# ORIGINAL ARTICLE

# CONVERGENT EVOLUTION OF RED CAROTENOID COLORATION IN WIDOWBIRDS AND BISHOPS (*EUPLECTES* SPP.)

**Maria Prager[1,2] and Staffan Andersson[1]**

[1]*Department of Zoology, University of Gothenburg, Box 463, 405 30 Göteborg, Sweden*

[2]*E-mail: maria.prager@zool.gu.se*

Avian carotenoid-based signals are classic examples of sexually selected, condition-dependent threat displays or mate choice cues. In many species, male dominance or mating success is associated with redder (i.e., longer wavelength) color hues, suggesting that red colors are either more efficient or more reliable signals than yellow colors. Few studies, however, have investigated selection for redness in a macroevolutionary context. Here, we phylogenetically reconstruct the evolution of carotenoid coloration in the African widowbirds and bishops (*Euplectes* spp.), for which agonistic selection for redder hues, as well as pigmentary mechanisms, is well documented. Using reflectance spectrometry for objective color quantification, and accounting for phylogenetic uncertainty, we find that yellow plumage color is a retained ancestral state in *Euplectes*, and that red color hues have convergently evolved two or three times. Results are discussed in relation to a known diversity in pigment mechanisms, supporting independent origins of red color, and suggesting that agonistic selection and physiological constraints have interacted to generate color diversity in *Euplectes*.

Extravagant color displays in birds are familiar examples of sexually selected signals, functioning in competition over mates, via intrasexual contests or intersexual attractiveness (Andersson 1994; Hill and McGraw 2006a). In a phylogenetic perspective, however, many questions remain regarding the evolution and diversification of color signals.

Brilliant red, orange, and yellow colors are typically achieved by deposition of carotenoid pigments into feathers, skin, or beak tissue (Fox and Vevers 1960; Brush 1978; Stradi 1998). Like all animals, birds must acquire carotenoids exclusively through the diet (Brockmann and Völker 1934; Goodwin 1984; Hill and McGraw 2006b), and carotenoid-based displays are therefore potentially limited by food intake (Endler 1980; Hill 1990). Furthermore, the uptake and expression of carotenoids can be affected by parasites (Hamilton and Zuk 1982; Milinski and Bakker 1990; Thompson et al. 1997; Zahn and Rothstein 1999) as well as direct allocation conflicts with immunological or antioxidant systems (Lozano 1994; von Schantz et al. 1999; Møller et al. 2000; Blount et al. 2003). For any or several of these reasons, striking carotenoid colors are presumed to indicate health or condition across a wide range of taxa (Olson and Owens 1998; McGraw 2006, but see, e.g., Hartley and Kennedy 2004).

In many carotenoid-pigmented birds (Shawcross and Slater 1983; Hansen and Rohwer 1986; Hill 1990; Evans and Hatchwell 1992; Wolfenbarger 1999; Pryke and Griffith 2006; Pryke 2007) and other vertebrates (Bakker and Sevenster 1983; Evans and Norris 1996), mating success or dominance is associated with redder (i.e., longer wavelength) color hues. As observed by Hill (1996), this suggests that red carotenoid-based signals generally are more efficient or more reliable (honest) than yellow signals (Schluter and Price 1993; Andersson 2000). For example, red signals may more efficiently exploit sensory or cognitive receiver biases (Endler and Basolo 1998), or be more honest quality advertisements due to higher production or maintenance costs (Hudon

1991; Hill 1996). In either case, exaggeration of carotenoid color signals toward redder hues is expected. This is true also for purely condition-dependent signals, given that senders evolve physiological or behavioral adaptations that reduce signaling costs (Hill 1994; Badyaev 2004).

In a macroevolutionary context, the above predicts directional and convergent evolution of red carotenoid coloration, but how can this be reconciled with the view of sexual selection as labile and divergent in many animals (Wiens 2001), including several bird groups (e.g., Kusmierski et al. 1997; Omland and Lanyon 2000; Kimball et al. 2001; Cardoso and Mota 2008; but see Prager and Andersson 2009)? Among these, only the study of New World orioles *Icterus* spp (Hofmann et al. 2006) concerned carotenoid coloration, and the lability of color observed in this genus was recently contrasted with convergent yellow to red evolution in the sister genus of caciques (*Cacicus,* Kiere et al. 2009). Further phylogenetic analyses of color evolution are clearly needed, especially in taxa where both signal selection and signal mechanisms are better understood.

Phylogenetic and comparative analyses of signal diversity may enable interesting inferences of the past evolution as well as the current function of carotenoid pigmentation. Ancestral character state reconstruction may reveal interesting macroevolutionary patterns, such as convergence and directional change in plumage signals (e.g., Omland and Lanyon 2000; Hofmann et al. 2006), and suggest key taxa for selection analyses and experiments (Omland and Hofmann 2006). This interaction between macro- and microevolutionary studies becomes a particularly powerful approach if also combined with data on the proximate mechanisms of coloration, which may help identify genetic and developmental constraints on signal evolution (Harvey and Pagel 1991). Knowledge of the proximate basis of coloration is also important for defining and scoring characters, as well as for selecting appropriate algorithms for ancestral state reconstruction (Hofmann et al. 2006). Yet, to date, few reconstructions of color evolution have been performed on taxa where the underlying pigment mechanisms have been analyzed in detail.

