Revista Brasileira de Ornitologia 26(4): 251–257.
December 2018

ARTICLE

Plumage polymorphism and variation in the *melanocortin-1 receptor* gene in the Fuscous Flycatcher, *Cnemotriccus fuscatus* (Wied, 1831)

Sandriéllem Natália Vieira[1], Juliana Araripe[1], Alexandre Aleixo[2] & Péricles Sena do Rêgo[1,3]

[1] Laboratório de Genética e Conservação, Instituto de Estudos Costeiros, Universidade Federal do Pará, 68600-000, Bragança, PA, Brazil.
[2] Coordenação de Zoologia, Museu Paraense Emílio Goeldi, Belém, PA, Brazil.
[3] Corresponding author: periclessena@yahoo.com.br

Received on 26 October 2017. Accepted on 03 December 2018.

ABSTRACT: We investigated the possible mechanisms behind the variation plumage color of the Fuscous Flycatcher, *Cnemotriccus fuscatus*, by sequencing the *melanocortin-1 receptor* (*MC1R*) gene, which has been associated with the variation in plumage coloration in birds. *C. fuscatus* is widely distributed in South America and includes seven subspecies, which differ in their plumage coloration. Here we tested the hypothesis that the variation in the *MC1R* gene explains the plumage polymorphism found in *C. fuscatus*. We sequenced the *MC1R* gene in six subspecies, representing two groups: group 1 (yellow morph), with three subspecies, *C. f. duidae*, *C. f. fumosus*, and *C. f. fuscatus*, and group 2 (white morph), with the remaining subspecies, *C. f. bimaculatus*, *C. f. beniensis*, and *C. f. fuscatior*. The only variation we found among the *C. fuscatus* sequences were six non-synonymous substitutions from 22 variable sites, none of which were associated systematically with either plumage morph. The result of the neutrality test indicated that the polymorphism of the *MC1R* gene is not suggestive of significant selection pressure. We conclude that variation in plumage coloration in *C. fuscatus* does not appear to be determined by the *MC1R* gene, and that it may be related to other loci or under the influence of environmental factors.

KEY-WORDS: birds, *MC1R* gene, mutation, pigmentation, Tyrannidae.

INTRODUCTION

The variation in plumage coloration has been studied from ecological, evolutionary and genetic perspectives (Hoekstra & Price 2004, Mundy 2005, Uy *et al.* 2016). Such diversity has been related to visual communication, and may have evolved in response to the evolution of the avian visual system (Osorio & Vorobyev 2008), although there is also some evidence that changes in plumage coloration may be a response to varying pressures in different types of habitat (Gomez & Théry 2004, McNaught & Owens 2002). Many questions remain unresolved, however, on the evolution of plumage coloration and its relation to speciation in birds (Stoddard & Prum 2011, Seddon *et al.* 2013), such as the mechanisms that mediate the change in coloration between juveniles and adults (Galván & Jorge 2015), and the factors determining changes in coloration despite the considerable energetic costs of this process (Legagneux *et al.* 2012, Mercadante & Hill 2014).

Previous studies (*e.g.*, Robbins *et al.* 1993, Vidal *et al.* 2010, Johnson *et al.* 2012) have suggested that the *melanocortin-1 receptor* (*MC1R*) gene may be involved in the differentiation of the plumage in avian species, due to the association between mutations in this gene and the phenotypic variation found in a number of different groups of wild birds (Johnson *et al.* 2012, Ran *et al.* 2016). For example, single non-synonymous mutations in the *MC1R* gene were associated with plumage polymorphisms in the bananaquit (*Coereba flaveola*) and the chestnut-bellied monarch, *Monarcha castaneiventris* (Theron *et al.* 2001, Uy *et al.* 2009). Studies in birds have also shown that the *MC1R* gene controls the amount of both eumelanin (brown/black) and pheomelanin (red/yellow) produced (Takeuchi *et al.* 1996, Wen *et al.* 2015). In particular, García-Borrón *et al.* (2005) showed that the yellow (pheomelanin) phenotype is produced by recessive *MC1R extension* (e) alleles.

