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Concordant evolution of plumage colour, feather microstructure and a melanocortin receptor gene between mainland and island populations of a fairy-wren

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Studies of the patterns of diversification of birds on islands have contributed a great deal to the development of evolutionary theory. In white-winged fairy-wrens, *Malurus leucopterus*, mainland males develop a striking blue nuptial plumage whereas those on nearby islands develop black nuptial plumage. We explore the proximate basis for this divergence by combining microstructural feather analysis with an investigation of genetic variation at the *melanocortin-1 receptor* locus (*MC1R*). Fourier analysis revealed that the medullary keratin matrix (spongy layer) of the feather barbs of blue males was ordered at the appropriate nanoscale to produce the observed blue colour by coherent light scattering. Surprisingly, the feather barbs of black males also contained a spongy layer that could produce a similar blue colour. However, black males had more melanin in their barbs than blue males, and this melanin may effectively mask any structural colour produced by the spongy layer. Moreover, the presence of this spongy layer suggests that black island males evolved from a blue-plumaged ancestor. We also document concordant patterns of variation at the *MC1R* locus, as five amino acid substitutions were perfectly associated with the divergent blue and black plumage phenotypes. Thus, with the possible involvement of a melanocortin receptor locus, increased melanin density may mask the blue-producing microstructure in black island males, resulting in the divergence of plumage coloration between mainland and island white-winged fairy-wrens. Such mechanisms may also be responsible for plumage colour diversity across broader geographical and evolutionary scales.

Keywords: feathers; structural colour; Fourier analysis; fairy-wrens; melanin; island

1. INTRODUCTION

Investigating patterns of phenotypic divergence of birds on islands has been central to the development of theories relating to the origin and maintenance of biological diversity (Grant 1965, 2001). Extensive research on Darwin's finches, for example, has provided convincing evidence that variation in beak size and shape has evolved in response to selective pressures posed by differences in food supply on different islands (Grant 1999). Insular variation in plumage patterns appears to be more widespread and of a greater magnitude than variation in morphometric traits (Grant 2001), yet we know comparatively little about the evolution of plumage coloration on islands. Of particular note is that conspicuousness and sexual dichromatism tend to be reduced among island species (Grant 1965; Peterson 1996; Omland 1997) and such reduced dichromatism has most often evolved from a sexually dichromatic ancestral state among birds in general (Peterson 1996; Price & Birch 1996; Omland 1997). Evolutionary explanations for this sort of change remain speculative, and no study, to our knowledge, has documented the proximate mechanisms responsible for generating these patterns.

In this study, we investigated the proximate basis for a dramatic divergence in sexual plumage ornamentation between mainland and island subspecies of the white-winged fairy-wren, *Malurus leucopterus*. In the mainland subspecies, *M. l. leuconotus*, which is distributed throughout much of Australia, males develop a striking electric blue nuptial plumage, whereas the males of two endemic oceanic island subspecies, *M. l. leucopterus* (on Dirk Hartog Island) and *M. l. edouardi* (on Barrow Island), moult into a black nuptial plumage (Schodde & Mason 1999). These islands are both closer to the mainland than to each other, and recent molecular evidence suggests that, despite their similar appearance, the two island subspecies are more closely related to the mainland subspecies than to each other (Driskell *et al.* 2002).

Our aim in this study was to identify the proximate mechanisms responsible for the striking divergence in nuptial plumage between one mainland and one island population of white-winged fairy-wrens. First, we used reflectance spectrometry and transmission electron microscopy to determine how phenotypic divergence between blue and black morphs related to variation in feather microstructure. Then, we investigated patterns of genetic variation at the *melanocortin-1 receptor* locus (*MC1R*). This gene is expressed in developing feathers and hair and plays a key role in the control of melanin synthesis in vertebrates (Jackson 1997). A single point

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mutation at *MC1R* has been implicated in the expression of a melanic plumage morph in polymorphic bananaquits, *Coereba flaveola*, on Caribbean islands (Theron *et al.* 2001). Variation at this locus has also recently been linked to melanic plumage polymorphisms in lesser snow geese, *Anser c. caerulescens*, and arctic skuas, *Stercorarius parasiticus*, where, remarkably, the degree of melanism corresponds to the copy number of variant *MC1R* alleles (Mundy *et al.* 2004).

