Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color

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Carotenoid-based plumage ornaments are typically considered to be sexually selected traits, functioning as honest condition-dependent signals of phenotypic quality, but few studies have addressed the function of carotenoid color variation in male contest competition. Using two experiments, we investigated the status signaling function of the variable (ranging from yellow to red) carotenoid throat patch (collar) in the polygynous, sexually dimorphic red-collared widowbird (*Euplectes ardens*). First, we tested if the red collar functions as a dominance signal by painting spectrometrically controlled collar patches onto the brown plumage of nonbreeding males and staging dyadic male contests over food resources. Red-collared males dominated orange males, which in turn dominated the control brown and novel blue collars. Red dominance persisted when the collar manipulations were reversed within dyads and also when tested against testosterone implanted males. In the second experiment the collar size and color of breeding males were manipulated in the field before and after territories were established. All males with enlarged red and most with enlarged orange or reduced red collars obtained territories, whereas most males with reduced orange and all with blackened (removed) collars failed to establish or retain territories. In addition, among the territorial males, those with reduced signals defended smaller territories, received more intrusions, and spent more time in aggressive interactions. Redness and, to a lesser extent, size of the carotenoid ornament both seem to independently indicate male dominance status or fighting ability in male contest competition. *Key words:* carotenoids, dominance, *Euplectes ardens,* male–male competition, plumage coloration, status signaling, widowbirds. *[Behav Ecol 13:622–631 (2002)]*

O ne of the most conspicuous ornamental traits in avian sexual dichromatism is carotenoid-based plumage coloration (i.e., red, orange, and yellow; Brush, 1978; Fox and Vevers, 1960). In addition, carotenoid pigmentation (either the color intensity or the extent of the colored area) has been demonstrated to correlate with some aspect of individual quality, such as the diet, immunocompetence, parasite resistance, and general health and vigor (Dufva and Allander, 1995; Hill, 1999; Hill and Montgomerie, 1994; Johnson et al., 1993; Linville et al., 1998; Sundberg and Larsson, 1994). In a number of bird studies, carotenoid pigmentation has been more or less conclusively shown to be a cue for female choice of males (reviewed in Hill, 1999). However, a relatively neglected possibility in recent studies of carotenoid signaling is that carotenoid ornaments are assessed in intrasexual agonistic conflicts. Although some studies suggest a primary function of carotenoid coloration in male competition—for example, in the northern cardinal *Cardinalis cardinalis* (Wolfenbarger, 1999) and redbilled quelea *Quelea quelea* (Dale, 2000; Shawcross, 1983)—the few experimental tests demonstrate no or even negative effects on male dominance (Belthoff et al., 1994; Brown and Brown, 1988; McGraw and Hill, 2000; Wolfenbarger, 1999).

In most avian mating systems, males compete for resources that are necessary for attracting females, such as territories. Sexual selection through male contest competition can thus be severe and may, like female choice, drive the evolution of conspicuous male plumage signals (Andersson, 1994). Many avian studies indicate that selection for male competitive ability may favor the evolution of conspicuous signals or "badges of dominance" in male plumage, especially in populations with frequent interactions (Guilford and Dawkins, 1995; Rohwer, 1975; Studd and Robertson, 1985). Such signals may help individuals to assess one another's competitive ability and avoid costly escalated interactions when establishing dominance relationships (Maynard Smith, 1988; Rohwer, 1982). However, most studies evaluating avian plumage variation in dominance interactions have focused on melanin-based coloration, where melanin-pigmented ornaments function as reliable indicators of social rank during the nonbreeding season (reviewed in Senar, 1999).

In this study we investigated whether the carotenoid throat patch (collar) displayed by male red-collared widowbirds (*Euplectes ardens*) determines the outcome of male contests. During the nonbreeding season these African weaverbirds (subfamily Ploceinae) are sexually monochromatic and drab brown. Before breeding, however, males molt into an ornate black nuptial plumage with long tail and a crescent-shaped red carotenoid collar (based mainly on lutein, zeaxanthin, and canthaxanthin; unpublished data). With fully developed collars but still growing tails, males compete fiercely to obtain and defend large breeding territories in grasslands, excluding a large number of males that remain in the area as floaters. Resident males signal their territories by perching prominently with head raised while ruffling their body feathers (especially the collar). This threat display is escalated to aggressive chases and physical combat when necessary (Pryke et al., 2001b). The collar is variable in both size and color, and descriptive results have shown that territorial males have larger and redder collars than floaters (Andersson et al., in press). Previous studies have also indicated an agonistic function of the collar by showing that the collar signal level (large/red

vs. small/orange) of model intruders affects the aggressive response of residents (Pryke et al., 2001b). The collar does not contribute to male attraction of breeding females, which instead is strongly determined by the other prominent visual ornament, the long tail (Pryke et al., 2001a). Yet to be demonstrated, however, is a direct effect of the collar signal in settling male dominance interactions and success in competition over territories. Moreover, in relation to carotenoid allocation as a potential honesty-maintaining cost, it is essential to identify an effect of color per se (redness) independent of patch size because the latter is only indirectly related to carotenoid investment.

To test the status signaling functions of the different visual aspects of the collar and to confirm the relevance of these signal functions in male contests, we conducted two manipulation experiments. First, in standardized dominance trials in captivity, we experimentally painted collars onto the brown plumage of captive, nonbreeding males and conducted paired trials that pitted two unfamiliar birds with different collar treatments against each other in competition over food. Because male aggression and threat signals are often regulated by elevated testosterone levels (Wingfield et al., 1990), we also tested the effect of artificially elevated testosterone levels (via subcutaneous implants) on aggression and success in contests with collar-manipulated males. Second, in a field experiment, we manipulated the collar color and size of male red-collared widowbirds upon their arrival to the breeding grounds (when male contest competition is probably the most intense) and after territory establishment to determine the effect of the collar signal on the competitive ability of males in acquiring and defending territories. Because territory acquisition and maintenance is limiting and presumably critical for male mating success, any signal trait that increases the efficiency (or decreases the cost) of the frequent contests will clearly be favored by sexual selection. Here we show that this is the likely context in which the carotenoid-based status signal of red-collared widowbirds has evolved.

