Journal of Heredity
doi:10.1093/jhered/esu006

Melanocortin 1 Receptor (*MC1R*) Gene Sequence Variation and Melanism in the Gray (*Sciurus carolinensis*), Fox (*Sciurus niger*), and Red (*Sciurus vulgaris*) Squirrel

Helen R. McRobie, Linda M. King, Cristina Fanutti, Peter J. Coussons, Nancy D. Moncrief, and Alison P.M. Thomas

From the Department of Life Sciences, Anglia Ruskin University, East Road, Cambridge CB1 1PT, UK (McRobie, King, Coussons, and Thomas); the University of East Anglia, Norwich, UK (Fanutti); and the Virginia Museum of Natural History, Martinsville, VA (Moncrief).

Address correspondence to Helen R. McRobie at the address above, or e-mail: helen.mcrobie@anglia.ac.uk.

Data deposited at Dryad: http://dx.doi.org/doi:10.5061/dryad.3h1c4

Sequence variations in the melanocortin 1 receptor (*MC1R*) gene are associated with melanism in many different species of mammals, birds, and reptiles. The gray squirrel (*Sciurus carolinensis*), found in the British Isles, was introduced from North America in the late 19th century. Melanism in the British gray squirrel is associated with a 24-bp deletion in the *MC1R*. To investigate the origin of this mutation, we sequenced the *MC1R* of 95 individuals including 44 melanic gray squirrels from both the British Isles and North America. Melanic gray squirrels of both populations had the same 24-bp deletion associated with melanism. Given the significant deletion associated with melanism in the gray squirrel, we sequenced the *MC1R* of both wild-type and melanic fox squirrels (*Sciurus niger*) (9 individuals) and red squirrels (*Sciurus vulgaris*) (39 individuals). Unlike the gray squirrel, no association between sequence variation in the *MC1R* and melanism was found in these 2 species. We conclude that the melanic gray squirrel found in the British Isles originated from one or more introductions of melanic gray squirrels from North America. We also conclude that variations in the *MC1R* are not associated with melanism in the fox and red squirrels.

Subject area: *Molecular adaptation and selection*

Key words: *Melanism, Melanocortin 1 receptor,* Sciurus carolinensis, Sciurus niger, Sciurus vulgaris, *squirrel*

Vertebrate pigmentation is the result of the coordinated action of many genes and cell types and more than 100 loci are thought to be involved (reviewed by Lin and Fisher 2007). Melanocytes, cells that synthesis pigment, produce 2 distinct forms of melanin: eumelanin, which is a dark brown/black pigment, and pheomelanin, which is a pale red/yellow pigment. Hair color depends on the distribution and relative amounts of these 2 pigments, where darker hairs contain a greater proportion of eumelanin (Robbins et al. 1993).

The genetic basis of melanic (dark) phenotypes has been described in a number of mammals, birds, and reptiles including mice (Robbins et al. 1993), rock pocket mice (Nachman et al. 2003), pigs (Kijas et al. 1998), fox (Vage et al. 1997), cattle (Klungland et al. 1995; Theron et al. 2001), dogs (Everts et al. 2000), chicken (Takeuchi et al. 1996), bananaquit (Theron et al. 2001), and wall lizards (Buades et al. 2013) (reviewed in Majerus and Mundy 2003). In the majority of reported cases, melanism is associated with variation in 1 gene, the melanocortin 1 receptor (*MC1R*) gene. This highly polymorphic gene encodes a 7-transmembrane G protein–coupled receptor, which is predominantly expressed in melanocytes. The MC1R plays a central role in regulating melanin production in these specialized cells. The MC1R is activated by its agonist, alpha melanocyte-stimulating hormone (α-MSH) (Donatien et al. 1992). When the MC1R is bound by α-MSH, intracellular levels of cyclic adenosine monophosphate (cAMP) are elevated through a G-protein signaling pathway and eumelanin is produced. However, if the MC1R is bound by its antagonist, agouti signaling protein (ASIP), α-MSH binding is blocked, cAMP levels are reduced

and pheomelanin is produced (Abdel-Malek et al. 2001; Barsh et al. 2000, reviewed by Garcia-Borron et al. 2005 and Mountjoy et al. 1992).