A genus for which both the adaptive function and proximate mechanisms of carotenoid coloration are unusually well documented are the African widowbirds and bishops (*Euplectes* spp.). These are sexually and seasonally dichromatic weaverbirds (subfam. Ploceinae), breeding semi-colonially and polygynously in grasslands of sub-Saharan Africa. Male nuptial plumages show remarkable interspecific variation in carotenoid color expression, ranging from the small yellow epaulettes of certain widowbirds to the extensive scarlet red body plumage of many bishop species. The bright carotenoid plumage patches are displayed against contrasting black melanin pigmentation, and are emphasized through posturing in agonistic and courtship interactions (Crook 1964). Behavioral observations and experiments in two phylogenetically distant *Euplectes* species have furthermore shown that males with redder (longer wavelength) carotenoid hues are more successful in male–male competition over territories (Pryke et al. 2001, 2002; Pryke and Andersson 2003a,b). In contrast, the more or less extremely elongated nuptial tails of widowbird males are selected by female mate choice (Andersson 1982, 1992; Pryke and Andersson 2002, 2005).

The presence of carotenoids in bishop feathers was established already by Kritzler (1943), and plumage as well as plasma pigments have recently been analyzed in several *Euplectes* species (Andersson et al. 2007; Prager et al. 2009). The results indicate that yellow feather colors primarily derive from direct deposition of the dietary yellow carotenoids lutein and zeaxanthin. Red color signals are, on the contrary, achieved by two distinctly different biochemical strategies: either addition of relatively small amounts of metabolically derived red C4-keto-carotenoids (mainly α-doradexanthin and canthaxanthin), or deposition of high concentrations of both dietary and derived yellow pigments (Andersson et al. 2007; Prager et al. 2009).

In this study, we use a subspecies-level phylogeny of *Euplectes* (Prager et al. 2008) and spectral reflectance data to reconstruct and explore two aspects of the evolution of avian carotenoid coloration: First, the much more common occurrence of yellow than red plumage colors, across the weaverbirds (Ploceinae, ca. 120 species in 17 genera), may indicate that red is a more derived state. If supported by rigorous ancestral state reconstruction, it would suggest directional evolution of the presumably more costly and honest red carotenoid displays (Hill 1996). Second, the two distinct "red mechanisms" that we have identified in *Euplectes* (Andersson et al. 2007) suggest convergent signal evolution. Like the convergent evolution of elongated tails in this genus (Prager and Andersson 2009), this would provide a contrast to the common view of sexual selection as labile and divergent (e.g., Wiens 2001). We test these hypotheses by objectively quantifying carotenoid color hues, and by phylogenetically reconstructing color evolution within the genus *Euplectes*, while accounting for uncertainty in phylogeny, and comparing results from different character coding schemes and reconstruction methods.

# Methods
## SAMPLES AND MEASUREMENTS
Carotenoid color hue was examined in 33 *Euplectes* taxa, representing all 17 *Euplectes* species, and eight outgroup taxa, all of which are included in the molecular phylogeny by Prager et al. (2008). Measurements were acquired from live birds in the field, and from skin specimens at the Natural History Museum at Tring, UK (Table 1). Although *Euplectes* color hues are generally homogeneous within and between brightly carotenoid-pigmented

**Table 1** Plumage coloration in *Euplectes*, i.e., widowbirds (w.) and bishops (b.), and outgroup genera *Amblyospiza (A.)*, *Foudia (F.)*, *Ploceus (P.)*, and *Quelea (Q.)*. Continuous hue (spectral position) was measured as the wavelength at which reflectance is halfway between its minimum and its maximum value ($\lambda_{R50}$). Discrete hue classes were derived by k-means clustering of taxon mean values, and complemented by a category for taxa lacking carotenoid pigmentation (non-car.), resulting in three- and four-state classifications, respectively.

