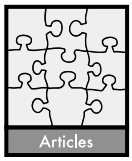

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Melanin coloration in New World orioles II: ancestral state reconstruction reveals lability in the use of carotenoids and phaeomelanins

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Although many animals use carotenoids to produce bright yellow, orange, and red colors, an increasing number of studies have found that other pigments, such as melanins, may also be used to produce bright colors. Yet, almost nothing is known about the evolutionary history of this colorful melanin use. We used reflectance spectrometry to determine whether colors in New World orioles were predominantly due to carotenoids, colorful melanins, or a mixture of both. We then used ancestral state reconstruction to infer the directionality of any pigment changes and to test for phylogenetic signal. We found that three oriole taxa likely switched from carotenoid- to melanin-based colors. Several other oriole taxa apparently gained localized melanin coloration, or had coloration that seemed to be produced by a mixture of carotenoids and melanins. We also found little phylogenetic signal on the use of carotenoids or melanins to produce color. However, all pigment changes occurred within one of three major clades of the oriole genus, suggesting there may be signal at deeper phylogenetic levels. These repeated independent switches between carotenoid and melanin colors are surprising in light of the important signaling role that color pigments (especially carotenoids) are thought to play across a wide range of taxa.

Carotenoid pigments are a well-studied means for producing many of the bright yellow, orange, and red colors found throughout the animal kingdom (reviewed in Fox 1976, Hill 2002, Hofmann et al. 2007). Considerable research has focused on understanding why sexual selection might favor the evolution of increasingly bright or intense carotenoid coloration (e.g., Hill 1991, 1994, Faivre et al. 2003, Blount et al. 2003, see also Olson and Owens 1998). However, such studies often make the implicit assumption that animals with these elaborate carotenoid colors are derived from ancestors with little (or no) carotenoid coloration. A phylogenetic perspective suggests that the reverse is possible, and carotenoid colors may also be reduced or lost (Omland and Hofmann 2006).

Several recent studies have found that melanins may produce bright, highly reflective colors that appear visually similar to carotenoids and were sometimes mistakenly considered carotenoids. These colors include the chestnut of the orchard oriole *Icterus spurius* (Hofmann et al. 2007), as well as various color elements of zebra finches *Taeniopygia guttata*, barn swallows *Hirundo rustica rustica* and *H. r. erythrogaster*, and red-winged blackbird epaulets *Agelaius phoeniceus* (McGraw et al. 2003, 2004 a, b, c). Although these individual occurrences of melanin-based colors have important implications for the role of melanins in color signaling, little is known about the evolutionary history of such colors.

New World orioles (genus *Icterus*) are an ideal system for studying the evolution of carotenoid and melanin colors. Orioles have colored plumage that ranges from lemon-yellow to red-orange, as well as chestnut or tan colors that are suggestive of the presence of melanins (for illustrations see Jaramillo and Burke 1999). Adult males of all species also have achromatic black or dark brown plumage, which is likely due to eumelanins and which differs from the colorful chestnut

or tan colors that are due predominantly to phaeomelanins. Previous research found that the bright orange plumage of the Baltimore oriole *I. galbula* is produced by a mixture of carotenoids (Hudon 1991). However, we have recently demonstrated that the chestnut color of the orchard oriole is produced by melanins, predominantly phaeomelanins, and the tan color found in the closely related Fuertes's oriole *I. fuertesi* is produced by a combination of carotenoids and melanins (Hofmann et al. 2007). In addition to having carotenoid and melanin colors, orioles also have a well-resolved molecular phylogeny that is supported by both mitochondrial DNA (Omland et al. 1999) and nuclear introns (Allen and Omland 2003). This phylogeny was previously used by Omland and Lanyon (2000) to study pattern evolution and suggested multiple instances of convergence and reversal in oriole pattern.

The first goal of this study was to use reflectance spectrometry to determine whether the coloration of different oriole taxa was due to carotenoids, melanins, or a mixture of both, across males within the oriole genus. We then used ancestral state reconstruction to infer the directionality of any changes that occurred and to investigate whether the pigments used to produce color were subject to strong or weak phylogenetic signal. In particular, was there a tendency to shift from non-carotenoid to carotenoid, or did the reverse occur? Understanding the directionality of carotenoid and melanin color evolution, as well as the role of phylogenetic signal, may have important implications for theories of color signaling. For example, strong phylogenetic signal might limit the type or direction of color evolution, even if that type or direction of color change was favored by sexual selection.