In this context, we investigated the variation in coloration found among the subspecies of the Fuscous Flycatcher, *Cnemotriccus fuscatus*, a monotypic genus widely distributed in South America (Fig. 1). There are seven *C. fuscatus* subspecies, which are differentiated not only on the basis of their morphological characters, but also their vocalizations and ecology (Fitzpatrick *et al.* 2004). These subspecies can be divided into two groups,

based primarily on the coloration of the belly, which is either white or yellow. These flycatchers can be found in a variety of habitats, including fluvial islands, rainforest, dry forests, riparian habitats, and lowland and secondary forests (Rasmussen & Collar 2002). It is thus important to understand which factors may influence the variation in the coloration of plumage found among the different subspecies of the Fuscous Flycatcher (Farnsworth & Lebbin 2017). In particular, if a relationship can be found between genotype and phenotype, it might represent evidence of the role of natural selection in the fixation of subspecific coloration patterns (Hewitt 1988, Chunco *et al.* 2007).

Here we investigated the possible mechanisms that determine differentiation in plumage amongst the subspecies of *C. fuscatus*. Specifically, we tested whether non-synonymous mutations in the sequence of the *melanocortin-1 receptor* (*MC1R*) gene were associated systematically with variation in plumage coloration amongst the six subspecies, and whether these mutations are suffering selection pressures.

METHODS

We sequenced 27 samples of *Cnemotriccus fuscatus* muscle tissue (Table 1), representing six of the seven described subspecies. The samples were provided by the Goeldi Museum (MPEG: Museu Paraense Emilio Goeldi) in Belém, and the National Museum (MNRJ) in Rio de Janeiro. We followed the classification of Fitzpatrick *et al.* (2004) to allocate the subspecies to two groups (yellow and white morphs). Group 1 (yellow morph) was composed of *Cnemotriccus fuscatus duidae* (*n* = 5 specimens), *Cnemotriccus fuscatus fumosus* (*n* = 7), and *Cnemotriccus fuscatus fuscatus* (*n* = 5), which are ventrally yellow to light yellow. Group 2 (white morph) contained the other three subspecies, *Cnemotriccus fuscatus bimaculatus* (*n* = 5), *Cnemotriccus fuscatus beniensis* (*n* = 3), and *Cnemotriccus fuscatus fuscatior* (*n* = 2), which are ventrally white or light gray. Both male and female specimens were included, as *C. fuscatus* is not dichromatic (Fitzpatrick *et al.* 2004).

Total DNA was isolated from the muscle tissue using the Wizard© Genomic DNA purification kit (Promega), following the manufacturer's instructions. To obtain a partial sequence of the *MC1R* gene, we amplified the samples by PCR using the primers described by Cheviron *et al.* (2006): lcorMSHR9 (5' – CTG GCT CCG GAA GGC RTA GAT – 3') and lcorMSHR72 (5' – AYG CCA GYG AGG GCA ACC A – 3'). The PCR conditions were the same as those used by Cheviron *et al.* (2006), and the PCR products were sequenced by Sanger's didesoxiterminal method (Sanger *et al.* 1977), using an ABI 3500 automatic sequencer.

The DNA sequences were aligned and their nucleotides were compared to those from the bananaquit, *Coereba flaveola* (GenBank access numbers AF362598 and AF362601) and *Gallus gallus* (AB201631) using Bioedit

