

**SEXUAL SELECTION OF MULTIPLE ORNAMENTS
IN THE
RED-COLLARED WIDOWBIRD**

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ABSTRACT

Although sexual selection often explains the evolution and maintenance of a single male ornament, it is unclear how the multicomponent nature of most sexual displays evolves. Theoretical models suggest that handicap signalling should converge on a single most informative quality indicator, whereas additional signals are more likely to be arbitrary Fisherian traits, amplifiers, or exploitations of receiver psychology. Despite the predictions that multiple handicap signals are unlikely to be stable, the male nuptial plumage of the highly polygynous (ca. 3, but up to 9 actively nesting females) red-collared widowbird *Euplectes ardens* comprises two classic quality-indicating avian ornaments (handicaps); a long graduated tail (22 cm) and a red carotenoid throat patch (collar). To investigate the evolution and maintenance of these handicaps in the red-collared widowbird, a population was studied in the Hilton district, KwaZulu-Natal, South Africa, where these small (males ca. 20 g) African weaverbirds (Ploceidae) inhabit a grassy valley during the breeding season.

Multivariate selection analyses, used to investigate net, direct and indirect female selection, demonstrated an unusually strong fitness effect of natural tail length (47%) on male reproductive success (the order and total number of nests acquired). There were no other effects of morphology, ectoparasite load, display rate, territory quality, tail asymmetry or collar measures (hue, brightness and size) on reproductive success, suggesting that females base their mate choice exclusively on only one handicap signal, extreme tail elongation.

Although ignored in mate choice, there is strong evidence that the variable carotenoid collar (ranging from yellow to red) functions as a status or agonistic signal in male contest competition for territories. Compared to 'floating' males (that did not establish a territory in the area), resident males had a 60% larger and 23 nm 'redder' (longwave hue) collar. Model presentations also corroborated the status signalling function of the collar as territorial males were less aggressive towards conspecific models with intense collar displays, and males with greater carotenoid investment responded more aggressively to the models. In captive experiments, non-breeding brown males painted with red 'collars' dominated

orange painted, control brown, novel blue collared and testosterone-implanted males in dyadic contests over food resources. In addition, experiments in the field demonstrated that males manipulated with larger and redder collars established and maintained territories in the area, whereas most males with small, orange or blackened collars failed to establish or retain territories. Thus the size and particularly redness of the costly carotenoid collar reliably signals male status and fighting ability in male contests.

The unique negative phenotypic relationship between the expression of tail length and carotenoid pigmentation, suggests strong overlapping developmental costs (and allocation conflicts) between the two handicap ornaments. This trade-off is predicted to be strongest between signals with the same or similar costs. Although current theory predicts that multiple handicaps should be evolutionary unstable, the coexistence of multiple costly ornaments in the red-collared widowbird is stable because of selection by different receivers, females and males (i.e., multiple receivers).

PREFACE

The data described in this dissertation were collected at Mondi Mountain Home Estate in Hilton (from December 1999 to April 2000 and December 2000 to January 2001) and on farms in the Balgowan district (April 2000), KwaZulu-Natal, South Africa. The study forms part of a STINT bilateral exchange programme and was carried out under the supervision of Professor Michael J. Lawes (School of Botany and Zoology, University of Natal, Pietermaritzburg) and Professor Staffan Andersson (Department of Animal Ecology, Göteborg University, Göteborg).

This study represents original work by the author. The dissertation is compiled as a collection of papers (apart from the Introduction; Chapter I) and each chapter is formatted in accordance with the journal it has been published in or submitted to (except that the tables and figures appear in the text and not at the end of each chapter). The chapters will be referred to in the text by their roman numerals (i.e., I to VI).



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CHAPTER I

INTRODUCTION

Sexual ornaments, to attract mates or deter rivals, comprise a large part of the variety of bizarre forms, dazzling colours, and elaborate displays in the animal kingdom. The diversity of sexual and agonistic signalling, from butterfly wings to the peacocks tail and the size dimorphism of elephant seals, have fascinated and inspired biologists for centuries. In particular, the last thirty to forty years has seen a proliferation of diverse theories to account for these extravagant ornaments. Yet our understanding of the evolution of elaborate secondary sexual characters is far from complete.

Sexual Selection Through Female Mate Choice

Since Darwin (1871) postulated his theory of sexual selection that exaggerated male ornaments evolve in response to female mate choice (inter-sexual selection) or male contest competition (intra-sexual selection), the former in particular, has provided a controversial issue with considerable interest from both theorists and empiricists (Kirkpatrick and Ryan 1991; Andersson 1994; Bradbury and Vehrencamp 1998). The debate ranges from whether female mating preferences exist at all, to their underlying genetics and sensory physiology.

Initially, conspicuous ornaments were largely explained by species recognition (e.g. Mayr 1942; Huxley 1938). Beyond what is needed to find a conspecific mate, female preferences for particular males were not considered a legitimate foundation for a truly scientific theory (Dawkins 1986). However, Fisher (1930) affirmed the importance of female choice to theories of behavioural ecology by recognising that female preferences are traits subject to selection and thus requiring evolutionary explanations. Fisher described how a novel genetic male character might become the object of a genetically determined female

mating preference. Females exercising the preference produce more 'attractive' sons because they have paired with males that have a genetically controlled attractive trait. Those males possessing the preferred trait by choosy females will gain a reproductive advantage, as they will be more likely to pair than males lacking the preferred character. Consequently, the female preference and the preferred male trait may coevolve to extremes through joint inheritance ('runaway sexual selection') until the cost of displaying the ornament prevents further exaggeration by balancing its mating advantage.

The initial establishment of the favoured trait and the female preference could potentially be brought about by several different situations. Initially, Fisher suggested that the male trait could be favoured by natural selection, and a female preference for this trait could subsequently evolve. As it undergoes the runaway process, however, the male trait is likely to be exaggerated to the point that it loses its natural selection advantage. An alternative possibility is sensory exploitation (e.g. Ryan 1990; Ryan and Rand 1993). If females have pre-existing sensory biases or hidden preferences arising from sensory recognition mechanisms, males that mimic these stimuli are more likely to attract the attention of females. A third possibility is that females initially evolve preferences for certain male traits because they distinguish conspecific males from closely related species and serve to prevent interspecific hybridisation (Halliday 1978; Lande 1982). Although Fisher initially invoked adaptive female choice (i.e., directed at fit males) to explain how the preference was carried to the critical frequency for the runaway process to take over, the conventional view is that ornaments are 'arbitrary' in the sense that the choosy female gets no other benefit than attractive sons (Majerus 1986; Maynard Smith 1991). The establishment of a female preference is still highly speculative, however, the general process of Fisherian runaway selection has subsequently been confirmed by genetic models (O'Donald 1980; Lande 1981; Kirkpatrick 1982; Pomiankowski et al. 1991), and although difficult to demonstrate, it is widely believed to be a potent evolutionary force (Andersson 1994).

Unlike Fisherian selection processes, the 'good genes' model (developed in the 1960's along with the adaptationists approach to evolutionary biology) argues

that exaggerated ornaments ('handicaps') evolve because they reflect heritable male fitness (quality) that the choosy female might confer to her offspring (Williams 1966; Zahavi 1975; Hamilton and Zuk 1982). They suggest that females should choose the most highly ornamented males as only high-quality males with good genes are able to produce the most extravagant secondary sexual characters (i.e. handicap male survival). Genetic models have confirmed this selection process based on the coevolution of the condition-dependent or indicator trait, intrinsic male quality, and female preference for the trait (e.g. Zahavi 1975; Maynard Smith 1985; Andersson 1986; Iwasa et al. 1991). In recent years, this mechanism has gained theoretical support both as an isolated factor (Andersson 1986; Grafen 1990) and as an influential process in concordance with the Fisherian process (e.g. Andersson 1982a; Pomiankowski 1988; Heywood 1989; Iwasa et al. 1991).

Another indicator mechanism is the direct benefits models where males vary in a nonheritable phenotypic trait that indicates potential direct benefits to the female (e.g., food resources, high quality breeding territories, protection from predators) and/or offspring (e.g., parental investment, control of essential resources). There is good evidence for fitness consequences of the direct benefits model in socially monogamous species with male paternal investment (Heywood 1989; Hoelzer 1989; Price et al. 1993; Andersson 1994), however, it appears less important in polygynous and lekking species, where the females and/or offspring obtain limited or no resources from the males.

These different models of female choice are not mutually exclusive; they may operate simultaneously or in an evolutionary sequence. Empirically, female choice based on male aesthetic traits has been demonstrated, more or less convincingly, in a large number of animal taxa (Andersson 1994; Bradbury and Vehrencamp 1998). However, much is unknown. Few studies have managed to estimate the importance of female choice in relation to the other, less controversial, process of male contest competition (Bradbury and Davies 1987; Andersson 1994) or the relative strength of selection on multiple male ornaments within a species (Bradbury and Vehrencamp 1998).

Sexual Selection Through Male Contest Competition

Another possible scenario for the evolution of secondary sexual ornaments is that they function to mediate male-male competition for access to limited resources, such as females and territories (Darwin 1871; Andersson 1994). Male contests may take many forms from improving male endurance (naturally selected traits such as foraging efficiency), and large body sizes and overt aggression along with the development of elaborate horns, tusks, spurs and other weapons that confer a superior fighting ability to their bearer (Darwin 1871; Andersson 1994), to conspicuous male signals that signal resource ownership (Slagsvold and Lifjeld 1988) or make good fighters recognisable and memorable (Rohwer 1975, 1982). The effective use of sexual traits in aggressive contests is a mechanism that maintains the honesty of traits such as weapons, but there is controversy over how this result is achieved for traits that merely act as a signal of fighting ability or dominance.

Rohwer (1975) proposed that conspicuous plumage signals or 'badges of status' may provide a means by which birds signal their social status to conspecifics. Such badges are likely to evolve when there are frequent interactions with unfamiliar birds and where there are perceivable differences in competitive ability. A high status or dominant individual thus benefits from improved access to limiting resources. Although all individuals display essentially the same badge, it is the variation in extent of the signal that enables status signalling to operate. The major advantage of these signals would be that contestants of unequal status competing for limited resources could settle potential fights without risking accidental injury or wasting energy assessing the relative fighting ability of potential opponents (Rohwer 1982). The empirical evidence generally supports the 'status signalling' hypothesis as a number of studies have demonstrated that plumage badges do settle intraspecific contests (reviewed in Senar 1999).

For status badges to reliably signal male fighting ability and to prevent 'cheats' from invading the signalling system, theoretical models suggest that they require both receiver-dependent (incidental) punishment costs and receiver-independent (necessary) costs of general aggressiveness to be evolutionary stable

(Owens and Hartley 1991; Johnstone and Norris 1993). A number of signal costs have been invoked for handicapping honest status badges, for example, increased predation risk (Fugle and Rothstein, 1987; Butcher and Rohwer, 1988), decreased susceptibility to parasites and pathogens by reducing immunocompetence as a consequence of increased testosterone levels (Zuk et al., 1990; Owens and Hartley, 1991) and decreased survival ability (Møller 1989; Veiga 1995; Griffith 2000). This situation could lead to a form of agonistic indicator or handicap process if a male in good condition benefits from having larger or more conspicuous ornaments than other males. The idea of condition-dependent dominance badges is free from the problems of 'Fisherian' and 'good genes' mechanisms for female choice as male fitness does not need to be heritable.

Although the selection process of male contest competition is relatively uncontroversial (compared to female choice), the generality of male-male competition contributing to the evolution and maintenance of sexual signalling is unknown. Contest competition also requires attention as regards the function of signal traits rather than the evident large body sizes and weapons used in male contests. Furthermore, as male dominance often reflects male phenotype or even genetic quality, male-male competition should often be an important factor in mate choices (Berglund et al. 1996), however, it is unknown whether agonistic signalling hinders or facilitates adaptive female choice. Finally, there is uncertainty about which male traits evolve and are maintained exclusively through male competition.

Sexual Selection of Multiple Ornaments

Although sexual selection often explains the evolution and maintenance of a single male ornament, the importance of multiple ornamentation in animal taxa has only recently been considered. Many sexual displays are examples of multi-component signalling (Rowe, 1999) with primarily polygynous males displaying exaggeration of several distinct signals even within a given modality (e.g., visual, acoustic). Why many species display more than a single ornament is an important

issue with regard to theories about the evolution and maintenance of male ornamentation.

Several recent studies have modelled the conditions that may lead to the evolution of multi-component displays. Pomiankowski and Iwasa (1993) demonstrated that the coexistence of multiple Fisherian traits is evolutionary stable. Other work on the handicap process showed that when traits are honest indicators of quality, females evolve preferences for a single ornament with the highest combination of honesty and detectability ('efficiency' Schluter and Price 1993), although multiple arbitrary Fisherian traits may easily evolve along with it (Iwasa and Pomiankowski 1994, 1995). Møller and Pomiankowski (1993a) outlined three hypotheses to explain why males evolve multiple ornaments. The 'multiple message' hypothesis predicts that various ornaments may convey different information of the overall quality to the receiver (e.g., signal different kinds of condition or reflect condition on different time scales). The 'redundant signal' hypothesis predicts that multiple ornaments more reliably signal overall quality (i.e., each ornament provides a partial representation of overall condition), and results consistent with it could imply that two or more advertisements may function together as a single ornament. The 'unreliable signal' hypothesis predicts that ornaments do not reliably signal male quality but are maintained because they are relatively uncostly, hence female preferences will be weak and may switch from one ornament to another. Recently, Johnstone (1996) developed a model that suggests multiple sexual signals could be evolutionary stable if the evaluation costs to female receivers are negligible and if additional displays serve as 'backup signals' to enhance the accuracy of the assessment, or if they signalled different qualities ('multiple messages').

Basically, these theoretical studies converge on the prediction that most multiple ornaments are not true handicaps but rather arbitrary Fisherian traits, amplifiers of single costly ornaments (Hansson 1989), or exploitations of receiver sensory biases and psychology (Ryan 1990; Ryan and Rand 1993; Rowe 1999). Subsequently, female preferences are thought to evolve for a single, reliable indicator of male quality, with weak or no preference for other ornaments. Furthermore, once a preference for a single indicator trait has evolved, additional

Fisherian traits can be evolutionary stable, but another handicap cannot invade as a single, costly and therefore reliable trait is better than two less-costly and less-reliable traits (Bradbury and Vehrencamp 1998). In accordance with the theoretical studies, empirical work on multi-ornamented species has shown female preference for only one, if any, handicap mechanism among the male traits (reviewed in Andersson 1994; Bradbury and Vehrencamp 1998). As the evolutionary models suggest that most multiple costly ornaments or handicaps are unstable because females will tend to place more weight on the most 'efficient' signal (Schluter and Price 1993), the maintenance of multiple handicaps is currently contentious.

However, recent literature on the coexistence of multiple ornaments has neglected male-male competition, which not only selects for male size dimorphism and weapons but also may also select for honest ornaments such as status signals (Andersson 1994). Separate agonistic and epigamic plumage signals have been identified in several bird species, for example, malachite sunbirds *Nectarinia johnstoni* (Evans and Hatchwell 1992a,b), ring-necked pheasants *Phasianus colchicus* (von Schantz et al. 1989; Mateos 1998), and the yellow-browed leaf warbler *Phylloscopus inornatus* (Marchetti 1998). In spite of this and several other examples of separate inter- and intra-sexual signals, multiple receivers (mates, rivals, predators etc.) have rarely been discussed in the context of multiple ornaments. If different displays are directed at separate receivers, these signals could be evaluated for honesty in their own context (i.e., not 'compete' in terms of 'efficiency' in the same response) and hence could explain why multiple handicaps coexist (Chapter III). Therefore, in species with multiple ornaments, it is possible that some characters have evolved through female choice, others through male-male competition, and others through both processes simultaneously. However, at present, there is insufficient evidence to resolve the evolution and maintenance of multiple ornaments, especially the coexistence of multiple handicaps.

Handicaps and the Expression of Individual Quality

Apart from the nature of the sexual selection process, an important part of understanding male ornaments is the selection pressures that have directed their evolution and constrained further exaggeration. The costs are essential for understanding why males, in spite of strong female choice or male contest competition, differ in ornament expression or display. The expression and type of cost (e.g., phenotypic or genetic) of bearing the ornamental handicap may also influence the mechanism of sexual selection on the trait (i.e., intra- versus inter-sexual selection).

Differential individual costs of ornamentation are central to the good genes models of sexual selection. Ornaments that are costly to produce, maintain, display or handicap male survival are thought to support the good genes models, however, runaway models also predict ornaments to be costly (at least at equilibrium; Balmford and Read 1991). In addition, relationships between ornament expression and aspects of male viability (e.g., body condition, parasite load, and energy rates) have been taken as support for the handicap models (Balmford and Read 1991; Kirkpatrick and Ryan 1991; Jones 1992). These relationships are also compatible with a Fisherian runaway process, because if ornaments end up being costly, their expression should be likely to depend on viability.

However, the good genes or handicap view of sexual signals is unique in that it assumes differential individual abilities to cope with the costs, and such differences could be reflected by ornament expression and have genetic viability advantages to offspring (Williams 1966; Zahavi 1975; Andersson 1982a; Hamilton and Zuk 1982; Grafen 1990; Johnstone 1995). This distinction may not exclude either of the two process, but with the increasing evidence of ornament expression relating to male viability (e.g., Milinski and Bakker 1990; Møller 1990; Zuk et al. 1990; Hill 1999) it at least indicates that, given a heritable component of viability, some handicap or good genes process is likely functioning, with or without Fisherian selection.

Birds are among the most brilliantly ornamented animal taxa (probably rivalled only by fishes), with primarily males displaying bizarre and extreme plumage ornaments along with dazzling and even gaudy coloration. In recent years, the research focus on the function of these ornaments has changed from 'aesthetic' displays to indicators of male quality and viability (Andersson 1994). Presently, three main types of avian visual ornamental signals are proposed as sexually selected handicaps; elaborate tail elongation, carotenoid-based plumage coloration and fluctuating asymmetry of feather ornaments.

Tail Elongation

Most birds possess tails well suited for an aerodynamic function, however in some species the tails have been modified to elaborate ornaments, from the few spiny barbs of the spinetail swifts (*Neafrapus cassini*) to the two metre Argus pheasant's tail (*Argusianus argus*) and the weird shapes of the marvellous spatula-tailed hummingbird (*Loddigesia mirabilis*).

Elongation of the tail for display purposes can affect the aerodynamic functions of tails during flight. Tail length has serious effects on flight economy: increasing the area of the tail from the base to the point of maximum width increases both lift and drag, but any further extension increases drag without increasing lift (Thomas 1993; Norberg 1995). In addition, increased length raises total body weight by adding mass to the tail as well as decreasing flight speed. The magnitude of the aerodynamic cost of tail elongation also depends on the details of the tail shape. Pintails (long central feathers) and deeply forked tails (longer outer feathers) do not increase drag and hence the power required for flight as severely as graduated tails (all feathers elongated) (Balmford et al. 1993; Thomas 1993; Norberg 1995). Tail elongation thus increases the energetic cost, and through effects on the turning moment and moment of inertia, alter manoeuvrability and agility possible in flight (Evans and Thomas 1992; Thomas 1993, 1996; Thomas and Balmford 1995). Subsequently, the aerodynamic cost of tails increases sharply with increased length (Balmford et al. 1993) and the marginal costs of tail elongation of graduated tails greatly exceeds that of other tail types (Thomas 1993).

The production of elongated tail ornaments is also likely to be stressful. This is suggested by seasonal variation of tail length in relation to food supply (Evans 1991), correlations between tail length and age over several years (Manning 1987), and stronger fluctuating asymmetry, indicative of developmental stress, in tail ornaments compared to non-ornamental tails of conspecific males and females of related species (Møller and Höglund 1991).

Honesty may also be imposed by the substantial physiological costs of tail growth, inferred from frequent growth and fault bars (translucent bars due to reduced deposition of keratin; Grubb 1989), which signal nutritional stresses during growth of tail feathers (S. Andersson 1994; Hill and Montgomerie 1994). In addition, the absence of melanin increases feather abrasion and weakens feather strength (Burt 1986; Bonser 1995), so tail markings could affect the ability of the tail to withstand abrasion and breakage. Feather quality (i.e., resistance to damage and abrasion) encapsulates the effects of diet, prior stresses, and environmental conditions on growth at the time of moult (Fitzpatrick 2000). As tail length, shape and markings are handicap signals of feather quality, and impose considerable developmental, energetic, flight and nutritional costs they should ensure an honest expression.

Carotenoid-based Plumage Coloration

Extravagant and sexually dimorphic bird colours have long been a focus for sexual selection studies, converging in recent years on quality-indicating ornaments (Hill 1999). Carotenoid-based coloration (yellow, orange and red pigmentation) is one of the best examples of condition-dependent ornaments that appear to serve as honest signals of male quality in birds and fish. Carotenoids are lipid-soluble, unsaturated C-40 hydrocarbons in a group of more than 600 different biochemicals that are widespread in plants (Møller et al. 2000). No vertebrates can synthesise carotenoid pigments *de nova*, so carotenoids must be ingested (Brush 1978; Goodwin 1984). Although energetically and physiologically costly, at least some vertebrates can modify the structure of the ingested pigments (e.g., yellow carotenes converted to red xanthophylls; Goodwin 1984; Brush 1990). Typically yellow pigments (mainly lutein) are highly available

in the diets of most terrestrial birds, whereas others such as red carotenoids are usually rare and instead are metabolically converted from yellow precursors (Hill 1996). Subsequently, the display of carotenoid-based pigmentation is often invoked as a general indicator of foraging ability (Endler 1980; Hill 1990, 1991; Møller et al. 2000), especially when colour displays demand large amounts of pigment, or pigments that are rare in the environment (Hill 1999). The access to carotenoid pigments may also be limited by direct interference competition among males for carotenoid-rich resources (e.g., arthropods). Therefore, the variation in carotenoid pigmentation is a result, at least partly, of the type and quantity of carotenoids ingested (Brush and Power 1976; Kodric-Brown 1989; Hill 1996).

In addition, after carotenoids are ingested, various parasites (e.g., cilia and coccidia) and pathogens (e.g., avian pox) can inhibit uptake, transport, or deposition of carotenoid pigments and can therefore alter the expression of plumage coloration independent of diet (Thompson et al. 1997; Hill and Brawner 1998). The physiological condition of the individual, independent of parasitic infection, has also been invoked as a factor in the efficiency with which ingested carotenoids might be used to pigment feathers (Bortolotti et al. 1996; Negro et al. 1998). Carotenoids exert immunomodulating, immunostimulating and antioxidant actions (Lozano 1994). Depletion of stored carotenoids through their mobilisation into nuptial coloration may compromise the immune system (Shykoff and Widmer 1996). Consequently, the expression of carotenoid-based nuptial coloration may represent the outcome of a prioritisation of allocation of scarce carotenoids between nuptial plumage, and the immune system, with potential consequences of increased susceptibility to parasites and pathogens. Individuals in good physical condition should therefore be better able to produce bright colours without compromising immune competence.

Carotenoid-based pigmentation thus represents an honest signal of male quality that is conditionally expressed as intense red carotenoid-based nuptial coloration may indicate a male's viability traits, such as the ability to forage for carotenoid-rich invertebrates and other scarce resources, fighting ability, resistance to parasites and pathogens, and superior health and condition.

Bilateral Symmetry of Ornamental Feathers

One of the more recently proposed and most controversial of the various handicap arguments relates to the phenomenon of fluctuating asymmetry (FA). The degree of asymmetry is argued to be indicative of a harmonious genome, since random deviations from symmetry in paired ornaments reflect how accurately the genome can maintain developmental homeostasis during environmental, developmental and genetic stress (Palmer and Strobeck 1986; Møller and Pomiankowski 1993b). The basis of FA analyses and interpretations are primarily founded on the relationship between the size of an ornamental character and the degree of FA (Møller 1991; Møller and Höglund 1991; Møller and Pomiankowski 1993b). In a non-sexually selected trait under strong stabilising selection, the degree of FA exhibited by a population or species, and thus the extent of developmental disruption, is thought to be greater at the upper and lower extremes of the trait's size distribution. In contrast, for costly ornamental traits a negative relationship between trait size and degree of FA is predicted, i.e., the larger the trait the more symmetrical it is expected to be. This is because elaborate sexual ornaments are thought to be more sensitive to mechanisms that causally mediate fitness (such as those underlying metabolic, immunological and developmental competence; Watson and Thornhill 1994) such that only superior quality males can produce both large and symmetric ornaments.

The proposed relationship between FA and tail length as a signal of genetic quality has been challenged on the basis that strong natural selection to minimise asymmetry due to the large aerodynamic and mechanical impairments on birds with long tails could also produce the negative relationship (e.g., Balmford et al. 1993; Evans and Hatchwell 1993; Evans et al. 1994; Thomas and Balmford 1995). In addition, FA as a condition-dependent signal is complicated by variation in ornament sizes of arbitrary Fisherian traits (thought to be low-cost) reflecting male viability at equilibrium (Balmford and Read 1991; Evans et al. 1995). Another possible challenge to the handicap signals of fluctuating asymmetry is based on the argument that sensory systems are designed to be sensitive to symmetry irrespective of mate quality, which may account for

reported female preference for more symmetrical mates (e.g., Enquist and Arak 1994; Johnstone 1994).

Consequently, FA as an indicator of male quality is still highly contentious as tests of the hypothesis have yielded contradictory results. In addition, female preference for condition-dependent FA has been conclusively documented in nature for only one avian species, the barn swallow *Hirundo rustica* (Møller 1990, 1991). Although FA may be important for signalling in some species (and may still prove to be), there is as yet, a general lack of consensus as to whether asymmetry is used in visual signalling (Swaddle 2000).

The *Euplectes*

The widowbirds (formerly *Coliuspasser*) together with the bishops form the genus *Euplectes*, a group of 16 small (13-45 g) African weaverbirds (family Ploceidae; Delacour and Edmond-Blanc 1933; Hall and Moreau 1970). The genus seems to have radiated at least as recently as within the Pleistocene (Crook 1964), and all species are closely related as judged from hybridisation in captivity (Colahan and Craig 1981) and similarities in general ecology and behaviour (Craig 1980). Habitat preferences range from reed beds and moist, open grassland through to savanna with sparse bush. Probably all are polygynous although data is insufficient for many species (Emlen 1957; Skead 1965; Craig 1980). Except in the lek-breeding Jackson's widowbirds (*E. jacksoni*), males defend nesting territories and participate to some extent in nest building, but not (or very rarely) in incubation or feeding. All species are strikingly sexually dimorphic in nuptial plumage. Breeding males have black body plumage with various amounts and combinations of white, yellow and red pigmentation. The male bishops are the most brilliantly coloured (either red or yellow on a black body) and have a short tail, while male widowbirds replace the tail feathers during pre- and post-breeding moult, and grow graduated tails of various lengths. Females are in most cases noticeably smaller, streaky brown or buff, and difficult to distinguish in the field. In the non-breeding season, males moult into their eclipse brown plumage that resembles the

females and juveniles, but in some species retain part of the nuptial coloration on the rump or wings. The male's eclipse plumage is also associated with a shift from granivory/insectivory in the breeding season to almost exclusive granivory during the winter months (Maclean 1990).

