Association of a Glu92Lys substitution in MC1R with extended brown in Japanese quail (Coturnix japonica)

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doi:10.1111/j.1365-2052.2006.01442.x

Association of a Glu92Lys substitution in *MC1R* with *extended brown* in Japanese quail (*Coturnix japonica*)

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Summary

We investigated *melanocortin 1 receptor* (*MC1R*) as a candidate locus for the *Extended brown* phenotype in quail, in which there is a general darkening throughout the plumage. An initial screen of variation in *MC1R* in *Extended brown* and in wild-type quails revealed two polymorphic non-synonymous sites. One of these sites, a G-to-A substitution leading to a Glu92Lys mutation, was perfectly associated with plumage phenotype; all *Extended brown* birds were homozygous for Lys92. Co-segregation of the Glu92Lys mutation with the *Extended brown* phenotype was confirmed in 24 progeny of an $E/e^+ \times E/e^+$ cross. Glu92Lys is likely to be the causative mutation for the increased melanism in *Extended brown*, given that the same mutation is associated with melanic plumage in many breeds of domestic chicken, as well as in a wild passerine bird (the bananaquit, *Coereba flaveola*) and laboratory mice. Interestingly, the increase in melanization with the Glu92Lys mutation is less marked in quails than in most other birds and mammals. Phylogenetic results indicate that the Glu92Lys mutation has independently occurred in quail and chicken lineages.

Keywords *extended brown*, *MC1R*, melanism, quail.

The *MC1R* (*melanocortin 1 receptor*) locus encodes a seven-transmembrane G-protein coupled receptor that plays a key role in the regulation of eumelanin/phaeomelanin production by feather bud and hair follicle melanocytes (reviewed in Jackson 1997). Variation in *MC1R* is associated with feather colour or hair colour variation in a wide variety of domesticated and wild species (e.g. Robbins *et al.* 1993; Klungland *et al.* 1995; Nachman *et al.* 2003). In birds, a potential role of *MC1R* in plumage colour was first documented in chickens (Takeuchi *et al.* 1996a,b). Subsequent studies have confirmed this initial finding, and shown that several amino acid variants in MC1R probably contribute to the plumage colour phenotype (Kerje *et al.* 2003; Ling *et al.* 2003). Variation in *MC1R* is also associated with melanic phenotypes in three wild bird species: bananaquit, lesser snow goose and arctic skua (Theron *et al.* 2001; Mundy *et al.* 2004).

The *Extended brown* (E) mutation in Japanese quail is an autosomal incomplete dominant mutation associated with feather darkening in both sexes (Cheng & Kimura 1990), and these features are consistent with an effect at *MC1R*. We therefore investigated whether *MC1R* variation was associated with *Extended brown*. Initial screens were carried out on two wild-type Japanese quail and three *Extended brown* quail: two from a French flock and one with a darker phenotype from a Japanese flock. A 859-bp segment of the 945-bp single coding exon of *MC1R* was amplified using primers MSHR72 (ATGCCAGTGAGGGCAACCA) and MSHR78 (CAGGAGCACAGCACCACCTC). Polymerase chain reaction (PCR) products were directly sequenced on both strands using the PCR primers and two internal primers: MSHR73 (GGCGTAGAAGATGGTGATGTAGC) and MSHR74 (GTGGACCGCTACATCACCAT). Sequences were edited in Seqman (DNASTAR, GATC Biotech, Konstanz, Germany) and manually aligned with other *MC1R* sequences.

Of two synonymous and two non-synonymous substitutions present in Japanese quails (Table 1), a single non-synonymous substitution (Glu92Lys) showed a perfect association with plumage phenotype. The three *Extended brown* individuals from French and Japanese sources were homozygous for Lys92 whereas the two wild-type Japanese birds were homozygous for Glu92. To assess this association further, we investigated co-segregation of the Glu92Lys mutation and plumage colour in 24 progeny from two $E/e^+ \times E/e^+$ crosses. Eight male progeny of each of the three

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Accepted for publication 13 February 2006

Table 1 *MC1R* variation in Japanese quail and domestic fowl.

Species	Phenotype	Allele	24	33	37	58	71	92	110	126	133	143	177	213	215	244	Accession no.
Japanese quail	Wild-type	e^+	T	C	D	V/I	M	**E**[1]	I	V	L	T	F	C	H	L	DQ395091
	Extended brown	*E*	–	–	–	V/I	–	**K**[1]	–	–	–	–	–	–	–	–	DQ395089/90
Red junglefowl	Wild-type	*E*N*	A	–	–	V	–	–	–	–	–	L	–	–	–	–	[2]AY220303
	White Leghorn	*E*	A	–	–	V	–	K	–	I	–	–	L	R	–	–	[2]AY220304
	Barred plymouth rock	*E*	A	–	–	V	T	K	T	–	–	–	L	R	–	–	[3]
	Fayoumi	$E^{R-Fayoumi}$	A	–	–	V	–	–	–	–	Q	–	L	–	–	–	[4]
	Buttercup	e^{bc}	A	–	–	V	T	K	–	–	–	–	L	–	P	–	[2]AY220305
	Nagoya Cortin	e^y	A	W	G	V	–	–	–	–	–	A	L	R	–	P	[3]