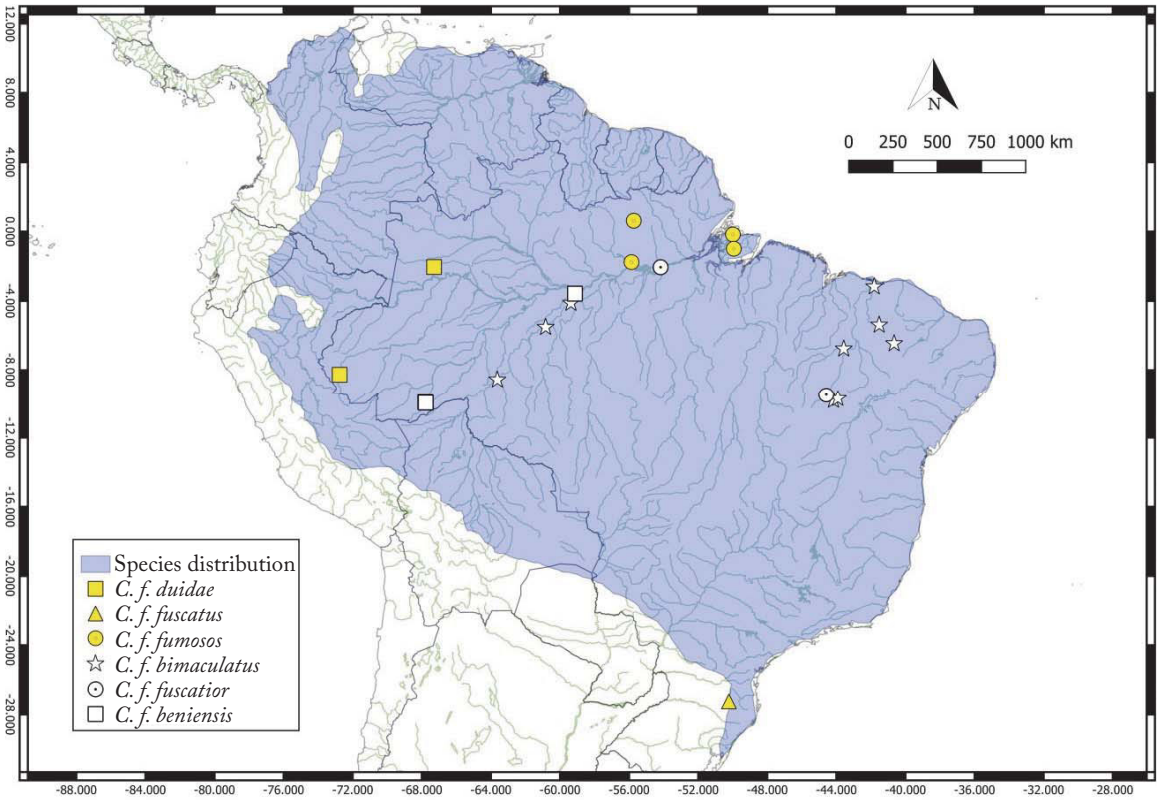

Figure 1. Map showing the distribution of samples of *Cnemotriccus fuscatus* sequenced in this study. Yellow points represent specimens with yellow bellies and white points denote specimens with white bellies.

Table 1. Location, coordinates, subspecies, voucher number, identification code, and source of tissue used in this study.

Locations (Coordinates)	Subspecies	Voucher number	Identification of the tissue	Institutions
Óbidos - PA (00°37'50''N; 55°43'40''W)	*C. f. fumosus*	CN1410, CN1378,	Cfu1410, Cfu1378	MPEG*
Chaves - PA 00°12'29.2''S; 49°58'39.2''W	*C. f. fumosus*	MARJ117, MARJ118	Cfu118, Cfu117	MPEG
Marajó - PA (00°59'21''S; 49°56'24''W)	*C. f. fumosus*	MAYA008	Cfu008	MPEG
Oriximiná - PA (1°45'36.89''S; 55°51'30.28''W)	*C. f. fumosus*	ORX336, ORX359	Cfu336, Cfu359	MPEG
Porto Walter - AC (08°20'35.7''S; 72°36'19.7''W)	*C. f. duidae*	UFAC1021	Cfu1021	MPEG
Japurá - AM (02°02'31.5''S; 67°17'16.6''W)	*C. f. duidae*	JAP225, JAP267, JAP270	Cfu225, Cfu267, Cfu270	MPEG
Porto Walter - AC (08°20'35.7''S; 72°36'19.7''W)	*C. f. duidae*	UFAC0976	Cfu0976	MPEG
Uruçuí - PI (07°14'2.00''S; 44°33'1.55''W)	*C.f. bimaculatus*	URC171	Cfu171	MPEG
Curimatá - PI (09°41'284''S; 44°14'200''W)	*C.f. bimaculatus*	SRV005	Cfu005	MPEG
Redenção do Gurgueia - PA (9°38'022''S; 44°08'807''W)	*C.f. bimaculatus*	SRV042	CfuS042	MPEG
Borba, Puruzinho, Ilha - AM 04°07'42''S; 59°21'55.4''W	*C. f. bimaculatus*	MAD500	Cfu500	MPEG
Autazes, Uricurituba, Ilha - AM 03°34'47''S; 59°07'50''W	*C.f. bimaculatus*	MAD608	Cfu608	MPEG
Santa Catarina - SC (27°14'32.42''S; 50°13'7.88''W)	*C. f. fuscatus*	TERNA210, TERNA398,TERNA1068, TERNA1349, Cachimbo470	Cfu210, Cfu398, Cfu1068, Cfu1349, Cfu470	MNRJ†
Rio Branco - AC (09°57'32.3''S; 67°43'57.2''W)	*C. f. beniensis*	UFAC1199, UFAC1297, UFAC273	Cfu1199, Cfu1297, Cfu273	MPEG
Monte Alegre - PA (2°3'14.72''S; 54°10'24.49''W)	*C. f. fuscatior*	PEMA042, PEMA037	Cfu042, Cfu037	MPEG