The tail ornaments vary greatly in size, from the comparatively short 45-65 mm tail in the male yellow-rumped widowbird (*E. capensis*) and red-shouldered widowbird (*E. axillaris*) to the half a metre plumes of the male long-tailed widowbird (*E. progne*), one of the most extreme avian ornaments. The striking sexual dimorphism and elaborate tail plumage of this genus have provided some of the most fascinating examples of sexual selection (Andersson 1982b; Andersson 1989, 1992; Savalli 1993, 1994). The long tails function in mate choice in the long-tailed widowbird (Andersson 1982b) and Jackson's widowbird (Andersson 1989, 1992), and in male-male competition for territories in the relatively shorter-tailed yellow-shouldered widowbird, *E. macrourus* (Savalli 1994). However, despite the *Euplectes* male's conspicuous display of bright nuptial coloration, the signal function of the colour patches has not yet been studied.

Red-collared Widowbirds

The red-collared widowbird (*Euplectes ardens*) is one of the most distinctive species in the group (formerly placed in a separate genus *Niobella*; Wolters 1975), as they show no obvious connection with any other species (Craig 1980). Red-collared widowbirds tend to favour drier habitats than the other species (e.g., open grasslands and sparsely bushed savannas or 'bushveld') in equatorial and southern Africa. These birds are small (males 17-25 g) and highly sexual dimorphic during the breeding season. Males in nuptial plumage are velvety black with a long, graduated tail (middle quills are the shortest) and red carotenoid chest patch ('collar'), which is completely absent in some populations, and extended over the nape and onto the crown in others. In contrast, the females are a streaky sparrowy brown with a short tail (ca. 4 cm).

Euplectes ardens, has three (possibly four) groups of subspecies separated mainly by differences in the nuptial plumage of the male and geographical separation of their ranges through Africa. The smallest red-collared widows, *E.a. ardens* (males ca. 20 g) are confined to grasslands and bushveld in southern and eastern Africa. Breeding males are black with a ca. 22 cm long, graduated tail and a crescent-shaped carotenoid collar on the lower throat. Juveniles and males during the non-breeding season resemble the females in plumage. The montane populations (commonly called red-naped widowbirds), *E. a. laticaudus* from Ethiopia and *E. a. suahelicus* from Kenya and Tanzania, are geographically separated and morphologically distinctive from *E. a. ardens* both in nuptial plumage (with red collars, napes and crowns) and in their measurements, with relatively shorter tails (ca. 140 and 170 mm, respectively; Craig 1993a) and larger body sizes (i.e., tarsi, wings and culmen). Both subspecies also differ in their moult patterns, since males during the post-nuptial moult appear to replace the long tail retrices of the breeding plumage with similar long feathers (Bowen 1931; Chapin 1954). Tail elongation within these three subspecies is not related to body size, as the largest birds (*laticaudus*) have the shortest tails, and the smallest males (*E.a. ardens*) have the longest tails. The fourth possible subspecies, the all black form *E. a. concolor* inhabiting West Africa and northern Zaire, is generally considered a melanistic version of *E. a. ardens* as both males and females cannot be separated satisfactorily on the basis of morphometric measurements (Neunzig 1927; Hall and Moreau 1970), although males tend to have longer tails than the other subspecies (ca. 250 mm; Craig 1993b). Occasional all-black males have been reported from Tanzania (Britton 1980), Malawi (Benson and Benson 1977) and Zambia (Benson et al. 1971; Tree 1972), therefore, although *concolor* is used to name the uniform populations in West Africa, the criteria of all-black males may not be sufficient to classify them as a subspecies (Craig 1993a).

The red-collared widowbird, *E.a.ardens* (hereafter *E. ardens*) was the subject of this study. Male tail length is proportionately about the same size as those of the highly exaggerated long-tailed widowbirds; average red-collared widowbirds' tail length, body mass and body length are about half the size of those in the long-tailed widowbirds (Maclean 1993). This species is also

distinctive among the *Euplectes* in that despite the male's very long tail, the sexual size dimorphism (male tarsus length and mass is about 11% larger and 20% heavier than females, respectively) is much smaller than other species, even those with medium and short tails (Craig 1978; Andersson and Andersson 1994). Details on the breeding patterns (Chapter II), and courtship (Chapter III) and territorial behaviour (Chapter IV, V) are provided in the chapters.

Study Area

Fieldwork was carried out in a grassland valley at Mondi Mountain Home Estate (29°43' S, 30°17' E) in the Hilton district of eastern KwaZulu-Natal, South Africa (Alt. ca. 1140 m). The area is isolated from other suitable grasslands by plantation (*Pinus patula*), extensive agricultural lands and a residential area bordering the clearing. The well-watered valley contains many reed-filled marshes and dams, but is dominated by open grasslands (mainly *Paspalum dilatatum* and *Eragrostis curvula*) interspersed with stretches of fairly dense scrub.

Rainfall is variable in amount, duration and intensity and is restricted to the summer season (September to March). The average annual rainfall for the area is about 800-1000 mm, and is coupled with high temperatures and high evaporation rates (Schultze et al. 1997). The widowbirds remain in the area for the breeding season but leave the area and form large, mixed species nomadic flocks for the winter season, returning again for breeding after the onset of the major rains (usually October to December).

Outline of the Study

This study deals primarily with the evolution and maintenance of the three commonly advocated avian visual handicaps (tail length, tail asymmetry and carotenoid coloration) in a single species, the red-collared widowbird. Apart from descriptions of the basic natural history of this species (Emlen 1957) and tests of

fluctuating asymmetry in tail length (Goddard 1997), no other work has been done on this species. The first chapter in this thesis explores the clumped patterns of nest distribution and the putative costs and benefits of polygyny to the red-collared widowbird. The second chapter investigates the relationships between signal expression and aspects of male fitness by investigating the targets of female selection from a variety of potentially sexually selected traits. The importance of the carotenoid collar in male contest competition is tested by model presentations (chapter IV) and experimental manipulation of the males themselves in the field and in captivity (chapter V). Finally, (chapter VI) the developmental trade-off between the two handicaps (tail length and carotenoid collar) is discussed in relation to life-history and the theoretical models of the coexistence of multiple ornaments. Taken together this thesis aims to investigate the relative importance of each ornament in female choice and male-male competition and whether the coexistence of multiple handicaps can be evolutionary stable.

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CHAPTER II

PATTERNS OF NEST DISTRIBUTION IN THE POLYGYNOUS RED-COLLARED WIDOWBIRD: WHY DO FEMALES CLUMP THEIR NESTS ON MALE TERRITORIES?

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(manuscript)

**PATTERNS OF NEST DISTRIBUTION IN THE
POLYGYNOUS RED-COLLARED WIDOWBIRD:
WHY DO FEMALES CLUMP THEIR NESTS ON MALE
TERRITORIES?**

ABSTRACT.—We explored the characteristics and patterns of nest distribution and their putative costs and benefits to breeding females in the polygynous red-collared widowbird (*Euplectes ardens*). The male builds ca. 13 simple nest rings ('cock's nests') on his territory, however, the females ignore these and alone builds the nest; a small oval nest well concealed in growing grasses and orientated away from the midday sun. Compared to other *Euplectes*, the male red-collared widowbird nest building has a much reduced role and is only part of a general courtship stimulus. Females used only 36% of the territory available for nesting and clumped their nests at about 15 m from each other, irrespective of the number of females on the territory and the size of the territory. Nest predation was high (70%), although predation intensity was independent of nest density. However, during synchronous nestling stages, nesting close to other birds incurred the cost of increased predation. The clumped, but relatively evenly spaced nest distribution may be to maintain a sufficient distance to reduce predation and intraspecific brood parasitism. Interestingly, nests were aggregated towards the center of the male's territory, possibly to take advantage of male vigilance from prominent central perches and/or to avoid harassment by neighbors. Sharing a territory has no obvious costs or benefits to females as males provide no food resources, paternal care or nest sites on their territories. As earlier work shows that females demonstrate strong selection for longer tailed males, potentially females could gain indirect genetic benefits of higher quality offspring (assuming there is some heritable component to tail length) without incurring high costs of polygyny.

IN POLYGYNOUS animals males typically benefit from acquiring multiple mates while females may suffer some cost from sharing males. As female choice is assumed to be adaptive, in that females make choices that maximize their fitness (Verner 1964; Orians 1969; Andersson 1994), females should determine whether or not polygyny occurs through the relative costs and benefits of their choice of mate. In spite of the considerable interest in polygynous avian mating systems, few studies have addressed the costs and benefits to females settling on male territories and most have involved the red-winged blackbird *Agelaius phoeniceus* (see Searcy and Yasukawa 1995; Beletsky and Orians 1996).

In this study, we examined the patterns of nest distribution in the polygynous red-collared widowbirds *Euplectes ardens*. Red-collared widowbirds are small (ca. 20 g) widowbirds restricted to open and shrubby savannas in eastern and southern Africa. Like other widowbirds, they are highly sexually dimorphic; breeding males are black with a long graduated tail (ca. 22 cm) and a red carotenoid collar on the lower throat, whereas females and non-breeding males are a streaky dull brown. At the beginning of the breeding season males establish exclusive breeding territories, which they aggressively defend from intruders (Emlen 1957; Pryke et al. 2001a; IV). The males typically provide no resources for the females apart from potential nest sites on their territories and the male does not participate in any form of paternal care (Skead 1965; Craig 1980; personal observations). Males are highly polygynous acquiring up to nine actively nesting females, although most males only attracted about three nesting females. The highly skewed mating success results from strong female choice for males with the longest tails (Pryke et al. 2001b; III).

As a consequence of directed mate selection and the polygynous mating system, females are forced to share a male's territory. The relative costs and benefits of polygyny to the females are likely to affect which male they choose to breed with and where they position their nests on the territory (i.e., clumped or dispersed). There are a number of potential benefits to polygynous and clumped nesting in birds, similar to those suggested to favor coloniality (reviewed in Alexander 1974; Wittenberger and Hunt 1985; Rolland et al. 1998).

First, high nesting densities could increase foraging efficiency with females acquiring information on the location of food by observing those females that have been successful in foraging (i.e., 'information centers' Ward and Zahavi 1973; Brown 1986). However, the information-sharing hypothesis is unlikely to affect the nesting densities and distributions of red-collared widowbirds on a territory as females commonly forage on communal feeding grounds away from the territory. Second, predation is the primary cause of nest mortality in most passerines (Ricklefs 1969; Filliater et al. 1994) and increased density and clumping of nests could result in lower nest predation (Fretwell 1972; Rolland et al. 1998). Females could improve anti-predator vigilance and defense through cooperation with their neighbors in the detection and mobbing of predators (Pulliam 1973; Andersson 1976; Curio 1978; Robinson 1985; Picman et al. 1988). In addition, colonial nesting may lower the impact of predators through predator dilution effects (Holling 1959; Pulliam and Caraco 1984), and by breeding in the center of a colony females may increase their success through geometric (i.e., selfish herd) effects (Hamilton 1971). This hypothesis predicts that nests in close association have lower predation rates (and hence higher reproductive success) than more dispersed nests.

Similarly, there are a number of potential costs to females sharing territories with other females. First, polygyny may be costly to females if male parental care affects reproductive success and is non-shareable (Trivers 1972). However, in the red-collared widowbird males provide no paternal care as the female alone builds the nest, incubates the eggs and feeds the young. Second, high nesting densities may increase competition for limited nest sites on the territories. As a consequence, birds may nest in clumps because suitable sites are limited and patchily distributed (Lack 1968; Snapp 1976) or the preferred high quality sites are clumped (Emms and Verbeek 1989). In addition, high nest densities and close neighbors may increase intraspecific parasitism (Møller 1989) as well as adversely affect the risk of nest detection if predators are localized (Croze 1970; Krebs 1971), return to areas where they were successful on previous visits (O'Reilly and Hannon 1989) or are attracted to the higher activity levels of colonial nesting

birds. Support for this hypothesis would come from increased predation rates on closely situated nests.

If nesting polygynously affords only costs and no or few benefits of nesting with conspecifics, females should settle in a way that maximizes their nearest neighbor distances within the territory. In contrast, if colonial nesting maximizes the benefits with few or no costs, females should clump their nests. In this study we explore the patterns of nest spacing on territories and the relative costs and benefits of nest distribution and polygyny to females sharing territories. In addition, we investigate characteristics of the nests and their influence on predation intensity, as well as the role and evolution of male participation in nest building.

STUDY AREA AND METHODS

Study site and population.— Field work was carried out from December 1999 to April 2000 in a grassy valley on Mondi Mountain Home Estate (29°43' S, 30°17'E), situated in the Hilton district, eastern KwaZulu-Natal, South Africa (altitude ca. 1100 m, Fig. 1). The area is isolated from other grasslands by plantation (*Pinus patula*), extensive agricultural lands and a residential area bordering the clearing. The well-watered valley contains many small reed-filled marshes and dams, but is dominated by open grasslands (mainly *Paspalum dilatatum* and *Eragrostis curvula*) interspersed with stretches of fairly dense shrub.

A population of red-collared widowbirds (*Euplectes ardens*) was studied during the breeding season (December 1999 to April 2000). Widowbirds were caught with mist nets either within their territories or at the different communal night roosts situated in reedbeds. All male widowbirds were marked with a numbered aluminum ring and a unique combination of three randomly assigned colored plastic bands to identify territory holders.

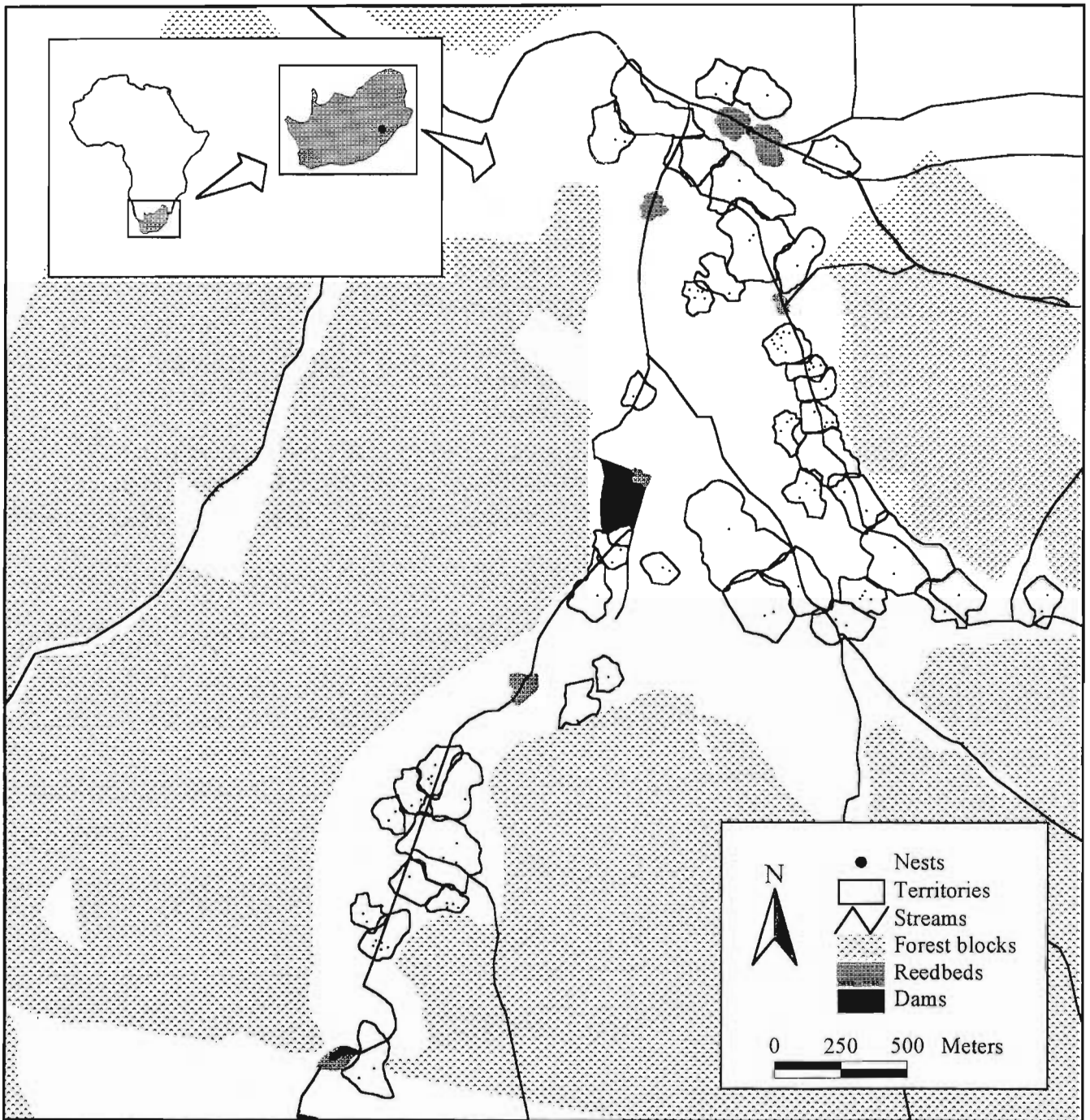


FIG. 1. Map of the study area at Mondi Mountain Home Estate (29°43' S, 30°17' E; eastern KwaZulu-Natal, South Africa) showing the location of the active nests ($n = 114$) on the male territories observed ($n = 43$).

Nesting characteristics.—As territorial males were the main focus in the study, the search for nests was restricted to territories defended by marked males. Throughout the breeding season, detailed observations of territorial behavior and aggressive interactions between males were made to obtain information on territory boundaries and the identities of the territory owners. Based on these data, territory maps were drawn using the convex polygon method (Odum and Kuenzler 1955) and updated every second week. The territory maps were digitized and ArcView Ver. 3.0 (ESRI, 1996) was used to estimate territory area.

The mean vegetation type was estimated as an indirect measure of the suitability of vegetation for nesting. The abundance of each vegetation type was measured at fixed sampling points situated 20 m apart on 50 m transects across the entire study area. The measurements were taken at two-weekly intervals and averaged across both the territories and the nesting season.

Nest searches were conducted daily to locate nests built by both males (simple 'cock's nests'; see below) and females. All nests were marked with numbered tape flags situated at about 5 m from the nest. For each nest found we recorded: (1) the nest condition (nest ring, unlined, complete or old), (2) nest content (presence and number of eggs and nestlings, and dimensions of the eggs), (3) nest height (the height to the nearest cm from the ground to the lower rim of the entrance hole), (4) nest vegetation (the species in which the nest was built and the vegetation height to the nearest cm), (5) vegetation cover (% cover of grass or shrub around the nest), (6) nest orientation (compass direction of the nest entrance), and (7) nest size (the maximum height, width and breadth of the nest, and the diameter of the entrance hole). The positions of the nests within the territories were mapped onto the digitized territory maps and the linear inter-nest distances were estimated.

Active nests were revisited every third day to determine the incubation period and nestling duration. However, due to the suspected heavy nest predation, a number of precautions were undertaken to minimize observer disturbance to the nests. First, visit duration at a nest was minimized (subsequent visits were usually less than 10 seconds). In addition, the time taken to measure the eggs was minimized (usually less than 3 minutes) and all vegetation measurements were

recorded after the completion of nesting or after the nest was predated or abandoned. Second, different paths were used to approach nests or during searching activities to avoid creating paths through the territories. Lastly, nests were not approached when potential visual predators (e.g., the highly vigilant fiscal shrike *Lanius collaris*) were sighted nearby.

Patterns of Nest Distribution.—Red-collared widowbirds are polygynous and the nest density per territory area is significantly more clumped than predicted from a random distribution of nests on the total available territory area (Pryke et al. 2001b; III). To estimate the relative proximity of nests on the territory, nearest neighbor distances were calculated for each nest on a territory. Territories that contained only one nest were excluded from the analyzes as they had no neighbors. To compare the mean nearest neighbor distances per territory with those expected if nests were randomly distributed we used the method of Sinclair (1985), which calculates expected nearest neighbor distances based on both nest density and the total area available, while correcting for edge effects. This method was repeated for both simultaneously active nests and all active nests within male territories.

The primary female settling on a territory may affect subsequent female settlement (Searcy 1986, 1988). To control for this possible bias, we also analyzed distances between primary nests and their nearest active neighbors.

To determine the relative position of nests on a male's territory we calculated the ratio of the nearest neighbor distance to the nearest territory boundary for each nest. Ratios close to one suggest a uniform nest distribution within the territory, whereas ratios greater than one suggest nests are closer to the perimeter, and ratios less than one indicate centralized nest distribution. The frequency of these ratios was tested against a Poisson distribution to determine if nest were positioned randomly on a territory with respect to the territory boundaries.

Nest Fate.—If a nest failed, the reason for the failure was recorded if known. A nest was considered abandoned if it was lined but remained empty, or was active on at least one visit but later found inactive with nest contents. Nests were also storm-destroyed if they were wind flattened, or badly damaged by hail

or rain. A nest was considered predated if eggs or nestlings were missing before the predicted date of fledgling when they had been present on the previous inspection. This may have included some nests deserted by adults and later found by predators. In addition, a nest was considered destroyed by a predator if it was found in disarray not caused by inclement weather.

Nest predation rates were estimated using the Mayfield method (1961, 1975), with variances calculated according to Johnson (1979) as it corrects for nests found at different times of the breeding cycle. The daily mortality rate (m) is estimated by dividing the total number of lost nests by the sum of days nests have been exposed to predation. Daily survival probability (s) is then $1-m$, and the survival chance through t days is s^t . Since the incubation period (t_i) and the nestling period (t_n) are known, the survival probability through both stages is estimated as $(s_i)^{t_i} * (s_n)^{t_n}$.

Logistic regression analyses were used to establish the relative influence of possible variables on nest desertion and nest predation. The Wald chi-square test was used to establish if each variable had a significant effect upon the dependent variable. For nest predation rates, the regressions were analyzed separately for the clutch, brood and total nesting periods (with no overlap in nest activity) since eggs and nestlings were often predated by different predators.

RESULTS

Nest Characteristics. —The male red-collared widowbirds construct several simple 'cock's nests' on their territories (mean \pm SD = 13.1 ± 4.73 nests, $n = 43$ territories, range: 3 – 22 nests per territory) by producing a loose structure of a few intertwined grass blades between upright grass or plant stalks. Building material usually comprises strips of grass detached from near the base of the grass stem. The female constructs the nest. Only one 'cock's nest' was used as a frame for subsequent nest building; all other females chose to nest either adjacent (< 1 m) to the cock's nest ($n = 19$), or somewhere else on the territory ($n = 123$).

Like other *Euplectes*, the red-collared widowbird builds a globular nest that is semi-closed with a side entrance; about 11.45 cm (± 0.59 , $n = 209$) high, 7.78 cm (± 0.56 , $n = 209$) wide and 7.19 cm (± 0.86 , $n = 209$) broad, with an entrance diameter of 3.87 cm (± 0.34 , $n = 209$). The nest is woven from growing or fresh grass; as the materials dry they shrink and strengthen the mesh of the woven structure. The nest is lined with fine grass and dry, soft seed heads that project to form a porch concealing part of the entrance. The nest is often further concealed by strands of green, growing grass that are bent and woven over the top of the nest.

The nests were usually well hidden in strong course grasses, leafy shrubs and shorter tussock grasses. Nests ($n = 209$) were most commonly built in grasses (80.5%), especially *Paspalum dilatatum* (38.7%), *Eragrostis curvula* (25.3%), *Pennisetum clandestinum* (8.1%), *Digitaria smutsii* (3.3%), *Harpechloa falx* (2.8%) and *Eragrostis plana* (2.3%), and less frequently in bushes and shrubs (19.5%) such as *Senecio serratuloides* (10.5%), *Rubus cuneifolius* (8.6%) and *Verbena bonariensis* (0.9%). Nests were built close to the ground (45.6 ± 15.7 cm measured to the bottom of the entrance, $n = 209$, range: 16 to 94 cm), but were placed higher in bushes than in grasses (shrubs 56.2 ± 9.3 cm, $n = 42$; grasses 39.8 ± 11.2 cm, $n = 167$; t -test = 8.3, $P < 0.001$) probably due to the greater structural support in bush foliage. The height of a nest was, however, independent of vegetation height (grasses $F_{1,167} = 1.32$, $P = 0.29$; shrubs $F_{1,167} = 1.08$, $P = 0.52$).

As nests are commonly placed in dense grass or shrubs, they are often sheltered and shaded. Nevertheless, more nestlings were lost on hot days ($> 35^{\circ}\text{C}$) through overheating (i.e., dead with stomach full and very hot) in exposed nests ($< 30\%$ vegetation cover; $n = 11$) than sheltered nests ($> 30\%$ vegetation cover; $n = 1$). The nests were also orientated more frequently to the east, southeast and south (i.e., $90^{\circ} - 225^{\circ}$) than expected from a random distribution ($\chi^2 = 15.8$, $df = 113$, $P < 0.005$): this sector of the compass is the most shaded, as in the southern hemisphere the sun passes to the north.

Many complete nests were deserted before egg-laying (31.2%, $n = 65$), so it is possible that deserted nests differed somehow in placement or degree of

protection from those nests in which eggs were laid ($n = 144$). However, none of the nest variables tested had a significant effect on nest desertion (Table 1).

TABLE 1. Logistic regression analysis to determine which characteristics were good predictors of nest desertion ($n = 65$).

Nest characteristics	Wald statistic	<i>P</i>
Nest height	1.37	0.30
Nest vegetation	0.93	0.66
Nest visibility cover	0.42	0.55
Nest orientation	0.86	0.42
Nest size	2.11	0.15
Number of nests on the territory	0.09	0.88
Nearest neighbor distance	0.73	0.41
Position relative to the territory boundary	0.04	0.73

Clutch Size and Incubation.—The eggs are pale blue-green, and usually heavily speckled with shades of gray and brown often forming a blotched cap at the larger end. Mean dimension of eggs, from 86 clutches, were 19.04 (± 0.66) mm long, and 13.98 (± 0.42) mm wide ($n = 242$). Clutch size ranged from 1 to 4 eggs, with about 2-3 eggs per nest (2.8 ± 0.92 eggs, $n = 86$). The incubation period estimated from nests visited from initiation to hatching was 13 to 14 days (13.3 ± 0.5 days, $n = 19$). There were two cases of apparent intraspecific nest parasitism. In one nest, a fourth egg was added to the clutch four days after the last egg; in all other nests visited during laying only one day separated egg-laying in each clutch. The egg hatched four days after the other nestlings and only fledged five days after the other nestlings had left the nest. In another nest, nine eggs were counted but they were predated before hatching.