| Taxon Scientific name[1] | Common name | Origin | Patch | n | Continuous hue ($\lambda_{R50}\pm$SE) | Discrete hue category 3-state | 4-state |
|---|---|---|---|---|---|---|---|
| A. albifrons albifrons | thick-billed weaver | – | – | – | – | non-car. | non-car. |
| E. afer afer | yellow-crowned b. | Cameroon & Nigeria[2] | rump | 10 | 520.3±1.3 | yellow | yellow |
| E. afer ladoensis | yellow-crowned b. | S Sudan to N Tanzania[2] | rump | 9 | 522.1±1.8 | yellow | yellow |
| E. afer taha | yellow-crowned b. | Wakkerstroom, South Africa | rump | 13 | 527.4±0.6 | yellow | yellow |
| E. albonotatus albonotatus | white-winged w. | E South Africa to Malawi[1] | lwc[3] | 10 | 525.7±1.3 | yellow | yellow |
| E. albonotatus asymmetrurus | white-winged w. | Sao Tomé, S.T. and Principe | lwc | 15 | 519.3±1.0 | yellow | yellow |
| E. albonotatus eques | white-winged w. | – | – | – | – | non-car. | non-car. |
| E. ardens ardens | red-collared w. | Pietermaritzburg, South Africa | breast | 34 | 568.0±1.7 | red | orange |
| E. ardens concolor | red-collared w. | – | – | – | – | non-car. | non-car. |
| E. ardens suahelicus | red-collared w. | S Kenya[2] | breast | 10 | 595.2±2.7 | red | red |
| E. aureus | golden-backed b. | Sao Tomé, S.T. and Principe | rump | 10 | 535.7±2.1 | yellow | yellow |
| E. axillaris axillaris | fan-tailed w. | Pietermaritzburg, South Africa | lwc | 14 | 590.0±2.4 | red | red |
| E. axillaris phoeniceus | fan-tailed w. | SW Uganda[2] | lwc | 10 | 586.3±1.4 | red | red |
| E. capensis approximans | yellow-rumped w. | KwaZulu-Natal, South Africa[2] | lwc | 10 | 519.8±0.8 | yellow | yellow |
| E. capensis crassirostris | yellow-rumped w. | S Kenya[2] | lwc | 10 | 521.2±0.7 | yellow | yellow |
| E. capensis phoenicomerus | yellow-rumped w. | W Cameroon[2] | lwc | 10 | 516.5±0.5 | yellow | yellow |
| E. diadematus | fire-fronted b. | SE Kenya | forehead | 3 | 584.0±9.0 | red | red |
| E. franciscanus E | northern red b. | N Uganda[2] | nape | 4 | 603.8±5.4 | red | red |
| E. franciscanus W | northern red b. | Nigeria to N Cameroon[2] | nape | 10 | 602.1±2.4 | red | red |
| E. gierowii ansorgei | black b. | NE DRC to SW Ethiopia[2] | breast | 15 | 560.2±1.9 | red | orange |
| E. gierowii friederichseni | black b. | N Tanzania[2] | breast | 4 | 575.0±3.8 | red | orange |
| E. hartlaubi humeralis | marsh w. | Entebbe, Uganda[2] | lwc | 2 | 555.0±1.0 | yellow | orange |
| E. hordeaceus craspedopterus | black-winged b. | Ethiopia[2] | crown | 10 | 589.8±2.1 | red | red |
| E. hordeaceus hordeaceus | black-winged b. | Sao Tomé, S.T. and Principe | crown | 10 | 585.9±3.6 | red | red |
| E. jacksoni | Jackson's w. | – | – | – | – | non-car. | non-car. |
| E. macrourus macrocercus N | yellow-mantled w. | Ethiopia[2] | lwc | 10 | 521.7±1.4 | yellow | yellow |
| E. macrourus macrocercus S | yellow-mantled w. | Uganda to W Kenya[2] | lwc | 5 | 513.6±2.2 | yellow | yellow |
| E. macrourus macrourus | yellow-mantled w. | Nigeria[2] | lwc | 10 | 519.7±1.0 | yellow | yellow |
| E. nigroventris | zanzibar b. | E Tanzania[2] | nape | 10 | 584.9±1.8 | red | red |
| E. orix nigrifrons | southern red b. | SW Uganda[2] | crown | 10 | 590.6±1.6 | red | red |
| E. orix orix | southern red b. | Pietermaritzburg, South Africa | crown | 22 | 580.1±1.7 | red | red |
| E. progne delamerei | long-tailed w. | S Kenya[2] | lwc | 5 | 591.8±3.7 | red | red |

Continued.

**Table 1** Continued.

| Taxon Scientific name[1] | Common name | Origin | n | Continuous hue ($\lambda_{R50}\pm$SE) | Patch | Discrete hue category 3-state | Discrete hue category 4-state |
|---|---|---|---|---|---|---|---|
| *E. progne progne* | long-tailed w. | NE South Africa[2] | 9 | 595.0±2.6 | lwc | red | red |
| *E. psammocromius* | mountain marsh w. | S Highlands, Tanzania[2] | 5 | 519.3±1.9 | lwc | yellow | yellow |
| *F. madagascariensis* | Madagascar red fody | Mahé, Seychelles | 18 | 582.7±2.2 | breast | red | red |
| *F. sechellarum* | Seychelles fody | Cousin Island, Seychelles | 3 | 528.2±3.1 | breast | yellow | yellow |
| *P. luteolus luteolus* | little weaver | Nigeria[2] | 4 | 513.0±0.9 | breast | yellow | yellow |
| *P. melanocephalus fischeri* | black-headed weaver | Uganda[2] | 6 | 513.8±3.5 | belly | yellow | yellow |
| *P. taeniopterus taeniopterus* | north. masked weaver | Sudan[2] | 6 | 535.8±4.2 | breast | yellow | yellow |
| *Q. cardinalis* | cardinal quelea | Uganda to W Kenya[2] | 5 | 598.8±2.2 | breast | red | red |
| *Q. quelea lathami* | red-billed quelea | Transvaal, South Africa[2] | 5 | 609.8±2.8 | breast | red | red |

[1]Taxonomic references are detailed in Prager et al. (2008).

[2]Specimens from the Natural History Museum at Tring, UK.

[3]Lesser wing coverts.

patches of individual male breeding plumages (Prager and Andersson, pers. obs.), two taxa (*E. diadematus* and *E. gierowii ansorgei*) have two differently colored carotenoid regions. Here, we measured and reconstructed the longest wavelength (i.e., "reddest") feather hue displayed by breeding males of each taxon.

Depending on the proximate mechanisms underlying color expression, color hue can vary continuously, as well as group into more or less discrete classes, such as yellow, orange, or red. Assumptions of the mechanistic basis of interspecific color variation, combined with observations of current color distributions, have accordingly been used to justify either continuous (Hofmann et al. 2006) or discrete (Wang and Shaffer 2008) treatment of color hue in ancestral state reconstructions. Since high-performance liquid chromatography (HPLC) carotenoid profiles of *Euplectes* feathers (Andersson et al. 2007; Prager et al. 2009) suggest that evolutionary changes in color hue may have resulted from both quantitative and qualitative changes in pigmentation (see Introduction), we treat and reconstruct color hue both as a continuous and discrete trait in this study.