* Museu Paraense Emilio Goeldi.
† Museu Nacional do Rio de Janeiro.

v. 7.2.5, to confirm the position of amplified fragment. We then assessed if aminoacid sequence presented stop codons and indels, which could indicate pseudogenes. The potential association of variable sites with the plumage morphotype of each species was confirmed by visual inspection. We calculated Tajima's D (Tajima 1989) in DnaSP (version 3.51, Rozas *et al.* 2003) to verify whether the *MC1R* gene was under selection pressure in the two groups.

RESULTS

A total of 744 base pairs were sequenced for each of the 27 *C. fuscatus* samples, representing nucleotides 129–873 of the *MC1R* gene of *Gallus gallus*, which includes all the sites known to be associated with plumage polymorphisms in birds (Theron *et al.* 2001). Only 21 samples were considered (pp >0.6) after the resolution of the gametic phases (Harrigan *et al.* 2008, Table 2). In the BLAST (NCBI: National Center for Biotechnology Information) analysis, the sequences were 95% similar to that of *Gallus gallus* (Kerje *et al.* 2003) and 97% similar to that of *Coereba flaveola* (Theron *et al.* 2001).

We identified 22 variable sites in the 21 *C. fuscatus* samples (Table 2), including all six subspecies. These variable sites of the *MC1R* locus determined six non-synonymous mutations for the codification of amino acids, A8G, S9R, S10N, S89N, V226I, and L240I (Table 3). None of these sites were associated with the coloration patterns of either the two groups or any of the subspecies. Tajima's D was not significant (-1.603, $P > 0.05$), indicating that the variation found in the study locus in *C. fuscatus* is neutral, with a signal of recent demographic expansion, against the constant demographic model. All the sequences generated in the present study were deposited in GenBank (www.ncbi.nlm.nih.gov) under access numbers MK102986 through MK103006 (Table 3).

DISCUSSION

Our study of *Cnemotriccus fuscatus* indicates that there is no clear association between the plumage polymorphism found in this species and mutations of the *MC1R* gene. As in previous studies of bird species such as *Phylloscopus toutinegras* (MacDougall-Shackleton *et al.* 2003), *Lepidothrix coronata* (Cheviron *et al.* 2006), *Dendrocolaptes platyrostris* (Corso *et al.* 2013), *Philomachus pugnax*

Table 2. MC1R variable sites found in the sequences of *Cnemotriccus fuscatus*.

Voucher – Subspecies	Nucleotidic sites																								Belly Plumage
	1	2	2	2	7	1 3	1 7	1 7	2 1	2 4	2 6	2 7	3 1	3 2	4 6	4 9	5 0	6 3	6 4	6 7	6 8	7 1	7 2		
	5	3	5	9	5	8	2	2	3	3	6	6	8	7	5	8	4	3	8	6	7	8	3		
Cfu008 - *C. f. fumosus*	G	C	A	G	A	C	C	C	G	C	G	C	C	T	C	C	C/G	T	C	G	C	C/A	C		Yellow
Cfu117- *C. f. fumosus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		Yellow
Cfu118 - *C. f. fumosus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		Yellow
Cfu1410 - *C. f. fumosus*	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	T	.	.	.	.		Yellow
Cfu359 - *C. f. fumosus*	.	.	.	.	A/G	.	.	.	.	.	.	.	.	.	T	.	.	.	T	G/A	T	.	T		Yellow
Cfu225 - C. *f. duidae*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	.	.	.	T		Yellow
Cfu267- *C. f. duidae*	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	T	.	.	.	C/T		Yellow
Cfu0976 - *C. f. duidae*	.	.	.	.	G/A	.	.	.	.	.	G/A	C/T	.	.	.	.	.	.	T	.	.	.	.		Yellow
Cfu1021 - *C. f. duidae*	.	.	.	.	A/G	.	.	.	.	.	.	.	.	.	.	.	.	.	C/T	.	.	.	.		Yellow
Cfu270 - *C. f. duidae*	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.		Yellow
Cfu210 - *C. f. fuscatus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		Yellow
Cfu398 - *C. f. fuscatus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C/T		Yellow
Cfu1068 - *C. f. fuscatus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		Yellow
Cfu273 - *C. f. beniensis*	.	G	C	G/A	.	.	.	.	.	.	.	.	.	.	.	C/A	.	.	.	.	.	.	.		White
Cfu1297 - *C. f. beniensis*	.	.	.	.	.	.	.	C/T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		White
Cfu500 - *C. f. bimaculatus*	A/G	.	.	.	.	.	.	C/T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		White
Cfu608 - *C. f. bimaculatus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		White
Cfu005 - *C. f. bimaculatus*	.	.	.	.	G	.	.	.	A/G	T/C	.	.	C/T	C	C/T	.	.	.	.	.	.	.	.		White
Cfu042 - *C. f. bimaculatus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		White
Cfu171 - *C. f. bimaculatus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		White
Cfu042 - *C. f. fuscatior*	.	.	.	.	.	T/C	T/C	.	.	.	.	.	.	.	.	.	.	C/T	.	.	.	.	C/T		White