The gray squirrel (*Sciurus carolinensis*) is a native of North America but has been repeatedly introduced to the British Isles where it has become a highly successful invasive species (Gurnell et al. 2004). These British gray squirrels have 3 distinct color morphs: wild-type gray, brown-black, and jet black. Brown-black and jet black morphs are both considered to be melanic (see Supplementary Materials online for images of all phenotypes). The first recorded sighting of a melanic gray squirrel in the British Isles was in Bedfordshire in 1912 (Middleton 1931). The range and population of these melanic squirrels have been increasing steadily over the last century and they can now be seen regularly in many areas across the south of England, particularly in the counties of Bedfordshire, Hertfordshire, and Cambridgeshire (McRobie 2012). We have previously reported that the genetic basis of melanism in these British gray squirrels is associated with a 24-bp deletion in the *MC1R*, where the gray phenotype is homozygous for a wild-type *MC1R* E^+ allele, the jet black is homozygous for the *MC1R* $\Delta 24$ E^B allele and the brown-black is heterozygous for the E^+ and E^B alleles (McRobie et al. 2009). Melanic gray squirrels are also common in North America where they live in mixed populations with gray squirrels. Following on from our previous work, here we investigate the origin of the melanic gray squirrel in Britain to establish if the 24-bp deletion is a new mutation that occurred since its introduction or whether melanic gray squirrels were introduced from North America.

Given the clear association between the 24-bp deletion and melanism in the British gray squirrel, we extended the study of variations in the *MC1R* to 2 other squirrel species with melanic variants, the fox squirrel (*Sciurus niger*) and the red squirrel (*Sciurus vulgaris*). All 3 squirrel species live in mixed populations where wild-type morphs interbreed freely with melanic morphs of the same species (Gurnell 1987). The fox squirrel is a native of North America and has a spectrum of color morphs ranging from grizzled russet-orange through various shades of gray to black (Moncrief et al. 2010). The red squirrel is native to Europe and also has a spectrum of color morphs ranging from russet-red, red-brown, brown, to black. Red squirrels can also be black with a gray dorsum and black with red flanks (Lapini L, personal communication). Given the previous finding of a mutation on the *MC1R* associated with melanism in the closely related gray squirrel and considering the widespread role of the *MC1R* in pigmentation, we chose this as the first candidate gene to investigate for the genetic basis of melanism in the fox and red squirrels. In this study, we sequenced the entire *MC1R* gene from both wild-type and melanic color morphs of the fox and red squirrels to test the hypothesis that *MC1R* variation is associated with melanism in these species.

Materials and Methods

Gray squirrel samples were obtained from Cumbria, West Yorkshire, Northamptonshire, and Cambridgeshire in Britain and from Massachusetts, Virginia, and British Columbia in North America. Of the 51 wild-type samples, 45 were from Britain and 6 from North America. Of the 44 melanic samples, 36 were from Britain and 8 were from North America. Forty-two of these melanic samples were brown-black and 2 were jet black. All melanic gray squirrel samples from Britain were obtained from Cambridgeshire, as melanic gray squirrels are absent from the other British locations tested. A total of 9 fox squirrel samples were obtained from Georgia, North America, 4 being wild type and 5 melanic. A total of 39 red squirrel samples were obtained, including 33 from Italy of which 10 were wild type and 23 melanic. All Italian samples were obtained from the Friuli-Venezia Giulia region in northeastern Italy: 30 from Udine, 1 from Pordenone, 1 from Gorizia, and 1 from Trento. A further 6 wild-type red squirrel samples were obtained from Inverness-shire and Cumbria in Britain.