Methods

Specimen analysis

We examined the colorful plumage of adult male oriole specimens from the Smithsonian National Museum of Natural History, the Delaware Museum of Natural History, and the Field Museum of Natural History (see Appendix 1 for voucher numbers). Reflectance spectra were measured from museum specimens from 300–700 nm using the methods of Hofmann et al. (2007). All measurements were taken relative to a diffuse white standard and the dark, in replicates of three. Raw spectral data were processed, replicates were averaged, and composite spectra were generated from each body region of each taxon for further scoring. In all, we scored color across 45 different taxa. Five different specimens were scored for each taxon (with the exception of six taxa for which only 1–4 specimens were available for measurement, see Appendix 1).

Plumage measurements were taken from seven body regions: the breast, belly, throat, rump, back, crown, and epaulet (a shoulder patch, formed by the median and lesser coverts). (Due to limitations of the equipment being used, we could only measure color from the epaulet when both median and lesser coverts were the same color). All orioles have color in at least one of these regions, although no single region is colored across all taxa (Table 1). For example, the epaulet oriole *I. cayanensis cayanensis* has colored epaulets but black plumage occurring across the remaining six body regions, whereas the yellow-backed oriole *I. chrysater* has a black epaulet but most other body regions are colored.

Character scoring

We defined colorful plumage as any plumage that had a maximum reflectance over 10%. When reflectance spectra were examined, there was a discrete gap in maximum reflectance around 10%, corresponding to the difference between black or dark brown plumage and all others (data not shown). In addition, plumage that had a maximum reflectance under 10% lacked the spectral characteristics used to classify color. Therefore, plumage with less than 10% reflectance was scored as black. Body regions having black plumage were treated as missing data when performing ancestral state reconstruction because of the possibility that carotenoids were being masked by the black color (Butcher 1991, also see Hofmann et al. 2007). The reflectance spectra of carotenoid and melanin colors have unique shapes due to biochemical differences between the two pigments (Bleiweiss 2004, McGraw et al. 2004a, Shawkey and Hill 2005, Hofmann et al. 2007). Carotenoid colors produce a complex reflectance spectrum which characteristically has a minor peak in the ultraviolet region of the spectrum and a rapid increase in reflectance occurring between 500–550 nm that plateaus at longer wavelengths (Fig. 1a; Bleiweiss 2004, see also examples in MacDougall and Montgomerie 2003, Mays et al. 2004, Hofmann et al. 2007). Melanin colors produce a reflectance spectrum which lacks all of these carotenoid characteristics and instead has a gradual increase in reflectance at longer wavelengths (Fig. 1b; McGraw et al. 2004a, Safran and McGraw 2004).

The class of pigment producing color was scored as a character with the following states: carotenoid (C) if all colorful plumage had reflectance spectra with characteristics of carotenoid colors (Fig. 1a); melanin (M) if all colorful plumage had characteristics of melanin colors (Fig. 1b); intermediate (I) if all colorful plumage

Table 1. Individual body regions and overall body color were scored as either carotenoid (C), melanin (M), intermediate (I), patch (P), or black (B). Presence of colorful melanins was scored as present (Y) or absent (N).