METHODS

Dominance experiments

Experiments on captive birds were performed during September and October 2000 at the University of Natal, Pietermaritzburg, South Africa. Birds used in these experiments were captured in mist nets from March to April 2000 at three localities in KwaZulu-Natal. At this time of the year individuals have completed breeding and begin to molt into their brown eclipse plumage, and they are easy to accurately age as adult breeding males. Birds were housed in three outside aviaries (1.5 m wide × 2.7 m long × 2.2 m high) visually isolated from each other over the winter months, so that individuals had 6 months to habituate to captivity before the experiments began. The potential for dominant males to control food resources was minimized by placing as many food dishes as birds within each cage.

Before experiments began, we took standard morphometrics of wing chord length (to the nearest 0.5 mm), culmen, tarsus, tail length (all to the nearest 0.1 mm), and body mass (to the nearest 0.5 g). From the three measures of body size (culmen, tarsus, and wing), we used a principal components analysis (PCA) to calculate an index of body size. The first principal component (PC1) accounted for 73.3% of the variation in the body size measurements of the captive birds, and PC1 was used as an index of body size. The experimental collar size (chest patch) was calculated (to the nearest 0.1 mm^2) as the product of the maximum width of the collar across the chest, and the average of three height measures when holding
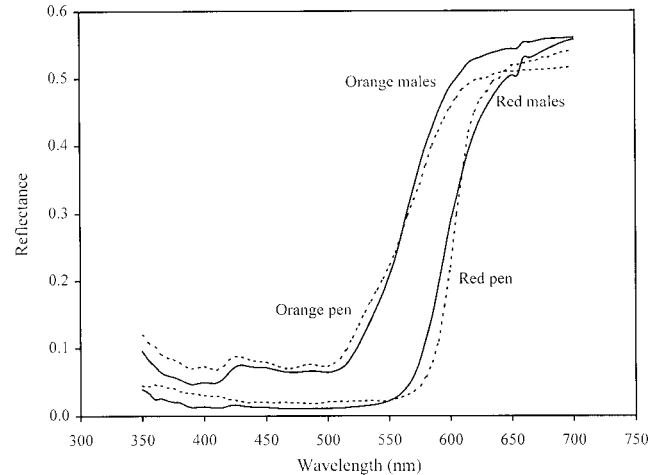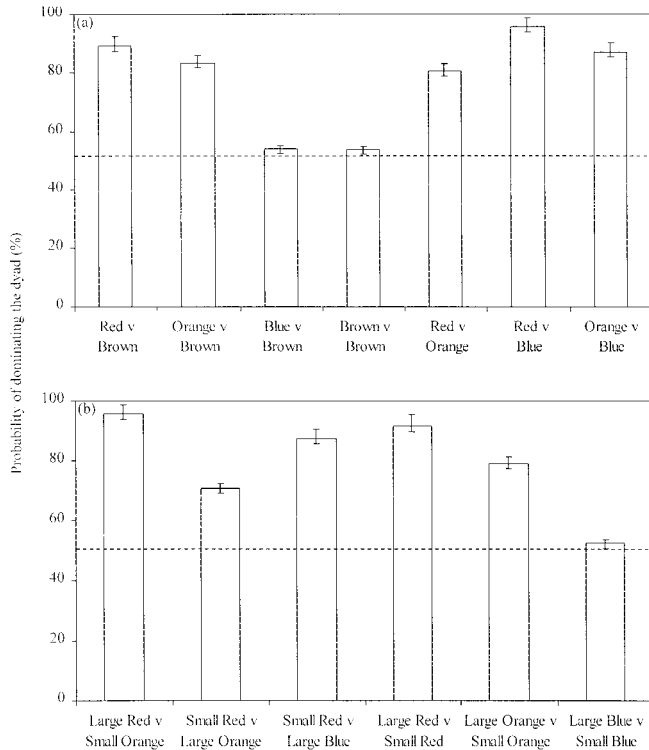

Figure 1
Reflectance spectra for the natural breeding collars and the manipulated plumage of brown, nonbreeding male red-collared widowbirds. Reflectance for the natural collars (solid line) show the most extreme red-collared male and the average (orange) collared males in the population ($n = 125$). The manipulated collars (dashed line) were painted with Copic orange and red pens selected to correspond closely to the natural red and orange collars of the birds.

the head in a normal position. Repeatabilities (Lessells and Boag, 1987) of the collar measurements were highly significant ($r = .88$, $F_{63,46} = 18.7$, $p < .001$).

In the experiments that follow, we tested if the collar color, size, or both were sufficient to establish dominance. We manipulated male birds before their nuptial molt to avoid confounding effects of other aspects of the breeding plumage and reproductive condition and to standardize the manipulation effect (which is more problematic when changing an existing color patch). Furthermore, nonbreeding males randomly given experimental collars, compared to nuptial males, are less likely to perceive and behave according to their own signal, which means that opponent responses and interaction outcomes are more directly affected by the signals than by associated behaviors. Copic pens (Too Marker Products, Tokyo) were used for painting the collars onto the brown throats of nonbreeding males. The orange (YR09) and red (R29) pens provided the best match to the natural variations in plumage reflectance (Figure 1). We painted control birds with a similar but transparent pen (code 0, colorless). To control for the novelty of adding a collar to the brown plumage of nonbreeding males, as well as a potentially independent effect of collar presence regardless of its color, we also included a blue (pen B29) collar in the experiments. Manipulated collars were painted onto the lower throat of males corresponding to the average size of red-collared widowbird collars (mean ± SD = 210.1 mm^2 ± 59.4, $n = 125$; collar treatments 208.3 mm^2 ± 4.7, $n = 192$), whereas small and large collars were painted to correspond to the lower (82 mm, $n = 125$; collar treatments 85.3 mm^2 ± 3.2, $n = 144$) and upper limit (328 mm, $n = 125$; collar treatments 321.7 mm^2 ± 3.6, $n = 144$), respectively, of the natural variation of collar size (Pryke et al., 2001b). Once the experiment ended, we removed the manipulated collars from the experimental bird (with alcohol) to prevent familiarization with the manipulations and returned all birds to their aviaries to maintain a standardized housing setting for all males.