By combining a detailed analysis of plumage reflectance, feather microstructure and genetic variation at a melanocortin receptor locus, our study takes a novel, integrative approach to investigating plumage divergence among island taxa.

2. MATERIAL AND METHODS

(a) *Sampling*

Between August and December 2000 and 2001, we studied one island population (Dirk Hartog Island; 25°53′ S, 113°06′ E) and one mainland population (Lancelin; 31°09′ S 115°26′ E) of white-winged fairy-wrens in Western Australia (see Rathburn & Montgomerie 2003). We captured birds by using mist nets, removed breast feathers for microstructural analysis, and collected small blood samples to obtain DNA from which to sequence the *MC1R* gene.

(b) *Spectrometry*

We measured the plumage reflectance of blue ($n = 8$) and black ($n = 7$) males by using an S2000 spectrometer and PX-2 pulsed xenon light source (Ocean Optics, Dunedin, FL, USA). We obtained three readings from the breast region of each bird, moving the probe by at least 3 mm between readings. All measurements were taken perpendicular to the feather surface using a metal-encased fibre optic probe in a black rubber holder that maintained the probe at a fixed distance (3 mm) from the feather surface and excluded external light. Reflectance was calculated as the percentage of light reflected from a Spectralon white standard (Ocean Optics) at wavelengths in the bird-visible spectrum from 300 to 700 nm (Cuthill *et al.* 2000). We calculated hue as the wavelength of maximum reflectance.

(c) *Transmission electron microscopy*

We cut the distal 1 cm of barbs from breast feathers ($n = 5$ blue and $n = 5$ black males), then embedded and sectioned them following Shawkey *et al.* (2003*b*). We examined barbs on a Phillips EM301 transmission electron microscope (Veeco FEI Inc, Hillboro, OR, USA) and took two to four micrographs per individual of whole barb cross-sections at magnification ×3400 or ×5700, and of the medullary keratin matrix (spongy layer) at magnification ×25 000 or ×57 000. We also took micrographs of a waffle-pattern diffraction grating (Ted Pella, Redding, CA, USA), accurate to 1 nm ± 5%, to calibrate the images.

To obtain digital images for analysis, we scanned TEM micrograph negatives at 400 d.p.i. When the images were too dark to be resolved accurately, we took digital photographs of micrograph negatives using the trans-illumination mode on a gel documentation system (Alpha-Innotech, San Landro, CA, USA; see Shawkey *et al.* 2003*a*).

(d) *Fourier analysis*

We used two-dimensional Fourier analysis of the digitized images of spongy layer to determine whether the medullary keratin matrix of feather barbs was sufficiently organized, and at an appropriate spatial scale, to produce colour by coherent light scattering alone (see Prum *et al.* 1998, 1999). The largest available square portion of pure spongy layer (uninterrupted by melanin granules or cell boundaries; 800–1048 pixels^2) was analysed using the Fourier tool for biological nano-optics (Prum & Torres 2003) in MATLAB v. 6.5. Radial averages of Fourier power spectra were multiplied by twice the mean refractive index (RI) of keratin (RI = 1.54) and air (RI = 1.0) to obtain a predicted reflectance curve and hue (wavelength at maximum reflectance) for each image (Prum *et al.* 1998, 1999).

(e) *Quantifying microstructural variation*

We used IMAGEJ v. 1.30 (Rasband 2002) to investigate microstructural differences between blue (mainland) and black (island) males. Using calibrated TEM images, we measured the total cross-sectional area of the barb section in the micrograph and the proportion of the barb cross-section composed of cortex and spongy layer. We also quantified the density of melanin granules by counting those present separately in the cortex and spongy layer and dividing this number by the total cross-sectional area of each region. To quantify the position of melanin relative to the spongy layer, we measured the perpendicular distance from the cortex to 10–15 melanin granules in the spongy layer and averaged this distance for each micrograph. Finally, we used CARNOY v. 2.0 (http://www.carnoy.org) to measure the proportion of the cross-sectional area of cortex and spongy layer that was composed of melanin.