Taxa	Epaulet	Back	Rump	Throat	Breast	Belly	Crown	Overall body color	Presence of colorful melanins
I. cayanensis cayanensis	C	B	B	B	B	B	B	C	N
I. cayanensis pyrrhopterus	M	B	B	B	B	B	B	M	Y
I. cayanensis periporphyrus	I	B	B	B	B	B	B	I	Y
I. chrysocephalus	C	B	C	B	B	B	C	C	N
I. chrysater chrysater	B	C	C	B	C	C	C	C	N
I. chrysater hondae	B	C	C	B	C	C	C	C	N
I. nigrogularis nigrogularis	C	C	C	B	C	C	C	C	N
I. nigrogularis trinitatis	C	C	C	B	C	C	C	C	N
I. leucopteryx leucopteryx	[1]-	[2]C	[2]C	B	C	C	[2]C	C	N
I. auratus	[1]-	C	C	B	C	C	C	C	N
I. mesomelas mesomelas	C	B	C	B	C	C	C	C	N
I. mesomelas salvinii	C	B	C	B	C	C	C	C	N
I. mesomelas taczanowskii	C	B	C	B	C	C	C	C	N
I. auricapillus	C	B	C	B	C	C	C	C	N
I. graceannae	C	B	C	B	C	C	C	C	N
I. pectoralis	C	B	C	B	C	C	C	C	N
I. gularis tamaulipensis	C	B	C	B	C	C	C	C	N
I. gularis yucatanensis	C	B	C	B	C	C	C	C	N
I. gularis gularis	C	B	C	B	C	C	C	C	N
I. pustulatus formosus	[1]-	[2]C	C	B	C	C	C	C	N
I. pustulatus sclateri	[1]-	[2]C	C	B	C	C	C	C	N
I. cucullatus nelsoni	[1]-	B	C	B	C	C	C	C	N
I. cucullatus igneus	[1]-	B	C	B	C	C	C	C	N
I. icterus ridgwayi	C	B	C	B	C	C	B	C	N
I. jamacaii croconotus	C	C	C	B	C	C	C	C	N
I. jamacaii strictifrons	C	B	C	B	C	C	C	C	N
I. galbula galbula	C	B	C	B	C	C	B	C	N
I. bullockii bullockii	[1]-	B	C	B	C	C	B	C	N
I. bullockii parvus	[1]-	B	C	B	C	C	B	C	N
I. abeillei	[1]-	B	B	B	C	C	B	C	N
I. spurius	M	B	M	B	M	M	B	M	Y
I. spurius fuertesi	I	B	I	B	I	I	B	I	Y
I. dominicensis prosthemelas	C	B	C	B	P	C	B	P	Y
I. dominicensis northropi	C	B	C	B	C	C	B	C	N
I. dominicensis melanopsis	C	B	C	B	B	B	B	C	N
I. dominicensis dominicensis	C	B	C	B	B	C	B	C	N
I. dominicensis portoricensis	C	B	C	B	B	B	B	C	N
I. wagleri wagleri	C	B	C	B	P	C	B	P	Y
I. laudabilis	C	B	C	B	B	P	B	P	Y
I. bonana	M	B	M	B	M	M	B	M	N
I. oberi	B	B	C	B	P	C	B	P	Y
I. graduacauda audubonii	C	[2]C	[2]C	B	C	C	B	C	N
I. graduacauda graduacauda	C	[2]C	[2]C	B	C	C	B	C	N
I. maculialatus	C	B	C	B	C	C	B	C	N
I. parisorum	C	B	C	B	C	C	B	C	N

[1]Epaulet could only be measured when both middle and lesser coverts were the same color.
[2]Indicates taxa with mottled or olive coloration which had carotenoid spectral shape but reduced reflectance.

had characteristics of both carotenoid and melanin colors (e.g., a reduced but not absent peak in the ultraviolet regions of the spectrum, and a slight leveling off at longer wavelengths that never completely plateaus) (Fig. 1c); finally, patch (P) if a small localized region of plumage had characteristics of a melanin color, but the remainder of the colorful plumage had carotenoid characteristics (e.g., a thin band of chestnut plumage between a black bib and a carotenoid breast).

Ancestral state reconstruction

All ancestral state reconstructions were performed in MacClade (Maddison and Maddison 2000) using the previously published oriole mitochondrial DNA phylogeny (Omland et al. 1999; Fig. 6). This phylogeny was previously used by Omland and Lanyon (2000) to investigate pattern convergence in orioles. We reconstructed the class of pigment producing color within