### Continuous hue

Objective and context-independent color measures were acquired using reflectance spectrometry. Reflectance was measured with a USB2000 spectroradiometer system (OceanOptics, Dunedin, FL), including a fiber-optic reflectance probe, a HL2000 tungsten-halogen light source, and using CSpec software (Ancal, Las Vegas, NV). We used a "coincident normal" measuring configuration (coaxial illumination and reading beams, perpendicular to the plumage plane), with a homemade probe holder fitted on the probe, taking three to five scans and removing the probe between each. The $\lambda_{R50}$ colorimetric (wavelength at which reflectance is halfway between its minimum and its maximum) was used as a measure of the spectral location, that is, hue of plumage colors. For these and other details, see Andersson and Prager (2006).

### Discrete hue

Following a procedure modified from Wang and Shaffer (2008), we used one-dimensional k-means clustering in the software "R" (R Development Core Team 2009) to partition the continuous $\lambda_{R50}$ values into hue categories. "k-means clustering" (Hartigan and Wong 1979) is an iterative process in which *n* observations are partitioned into *k* clusters, while minimizing distances between sample points and cluster centroids. Initial values for centroids are randomly assigned, and new values are calculated as cluster means after each iteration, until cluster assignments no longer change. To find the most robust assignment of carotenoid hue classes in our dataset, three trials of k-means clustering with $k = 2$, $k = 3$, and $k = 4$, respectively, were each run on 1000 bootstrapped samples of taxon mean values. The percentage of runs in which

original data points would fall into the same (best) clusters was then compared between the three different trials.

In trials with $k = 2$ and $k = 3$, 100% of the runs returned the same clusters, loosely corresponding to the human-subjective classes "yellow" ($\lambda_{R50} = 513$–555 nm)/"red" (560–610 nm) and "yellow" (513–536 nm)/"orange" (555–575 nm)/"red" (580–610 nm), respectively. In contrast, clustering with $k = 4$ was less robust, giving identical results in only 43.8% of the runs. Ancestral reconstructions of discrete color hue were thus based on the former two classifications (i.e., $k = 2$ and $k = 3$). In order to include taxa lacking carotenoid pigmentation (and thus $\lambda_{R50}$ value) in the analyses, a "non-carotenoid" class was added to each of these classification schemes (hereafter called "three-state" and "four-state" color hue).

### RECONSTRUCTION OF COLOR EVOLUTION

We reconstructed continuous and discrete measures of color hue in *Euplectes* and outgroups.

#### Continuous hue

Reconstructions of continuous hue states were performed in the software Mesquite (Maddison and Maddison 2004) version 2.71, using the fully resolved ("allcompat") version of the Bayesian consensus tree in Prager et al. (2009). Before reconstruction of continuous hue, alternative models of character evolution were compared using the Akaike Information Criterion (AIC; Akaike 1974) in the Mesquite module CoMET (Oakley et al. 2005; Lee et al. 2006), varying the punctuation asymmetry threshold between 5 and 1000. We found that color hue evolution in the genus is best described by a "punctuated avg/equal" model, although this is not significantly better ($\Delta$AIC $<$ 2) than a "punctuated avg/distance" model. Both models limit trait change to one of each pair of descendents of a node, but whereas the former assumes that the amount of change between subsequent nodes is equal across the tree, the latter expects it to be proportional to genetic distance, or time, separating these nodes.

Among available options for reconstruction of continuously varying characters in Mesquite, that is, linear parsimony (Farris 1970; Swofford and Maddison 1987) and squared-change parsimony (Rogers 1984; Huey and Bennett 1987), we follow Hofmann et al. (2006) and Prager et al. (2009) in considering linear parsimony to be the best correspondence to the punctuated models suggested by CoMET. However, in order to test the sensitivity of our results to choice of method, we also reconstructed color hue evolution using squared-change parsimony.

#### Discrete hue

Discrete color hues of ancestral nodes were mapped onto the Bayesian consensus tree in Mesquite, using ordered maximum

parsimony and maximum-likelihood (Markov k-state 1 parameter model, Lewis 2001) methods. To account for uncertainty in the estimated tree, when making inferences about ancestral states, we also applied stochastic character mapping (Nielsen 2002; Huelsenbeck et al. 2003) of four-state color hue to the "post-burnin" posterior sample of 10,000 trees in Prager et al. (2009), using SIMMAP (Bollback 2006) version 1.0 beta 2.0.5.

Stochastic character mapping is a Bayesian reconstruction method, in which ancestral character states are sampled in proportion to their posterior probabilities, which are probabilities conditional on the observed distribution of states among extant taxa, and a stochastic model of character transformation. Markov chain Monte Carlo (MCMC) sampling of both alternative trees and model parameters, moreover, enables SIMMAP to calculate marginal posterior probabilities of each character state at selected ancestral nodes, i.e., probabilities that are integrated over alternative tree topologies, branch lengths, and transformation rates. For multistate characters, SIMMAP implements only a single-rate model for character state change, meaning that all state transitions are assumed to be equally likely, for a given branch length or time interval. To sample overall rate values (or "tree lengths") in proportion to posterior probability, SIMMAP requires prior knowledge of the probability of different rate values to be specified, in the form of a discrete gamma ($\Gamma$) distribution. The gamma distribution is defined by its "shape" and "rate" parameters, $\alpha$ and $\beta$ [mean, $E(\Gamma) = \alpha/\beta$, and standard deviation, $SD(\Gamma) = \sqrt{(\alpha/\beta^2)}$], and is discretized by SIMMAP using $k$ categories.