Total genomic DNA was extracted from muscle tissue using Qiagen DNeasy extraction kits. The *MC1R* was amplified by polymerase chain reaction (PCR) in 2 stages: 90% of the gene, including the start codon at the beginning of the gene, was amplified using the primers MSHR4F (5'-TGC TTC CTG GAC AGG ACT ATG-3') and MC1R11R (5'-TCG TGT CGT YGT GRA GGA AC-3'). The rest of the gene, including the 3' end, was amplified using MC1Rdel (5'-AAC GCA CTG GAG ACG ACC ATC-3') and MC1Rer6 (5'-CTG GGC TTG AGA CCA GA-3'). A forward primer MC1RBF (5'-CTG GTG AGC ACC TTC CTA CTG-3') and reverse primer MC1RBR (5'-CCA GCA GTA GGA AGG TG-3') were used to sequence the *MC1R*$\Delta 24$ allele of the gray squirrel. All PCR reactions were carried out in duplicate on a DNA thermocycler (Techne touchgene gradient) in a total volume of 25 μL using approximately 25 ng template DNA, 1× TopTaq PCR buffer, 3 mM $MgCl_2$, 0.2 mM deoxyribonucleotide triphosphates, 0.4 μM primers, and 1× TopTaq polymerase. The following PCR conditions were used: initial denaturation 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 1 min followed by a final extension at 72 °C for 5 min. PCR products were purified and sent for sequencing at Source BioScience, Cambridge. Chromatograms were examined by eye to identify heterozygotes and sequences were aligned using ClustalW to obtain the full *MC1R* sequence of the gene for each sample. Sequences were compared with the *MC1R* sequences on GenBank to confirm that this was the *MC1R* gene. In fulfillment of data archiving guidelines (Baker 2013), we have deposited the primary data underlying these analyses with Dryad. Sequences have been deposited with GenBank.

Results

Our results showed that the melanic gray squirrels of North America and the British Isles had an identical 24-bp deletion in the *MC1R*. Figure 1 shows a schematic diagram of the MC1R protein with deleted amino acids indicated. Of the 44 melanic samples tested, the 42 brown-black samples were heterozygous and the 2 jet black samples were homozygous