Table 3. Position of non-synonymous variations within the amino acid *Cnemotriccus fuscatus*. GenBank access numbers for the samples analyzed.

Voucher – Subspecies	Amino acid positions						Belly Plumage	Access number GenBank
	8	9	1 0	8 9	2 2 6	2 4 0		
Cfu008 - *C. f. fumosus*	A	S	S	S	V	L/I	Yellow	MK102986
Cfu117 - *C. f. fumosus*	.	.	.	.	.	.	Yellow	MK102987
Cfu118 - *C. f. fumosus*	.	.	.	.	.	.	Yellow	MK102988
Cfu1410 - *C. f. fumosus*	.	.	.	N	.	.	Yellow	MK102989
Cfu359 - *C. f. fumosus*	.	.	.	.	V/I	.	Yellow	MK102990
Cfu225 - C. *f. duidae*	.	.	.	.	.	.	Yellow	MK102991
Cfu267 - *C. f. duidae*	.	.	.	N	.	.	Yellow	MK102992
Cfu0976 - *C. f. duidae*	.	.	.	S/N	.	.	Yellow	MK102993
Cfu1021 - *C. f. duidae*	.	.	.	.	.	.	Yellow	MK102994
Cfu270 - *C. f. duidae*	.	.	.	.	.	.	Yellow	MK102995
Cfu210 - *C. f. fuscatus*	.	.	.	.	.	.	Yellow	MK102996
Cfu398 - *C. f. fuscatus*	.	.	.	.	.	.	Yellow	MK102997
Cfu1068 - *C. f. fuscatus*	.	.	.	.	.	.	Yellow	MK102998
Cfu273 - *C. f. beniensis*	G	R	S/N	.	.	.	White	MK102999
Cfu1297 - *C. f. beniensis*	.	.	.	.	.	.	White	MK103000
Cfu500 - *C. f. bimaculatus*	.	.	.	.	.	.	White	MK103001
Cfu608 - *C. f. bimaculatus*	.	.	.	.	.	.	White	MK103002
Cfu005 - *C. f. bimaculatus*	.	.	.	.	.	.	White	MK103003
Cfus042 - *C. f. bimaculatus*	.	.	.	.	.	.	White	MK103004
Cfu171 - *C. f. bimaculatus*	.	.	.	.	.	.	White	MK103005
Cfu042 - *C. f. fuscatior*	.	.	.	.	.	.	White	MK103006

(Farrell *et al.* 2014) and the genus *Antilophia* (Luna *et al.* 2018), our findings reinforce the conclusion that this gene does not always play a role in the variation in plumage coloration found among populations or species. In this case, other genes or mechanisms may determine this variation, as observed in a number of birds (McLean & Stuart-Fox 2014).