Since rates of morphological evolution are generally estimated from data on a single or a few characters, rather than thousands of nucleotides in a typical DNA sequence alignment, these estimates are potentially more sensitive to choice of priors. Therefore, after scaling all tree lengths to one, we compared the results from four different priors on the overall transformation rate: (1) an empirical prior set to mimic the distribution of tree lengths derived from maximum likelihood reconstruction of discrete color hue on the posterior sample of trees in Mesquite ($\alpha = 100$, $\beta = 6.7$, i.e., $E[\Gamma] = 15$, $SD[\Gamma] = 1.5$); (2) a less informative prior with the same mean ($\alpha = 3$, $\beta = 0.2$, i.e., $E[\Gamma] = 15$, $SD[\Gamma] = 8.7$); (3) a prior of intermediate spread, with mean set to the observed low extreme of the likelihood distribution ($\alpha = 5$, $\beta = 0.5$, i.e., $E[\Gamma] = 10$, $SD[\Gamma] = 4.5$); and (4) a prior of intermediate spread, with mean set to the high extreme of the likelihood distribution ($\alpha = 10$, $\beta = 0.5$, i.e., $E[\Gamma] = 20$, $SD[\Gamma] = 6.3$). The gamma distribution was discretized using 50 categories, and the number of draws made from the prior distribution of rates was set to 50 per tree. Following each analysis, the posterior distribution of the rate parameter was inspected to make sure that it was not truncated at either end, thus warranting limits on the prior to be changed.

## *Results*

### SAMPLES AND MEASUREMENTS

Mean spectral reflectance of the carotenoid-pigmented plumage patches in 33 *Euplectes* taxa are shown in Figure 1. Objective hue colorimetrics, and discrete color scores derived from these, are listed for *Euplectes* and outgroups in Table 1.

### RECONSTRUCTIONS OF COLOR EVOLUTION

#### *Continuous hue*

Linear parsimony reconstruction of continuous color hue suggested that extant widowbirds and bishops likely derive from a common ancestor with yellow ($\lambda_{R50} = 522\text{–}536$ nm; Fig. 2) plumage coloration. These results furthermore imply that red coloration (i.e., $\lambda_{R50} >$ ca. 555 nm) has evolved repeatedly and independently in the most recent common ancestor of the "red bishop" clade, and in the ancestors of current *E. axillaris* (fan-tailed widowbird) and *E. progne* (long-tailed widowbird) subspecies. In addition, one or two independent "gains" of red carotenoid coloration, that is, large ($>$30 nm) increases in hue, has occurred in the *Euplectes* sister group of *Quelea* and *Foudia* (see Appendix S1). Based on midpoints of ranges estimated by linear parsimony, slight decreases in hue ($<$15 nm) have also occurred in the ancestors of current *E. gierowii* (black bishop), *E. ardens* (red-collared widowbird), and *E. albonotatus* (white-winged widowbird) subspecies.
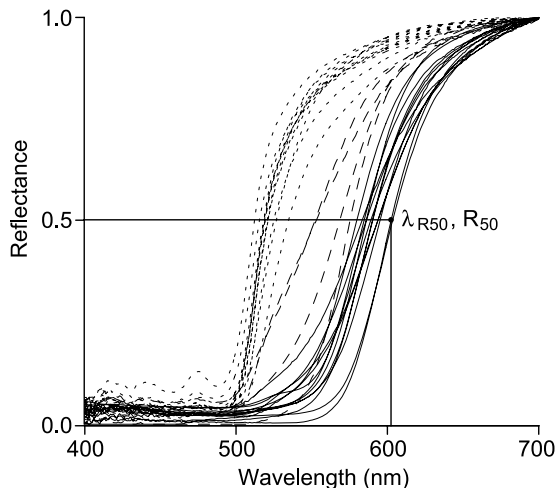


**Figure 1.** Mean spectral reflectance of the carotenoid-pigmented plumage patches in 33 *Euplectes* taxa. To illustrate differences in spectral position (hue), independent of variation in brightness, reflectance spectra have been normalized to equal maxima. Taxon spectra are sorted into the classes "yellow" (dotted lines), "orange" (dashed) and "red" (solid), based on "k-means" clustering (*k* = 3) of the objective hue colorimetric $\lambda_{R50}$ (i.e., wavelength of the reflectance midpoint [$R_{50}$], see Methods, illustrated for the rightmost curve in the graph).

Ancestral reconstruction with squared-change parsimony (Appendix S2) supported a slightly longer wavelength, but still yellow or golden, hue ($\lambda_{R50} = 544$ nm) of the *Euplectes* ancestor. The same three independent origins of red hues were again suggested within the genus, as well as one additional gain in the *Quelea* plus *Foudia* clade. Finally, minor decreases ($<$15 nm) in color hue were mapped onto branches leading to most yellow groups of the tree, for example, to clades of *Ploceus*, *E. afer* (yellow-crowned bishop), and *E. macrourus* (yellow-mantled widowbird).

#### *Discrete hue*

With varying confidence, all analyses of discrete color hue supported the main results derived from continuous reconstructions, that is, a yellow ancestor to extant widowbirds and bishops, and three independent origins of red coloration in the genus (Fig. 2). Inclusion of non-carotenoid pigmented taxa in discrete analyses furthermore showed that complete loss of carotenoid pigmentation has occurred independently in *E. ardens concolor* (the all-black form of red-collared widowbird), the East-African *E. albonotatus eques*, and *E. jacksoni* (Jackson's widowbird). In addition, likely reversals from red to orange or yellow were suggested to have occurred in the ancestors of *E. gierowii* (black bishop), in *E. ardens ardens* (red-collared widowbird), and, possibly, in *E. albonotatus*.

