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6 ***MC1R* polymorphism associated to plumage color variations in *Coturnix chinensis*.**

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26 SUMMARY

27 The melanocortin 1 receptor (*MC1R*) gene was investigated as a candidate for plumage
28 variations in Chinese painted quail, *Coturnix chinensis*. Four silent and two missense
29 nucleotide polymorphisms were identified. The correspondent amino acid changes,
30 p.Glu92Lys and p.Pro292Leu were found in *Blue Face* and *Red Breasted* animals,
31 respectively. *Blue Face* is a melanic phenotype similar to the co dominant *Extended*
32 *Brown* of Japanese quail, and both share the p.Glu92Lys mutation. The association of
33 p.Pro292Leu with the recessive *Red Breasted* was confirmed in 23 animals from an
34 experimental F2 cross.

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40 KEYWORDS

41 Quail, MC1R, plumage, color variation.

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51 The melanocyte production of black eumelanins and red phaeomelanins is mainly
52 regulated by the activity of the melanocortin 1 receptor (MC1R), and has great influence
53 in fur or plumage color. The *MC1R* gene has only one exon and has been reported to
54 cause color variation in both mammals and birds (Andersson 2003). In chicken (Kerje et
55 al. 2003), quail (Nadeau et al. 2006) and bananaquit (Theron et al. 2001) the same
56 polymorphism, p.Glu92Lys, causes dominant black plumages likely through a MC1R
57 constitutive activation (Robbins et al. 1993). In contrast, recessive pheomelanic colors
58 have been linked to the inactivation of the receptor, at least in mammals (Kijas et al.
59 1998).

60 Chinese painted quail (*Coturnix chinensis*) is the smallest of the domesticated quail
61 species and has an accentuated sexual dimorphism in plumage color. While *Wild*
62 females display a brown coloration similar to that of Japanese quail, *Wild* males show a
63 general blue pattern on its back and a red tail (Figure 1). Because breeders value the
64 colors of these animals, several plumage varieties have been already established. Some
65 of these are compatible with variations in melanins content, such as the incomplete
66 dominant *Blue Face* and maybe the recessive *Red Breasted*. Interestingly, although *Blue*
67 *Face* males and females have darkened eumelanic plumages, there is a strong contrast
68 among the *Red Breasted* sexes: females display lighter brown colors while males display
69 a completely blackened face and a ventral extended red patch (Figures 1).

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75 Thirteen quails of two different origins displaying *Wild* (5), *Blue Face* (3) and *Red*
76 *Breasted* (5) plumages were sampled to assess *MC1R* variability. A *Wild* male and a
77 *Red Breasted* female were used to generate an experimental cross and 15 *Wild* and 8
78 *Red Breasted* F2 individuals were analyzed. In all cases, DNA was extracted from
79 growing feathers as described in Vidal et al. (2010a).

80 Primers MC1R-F1 5'- ACGGCCCCAGCCAGGGGTCCT-3' and MC1R-R1 5'-
81 AGGCACACATCACTGCAAAG-3' were designed from published sequences of
82 *Coturnix chinensis* (AB201632) and *Gallus domesticus* (D78272.1) to amplify 1200 bp
83 including the full coding region of the gene. Two internal primers (MC1R-F2 5'-
84 CCTCATCCTCATCGTCACCT-3' and MC1R-R2 5'-
85 TACCAGGAGCACAGCACCAC-3') were designed to fully sequence the 942 bp of
86 the coding region of the *MC1R* gene. PCR products were purified with the ExoSAP-IT
87 PCR Product Cleanup (Thermo Fisher Scientific) and sequenced with the BigDye
88 Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific). Sequences were
89 aligned using the Multalin software (Corpet 1988). The effects of new non-silent
90 polymorphisms were assessed with Panther-Psep (Tang & Thomas 2016).

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92 The sequencing of 13 individuals allowed the identification of 6 polymorphisms
93 conforming 5 haplotypes (see Table 1). Two of the polymorphisms are missense, and
94 cause the amino acid changes p.Glu92Lys (haplotype H3) and p.Pro292Leu (haplotypes
95 H4 and H5).

96 The p.Glu92Lys change has been demonstrated to cause dominant melanism in mice
97 (Robbins et al. 1993), chicken (Takeuchi et al. 1996) and bananaquit (Theron et al.
98 2001). This melanism is related to a constitutive activation of the *MC1R* receptor,

99 which increases the concentration of cAMP inside the melanocytes, and eumelanins are
100 synthesized almost exclusively (Robbins et al. 1993; Ling et al. 2003).