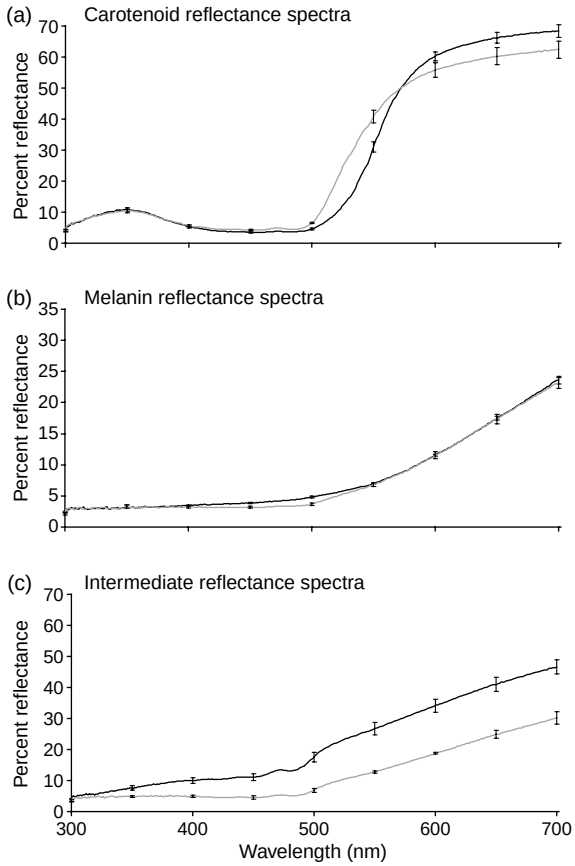

Fig. 1. Representative spectra from carotenoid (a), melanin (b), and intermediate (c) color plumage. All spectra were measured from the breast and are averages from five different specimens except for the tawny-shouldered oriole, for which the epaulets of two different specimens were measured. The carotenoid spectra are from the Baltimore *I. galbula* (black) and Altamira orioles *I. gularis gularis* (gray), the melanin from the orchard *I. spurius* (black) and Martinique orioles *I. bonana* (gray), and the intermediate from the Fuertes's *I. fuertesi* (black) and tawny-shouldered orioles *I. c. periporphyrus* (gray). Bars represent standard error (error bars are barely visible for melanin because of low variation).

each body region as well as overall body color (see Discussion). Color pigment class was treated as a reversible, discrete ordered character with I and P being intermediates between C and M (Fig. 2) (see Discussion). Finally, we examined how changing the weight of gaining any type of melanin color altered the ancestral state reconstruction. We scored the presence or absence of any type of colorful melanin as a discrete binary character with two states: present (Y), or absent (N), and examined how altering the cost of gaining colorful melanins influenced the reconstruction of ancestral states (Maddison and Maddison 1992, Omland 1997).

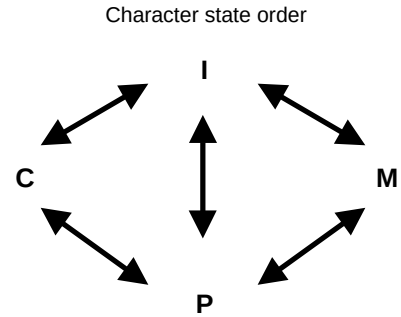

Fig. 2. Character state ordering used to reconstruct body color. All changes were considered a single step with the exception of C – M which required going through other character states thus requiring two steps.

Testing phylogenetic signal

We calculated the retention index (RI) and the consistency index (CI) for the body pigment class reconstruction (Maddison and Maddison 1992) and also compared the observed number of steps from this reconstruction to a randomly generated distribution. The shuffle command in MacClade was used to randomly shuffle the observed character states 100 times across the oriole phylogeny (Maddison and Slatkin 1991). Both the number of steps and the number of clades in which melanin pigments occur were recorded from the resulting reconstructions.

Results

Character scoring

We found three oriole taxa in which all male color plumage had spectral characteristics of melanins: *I. spurius*, *I. bonana* and *I. cayanensis pyrrhopterus* (note that the epaulet is the only colored body region in all three subspecies of *I. cayanensis*; Table 1). Two oriole taxa had intermediate color plumage: *I. fuertesi* and *I. c. periporphyrus* (Table 1). Although we observed changes in brightness, we did not detect gradation between melanin and intermediate plumage (Fig. 1). Four oriole taxa had melanin patches: *I. oberi*, *I. laudabilis*, *I. d. prosthemelas* and *I. wagleri*. The remainder of the oriole taxa had overall body colors that were characteristic of carotenoids (Table 1).

Ancestral state reconstructions

When we examined pigment class within body regions we found that changes in color plumage pigmentation

occurred in the breast, belly, rump and epaulet (Table 1). Each body region produced a slightly different ancestral state reconstruction because different taxa had black plumage at different places on the body, for which color could not be determined. However all reconstructions suggested independent gains of melanin based colors had occurred (e.g., epaulet, Fig. 3). Reconstructing overall body pigment class suggested that melanin-colored plumage arose independently three times. Intermediate color plumage arose two different times, both times in sister taxa to orioles with phaeomelanin color plumage. In addition, localized melanin color patches appear to have evolved independently one to four times (Fig. 4).