Reconstructions of three- and four-state hue (Table 1), respectively, were highly concordant, and results are thus only illustrated for the latter, more fine-scaled classification scheme (Fig. 2). The likelihood of yellow ancestors to (1) all *Euplectes*, (2) *Euplectes* minus *E. afer* and *E. aureus*, (3) "true widowbirds" (*E. capensis* to *E. progne*; Fig. 2), (4) and "true widowbirds" minus *E. capensis* and *E. macrourus*, however, received 0.17–0.21 higher likelihood support from reconstruction of three-state hue (see Appendix S2), compared to four-state hue.

Within *Euplectes*, parsimony reconstruction was unambiguous and consistent with maximum likelihood and stochastic character mapping, with two exceptions: First, in the ancestor of the "red bishop" clade (*E. gierowii* to *E. orix*; Fig. 2), the likelihood and posterior probability of red coloration (0.56 and 0.75–0.78, depending on prior settings) was higher than for orange (0.26 and 0.20), which was found to be the most parsimonious state. Second, proportional likelihood support for the most parsimonious state in the common ancestor of *E. axillaris* and *E. albonotatus* (yellow; 0.52) was very similar to that of an alternative state (red: 0.47), and posterior probabilities for these were reversed (0.17–0.19 vs. 0.80–0.82).

Stochastic mappings based on different priors settings for overall transformation rate consistently favored the same ancestral states, although posterior probabilities (integrated over alternative tree topologies) varied somewhat between different prior settings.
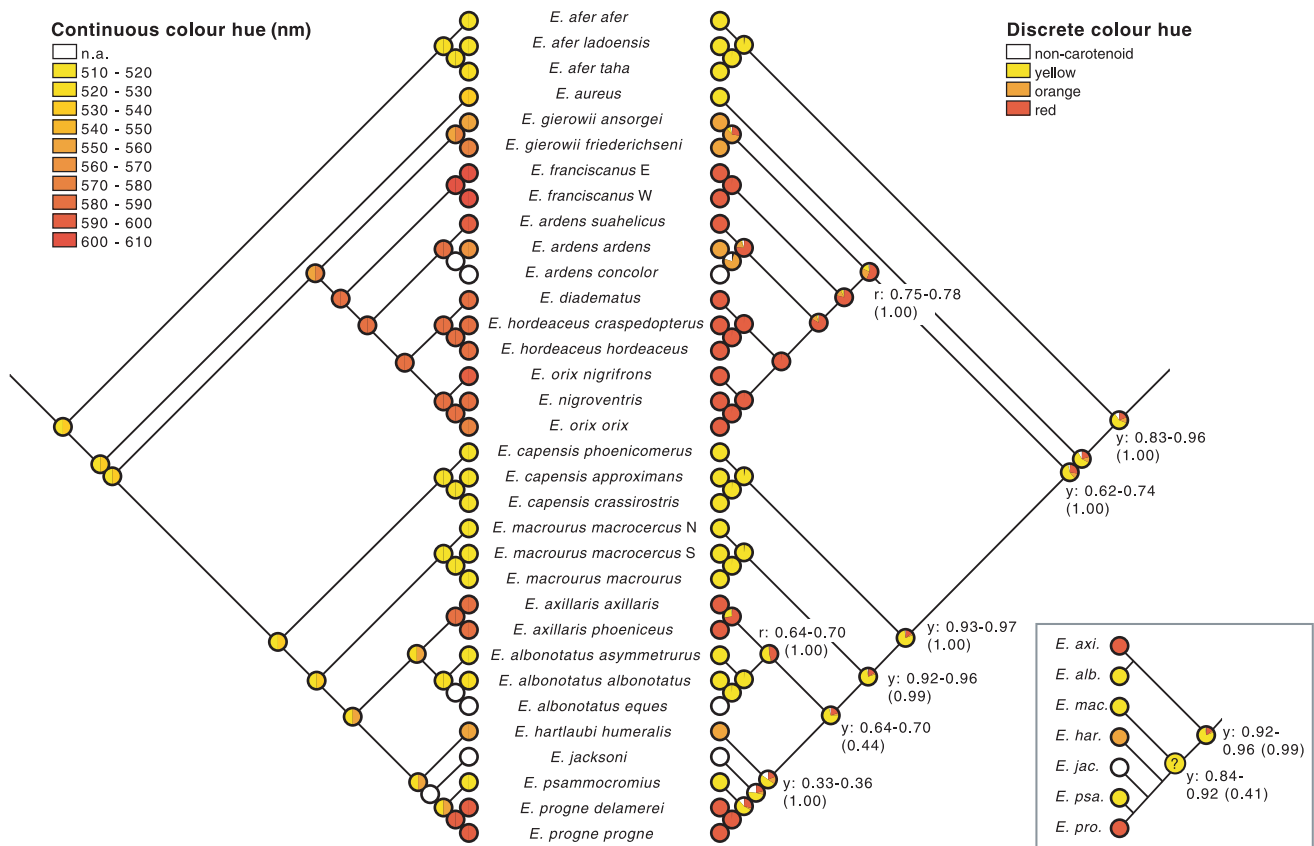
**Figure 2.** Reconstruction of ancestral carotenoid color hues in *Euplectes*. Circles in the left tree represent linear parsimony estimates of continuous hue states (single, or intervals of equally parsimonious, states). Pie charts in the right tree show proportional likelihoods of alternative discrete hue states. For selected nodes, numbers additionally state the posterior probability of favored states (y = yellow, o = orange, r = red), and of the node itself (in parenthesis; from Prager et al. 2009). The inserted box illustrates an alternative topology for a poorly resolved clade, of importance for conclusions about the homology of red coloration in the group.