101 Interestingly, in Japanese quail, this same polymorphism has been associated to the
102 darker *Extended Brown* plumage (Nadeau et al. 2006), an autosomal incomplete
103 dominant mutation. The constitutive activation of MC1R has been suggested as the
104 molecular mechanism for this phenotype as well (Nadeau et al. 2006).

105 We have found that all *Blue Face* animals carried one copy of haplotype H3, while none
106 of the others animals did. Because the pattern of inheritance of this melanic plumage
107 mimics the *Extended Brown* of Japanese quail, and although the number of available
108 samples is low, we suggest that an activation of MC1R caused by p.Glu92Lys is a likely
109 mechanism explaining this phenotype in Chinese painted quail.

110 The sequencing of 5 *Red Breasted* animals showed that three of them were homozygote
111 H4 and two were homozygote H5. These haplotypes differ in one silent position,
112 g.C601T, and both carry the g.C872T missense polymorphism causing a p.Pro292Leu
113 amino acid change (see Table 1). An experimental F2 cross was generated to confirm its
114 association with the *Red Breasted* plumage, and 15 *Wild* and 8 *Red Breasted* F2 animals
115 were genotyped. As expected all the *Red Breasted* animals were homozygote H4, while
116 of the *Wild* animals 10 were heterozygote H1H4 and 5 were homozygote H1.

117 The analysis of the p.Pro292Leu polymorphism using Panther-Psep (Tang & Thomas
118 2016) suggests a high probability of deleterious effects. So far, deleterious mutations of
119 this gene in mammals have been related to an increase in phaeomelanin production
120 caused by the receptor's inactivation. This mechanism has been described in yellow
121 mice (Robbins et al. 1993) and it is likely to explain red coloration in other mammals,
122 such as pig (Kijas et al. 1998). In humans, MC1R mutations causing red headedness are
123 considered to act in this same way, through deleterious mutations. Interestingly, a

124 polymorphism with very intense effects, p.Asp294His, has been located in the
125 transmembrane domain 7 (Lightner 2008) which is therefore suspected to be
126 functionally relevant. Both p.Asp294His and p.Pro292Leu are thus highly likely to be
127 deleterious by altering this transmembrane domain.

128 In this sense, a hypothetical loss-of-function caused by p.Pro292Leu would be
129 compatible with *Red Breasted* being recessive; however, the colors found in *Red*
130 *Breasted* (especially males) do not match other typical *MC1R* phenotypes. In fact, clear
131 *MC1R* phaeomelanic phenotypes have not been found in birds yet (Roulin & Ducrest
132 2013), and brown and red plumages have been associated to *Sox10* in chickens
133 (Gunnarsson et al. 2011) and pigeons (Domyan et al. 2014).

134 Interestingly enough, *MC1R* polymorphisms that could be inactivating the receptor have
135 been linked to recessive dark phenotypes in guinea fowl (Vidal et al. 2010b) and turkey
136 (Vidal et al. 2010a). These phenotypes do not display the typical white dots found in
137 *Wild* guinea fowl nor the white bars of *Wild* plumage turkeys, thus resulting in a darker
138 general appearance. It could be possible then that these colorations are not related to
139 neat increases in eumelanin synthesis.

140 In this context, the association of the putatively deleterious p.Pro292Leu mutation to the
141 *Red Breasted* quail could be in consonance with the inactivation of the receptor in birds
142 not directly affecting eumelanin/phaeomelanin balance, but plumage pattern and/or
143 color distribution. This could imply a significant difference in the function of *MC1R* of
144 mammals and birds that should be further studied.

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228 Table 1. MC1R variation in Chinese painted quail.

Haplotype	Genbank	Allele	DNA (Amino acid) ¹					
			117	271 (92)	601	633	714	872 (292)
H1	MG520490	<i>Wild</i>	C	G (Glu)	C	G	G	C (Pro)
H2	MG520492	<i>Wild</i>	T	-	-	A	-	-
H3	MG520491	<i>Blue Face</i>	-	A (Lys)	-	-	A	-
H4	MG520493	<i>Red Breasted</i>	-	-	-	-	-	T (Leu)
H5	MG520494	<i>Red Breasted</i>	-	-	T	-	-	T (Leu)

229 ¹Position in Japanese quail protein sequence BAD91489.1.