In the seven successful nests located before hatching, the nestlings fledged at about 13 days (13.1 ± 0.3 days). However, in each of the five nests containing

three young, one nestling was perched on or next to the nest by the 11th day. Although, the young vacated the nest by the 13th day they tended to remain hidden in the foliage near the nest for a few days.

Patterns of Nest Distribution.—For the nearest neighbor analyses we assumed that the entire territory was suitable for nesting. This seemed justified as if nest sites are limited the vegetation structure at nest sites should be different from that of the remaining territory. However, all vegetation types present on the territories were used in nesting, and the females nested in the most abundant grasses or shrubs within the territory ($r_s = 0.34 - 0.78$, $P < 0.05$ for all comparisons). Therefore, the vegetation composition used for nest sites was not different from that on the territory in general, and hence is unlikely to influence nest placement.

Using the area of nesting site polygons, females nested in only 36% ($\pm 8.1\%$, $n = 32$ territories) of the total area available on the territory. Nearest neighbor distances for active nests on a territory varied from 3.1 to 24.6 m with most nests 14.7 m from a neighbor (± 5.3 , $n = 114$). Both simultaneously active nests ($n = 87$) and all active nests ($n = 114$) were significantly clumped on the territories (simultaneously active nests: $\chi^2 = 317.5$, $df = 27$, $P < 0.001$; active nests: $\chi^2 = 328.9$, $df = 32$, $P < 0.001$). In addition, the distances between the primary nests and their nearest active nests on different male territories were shorter than expected ($\chi^2 = 305.2$, $df = 25$, $P < 0.001$), suggesting that females clump their nests and that the first female to settle does not adversely affect subsequent female settlement patterns. Interestingly, nearest neighbor distances were independent of nest density ($F_{1,26} = 0.44$, $r^2 = 1.1\%$, $P = 0.51$; Fig. 2a) and territory size ($F_{1,26} = 0.11$, $r^2 = 0.4\%$, $P = 0.74$; Fig. 2b) indicating that female's nesting on a territory maintain a relatively consistent distance between nests. The majority of nests on a territory were clustered towards the center (see Fig. 1) since distances between neighboring nests were significantly shorter than the distances to the territory boundaries ($\chi^2 = 76.3$, $df = 113$, $P < 0.001$).

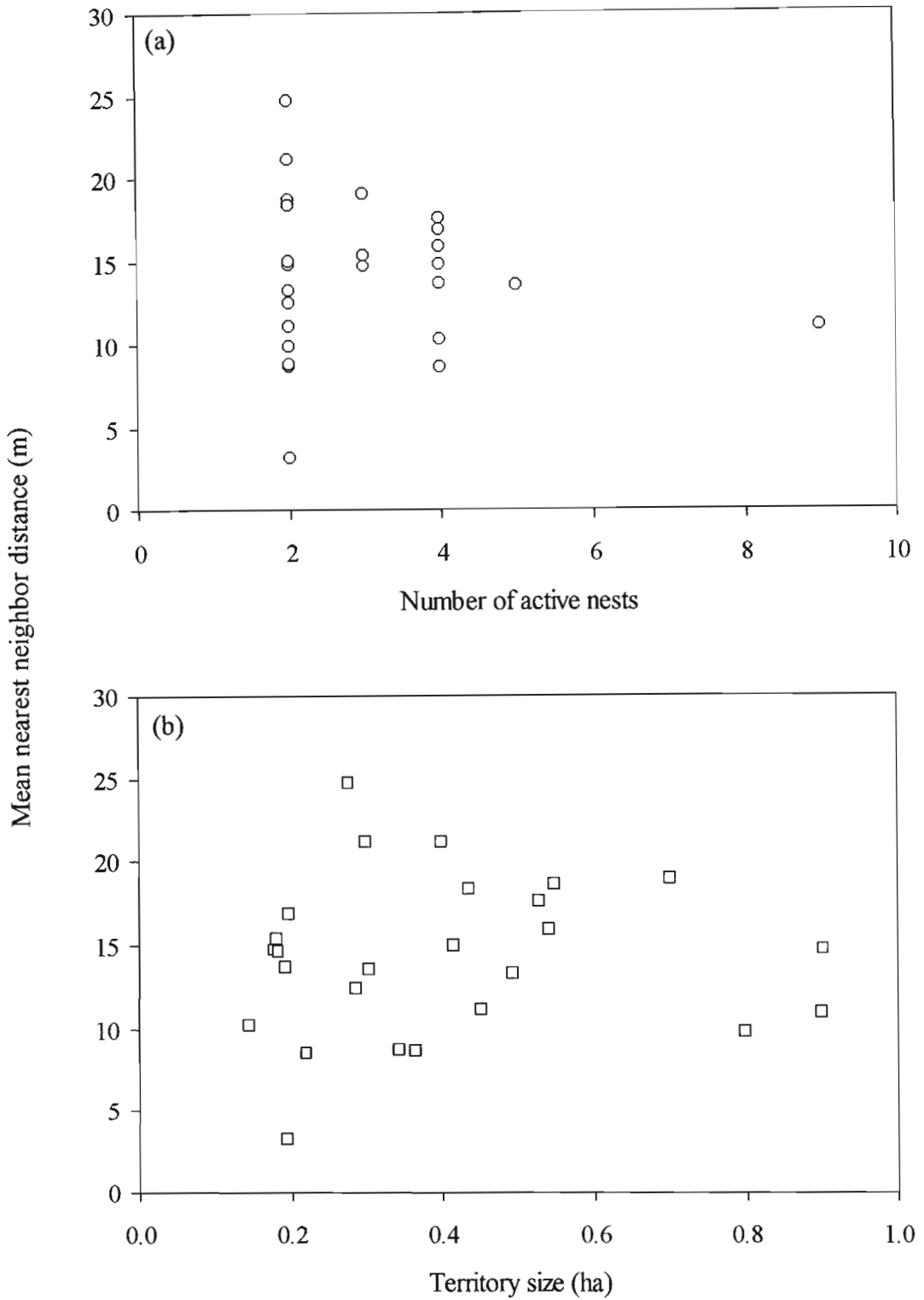


FIG. 2. The average nearest neighbor distances per territory (m) plotted against the (a) number of nests per territory; $y = -0.31x + 15.24$, $F_{1,26} = 0.44$, $R^2 = 1.1\%$, $P = 0.51$, and the (b) size of the territory (ha); $y = 1.41x + 13.68$, $F_{1,26} = 0.11$, $R^2 = 0.4\%$, $P = 0.74$.

Nesting Success and Predation.—Of 86 egg clutches, 63 (73%) were not successful (i.e., did not hatch young); the eggs were either predated ($n = 59$), or abandoned ($n = 4$). Ants may have caused the desertion of two of these nests. Twenty-three clutches subsequently hatched and 28 nests were found with nestlings. In forty-two of the remaining 49 nests nestlings did not fledge (85%); 25 broods were predated, 12 broods overheated in the nest, two were abandoned (starved), and three were destroyed by storm damage and the nestlings. The overall success of the nests was thus 7 of 114 nests (6%) fledging young, which is commensurate with the low nesting success reported for other *Euplectes* species (2 – 27%; Craig 1982; Savalli 1991; Andersson 1992; Hake and Andersson 1992).

The probability (estimated using the Mayfield 1969, 1975 and Johnstone 1979 method – see Methods) of a nest surviving predation through the 13 day incubation period was 65%, and through a 13 day nestling period until fledgling 47%. Therefore, the overall predation rate was very high as 70% of the active nests were destroyed. Logistic regression analyses were used to determine which nest characteristics affected predation rates during the egg, nestling and total nesting stages. Only the distances between nearest neighbors significantly predicted whether a nest survived during synchronous nestling stages (Table 2). Therefore, predation rates were more pronounced during the nestling stage and decreased with increasing distances between the nests on a territory.

The predator was rarely sighted, but in one case a common egg eater snake (*Dasypeltis scabra*) was found in the nest feeding on the eggs. The nest was left intact after the eggs had been removed, and such nests were assumed to be indicative of snake predation, which accounted for 28% of the predated nests. At another nest a fiscal shrike (*Lanius collaris*) tore apart the nest and killed the nestlings. Similar ripped apart nests were also considered to be due to bird predators and accounted for 54% of the predated nests. Predated nests with holes in the bottom were assumed to be black rats, *Rattus rattus* (Craig 1982; Friedl and Klump 1999) and were responsible for 14% of the predation losses. Two nests were found with families of rats living in them (females with three and six offspring, respectively). Some nests were completely destroyed (4%) and could not be attributed to any specific predators, but may have been one of the other

potential nest predators common in the study site such as slender mongooses (*Galerella sanguinea*) and feral cats (*Felis catus*).

Male red-collared widowbirds would frequently give alarm calls when predators approached, but no red-collared widowbird was ever sighted mobbing a potential predator. Males responded to avian predators (e.g., Jackal buzzards *Buteo rufoguscus*, Black sparrowhawks *Accipiter melanoleucus*, Gymnogenes *Polyboroides typus* and Longcrested eagles *Lophaetus occipitalis*) by either moving down into the grass or rapidly taking flight if the raptor came too close.

TABLE 2. Logistic regression analyses to determine which characteristics were good predictors of nest success or failure, during the egg stage ($n = 27$), nestling stage ($n = 25$) and the entire nesting period ($n = 30$).

Nest characteristics	Egg period		Nestling period		Nesting period	
	Wald statistic	<i>P</i>	Wald statistic	<i>P</i>	Wald statistic	<i>P</i>
Nest height	1.18	0.62	0.03	0.82	1.92	0.17
Nest vegetation	0.02	0.84	1.11	0.34	0.02	0.88
Nest visibility cover	1.21	0.69	2.80	0.10	2.51	0.10
Nest orientation	0.12	0.31	2.13	0.19	0.01	0.92
Nest size	0.63	0.75	2.68	0.12	1.78	0.19
Number of nests on the territory	0.82	0.48	1.73	0.28	0.93	0.35
Nearest neighbor distance	0.98	0.53	10.2	0.002	6.93	0.07
Position relative to the territory boundary	0.75	0.41	0.44	0.54	2.78	0.16

DISCUSSION

The nesting biology of the red-collared widowbird appears similar to the other species in the genus that have been studied. All species have similar ecologies; mainly granivorous birds inhabiting marsh and grasslands. The most obvious difference between the species is their breeding dispersion, ranging from lek-breeding in Jackson's widowbird, *E. jacksoni*, (Andersson 1992) to the highly colonial red bishops, *E. orix*, inhabiting reedbeds (Craig 1982; Friedl and Klump 1999) and the fairly widely dispersed breeding of the widowbirds inhabiting open grasslands and savannas (Craig 1980; Savalli 1993).

Patterns of Nest Distribution.—Through their strong mate selection for males with the longest tails female red-collared widowbirds are forced to share territories with other nesting females. But why do the females nest closer together than necessary based on the available space on a territory? Although we can only speculate here, there are a number of potential explanations for the observed nest distribution in the red-collared widowbird.

First, spatial variability in nest site availability may affect nest distribution with birds nesting in clumps because preferred nest sites are limited or patchily distributed (Lack 1968; Emms and Verbeek 1989). However, suitable nest sites are unlikely to affect nest placement on a red-collared widowbird territory, as females tended to ignore the male's cock's nests and build their own nests. In addition, the habitat is relatively uniform, and even if nest site quality varied between areas within territories, a given area usually provides numerous potential sites of similar quality. Furthermore, the birds nested in the most abundant vegetation on the territories, suggesting that nest site availability is not a limited resource and is therefore unlikely to explain the extent of nest clumping in the red-collared widowbird.

Second, the extremely high predation rate on the red-collared widowbird's nests (70%) may alter the costs or benefits to females of clumping their nests. For example, through combined vigilance, active aggressive defense and predator dilution effects, the North American red-winged blackbirds (*Agelaius phoeniceus*) clump their nests as an adaptive strategy to reduce predation by Marsh wrens

Cistothorus palustris (Picman 1980; Picman et al. 1988). In contrast, the probability of red-collared widowbird nests been predated on a territory during this season was higher for closely clumped nests during the nestling period. The most important predator in the area was the fiscal shrike, which are highly vigilant birds and may have been attracted to the increased activity caused by closely situated nests on a territory. Red-collared widowbird females are unable to displace the shrikes and no physical attacks or mobbing behavior was ever observed. Therefore, although predation rates were not density dependent, there may be predation costs to closely nesting females on a territory.

Interestingly, female red-collared widowbirds tend to clump their nests towards the center of a male's territory. Vigilant male red-collared widowbirds commonly sit on a high, exposed perch near the center of their territories (one central perch was favored in over 75 % of the observed behavior times). Such perches are probably primarily used for sighting potential females and guarding territories against intruding conspecifics. However, they may also function for locating predators, as vigilance is often related to the conspicuousness of the vigilant individual (Lendrem 1983). Possibly, the red-collared widowbirds may parallel the ecologically similar red-winged blackbirds, where females tend to build their nests close to tall perches which through male vigilance are safer from predation than nests with no nearby perches (Yasukawa 1989; Yasukawa et al. 1992). Although male red-collared widows were never seen approaching or mobbing a predator, they would give alarm calls when potential predators approached, which may alert the females nesting on a male's territory. Therefore, if the presence of a nearby male on a conspicuous perch decreases predation, female red-collared widowbirds may prefer to nest in close proximity to the male's central prominent perch. Alternatively, or in addition to, females may also nest centrally on a male's territory to avoid harassment by neighboring males. Male red-collared widowbirds aggressively display to any female visiting the territory; females foraging on a neighboring territory are often harassed and even chased by the displaying males. Thus, by placing their nests towards the center of the territory, females may be less hassled by neighboring males when arriving and departing from their nests.

Although females clumped their nests centrally within a territory, the nearest neighbor distances were independent of both territory size and nest density, indicating that the birds maintained essentially the same nesting distance regardless of the number of nesting females and the amount of nesting habitat available. There may, therefore, be an optimal nesting distance required for successful nesting. There are predation costs to females nesting closely to other active nests (see above), so females may maintain a sufficient distance to reduce nesting activities that may alert potential predators, avoid area-restricted predators and those returning to areas where they had been successful previously. Another possible explanation for the relatively constant nest spacing is interference from other females. Female red-collared widowbirds are not aggressive towards other females on the territory and have not been observed destroying each other's eggs or young (unlike, e.g., House sparrows *Hirundo rustica* - Veiga 1990, and Marsh wrens - Picman 1977, mated to the same male). However, there may be costs in terms of intraspecific brood parasitism ('egg dumping'). For example, house swallows nesting synchronously with conspecifics were more often parasitized if their nests were placed close to neighbors (Møller 1989). Two apparent cases were found during this season suggesting that this may be a potential cost to closely nesting females.

Polygyny in the red-collared widowbird.—Polygyny is a relatively rare mating system in birds (Moller 1986) and few polygynous species show the degree of polygyny exhibited by the *Euplectes* (Maclean 1990). Polygyny is always advantageous to the male, as the more females he acquires, the more young he is likely to produce, especially if the female alone raises the offspring. However, a female has more to lose in terms of reproductive effort, either in sharing a male with other females or mating with an inferior male, and so it is usually the female that plays the largest part in mate selection. The evolution of polygyny will therefore depend primarily on the advantages and disadvantages to the female.

Polygyny has no obvious costs to red-collared widowbird females selecting already mated males for a number of reasons. First, females choosing already mated males pay no cost through reduced male assistance (paternal care)

as in many other polygynous systems where males provision at least some of the young. Second, females do not compete for territorial resources as they feed off the territory and build their own nests. Third, the number of female's settling on a male's territory does not influence female reproductive success, at least not in terms of increased predation risk. Lastly, the presence of nesting females on a territory does not discourage further settlement on the territory. Similarly, there are few obvious benefits to females sharing a territory with other nesting females. Females acquire no benefits through increased foraging efficiency or decreased predation by nesting with other females.

In species, with relatively few costs or benefits to polygyny, female selection may depend mostly, if not entirely, on attributes of the male and/or the quality of his territory (Andersson 1994; Searcy and Yasukawa 1995). As female red-collared widowbirds show directed mate choice for longer tailed males, potentially they could gain indirect genetic benefits in terms of higher quality offspring (assuming that there is some heritable component to tail length) without incurring high costs from sharing the territory with other females.

Evolution of Nest Building.—There is some uncertainty about the extent to which the male *Euplectes* contribute to nest building (Collias and Collias 1964; Crook 1964; Skead 1965; Craig 1980). Phylogenetically, male nest building is regarded as a primitive condition among the weaverbirds (Ploceidae), from which the reduction in male involvement in the *Euplectes* is believed to be derived (Collias and Collias 1964; Craig 1980). In the bishop and widowbirds, the males build the initial structure (cocks nests), varying from only the first vertical ring in some species, to the complete outer structure in others. Although the initial mate attraction in *Euplectes* seems to be aerial displays, a form of nest display appears to be retained in female courtship. In most species, the male displays to visiting females while she inspects the cock's nest and the male has been observed to display directly to females at the nest in the white-winged widow *E. albonotatus*, and the red-shouldered widow *E. axillaris* (Mitchell 1966). The female may then use the cock's nest for subsequent nest building (most species) or build a new nest somewhere else on the territory.

In the red-collared widowbird, males construct only the initial vertical nest ring (usually only a few minutes per ring). The large number of these cock's nests on each male's territory and the lack of intermediate stages suggest that this is the male's only participation in nest building. During the courtship display, the male may lead the female to the nest rings while displaying. However, females tend to ignore these cock's nests and build their own nests somewhere else on the territory. This suggests that the cock's nest has only a symbolic value in this species, similar to the display courts of the lekking Jackson's widowbird (Andersson 1991), but without the inter-male variation on which female's base their choice. Earlier research shows that the quality of the male (i.e., his tail length, Pryke et al. 2001b; III) is more important to the female than the features of his territory. Thus nest building by males has a much reduced role in sexual selection in red-collared widowbirds and is only a small part of a more general courtship stimulus. The latter does not apply to all *Euplectes* species, as the number of cock's nests built are an important feature for female choice in the shorter tailed yellow-shouldered widowbird *E. macrourus* (Savalli 1993), and the red bishop *E. orix* (Friedl and Klump 1999).

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CHAPTER III

SEXUAL SELECTION OF MULTIPLE HANDICAPS IN THE RED-COLLARED WIDOWBIRD: FEMALE CHOICE OF TAIL LENGTH BUT NOT CAROTENOID DISPLAY

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SEXUAL SELECTION OF MULTIPLE HANDICAPS IN THE RED-COLLARED WIDOWBIRD: FEMALE CHOICE OF TAIL LENGTH BUT NOT CAROTENOID DISPLAY

Abstract.—Although sexual selection through female choice explains exaggerated male ornaments in many species, the evolution of the multicomponent nature of most sexual displays remains poorly understood. Theoretical models suggest that handicap signaling should converge on a single most informative quality indicator, whereas additional signals are more likely to be arbitrary Fisherian traits, amplifiers, or exploitations of receiver psychology. Male nuptial plumage in the highly polygynous red-collared widowbird (*Euplectes ardens*) comprises two of the commonly advocated quality advertisements (handicaps) in birds; a long graduated tail and red carotenoid coloration. Here we use multivariate selection analysis to investigate female choice in relation to male tail length, color (reflectance) of the collar, and other aspects of morphology, ectoparasite load, display rate and territory quality. The order and total number of active nests obtained are used as measures of male reproductive success. We demonstrate a strong female preference and net sexual selection for long tails, but marginal or no effects of color, morphology, or territory quality. Tail length explained 47 % of male reproductive success, an unusually strong fitness effect of natural ornament variation. Fluctuating tail asymmetry was unrelated to tail length, and had no impact on mating success. For the red collar, there was negative net selection on collar area, presumably via its negative relationship with tail length. None of the color variables (hue, chroma and brightness) had significant selection differentials, but a partial effect (selection gradient) of chroma might represent a color preference when tail length is controlled for. We suggest that the red collar functions in male agonistic interactions, which has been strongly supported by subsequent work. Thus, female choice targets only one handicap, extreme tail elongation, disregarding or even selecting against the carotenoid display. We discuss whether long tails might be better indicators of genetic quality than carotenoid pigmentation. As regards the evolution of multiple ornaments, we propose that multiple handicap signaling is stable not because of multiple messages but because of multiple receivers, in this case females and males.

Key words. —Carotenoid coloration, *Euplectes ardens*, female choice, handicaps, long tails, multiple ornaments, sexual selection.

The extreme diversity in design and expression of epigamic traits, among species as well as among multiple ornaments within species, remains largely unexplained. Many sexual displays are examples of multicomponent signaling (Rowe 1999), with exaggeration of several distinct signals even within a given modality (e.g., visual, acoustic). Sexual selection theory, particularly the handicap principle (Zahavi 1975) of costly and thus reliable (honest) quality advertisements proposes that ornamental signals are exaggerated to a point where viability costs balance the mating advantage (M. Andersson 1994). However, it is not clear why so many species have multiple ornaments (Møller and Pomiankowski 1993), especially because honest advertising is theoretically expected to reinforce a single most informative signal at the expense of others (Schluter and Price 1993; Johnstone 1996). Although multiple signals may include arbitrary Fisherian traits, amplifiers, and the exploitation of receiver sensory capabilities (e.g., Iwasa and Pomiankowski 1994; Rowe and Guilford 1999), the coexistence of handicaps (the redundant, or back-up, signal hypotheses; Møller and Pomiankowski 1993; Johnstone 1996) remains a contentious issue.

In avian ornamental plumage three types of visual signals are often proposed as sexually selected quality advertisements: (1) ontogenetically or aerodynamically costly tail elongation (Balmford et al. 1993; S. Andersson 1994; Norberg 1995); (2) bilateral symmetry as an indicator of developmental homeostasis (Parsons 1990; Møller and Höglund 1991); and (3) red carotenoid coloration revealing nutritional status or immune stress (Olson and Owens 1998; Hill 1999). We investigate the coexistence of all of these advocated handicap signals in a wild population of red-collared widowbirds, *Euplectes ardens*.

The long tails of breeding male widowbirds are well-known examples of costly traits (S. Andersson 1994; Norberg 1995) selected by female choice in two species (*Euplectes progne*, M. Andersson 1982; *E. jacksoni*, S. Andersson 1989, 1992), but suggested to function in male-male competition in a third (*E. macrourus*, Savalli 1994). Savalli (1993) and Craig and Villet (1998), in contrast, propose that the extreme tail ornament variation in *Euplectes* spp. (from almost no elongation to about 50 cm) can be attributed to a model of genetic drift. To help

resolve these contrasting views we examined the exaggerated tail ornamentation of another, largely unstudied, widowbird species. Most *Euplectes* spp. have conspicuous yellow or red color patches on their wings or body (Craig 1980), based on carotenoid pigments (Kritzler 1943; S. Andersson, unpubl. data), which because of their dependence on diet and health have become prime examples of honest quality advertisements (Hill 1999). Contrary to the intensely studied, and in some ways very similar, North American redwinged blackbird, *Agelaius phoeniceus* (Searcy and Yasukawa 1995), the signal functions of widowbird coloration have not been examined before. In addition, very few studies of carotenoid color variation use objective color quantification (Endler 1990; Bennett et al. 1994). A third independent signal of male quality in the red-collared widowbird may be the symmetry of the long tail feathers, which are conspicuously spread in perch displays. Fluctuating asymmetry (FA) of bilateral traits may indicate epigenetic stress (Parsons 1990; Møller and Höglund 1991); however, a recent study in red-collared widowbirds does not support this putative signal function (Goddard and Lawes 2000).

In addition to the potential quality advertisements above, female choice of breeding situation might target a variety of other male phenotypic traits or territory characteristics. For example, females may be choosing males on the basis of body size, dominance, display behavior, territory size, or territory quality. In *Euplectes* (and related Ploceinae), in particular, male-built nest frames (cock's nests) are an important display feature (Crook 1964; Craig 1980; Andersson 1991), the number and quality of which might be subject to female choice (Collias and Victoria 1978; Craig 1982; Andersson 1991; Savalli 1994; Friedl and Klump 1999).

In this paper we use multivariate selection analysis (Lande and Arnold 1983) to investigate the strength of female choice and net sexual selection on the multiple ornaments and other phenotypic and territory characteristics. The potential for honest quality advertisement is tested by analyzing the relationships between ornamental traits and body condition, and between trait expression and symmetry. Finally, we discuss the results in relation to hypotheses for the evolution of multicomponent sexual signals in birds.

MATERIALS AND METHODS

Study Species

The red-collared widowbird, *E. ardens*, is a polygynous, granivorous weaverbird (subfamily Ploceinae) of shrubby grassland and bush clearings in eastern and southern Africa. Like other widowbirds, males molt into nuptial plumage prior to the breeding season (October to April; Emlen 1957; Skead 1965). Male nuptial plumage is black except for a crescent-shaped red collar on the chest. Males also grow a long, graduated tail (~ 22 cm). Male plumage is in striking contrast to the drab brown plumage and short tail ($\bar{x} \pm \text{SD} = 44 \pm 24$ mm, $n = 241$) of females and non-breeding males. There is also a sexual size dimorphism (male tarsus: $\bar{x} \pm \text{SD} = 24.07 \pm 0.57$ mm, $n = 209$; female: $\bar{x} \pm \text{SD} = 21.88 \pm 0.66$ mm, $n = 249$; t -test = 37.8, $df = 207$, $P < 0.001$), but it is the least pronounced sexual size dimorphism among the widowbirds (Andersson and Andersson 1994; Craig 1998).

Males establish and defend relatively large ($\bar{x} \pm \text{SD} = 0.44 \pm 0.29$ ha, $n = 43$, range: 0.14 - 1.51 ha) and exclusive breeding territories from conspecifics and other *Euplectes* intruders (Emlen 1957). Male competition to acquire and maintain territories is high as judged by a large population of 'floaters' (males in nuptial plumage that do not establish territories) that frequently intrude onto occupied territories to attract females (Pryke et al. 2001; IV). Both sexes feed away from the breeding territories (Craig 1980; pers. obs.). The females visit male territories solely to secure mating opportunities and nest sites. During courtship the males display their long prominent tail in a slow smooth flight with exaggerated wing-beats. During this display the central rectrices are depressed and keeled to form an inverted sickle shape, and the head feathers are erected in a hood. The male circles a visiting female, landing near her with tail askew and wings outspread while uttering a characteristic hissing sound. The female may either fly off or stay in the displaying male's territory, but she usually moves away from the male. The display is repeated to a female until she retreats or solicits copulation.