We tested 13 different combinations of collar treatments (see Figure 2) with 12 different individual dyads within each combination (i.e., a total of 156 trials using 24 birds). In each

Figure 2
Within a dyad, the vertical axis gives the probability (%) of the male with the collar treatment mentioned first (on the bottom axis) dominating the male with the collar treatment mentioned second. Probabilities are calculated from the coefficients of the best-fit generalized linear model (GLM); Probability = exp(coefficient)/1 + [exp(coefficient)] for (a) different-colored but similar-sized collar treatments, and (b) different-sized and different-colored collar treatments (see Methods). The error bars are the 95% confidence intervals, calculated from the SE of the coefficients [upper CI = coefficient + (2 × SE); lower CI = coefficient − (2 × SE)]. The dashed lines indicate where the two males have an equal chance (50%) of dominating in the dyad.

trial a male from one cage was tested against a randomly chosen, unfamiliar male from another cage (i.e., excluding previously used dyads). This means that a given bird was used in all 13 experiments, but never more than once against the same opponent. In each trial we chose the member of a pair to be manipulated at random, and to further remove confounding individual differences, we later repeated each trial with the collar treatments reversed between the pair.

The evening before a trial, all food was removed to standardize and maximize the motivation of individuals to compete for food resources during trials (Andersson and Åhlund, 1991; Lemel and Wallin, 1993; McGraw and Hill, 2000). We conducted dominance trials in the first four hours after sunrise. The two males were transferred to an unfamiliar experimental cage (0.8 m wide × 2.1 m long × 0.9 m high), visually isolated from all other cages and containing perches on either side of a central feeder with visible food, but constructed so that only one bird could feed at a time. In addition, dropped food passed through the wire floor of the suspended test cage so that food remained available only at the feeder.

The birds were released simultaneously into the experimental cage, and all dyads began feeding within 4 min. Trials were run for 15 min, during which we recorded all interactions. The bird dominating the food bowl, the first bird to feed, and the number of supplants were extracted for analysis. The winner within a dyad was scored as the first bird to feed or the

bird dominating the food dish more than 75% of the time (these measures coincided in 94% of the cases; see Results). We scored supplants or the displacement of an individual from the food dish as active or passive. In active supplants the winning bird aggressively displaced the other, and in passive supplants the losing bird merely moved away at the passive approach of the winner.

Testosterone treatments
We implanted 24 males with 8-mm implants made from silastic tubing (Dow Corning; internal diam 1.47 mm, external diam 1.96 mm) sealed at each end with silastic glue. Birds were randomly chosen to receive either the testosterone treatment (Sigma Chemical T1500) or the control treatments (empty tubes). The birds were anesthetized by inhalation of isofluorane in oxygen (induction 2%, maintenance 1.5%, flow rate 0.3 ml/min). We made a small incision in the skin of the bird's abdominal cavity and inserted the implant to lie along the flank before closing the skin with a surgical suture (Mersilk, Ethicon). Birds awoke within 3 min and were placed into small recovery cages before being released into their housing aviaries. Testosterone-implanted males (with a control collar) were trialed against males with red, orange, blue, and control brown collars. These four experiments were repeated 12 times with randomly chosen males in each trial. Although there are no data for the natural or elevated testosterone levels, the aim of the implantations was to increase testosterone levels beyond the natural levels to determine whether the higher aggression of artificially implanted males could override the collar signal.

Territory experiments

The field study was conducted at Hilton, KwaZulu-Natal, South Africa (29°43′ S, 30°17′ E; elevation approximately 1140 m). We captured males using mist nets at communal roosts between 16 November and 3 December 2000 as they began to establish breeding territories in the area. Captured males were measured and color banded. We measured aspects of body and collar size as estimates of male competitive ability (see above). The first axis of the PCA for body size (as detailed above) among free-ranging birds accounted for 77.2% of the variation in the three body size measures.

We experimentally manipulated collars to test if the size or the color of the collar is the focus of male competition. Males were alternately assigned a treatment as they were captured so that a collar treatment was randomly assigned with respect to all other attributes. We manipulated collars by bleaching the feathers surrounding the collar and repainting the collar in a different size or color. Feathers were bleached by massaging 85% hydrogen peroxide into the feathers until most of the black feather pigment was removed. The feathers were then rinsed with warm water and dried. To paint the collar signal, we applied orange or red Copic pens to all the feathers starting close to the skin and working to the surface, making sure that each feather was marked completely. The color treatment was applied to the bleached feathers and over the original collar (Table 1).

Males were assigned to one of six experimental collar groups (Table 1): (1) *Reduced orange collar group* ($n = 14$). The bleached feathers around the collar and the collar area were blackened (Copic black 100) to reduce the size of the collar to about 82 mm². The size of the collar was at the lower limit of the natural variation of collar sizes in the population (see above). The collar was also colored orange to conceal the natural color. (2) *Reduced red collar group* ($n = 14$). The collar was similarly reduced but the reduced collar was colored red. (3) *Enlarged orange collar group* ($n = 14$). The collar was enlarged to about 335 mm² by painting the bleached

Table 1

Mean (± SD) male collar size and color (hue) for the field experimental collar treatments before and after the manipulations, for males both manipulated at the beginning of the breeding season (before they established territories) and for those defending already established territories