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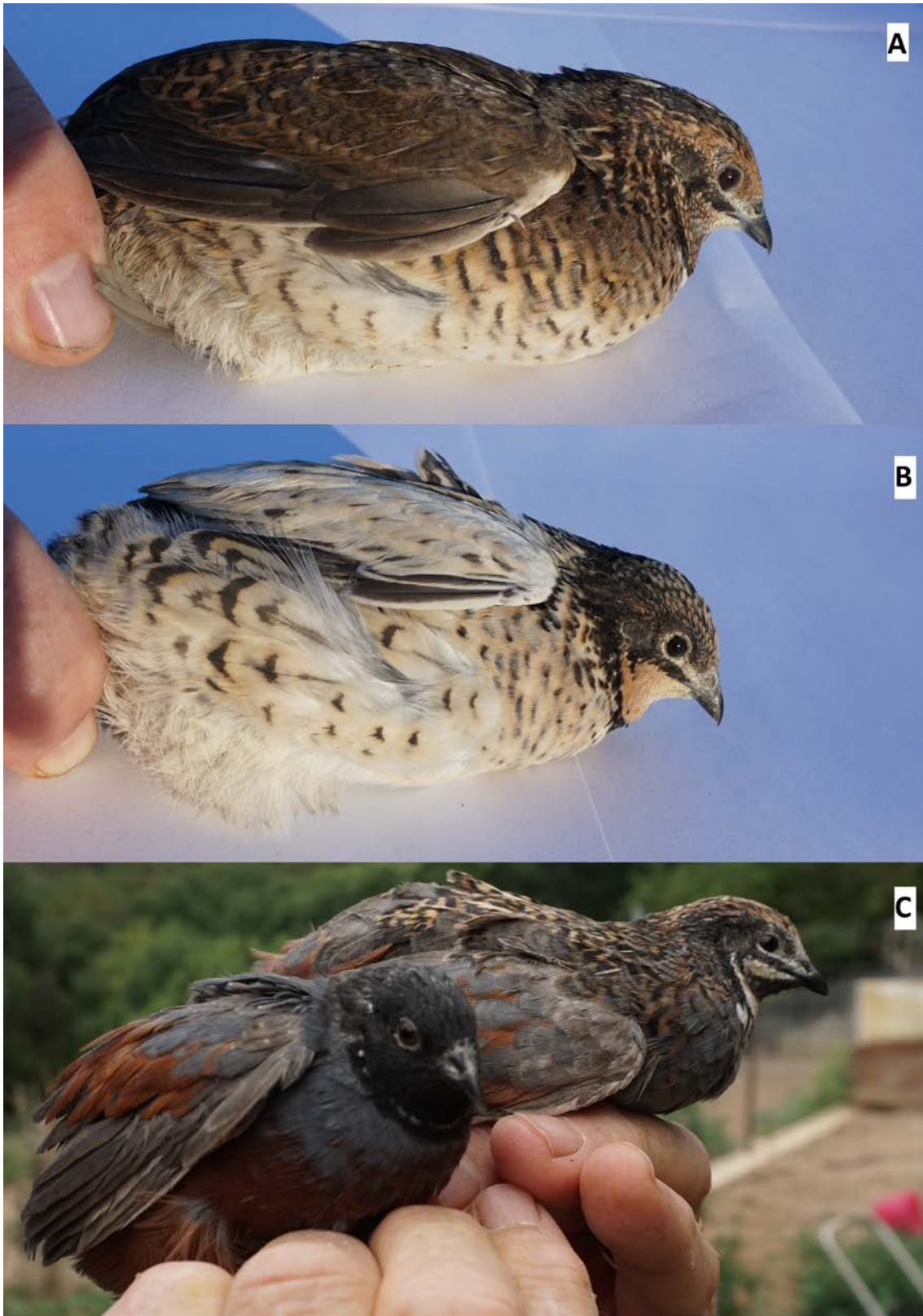
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242 Figure 1. Plumages of *Wild* and *Red Breasted* quail. (A) *Wild* female, (B) *Red Breasted* female,
243 (C) *Red Breasted* –front- and *Wild* –back- males. A complete color chart can be found at
244 http://www.featheredobsessions.com/Button_Quail_Colors.php.

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