(f) *Sequencing* MC1R

DNA was isolated from blood stored on filter paper using an ammonium acetate-based extraction protocol. Using primers based on the 5′ end of the chicken, *Gallus gallus*, *MC1R* sequence (MSH 1; Takeuchi *et al.* 1996) and three internal primers based on the chicken sequence (MSH 8, 9 and 10), we amplified two overlapping fragments that contained most (887 out of 942 base pairs) of the *MC1R* coding sequence of white-winged fairy-wrens. All reactions were performed in 25 or 50 µl volumes and included 10–40 ng of genomic DNA, 0.5 µM of forward and reverse primers, 10 mM of dNTPs (Promega, Madison, WI, USA), 2 mM $MgCl_2$, 2.5 mM 10× PCR buffer, and 0.75 units µl^{-1} of High Fidelity Taq polymerase (Applied Biosystems, Foster City, CA, USA). Reactions were run on a PTC-100 thermocycler (MJ Research, Waltham, MA, USA) using the following procedure: denaturation for 4 min at 94 °C, 1 min at 58 °C, 2 min at 72 °C, and 29 cycles of 1 min each at 94 °C, 1 min at 58 °C, and 2 min at 72 °C, and a final extension step of 10 min at 72 °C. PCR products were then run on 1.5% agarose gels and purified using QIAquick Gel Purification Kit (QIAGEN, Valencia, CA, USA). Both strands were directly sequenced in both directions with forward and reverse primers using a BigDye sequencing kit and separated on a 3100 Genetic Analyzer (Applied Biosystems). We compiled our sequences with known *MC1R* sequences for *G. gallus*, *Tangara cucullata*, *Phylloscopus trochiloides*, *P. humei*, and yellow and black morphs of *C. flaveola* (all available on GenBank) by using MACCLADE v. 4.0 (Maddison & Maddison 2000) and aligned them using CLUSTALX v. 1.83 (Thompson *et al.* 1997). We assumed that individuals were heterozygous at a site when chromatographs for both strands showed two peaks of roughly equal intensity for different nucleotides. Sequences have been deposited in GenBank (accession numbers AY614599–AY614611).

(g) *Statistical analysis*

We tested all variables for normality (Shapiro–Wilks' tests) and applied standard transformations to normalize the distribution of variables that were significantly non-normal. All proportional data were arcsine transformed to improve the fit to normality. Descriptive statistics are reported as mean ± s.e.m.

3. RESULTS

(a) *Spectrometry*

As expected from their blue colour, the feathers of nuptial males from the mainland exhibited peak reflectance at 419.2 ± 19.15 nm (figure 1*a*). By contrast, the reflectance of feathers from black nuptial males from the island was uniformly low across the bird-visible spectrum (figure 1*b*).

(b) *Fourier analysis*

Fourier power spectra generated from the spongy keratin matrix of blue feather barbs (figure 1*c*) were circular and uniform in all directions (figure 1*e*), suggesting that the keratin matrix of these barbs was highly organized (Prum *et al.* 1998, 1999). Further, the Fourier power was greatest at intermediate spatial frequencies, and the predicted hue (wavelength at peak reflectance) generated from Fourier analyses (425 ± 20.9 nm; figure 1*g*) closely matched the hue estimated from reflectance spectrometry, suggesting that the organization of keratin is at the appropriate spatial scale to produce this blue colour by coherent light scattering alone.

Fourier analysis of sections of pure spongy keratin matrix of black feather barbs (figure 1*d*) also produced circular power spectra (figure 1*f*), and a similar predicted hue of 452 ± 7.09 nm (figure 1*h*). The spongy layer of black feather barbs, however, was notably degraded by holes (absence of keratin) and was often interrupted by melanin granules (figure 1*d*).

(c) *Microstructural variation*

Qualitative examination of electron micrographs revealed extensive and consistent differences in feather microstructure between insular (black) and mainland (blue) nuptial males (figure 1*c*,*d*). The blue barbs of mainland nuptial males had a thin cortex beneath which lay an extensive spongy layer composed of irregularly shaped keratin rods and air spaces. As is typical of this type of microstructure, the spongy layer surrounded a layer of melanin granules distributed around large central vacuoles (figure 1*c*). This layer of melanin is thought to absorb incident light, thereby increasing the saturation of the structural blue colour (Prum 1999). By contrast, the black barbs of island nuptial males had an enlarged cortex that contained numerous melanin granules, and melanin granules were distributed both within and beneath the spongy layer (figure 1*d*).