Collar treatment	Size (mm²)		Hue (nm)		
	Before	After	Before	After	n
Males establishing territories					
Reduced orange collars	218.6 (±60.3)	81.4 (±3.4)	561.8 (±29.5)	568.5 (±2.7)	14
Reduced red collars	211.9 (±81.2)	82.9 (±4.8)	569.4 (±37.2)	591.5 (±3.1)	14
Enlarged orange collars	229.3 (±72.7)	339.8 (±3.7)	558.7 (±35.2)	571.2 (±2.9)	14
Enlarged red collars	216.1 (±68.2)	333.4 (±5.8)	553.9 (±41.3)	596.2 (±2.2)	14
Blackened collars	223.7 (±82.5)	0	563.5 (±32.1)	0	15
Control collars	203.4 (±78.3)	203.4 (±78.3)	569.9 (±24.8)	569.9 (±24.8)	14
Males on established territories					
Orange collars	224.1 (±60.3)	210.5 (±3.7)	571.3 (±42.1)	570.4 (±3.1)	12
Red collars	218.6 (±85.2)	208.3 (±4.5)	568.2 (±21.5)	597.3 (±2.5)	12
Control collars	216.4 (±71.5)	216.4 (±71.5)	567.8 (±36.1)	567.8 (±36.1)	11

feathers orange. This collar area corresponded to the upper limit of the natural variation of collar area in the population (see above). (4) *Enlarged red collar group* ($n = 14$). The bleached feathers were colored red to increase the collar size. (5) *Blackened collar group* ($n = 15$). The collars in this group were completely blackened. Although phenotypic manipulations that exceed the range of natural variation can potentially introduce problems (e.g., an abnormal stimulus or affect species recognition; Grether, 1996), we wanted to test whether the presence of the collar was essential to territory establishment. (6) *Control collar group* ($n = 14$). The collar was bleached and the whitened feathers were blackened to the original size of the collar. In addition, the transparent Copic pen was painted onto the collar to determine if the painting procedure, without any color or size change, affected territory acquisition and defense. This control treatment did not produce any visible change in the appearance of the males. The collar sizes of the males before manipulation in the six treatment groups were not significantly different (Table 1; ANOVA: $F_{5,87} = 2.26$, $p = .52$).

We mapped territories using the minimum convex polygon method (Odum and Kuenzler, 1955) about 6 days after manipulation. We estimated territory boundaries by mapping the perches of displaying owners and locations of aggressive interactions between territorial males. The territory maps were digitized, and ArcView Ver. 3.1 (ESRI, 1996) was used to estimate territory area. Over 6 weeks, we observed each experimental and control male weekly for a 15-min period (i.e., a total of 90 min per male) during the first 4 h of daylight (when activity is the highest) to quantify the frequency and duration of aggressive interactions with other males.

Manipulation of males on established territories
We also manipulated collars of males after territory establishment to test whether a signal change at this stage would affect male ability to retain and defend his territory. The territory areas of returning territorial males that were captured in the previous season (December 1999 to April 2000) were mapped once the males had reestablished territories in the area (from 11 to 28 December 2000). During this time, we recorded behavioral observations for 15-min periods daily to determine the effort and time spent in territorial activities. These males were then recaptured and sequentially assigned to one of three treatment groups (i.e., randomly assigned to a collar treatment with respect to their location in the grassland; see Table 1): (1) *control collar group* ($n = 11$), in which the feath-

ers were bleached and then repainted with black and clear pens to their original size and color; (2) *orange collar group* ($n = 12$), in which the feathers were bleached before average-sized orange collars of about 210 mm² were painted onto the males; and (3) *red collar group* ($n = 12$), in which the feathers were bleached before average-sized red collars were painted onto the males.

About 6 days after the manipulation, we remapped the territories and repeated the behavioral observations. Treatments were randomized between all males, so each male acted as its own control. Because the males do not participate in any breeding activity (such as incubation and feeding nestlings) and because the birds had not yet begun breeding, no changes in territorial behaviors were expected from one breeding activity to another between the two observational times.

Reflectance spectrometry and colorimetrics

We measured spectral reflectance (at ±2 nm resolution) from the experimentally manipulated and natural collars using a PS1000 miniature diode-array spectrometer (Ocean Optics), HL2000 halogen light source, and a fiber-optic reflectance probe from a 4-mm wide measuring spot. The probe was held at 90° to the plumage, and five consecutive scans (removing the probe between each scan) were taken from the center of the collar patch and averaged for each individual. We measured reflectance using the C-spec software (Ancal, Las Vegas, Nevada, USA) and in relation to a WS-2 white standard (>98% reflectance across the measuring range) that was scanned before each individual was measured.

Only the color signal indices for redness or hue was computed for these experiments from the raw spectral reflectance data, which was then averaged for each individual. As a measure of spectral location or hue of the reflectance, we computed λR_{50}, which is the wavelength at which reflectance is halfway between its minimum (R_{min}) and its maximum (R_{max}). Additional details on the methods used to collect and analyze reflectance are described in Pryke et al. (2001a).

Statistical analyses

Dominance experiments
To avoid type I errors associated with multiple pairwise comparisons, we developed a suite of explanatory models for the dominance experiments. In every dyad each bird was scored as either winning or losing (see Methods), coded as 1 and 0,

respectively. These binary outcomes were modeled as the Bernoulli dependent variables in a generalized linear model (GLM) with a logit link function. All modeling was performed with GENSTAT 5.1 (GENSTAT 5 Committee, 1987) using the MODEL and FIT directives to fit the generalized linear models. All possible effects and combinations were included in the models. To objectively select the most parsimonious model, we used Akaike's Information Criterion (AIC), which balances the fit of the model against the number of parameters used in the model (Anderson and Burnham, 2001). The model with the lowest AIC value (and a difference of at least two AIC units from the other models) is accepted as the model best fitting the data.