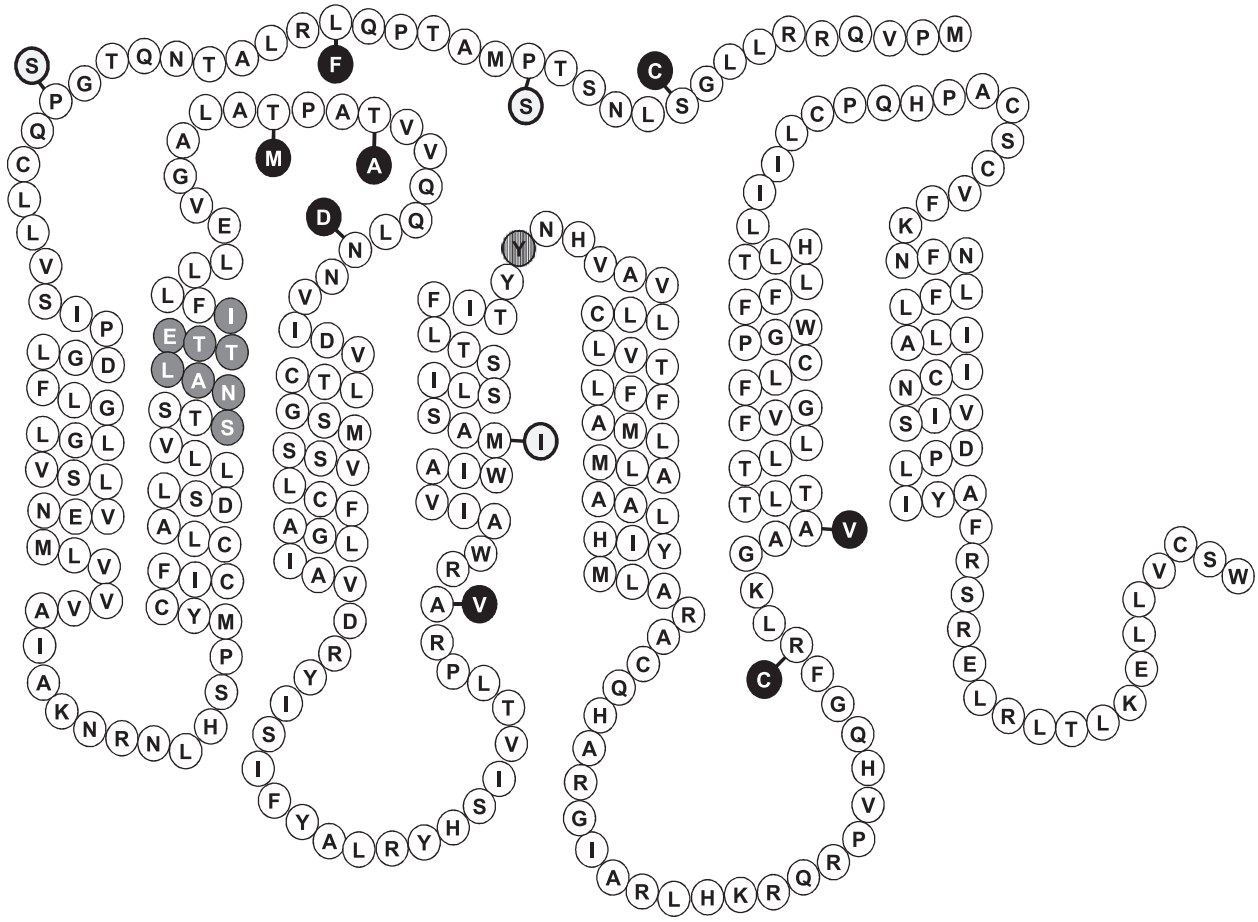

Figure 1. Schematic diagram of the MC1R showing amino acids of the wild-type gray squirrel (*Sciurus carolinensis*). Dark gray circles with white lettering indicate the 8 amino acids deleted in the *MC1R-⊥24 E*^{B} allele. Light gray circles and black circles indicate amino acids in the fox (*Sciurus niger*) and red (*Sciurus vulgaris*) squirrels, respectively, that differ from those of the gray squirrel. The circle with vertical stripes is the amino acid deleted in allele 2 of the red squirrel MC1R. Information for the predicted structure of the MC1R protein was obtained from Mundy (2005).

for the *MC1R-⊥24 E*^{B} (melanic) deletion. Information about phenotypes, genotypes, and allele frequencies for all 3 species is summarized in Table 1.

Our results show no association between variation in the *MC1R* and melanism in either fox or red squirrel. The *MC1R* in the fox squirrel has 945 bp, giving a 314 amino acid receptor. Two alleles were detected with 3 nonsynonymous substitutions. The substitutions were as follows with the wild-type gray squirrel MC1R as reference. Allele 1: P15S and M167I (accession number KF052119). Allele 2: P30S (accession number KF052120). Positions of these amino acids are shown on the schematic diagram of the MC1R in Figure 1. No observable differences in phenotype were identified between these alleles.

Two alleles of the *MC1R* of the red squirrel were also identified. The first allele (accession number KF188571) has 942 bp giving a 314 amino acid receptor with the following substitutions compared with the wild-type gray squirrel MC1R: S10C, L21F, T105M, T108A, N114D, A158V, R233C, and A238V. The second allele (accession number KF188572) has 939 bp giving a 313 amino acid receptor with the same substitutions compared with the gray squirrel but also a single amino acid deletion, Y180del. Positions of these amino acids are shown on the schematic diagram of the MC1R in Figure 1. All 33 Italian red squirrel samples (both melanic and wild type) were homozygous for allele 1. Five of the 6 British samples of the red squirrel (all wild type) were homozygous for the allele 2 and the other was heterozygous.