After the main rains the males construct several very simple cock's nests ($\bar{x} \pm \text{SD} = 13.1 \pm 4.73$ nests, $n = 43$ territories, range: 3 - 22) by producing a loose structure of a few intertwined grass blades between upright plant stalks (Skead 1965; pers. obs.). Interestingly, females rarely use the nest frames and rather build fresh nests close by or elsewhere on the territory (S. R. Pryke, unpubl. data; II). Red-collared widowbirds build a typical *Euplectes* nest that is semiclosed with a side entrance, and woven from fresh grass in dense grass or leafy shrub close to the ground ($\bar{x} \pm \text{SD} = 45.5 \pm 15.7$ cm above the ground, $n = 144$, range = 16 - 88 cm). Male parental care has not been recorded in this species.

A population of red-collared widowbirds was studied in the Hilton district, KwaZulu-Natal Province, South Africa (29°43' S, 30°17'E; elevation ~ 1100 m) between December 1999 and April 2000. The study area is a mosaic of reed-filled marshes and open grasslands (dominated by *Paspalum dilatatum* and *Eragrostis curvula*) interspersed with shrubs.

Morphometric Data

All widowbirds, mist-netted either on their territories or at communal night roosts nearby, were uniquely color-banded. Red color bands were not used, because they might influence male attractiveness (e.g. Burley 1985; Metz and Weatherhead 1991). Right and left tarsus and culmen were measured to the nearest 0.1 mm and wing length to the nearest 0.5 mm. The three linear dimensions of body size (tarsus, culmen, and wing) were highly intercorrelated, so the first component derived from a principal components analysis (PCA), which explained 68.8% of the variance, was used as a measure of body size. Body mass was measured to the nearest 0.5 g (50 g Pesola spring-balance). Although male body mass might change temporally throughout the day due to differences in crop contents, there was no relationship between body mass and the time of capture in this sample ($r_s = 0.17$, $n = 185$, $P = 0.27$).

Using tarsus length, which is one of the most reliable univariate measure of skeletal size in birds (Freeman and Jackson 1990), we computed a body condition

index as the residuals from a linear regression of $\log(\text{body mass})$ on $3 \times \log(\text{tarsus length})$ (S. Andersson 1994). Ectoparasite load (e.g., lice, ticks, and mites) on tail and wing feathers was scored from zero to three (none to heavy load).

All tail feathers were measured with a ruler to the nearest 0.1 mm from the point of insertion (base of the tail) to the tip of the feather. Total tail length was estimated from the longest rectrix (i.e., feather number 2). The number of tail feathers with blood quills (i.e., still growing) was counted. Missing, growing, and damaged feathers were excluded from the subsequent analyses. Tail asymmetry was calculated as the absolute difference between the length of the left (L) and right (R) characters (e.g., each pair of rectrices in the tail; Palmer and Strobeck 1986; Møller and Höglund 1991). A mean fluctuating asymmetry (FA) value was calculated for tail length as the mean of the absolute differences between each pair of rectrices. Birds with broken, missing, or growing feathers were excluded from the FA analyses.

Collar patch size was measured using calipers to the nearest 0.1 mm by taking the maximum horizontal measure (width) and three vertical (height) measures along the patch. Collar width and length were significantly correlated ($r_s = 0.82$, $n = 125$, $P < 0.0001$) and collar area was calculated as the product of the width and the average height.

Because many individuals were recaptured, repeatabilities (Lessells and Boag 1987) were calculated for each of the morphological characters, and these were all highly significant ($r_s = 0.81-0.96$, $n = 57$, $P < 0.001$).

Reflectance Spectrometry and Colorimetrics

Spectral reflectance (at ± 2 nm resolution) from the red collar was measured using a PS1000 miniature diode-array spectrometer (Ocean Optics, Inc., Dunedin, FL), HL2000 halogen light source, WS-2 white reference, a fiber-optic reflectance probe (Avantes, Eerbeek, Netherlands), and C-spec software (Ancal, Inc., Las Vegas, NV). The probe was fitted with a matte black tube that shielded

out external light and standardized the measuring distance. The probe was held (not pressed) perpendicularly against the plumage, scanning the direct (specular) reflectance from a 4 mm wide spot. Preceded by a reference scan for each individual, five consecutive scans (removing the probe between each) were taken from the center of the collar patch. Colorimetric analyses were made over the 350-700 nm spectral range. Although bird vision usually extends down to about 320 nm (Burkhardt 1989), UV/VIS measurements of captive birds and museum specimens have confirmed that the flat, weak reflectance below 400 nm continues down to 300 nm. Consequently, the contribution of the unmeasured 320-350 nm segment to the signal variation should be minimal.

Objective indices of the three main dimensions of color signals, spectral intensity, location, and purity (Hailman 1977) were computed from the five scans and averaged for each individual. From 5 nm segmented average spectra in the range 380-700 nm, we also computed coefficients in the uniform human CIELAB color space (International Commission on Illumination 1971) for 10 degree observer and the D65 (daylight) illuminant. These were used to confirm that our objective measures strongly predicted the intended colorimetric in human color vision, and thereby probably a corresponding signal dimension in avian color space. Three color variables were derived. Brightness (spectral intensity) was estimated by $R_{350-700}$, the sum of reflectance from 350-700 nm. Hue (spectral location, here referred to as redness) was estimated as $\lambda(R_{50})$, the wavelength at which reflectance is halfway between its minimum (R_{\min}) and its maximum (R_{\max}). $\lambda(R_{50})$ explained 75% of human-perceived hue variation (linear regression: CIE h_{ab} vs. $\lambda[R_{50}]$, $F_{1,126} = 445.8$, $P < 0.001$, $r^2 = 77.9\%$). Chroma' (spectral purity) has a more complex dependence on several reflectance shape aspects, such as slope height and steepness. A general way to capture this variation is to use the difference between two spectral segments (which ideally should correspond to cone absorbance spectra when these are known), divided by the total reflectance (see Endler 1990). To avoid a fixed arbitrary spectral segment, which confounds chroma with hue variation, we used individual $\lambda(R_{50})$ as the segment divider, and computed C_{R50} as $[R_{350-\lambda(R50)} - R_{\lambda(R50)-700}]/R_{350-700}$. Like the hue measure, C_{R50}

strongly predicted the corresponding variation in human color space, explaining 88% of the CIELab chroma measure (linear regression: CIE C_{ab} vs. C_{R50} , $F_{1,126} = 893.6$, $P < 0.0001$, $r^2 = 89\%$).

After thus mathematically disentangling three perceptually relevant aspects of reflectance variation, the three color dimensions remained significantly correlated (hue and chroma, $r = 0.33$, $n = 127$, $P < 0.001$; hue and brightness, $r = -0.53$, $n = 127$, $P < 0.001$; brightness and chroma, $r = -0.52$, $n = 127$, $P < 0.001$). These are expected relationships in pigment colors (subtractive reflectance), however, because absorptance (i.e., pigment concentration) by necessity increases chroma at the expense of brightness, and also (unless the slope of absorptance cut-off is vertical) shifts the hue (S.Andersson, unpubl. data). Uncorrelated measures of reflectance variation can be obtained by, for example, PCA (Endler 1990; Cuthill et al. 1999), which by definition creates uncorrelated, orthogonal, variance axes. However, this also complicates the interpretation in terms of color perception compared to segment-based measures. For example, although PC1 typically captures brightness variation, PC2 and PC3 are not easily interpreted in terms of hue and chroma (Grill and Rush 2000). In addition, when tested on the present data, PCA scores explained less of the corresponding CIELab measures than the above variables (S. Andersson unpubl. data).

Behavioral Observations

Each resident male was observed daily between dawn and dusk when the males defended their breeding territories. A random-number table was used to determine the order in which males were observed. All activities and their durations were continuously recorded on a dictophone with time given to the nearest five seconds. Male display rates were extracted for analysis. Display rate was calculated as the total time a male spent in courtship display divided by the time (in minutes) he was present during the territory observations. As female visitation rate is likely to affect display intensity and a higher display rate contribute

to female attraction, a correction for female movement should be made (e.g., Gibson and Bradbury 1985). However, the red-collared widowbird males display to all eclipse (brown) birds of both their own and other species (Craig 1980; S. R. Pryke, unpubl. data), and therefore it was not always possible to determine whether the displays were directed toward the female red-collared widowbirds. But this cause and effect problem is unlikely to substantially affect the results, because males perform their courtship displays irrespective of female proximity (S. R. Pryke, unpubl. data).

Territory Characteristics

Territorial males restricted their activities to a well-defined area, did not allow other males to trespass the boundaries, and actively defended the territory from other males (i.e., aggressive displays, chasing, parallel flight displays and direct attacks). Territory boundaries were mapped from direct observations of individually marked males and defined using the convex polygon method (Odum and Kuenzler 1955). The map of the territories was digitized and ArcView Ver. 3.0 (ESRI 1996) was used to estimate territory area.

To ensure that no male replacement had taken place, the presence of a male on a territory was monitored weekly throughout the study. Only one male lost his territory two weeks before nesting began. Territory size and locality was updated every three weeks. Differences in territory size between updates were so slight that area parameters were not significantly altered (estimated change in area: $\bar{x} \pm SD = 0.052 \pm 0.021$ ha, $n = 43$; paired t -test: $t = 0.76$, ns).

The mean foliage density and height within each territory was estimated as an indirect measure of the suitability of vegetation for nesting. Foliage height (maximum plant height) and density (on a rank scale from zero to 10) was measured at fixed sampling points situated 20 m apart on 50 m transects across the entire study area. Vegetation measurements were taken at two-week intervals and averaged across both the territories and the nesting season.

Nest locations were marked with a small numbered flag and plotted onto the territory maps. Nests were inspected every third day until the nest was abandoned, nestlings had fledged, or the nest was lost to predators.

Male Reproductive Success

Neither the small clutch size ($\bar{x} \pm \text{SD} = 2.7 \pm 0.63$ eggs, $n = 93$, range = 2-4 eggs), nor the low fledging success due to 70% predation (S. R. Pryke, unpubl. data; II), were suitable measures of male fitness. In this study we are concerned with selection resulting from female choice, thus we used two measures of male reproductive success that are directly influenced by female mating preferences: (1) the number of active nests; and (2) the clutch initiation date (pairing order).

Active nests were those nests containing eggs or nestlings. The number of active nests on a male's territory is likely to be a strong indication of female mating preferences in this system, unless they are counteracted (rather than inflated) by genetic extrapair copulation mate choice. Extrapair copulation frequency is unknown in this species, but is relatively low in the related polygynous red bishop (*E. orix*), where the number of nestlings sired is strongly related to the number of nests (Friedl and Klump 1999). In addition, females obtain little more than genes from their social mate choice, so it is unlikely that their criteria for extrapair copulation mate choice should be different.

The clutch initiation date was the date of the first egg that a female laid in the breeding season. The average date for all nests within a male's territory was calculated as the estimate of pairing order. The date of first breeding in birds may greatly affect the probability of offspring surviving to adulthood (e.g., Norris 1993). There may, therefore, be a fitness component of sexual selection on male traits, if males with larger trait values tend to mate with early breeding females. In all nests visited during laying, only one egg per day was added to the clutch. If a nest with a completely hatched brood was found, first clutch initiation date was calculated by subtracting the average incubation period ($\bar{x} \pm \text{SD} = 13.3 \pm 0.5$ days,

$n = 19$, range = 13-14 days) and the appropriate number of days for egg laying from the day of hatching. Clutches preyed upon or abandoned during the incubation period were excluded.

Selection Analysis

To estimate net intersexual selection on male ornaments and related traits, and to identify the direct and indirect effects of female choice, we used standardized multivariate selection analysis (Lande and Arnold 1983; Arnold and Wade 1984). Fitness measures were transformed to relative male fitness by dividing the estimate of male reproductive success by the population mean (Lande and Arnold 1983). All included independent variables were standardized (mean = 0, variance = 1) before analyses. Standardizing removes the effects of differential scaling and therefore allows a comparison (in standard deviation units) of the relative importance of each variable (Gibson 1987).

Directional selection differentials were calculated as the covariances between relative mating success and the standardized male characters. Selection differentials estimate the net selection (combined direct and indirect effects via correlated traits) acting on a trait by measuring the relative strength of bivariate relationships between each trait and relative reproductive success (Gibson 1987). Selection gradients were calculated as partial linear regression coefficients (β) from multiple regression of relative fitness to the standardized value of the trait. Selection gradients quantify the strength of selection acting on a trait independently of variation in the other characters included in the regression model (Lande and Arnold 1983). With mating success as a dependent fitness measure, selection gradients thus identify the probable cues used in mate choice (Andersson 1989).

Because regression residuals were approximately normal with homoscedastic variance, significance tests were based on Pearson correlation coefficients for selection differentials and ANOVA for selection gradients. In multivariate regression strong correlations among the independent variables

(multicollinearity) can lead to an overestimation of the standard errors of the regression coefficients, reducing the power of the analysis (Mitchell-Olds and Shaw 1987). To assess the influence of multicollinearity on the results, a PCA correlation matrix of the standardized variables in each regression was examined. Condition numbers (square root of the ratio of the largest eigenvalue to the smallest) below 10 are generally considered acceptable (Fry 1993). The condition numbers from all selection gradients were less than four, indicating that the correlations among the variables were not sufficiently high to adversely affect the significance tests.

RESULTS

Variation in Male Traits

Female choice of males depends on sufficient intrapopulation variation for females to visually perceive differences between male characteristics. Coefficients of variation (CV) were calculated to compare variability within the territorial male characters (Table 1). Body size traits exhibited CVs of 1.3% to 5.4% and were comparable with those of other studies of avian intrapopulation morphological variation (e.g., Alatalo et al. 1988; Barnard 1991; Evans and Barnard 1995). However, territory characteristics and other potentially sexually selected characters were considerably more variable, fitting the expectations for a highly dimorphic and polygynous species (Alatalo et al. 1988). Such high variation may provide potential cues for mate choice. Interestingly, variation in tail length (about 17%) was substantially higher than those of congeners: 6.0-9.4% in the longtailed widowbird, *E. progne* (Andersson 1982; Craig 1989), 12.5% in Jackson's widowbird, *E. jacksoni* (Andersson 1993) and 5-8% in the yellow-shouldered widowbird, *E. macrourus* (Savalli 1994).

TABLE 1. Means (\bar{x}), standard deviations (SD) and coefficients of variation (CV) for measured variables in male red-collared widowbirds (*Euplectes ardens*).

Male variable	\bar{x}	SD	CV	<i>n</i>
Culmen (mm)	14.2	0.38	2.66	43
Tarsus (mm)	24.7	0.32	1.31	43
Wing (mm)	77.3	1.26	1.63	43
Body mass (g)	21.6	1.16	5.36	43
Collar area (mm ²)	282.1	23.57	8.35	43
Tail length (mm)	213.5	35.91	16.85	43
Mean absolute tail asymmetry	5.5	3.21	58.76	43
Ectoparasites (0-3)	1.3	0.29	22.67	43
Collar brightness ($R_{350-700}$)	80.9	26.16	32.35	33
Collar hue ($\lambda[R_{50}]$)	581.2	13.27	2.28	33
Collar chroma (C_{R50})	0.6	0.09	15.81	33
Grass density (%)	61.6	18.37	29.20	43
Territory area (ha)	0.4	0.27	62.12	43
Number of cock's nests	13.1	4.73	36.16	43
Display rate (displays/min)	0.4	0.24	58.62	43

Color Variation

The red, crescent-shaped collar patch was highly variable in both size (area) and color. The CV for collar area within the population was 17% ($\bar{x} \pm SD = 233.9 \pm 41.9 \text{ mm}^2$, $n = 126$), which was the same magnitude as tail length. CVs of the three colorimetrics (brightness, hue, and chroma) were also substantial but are difficult to interpret without comparable data from other species. Reflectance variation among the territorial males is illustrated in Figure 1 by the average reflectance and two extreme males with respect to $\lambda[R_{50}]$ (hue). In addition to this variation, territorial males differed from males that did not establish a territory (at least not in the study area), by having larger and redder collar patches, suggesting that these traits are intrasexually selected signals in competition over territories (Pryke et al. 2001; IV; S. Andersson, S. R. Pryke, J. Örnborg, M J. Lawes, unpubl. data; VI).

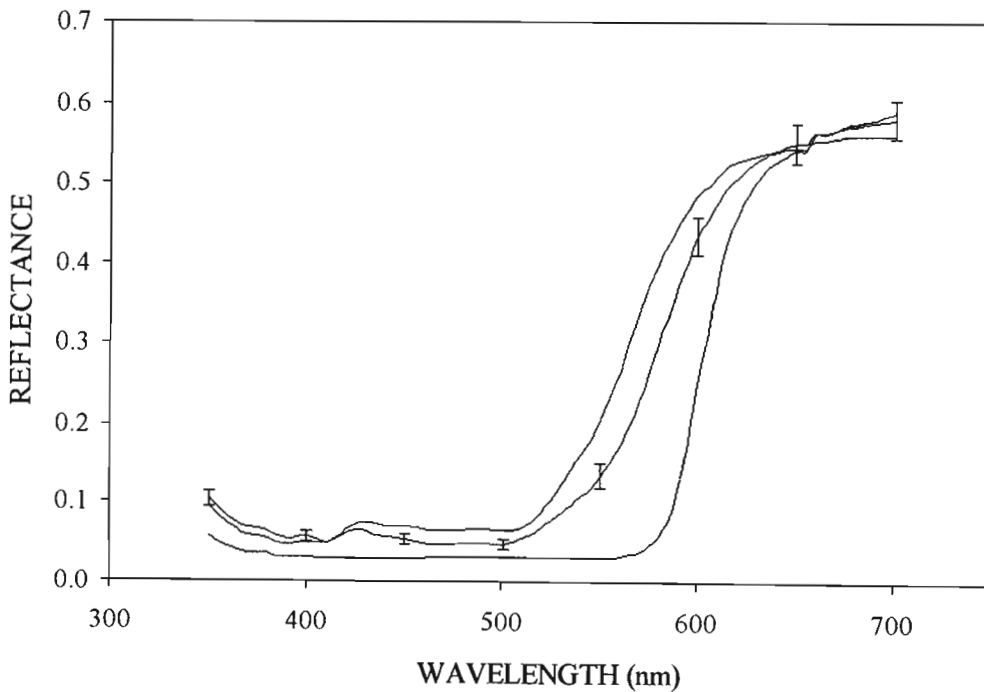


FIG. 1. Average reflectance (with standard error bars at 50 nm intervals) of the red collar patch for the 43 territorial males. The range of the hue $\lambda[R_{50}]$ variation is indicated by the reflectances with the most shortwave and the most longwave $\lambda[R_{50}]$ values, respectively.

Fluctuating Asymmetry

Tail asymmetry was normally distributed ($DN = 0.19$, $n = 126$, $P = 0.68$) and mean values did not deviate from zero ($t = 1.87$, $n = 126$, $P = 0.43$) demonstrating that this trait meets the criteria for exhibiting FA (Palmer and Strobeck, 1986). The degree of fluctuating asymmetry was not correlated with tail length ($F_{1,126} = 0.08$, $P = 0.48$, $R^2 = 0.08\%$) and males with longer tails did not demonstrate lower levels of asymmetry as predicted by the FA hypothesis (Møller 1990). In addition, no significant second-order polynomial relationship existed between tail size and FA ($F_{1,126} = 1.06$, $P = 0.79$, $R^2 = 0.06\%$), and therefore the relationship between tail size and asymmetry is not described by stabilizing selection (i.e., U-shaped function).

Variation in Male Reproductive Success

The distribution of 114 active nests (containing eggs or nestlings) among the 43 territorial males was highly skewed, with the most successful male recruiting nine nests (Fig. 2). Nest density (nests per territory area) was also significantly more clumped than predicted from a uniform (ideal free) distribution of nests on total available territory area ($\chi^2 = 42.62$, $df = 42$, $P < 0.001$). The second male fitness measure, clutch initiation date, deviated significantly from a Poisson distribution of random territory choice by the first females to settle ($\chi^2 = 14.64$, $df = 42$, $P < 0.005$). The significantly skewed distributions of nests, nest density, and settlement of early females, indicate strong female choice and inter-sexual selection pressure.

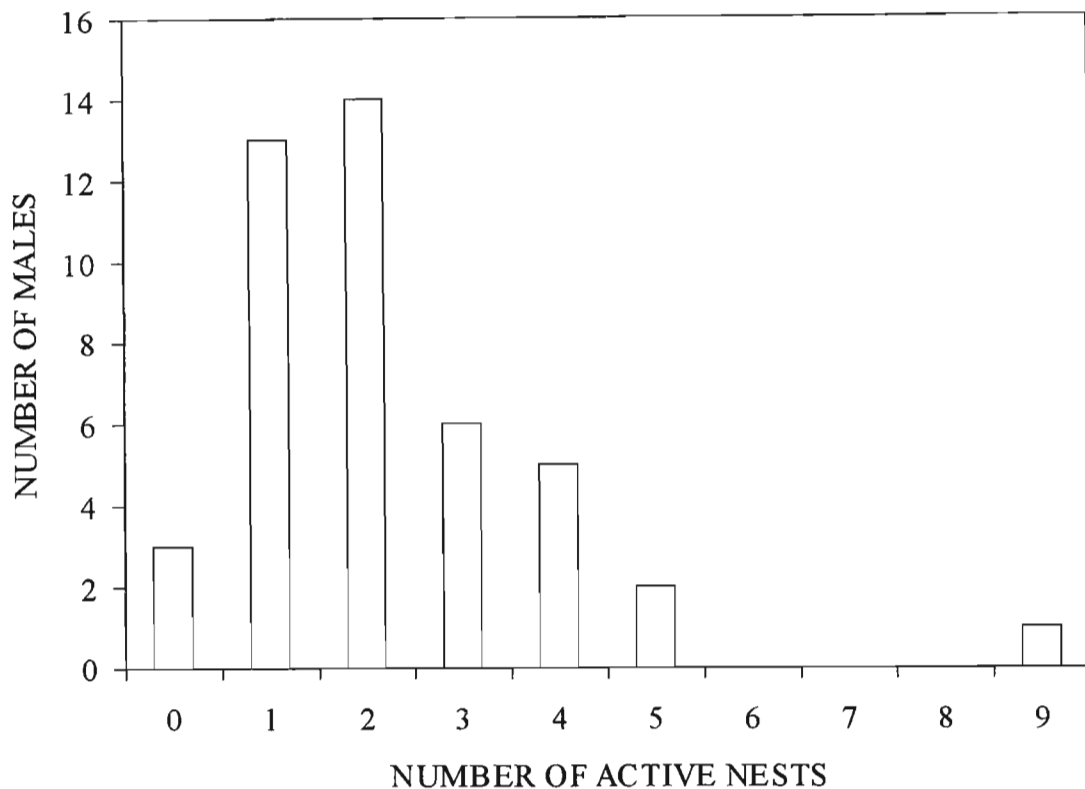


FIG. 2. Distribution of male reproductive success (number of active nests) among 43 male red-collared widowbirds.

Male Fitness Measures

Both an earlier breeding date and greater female settlement are associated with males producing more offspring. The date of initiation of breeding was a good estimate of male reproductive success because early breeding birds had higher reproductive success over the entire breeding season (total number of nestlings: $r_s = 0.63$, $n = 76$, $P < 0.001$; successful fledglings: $r_s = 0.71$, $n = 18$, $P < 0.001$). Similarly, males with more active nests on their territory had significantly more nestlings ($r_s = 0.91$, $n = 76$, $P < 0.001$) and fledglings ($r_s = 0.79$, $n = 18$, $P < 0.001$). Thus, although we use attraction of nesting females as the fitness measure in order to identify mate choice cues, the strong correlation to the final number of offspring confirms that female choice exerts a real selection pressure, at least within this particular breeding season.

TABLE 2. Standardized selection differentials for sexual selection on 12 traits in male red-collared widowbirds. The number of active nests and egg-laying date were used as the relative fitness measure of mating success. Selection differentials include both the direct and indirect effects of correlated traits on male reproductive success. Probability values are based on Pearson correlation coefficients.

Male Trait	Clutch					
	Active nests	<i>n</i>	<i>P</i>	Initiation date	<i>n</i>	<i>P</i>
Tail length	0.47	40	<0.001	0.30	40	<0.001
Collar area	-0.28	42	0.002	-0.19	42	< 0.001
Body size	-0.27	43	0.03	-0.11	43	0.05
Collar chroma	0.02	33	ns	0.09	33	ns
Collar brightness	0.13	33	ns	0.08	33	ns
Collar hue	-0.20	33	ns	-0.05	33	ns
Tail asymmetry	0.08	40	ns	0.03	40	ns
Ectoparasites	0.01	43	ns	-0.01	43	ns
Territory area	-0.15	43	ns	0.04	43	ns
Grass density	0.03	43	ns	0.01	43	ns
Cock's nests	0.08	43	ns	0.02	43	ns
Display rate	0.03	43	ns	0.05	43	ns

Selection Differentials: Estimating Sexual Selection

Selection differentials (s) are the bivariate covariances between relative fitness and the male trait, representing the combined effect of direct selection and indirect selection via correlated traits. Selection differentials for both the number of active nests and clutch initiation date produced similar covariances and significance levels (Table 2). The largest significant differential for both fitness measures was found for tail length, followed by collar area and body size. This suggests strong net directional sexual selection for longer tail length and, interestingly, against male body size and the area of the red collar patch.

TABLE 3. Estimates of standardized linear (β) selection gradients for 12 traits in male red-collared widowbirds. The number of active nests and egg-laying date were used as the measure of relative reproductive success. Due to the small sample size, the selection gradients (β_1) could not be tested; therefore a reduced model of five gradients was recalculated (β_2). Standard errors for the selection coefficients are in parentheses.

Male traits	Number of active nests			Clutch initiation date		
	β_1	β_2	P	β_1	β_2	P
Tail length	1.63 (± 0.38)	1.67 (± 0.36)	<0.001	0.76 (± 0.46)	0.81 (± 0.24)	<0.001
Collar chroma	0.51 (± 0.28)	0.53 (± 0.17)	0.005	0.44 (± 0.16)	0.32 (± 0.09)	0.081
Collar brightness	0.29 (± 0.21)	0.21 (± 0.16)	ns	0.21 (± 0.16)	0.18 (± 0.07)	ns
Collar hue	-0.04 (± 0.27)			-0.16 (± 0.19)		
Body size	-0.27 (± 0.19)	-0.33 (± 0.16)	ns	-0.36 (± 0.14)	0.29 (± 0.13)	0.032
Collar area	-0.91 (± 0.62)	-0.49 (± 0.48)	0.07	-0.59 (± 0.77)	0.36 (± 0.12)	0.009
Tail asymmetry	-0.05 (± 0.20)			-0.02 (± 0.15)		
Ectoparasites	0.11 (± 0.14)			-0.13 (± 0.22)		
Grass density	0.04 (± 0.07)			0.03 (± 0.01)		
Territory area	0.31 (± 0.20)			-0.19 (± 0.16)		
No. of cock's nests	0.07 (± 0.14)			-0.01 (± 0.11)		
Display rate	0.03 (± 0.11)			0.02 (± 0.17)		

Selection Gradients: Identifying Cues for Female Choice

The directional selection gradients (β) estimate the partial effect of each trait on fitness, here measured by the number of active nests as fitness measure (Table 3). This analysis indicates which of the ornamental traits had the strongest direct influence on male attractiveness. To reliably test their significance, the multiple regression was recalculated for the five strongest gradients. The selection of the five variables in the reduced model was based on both a backward and

forward stepwise regression to determine the amount of explained variance (R^2) that was removed or added, respectively, by each variable. The reduced model was highly significant ($P < 0.001$) and increased the variance accounted for in the model by 4% (to 62%).