Territory experiments
To prevent inflating comparison-wise errors and avoid type I errors induced in repeatedly testing the same hypothesis, we used a multiple analysis of variance (MANOVA) to test the effects of the collar treatments on territorial and behavioral activities. The test statistic Wilks's λ (likelihood ratio criterion) tests the overall significance of the effects of the treatments on the dependent variables. If this test is significant, the univariate F tests are consulted to ascertain which of the factors causes the overall significant effects. When the univariate F tests indicated significance, we used Scheffè's multiple comparison tests to determine which treatments contributed to the significance. Only significant interaction terms ($p < .05$) were included in the models. No transformations were required because the MANOVA residuals were roughly normal with homoscedastic variances.

To maintain the pairwise design in the analysis of the collar manipulations of males on established territories, the changes in territory size and behavioral activities were compared by assigning a zero, positive, or negative effect of the collar treatments for each male (resulting from a change from before and after treatment), tested within each treatment group using Wilcoxon's signed-rank tests.

RESULTS

Dominance experiments

Effect of collar manipulation on male dominance
The GLM model of the binary (loss/win) response variable that best fit the combined data (13 treatment combinations, 156 trials), showed a strong effect of collar manipulation (AIC = 283.5 and a weight of 88% compared to the other models; $\chi^2 = 86.12$, df = 311, $p < .001$). This statistical model identifies three control groups, brown versus brown, blue versus brown, and large blue versus small blue collars, suggesting that individuals in these dyads had equal probabilities of dominating the other (i.e., a random outcome). Red color had the strongest effect on dominance; red collars dominated all other treatments, regardless of size (Figure 2). However, there was also an effect of collar size because, within color treatments (i.e., red and orange), large collars dominated small ones (Figure 2b). Consistent with a stronger effect of redness than of badge size, orange collars dominated the controls, and large orange dominated small orange to a lesser extent than large red dominated small red. Collar redness thus had the strongest effect on the outcome of contests. None of the possible interacting effects such as body size (PC1), body mass, capture locality, housing cage or trial sequence (i.e., the initial or reversed collar treatment of the pair) had any effect on the outcome, except for one bird that was subordinate in all contests. Therefore, regardless of second-order effects, red-collared males were more likely to win contests.

The collar treatments also affected the type of interaction,
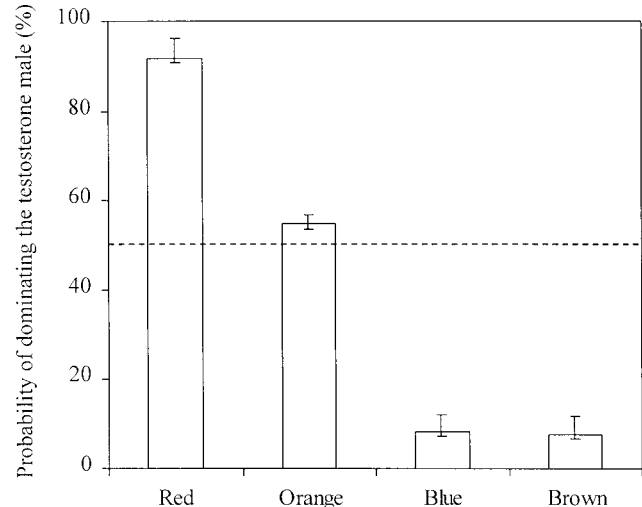
Figure 3
The probability (%) of the color treated males dominating the testosterone implanted brown males in the dyad. Probabilities are calculated from the GLM model best fitting the data (see Methods and Figure 2) with error bars for the 95% confidence intervals of the coefficient. The dashed lines indicate where the two males have an equal chance (50%) of dominating in the dyad.

active or passive, but due to the low sample sizes in many experiments (< 7) they could not be modeled with GLM. However, most supplants ($> 83\%$) among the three control groups were active, with males often pecking and fighting at the food bowl. In contrast, only 3 and 9 active supplants (1.6% and 4.8%) occurred in trials involving red-collared or orange-collared males, respectively. In 87% of the cases, if the red-collared bird left the food dish, it would passively (rather than actively) supplant the other bird feeding when returning to feed. Red-collared males were thus challenged less and settled contests without escalating conflict more than the control birds.

The male dominating the feed bowl was also generally the first bird to feed in a dyad (94% of the experiments). The most parsimonious GLM model, using the order of feeding as the binary response variable, identified the same model as described above (AIC = 281.7 and a weight of 91% compared to the other models; $\chi^2 = 84.31$, df = 311, $p < .001$). This shows that males with experimental red collars were the first to feed, dominated the feed bowl, and were rarely challenged by the other bird.

Effect of collar manipulation and testosterone-implanted males
The GLM model best explaining the outcome of trials between each of the four experimental collar colors and testosterone-implanted brown males showed that red-collared males remained dominant (AIC = 67.46 with a weight of 81% compared to the other models; $\chi^2 = 81.2$, df = 47, $p < .001$). Orange-collared and testosterone-implanted brown males had a similar probability of dominating in a dyad, whereas the testosterone-implanted males strongly dominated the brown- and blue-collared males (Figure 3). Few supplants were recorded during these experiments; the red-collared males tended to dominate the food bowl without being challenged, and in other experiments after an initial (usually active) supplant, the testosterone-implanted male was so aggressive at the food bowl that the other male rarely approached him.

Table 2

MANOVA to determine the effect of the collar treatments on territorial behaviors

Territorial behaviors	F	df	p
Males establishing territories			
Territory size	2493.3	24,116	<.001
Time spent on the territory	3.5	24,116	.45
Time spent signaling boundaries	4.2	24,116	.21
Intrusion rate	2418.7	24,116	<.001
Encounter rate	2437.6	24,116	<.001
Encounter duration	1934.1	24,116	.005
Males on established territories			
Territory size	3209.4	18,79	<.001
Time spent on the territory	6.2	18,79	.33
Time spent signaling boundaries	2.9	18,79	.61
Intrusion rate	3109.8	18,79	<.001
Encounter rate	2917.5	18,79	<.001
Encounter duration	2491.7	18,79	.002

Effects of collar treatments on males manipulated before establishing territories (Wilks's λ = 0.41, $F_{24,116}$ = 2519.3, p < .001) and males manipulated after territorial establishment (Wilks's λ = 0.56, $F_{18,79}$ = 3317.1, p < .001). For males establishing territories, the effects of all the collar treatments were tested (i.e., enlarged red, reduced red, enlarged orange and control collar treatments), except for the blackened and reduced orange-collared group due to the small number of males establishing territories (n = 0 and n = 3, respectively; see Figure 4). The effects of all the collar treatments were tested for males on established territories (i.e., red, orange, and control collar treatments). See text for details on which treatments contributed to the significant F tests.