Discussion

Our results strongly support the conclusion that the presence of melanic gray squirrels in Britain is the result of one or a few introductions from North America and not the result of a new mutation. Squirrels from both Britain and North America showed the same phenotypic and corresponding genotypic variation where wild-type squirrels were gray and homozygous for the wild-type E^{+} allele, brown-black squirrels were heterozygous for the wild-type E^{+} and *MC1R-⊥24*

Table 1 Variations in amino acids of the MC1R between *Sciurus carolinensis*, *Sciurus niger*, and *Sciurus vulgaris* with *MC1R* E^+ (GenBank accession number EU604831.2) as the reference sequence

Species	Amino acids															Allele frequency	Location / Phenotype/ genotype	Friuli, Italy		Inv, UK	Cu, UK	Nor, UK	WY, Cambs, UK		BC, Canada		MA, USA	VA, USA	GA, USA	
	10	15	21	30	87–94	105	108	114	158	167	180	233	238	Allele				wt	mel	wt	wt	wt	wt	bb	jb	bb	jb	wt	bb	wt	mel
S. carolinensis	S	P	L	P	SNALETTI	T	T	N	A	M	Y	R	A	E+	144	E+/E+				4	7	6	28		3			3		
																E+/EB								35		5			2	
		—	—	—	■		—	—	—	—	—	—	—	EB	46	EB/EB									1		1			
S. niger		—	S	—	—		—	—	—	I	—	—	—	N1	15	N1/N1													3	3
																N1/N2													1	2
		—	—	—	S	—		—	—	—	—	—	—	N2	3	N2/N2														
S. vulgaris	C	—	F	—	—		M	A	D	V	—	—	C	V	V1	67	V1/V1		10	23										
																V1/V2				1										
	C	—	F	—	—		M	A	D	V	—	■	C	V	V2	11	V2/V2			1	4										

Dashes indicate identity with the reference sequence and squares represent deleted amino acids. N1 and N2 refer to alleles 1 and 2 of *S. niger* and V1 and V2 refer to alleles 1 and 2 of *S. vulgaris*, respectively. Allele frequencies refer to the number of each allele observed in the samples tested. The numbers under locations refer to numbers of squirrels of each phenotype tested. Inv = Inverness-shire, Cu = Cumbria, Nor = Northamptonshire, WY = West Yorkshire, Cambs = Cambridgeshire, BC = British Columbia, MA = Massachusetts, VA = Virginia, GA = Georgia, wt = wild type, mel = melanic, bb = brown black, and jb = jet black.

E^B allele, and the jet black squirrels were homozygous for the *MC1R*-⊥*24* E^B allele.

Our results show no association between variation in the *MC1R* and melanism in either fox or red squirrel. In cases where melanism is associated with variations in this gene, phenotypic differences are often discrete and relatively large as demonstrated in the gray squirrel (McRobie et al. 2009), bananaquit (Theron et al. 2001), chicken (Takeuchi et al. 1996), mice (Robbins et al. 1993), jaguars (Eizirik et al. 2003), and pigs (Kijas et al. 1998). In both the fox and red squirrels, however, there are no such clear distinctions between phenotypes and the color differences are more subtle, presenting a continuous spectrum of color variation. A similar spectrum of variation is also observed in the gopher (Wlasiuk and Nachman 2007), 3 mustelid lineages (Hosoda et al. 2005), Old World leaf warblers (MacDougall-Shackleton et al. 2003), and the blue-crowned manakin (Cheviron et al. 2006). In all of these cases, where there is a wide spectrum of color morphs, melanism is not associated with variations in the *MC1R*. These findings suggest that cases where melanism is graduated across a species, genes other than the *MC1R* may be responsible.

