resistance to parasites and pathogens.

In conclusion, the carotenoid collars of male red-collared widowbirds are agonistic signals of their ability to defend a territory. Competitively dominant males are thought to benefit from their higher status, either in agonistic interactions with other males, or in the rela-tively less effort expended in territorial defence and maintenance. Thus males with a greater carotenoid signal obtain an advantage over males with a relatively lower carotenoid expression. The importance of carotenoid-based coloration may be more common in agonistic signalling than previously thought. Many studies on carotenoid-based ornaments have concentrated on mate choice, while largely ignoring sexual selection through male contest competition. Furthermore, many earlier studies have been restricted to presence/absence effects or the size of colour patches (e.g. red-winged blackbirds, Hansen & Rohwer 1986; Eckert & Weatherhead 1987; Metz & Weatherhead 1992). Natural and experimental colour variation based on objective reflectance measure-ments may provide better resolution of intra- as well as intersexual signal functions of carotenoid displays.

Acknowledgments

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