Territory experiments

Effect of collar manipulations on territory establishment

After the collar manipulations, 8 of the 14 control males acquired territories, which is about the expected number from our earlier studies of the natural variation. Against this frequency we tested the success of the other treatment groups in establishing territories. None of the 15 males with removed (blackened) collars established territories (Fisher's Exact test, p = .01), and, although not significant when tested against the control group, only 3 of the 14 reduced orange-collared males (p = .29), all 14 males with enlarged red collars (p = .40), 9 of the 14 with enlarged orange collars (p = .99), and 11 of the 14 with reduced red collars (p = .77) acquired territories. However, the number of black-collared males establishing territories was significantly different from the enlarged red (p < .001), enlarged orange (p = .006), and reduced red (p = .003) collared males, and significantly more males with enlarged red collars acquired territories than reduced orange-collared males (p = .05). The observed differences between treatments did not seem to result from differential predation because one enlarged orange, four reduced orange, and six of the blackened males that did not obtain territories were subsequently recaptured at communal night roosts in the area.

The collar-treated males also differed significantly in territorial and behavioral activities (MANOVA: Wilks's λ = 0.41, $F_{24,116}$ = 2519.3, p < .001; Table 2). Males with enlarged red collars defended significantly larger territories (Scheffè test, p < .001) than reduced red-collared males, which in turn held larger territories than the other manipulated males (Scheffè test, p < .005; Figure 4a).

We also tested whether the loss of territories and the alteration in their size could be due to the indirect effects of the treatments on a male's behavior. No significant effects were found for the active time spent on a territory or for the time signaling boundaries. However, the rate of intrusions by other males onto the territory (intrusions per minute) were highest in the reduced orange treatment group, while the enlarged red-collared and reduced red-collared males received significantly fewer male intrusions compared to all other groups (Scheffè test, p < .001 for both; Figure 4b).

The collar manipulations also significantly affected the number of aggressive interactions but not the duration of each of these encounters (Table 2). Enlarged red-collared males were involved in significantly fewer aggressive encounters than the other collar-manipulated groups (Scheffè test, p < .001; Figure 4c) and showed a decrease in interaction duration (Scheffè test, p < .01; Figure 4d). Therefore, males manipulated with large red collars established larger territories but spent proportionately less time in territorial defense.

### Effect of collar manipulations on males holding territories

After the second round of collar manipulations on males that had already established territories, 5 of the 12 orange-collared males but only 1 of the 12 red males lost their territories within 6 days and were replaced by a new male (n = 5) or by neighbors that expanded their territories (n = 1).

Among the males that retained their territories, the sizes of the defended territory area were affected by the collar manipulations (Figure 5): Red-painted males expanded their territories (Wilcoxon's signed-ranks test, two-tailed z = 2.03, n = 11, p < .001), whereas orange-collared males lost territory area after the treatment (z = 2.66, n = 7, p < .001). The control treatment had no affect on territory size (z = 0.19, n = 11), although two control males increased the size of their territories, in both cases at the expense of neighboring orange treated males, which consequently lost territory area.

Differences in territorial behaviors between the treatments, similar to those observed in males manipulated before territory establishment, suggest how the changes in territory size occurred. The three collar treatment groups differed significantly in territorial behaviors (MANOVA: Wilks's λ = 0.26, $F_{18,79}$ = 3317.1, p < .001; Table 2). Although there were no differences in the amount of time spent on the territory or in boundary signaling, there were significant differences in the number of aggressive interactions (Table 2). This was mainly due to the red group receiving fewer intrusions than the other males (Scheffè test, p < .001) and spending less time in aggressive encounters compared to the orange-collared males (Scheffè test, p < .001) but not compared to the control group (Scheffè test, p = .15).

Changes in territorial activities before and after the collar manipulations show that males responded behaviorally to the treatments. Although there were no significant changes in time spent on the territory in any of the groups (Wilcoxon's signed-ranks, two-tailed, control z = 1.42, n = 11, orange z = 0.92, n = 7, red z = 1.86, n = 11), the red-collared group spent less time in boundary signaling (control z = 0.76, n = 11, orange z = 1.53, n = 7, red z = 2.97, n = 11, p < .05), were involved in fewer aggressive interactions (z = 3.11, n = 11, p < .001), and spent less time in each encounter (z = 2.98, n = 11, p < .05) compared to before the manipulations. Conversely, orange-treated males became more involved in aggressive interactions after the treatment (number of encounters, z = 2.76, n = 7, p < .001; encounter duration, z = 1.97, n = 7, p = .09), but there were no such changes detected in the control group (number of encounters z = 1.22, n = 11; encounter duration, z = 0.85, n = 11).