Our measurements of feather microstructure showed that these observed differences were consistent among individuals within each population, as blue nuptial males had a significantly thinner cortex (i.e. smaller proportional cortex area) and a thicker spongy layer (i.e. larger proportional spongy layer area) than black males (table 1). In addition, blue males had both lower densities and a smaller proportional surface area of melanin granules in their barb cortex (table 1). Blue males also had lower densities of melanin granules in their spongy layer, although these occupied a comparable proportion of the surface area of the spongy layer to that in black males (table 1). Finally, melanin granules were located significantly farther away from the cortex in blue males than in black males (table 1).

(d) *Association of* MC1R *genotype with plumage colour*

The 887 base pair portion of the white-winged fairy-wren *MC1R* locus that we sequenced showed 92% nucleotide identity with the chicken *MC1R* and 96–97% nucleotide identity with that of other passerine birds (table 2). Among the 13 white-winged fairy-wrens that we sampled, there were 34 variable sites at the *MC1R* locus, of which 23 were synonymous and 11 were non-synonymous substitutions. Five of these non-synonymous substitution sites were perfectly associated with the divergence in plumage phenotypes between mainland and island birds. First, a guanine-to-adenine (G–A) transition at site 46 caused an alanine-to-threonine substitution at amino acid position 16 (table 2). Four out of seven island males were homozygous for guanine whereas three were heterozygous (A/G). By contrast, all six mainland males were homozygous for adenine. Second, a tyrosine-to-adenine (T–A) transition at site 113 caused an isoleucine-to-asparagine substitution at amino acid position 38 (table 2). All seven island males were homozygous for tyrosine whereas five of the mainland males were homozygous for adenine and one was heterozygous (A/T). Third, a G–A transition at site 331 caused a valine-to-isoleucine substitution at amino acid position 111 of *MC1R* (table 2). All seven island males were homozygous for guanine whereas five of the mainland males were homozygous for adenine and one was heterozygous (A/G). Fourth, an A–G substitution at site 470 caused a glutamine-to-arginine substitution at amino acid position 157 (table 2). Mainland males were either heterozygous (A/G; $n = 3/6$) or homozygous for guanine (3/6) whereas all seven island males were homozygous for adenine. Finally, a G–A transition at site 496 caused a valine-to-isoleucine substitution at amino acid position 166. Mainland males were homozygous for guanine (4/6) or heterozygous (A/G; 2/6), whereas all seven island males were homozygous for adenine (table 2). One of the synonymous substitutions we observed, a C–T transition at site 711, was also associated with the divergent phenotypes. Mainland males were either heterozygous (C/T; 1/6) or homozygous for cytosine (5/6), whereas all island males were homozygous for tyrosine.

In total, base pair transitions at 40% (8/20) of sites among mainland blue individuals resulted in non-synonymous substitutions, whereas only 25% (6/24) transitions among black island individuals lead to amino acid replacement. Accordingly, McDonald–Kreitman (McDonald & Kreitman 1991) tests comparing island individuals with four outgroup sequences (*G. gallus*, *T. cucullata*, *P. trochiloides* and *C. flaveola*) suggest that there is a deficiency of non-synonymous substitutions among island birds ($p = 0.04$, 0.07, 0.04 and 0.11, respectively). No significant differences were found in McDonald–Kreitman tests comparing either mainland birds or all birds with outgroup sequences (all $p > 0.3$).