When we reconstructed the use of any colorful melanins as a discrete binary character that was either present or absent, we found that melanin colors seem to have arisen at least twice and as many as six times independently within the oriole genus. Weighting a gain of colorful melanins as $2\times$ more difficult than a loss altered the ancestral state reconstruction such that only two independent gains were reconstructed (all branches that were previously equivocal were now melanin). When gains of colorful melanins were weighted as $2.5\times$ more difficult, colorful melanins were reconstructed as having arisen once.

Testing phylogenetic signal

When the pigment basis of overall body color was reconstructed as an ordered character the retention index (RI) was 0.22, and the consistency index (CI) was 0.30 (both values range from 0–1). The most parsimonious reconstruction of body pigment class produced a 10 step reconstruction with all changes occurring within a single clade (Fig. 4). Randomly shuffling characters produced a reconstruction with 10 steps or less 11% of the time ($P = 0.11$). A 12 step reconstruction with the maximum number of changes was most common, occurring 55% of the time. Randomly shuffling characters never produced a reconstruction with all changes occurring in a single clade, unlike the real data for which all changes occur in clade A. Thus the probability of this distribution occurring across clades is significantly different from random ($P < 0.01$)

Discussion

Ancestral state reconstruction

Many behavioral ecology studies have examined the proximate mechanisms underlying carotenoid colors

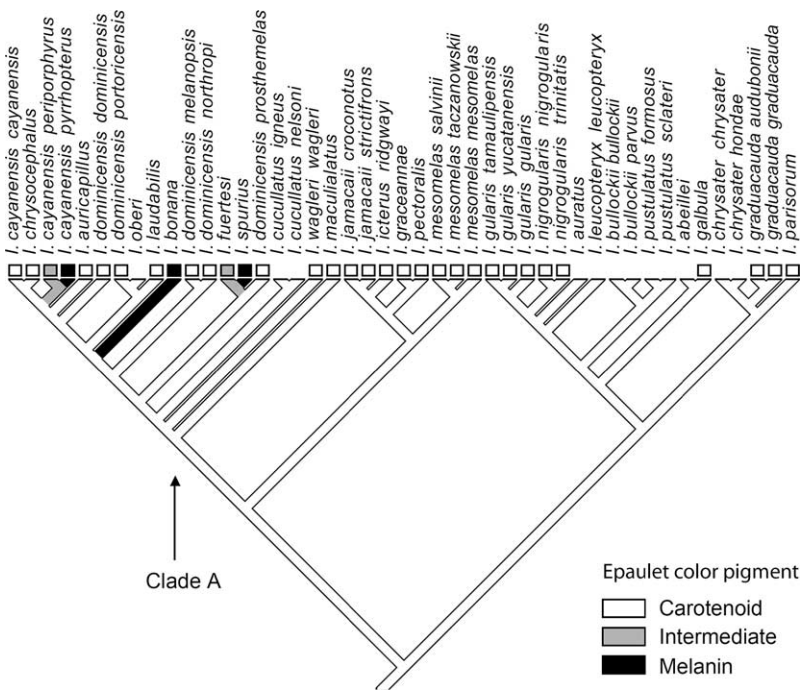

Fig. 3. Reconstruction of the pigment used to produce color within a single body region, the epaulet. The pigment used to produce color is reconstructed as a discrete ordered character using parsimony in MacClade (Maddison and Maddison 2000). Taxa for which the epaulet was black or could not be measured were treated as missing data and lack a box beneath the taxon name.