In conclusion, red-manipulated males expanded their territories, received fewer aggressive interactions, and spent less time in these interactions. In contrast, males with orange col-
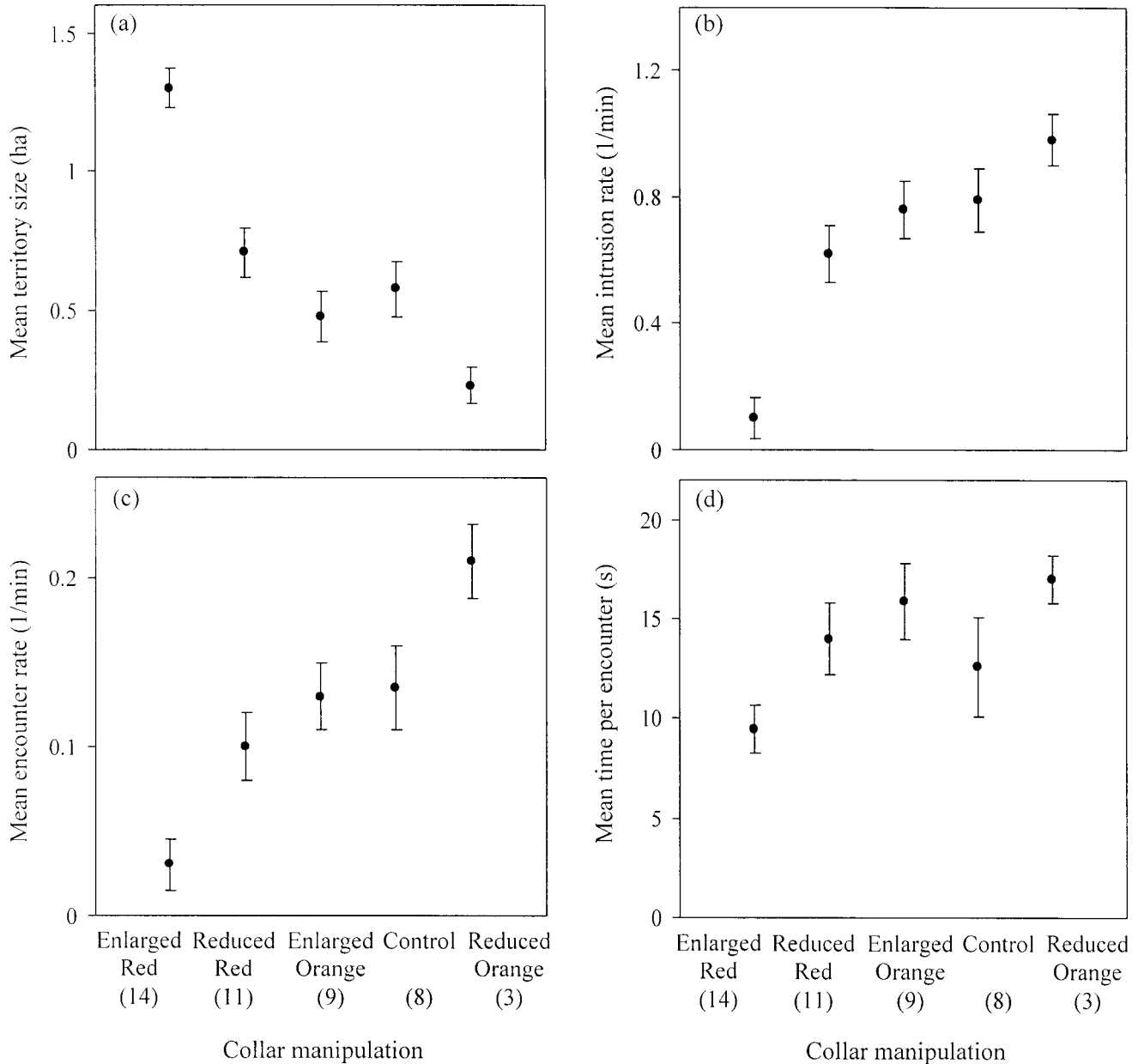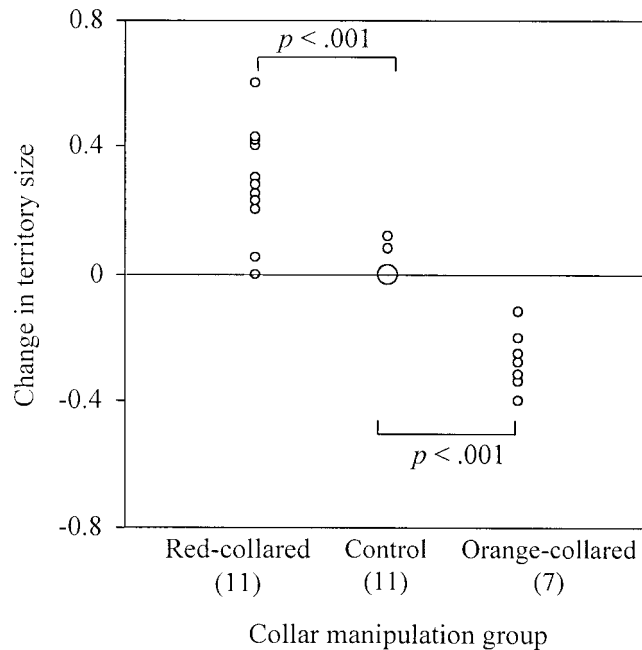
Figure 4
The significant effects of the collar manipulations (see Results and Table 2 for significance tests) on the mean (± SD) (a) territory size, (b) male intrusion rate onto the territory, (c) number of aggressive interactions, and (d) time spent in each aggressive interaction for males establishing territories. Sample sizes are provided in parentheses below the collar treatment groups.

lars lost territory area and received more and slightly longer aggressive encounters. Taken together with the other results from manipulations in captivity and before territory establishment, the red carotenoid color signal is fundamental in male contest competition over territories.