The *MC1R* gene is well characterized in a wide variety of species and the number of cases reporting the association of the *MC1R* to melanism indicates that this is a good candidate gene (Hoekstra 2006). However, it is likely that there is an ascertainment bias where positive results are more likely to be reported in this intronless gene, which is relatively easy to sequence and analyze (Mundy 2005). Other key genes involved in pigmentation are more complex, for example, *ASIP*, which has 3 coding exons (Abdel-Malek et al. 2001) and is considerably harder to work with and therefore less likely to be reported. A number of other studies investigating the *MC1R* have highlighted the complex nature of the genetics of pigmentation. For example, large deletions in the first extracellular region of the receptor are associated with melanism in the jaguar (15 bp), jaguarundis (24 bp) (Eizirik et al. 2003), and gray squirrel (24 bp) (McRobie et al. 2009). In contrast, deletions almost identical to these are not associated with melanism in the wolverine (15 bp), stone marten (28 bp), 4 species of martens (Hosoda et al. 2005) (45 bp), and the gopher (Wlasiuk and Nachman 2007) (21 bp). Further analysis of protein expression and function would be necessary to elucidate the effect of these deletions.

The study of melanism has provided many examples of convergent evolution. In some cases, the same genes are associated with melanism in distantly related taxa and conversely different genes produce similar phenotypes in closely related taxa. Thus, similar phenotypes can be the result of different underlying mechanisms (Gompel and Prud'homme 2009 and reviewed by Manceau et al. 2010). Rosenblum et al. (2010) demonstrated that independent mutations on the *MC1R* were associated with similar phenotypes in 3 species of white lizards (eastern fence lizard, little striped whiptail, and lesser earless lizard). A number of studies have identified mutations in *cis*-regulatory elements involved in pigmentation (Gompel et al. 2005 and reviewed by Hoekstra 2006). These studies highlight the importance of investigating protein function

as well as regulatory regions in elucidating the genetic and molecular basis of melanic phenotypes.

The genetics and molecular cell biology of pigmentation is clearly complex and it seems likely that melanism is polygenic in many cases, particularly where differences between phenotypes are relatively small as suggested by Wlasiuk and Nachman (2007). Given the wide spectrum of color morphs in the fox and red squirrels, and the complex phenotypes in the red squirrel where different body parts are differently colored, it seems possible that coloration is polygenic and may involve genes in the early stages of development, in the regulation of hormones or indeed the regulation of receptors. There are likely to be many more as-yet unidentified loci involved in pigmentation. The phenotypes observed in the fox and red squirrels may be associated with mutations in one or a few of these other candidate genes or in regulatory sequences of genes.

Overall our results confirm the polymorphic nature of the *MC1R* gene with even small sample sizes revealing substitutions and deletions. We conclude that the melanic gray squirrel found in the British Isles originated from one or more introductions of melanic gray squirrels from North America. We also conclude that variations in the *MC1R* are not associated with melanism in the fox and red squirrels. Future studies exploring other candidate genes or regulatory regions of genes, for example, *ASIP*, would no doubt provide insight into the genetics of melanism in the fox and red squirrels.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

Funding

Anglia Ruskin University.

Acknowledgments

We are very grateful to L. Lapini from the Friuli Museum of Natural History, Udine, Italy, for providing samples of red squirrel tissue from Italy; to Professor B. Hoagland from Westfield State College, Department of Biology, Massachusetts, for samples of gray squirrels; to Dr S. Pankhurst of Anglia Ruskin University for samples of red squirrels; to N. Wain for experimental work; and to M. D'Arcy for IT support. Permission was granted to import squirrel tissue to the United Kingdom from the United States of America by the Department for Environment, Food and Rural affairs: authorization number POAO/2009/297. Squirrel tissue was obtained from animals that died of natural causes in the wild and no ethical protocols were required for their collection.

References

Abdel-Malek ZA, Scott MC, Furumura M, Lamoreux ML, Ollmann M, Barsh GS, Hearing VJ. 2001. The melanocortin 1 receptor is the principal mediator of the effects of agouti signalling protein on mammalian melanocytes. J Cell Sci. 114:1019–1024.

Baker CS. 2013. Journal of Heredity adopts joint data archiving policy. J Hered. 104:1.

Barsh G, Gunn T, He L, Schlossman S, Duke-Cohan J. 2000. Biochemical and genetic studies of pigment-type switching. Pigment Cell Res. 13:48–53.