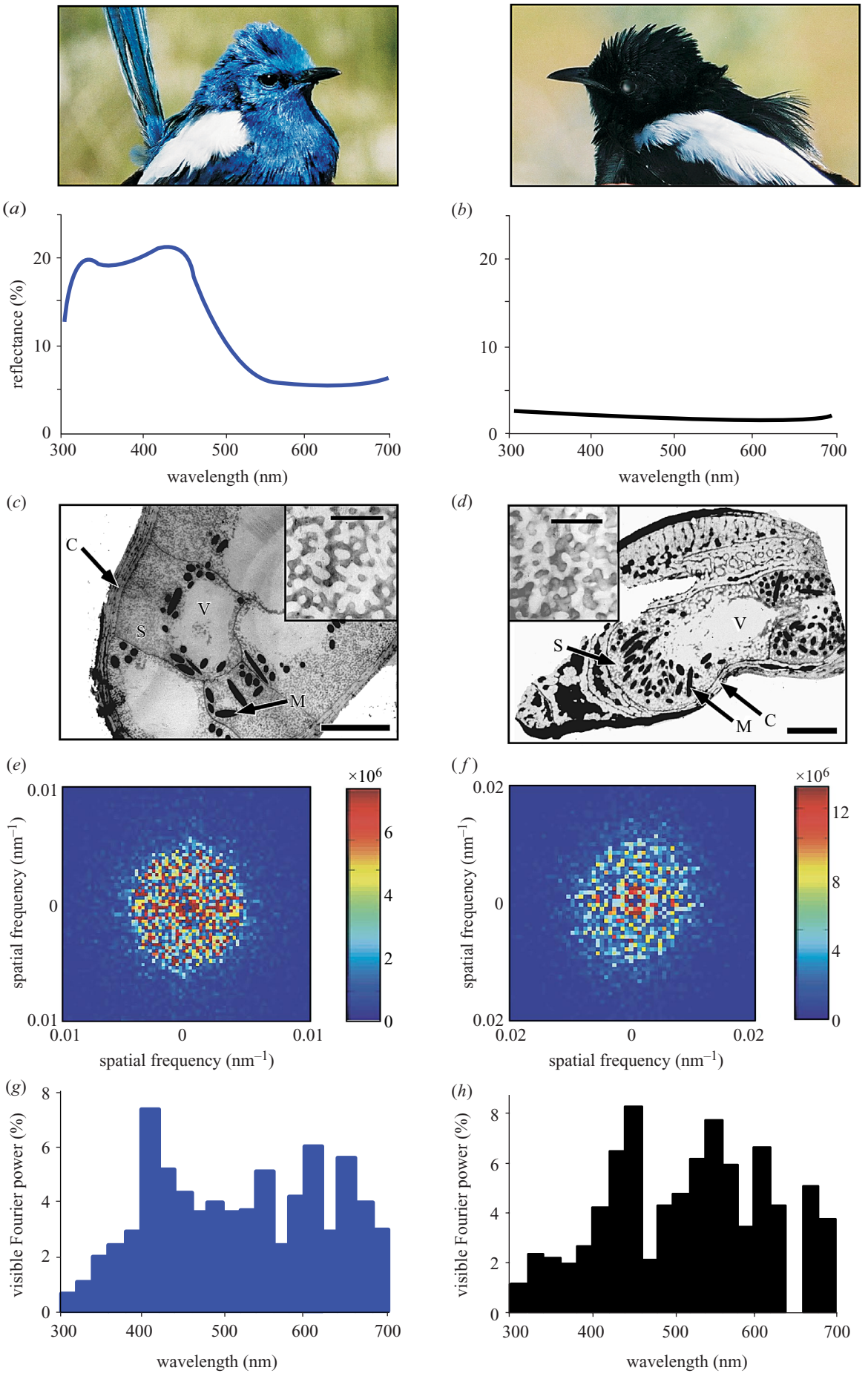

Figure 1. (*Caption opposite*.)

Figure 1. Feather microstructure and reflectance spectra of blue- (*M. l. leuconotus*; *a*,*c*,*e*,*g)* and black-plumaged males (*M. l. leucopterus*; *b*,*d*,*f*,*h*) of the white-winged fairy-wren. Shown are photographs of the two nuptial plumage types, followed by typical reflectance spectra (*a*,*b*), TEM micrographs (*c*,*d*), 2D Fourier power spectra (*e*,*f*), and predicted reflectance spectra based on Fourier analysis (*g*,*h*; see text for details). TEM micrographs (*c*,*d*) include whole barb sections (scale bars: 5 μm) and details of spongy layer (insets; scale bars: 0.5 μm). M: melanin, C: cortex, S: spongy layer, V: central air vacuole. Note that males in nuptial plumage differ in colour but not in patterning.