[1]Bold letters indicate amino acid site associated with *Extended brown*.
[2]Sequences reported in Kerje *et al.* (2003).
[3]Sequences reported in Takeuchi *et al.* (1996b).
[4]Sequence reported in Takeuchi *et al.* (1996a).

phenotypes (*Extended brown*, *wild-type* and *intermediate*) were genotyped, and there was perfect and significant association between the number of Lys92 alleles and plumage coloration ($P < 0.01$, Fisher's exact test).

The Glu92Lys mutation in *MC1R* has been previously associated with melanism (an overall increase in eumelanin deposition in hairs or feathers) in mice, chickens and bananaquits. Therefore, it is likely that this is the causative mutation of *Extended brown* in quails. *In vitro* studies in mice and chickens have shown that this mutation causes constitutive activation at *MC1R* (Robbins *et al.* 1993; Ling *et al.* 2003). The phenotypic effect of the presence of 92Lys is relatively mild in quail. In mice and bananaquits, the presence of a single 92Lys allele leads to eumelanin deposition throughout most hairs or feathers respectively. The situation in chickens is more complex and suggests intragenic epistatic effects on the Glu92Lys mutation. Although most *MC1R* alleles causing increased melanin deposition encode for 92Lys, they usually also contain at least one other substitution such as 71Thr or 126Ile (see Table 1; Takeuchi *et al.* 1996a,b; Kerje *et al.* 2003; M. Tixier-Boichard, pers. comm.). In addition, the phenotypic effect of Glu92Lys is abrogated by a second substitution in MC1R, His215Pro, as occurs in the *buttercup* allele (Kerje *et al.* 2003). In quail, the mild phenotypic effect of 92Lys may be due either to the absence of a positively epistatic mutation at *MC1R*, reflecting shared functional similarities of *MC1R* in galliforms, or to epistasis at another locus.

To determine whether the Glu92Lys mutation in quail and chicken reflected independent events or an ancestral polymorphism, we estimated phylogenies of their *MC1R* alleles using guineafowl *MC1R* as an outgroup. Phylogenetic reconstructions were performed using neighbour-joining (with HKY85 distances) and maximum likelihood (with an HKY85 model) methods in PAUP* v. 4.0 (Swofford 1998). Results from these methods were consistent in showing that the Glu92Lys substitution occurred independently in the quail and chicken lineages (Fig. 1).

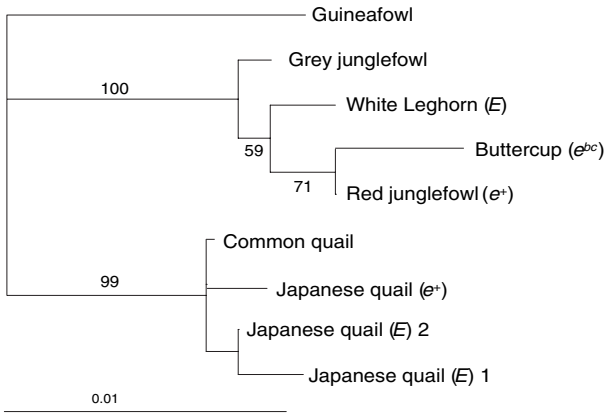

Figure 1 Maximum likelihood of phylogenetic reconstruction of galliform *MC1R* based on nucleotide sequences. Numbers above branches indicate percent bootstrap support in neighbour-joining analyses with 1000 replicates. The Japanese quail E1 (DQ395089) and E2 (DQ395090) sequences were obtained from French and Japanese flocks respectively. Other species are common quail (*Coturnix coturnix*; DQ395093), grey junglefowl (*Gallus sonneratii*; DQ395092) and vulturine guineafowl (*Acryllium vulturinum*; DQ395094), which was used as an outgroup.

Preliminary data from a survey of the molecular evolution of *MC1R* across galliforms show that this mutation has in fact occurred independently at least two other times in this group, including in curassows (*Crax*) and in peacock pheasants (*Polyplectron*) (N. Nadeau, unpublished results). The presence of four independent occurrences of the Glu92Lys mutation in galliforms, each associated with the same G272A transition, suggests high mutability at this nucleotide site in *MC1R*.

Acknowledgements

We thank David Gourichon and Chantal Moussu (INRA-UGFA, Nouzilly) for taking care of the experimental quail

and collecting the samples. Japanese stock with the *Extended brown* mutation were kindly provided by Professor S. Ito from Gifu University (Japan). We thank Michèle Tixier-Boichard for comments on the manuscript. This work was supported by the NERC.

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