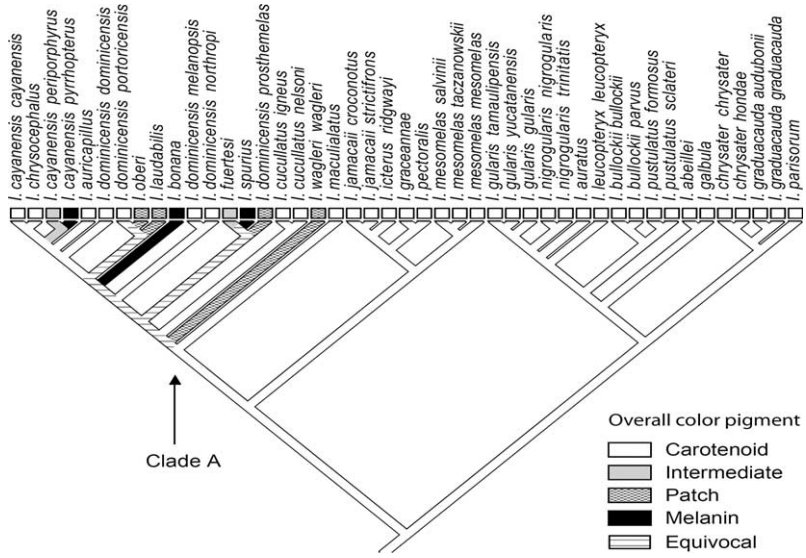

Fig. 4. Reconstruction of body color as a discrete ordered character using parsimony in MacClade (Maddison and Maddison 2000). Equivocal branches in this reconstruction represent nodes for which both carotenoid and intermediate states are equally parsimonious.

and the role of such colors in mate choice. These studies have focused, often implicitly, on understanding why carotenoid-based colors may become increasingly bright or intense (Omland and Hofmann 2006). Our findings suggest that the reverse may also happen, and that these elaborate carotenoid colors may be masked or lost. In three oriole taxa: the orchard oriole, the Martinique oriole, and the chestnut-shouldered oriole, all adult male color appears to be due to melanins (likely phaeomelanins). Ancestral state reconstruction suggests that these three gains of melanin color occurred independently, although whether from a carotenoid, patch, or intermediate ancestral state in many cases was equivocal with the assumptions we used (Fig. 4). However, the common ancestors at deeper phylogenetic levels are reconstructed as having carotenoid based color, strongly suggesting that multiple gains of melanin color have occurred (Fig. 4).

Ancestral state reconstruction also suggests that the two oriole taxa with intermediate plumage are both sister taxa to orioles with melanin-based color. It will be interesting to see if future studies find similar associations in other taxa that have carotenoid and melanin colors outside the genus *Icterus*. Although we did not observe overlap between melanin and intermediate colors in orioles, such overlap may occur in other genera, and represents another avenue of future research. Finally, the ancestral state reconstruction also suggested that localized regions of phaeomelanin coloration arose multiple times, either independently or in sister taxa to orioles having melanin and intermediate colors (Fig. 4).

To our knowledge this is the first study that has reconstructed the evolution of carotenoid and melanin colors among closely related taxa. Previous studies have used ancestral state reconstruction to examine the evolution of various elements of color and pattern. Hill and McGraw (2004) reconstructed yellow and red carotenoid coloration in cardueline finches, and numerous studies have reconstructed the evolution of various pattern elements (e.g., eye stripe, Price and Pavelka 1996; or colorful epaulets, Johnson and Lanyon 2000), or overall pattern (Omland and Lanyon 2000, Dumbacher and Fleischer 2001). However, no previous phylogenetic studies have addressed the evolution of different types of color pigments.

Phylogenetic signal

When the pigment basis of oriole color plumage was reconstructed as an ordered character both the retention index ($RI = 0.22$) and the consistency index ($CI = 0.30$) were low (both range from 0–1, with 0 being no agreement with the phylogeny and 1 being complete agreement) suggesting that strong phylogenetic signal was not present (Maddison and Maddison 1992, also see Omland and Lanyon 2000). Reconstructing the pigment basis of oriole color plumage produced a 10 step reconstruction. Randomly shuffling characters across the oriole phylogeny produced a reconstruction with 10 steps or less 11% of the time (Maddison and Slatkin 1991). While these results are not significantly different from random ($P = 0.11$), they suggest that

weak levels of signal might be present. This weak signal among terminal taxa is important because it suggests that the pigments used to produce coloration are open to selective pressures (such as sexual selection) or genetic drift, without the limitations of strong phylogenetic signal.

Although there appears to be little constraint on the use of carotenoids versus melanins to produce color among extant orioles, all pigment changes occur in only one of three major oriole clades. Random shuffling never produced a tree with all changes occurring within a single clade ($P < 0.01$), suggesting that phylogenetic signal is present at deeper phylogenetic levels. The clade in which pigment changes occur, referred to as clade A (Omland et al. 1999, Omland and Lanyon 2000), contains several taxa having typical oriole colors and patterns (e.g., *I. cucullatus*); however this clade also contains several oriole taxa with little color and mostly eumelanin (black) plumage (e.g., *I. cayanensis* ssp., and *I. dominicensis melanopsis*; Table 1, also see Omland and Lanyon 2000). These highly melanistic patterns, combined with repeated gains of melanin color, are suggestive of a tendency towards increased use of melanins, rather than carotenoids, within clade A.