A number of new genes associated with plumage coloration have been identified in recent years, although they have been analyzed in only a few species (Oribe *et al.* 2012, Bourgeois *et al.* 2016). Miwa *et al.* (2007), for example, found an association between mutations of the *endothelin receptor B2* (*EDNRB2*) gene and the coloration of *Cortunix japonica*, with a non-synonymous substitution that alters an amino acid (*R332H*) being associated with the "panda" pattern, in contrast with the standard "dotted white" pattern. Other genes that may be involved in pigmentation in birds include the *tyrosinase-related protein 1*, *TYRP1* (Xu *et al.* 2013, Bourgeois *et al.* 2016), *SRY-Box containing 10*, *SOX10* (Gunnarsson *et al.* 2011), Agouti protein, ASIP (Oribe *et al.* 2012, Zhang *et al.* 2013), and *Corin* (Bourgeois *et al.* 2016) genes, and the proopiomelanocortin (*POMC*) gene cluster, which includes *MC1R* (Kang & Kim 2015).

In addition to genetics, the variation found in the coloration of *C. fuscatus* may be related to environmental factors, given the diversity of habitats occupied by the species (Fig. 1). Uy *et al.* (2009), for example, found that natural selection may favor distinct coloration in different habitats based on the existence of several population patterns, with habitats dominated by short-wavelength light (*e.g.*, shaded woodland) favoring darker birds, and habitats rich in long-wavelength light (*e.g.*, forest clearings with direct sunlight) favor lighter-colored species.

Furthermore, the studied part of the gene *MC1R* includes all the main sites that were showed in previous research with plumage polymorphism of birds (Mundy 2005, Cheviron *et al.* 2006). Overall, our results reinforce

the conclusion that understanding the evolution of plumage coloration in *C. fuscatus* with varying patterns of eumelanin/pheomelanin pigmentation requires a more profound investigation of the genes in the melanocortin pathway and their potential variation, as well as other loci and environmental factors. Unlike many other bird species (see *e.g.*, Cheviron *et al.* 2006, Corso *et al.* 2013, Farrell *et al.* 2014, Luna *et al.* 2018), the variation in the plumage coloration of *C. fuscatus* does not appear to be related to mutations of the *MC1R* gene.

ACKNOWLEDGEMENTS

We thank the Goeldi Museum (MPEG) and National Museum (MNRJ) for providing samples. We are also grateful to the Federal University of Pará (UFPA) for infrastructure, the Institutional Program of Scientific Initiation Scholarships (PIBIC) for granting an undergraduate scholarship, the CAPES Science Without Borders (CSF) program for supporting overseas academic exchange and Stephen Ferrari for the revision of the manuscript. A.A. is supported by a CNPq research productivity fellowship (# 306843/2016-1).

REFERENCES

Bourgeois Y.X.C., Bertrand J.A.M., Delahaie B., Cornuault J., Duval T., Milá B. & Thébaud C. 2016. Candidate gene analysis suggests untapped genetic complexity in melanin-based pigmentation in birds. *Journal of Heredity* 107: 327–335.

Cheviron Z.A., Hackett S.J. & Brumfield R.T. 2006. Sequence variation in the coding region of the melanocortin-1 receptor gene (MC1R) is not associated with plumage variation in the Blue-crowned Manakin (*Lepidothrix coronata*). *Proceedings of the Royal Society of London B: Biological Sciences* 273: 1613–1618.

Chunco A.J., McKinnon J.S. & Servedio M.R. 2007. Microhabitat variation and sexual selection can maintain male color polymorphisms. *Evolution* 61: 2504–2515.

Corso J., Cabanne G.S., d'Horta F.M., Loreto É.L.S. & Miyaki C.Y. 2013. Plumage variation in the Planalto Woodcreeper (*Dendrocolaptes platyrostris*) and the melanocortin-1 receptor gene (MC1R). *Revista Brasileira de Ornitologia* 21: 1–4.

Farnsworth A. & Lebbin D. 2017. Fuscous Flycatcher (*Cnemotriccus fuscatus*). In: del Hoyo J., Elliott A., Sargatal J., Christie D.A. & de Juana E. (eds.). *Handbook of the birds of the world alive*. Available in http://www.hbw.com/node/57348 (access on 27 September 2017).

Farrell L.L., Küpper C., Burke T. & Lank D.B. 2014. Major breeding plumage color differences of male Ruffs (*Philomachus pugnax*) are not associated with coding sequence variation in the MC1R gene. *Journal of Heredity* 106: 211–215.

Fitzpatrick J.W., Bates J., Bostwick K., Caballero I., Clock B., Farnsworth A., Hosner P., Joseph L., Langham G., Lebbin D., Mobley J., Robbins M, Scholes E., Tello J., Walther B. & Zimmer K. 2004. Family Tyrannidae (tyrant-flycatchers), p. 170–462. In: del Hoyo J., Elliott A. & Christie D.A. (eds.). *Handbook of the birds of the world, v. 9 (cotingas to pipits and wagtails).* Barcelona: Lynx Editions.

