

Plumage variation in the Planalto Woodcreeper (*Dendrocolaptes platyrostris*) and the *melanocortin-1 receptor* gene (*MC1R*)

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ABSTRACT: The Planalto Woodcreeper (*Dendrocolaptes platyrostris*) presents “pale” and “dark” plumage variants, which are distributed throughout the Cerrado and Caatinga, and throughout the Atlantic Forest, respectively. To understand the genetic nature of the plumage variation in the species, we partially sequenced the *melanocortin-1 receptor* (*MC1R*) gene, which is associated with melanic phenotypes in vertebrates. We found no correlation between variation at *MC1R* sequences and plumage color in *D. platyrostris*. Aminoacid sites that were correlated with variation in melanic plumage in other bird species were monomorphic in *D. platyrostris*. Our results suggested that *MC1R* seems not to be involved in controlling plumage variation in *D. platyrostris*.

KEY-WORDS: plumage color polymorphism; melanocortin-1 receptor, *Dendrocolaptes*

INTRODUCTION

The genetic basis of phenotypic diversity within species is of great interest to evolutionary biologists because adaptive evolution depends on selection of genetic variants (Theron *et al.* 2001). Genetic changes resulting in color and pigmentation variation among closely related taxa might represent important evolutionary events. However, the molecular basis and developmental pathways responsible for phenotypic difference are unknown in most cases (Cheviron *et al.* 2006). Recently, it has been suggested that the locus encoding the *melanocortin-1 receptor* (*MC1R*) may cause color polymorphisms in wild populations (Mundy 2005). This gene encodes the MC1R protein, which is expressed in the melanocytes of developing feathers and hair follicles and plays a critical role in the control of melanin synthesis (Theron *et al.* 2001, Baião *et al.* 2007). Point mutations in this locus were associated with color polymorphisms based on melanin and can cause changes from light to dark color all over the body in a variety of taxa (revision in Corso *et al.* 2012).

The planalto woodcreeper (*Dendrocolaptes platyrostris*) is an endemic bird of the Atlantic forest that inhabits forest enclaves and gallery forests within the Cerrado, Chaco, and Caatinga (Figure 1). As many other woodcreepers, *D. platyrostris* does not have plumage sexual dimorphism. It has two parapatrically distributed subspecies: *D. p. platyrostris* – dark plumage morph - individuals are predominantly streaked buff, have blackish crown, dull chestnut wings and tail, and brown underparts, and inhabit the Atlantic Forest domain in southeastern Brazil, eastern Paraguay and extreme northeastern Argentina; *D. p. intermedius* – pale plumage morph - individuals have browner crown, almost no streaking on mantle, paler and brighter rufous wings and tail, and slightly paler underparts, and inhabit gallery forests of Cerrado / Caatinga domains (Ridgely & Tudor 1994, Willis & Oniki 2001, Cabanne *et al.* 2011; Figure 1). The study of Cabanne *et al.* (2011) indicated that plumage type in *D. platyrostris* (i.e., dark and pale morphs) was not correlated with neutral genetic divergence (mitochondrial DNA) but confirmed that it

was correlated to different types of habitats, with dark and pale morphs occurring respectively in more humid (Atlantic Forest) and dry (Cerrado / Caatinga) habitats.

In this study we examined the *MC1R* sequence variation of *D. p. platyrostris* and *D. p. intermedius*.

Specifically, we looked for fixed non-synonymous differences in *MC1R* between plumage morphs. Our main question was whether the light and dark phenotypes of this species could be related to changes in the *MC1R* coding region.



FIGURE 1. Distribution of biomes and of the two subspecies of *Dendrocolaptes platyrostris*. Modified from Cabanne *et al.* (2011).

MATERIAL AND METHODS

We sampled five adult individuals of *D. p. platyrostris intermedius* and five *D. p. platyrostris*, from localities that are 1600 km apart (Table 1). Total genomic DNA was isolated from blood or muscle samples by a standard phenol/

chloroform extraction protocol (Sambrook *et al.* 2001). A fragment of approximately 500 bp of the avian *MC1R* gene was amplified by PCR using the following primers: lcorMSHR72 – 5' AYGCCAGYGAGGGCAACCA 3' (Cheviron *et al.* 2006) and MC1RIntRev – 5' AACATGTGRATGTAGAGCACC 3'. PCR

conditions were: initial denaturation for 3 min at 94°C, followed by 45 cycles (denaturation at 94°C for 45 s, annealing at 50–60°C for 60 s and extension at 72°C for 90 s) and a final extension at 72°C for 5 min. This *MC1R* fragment includes main sites previously shown to be associated with melanic phenotypes in birds (Cheviron *et al.* 2006). Each specimen was submitted to at least two independent amplification and sequencing reactions (using the same primers used in the PCR) to confirm the sequences. Consensus sequences were obtained and

deposited in GenBank under the accession numbers: FJ985683–FJ985688, JN224986–JN224989.

DNA sequences were aligned and their nucleotides and deduced amino acids were compared to those from bananaquits (*Coereba flaveola*; GenBank number AF362605 and AF362598) using BIOEDIT v. 7 (Hall, 1999; Table 2). We tested for evidence of selection at *MC1R* by calculating Tajima's D statistic using MEGA 5 (Tamura *et al.* 2011).