4. DISCUSSION

The strikingly different plumage hues of nuptial-plumaged males of mainland and island subspecies of white-winged fairy-wrens probably result from differences in the density and spatial arrangement of melanin granules within their feather barbs. However, Fourier analysis reveals that both blue and black feather barbs contain a spongy layer capable of producing a blue hue by coherent light scattering. Moreover, the presence of this spongy layer in the black plumage of island males suggests that they evolved from a blue-plumaged ancestor. Five non-synonymous amino acid substitutions at the *MC1R* locus correspond perfectly with these divergent blue and black plumage phenotypes, suggesting that this gene may play a role in the observed differences in melanin density between blue and black males. Our findings thus have broad implications for investigating the evolution of plumage colour in birds and may provide insight into the genetic basis for some forms of sexual plumage ornamentation.

(a) *Microstructural differences*

The spongy layer of blue feather barbs of mainland nuptial males is composed of irregularly shaped keratin bars and air spaces, creating a medullary keratin matrix similar in appearance to that which produces a blue colour by coherent light scattering in the feather barbs of eastern bluebirds, *Sialis sialia* (Shawkey *et al.* 2003*b*), psittacids (Prum *et al.* 1999) and *Myiophonus* whistling thrushes (Andersson 1999; Prum *et al.* 2003).

Interestingly, Fourier analysis of the black feather barbs of island males revealed that, despite their black appearance, the spongy layer of these barbs could produce a structural blue plumage colour similar to that of mainland males. The discrepancy between the Fourier-predicted blue hue and the actual black hue of the plumage of island males most probably results from large differences between blue and black males in other aspects of barb microstructure. For example, black males had a thicker and more heavily melanized cortex, as well as a reduced spongy layer that contained a greater density of melanin granules than blue males. Moreover, melanin granules appeared to be incorporated directly into the spongy layer in black males, whereas they were located in the centre of the barb beneath the spongy layer in blue males. Thus, the thick, melanized cortex of black males' feather barbs probably reduces the amount of light that can penetrate the barb (Finger 1995; Shawkey *et al.* 2004), while the layer of melanin granules just beneath the cortex absorbs much of the light that does penetrate the barb before it can be scattered by the spongy layer. As a result, the feathers appear black. The keratin matrix of black males' feather barbs was also notably more degraded than that of blue males, which would further reduce the total volume of spongy layer available for coherent light scattering.

Black feather barbs in other species do not typically contain a spongy layer (Dyck 1978; M. D. Shawkey and S. M. Doucet, unpublished data), so the presence of a spongy layer in the black feather barbs of insular *M. l. leucopterus* suggests that these birds evolved from a blue-plumaged ancestor, a finding also supported by a recent phylogeographic study of this group (Driskell *et al.* 2002). Relatively minor changes in the size of light-scattering elements (Prum *et al.* 2003; Shawkey *et al.* 2004), the size and arrangement of other microstructural barb constituents (Finger 1995; Shawkey *et al.* 2004; this study), as well as pigment type or concentration (Dyck 1978; Hill 2002) can lead to a substantial phenotypic variation in plumage colour. As we have demonstrated, changes in some aspects of feather microstructure may lag behind dramatic changes in appearance and thus allow us to infer ancestral plumage character states. Thus, microstructural feather analysis may be a useful tool for reconstructing patterns of plumage colour evolution.

(b) *Association between* MC1R *and plumage colour*

Five non-synonymous substitutions at the *MC1R* locus were perfectly associated with the divergent nuptial plumage phenotypes of male white-winged fairy-wrens. For four of these base pair substitutions, A16T, I38N, I111V and Q157R, mainland (blue) birds showed the associated nucleotide transition while island (black) birds shared the (presumed) ancestral genotype found in the chicken, *Phylloscopus* warblers (MacDougall-Shackleton *et al.* 2003) and the bananaquit (Theron *et al.* 2001). By contrast, the fifth substitution, V166I, was found only in island fairy-wrens, whereas mainland birds expressed the ancestral genotype. If variation at *MC1R* acts to modulate melanosynthesis by way of dominant alleles, as has been shown in other species (Robbins *et al.* 1993; Theron *et al.* 2001), the substitution A16T fits the expected pattern, with melanic expression in both homozygotes and heterozygotes. Black island males have an apparently ancestral amino acid at that site (table 2); however, the closest relatives of white-winged fairy-wrens have predominantly black plumage (Christidis & Schodde 1997), suggesting that they evolved from a black-plumaged ancestor. Thus, island males may simply have reverted to an ancestral black plumage state. A second possibility is that some of the substitutions we documented are actually linked with variation at other loci involved in peripheral melanocortin systems. One possible candidate is the avian *Agouti-related protein* gene (Takeuchi *et al.* 2000). The *Agouti* locus in rodents, for example, is known to influence spatial and temporal control of melanin synthesis (Robbins *et al.* 1993). Finally, we cannot exclude the possibility that this divergence at *MC1R* could have evolved by the accumulation of neutral substitutions. Although our findings are suggestive, further work, including broader sampling within white-winged fairy-wrens and among closely related species, will be required to address this question fully.