As suspected from the strong bivariate relationship between tail length and fitness (Table 2, Fig. 3), tail length remained the most important male trait when other traits were held constant. Over 47% of the variance in male fitness (number of nests) was accounted for by tail length alone (Fig. 3). The standardized selection gradient of 1.67 for this trait was approximately three times greater than the next largest, and indicate that an increase in tail length of one standard deviation was associated with a 167% increase in relative fitness.

There was also a significant selection gradient on collar chroma (spectral purity). This suggests that, when tail length and other traits are held constant, females show a preference for more vividly colored (but not necessarily redder) male collars, but that this partial effect is counteracted so that there is no net selection on collar chroma or any other color parameters (Table 2). An almost significant ($P = 0.07$) negative direct effect of collar area (Table 3), combined with an indirect effect through its negative relationship with tail length (S. Andersson, S. R. Pryke, J. Örnborg, M. J. Lawes, unpubl. data; VI), seem to be the primary reasons for the significantly negative net selection on collar area (Table 2). When we repeated the preceding analysis with the start of egg laying as the dependent variable, results were similar (Table 3). The same five traits were identified as the most important factors and used in the reduced model (decreasing R^2 from 58% to 51% in the model). In addition to the predominant effect of tail length ($R^2 = 30\%$, Fig. 4), the weak negative selection gradients for collar area and body size were significant, whereas the effect of collar chroma was not ($P = 0.08$).

We conclude that, unless there is some highly correlated trait that we have failed to measure, tail length is the predominant cue for female choice in red-collared widowbirds, explaining close to 50% of the number of nesting females a male attracts to his territory.

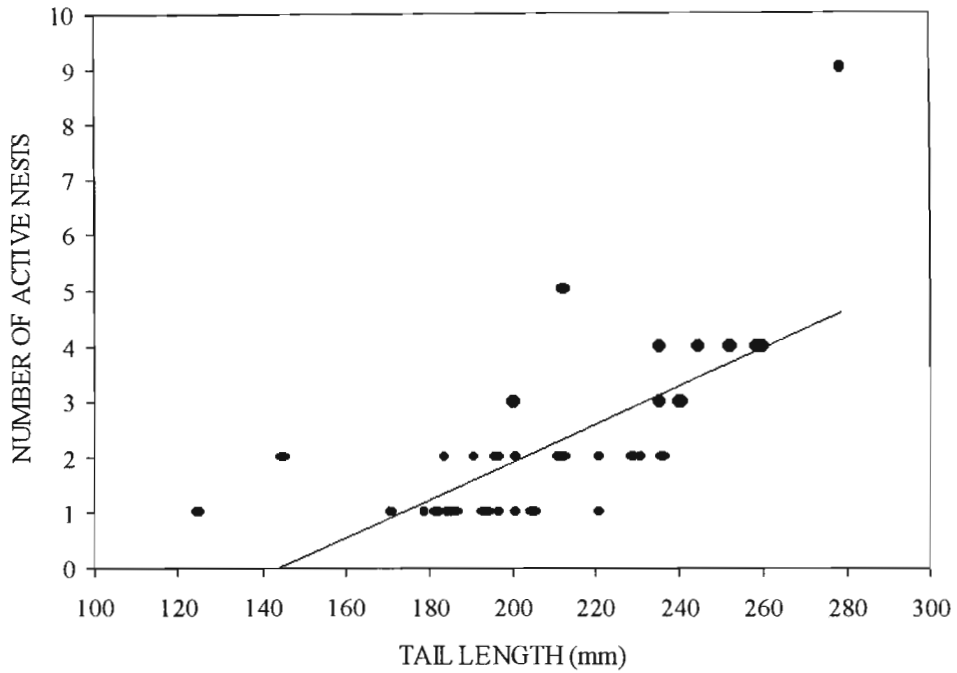


FIG. 3. Mating success (the total number of active nests) plotted against tail length ($y = 0.038x - 4.84$, $F_{1,40} = 17.3$, $r^2 = 47.4\%$, $P < 0.001$).

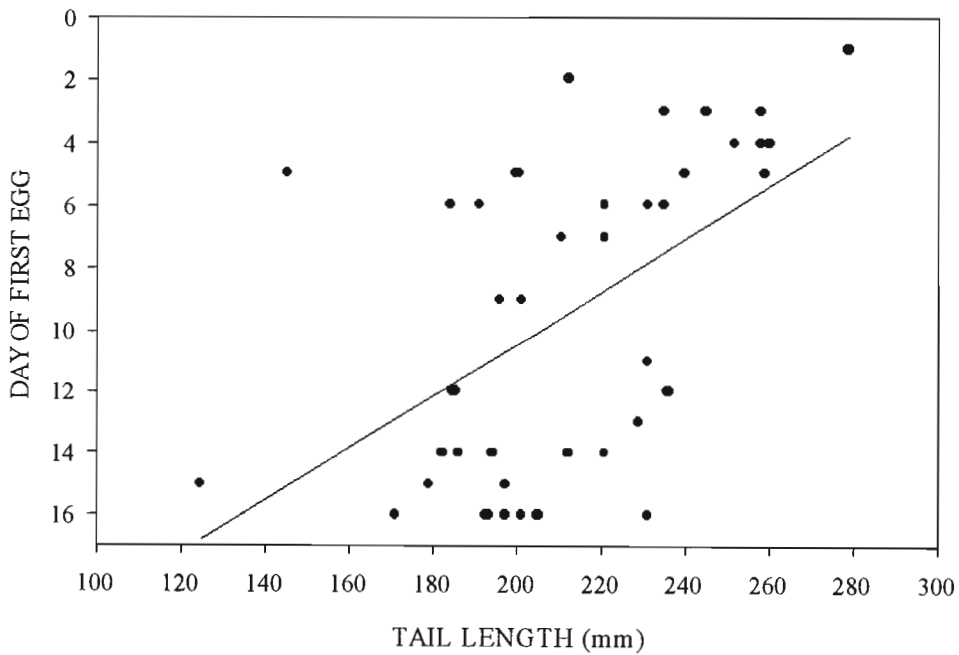


FIG. 4. Average date of egg-laying plotted against tail length ($y = -0.085x + 27.32$, $F_{1,40} = 9.3$, $r^2 = 30.0\%$, $P = 0.0003$). Day one represents the first egg of the nesting season (15 January 2000).

Male Characters and Body Condition

To determine whether the variation in tail length was explained by differences in body condition, the tail length of all full-grown males (no blood quills) captured in the area (including 50 males that were not observed to hold a territory), were regressed onto the body condition index. The significantly positive slope (Fig. 5) indicates an increase in tail length of about 4 cm from the lowest to the highest body condition estimate. There was also a just significant relationship between body condition and collar area ($F_{1,89} = 4.0$, $P = 0.049$, $r^2 = 2.5\%$), but not with other signaling traits.

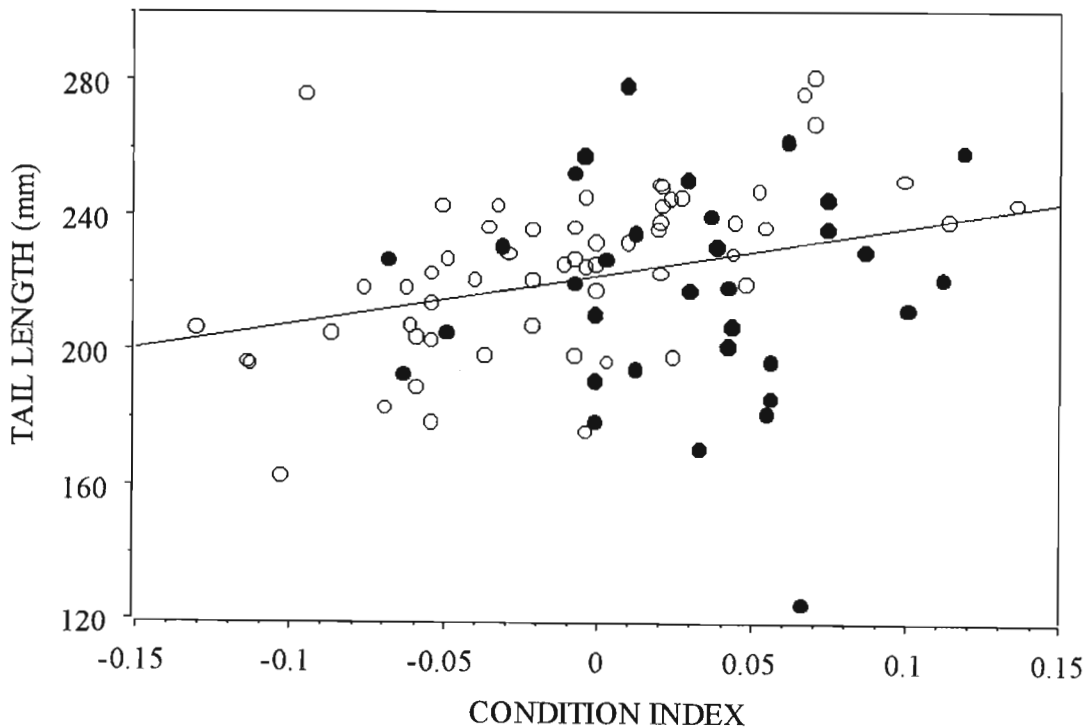


FIG. 5. Linear regression of full-grown tail length (no blood quills) on an index of body condition (residual body mass; see Materials and Methods; $F_{1,90} = 8.49$, $r^2 = 7.9\%$, $P = 0.004$). The sample includes territory-holding males from the selection analysis (filled circles) as well as males captured in the area but not found to hold a territory (open circles).

DISCUSSION

Sexual Selection and Tail Length in Widowbirds

Among the three strikingly displayed plumage ornaments in male red-collared widowbirds (tail length, tail symmetry, and the red collar), as well as other measures of male morphology and territory quality, the selection analyses singled out tail length as the most important correlate of mating success. Although the result is correlational, there are strong indications that female choice is responsible for this unusually strong sexual selection pressure. First, territory size and quality were included in the selection model but were irrelevant. Second, other results and experiments (Pryke et al. 2001; VI; Pryke et al. in press; V) show that the carotenoid collar signal, but not the tail ornament with which redness is negatively correlated (S. Andersson, S. R. Pryke, J. Örnborg, M J. Lawes, unpubl. data; VI), functions as an agonistic signal. Third, the agreement with mate choice experiments in other widowbirds (see below). Fourth, behavioral observations refute the function of tails in male contest competition; for example, two males without tails (lost at capture) had zero mating success (nests) but retained their territories throughout the season.

The selection on tail length was true whether total number of active nests or the order of female settlements were used as a male fitness measure. Our results thus confirm the role of sexual selection through female choice in the evolution of elaborate widowbird tails, and support the findings of a similar sexual selection analysis and a field experiment in Jackson's widowbird, *E. jacksoni* (Andersson 1989, 1992), and the pioneering experiment on longtailed widowbirds, *E. progne* (Andersson, 1982). Savalli (1994), however, found that in the case of the relatively less extreme tails (~ 10 cm) of the yellow-shouldered widowbird (*E. macrourus*), there was no effect of tail length on female choice. Tail plumes instead seemed important in male-male competition, because tail-shortened males were less successful in maintaining their territories (Savalli 1994). One possible explanation is that the substantially shorter tails in this widowbird represent a phylogenetically

older condition (Craig 1980), from which the more extreme widowbird tails have evolved secondarily through female choice (Berglund et al. 1996).

The idea that widowbird tails have evolved through sexual selection has been questioned from the standpoint that genetic drift is sufficient to explain the interspecific variation in tail length among the *Euplectes* (Savalli 1993; Craig and Villet 1998). For several reasons, however, it seems unlikely that the extreme ornamental variation (i.e., from almost no tail elongation in the redshouldered widow, *E. axillaris*, to the 50-60 cm long plumes of the longtailed widow) is the result of random processes. Including this study, evidence for directional sexual selection on tail length has been found in four *Euplectes* species (*E. progne*, *E. jacksoni*, *E. macrourus*, and *E. ardens*). Drift might well have played a role in the history of widowbird tail divergence, but it is reasonable to assume that sexual selection has also been at work, as it clearly is today. In addition, the neutral model here makes the implicit assumption that a major anatomical trait can drift considerably in size (in this case by an order of magnitude) unconstrained by natural selection from, for example, production costs or hampered locomotion. The many translucent stress bars on widowbird tail feathers (S. Andersson 1994) and the laborious flight of male widows in windy conditions, suggest that costs are steep enough to only allow change through persistent directional selection, such as that arising from open-ended mating preferences. It seems likely that sexual selection has played a major role in the divergence of tail elongation within the *Euplectes*, striking different balances between sexual and natural selection in different species.

Costs of Tail Elongation

Long graduated tails are aerodynamically costly, especially in terms of the increased drag from a larger tail area (Norberg 1995; Thomas and Balmford 1995), suggesting that these tail types are reliable signals of male quality (Balmford et al. 1993). Honesty may also be imposed by the substantial physiological costs of tail

growth, as has been argued for Jackson's widowbird, *E. jacksoni*, in which the rapid tail growth starts several weeks before food conditions improve (S. Andersson 1994). Frequent fault bars in rectrices (S. Andersson 1994), present also in red-collared widows (pers. obs.) and longtailed widows, *E. progne* (M. Andersson pers. comm.), and the extremely variable timing of tail completion in all three species are further support for a cost of tail growth (for a detailed discussion, see S. Andersson 1994). Like in Jackson's widow (S. Andersson 1989, 1994), we found a positive relationship between full-grown tail length and body condition in red-collared widows, suggesting that females attracted to long tails also choose viable males.

Signal Content of Long Tails and Carotenoid Coloration?

A recent handicap model suggests that when several traits are honest indicators of quality, females should evolve preferences for a single ornament with the highest combination of honesty and detectability (Schluter and Price 1993). Is the strong female preference for long tails rather than carotenoid displays related to a difference in honesty between the two plumage signals? Further research into the reliability of the signal types is needed, but there are some possibilities in this case. A first possibility is simply that carotenoid pigmentation is not a costly and honest indicator in this species, as suggested for another African weaverbird, the red-billed quelea (*Quelea quelea*), in which Dale (2000) found no relation between red facial plumage and condition or reproductive success. However, bill redness was a strong predictor of male condition and has previously been found to signal dominance (Shawcross and Slater 1983), the same signal function that we argue for the carotenoid display in red-collared widowbirds (see below). In addition, compared to the quelea the carotenoid plumage display is highly variable in redcollared widowbirds (Table 1), and there is evidence for condition-dependence (residual body mass) of both its size and redness in a large sample of territorial and floater males (S. Andersson, S. R. Pryke, J. Örnberg, M J. Lawes, unpubl. data; VI). In

sum, there clearly seems to be scope for costs and honesty of carotenoid signaling in red-collared widowbirds. The question thus remains why females seem to ignore this information in their choice of nesting partner?

Another possibility is that the two ornaments differ in the quality type that they indicate. In contrast to the classic example of female house finches (*Carpodacus mexicanus*) seeking immediate resources and paternal effort (Hill 1991, 1999), good genes are more likely the primary benefit of female choice in red-collared as well as in other widowbirds with uniparental care and minimal or no resources provided by the male. An interesting possibility might then be that the good-genes benefit is larger for tail length compared to carotenoid pigmentation. This means that even if current nutrition or condition would be better predicted by collar redness, the genetic component of viability could be more strongly related to tail length, which thereby should be the target for 'gene-shopping' females. Until ornament heritabilities and their genetic correlations with viability can be estimated and compared, this is of course a highly speculative explanation, but it is important to keep the distinction between direct and genetic signal content in mind. More studies of direct and indirect benefits of different handicap signals are clearly needed.

A third alternative explanation for why females focus on the long tail rather than the red collar might be its higher detectability. In contrast to the relatively small collar patch, the elongated tail plumes enlarge the visual surface of the male by two to three times, making it visible from much farther away than the red collar. To the human observer, the tail display (in flight) is easily detected from at least 100-200 m whereas the red collar is visible only in close proximity (less than 20 m; pers. obs.). Therefore, even if both plumage signals are equally reliable indicators of the same male quality, the strong female preference for exaggerated tails may relate to higher detectability and thus signal efficiency. Building on the model by Schluter and Price (1993), this implies that there is no net benefit (fitness gain minus search cost) for females to also assess the carotenoid display.

Female Preference and Fluctuating Asymmetry

In common with several other recent studies (e.g., Oakes and Barnard 1994; Evans and Barnard 1995; Kimball et al. 1997) our data failed to support the predictions of the sexual selection hypothesis of FA (Møller and Höglund 1991). Although there was strong and presumably perceivable variation in tail asymmetry (Table 1), tail FA was not under directional selection through female choice. Furthermore, asymmetry was not lower in longer tails, nor was tail symmetry related to male condition (see also Goddard and Lawes 2000). The potential reproductive benefits of producing large ornaments may surpass the costs of asymmetrical development and therefore males may attempt to maximize tail ornamentation (Goddard and Lawes 2000). In addition, recent studies have demonstrated that trait size is more responsive to stress than FA (Hunt and Simmons 1997; David et al. 1999; Woods et al. 1999; Björksten et al. 2000), suggesting that tail length is a more reliable quality-indicator than tail FA.

Female Preference and Territory Quality

Females in many polygynous bird species choose males on the basis of their territories or a combination of male and territory characteristics (M. Andersson 1994; Searcy and Yasukawa 1995), but there was no evidence in this study that territory quality or size affected mating success. Neither did the number of cock's nests on a male's territory predict male reproductive success, unlike other studies on related *Euplectes* (Savalli 1994; Friedl and Klump 1999). The male nest building in red-collared widowbirds is restricted to a quickly produced, loosely woven ring of a few grass blades (Craig 1980; pers. obs.). Although females may inspect the nest frames, few or none are used in subsequent nesting. They might constitute a necessary courtship stimulus, similar to the cavities in the display structures of the lekking *E. jacksoni* (Andersson 1991), but without any inter-male variation on which female choice is based.

The Coexistence of Multiple Costly Ornaments

A question with regard to the evolution of multiple ornaments (Møller and Pomiankowski 1993) is how several handicap signals can be maintained rather than converging on a single most efficient (informative and detectable) trait (Schluter and Price 1993; Johnstone 1996). Although multiple good-genes criteria can be stable under special circumstances (Iwasa and Pomiankowski 1994; Johnstone 1995; Johnstone 1996), these theoretical models suggest that multiple ornaments usually are not true handicaps but rather arbitrary Fisherian runaway traits, exploitations of receiver psychology (Rowe 1999) or perhaps amplifiers of a single handicap message (Hasson 1989). In accordance with this, most studies of multi-ornamented species demonstrate or suggest only one, if any, handicap mechanism among the traits under study (for reviews, see M. Andersson 1994; Bradbury and Vehrencamp 1998, for reviews). Similarly in the red-collared widowbird, female mate choice appears almost exclusively directed at male tail length, despite the presence of another strong handicap candidate, carotenoid coloration, as well as a suite of other morphological and behavioral traits.

Our results thus do not support multiple choice criteria (redundant signal hypothesis) for combined assessment of male quality (e.g., Zuk et al. 1990a, 1992; Kodric-Brown 1993; Møller and Pomiankowski 1993) or different aspects of male quality (multiple message hypothesis; Zuk et al. 1990b; Møller and Pomiankowski 1993). Until further breeding seasons are included, we can not rule out the possibility that female choice criteria vary between years as in Marchetti (1998), but like most other mate choice studies we assume that our snapshot of sexual selection is representative.

From field and captive dominance experiments (Pryke et al. 2001; IV; Pryke et al. in press; V), the red carotenoid display appears to function primarily in male contest competition for territories. This is similar to the agonistic function of red epaulets in redwinged blackbirds (*A. Phoeniceus*) and longtailed widowbirds, as well as the red pectoral feathers in scarlet-tufted malachite sunbirds (*Nectarinia johnstoni*; see review by M. Andersson 1994). In this situation, multiple handicaps

may result by neither the redundant signaling nor the multiple message hypotheses, but from selection by different receivers (the multiple receiver hypothesis). Separate agonistic and epigamic plumage signals have been identified in several bird species, for example, malachite sunbirds (Evans and Hatchwell 1992a,b), ring-necked pheasants (*Phasianus colchicus*; von Schantz et al. 1989; Mateos 1998), and the yellow-browed leaf warbler (*Phylloscopus inornatus*; Marchetti 1998). With different receivers 'locking in' to different signals, these signals do not 'compete' over the same response and could thus be selected for efficiency (honesty X detectability sensu Schluter and Price 1993) within their own context. They might, however, compete in terms of sender investment, as indeed seems to be the case in red-collared widowbirds (S. Andersson, S. R. Pryke, J. Örnborg, M. J. Lawes, unpubl. data; VI). The important point, however, is that two handicap signals can coexist because they are selected by different receivers, in this instance female and male conspecifics, but it could also be, for example, different age classes, predators, or prey. The multiple receiver hypothesis is by no means a new idea for multiple ornamentation (e.g., Butcher and Rohwer 1988; M. Andersson 1994; Savalli 1995), but it has been neglected in the recent literature on multiple ornaments (e.g., Møller and Pomiankowski 1993; Johnstone 1996).

Conclusions

Despite theoretical predictions that multiple handicap signals are unlikely to be stable (Schluter and Price 1993; Iwasa and Pomiankowski 1994; Johnstone 1996), the red-collared widowbird possesses two classic quality-indicating avian ornaments; a long graduated tail and a red carotenoid display, as well as a number of other potential signal traits. We have shown that females base their mate choice almost exclusively on tail length, which explains a uniquely large proportion (47%) of the polygynous mating success of males. The red color signal is instead likely to function as an honest status signal in male contest competition (Pryke et al. 2001; IV). We propose that the multiple receiver hypothesis, in which multiple handicaps

coexist because they are selected by different receivers, might be a common explanation for multiple ornaments in birds. We have also argued that in systems like this, where females receive little or no direct benefits from choosing high-quality males, exaggerated anatomical traits such as long graduated tails might be a more reliable indicator of heritable genetic quality than carotenoid coloration. In status signaling, heritability is not an issue and signal expression (the carotenoid display in this case) need only be a phenotypically honest signal of, for example, fighting ability or (when applicable) other direct benefits such as parental investment. Studies of genetic versus phenotypic signal content of different types of ornamentation are needed.

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CHAPTER IV

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

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 keeled tail 'flying' models (i.e., courtship display) than folded tail 'flying' models suggest the tail display is used to assess the intention of intruding males. Territory owners were less aggressive towards models with intense collar displays, 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.

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, due 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 and Rohwer 1986; ring-necked pheasants *Phasianus colchicus*: Mateos & Carranza 1997; red jungle fowl *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 sparrowy-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. 2001; III). 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 onto 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, among the territorial males, mating success (number of nests) was found to be strongly determined by tail length, whereas collar size and colour had no or even negative effects on the attraction of nesting females (Pryke et al. 2001; III). Second, floaters had significantly smaller and yellower collars than the territory owners (Andersson, S., Pryke, S. R., Örnborg, J. & Lawes, M. J. unpublished manuscript; VI). However, the above results were purely correlational, which is why experimental tests of the signal function are required.

To demonstrate agonistic signalling, it is necessary to 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 bird species. However, most 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 only affect the behaviour of the target individual. In this study, 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 is 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 mistnets 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) length 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 inter-correlated, we used the first components of a principal component 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 ($r_s=0.34$, $N=185$, $P<0.001$), an index of body condition was calculated based on tarsus length, which is a good indicator of skeletal size (Freeman & Jackson 1990). Body condition was expressed as the residuals from $\log(\text{body mass})$ linearly regressed on $3\log(\text{tarsus length})$ (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 present on the wing and tail feathers.

Collar Measurement and Colourimetrics

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 $\pm 2\text{nm}$ resolution) from the red collar was measured with a PS1000 spectrometer (Ocean Optics Inc., Dunedin, FL), using a HL2000 halogen light source, and a fibre-optic 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, NV), five consecutive scans (removing the probe between each scan) were taken from the centre of the collar patch and averaged 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 350-700 nm; (2) 'Hue' (λ ; spectral location) estimated as $\lambda(R_{50})$, the wavelength at which reflectance is halfway between its minimum (R_{\min}) and its maximum (R_{\max}); and (3) 'Chroma' (c ; 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. (2001; III).

Territorial Behaviour

We mapped the territories of 43 colour-banded males using 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. Non-territorial 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 onto 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 minutes) when males defended territories aggressively from conspecifics. A random-number table was used to determine the order in which males were observed. All activities and their durations were continuously recorded onto a dictophone with the time given to the nearest five seconds. Data on the time spent active on a territory, the time in territory defence, the number of aggressive interactions, encounter length and fights were extracted for analysis.

Model Experiments

We made nine models from skins of adult male red-collared widows. The nine males were captured from the study population (ca. 900 widowbirds) and

euthanised using affixation (i.e., rapid euthanasia within ca. 10 s with an increasing concentration of carbon dioxide gas) at the University of Natal, South Africa. This procedure was carried out in accordance with the guidelines proposed by the University of Natal Ethics Committee on approved protocols for animal research and with permission from the KwaZulu-Natal Conservation Services, South Africa. The first set of experiments was 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 are highly correlated (Fig. 1), with the product of these two dimensions contributing to the greatest difference between floating and territory owners (Andersson, S., Pryke, S. R., Örnborg, J. & Lawes, M. J. unpublished manuscript; VI). Therefore, the model collars were selected from the natural variation in this combined signal measure. Separating the independent effects of colour versus size was not our goal in this study, but rather to mimic the natural variation in carotenoid display intensity. In other words, one model category displayed a small yellow collar (S_Y), the other an average sized orange collar (A_O) and the last a large, red collar (L_R). S_Y collars were made by blackening the yellow feathers with a marker pen (Sanford Sharpie permanent marker, Mesa, Arizona) to reduce the collar area to the lower limit of natural variation collar size (Fig. 1). A_O collars were chosen from the population to represent the average collar area and colour (Fig. 1). The model with a L_R was constructed by gluing supplemental feathers taken from other live birds with similar red coloured collars onto 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' (either with the tail 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. These models were fastened onto wooden doweling that could be inserted onto 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). Keeled tail models were made by setting and gluing the tail feathers in a deep vertical keeled position during the taxidermic procedure, and folded-tailed models by positioning the tail feathers directly behind the body. The tail was shortened (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.