TABLE 1. Specimens sequenced, sampling localities, sample identification, and voucher identification of tissue samples.

Taxon ^a	Locality (habitat)	Tissue identification ^b	Vouchers ^c
<i>D. p. platyrostris</i>	Pinhalão, Paraná (PR). 23°46'S; 50°3'W (Atlantic forest)	LGEMA P885	MZUSP 75622
	Wenceslau Braz, PR. 22°5'S; 48°47'W (Atlantic forest)	LGEMA P957	MZUSP 75690
	Morro Grande State Park, São Paulo (SP). 23°42'S; 46°59'W (Atlantic forest)	LGEMA P2480, P2482	-
	Barreiro Rico, SP. 22°38'S; 48°13'W (Atlantic forest)	LGEMA P1696	-
<i>D. p. intermedius</i>	National Park of Serra das Confusões, Piauí. 9°40'S; 44°8'W (Caatinga)	LGEMA P2277, P2278, P2329, P2379, P2418	MZUSP 77719, 77720, 77721, 77722

^a *D. p.* = *Dendrocolaptes platyrostris*.

^b Samples are deposited at the Laboratório de Genética e Evolução Molecular de Aves (LGEMA), Universidade de São Paulo, São Paulo, Brazil.

^c Voucher specimens housed at MZUSP: Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil. A dash indicates that a voucher specimen does not exist.

TABLE 2. *MC1R* sequences of *Dendrocolaptes platyrostris*. Sites are numbered based on the alignment with bananaquit (*Coereba flaveola*) *MC1R* sequences (see methods). Sites in bold are associated with color polymorphism in other avian taxa (amino acid replacement), all *D. platyrostris* individuals are monomorphic at these sites. Variable sites in *D. platyrostris* that resulted in putative aminoacid substitution are underlined. Asterisks show sites that are identical to those of *Coereba flaveola*.

Taxa	Nucleotide site					Aminoacid position										Plumage
	1	1	2	2	5	1	3	6	8	9	1	1	1	1	2	
	1	2	0	7	1	6	8	8	5	2	1	5	6	7	0	
	3	0	3	4	4						1	7	6	2	7	
<i>C. flaveola</i> M5	T	C	A	A	G	A	I	H	V	K	V	Q	V	V	H	melanic
<i>C. flaveola</i> Y24	*	*	*	G	*	*	*	*	*	E	*	*	*	*	*	yellow
<i>D. p. platyrostris</i> P885; P2480; P2482; P1696	*	T	*	G	*	.	*	*	*	E	*	*	*	*	*	dark
<i>D. p. platyrostris</i> P957	*	T/C	*	G	*	.	*	*	*	E	*	*	*	*	*	dark
<i>D. p. intermedius</i> P2278; P2379; P2418	*	T	*	G	<u>A</u>	.	*	*	*	E	*	*	*	I	*	pale
<i>D. p. intermedius</i> P2329	T/A	T	*	G	<u>A</u>	.	*	*	*	E	*	*	*	I	*	pale
<i>D. p. intermedius</i> P2277	*	T	<u>G</u>	G	*	.	*	R	*	E	*	*	*	*	*	pale

RESULTS

The alignment matrix presented 525 characters encompassing sites 100 to 624 of the *MC1R* gene. It had 96–97% nucleotide identity with sequences from other passerine birds such as *Coereba flaveola* (Theron *et al.* 2001), *Phylloscopus* warblers (MacDougall-Shackleton *et al.* 2003), and *Lepidothrix coronata* (Chevion *et al.* 2006). Two *D. platyrostris* individuals presented heterozygous sites at positions 113 and 120 (Table 2). There were three variable sites that resulted in non-synonymous substitutions (amino acid positions 38, 68, and 172), which allowed us to identify three polymorphic DNA sites in pale morph birds: c.113 T > A, c.203 A > G, and c.514 G > A (Table 2). These changes could not be correlated with phenotype differences, once those changes maintain the same polarities of original aminoacids. As the number of variable sites observed was very low, our statistical power to detect selection was also low (Tajima's D: -0.78, not significant at P > 0.10).

DISCUSSION

The results obtained are in contrast with some studies of vertebrate taxa, including birds (reviewed in Mundy 2005), which observed a linkage between *MC1R* mutations and the appearance of melanic phenotype. In *Coereba flaveola*, a non-synonymous substitution (E92K) was associated with melanic plumage (Mundy *et al.* 2004). In the red-footed booby (*Sula sula*), the white/melanic polymorphism observed was associated to two point substitutions, V85M and H207R (Baião *et al.* 2007). These three sites were monomorphic in *D. platyrostris* (Table 2). However, these results should be interpreted with caution, as our sample size is small and it may not have allowed the identification of association between phenotypic and genotypic variations. However, even though we did not completely sequence the *MC1R*, the segment studied contained the majority of sites previously shown to be correlated with plumage differences in birds (Mundy *et al.* 2004, Mundy 2005, Chevion *et al.* 2006). Therefore, *D. platyrostris* seems to be another instance of a bird species that does not present any evidence that *MC1R* is correlated with pigmentation differences as documented previously in *Phylloscopus* warblers (MacDougall-Shackleton *et al.* 2003) and *Lepidothrix coronata* (Chevion *et al.* 2006), the latter belonging to the same Passeriformes suborder (Tyranni) as *D. platyrostris*.

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