Table 1. Comparison of measurements of microstructural feather elements in the feather barbs of blue (mainland; $n = 5$; *M. l. leuconotus*) and black (island; $n = 5$; *M. l. leucopterus*) nuptial-plumaged male white-winged fairy-wrens.
(Data were calculated from micrographs of barb cross-sections. Means ± s.e.m. are shown; *t*-tests were performed on transformed data in some cases.)

measurement	blue barbs	black barbs	*t*	*p*
cortex				
proportional area	0.27 ± 0.05	0.51 ± 0.07	2.8	0.02
proportional melanin area	0.007 ± 0.004	0.10 ± 0.02	3.8	0.006
melanin density (granules μm^{-2})	0.10 ± 0.06	0.64 ± 0.19	3.5	0.008
melanin distance from cortex (μm)	2.42 ± 0.48	0.36 ± 0.03	6.1	0.0003
spongy layer				
proportional area	0.50 ± 0.03	0.34 ± 0.04	3.1	0.01
proportional melanin area	0.14 ± 0.02	0.14 ± 0.03	0.1	0.94
melanin density (granules μm^{-2})	0.55 ± 0.06	0.87 ± 0.11	2.6	0.03

Table 2. Variable sites leading to non-synonymous amino acid substitution in the *MC1R* sequence of white-winged fairy-wrens.
(Individuals of other species with known *MC1R* sequences available on GenBank are included for comparison. Dashes indicate missing data.)

	site on *MC1R* sequence										
	25	26	46	82	113	331	470	496	544	545	574
G. gallus	C	T	G	G	T	G	A	G	T	G	G
P. trochiloides	—	—	—	G	T	G	A	G	T	G	G
P. humei	—	—	—	G	T	G	A	A	T	G	G
C. flaveola Y23	C	T	G	G	T	G	A	G	T	G	G
C. flaveola M4	C	T	G	G	T	G	A	G	T	G	G
T. cucullata	C	T	G	G	T	G	A	G	T	G	G
M. l. leuconotus 60465	C	T	A	G	A	A	G	G	T	G	G
M. l. leuconotus 60478	C	T	A	G	A	A	G	G	T	A	G
M. l. leuconotus 60482	C	T	A	G	A	A	G	G	T	A	G
M. l. leuconotus 60495	C	T	A	G	A	A	A/G	G/A	T/C	G	G
M. l. leuconotus 60457	C	T/C	A	G	A	A	A/G	G	T	A	G
M. l. leuconotus 60473	C	T/C	A	A	A/T	A/G	A/G	G/A	T	G	G
M. l. leucopterus 60004	G	T	G	A	T	G	A	A	T	G	G
M. l. leucopterus 60367	C	T	G/A	G	T	G	A	A	T	G	G
M. l. leucopterus 60372	C	T	G	G	T	G	A	A	T	G	G
M. l. leucopterus 60374	C	T	G	A	T	G	A	A	T	G	G
M. l. leucopterus 60418	C	T	G	A/G	T	G	A	A	T	G	G
M. l. leucopterus 60419	C	T	G/A	A	T	G	A	A	T	G	G
M. l. leucopterus 60026	C	T	G/A	G	T	G	A	A	T	G/A	G/A