Although reconstructions showed considerable uncertainty, the posterior probabilities for yellow ancestors to (1) all *Euplectes* and (2) true widowbirds (see above) were high (0.83–0.96 and 0.93–0.97), suggesting that red coloration has been gained separately in bishops and true widowbirds. Less likely, but implied by lower support for yellow color in the ancestor of *Euplectes* minus *E. afer* and *E. aureus* (0.62–0.74), red color has been lost and subsequently regained in the widowbird clade. Stochastic character mapping on the two alternative topologies within the poorly resolved widowbird clade (Fig. 2 and insert), likewise showed some support (0.64–0.70 or 0.92–0.96) for yellow plumage in the most recent common ancestors of *E. axillaris* and *E. progne*, suggesting independent gains of red coloration in these taxa as well.

## Discussion

Ancestral state reconstructions of both discrete and continuous color hue in *Euplectes* suggest a rather directional and convergent evolution of red carotenoid coloration: Extant widowbirds and bishops derive from a yellow ($\lambda_{R50}$ = 522–544 nm) ancestor, and during the diversification there have been at least two, probably three, convergent gains of red coloration (i.e., $\lambda_{R50}$ > ca. 555 nm), after which there are no strongly supported reversals to yellow and only a single loss of carotenoid coloration (in *E. ardens concolor*). According to the most likely scenario, red hues have evolved independently in the most recent common ancestor of the "red bishop" clade, and in the ancestors of extant *E. axillaris* (fan-tailed widowbird) and *E. progne* (long-tailed widowbird) subspecies.

That the similarity in color hue between "red bishops" (*E. gierowii* to *E. orix*; Fig. 2) and "true widowbirds" (*E. capensis* to *E. progne*) is due to convergent evolution, rather than shared ancestry, is further supported by HPLC analyses of plumage pigments, showing distinct differences in feather pigmentation between the two groups: First, *E. orix* (southern red bishop) and *E. ardens* (red-collared widowbird), both belonging to the "red bishop" clade, achieve orange-red color by adding relatively small amounts of metabolically derived red C4-keto-carotenoids (mainly α-doradexanthin and canthaxanthin) to feathers.

In contrast, the longwave hues of *E. axillaris* (fan-tailed widowbird) derive from deposition of high concentrations of dietary and derived yellow pigments (Andersson et al. 2007; Prager et al. 2009). Importantly, these mechanistic differences support homoplasy in color between these clades, even if their most recent common ancestor (third node from right in right-hand tree, Fig. 2) was not yellow, in which case red coloration has been lost and later regained in the "true widows." The support for a third independent origin of red plumage color in *E. progne* (long-tailed widowbird) varies considerably with alternative tree topologies (Fig. 2 and insert), but may be resolved by future pigment analyses in this species, and by better phylogenetic resolution in this part of the tree.

## RECONSTRUCTION METHODS

Given that evolutionary processes include a stochastic component, ancestral state reconstructions are inevitably uncertain, especially for rapidly evolving traits (Schluter et al. 1997; Losos 1999). Whereas parsimony methods only reflect this uncertainty by displaying equally parsimonious states, maximum likelihood and Bayesian methods provide estimates of accuracy for all reconstructed ancestral states. Following Edwards (1972), Pagel (1999) suggested that the difference in log likelihood between states should be at least two, before one concludes that one is significantly more likely than another. Applying this strict likelihood requirement to color evolution in *Euplectes* would mean that most internal nodes are considered equivocal, at least in terms of four-state hues. However, many of these nodes received close to 0.95, i.e., high, posterior probability in stochastic character mapping. Although the support for reconstructed values varied somewhat between methods, reconstructions of discrete and continuous color gave highly concordant results, regardless of whether parsimony, likelihood, or (Bayesian) stochastic methods were used. This is particularly reassuring because state probabilities in the latter method are integrated over alternative tree topologies, branch lengths, and transformation rates, and because rate priors were intentionally varied within quite wide intervals. Below, we discuss the implications of these results and, for poorly supported nodes, the extent to which alternative reconstructions would lead to different interpretations of the evolutionary history of carotenoid coloration in widowbirds and bishops.

## SIGNAL SELECTION AND COSTS

Although multiple gains of a signal selection pressure for red is entirely possible, it is tempting to interpret the convergence of red coloration as a result of a universal and ancestral selection pressure for ever redder and more honest carotenoid displays. As regards the agonistic function, the importance of redness for social status has been established for the phylogenetically quite distant *E. axillaris* and *E. ardens* (Pryke et al. 2001, 2002; Pryke and

Andersson 2003a,b). The generality of red dominance and honesty in other weaverbirds needs further investigation, however, because, for example, red bill color (Shawcross and Slater 1983) but not plumage redness (Dale 2000) was found to be related to individual quality in *Quelea quelea*. Furthermore, color manipulations in a captive *E. orix* population had no consistent effect on social status (Edler and Friedl 2010). Finally, females mated to males with brighter (i.e., likely less carotenoid-pigmented) plumage produced larger clutches in *Foudia madagascariensis* (Estep et al. 2006).