Galván I. & Jorge A. 2015. The rusty plumage coloration of juvenile Gyrfalcons is produced by pheomelanin and its expression is affected by an intracellular antioxidant. *Journal of Raptor Research* 49: 59–65.

García-Borrón J.C., Sánchez-Laorden B.L., Jiménez-Cervantes C. 2005. Melanocortin-1 receptor structure and functional regulation. *Pigment Cell and Melanoma Research* 18: 393–410.

Gomez D. & Théry M. 2004. Influence of ambient light on the evolution of colour signals: comparative analysis of a Neotropical Rainforest bird community. *Ecology Letters* 7: 279–284.

Gunnarsson U., Kerje S., Bed'hom B., Sahlqvist A.-S., Ekwall O., Tixier-Boichard M., Kämpe O. & Andersson L. 2011. The dark brown plumage color in chickens is caused by an 8.3-kb deletion upstream of SOX10. *Pigment Cell and Melanoma Research* 24: 268–274.

Harrigan R.J., Mazza M.E. & Sorenson M.D. 2008. Computation *vs.* cloning: evaluation of two methods for haplotype determination. *Molecular Ecology Resources* 8: 1239–1248.

Hewitt G.M. 1988. Hybrid zones: natural laboratories for evolutionary studies. *Trends in Ecology and Evolution* 3: 158–167.

Hoekstra H. & Price T. 2004. Parallel evolution is in the genes. *Science* 303: 1779–1781.

Johnson J.A., Ambers A.D. & Burnham K.K. 2012. Genetics of plumage color in the Gyrfalcon (*Falco rusticolus*): analysis of the melanocortin-1 receptor gene. *Journal of Heredity* 103: 315–321

Kang D.Y. & Kim H.C. 2015. Functional relevance of three proopiomelanocortin (POMC) genes in darkening camouflage, blind-side hypermelanosis, and appetite of *Paralichthys olivaceus. Comparative Biochemistry and Physiology B: Biochemistry and Molecular Biology* 179: 44–56.

Kerje S., Lind J., Schutz K., Jensen P. & Andersson L. 2003. Melanocortin 1-receptor (MC1R) mutations are associated with plumage colour in chicken. *Animal Genetics* 34: 241–248.

Legagneux P., Clark R.G., Guillemain M., Eraud C., Théry M. & Bretagnolle V. 2012. Large-scale geographic variation in iridescent structural ornaments of a long-distance migratory bird. *Journal of Avian Biology* 43: 355–361.

Luna L.W., Silva W.A.G., Araripe J., Pereira I.T.F., d'Horta F.M., Sampaio I., Schneider H. & Rêgo R.S. 2018. Mutations in the melanocortin-1 receptor (*MC1R*) gene have no influence on the distinct patterns of melanic plumage found in the manakins of the genus *Antilophia* (Aves: Pipridae). *Anais da Academia Brasileira de Ciências* 90: 2873–2879.

MacDougall-Shackleton E.A., Blanchard L. & Gibbs H.L. 2003. Unmelanized plumage patterns in Old World Leaf Warblers do not correspond to sequence variation at the melanocortin-1 receptor locus (MC1R). *Molecular Biology and Evolution* 20: 1675–1681.

McLean C.A. & Stuart-Fox D. 2014. Geographic variation in animal colour polymorphisms and its role in speciation. *Biological Reviews* 89: 860–873.

McNaught M.K. & Owens I.P.F. 2002. Interspecific variation in plumage colour among birds: species recognition or light environment? *Journal of Evolutionary Biology* 15: 505–514.

Mercadante A. & Hill G.E. 2014. An experimental test of the role of structural blue and melanin-based chestnut coloration in aggressive contest in male Eastern Blue Birds. *Frontiers in Ecology and Evolution* 2: 24.

Miwa M., Inoue-Murayama M., Aoki H., Kunisada T., Hiragaki T., Mizutani M. & Ito S. 2007. Endothelin receptor B2 (EDNRB2) is associated with the panda plumage colour mutation in Japanese Quail. *Animal Genetics* 38: 103–108.

Mundy N. 2005. A window on the genetics of evolution: MC1R and plumage colouration in birds. *Proceedings of the Royal Society of London B: Biological Sciences* 272: 1633–1640.