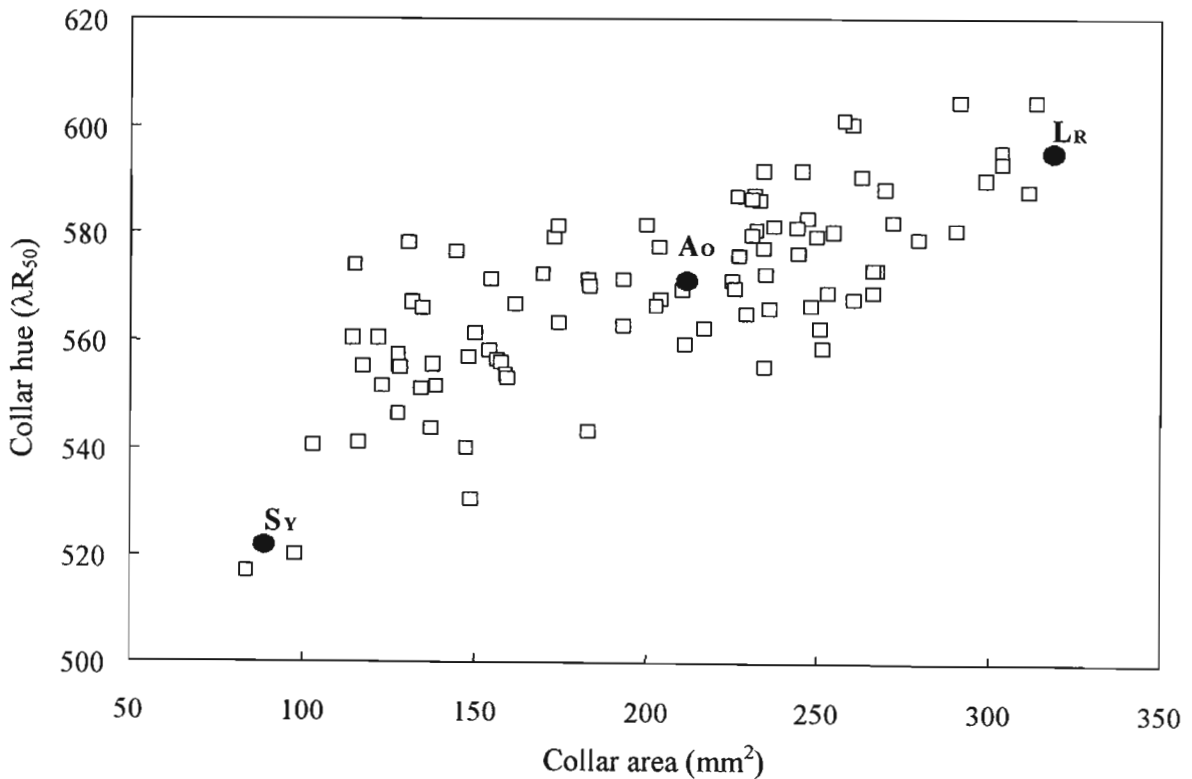


Figure 1. Correlation between collar area (mm^2) and colour (hue; $\lambda[\text{R}_{50}]$) for the population ($r_s=0.76$, $P<0.001$, $N=93$, $R^2=57.2\%$), showing the three collar categories (S_Y : small yellow, A_O : average orange, L_R : large red) for the models.

We randomly presented the nine models (i.e., three perched models, three folded-tail models and three keeled-tailed 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 at three different temporal intervals in the breeding season. The first coincided with the pre-nesting period about two weeks before the first nest was located (20 December 1999 to 11 January 2000), the second during the most intensive nesting period when the majority of nests had their first eggs (12 February to 4 March 2000), and the last at the end of the breeding season, when most of the nestlings had fledged (2 to 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 three to four hours following 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 the model was detected. We recorded ten-minute 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.). 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 an hour before the experiment began to minimise 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 the territory. 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, the distance of the closest approach and the response time (the time in seconds from when the model was displayed until the territory owner approached the model) was measured. 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 a period of 1-2 days to minimise the possibility of habituation. Randomising the order of presentation for both the posture and collar parameter model categories also experimentally controlled the variation in response due to 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 Results). 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 pre-nesting, nesting and post-nesting phase). Although red-collared widow males do occasionally chase red-shouldered widows (and vice versa), these two species defend overlapping territories in the area 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 inter-related 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 using 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 (pre-nesting, nesting and post-nesting). 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 Scheffè'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 is related to the size ($F_{1,42}=32.19$, $P<0.0001$, $R^2=45.9\%$) and colour ('hue'; $F_{1,32}=21.52$, $P<0.0001$, $R^2=41.1\%$) of the resident male's collar. Resident males signalled territory boundaries by sitting prominently on the boundary with their culmen directed forward and upwards, body feathers slightly ruffed (especially on the chest) and tail partially spread. Intrusions by other males onto occupied territories were common. Territory owners tended to focus all attention on intruders and commonly performed boundary displays, with their chest (and collar) ruffed 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 toward intruders within 28.5 ± 79 s ($\bar{X}\pm SE$, $N=486$) after the intruder had landed. Following attack the resident male may pursue 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.

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

Male trait	Territorial behaviour variables					<i>N</i>
	Active time on territory	Time spent conspicuous	Time in territory defence	Number aggressive interactions	Encounter length	
Territory area	-0.32	-0.25	-0.59***†	-0.37	-0.33	43
Tail length	0.04	0.07	0.13	0.17	0.08	43
Body size (PC1)	-0.28	-0.11	-0.22	-0.31	-0.36	43
Collar area	-0.73***†	-0.52***†	-0.44***†	-0.65***†	-0.46***†	43
Collar chroma	-0.17	-0.21	-0.11	-0.14	-0.41*	33
Collar hue	-0.51**†	-0.41*	-0.38*	-0.47***†	-0.48*	33
Collar brightness	0.08	-0.02	0.09	-0.23	0.06	33
Body condition	-0.22	-0.17	-0.21	-0.43*	-0.34	43
Ectoparasite load	-0.13	-0.19	-0.24	-0.29	-0.28	43

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

†Significant after Bonferroni adjustment at $\alpha = 0.05$

The four measures of territorial behaviour provided an indication of the relative amount of time spent by residents 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). 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, redder and larger collared males spend less time in aggressive encounters and receive 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 date of breeding during which it was presented (Table 2). For all model types, males were significantly more aggressive during the nesting period compared to the pre-nesting (Scheffè test, $P < 0.001$) and post-nesting (Scheffè test, $P < 0.001$) stage. 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 presentation time*collar 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. Likewise, the 'flying' red-shouldered widowbird model was generally ignored and although four males did react (9%), none of them approached the model closer than three metres. The keeled-tail models were approached significantly quicker than were 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. 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 (Scheffè test, $P < 0,001$) and attacked more than the flying ones. In addition, although not significant, males approached keeled tailed models with smaller yellow collars closer than other collar types (collar*model interaction; Table 2). Several owners (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 significant 2- or 3-way interaction terms involving model type.

Table 2. Repeated measures analysis of variance (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. Only significant interactions are reported

Source of variation	Reaction time			Approach distance		
	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
<i>Between males</i>						
Presentation time (pre-nesting, nesting and post-nesting)	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 _V), 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
<i>Within males</i>						
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

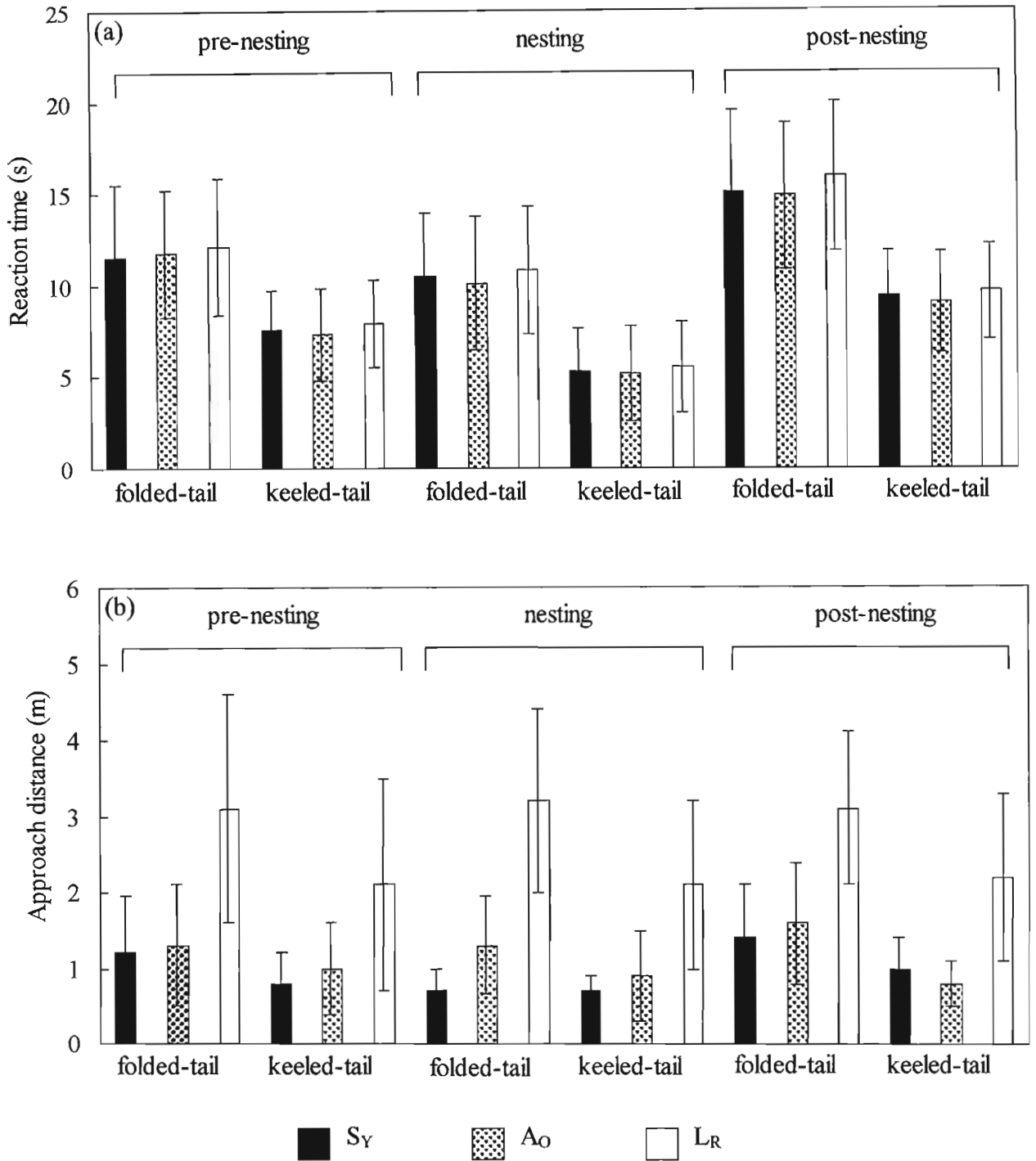


Figure 2. The mean (±SD) residents (a) reaction time and (b) distance approached to the different model postures (folded-tail and keeled-tail) and model collars (S_Y: small yellow, A_O: average orange, L_R: large red) during the three presentation times (pre-nesting, nesting and post-nesting).

Effect of colour signal

There was no significant difference within the three different 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' $\lambda=0.71 - 0.93$, $F_{6,8} = 1.3 - 2.9$, *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) so that males approached the S_Y and A_O collars closer than the maximum colour signal (Scheffè 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 owners 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 models 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) was 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.

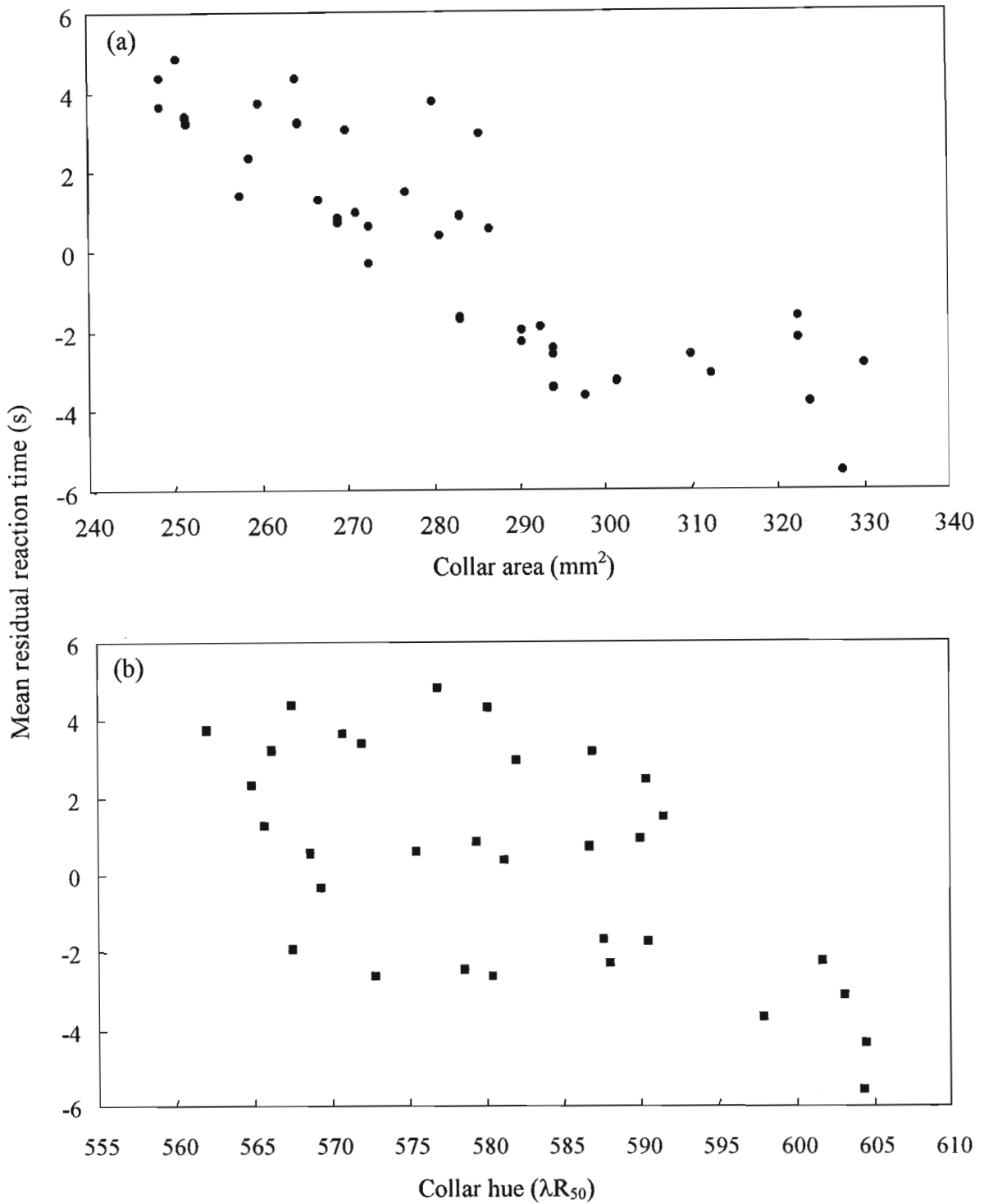


Figure 3. The mean residual reaction time of each male to the models in relation to the (a) size ($F_{1,42}=102.39$, $P<0.0001$, $R^2=72.9\%$) and (b) colour ($F_{1,32}=18.84$, $P<0.0001$, $R^2=38.8\%$) of the resident male's collar. The mean residual measures for each male were derived while controlling for the effect of presentation time, model type and model collar (i.e., residuals derived from the RM-ANOVA model; see Table II). The higher residual values indicate a relatively longer time taken to react to the models.

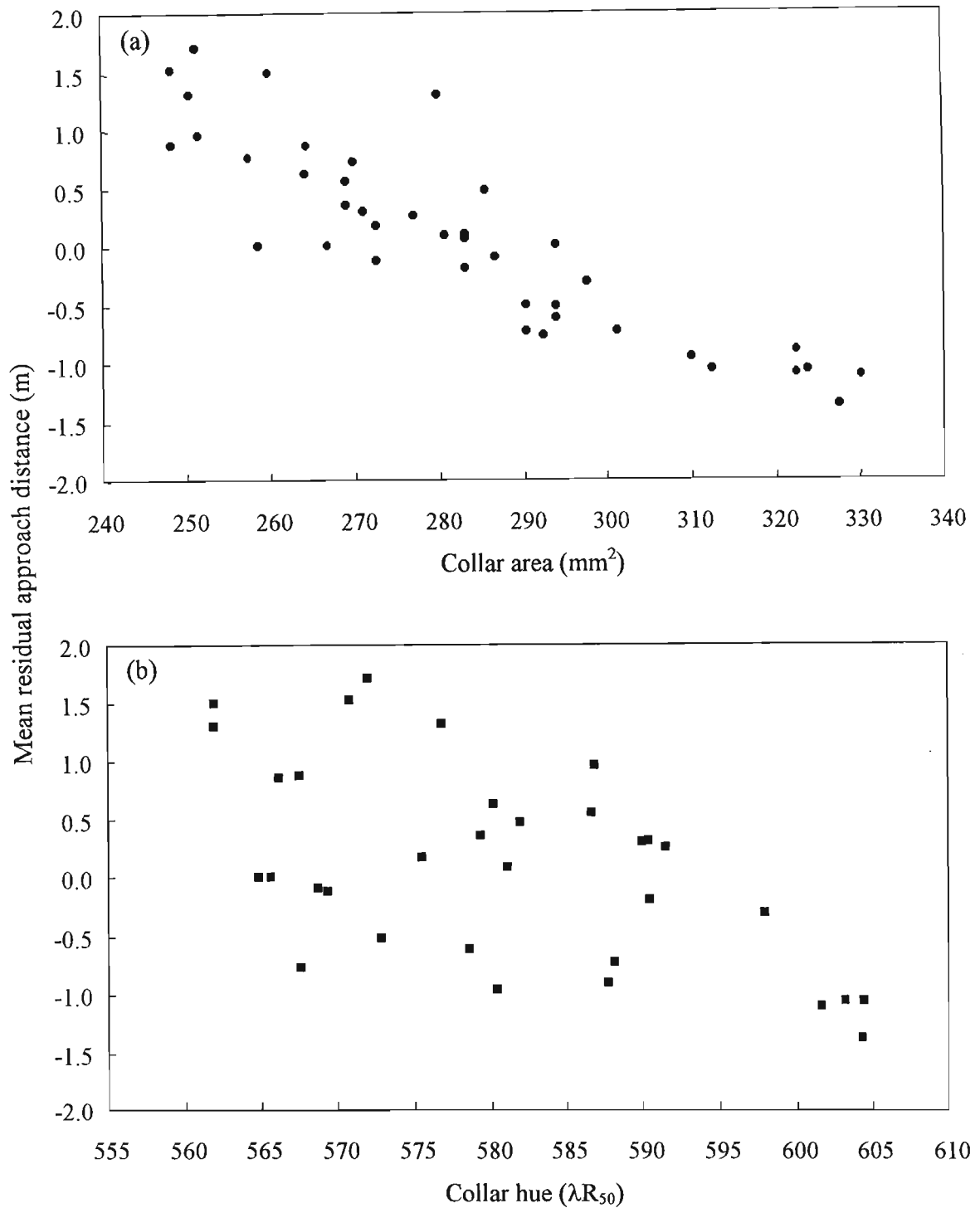


Figure 4. The mean residual proximal distance of each male to the models in relation to the (a) size ($F_{1,42}=143.43$, $P<0.0001$, $R^2=79.1\%$) and (b) colour ($F_{1,32}=15.17$, $P<0.0005$, $R^2=32.9\%$) of the resident male's collar. The mean residual measures for each male were derived while controlling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

Response in relation to morphometrics and aggression

Several of the male morphometric characteristics were inter-related and therefore a step-wise multivariate regression analysis was used 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 standardised (mean 0, variance 1) to obtain comparable parameter estimates (β) and the residuals did not depart from a normal distribution in any of the models (Komogorov-Smirnov tests; $D=0.43 - 0.97$, $P=0.3 - 0.8$). Reaction time (as the dependent variable) was log-transformed and primarily related to collar area, which accounted for more than 36% of the variation (Table 3). 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 lower levels of effort in territorial maintenance, advertisement of residency, and agonistic interactions but displayed a higher aggressive response to the model presentations.

Table 3. Multiple step-wise regression model of the best combination of characters for predicting aggression (reaction time ($P < 0.001$, $R^2 = 67.3\%$) and closest distance approached ($P < 0.001$, $R^2 = 69.1\%$) to the models) and dominance ($P < 0.001$, $R^2 = 70.8\%$). All measurements were standardised to obtain comparable parameter estimates (β)

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

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

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, the experimental results showed 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 the relatively 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 conflicts than males with

relatively smaller collars. The increase in male aggressiveness is likely 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 and/or 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 1975, 1982; Senar 1999). Status signalling is likely to be advantageous between strangers in territorial systems when there are sufficiently high rates of intrusion by strange birds, since neighbours supposedly have knowledge of each other (Rohwer 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 the large-collared models were more successful in deterring aggressive interactions from resident males, birds with stronger (larger and redder) carotenoid signals appear to more effectively defend their territories. This suggests that there should be strong directional selection for enlarged badge size. What 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; III), suggests a developmental trade-off between the two costly plumage signals (Andersson, S., Pryke, S. R., Örnborg, J. & Lawes, M. J. unpublished manuscript; VI).

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 (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 destabilise 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 colouration has potential direct honest-enforcing production costs. As carotenoid pigments cannot be synthesised and have to be acquired from the diet, carotenoid-based trait expression is related to the males 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 males foraging ability, nutritional condition, parasite susceptibility and immunocompetence ability could

all influence carotenoid expression (Lozano 1994; Olson & Owens 1998; Hill 1999). Carotenoid coloration might be a good indicator of the fighting ability since carotenoid pigmentation contains information about several aspects of male vigour (Dufva & Allander 1995; Bortolotti et al. 1996; Endler & Houde 1995; 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 colouration 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 the 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 and 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 an agonistic signal 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 relatively lower amount of 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 measurements may provide better resolution of intra- as well as intersexual signal functions of carotenoid displays in the future.

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CHAPTER V

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

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

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 non-breeding 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 prior to and after territory establishment. 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*: dominance, carotenoids, status signaling, male-male competition, plumage coloration, *Euplectes ardens*.

One 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; Shrawcross 1983), the few experimental tests demonstrate no or even negative effects on male dominance (Belthoff et al. 1994; 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 non-breeding 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 non-breeding season these African weaverbirds (subfamily Ploceinae) are sexually monochromatic and drab brown. Prior to 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., 2001a; IV). 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 prep; VI). 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., 2001a; IV). 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., 2001b; III). 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, since 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 territorial contests, we conducted two manipulative experiments. First, in standardized dominance trials in captivity, we experimentally painted ‘collars’ onto the brown plumage of captive, non-breeding males and conducted paired trials that pitted two unfamiliar birds with different collar treatments against each other in competition over food. As 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 (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 onto the breeding grounds (i.e. 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. Since 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 are easy to accurately age as adult breeding males. Birds were housed in three outside aviaries (1.5 m wide x 2.7 m long x 2.2 m high) visually isolated from each other over the winter months, so that individuals had six 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), a principal component analysis (PCA) was used 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 condition. The experimental collar size (chest patch) was calculated (to the nearest 0.1 mm²) as the product

of the maximum width of the collar across the chest, and the average of three height measures when holding the head in a normal position. Repeatabilities (Lessells and Boag 1987) of the collar measurements were highly significant ($r = 0.88$, $F_{63,46} = 18.7$, $p < .001$).

In the experiments that follow we tested if the collar color, size or both were sufficient to establish dominance. Male birds were manipulated prior to 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, non-breeding 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 Inc., Tokyo, Japan) were used for painting the collars onto the brown throats of non-breeding males. The orange (YR09) and red (R29) pens provided the best match to the natural variations in plumage reflectance (Figure 1). Control birds were painted with a similar but transparent pen (Code 0 colorless). To control for the novelty of adding a collar to the brown plumage of non-breeding males, as well as a potentially independent effect of collar presence irrespective 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 \pm SD = $210.1 \text{ mm}^2 \pm 59.4$, $n = 125$; collar treatments $208.3 \text{ mm}^2 \pm 4.7$, $n = 192$), whereas small and large collars were painted to correspond to the lower (82 mm, $n = 125$; collar treatments $85.3 \text{ mm}^2 \pm 3.2$, $n = 144$) and upper limit (328 mm, $n = 125$; collar treatments $321.7 \text{ mm}^2 \pm 3.6$, $n = 144$), respectively, of the natural variation of collar size (Pryke et al., 2001a; IV). Once the experiment was halted, the manipulated collars were removed from the experimental bird (with alcohol) to prevent familiarization with the manipulations and all birds were returned to their aviaries to maintain a standardized housing setting for all males.