In addition to the convergence of redness, strongly supported by the different underlying mechanisms, ancestral state reconstructions also suggest evolution toward shorter wavelength hues in some branches of the *Euplectes* tree, and even complete loss of carotenoid pigmentation in three taxa: Jackson's widowbird (*E. jacksoni*), the East African subspecies of white-winged widowbird (*E. albonotatus eques*), and the all-black form of the red-collared widowbird (*E. ardens concolor*). These losses seem independent of each other and all are taxa with elongated nuptial tails, exaggerated directionally (Prager and Andersson 2009) in response to female mate choice (Andersson 1982, 1992; Pryke and Andersson 2002, 2005). A trade-off between tail length and carotenoid investment has previously been demonstrated intraspecifically in *E. ardens ardens* (Andersson et al. 2002), but may also contribute to the apparent negative relationship between nuptial tail elongation and coloration in the genus as a whole (S. Andersson et al., unpubl. data).

## GENETIC CONSTRAINTS

Given consistent selection for redness and limited variation in ecological constraints (diet, habitat, visual predators), one likely explanation for yellow or no carotenoid coloration in some taxa seems to be genetic or physiological constraints. Indeed, diet manipulation showed that *E. afer* (yellow-crowned bishop) not only lacked red C4-keto-carotenoids (α-doradexanthin and canthaxanthin) in their feathers, but, unlike *E. orix*, were also unable to manufacture these from yellow dietary precursors (lutein and β-carotene, respectively; Prager et al. 2009). Whether this means that *E. afer*, and presumably other yellow *Euplectes*, lack the actual gene for the hypothesized enzyme, or just its expression (due to, e.g., energetic constraints) remains to be investigated.

## IMPLICATIONS FOR SEXUAL SELECTION AND SIGNAL DIVERSIFICATION

As in our reconstruction of male tail elongation (Prager and Andersson 2009), the evolutionary pattern for carotenoid coloration seems to be one of directional and convergent signal exaggeration. There are several reasons why we think this is the expected footprint of sexual selection, despite the many studies indicating lability and divergence (in birds, e.g., Kusmierski et al.

1997; Prum 1997; Kimball et al. 2001; Ödeen and Björklund 2003; Hofmann et al. 2006; Price et al. 2007; Cardoso and Mota 2008):

- We analyze a taxon and signal traits for which sexual selection pressures are uniquely well documented. These signals are furthermore measured in dimensions (i.e., tail length and color hue) that are both targeted by signal receivers, and closely related to honesty-maintaining mechanisms (Andersson 2000; Andersson and Prager 2006). In contrast, when little is known about the details of sexual selection, missing or mismeasuring its targets may invalidate conclusions about its macroevolutionary consequences.

- Younger and less ecologically diverse radiations like this one may present cleaner outcomes of sexually selected signal diversification, not drowning in a multitude of phylogenetically and historically varying constraints. In fact, we think the view of labile and divergent sexual selection partly derives from ascribing reconstructed patterns of signal evolution to only one of several underlying selection processes. Ecological divergence in signal conditions and costs, or conflicts with species recognition, can reverse or redirect signal evolution, but this does not mean that sexual selection per se is an inherently labile and divergent force.

- The distinction between signal selection and constraints highlights the importance of understanding the underlying mechanisms. Without knowing the biochemistry behind red coloration in the two clades (Andersson et al. 2007; Fig. 2), the case for convergence (and perhaps a pre-existing sensory bias) would have relied only on the ancestral state reconstruction and had been much weaker.

## CONCLUSION

Conspicuous avian plumage colors have been discussed and intraspecifically tested with respect to social and sexual selection pressures for more than a century. Yet, few investigations have targeted interspecific color diversity by combining data on proximate mechanisms and adaptive functions of coloration in a robust phylogenetic framework. Our reconstruction of the evolution of sexually selected carotenoid coloration in the African widowbirds and bishops (*Euplectes* spp.) provides a valuable perspective on sexually selected signal diversification, and for exploring genetic and physiological constraints on carotenoid coloration in birds. We conclude that extant widowbirds and bishops likely derive from a yellow ancestor, and that redder (longer wavelength) color hues have been repeatedly gained, via different pigment mechanisms. The directional and convergent sexual selection scenario may be more representative than it seems in the literature, but only detected where signal functions and mechanisms are well

known, and social and ecological diversification is limited. These results should guide further experimental research, underlining the important synergy of intra- and interspecific studies for understanding the evolutionary origins of biodiversity.

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## Supporting Information

The following supporting information is available for this article:

**Appendix S1.** Reconstruction of ancestral carotenoid color hues in the *Euplectes* and outgroups (*Amblyospiza*, *Ploceus*, *Foudia*, and *Quelea*). Circles in the left tree represent linear parsimony estimates of continuous hue states (single, or intervals of equally parsimonious, states). Pie charts in the right tree show proportional likelihoods of alternative discrete (four-state) hue.

**Appendix S2.** Reconstruction of ancestral carotenoid color hues in the *Euplectes* and outgroups (*Amblyospiza*, *Ploceus*, *Foudia*, and *Quelea*). Circles in the left tree represent squared-change parsimony estimates of continuous hue states. Pie charts in the right tree show proportional likelihoods of alternative discrete (three-state) hue.

Supporting Information may be found in the online version of this article.

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