The deficiency of non-synonymous mutations that we found among black island males suggests that the *MC1R* locus has been under selective constraint in island birds. This stands in contrast to the findings of Theron *et al.* (2001), who showed that selective constraint operates on yellow rather than melanic morphs in bananaquits. Instead, our findings parallel those of Harding *et al.* (2000) who found that in humans, African *MC1R* haplotypes are under strong selective constraint whereas European haplotypes experience little or no selective constraint. We can potentially account for this variation in selection by considering how *MC1R* function is influenced by amino acid substitutions. For example, the G92L substitution implicated in the expression of melanic plumage in bananaquits is known to result in the constitutive activation of *MC1R* in mice (Robbins *et al.* 1993). Because constitutively active melanic *MC1R* haplotypes do not need to bind MSH, they may be less sensitive to changes in amino acid composition than yellow haplotypes (Theron *et al.* 2001). By contrast, variation at the *MC1R* locus of fairy-wrens may alter the responsiveness of its receptors to MHS, as has been shown in the tobacco mouse, *Mus poschiavinus* (Robbins *et al.* 1993). Such variation in *MC1R* function would be sufficient to explain differences in selective constraint in bananaquits and fairy-wrens, though further research is necessary to determine conclusively how variation at *MC1R* modulates melanin synthesis in these species.

(c) *Implications*

Although we have made considerable progress in identifying the proximate basis for divergence in nuptial plumage between mainland and islands subspecies of white-winged fairy-wrens, the selective pressures driving this divergence remain unclear. Fairy-wrens (Aves: Maluridae) have among the highest reported rates of extra-pair paternity in birds (Mulder *et al.* 1994), and male plumage appears to be sexually selected in this family, as most

species are highly sexually dichromatic (Schodde & Mason 1999), timing of moult is associated with male quality and female mate choice (Mulder & Magrath 1994) and, in white-winged fairy-wrens, male plumage colour is correlated with indicators of condition (Rathburn 2004). Yet, because females of both subspecies have melanin-based brown plumage, the black nuptial plumage of island birds results in reduced sexual dichromatism as measured by reflectance contrast (*sensu* Endler 1990; M. K. Rathburn and R. Montgomerie, unpublished data), and this reduced dichromatism is accompanied by a parallel reduction in sexual-size dimorphism (Rathburn & Montgomerie 2003). Reduced sexual dichromatism appears to be a widespread phenomenon among island taxa (Peterson 1996; Omland 1997), yet the evolution of this reduced dichromatism remains puzzling (Badyaev & Hill 2003).

One possible reason for decreased dichromatism on islands is that decreased genetic variability among small, isolated (island) populations (Frankham 1997) may lead to a corresponding reduction in the strength of sexual selection (Griffith 2000; Rathburn & Montgomerie 2003). Thus, genetic drift (Mayr 1942; Peterson 1996), inbreeding and a limited choice of mates may reduce the strength of selection on male traits and/or female preference for those traits (Omland 1997). Moreover, complex traits comprising several functionally related elements are more likely to be lost than gained, because a mutation affecting any of the critical elements can remove the character entirely (Omland 1997). Thus, as may be the case in white-winged fairy-wrens, a simple mutation at a melanocortin system locus could change the amount and/or arrangement of melanin granules within a feather and effectively mask the original structural blue colour. Subsequent degradation of the spongy keratin matrix suggests that there may be a release of selective pressure for this highly organized nanostructure, or selection against it if is costly to produce. Another possibility is that insular environments may be harsher, and the sexually selected benefit of displaying a highly elaborate ornament is outweighed by the costs of producing or maintaining such an ornament. In white-winged fairy-wrens, for example, island birds had significantly higher rates of predation and brood parasitism, smaller clutch sizes, longer nestling periods and lower fledging success than mainland birds (Rathburn & Montgomerie 2003). Finally, island plumage variation may actually be more strongly influenced by natural than sexual selection. For example, melanized black plumage may confer a selective advantage through abrasion resistance (Bonser 1995), resistance against bacterial degradation (Goldstein *et al.* 2004) or crypsis (Endler 1990).

In addition to their critical role in producing black, grey and earthy shades of plumage, melanin pigments are an important accessory component of both non-iridescent structural colours (Prum 1999) and some carotenoid-based colours (Hopenstand & Johnson 2001), and are essential for the production of iridescent structural colours (Prum 1999). Thus, mechanisms that affect melanin synthesis and deposition could potentially influence most types of plumage coloration. Future studies addressing how genetic variation at melanocortin system loci relates to variation in pigment deposition in feathers at a range of evolutionary scales will determine to what extent this pathway may have shaped the evolution of avian plumage ornaments.

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