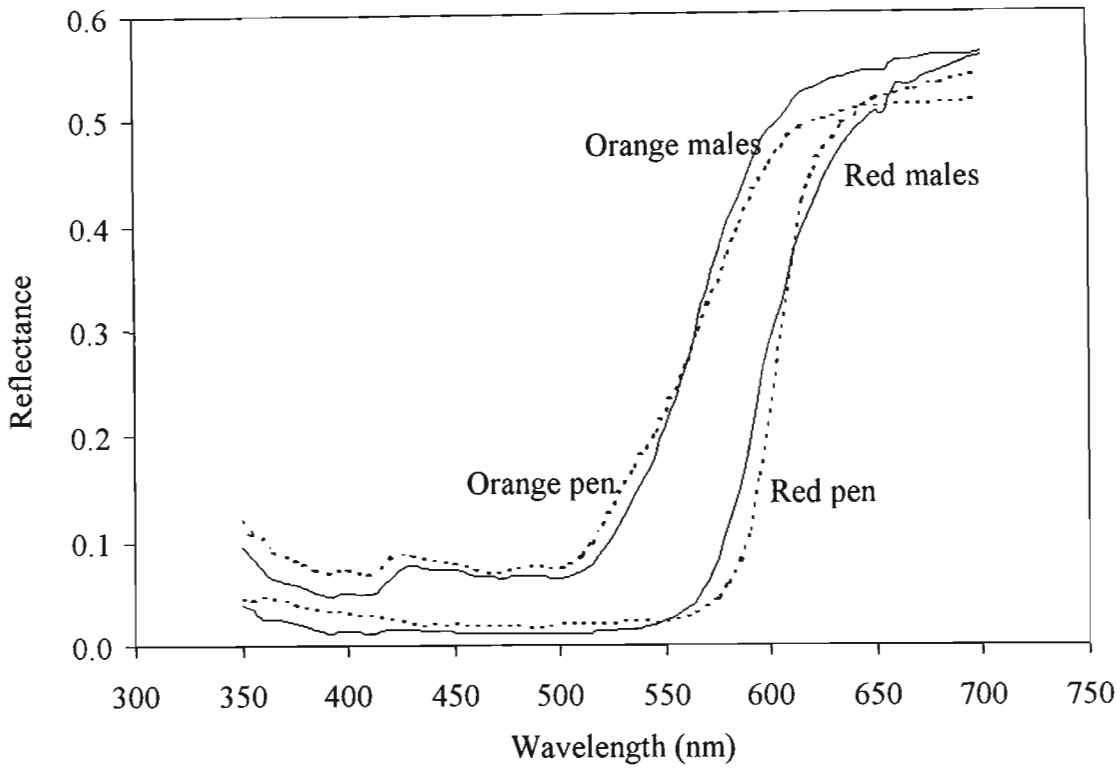


Figure 1

Reflectance spectra for the natural breeding collars and the manipulated plumage of brown non-breeding male red-collared widowbirds. Reflectance for the natural collars (—) shows the most extreme ‘red’ collared male and the average (‘orange’) collared males in the population ($n = 125$). The manipulated collars (---) were painted with Copic orange and red pens selected to correspond closely to the natural red and orange collars of the birds.

Thirteen different combinations of collar-treatments were tested (see Figure 2) with 12 different individual dyads within each combination, i.e. a total of 156 trials (using 24 birds). In each 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 thirteen experiments, but never more than once against the same opponent. In each trial the member of a pair to be manipulated was also chosen at random, and to further remove confounding individual differences, each trial was later repeated 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). Dominance trials were conducted in the first four hours following sunrise. The two males were transferred to an unfamiliar experimental cage (0.8 m wide x 2.1 m long x 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 four minutes. Trials were run for 15 minutes, during which all interactions were recorded. 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). Supplants or the displacement of an individual from the food dish were scored 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

Twenty-four males were implanted with 8 mm implants made from silastic tubing (Dow Corning; internal diameter 1.47 mm, external diameter 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 anaesthetized by inhalation of Isoflurane in oxygen (induction 2 %, maintenance 1.5 %, flow rate 0.3 ml.min⁻¹). A small incision was made in the skin of the abdominal cavity, and the implant was inserted to lie along the flank before the skin was closed with a surgical suture (Mersilk, Ethicon). Birds awoke within three minutes 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 ca. 1140 m). Males were captured using mist nets at communal roosts between the 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 principal component analysis for body size (as detailed above) among free-ranging birds accounted for 77.2 % of the variation in the three body size measures.

Collars were experimentally manipulated 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. Collars were manipulated 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, orange or red Copic pens were applied 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* ($n = 14$). Here the collar was enlarged to about 335 mm² by painting the bleached 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 e.g. Grether, 1996), we wanted to test whether the presence of the collar was essential to territory establishment. (6) *Control collar group* ($n = 14$). Here 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: $F_{5,87} = 2.26$, $p = .52$).

Territories were mapped using the minimum convex polygon method (Odum and Kuenzler, 1955) about 6 days after manipulation. Territory boundaries were estimated by mapping the perches of displaying owners and locations of aggressive interactions between territorial males. The territory maps were digitized and ArcView Ver. 3.0 (ESRI, 1993) was used to estimate territory area. Over six weeks, each experimental and control male was observed weekly for a 15-minute period (i.e. a total of 90 minutes per male) during the first four hours of daylight (when activity is the highest) to quantify the frequency and duration of aggressive interactions with other males.

Table 1

Mean (\pm 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 those defending already established territories

Collar treatment	Size (mm ²)		Hue (nm)		<i>n</i>
	Before	After	Before	After	
<i>Males establishing territories</i>					
Reduced orange collars	218.6 (\pm 60.3)	81.4 (\pm 3.4)	561.8 (\pm 29.5)	568.5 (\pm 2.7)	14
Reduced red collars	211.9 (\pm 81.2)	82.9 (\pm 4.8)	569.4 (\pm 37.2)	591.5 (\pm 3.1)	14
Enlarged orange collars	229.3 (\pm 72.7)	339.8 (\pm 3.7)	558.7 (\pm 35.2)	571.2 (\pm 2.9)	14
Enlarged red collars	216.1 (\pm 68.2)	333.4 (\pm 5.8)	553.9 (\pm 41.3)	596.2 (\pm 2.2)	14
Blackened collars	223.7 (\pm 82.5)	0	563.5 (\pm 32.1)	0	15
Control collars	203.4 (\pm 78.3)	203.4 (\pm 78.3)	569.9 (\pm 24.8)	569.9 (\pm 24.8)	14
<i>Males on established territories</i>					
Orange collars	224.1 (\pm 60.3)	210.5 (\pm 3.7)	571.3 (\pm 42.1)	570.4 (\pm 3.1)	12
Red collars	218.6 (\pm 85.2)	208.3 (\pm 4.5)	568.2 (\pm 21.5)	597.3 (\pm 2.5)	12
Control collars	216.4 (\pm 71.5)	216.4 (\pm 71.5)	567.8 (\pm 36.1)	567.8 (\pm 36.1)	11

Manipulation of males on established territories

Male collars were also manipulated after territory establishment to test if 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 re-established territories in the area (from 11 to 28 December 2000). During this time behavioral observations for 15-minute periods were recorded 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 feathers 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 mm^2 were painted onto the males. (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 the territories were remapped and the behavioral observations repeated. Treatments were randomized between all males so each male acted as its own control. Since 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

Spectral reflectance (at $\pm 2 \text{ nm}$ resolution) from the experimentally manipulated and natural collars were measured using a PS1000 miniature diode-array spectrometer (Ocean Optics Inc., Dunedin, USA), 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. Reflectance was measured using the C-spec software (Ancal Inc., Las Vegas, 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. (2001b).

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 the territorial and behavioral activities. The test statistic Wilks' lambda (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, Scheffè's multiple comparison tests were used to test which treatments contributed to the significance. Only significant interaction terms ($p <$

.05) were included in the models. No transformations were required as 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 change 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, namely 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 as red collars dominated all other treatments, irrespective of size (Figure 2). However, there was also an effect of collar size since 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, trial sequence (i.e. the initial or reversed collar treatment of the pair) had any effect on the outcome, except for one bird which was subordinate in all

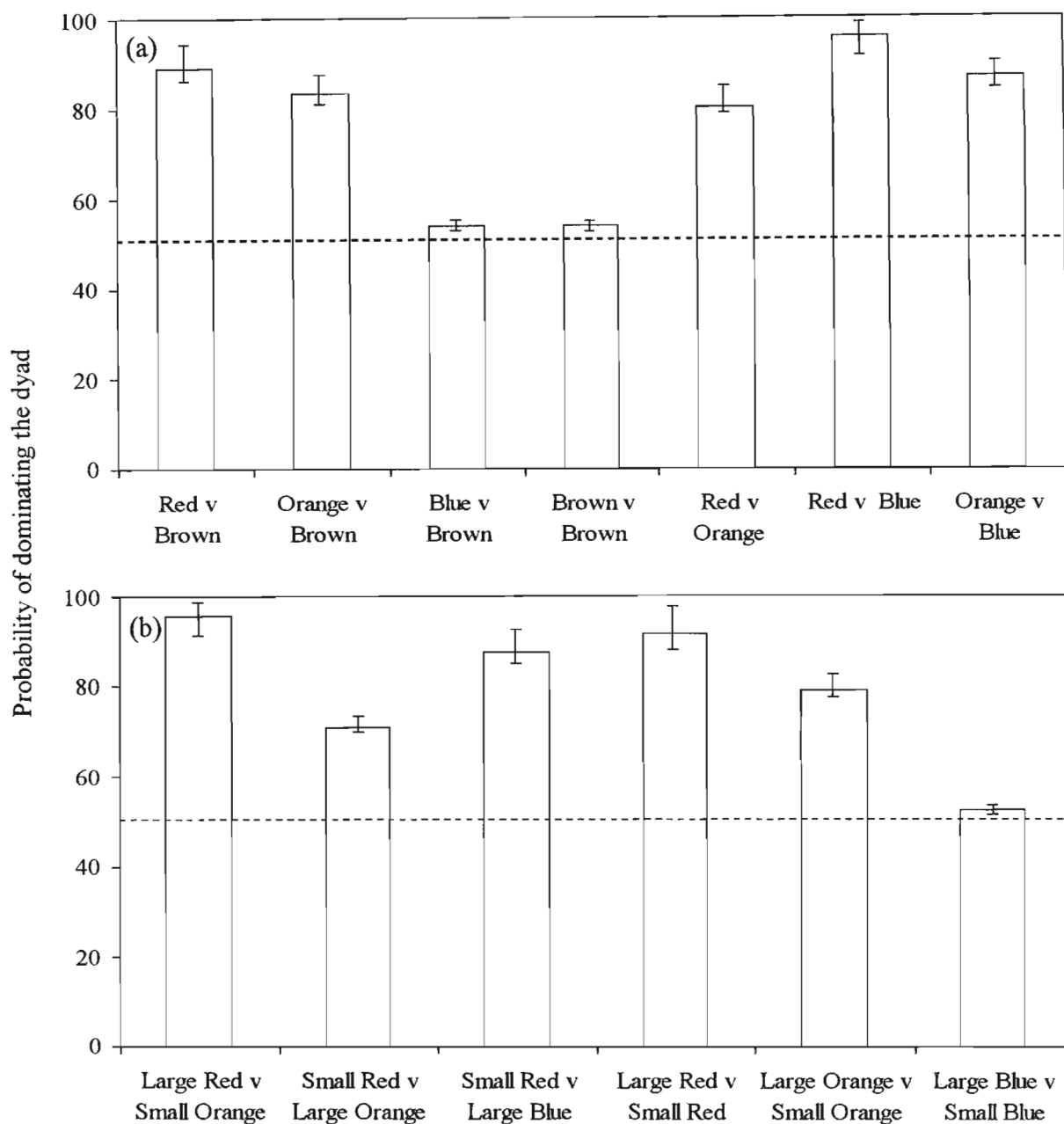


Figure 2

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; $\text{Probability} = \text{EXPONENT}[\text{coefficient}] / [1 + (\text{EXPONENT}(\text{coefficient}))]$) for (a) different colored but similar sized collar treatments, and (b) different sized and 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.

contests. Therefore, irrespective of second order effects, red-collared males were more likely to win contests.

The collar treatments also affected the type of interaction, 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 brown males had a similar probability of dominating in a dyad, whereas the testosterone males strongly dominated the brown- and blue-collared males (Figure 3). Very 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.

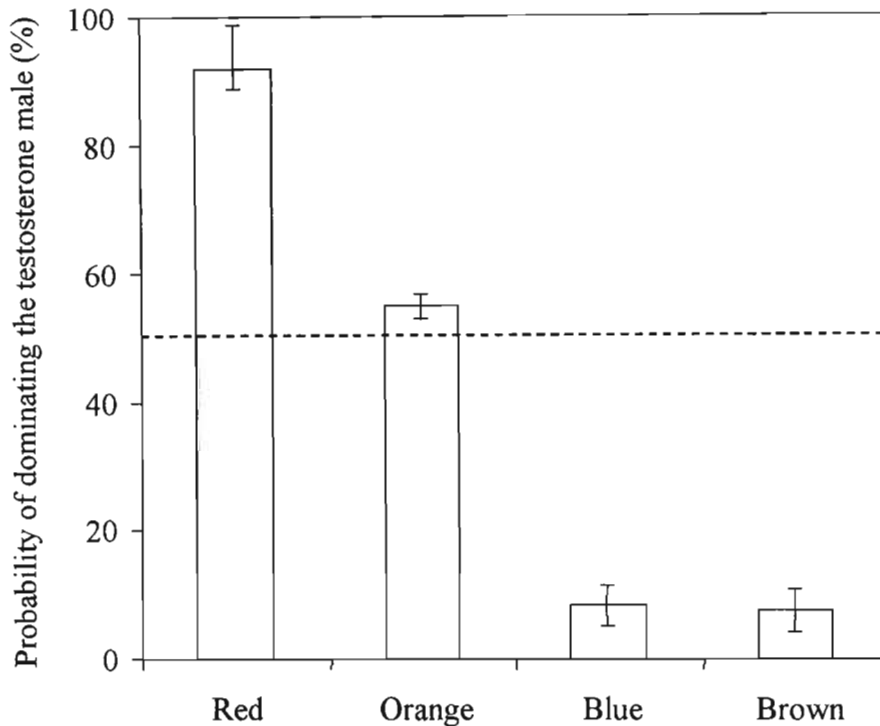


Figure 3

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.

Territory experiments

Effect of collar manipulations on territory establishment and size

After the collar manipulations, eight 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 three of the 14 reduced orange-collared males ($p = .29$), all 14 males with enlarged red collars ($p = .40$), nine of the 14 enlarged orange collars ($p = .99$) and 11 of the 14 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 as 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' Lambda = 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 or not 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 treated 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.

Table 2
MANOVA to determine the effect of the collar treatments on territorial behaviors

Territorial behaviors	<i>F</i>	<i>df</i>	<i>p</i>
<i>Males establishing territories</i>			
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
<i>Males on established territories</i>			
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 prior to establishing territories (Wilks' Lambda = 0.41, $F_{24,116} = 2519.3$, $p < .001$) and males manipulated after territorial establishment (Wilks' Lambda = 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.

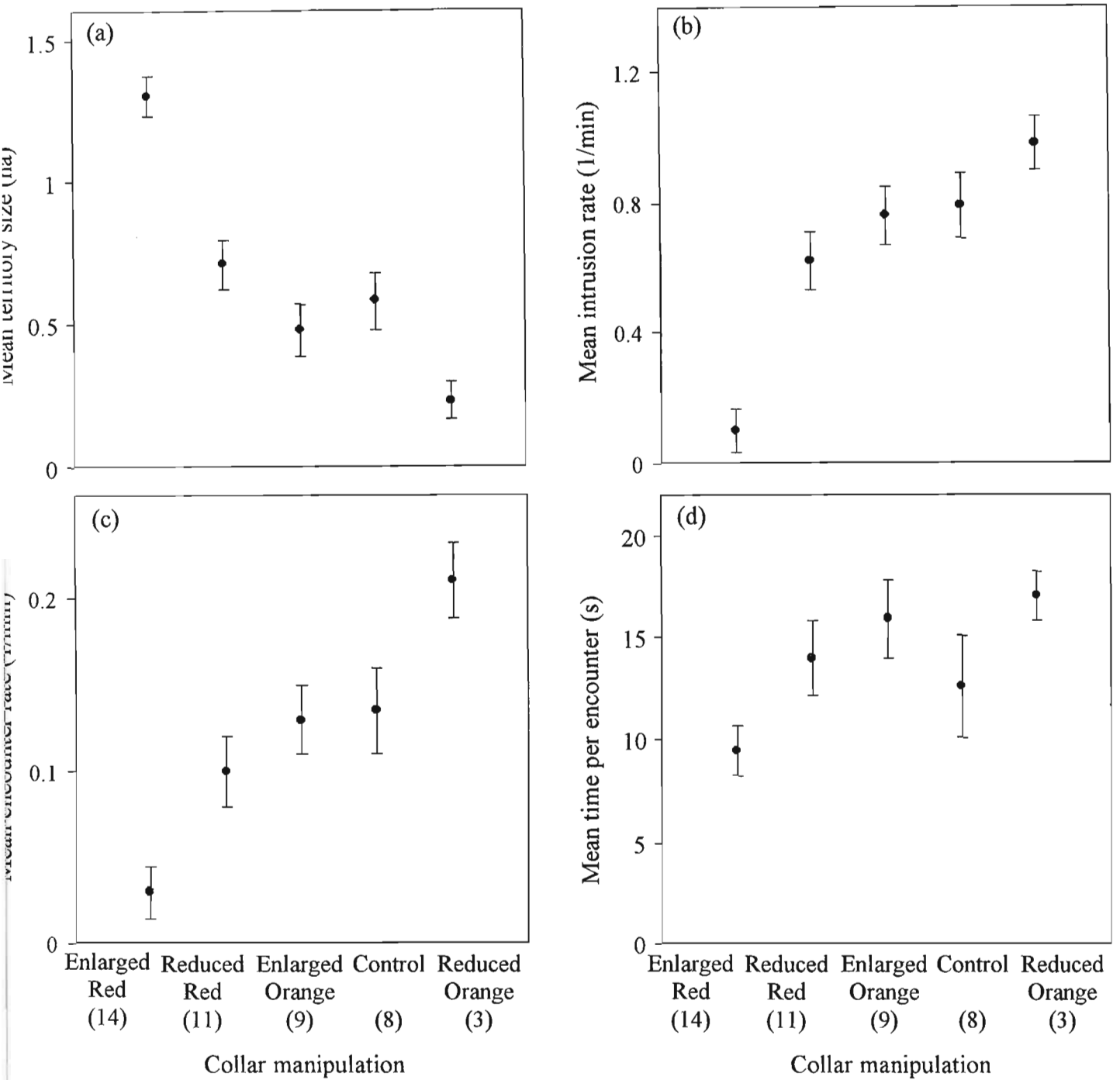


Figure 4

The significant effects of the collar manipulations (see Results and Table 2 for significance tests) on the mean (\pm 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.

Effect of collar manipulations on males holding territories

After the second round of collar manipulations on males that already had established territories, five of the 12 orange-collared males but only one of the 12 red males lost their territories within six 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-rank test 2-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 ($z = 0.19$, $n = 11$, *NS*), 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.

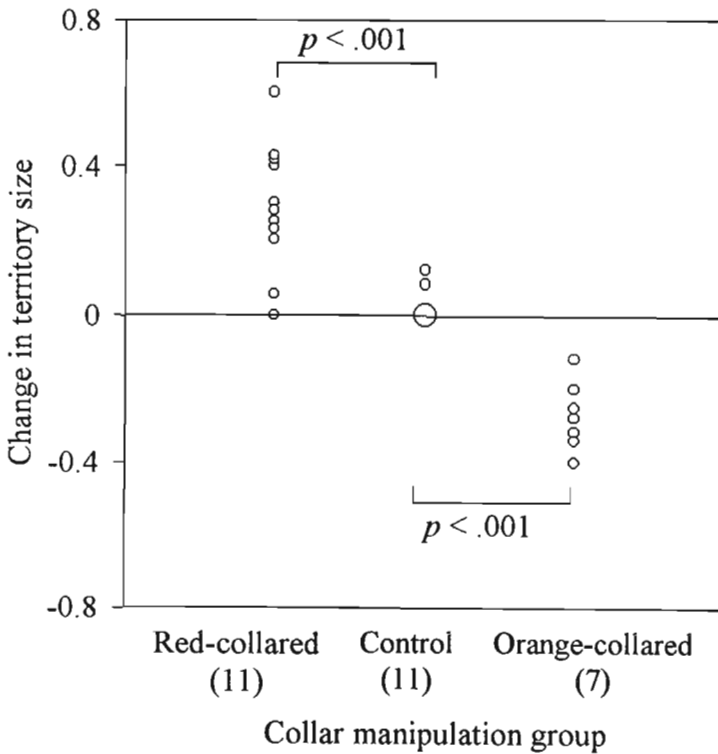


Figure 5

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).

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' Lambda = 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 between 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-rank 2-tailed, control $z = 1.42$, $n = 11$, *NS*, orange $z = 0.92$, $n = 7$, *NS*, red $z = 1.86$, $n = 11$, *NS*), the red-collared group spent less time in boundary signaling (control $z = 0.76$, $n = 11$, *NS*, orange $z = 1.53$, $n = 7$, *NS*, 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, $z = 1.97$, $n = 7$, $p = .09$), while there were no such changes detected in the control group (number of encounters $z = 1.22$, $n = 11$, *NS*, encounter duration, $z = 0.85$, $n = 11$, *NS*).

In conclusion, red-manipulated males thus expanded their territories, received fewer aggressive interactions and spent less time in these interactions. In contrast, males with orange collars lost territory area and received more and slightly longer aggressive encounters. Taken together with the other results from manipulations in captivity and prior to 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; IV; b; III) but, most importantly, also show that color (i.e. 'redness') per se is a crucial signal component in addition to 'badge size'. In this case, it provides evidence that it is the concentration rather than the patch size of carotenoid pigmentation that is the primary message of the signal. In particular, 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 Olwens, 1998). Recent chromatography analyses have also shown a strong correlation between reflectance-based 'redness' and the carotenoid-concentration in red-collared widowbird feathers (unpublished data). The red-collared widowbird thus seems a likely case of reliable signaling of status or 'fighting ability' with carotenoid investment as the 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. (2001b; III). 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., 2001b; III).

In the dyadic contests, rival males tended to avoid red collars even when painted onto the plumage of a non-breeding male. Since 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 since 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 non-breeding plumage (approximately one 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 redder collars than floaters (Andersson et al., in prep; VI) 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., 2001a; VI). 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., 2001a; VI). Males with reduced collar signals suffered from higher intrusion rate 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 non-breeding 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* (Røskraft and Rohwer, 1987; Hansen and Rohwer, 1986), scarlet-tufted malachite sunbirds *Nectarinia johnstoni* (Evans and Hatchwell, 1992), ring-necked pheasants *Phasianus colchicus* (Mateos and Carranza, 1997) and collared flycatchers *Ficedula albicollis* (Pärt and Qvarström, 1997). Our experiments also demonstrate this as 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 size 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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CHAPTER VI

Agonistic Carotenoid Coloration, Attractive Tails, and a Trade-off between Multiple Ornaments

Reworked from a manuscript by

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(submitted manuscript)

Agonistic Carotenoid Coloration, Attractive Tails, and a Trade-off between Multiple Ornaments

ABSTRACT: The nuptial plumage of male red-collared widowbirds *Euplectes ardens* features a long, graduated tail and a red carotenoid ‘badge’. Here we explore signal functions, signal content and trade-offs between these ornaments by comparing morphometry and color reflectance of non-breeding ‘Floaters’ to territory-holding ‘Residents’. Tails are shorter in Residents, but their carotenoid signal is 60% larger and has 23 nm more longwave (‘redder’) hue than in Floaters. The carotenoid signal has the strongest partial effect on the probability of territory ownership, but Residents are also larger and in better condition. We demonstrate a negative phenotypic correlation between the two signals: tail length is negatively associated with the carotenoid signal, which together with body size and condition explains 55% of the variation in tail length. The trade-off is steeper among Residents, suggesting an interaction with costs of territory defense. Trade-offs between multiple signals are rarely explored in the literature, but may help distinguish between the content-based hypotheses for multiple ornament evolution. From a life history perspective on multiple signals, we predict that allocation conflicts and negative phenotypic correlations will be strongest between ‘backup signals’ (same costs), less pronounced between ‘multiple messages’ (different costs) and weak between ‘unreliable signals’ (negligible costs). The trade-off between tail growth and carotenoid pigmentation in red-collared widowbirds thus suggests that a similar quality message is conveyed to mates and rivals, respectively. The trade-off might also explain the negative interspecific association of nuptial tail elongation and coloration in the *Euplectes* (widowbirds and bishops).

Keywords: multiple ornaments, sexual selection, carotenoid coloration, signal, trade-off, reflectance.

Co-occurrence in a species of several different signals or signal components that each advertise individual quality ('handicaps' or 'indicators') is expected to be rare, since theoretically such communication should converge on a single most efficient signal (Schluter and Price 1993; Johnstone 1996). Additional ornaments are instead likely to be arbitrary ('Fisherian') traits (Møller and Pomiankowski 1993; Iwasa and Pomiankowski 1994), amplifiers (Hasson 1990) or other exploitations of receiver neural systems (Ryan 1990; Rowe 1999). This explanation may apply to many multiple ornaments without obvious costs, but some species defy the rule by displaying two or more costly signals. The red-collared widowbird *Euplectes ardens* is a striking example. In nuptial plumage, the male has bright red carotenoid chest plumage and a long graduated tail, two signal traits with well-documented costs (Balmford et al. 1993; M. Andersson 1994; S. Andersson 1994; Hill 1996; Hill 1999; von Schantz et al. 1999; Møller et al. 2000). The most plausible reason for the maintenance of both these signals in the red-collared widowbird is that they are selected by different receivers; carotenoid colors by male contest competition (Pryke et al. in press a; III) and the long tail by female choice of mate (Pryke et al. in press b; IV).

Here we explore the natural variation and condition-dependence of the two presumed handicap signals in red-collared widowbirds. A strong female preference for long tails (like in the related *E. progne* and *E. jacksoni*; Andersson 1982; Andersson 1989, 1992, 1993) was shown by selection analyses among territorial males (Pryke et al. 2001b; III), but the weaker effects of the carotenoid display were partly contradictory and difficult to interpret. For instance, there was negative net selection on collar area but a positive partial effect of red 'chroma'. Experiments have strongly suggested an agonistic function of the collar (Pryke et al. 2001a; IV; Pryke et al. in press; V), but there are no documented fitness consequences in the wild of natural collar variation.

Our first goal is to test whether carotenoid signal expression (size and redness) is associated with male success in obtaining a territory. We do this by comparing the ornamentation and morphology of 'Resident' males with that of 'Floaters' that roamed the breeding grounds but never obtained a territory.

Second, we explore the signal content of the multiple displays via their relationships with body condition (residual body mass) and body size. Although these are rather unrefined indices of male quality, they provide some initial insight into signaling costs. Thirdly, we investigate correlations between signal expressions and putative fitness benefits. In classical life-history theory, trade-offs between investment in growth, survival and reproduction are central concepts (e.g., Williams 1966; Stearns 1992). Costly sexual displays also represent reproductive investments, potentially subject to short- or long-term allocation conflicts (Williams 1966; Andersson 1982; 1994; Höglund and Sheldon 1998;).

Several fascinating trade-offs between secondary sex traits and other life-history traits have been revealed (e.g., Møller 1989; Gustafsson et al. 1995; Zuk 1996; Zuk et al. 1998; Brooks 2000; Galen 2000), but little attention has been given to such trade-offs in the context of multiple ornamentation. Johnstone (1996) noted that the level of one display component often influences the cost of another, and investment conflicts seem obvious for certain trait combinations, such as acrobatic flight displays and elaborate plumage structures. Yet, the nature of such interactions has been little studied (but see Evans 1993), especially as regards the evolution of multi-component displays. As trade-offs among other reproductive investments (e.g., offspring size and number) shed light on the evolution of life histories (e.g., Stearns 1992), trade-offs between signals may do the same for 'signal histories'.