Assumptions of ancestral state reconstruction

Assumptions about character evolution are always made during ancestral state reconstruction (Omland 1997, 1999, Cunningham 1999, Omland and Hofmann 2006). Even the simplest method of reconstructing characters (discrete unordered), makes the assumption that gains and losses are equally probable and that there are not (or historically were not) any intermediate ancestral states (Omland 1997, Omland and Hofmann 2006). Since we found that many changes in color pigment use tended to occur across all colored regions (for example there were no taxa with melanin rumps and carotenoid breasts), we chose to reconstruct overall body color in addition to the color of individual body regions. Using this approach, all orioles receive a colored pigment state because all orioles have at least one colored body region (even though no one body region is colored in all orioles).

We chose to reconstruct the pigment basis of color plumage as an ordered character. The intermediate (I) and patch (P) character states were considered a single step between the carotenoid (C) and melanin (M) states (Fig. 2). This assumption is supported by biochemical data. The concentration of phaeomelanins found in the intermediate plumage of the Fuertes's oriole is between that found in carotenoid and melanin plumage (Hofmann et al. 2007). When we reconstructed the presence (Y) or absence (N) of any melanin color we found that changing the weight of a gain of melanin color significantly altered the reconstruction (a 2.5× weight of gaining any type of melanin color altered the reconstruction such that a single gain occurred in clade A). However, regardless of the assumptions or weighting used, clearly there have been changes between pigment types.

Loss of color versus loss of pigment

When we say that orioles have lost carotenoid coloration, we do not necessarily mean that there are no carotenoid pigments present. Biochemical analysis of the melanin-based color plumage of adult male orchard orioles found that carotenoids were still present, although there were no spectral (or visual) characteristics of carotenoids (Hofmann et al. 2007). In this case, one might conclude that a high concentration of phaeomelanins was masking the presence of carotenoids. These observations leave open the possibility that carotenoids may still be present in all of the oriole taxa we scored as having melanin coloration. Thus, we are not claiming to reconstruct losses of carotenoid pigments, simply losses of carotenoid color. However, from a signaling perspective, carotenoid coloration has been functionally lost if there is no spectral (or visual) appearance, regardless of whether carotenoids are still present.

Why switch pigments?

We were surprised to find repeated losses of carotenoid coloration and high lability between carotenoid and melanin colors given the important signaling roles that pigment based colors (especially those due to carotenoids) are thought to play. A strong selective pressure due to life history or environment might account for these multiple switches in pigmentation. However, we could find no common factor that explains why the orioles we examined would switch from a carotenoid to a melanin color. The orchard oriole is a long distance migrant that breeds throughout eastern North America and winters in Mexico and Central America (Scharf and Kren 1996, Baker et al. 2003). The chestnut-shouldered oriole is non-migratory and found throughout South America, particularly in Brazil (Jaramillo and Burke 1999). Both the orchard oriole and the chestnut-shouldered oriole are sympatric with other orioles that have very similar patterns: the orchard oriole is sympatric with the larger Baltimore oriole (Scharf and Kren 1996, Rising and Flood 1998), and the chestnut-shouldered oriole may be sympatric with other epaulet oriole subspecies (Jaramillo and Burke 1999). In contrast, the Martinique oriole is endemic to the island of Martinique (where no other orioles breed; Jaramillo and Burke 1999). Thus, these oriole taxa have different

life histories, inhabit a range of physical environments, and have varying degrees of sympatry with other orioles. We are also not aware of any differences in the diet or light environment that might suggest why these orioles have unique plumage.

In summary, we found three oriole taxa that had melanin-colored, rather than carotenoid-colored plumage. Ancestral state reconstruction suggests that these changes occurred independently three different times. We also found multiple instances of colorful melanin patches and intermediate plumage. Our findings do not suggest directional evolution from non-carotenoid to carotenoid coloration. Rather, in orioles there appears to be lability between carotenoid- and melanin-based coloration, and perhaps even directionality towards an increasing use of melanins in one clade of orioles. Finally, this study highlights that the causes of such interspecific differences in colors and patterns are not well understood. While sexual selection clearly leads to elaborate colors and patterns in many species, we do not know why one species has yellow carotenoids, a second has orange carotenoids, yet a third may have chestnut-colored phaeomelanins. Further research across a range of taxa using phylogenetic approaches is necessary to better understand these differences in color.