DISCUSSION

Our results not only confirm the previously indicated agonistic signal function of the red collar in red-collared widowbirds (Pryke et al., 2001a,b) but, most important, also show that color (redness) per se is a crucial signal component in addition to badge size. Our results provide evidence that it is the concentration rather than the patch size of carotenoid pigmentation that is the primary message of the signal. In par-

ticular, the dominance of red over orange (both closely mimicking the natural reflectance variation; Figure 1) is in line with carotenoid investment as the basis for content-based rather than efficacy-based signal selection (Andersson, 2000). Carotenoid-based coloration has many direct potential production costs such that during molt the bird's foraging ability, current nutritional condition, parasite load, and the allocation between competing physiological functions can all influence carotenoid deposition and intensity (Hill, 1999; Olson and Owens, 1998). Recent chromatography analyses have also shown a strong correlation between reflectance-based redness and the carotenoid-concentration in red-collared widowbird feathers (Andersson et al., unpublished data). The red-collared widowbird thus seems a likely case of reliable signaling of status or fighting ability, with carotenoid investment as the

Figure 5
Changes in the territory size of experimentally manipulated males (i.e., the territory size before collar treatment subtracted from the size of the territory after manipulation). Each point represents one individual and (O) represents nine control males that did not change territory size. (See Results for significance tests.)

honesty-maintaining cost. Why females seem to neglect this likely quality advertisement in their polygynous mate choice and instead focus almost entirely on the long tail is discussed in Pryke et al. (2001a). In short, we speculate that the genetic component of quality might be larger in the tail length variation, while carotenoid pigmentation might be more environmentally dependent (which would deflate its value as a good genes indicator but not as an honest signal of fighting ability and dominance).

In addition, redder males also tend to be more aggressive in, for example, sticklebacks *Gasterosteus aculeatus* (Bakker and Milinski, 1993; McLennan and McPhail, 1989) and cichlids *Cichlasoma meeki* (Evans and Norris, 1996), so there may be some intimidation value to red coloration. This may be true for red-collared widowbirds, where females ignore or even select against the collar signal (Pryke et al., 2001a).

In the dyadic contests, rival males tended to avoid red collars even when they were painted onto the plumage of a nonbreeding male. Because no other differences (random or artifact) were detected among the dyads, the red collar in isolation seems to contribute to dominance. The artificial collars did not affect species recognition in an appreciable way because the unnatural but equally conspicuous blue collars had no effect of dominance, and the males responded to them as strongly as they did to control brown birds. Adding the collar signal to males in nonbreeding plumage (approximately 1 month before the nuptial molt) removed many potential confounding effects of the males' own signals and experiences thereof. Together with reversing the treatments within dyads, we were able to maximally uncouple the effect of the signal from the many behavioral and morphological factors that often complicate studies of dominance interactions (Jackson, 1991; Senar, 1999).

The field experiments clearly demonstrate the function of the collar in male–male competition for territories. Previous studies have shown that territory owners have larger and red-

der collars than floaters (Andersson et al., in press) and that, among the residents, the level of aggression is affected by the signal expression of an artificial intruder as well as of the collar size and redness of the responding resident (Pryke et al., 2001b). Here we show experimentally that reduced collar signals lead to failure in obtaining a territory, as well as maintaining an established territory, again with both redness and badge size as the important signal parameters.

The manipulations also influenced the aggressive interactions among males, in line with previous correlational data (Pryke et al., 2001b). Males with reduced collar signals suffered from higher intrusion rates and prolonged aggressive interactions, whereas males with boosted signals received fewer intrusions and spent less time in defense and aggressive interactions. Although the exact mechanism through which males lost territories or territory area was not observed, the behavioral evidence suggests that males with smaller collar signals lost encounters with intruding males displaying larger collar signals. Indirect evidence supports this, as all territories were replaced with new owners or included into a neighboring territory.

The status-signaling hypothesis was originally proposed to account for plumage variability (related to dominance) in flocking birds during the nonbreeding season (Rohwer, 1975). However, the hypothesis is equally applicable to contest competition over breeding resources (or directly over access to females) during the breeding season. Agonistic sexual selection of avian male plumage ornaments have been demonstrated in several species, such as red-winged blackbirds *Agelaius phoeniceus* (Hansen and Rohwer, 1986; Røskraft and Rohwer, 1987), scarlet-tufted malachite sunbirds *Nectarinia johnstoni* (Evans and Hatchwell, 1992), ring-necked pheasants *Phasianus colchicus* (Mateos and Carranza, 1997), and collared flycatchers *Fidecula albicolis* (Pärt and Qvarström, 1997). Our experiments also demonstrate agonistic sexual selection: Male red-collared widowbirds are able to assess the competitive ability of the manipulated male without any aggression. When redder-collared or larger-collared males fed, the brown males would rarely challenge these birds (males with smaller collar signals). However, brown control (with similar badges) and blue-collared males had more and longer interactions (active supplants), suggesting that neither individual considered himself inferior and did not show submissive or avoidance behaviors to end the conflicts. Similarly, other status signaling studies have shown that birds with similar-sized badges were more likely to fight with each other (e.g., dark-eyed juncos, *Junco hyemalis*: Balph et al., 1979; house sparrows, *Passer domesticus*: Møller, 1987). Although the red-collared males dominated the testosterone-implanted males, brown birds rarely approached these males. The testosterone levels may therefore override the normative response behavior of brown males, breaking down the stable strategy that prevents conflict escalation. The red-collared males' domination over the testosterone-implanted males (with few supplants) also emphasizes the strength and efficiency of the badge/collar signal in resolving potentially costly conflict.

Status signaling in birds has primarily been demonstrated for structural or melanin-based plumage signals (see Senar, 1999), for which honesty-enforcing production costs are not well understood. The honesty of such signals are instead often argued to be socially mediated, with some striking examples of costs in terms of trade-offs between signal investment and reproductive effort (e.g., Griffith, 2000; Gustafsson et al., 1995). Under this scenario, only males with superior condition or fighting ability can bear the cost of carrying an elaborate signal (Rohwer and Rohwer, 1978; Senar, 1999). In contrast, the honesty of carotenoid-based status signals may be

more likely to be enforced by an immediate cost of producing the signal.

In conclusion, the red collars of male red-collared widowbirds are used to signal their relative dominance status and ability to defend a territory. The honesty of the signal is maintained through the various costs involved in producing intense red carotenoid pigmentation. Most studies have focused exclusively on the epigamic function of conspicuous coloration. However, traits that have direct honest-enforcing signal costs, such as carotenoid coloration, may be more important in male contests than previously thought. Further studies with spectrometrically controlled color variation and color manipulations will tell.

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