Materials and Methods

Study Species

Red-collared widowbirds are small (20 g) weaverbirds (subfamily Ploceinae) of shrubby grassland in equatorial and southern Africa. At the onset of breeding, October to April in South Africa, males molt from the sparrow-like plumage of non-breeding males and females into a velvety black nuptial plumage, with a crescent-shaped red collar on the chest and a long (22 cm) graduated tail (the

growth of which lasts for about six weeks; Goddard and Lawes 2000). With fully developed body plumage but incomplete tails males establish large (mean = 0.44 ha) breeding territories that are aggressively defended from conspecifics as well as congeners (Emlen 1957). Both sexes feed outside territories. Like other *Euplectes* species, RCW are highly polygynous, with up to nine females per territory and strongly skewed variation in male success (Pryke et al. 2001b; III). During male courtship the long tail is keeled and displayed in slow flight with exaggerated wing-beats. The male circles a visiting female if she lands, and performs a perched display in front of her with tail and wings spread.

The loosely woven nest frames constructed by the male ('cock's nests') are rarely used by the female, who alone builds the nest, a ball nest with a side entrance, in dense grass or herbs, and performs all incubation and feeding of young. In defense against intruders, the resident male typically responds with a perched boundary display with upright posture and ruffled chest feathers exposing the red collar. If the intrusion persists, he shifts to direct chase flight (tail not keeled), which usually deters the intruder or, rarely (4% of all observed chases) ends in a physical fight (Pryke et al. 2001a; IV).

Study Populations and Morphometrics

Red-collared widowbirds were studied in the Hilton district, KwaZulu-Natal Province, South Africa (29°43'S, 30°17'E) during December 1998 ($n = 41$) and between December 1999 and April 2000 ($n = 116$), and in the Balgowan area (29°35'S, 30°04'E) during April 2000 ($n = 22$). Males were mist-netted either on their territories or at communal night roosts nearby, and uniquely color-banded, excluding red bands to minimize potential effects on color communication (Burley et al. 1982; Metz and Weatherhead 1991). Tarsus length and culmen length were measured to the nearest 0.1 mm with vernier calipers, wing length to the nearest 0.5 mm with a ruler, and body mass to the nearest 0.5 g with a 50 g Pesola spring-balance.

As a measure of overall body size, we used the first principal component ('size PC1') derived from and explaining 47% of the variation in tarsus length, wing length and culmen length. An index of body condition was computed as the standardized residuals from a linear regression of log (body mass) on 3*log (tarsus length) (S. Andersson 1994), using tarsus length rather than 'size PC1' as the best measure of skeletal size (Freeman and Jackson 1990). Since the mass/tarsus relationship differed between the three populations (ANCOVA of body mass, tarsus by population interaction; $F_{2,188} = 6.7$, $P = 0.002$), we computed a body condition index for each population before pooling, making the measure relative to the population mean rather than the overall mean.

Tail length was measured to the nearest 0.1 mm as the maximum length of the stretched and folded tail. Fluctuating asymmetry (FA) of the longest pair of rectrices was calculated as the absolute left-right difference divided by their average length (Palmer and Strobeck 1986; Møller and Höglund 1991). Birds with broken, missing or growing feathers were excluded from the FA analyses. Collar area was estimated as the product of the maximum width and the average of three height measures (left, central, and right). Collar asymmetry was computed as above for the left and right side height measures. In the Hilton 1999/2000 population, where 57 males were captured more than once and remeasured, repeatabilities (Lessells and Boag 1987) were highly significant for all measurements ($r = 0.81-0.96$, $P < 0.001$).

Reflectance Spectrometry and Colorimetrics

Spectral reflectance (at ± 2 nm resolution) from the red collar was measured using a PS1000 spectrometer (Ocean Optics Inc., Dunedin, Florida), HL2000 halogen light source, WS-2 white reference, a fiber-optic reflectance probe (Avantes, Eerbeek, Netherlands), and C-spec software (Ancal Inc., Las Vegas). The probe was fitted with a matte black tube that shielded out external light and standardized the measuring distance. The probe was held (not pressed) against the plumage,

scanning specular reflectance from a 4 mm wide spot. Preceded by a reference scan for each individual, five consecutive scans (removing the probe between each) were taken from the center of the collar patch.

Objective indices of the three main dimensions of color signals (spectral intensity, location and purity) (Hailman 1977) were computed from the five scans and averaged for each individual. Spectral intensity ('brightness') was estimated by $R_{350-700}$, the sum of reflectance from 350-700 nm. Spectral location ('hue' here referred to as 'redness') was estimated as $\lambda(R_{50})$, 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 reflectance shape aspects, such as slope height and steepness. To avoid a fixed arbitrary spectral segment, we used $\lambda(R_{50})$ as the segment divider, and computed chroma ($C[R_{50}]$) as $R_{[\lambda_{350}-\lambda(R_{50})]} - R_{[\lambda(R_{50})-\lambda_{700}]} / R_{320-700}$. The details on the methods used to collect and analyze reflectance indices are described in Pryke et al. (2001b; III).

Statistical Methods

For relationships involving the strongly skewed fluctuating asymmetry measures, Spearman rank correlations were used. In all other cases, parametric tests were used since variables were approximately normal and residual variances reasonably uniform (and not extra-binomially distributed in the case of logistic regressions). In multiple regressions, multicollinearities were not substantial enough to affect the analyses (Mitchell-Olds and Shaw 1987), as judged by the ratios of eigenvalues from the covariance matrix of the standardized independent variables (Fry 1993). Two-tailed significance tests were used throughout.

Results

Variation between Residents and Floaters

The variation and differences in plumage signals and other morphology between Floaters and Residents are summarized in Table 1. Whereas tail length which was strongly sexually selected among Resident males (Pryke et al. 2001b; III), and tail asymmetry, did not differ significantly between Floaters and Residents, the carotenoid signal expression differed in several respects. In Residents, the collar was 60% larger, tended to be more symmetric, and was significantly 'redder' through 23 nm more longwave 'hue' (spectral position of the slope, $\lambda(R50)$; see Methods). When mathematically translated via human CIE-Lab color space coefficients (International Commission on Illumination 1971) to the closest notation in the NCS Color system (Scandinavian Color Institute, Stockholm Sweden), the average Floater and Resident coloration correspond to notations that are two hue steps and one chroma step apart (2060-Y40R and 2070-Y60R, respectively). There were also less marked differences in objective chroma and brightness (Table 1), both of which were correlated with hue ($r = 0.40$ and -0.59 , respectively, $n = 71$, $P < 0.001$). In the following results, we will focus on $\lambda(R50)$ ('hue' or 'redness'), since this has proven to be the most important aspect of the agonistic signal variation (see below).

In non-signaling aspects of morphology (Table 1) Residents were larger (tarsus length and PC1 of tarsus, wing and culmen) and heavier, both absolutely (body mass) and relatively (i.e., condition; see Methods). Several of the traits separating Floaters from Residents in the Hilton 1999/2000 sample were intercorrelated. Most notably, collar area was positively correlated with redness ($r = 0.75$, $n = 68$, $P < 0.001$), body mass ($r = 0.29$, $n = 118$, $P = 0.001$), and body size PC1 ($r = 0.19$, $n = 118$, $P = 0.03$), while negatively related to tail length ($r = -0.42$, $n = 114$, $P < 0.001$) and to collar asymmetry ($r = -0.36$, $n = 118$, $P < 0.001$). To identify independent effects, we explored multiple logistic regressions with male status as Floater or Resident as the response variable and combinations of up

Table 1: Variation in ornamental, morphology and body condition among and between Floater and Resident red-collared widowbird males. F-values and significance levels are derived from one-way ANOVA's

Trait	Measure or unit	Floaters			Residents			F	P
		Mean	±SD	n	Mean	±SD	n		
<i>Red chest patch</i>									
Area	width*height (mm ²)	195	±34.8	78	274.2	±25.1	40	163.3	<0.001
Asymmetry	(L-R) /mean height	0.014	±0.016	78	0.009	±0.007	40	3.9	0.051
Hue ('redness')	λ(R50)	557	±14.7	38	580.3	±12.7	33	50.1	<0.001
Chroma	R50 chroma	0.54	±0.10	38	0.59	±0.09	33	5.3	0.025
Brightness	R(350-700)	94.6	±18.0	38	82.1	±25.6	33	5.8	0.019
<i>Tail</i>									
Tail length	mm	225.5	±24.6	76	215.6	±31.9	40	3.5	0.065
Tail asymmetry	(L-R) /mean length	0.100	±0.224	69	0.068	±0.166	37	0.6	NS
<i>Morphology</i>									
Tarsus length (TL)	mm	23.72	±0.42	81	24.48	±0.29	41	110.4	<0.001
Wing length (WL)	mm	76.60	±1.80	81	77.30	±1.30	41	5.9	0.016
Culmen length (CL)	mm	14.11	±0.37	81	14.17	±0.38	41	0.7	NS
Body size	PC1 (TL/WL/CL)	-0.24	±0.83	81	0.68	±0.86	41	33.8	<0.001
Body mass	g	20.20	±1.10	81	21.60	±1.00	41	42.9	<0.001
Body condition	Residual mass index	-0.009	±0.053	81	0.02	±0.04	41	6.6	0.012

to three independent variables, until a model with high explanatory power and three significant independent effects was found. The best model (Table 2) included a dominating effect of collar area, a highly significant but weaker effect of body size, and a just significant effect of condition ($P = 0.037$). As in selection analysis (Lande and Arnold 1983), variables were standardized to zero mean and unit variance, so that coefficients denote the effect of a change corresponding to one

standard deviation (SD) of the variable on the odds of territory ownership. Thus, a change of one SD in collar area is associated with 120 times greater probability that a male is a territorial Resident, as compared to 9 times for body size and 2.7 times for body condition.

Table 2: Traits affecting male status as Floater or Resident, tested by logistic regression of male status on three standardized (zero mean and unit variance) traits

Trait (standardized)	Exp[coeff] [†]	χ^2
Collar area	120.4	87.9***
Body size PC1	9.1	17.1***
Condition	2.7	4.3*

[†]Exp[coeff] values can be interpreted as the increased odds of territory ownership associated with a trait change of one standard deviation. Log likelihood ratio χ^2 : *** $P < 0.001$, and * $P = 0.037$, $n = 118$. Full model $R^2 = 81\%$.

Condition-dependence and Signal Content

We investigated condition-dependence with bivariate linear regressions of signal traits on the standardized condition index. The sample used was all males captured at the Hilton site 1998/99 ($n = 41$) and 1999/2000 ($n = 116$), and at Balgowan 1999/2000 ($n = 22$). We included only the last season's measurements from five males that were recaptured between years. Although the amount of variance explained was small (6%), tail length was significantly related to the condition index ($F_{1,174} = 14.3$ $P < 0.001$, $R^2 = 7.1\%$), and the relationship did not differ between populations (ANCOVA, population by condition interaction, $F_{2,171} = 0.53$,

$P = 0.59$). Tail length was also positively related to overall body size, and both body size and condition independently predicted tail length in a multiple regression ($\beta_{\text{BODY SIZE}} = 0.34$, $t = 4.9$, $P < 0.001$, $\beta_{\text{CONDITION}} = 0.23$, $t = 3.4$, $P < 0.001$, $F_{2,173} = 19.9$, $P < 0.001$, $R^2 = 18.9\%$). As previously reported for this species (Goddard and Lawes 2000; Pryke et al. 2001b; III), tail asymmetry showed no relationship with tail length ($r_s = -0.06$, $n = 157$, $P = 0.48$) or any other morphological trait ($r_s = -0.13 - 0.09$, $n = 129-157$, $P = 0.17-0.81$). However, tail asymmetry tended to be negatively correlated with condition ($r_s = -0.15$, $n = 157$, $P = 0.054$) and with absolute body mass ($r_s = -0.14$, $n = 157$, $P = 0.069$).

For the carotenoid color signal, redness had a weak, non-significant positive association with condition (linear regression, $F_{1,126} = 2.3$, $P = 0.12$), while all other aspects of collar color, area and symmetry were unrelated to condition ($P = 0.27 - 0.66$). Interestingly, however, collar asymmetry (measured in the Hilton 1999/2000 sample) was negatively correlated with both collar area (see above) and redness ($r_s = -0.26$, $n = 68$, $P = 0.031$), which might be interpreted as support for a quality-indicating mechanism (Møller and Pomiankowski 1993).

The much stronger effect of collar area than of body size on territory ownership (Table 2) suggests a direct signal function of the collar in territory defense, not just a correlated effect of male size. This was also true when replacing collar area with redness in the logistic regression ($\exp[\text{coeff}] = 17.1$, $\chi^2 = 13.9$, $P < 0.001$; size PC1, $\exp[\text{coeff}] = 6.4$, $\chi^2 = 9.6$, $P = 0.002$). A status signaling function of both collar area and redness has also been experimentally demonstrated (Pryke et al. 2001a; IV; Pryke et al. in press; V).

Signal Trade-offs

The presumably costly acquisition, modification and internal allocation conflicts of carotenoid pigments should lead to a trade-off between the size and coloration of a color signal. In other words, a given amount of pigment can be used for a small, intensely colored patch or a large patch with more diluted coloration. This trade-

off is not exerted as a negative phenotypic correlation in the red-collared widowbirds. Instead, as expected if total investment (here, carotenoids) is more variable than individual allocation rules (van Noordwijk and de Jong 1986), collar area was positively correlated with redness ($r = 0.75$, $n = 90$, $P < 0.001$) as well as with chroma ($r = 0.33$, $n = 90$, $P = 0.001$). In contrast, there were significant negative correlations between tail length and both collar area ($r = -0.40$, $n = 135$, $P < 0.001$) and collar redness ($r = -0.31$, $n = 119$, $P < 0.001$). Examining the sample populations separately, these negative correlations were present in the large Hilton 1999/2000 sample (tail length and collar area, $r = -0.42$, $n = 114$, $P < 0.001$; tail length and redness, $r = -0.47$, $n = 68$, $P < 0.001$), and similar in the small Balgowan sample (tail length and collar area, $r = -0.58$, $n = 21$, $P = 0.005$; tail length and redness, $r = -0.35$, $n = 21$, $P = 0.11$). In the Hilton 1998/1999 sample, only hue (redness) was measured and showed no relationship with tail length ($r = 0.06$, $n = 31$, *NS*). To control for confounding variables behind the apparent collar/tail trade-off, and to clarify which of the two correlated aspects of the carotenoid display (area and redness) had the strongest effect, we ran stepwise multiple regressions of tail length against all other signal and morphological traits (listed in Table 1). Both forward and backward stepwise procedures (F -to-enter = 4, F -to-remove = 3.9) resulted in the same model with three independent standardized variables ($F_{3,133} = 55.3$, $P < 0.001$, $R^2 = 55.1\%$); wing length ($\beta = 0.57$, $t = 9.5$, $P < 0.001$), collar area ($\beta = -0.37$, $t = 6.4$, $P < 0.001$), and body condition ($\beta = 0.19$, $t = 9.5$, $P = 0.002$). Thus the trade-off between tail length and carotenoid display remained strong also when correlated traits were held constant.

If redness, was entered instead of collar area in the above model, it had nearly as strong a negative effect ($\beta = -0.34$, $t = -4.5$, $P < 0.001$) on tail length, and the amount of variance explained by the model dropped only three percent ($F_{3,87} = 33.0$, $P < 0.001$, $R^2 = 52.5\%$). Patch size and color are thus closely related indices of carotenoid signal investment. As a combined measure of carotenoid investment, we therefore also calculated the product between area and redness (both first standardized to a mean of 10, to avoid negative values, and unit standard deviation). When entered into the model, the amount of tail variation

explained increased to 58%. The signal trade-off was not just an effect of Floaters and Residents adopting different strategies (i.e., small-collared, long-tailed Floaters and large-collared, short-tailed Residents). When territory ownership status was entered as a cofactor, the negative effect of collar area on tail length remained the most significant factor (ANCOVA, Collar area, $F_{1,110} = 36.8$, $P < 0.001$; Territory ownership, $F_{1,110} = 9.2$, $P = 0.003$; Fig. 1). This means that within each category (Floaters and Resident), collar and tail length were also negatively related. Interestingly, the interaction term was also significant (ANCOVA, 'Territory ownership status' by 'Collar area' interaction, $F_{1,110} = 6.8$, $P = 0.010$), with Residents showing a significantly steeper trade-off slope (Floaters $\beta = -0.43$, $t = -4.0$, $P < 0.001$; Residents $\beta = -0.59$, $t = -4.4$, $P < 0.001$; Fig. 1).

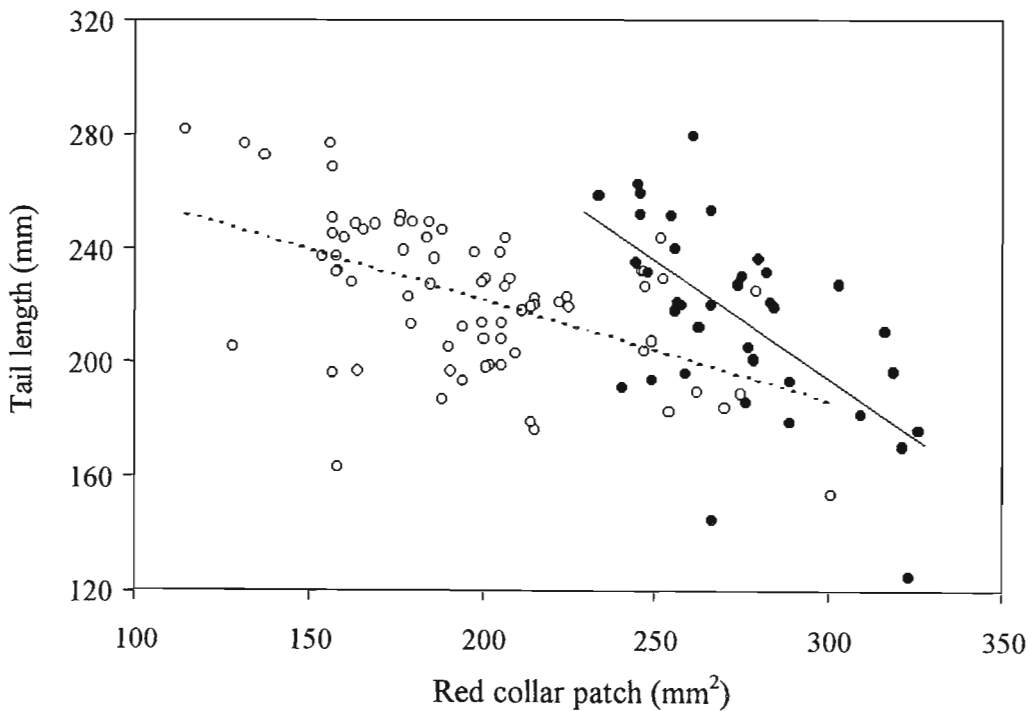


Figure 1: Signal trade-off (negative phenotypic correlation) between tail length and collar area in red-collared widowbirds. The negative relationship is found both in the group of Floaters (empty circles) and in Residents (filled circles). Linear regression lines: Floaters, Tail length = $283.8 - 0.30 \times$ Collar area, $F_{1,74} = 16.2$, $P < 0.001$, $R^2 = 17.1\%$; Residents, Tail length = $421.1 - 0.75 \times$ Collar area, $F_{1,38} = 19.3$, $P < 0.001$, $R^2 = 2.5\%$.

Discussion

Agonistic Sexual Selection of Carotenoid Coloration

Although there has been an emphasis on mate choice in the study of carotenoid signals in recent years (see e.g., Hill 1999), there are many cases where red carotenoid displays function in aggressive competition instead of, or in addition to, mate attraction. Epaulets in redwinged blackbirds, pectoral tufts in malachite sunbirds, combs and wattles in galliform birds, and skin color in sticklebacks are prominent examples (reviewed by M. Andersson 1994). More recently, contest competition rather than mate choice has been suggested to be the primary context of carotenoid signaling in northern cardinals *Cardinalis cardinalis* (Wolfenbarger 1999) and redbilled queleas *Quelea quelea* (Dale 2000; Shawcross and Slater 1983). The present result from red-collared widowbirds, with Floaters and Residents differing markedly in both size and objective redness of a carotenoid badge, reveal a strong effect of natural carotenoid signal variation in male contest competition in the wild. This status signaling function has subsequently been confirmed by manipulative experiments in both captive dominance interactions (Pryke et al. in press; V) and in the context of territory acquisition and maintenance in the field (Pryke et al, 2001a; IV; in press; V). In addition, a direct (rather than an indirect, correlated) fitness effect of ornament variation is indicated by the much stronger independent effect on resident status of the collar than of body size or condition (Table 2).

Are there alternative interpretations of the results? Potentially reversed causation could have produced these results, i.e., that dominance affects or allows strong signal expression through resource access, rather than the other way around. The lack of body size effects speaks against this, but if food (mainly grass seeds) is scarce before or during the nuptial molt, there might be direct resource competition through which superior individuals (for other reasons than size) obtain a territory as well as nutrition for carotenoid coloration. This mechanism seems,

however, more likely to be a part of, rather than preempting, a status signaling function of the collar, which in addition has been experimentally demonstrated.

Signal Trade-offs and the Evolution of Multiple Ornaments

Sexually selected signals often represent considerable reproductive investments, and are therefore expected to follow similar patterns as other life history traits as regards allocation and trade-offs (M. Andersson 1994; Höglund and Sheldon 1998). In observational data, however, trade-offs are rarely revealed as negative phenotypic correlations, because large variation among individuals in levels of available resources (genetic or environmental) may often create positive associations between traits in the population (see e.g., van Noordwijk and de Jong 1986; Stearns 1992). In addition, even if an observable trade-off is present in the population, costs can often be deferred to another time (e.g., next breeding season), making the negative correlation detectable between seasons (see for e.g., Møller 1994; Gustafsson et al. 1995; Griffith 2000). Taken together, it is therefore not surprising that multiple ornamental traits are usually positively correlated within seasons (e.g., Evans and Hatchwell 1992b; Kodric-Brown 1985; Zuk et al. 1990). Viewed against this background, the negative within-season correlation between carotenoid coloration and tail length in red-collared widowbirds is especially interesting; it is the first such example from sexually selected signals that we are aware of.

There are at least three possible interpretations. First, individual variation in relative allocation between the two ornaments might be particularly wide, exceeding the variation in optimal total investment mentioned above. Frequency-dependent or fluctuating selection pressures could be involved in maintaining a large variation in signal allocation, but we see no reason why this should apply more to this than to other multi-ornamented species. Second, variation in male quality or resources might be exceptionally small (constraining ornament variation to a narrower range in the trade-off). This is refuted by the large coefficients of

variation (CV; 12-18%) in both ornaments (Pryke et al. 2001b; III), and also when controlling for their negative association. For example, fitted values from a linear regression of tail length on collar redness retain a CV of 5.1%, still more than twice the variation in non-ornamental traits such as wing length.

Third, and the explanation we find most likely, is that the trade-off itself might be particularly strong relative to the variation in available resources (e.g., creating a pattern similar to that illustrated in Stearns 1992, Figure 4.8). In the red-collared widowbirds and its congeners, the molt to an elaborate nuptial plumage, partly overlapping with the intense competition for territory acquisition and defense, is likely to be stressful, and there are several ways by which nutrition and metabolism can mediate a conflict between costly carotenoid pigmentation (e.g., Olson and Owens 1998; Hill 1999; Møller et al. 2000) and costly tail growth (e.g., Lindstrom et al. 1993; Schieltz and Murphy 1997; Svensson and Nilsson 1997). For example, vitamin A and other retinoids are important growth factors (Goodwin 1986), irreversibly metabolized from ingested carotenoids that are also required for red plumage pigmentation. Parasites and other immune-challenges are also likely to affect the rapid tail growth as well as carotenoid uptake and use (Møller et al. 2000), and can further sharpen the conflict between the two signal investments.

This apparent intraspecific conflict between carotenoid coloration and tail length is also strikingly expressed in a comparison among the other species in the *Euplectes*; the 'widowbirds' have elongated tails but little or no carotenoid pigmentation, whereas the 'bishops' have normal tails but extensive yellow or red plumage coloration (A Craig 1982; Andersson and Andersson 1994). We are currently investigating other phylogenetic and ecological components of this variation, but it seems likely that a costly trade-off between the two ornaments has been one of the important constraints.

Signal trade-offs in general might also tell us something about multiple ornament evolution. Johnstone (1996) found that multiple honest signals could be stable only if signal costs are so strongly accelerating that it pays to shift the marginal investments to a second signal. But to be an honest indicator this additional signal must also be costly and traded off against the investment in the

first signal. We suggest that the nature and strength of such signal trade-offs can help distinguish between the debated mechanisms behind multiple ornament evolution, especially the three initial hypotheses proposed by Møller and Pomiankowski (1993) that are based on signal content: 'Backup (or Redundant) signaling', 'Multiple messages', and 'Unreliable signaling' (Møller and Pomiankowski 1993; Iwasa and Pomiankowski 1994; Johnstone 1996).

First, the 'Backup signaling' hypothesis implies that multiple signals have qualitatively similar messages and thus identical or overlapping costs. This idea predicts strong trade-offs between ornaments, for instance in simultaneous growth of two energetically or nutritionally demanding ornaments, as we suggest is the case for the carotenoid coloration and long tail in the red-collared widowbird. The 'Multiple messages' hypothesis, on the other hand, suggests that the different signals have different messages as well as costs and thus are more independent. Immunocompetence and physical strength might exemplify such largely independent costs, and involve indicator traits that are not in immediate conflict. They should be less likely than 'Backup signals' to result in negative phenotypic correlations between ornaments. Finally, the 'Unreliable signals' hypothesis suggests that multiple ornaments are not particularly costly (Møller and Pomiankowski 1993), having evolved through Fisherian runaway selection or as exploitations of pre-existing sensory biases (reviewed e.g., in M. Andersson 1994). Such multiple signals should be least constrained by signal trade-offs, and most likely to covary with individual variation in total investment.

In conclusion, positive correlations between traits in studies of multiple secondary sex characters, are often taken as support for a function as costly indicators ('handicaps' sensu Zahavi and Zahavi 1997). It is, however, also possible to apply a life-history perspective on investments in sexual and other signals (e.g., Williams 1966; Partridge and Endler 1987; M. Andersson 1994; Höglund and Sheldon 1998). We propose that the most costly signals (typical handicaps) may show the strongest trade-offs and therefore may be most likely to show negative phenotypic correlations. Traits such as the elongated tail and the red carotenoid patch in red-collared widowbirds may therefore be evidence for

multiple advertisements of a similar quality (i.e. 'backup signals'). This may be the case whether or not the multiple signals are addressing the same or, as in the present case, different receivers.

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