Oribe E., Fukao A., Yoshihara C., Mendori M., Rosal K.G., Takahashi S. & Takeuchi S. 2012. Conserved distal promoter of the agouti

signaling protein (ASIP) gene controls sexual dichromatism in chickens. *General and Comparative Endocrinology* 177: 231–237.

Osorio D. & Vorobyev M. 2008. A review of the evolution of animal colour vision and visual communication signals. *Vision Research* 48: 2042–2051.

Ran J.S., You X.Y., Jin J., Zhou Y.G., Wang Y. Dan L., Ren P. & Liu Y.P. 2016. The relationship between MC1R mutation and plumage color variation in Pigeons. *BioMed Research International* 2016: 3059756.

Rasmussen P.C. & Collar N.J. 2002. Family Bucconidae (puffbirds), p. 102–138. In: del Hoyo J., Elliott A. & Christie D.A. (eds.). *Handbook of the birds of the world, v. 7 (jacamars to woodpeckers).* Barcelona: Lynx Editions.

Robbins L., Nadeau J.H., Johnson K.R., Kelly M.A., Roselli-Rehfuss L., Baack E., Mountjoy K.G. & Cone R.D. 1993. Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. *Cell* 72: 827–834.

Rozas J., Sánchez-DelBarrio J.C., Messeguer X. & Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19: 2496–2497.

Sanger F., Nicklen S. & Coulson A.R. 1977. DNA sequencing with chain-termination inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* 74: 5463–5468.

Seddon N., Botero C.A., Tobias J.A., Dunn P.O., MacGregor H.E.A., Rubenstein D.R., Uy J.A.C., Weir J.T., Whittingham L.A. & Safran R.J. 2013. Sexual selection accelerates signal evolution during speciation in birds. *Proceedings of the Royal Society of London B: Biological Sciences* 280: 20131065.

Stoddard M.C. & Prum R.O. 2011. How colorful are birds? Evolution of the avian plumage color gamut. *Behavioral Ecology* 22: 1042–1052.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585–595.

Takeuchi S., Suzuki H., Yabuuchi M. & Takahashi S. 1996. A possible involvement of melanocortin 1-receptor in regulating feather color pigmentation in the chicken. *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression* 1308: 164–168.

Theron E., Hawkins K., Bermingham E., Ricklefs R.E. & Mundy N.I. 2001. The molecular basis of an avian plumage polymorphism in the wild: a melanocortina-1-receptor point mutation is perfectly associated with the melanic plumage morph of the Bananaquit, *Coereba flaveola. Current Biology* 11: 550–557.

Uy J.A.C., Moyle R.G., Filardi C.E. & Cheviron Z.A. 2009. Difference in plumage color used in species recognition between incipient species is linked to a single amino acid substitution in the melanocortin-1 receptor. *American Naturalist* 174: 244–254.

Uy J.A.C., Cooper E.A., Cutie S., Concannon M.R., Poelstra J.W., Moyle R.G. & Filardi C.E. 2016. Mutations in different pigmentation genes are associated with parallel melanism in island flycatchers. *Proceedings of the Royal Society of London B: Biological Sciences* 283: 20160731.

Vidal O., Araguas R.M., Fernández R., Heras S., Sanz N. & Pla C. 2010. Melanism in Guinea Fowl (*Numida meleagris*) is associated with a deletion of phenylalanine-256 in the MC1R gene. *Animal Genetics* 41: 656–658.

Wen L., Fu Y., Dai B. & Antalffy J. 2015. Polymorphism in some birds of Galliformes. *Acta Ecologica Sinica* 35: 103–106.

Xu Y., Zhang X.H. & Pang Y.Z. 2013. Association of tyrosinase (TYR) and tyrosinase-related protein 1 (TYRP1) with melanic plumage color in Korean Quails (*Coturnix coturnix*). *Asian-Australasian Journal of Animal Sciences* 26: 1518–1522.

Zhang X.H., Pang Y.Z., Zhao S.J., Xu H.W., Li Y.L., Xu Y., Guo Z. & Wang D.D. 2013. The relationship of plumage colours with MC1R (melanocortin 1 receptor) and ASIP (agouti signaling protein) in Japanese Quail (*Coturnix coturnix japonica*). *British Poultry Science* 54: 306–311.

Associate Editor: Fábio R. Amaral.