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Appendix 1. Museum voucher numbers for taxa measured.

I. cayanensis cayanensis	S-625749	F-208381	F-278672	F-258299	F-28104
I. cayanensis periporphyrus	F-110708	F-110709	–	–	–
I. cayanensis pyrrhopterus	S-173417	S-284236	S-16463	S-284240	S-253093
I. chrysocephalus	S-515442	S-625748	S-326500	S-610174	S-586488
I. chrysater chrysater	S-144406	S-144400	S-144407	S-1444399	F-186267
I. chrysater hondae	F-111598	F-111600	F-111596	–	–
I. nigrogularis nigrogularis	S-627066	S-610091	S-626227	S-626192	S-610092
I. nigrogularis trinitatis	S-355959	–	–	–	–
I. leucopteryx leucopteryx	S-310424	S-355952	F-331145	F-331142	F-331144
I. auratus	S-106267	S-129822	S-106268	S-129814	S-129820
I. mesomelas mesomelas	S-357113	S-360309	S-371974	S-371973	S-371975
I. mesomelas salvinii	S-220785	S-607084	S-469147	S-608015	S-469146
I. mesomelas taczanowskii	S-526244	–	–	–	–
I. auricapillus	S-484858	S-403475	S-470347	S-427431	S-427430
I. graceannae	S-169157	F-222395	F-299950	F-50151	F-296792
I. pectoralis pectoralis	S-361363	S-472494	S-361364	S-396574	S-582310
I. gularis tamaulipensis	S-158891	S-363584	S-531621	S-593407	S-525213
I. gularis yucatanensis	S-166729	S-166730	S-167622	S-167623	S-147140
I. gularis gularis	S-29239	S-525212	S-57592	S-54158	S-144397
I. pustulatus formosus	D-44131	S-525209	S-194172	S-144410	F-375083
I. pustulatus sclateri	S-361734	S-361738	S-361739	S-109624	S-109629
I. cucullatus nelsoni	D-60452	D-45130	D-9950	D-44217	D-35392
I. cucullatus igneus	S-167640	S-167641	S-167637	S-167636	S-167635
I. icterus ridgwayi	S-384332	S-370031	S-384327	S-384330	S-370037
I. icterus croconotus	S-370508	F-252232	F-222394	F-252235	F-281442
I. jamacaii strictifrons	F-152692	F-155436	F-155853	F-153063	–
I. galbula	S-528135	S-528133	S-528161	S-528163	S-567360
I. bullockii bullockii	S-465249	S-563312	S-418277	S-419275	S-528154
I. bullockii parvus	F-29578	F-29595	F-93105	F-29598	F-5353
I. abeillei	S-144354	S-126639	S-144358	S-144359	S-144353
I. spurius	S-593363	S-593366	S-422083	S-394401	S-565182

Appendix (*Continued*)

I. fuertesi	D-44149	D-44150	D-44147	D-44146	D-40009
I. dominicensis prosthemelas	S-360300	S-371979	S-371981	S-371984	S-357107
I. dominicensis northropi	F-29821	F-29819	–	–	–
I. dominicensis melanopsis	S-177866	S-395831	S-171452	S-171453	S-395830
I. dominicensis dominicensis	S-264790	S-327892	S-280460	S-305585	S-280459
I. dominicensis portoricensis	S-231651	S-355981	S-231649	S-231642	S-231637
I. wagleri wagleri	D-44602	D-23362	D-23363	D-34828	D-34827
I. laudabilis	S-176726	S-176728	S-80899	F-29767	F-29765
I. bonana	S-355956	S-355957	S-355958	F-29800	F-29798
I. oberi	S-355967	S-355961	S-355963	S-355964	F-29834
I. graduacauda audubonii	D-44410	D-44412	F-172474	F-118925	F-375123
I. graduacauda graduacauda	F-12458	S-601204	S-601200	S-601203	F-102868
I. maculialatus	D-27423	D-27422	F-153249	F-153250	F-109640
I. parisorum	S-564602	S-531620	S-364318	S-196831	S-567607

S = Smithsonian National Museum of Natural History, D = Delaware Museum of Natural History.
F = Field Museum of Natural History.