Buades JM, Rodríguez V, Terrasa B, Pérez-Mellado V, Brown RP. 2013. Variability of the *mc1r* gene in melanic and non-melanic *Podarcis lilfordi* and *Podarcis pityusensis* from the Balearic archipelago. PLoS ONE. 8:e53088.

Cheviron ZA, Hackett SJ, Brumfield RT. 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*). Proc R Soc B. 273:1613–1618.

Donatien PD, Hunt G, Pieron C, Lunec J, Taieb A, Thoday AJ. 1992. The expression of functional MSH receptors on cultured human melanocytes. Arch Dermatol Res. 284:424–426.

Eizirik E, Yuhki N, Johnson WE, Menotii-Raymond M, Hannah SS, O'Brien SJ. 2003. Molecular genetics and evolution of melanism in the cat family. Curr Biol. 13:448–453.

Everts RE, Rothuizen J, van Oost BA. 2000. Identification of a premature stop codon in the melanocyte-stimulating hormone receptor gene (MC1R) in Labrador and Golden retrievers with yellow coat colour. Anim Genet. 31:194–199.

Garcia-Borron JC, Sanchez-Laorden BL, Jimenez-Cervantes C. 2005. Melanocortin-1 receptor structure and functional regulation. Pigment Cell Res. 18:393–410.

Gompel N, Prud'homme B. 2009. The causes of repeated genetic evolution. Dev Biol. 332:36–47.

Gompel N, Prud'homme B, Wittkopp PJ, Kassner VA, Carroll SB. 2005. Chance caught on a wing: cis-regulatory evolution and the origin of pigment patterns in *Drosophila*. Nature. 433:481–487.

Gurnell J. 1987. The natural history of squirrels. London: Christopher Helm.

Gurnell J, Wauters LA, Lurz PWW, Tosi G. 2004. Alien species and interspecific competition: effects of introduced eastern grey squirrels on red squirrel population dynamics. J Anim Ecol. 73:26–35.

Hoekstra HE. 2006. Genetics, development and evolution of adaptive pigmentation in vertebrates. Heredity. 97:222–234.

Hosoda T, Sato JJ, Shamada KL, Campbell KL, Suzuki H. 2005. Independent nonframeshift deletions in the MC1R gene are not associated with melanistic coat colouration in three mustelid lineages. J Hered. 96:607–613.

Kijas JMH, Wlaes R, Tornsten A, Chardon P, Moller M, Andersson L. 1998. Melanocortin receptor 1 (MC1R) mutations and coat colour in pigs. Genetics. 150:1177–1185.

Klungland H, Vage DI, Gomez-Raya L, Adalsteinsson S, Lien S. 1995. The role of the melanocyte-stimulating hormone (MSH) receptor in bovine coat colour determination. Mamm Genome. 6:636–639.

Lin J, Fisher D. 2007. Melanocyte biology and skin pigmentation. Nature. 445:843–850.

MacDougall-Shackleton EA, Blanchard L, Gibbs HL. 2003. Unmelanized plumage patterns in old world leaf warblers do not correspond to sequence variation at the melanocortin-1 receptor locus (MC1R). Mol Biol Evol. 20:1675–1681.

Majerus MEN, Mundy NI. 2003. Mammalian melanism: natural selection in black and white. Trends Genet. 19:585–588.

Manceau M, Domingues VS, Linnen CR, Rosenblum EB, Hoekstra HE. 2010. Convergence in pigmentation at multiple levels: mutations, genes and function. Phil Trans R Soc B. 365:2439–2450.

McRobie H. 2012. Black squirrel: genetics and distribution. Q J Forest. 106:137–141.

McRobie H, Thomas A, Kelly J. 2009. The genetic basis of melanism in the gray squirrel (*Sciurus carolinesis*). J Hered. 100:709–714.

Middleton AD. 1931. The grey squirrel. London: Sidgwick and Jackson.

Moncrief ND, Lack JB, Van Den Bussche RA. 2010. Eastern fox squirrel (*Sciurus niger*) lacks phylogeographic structure: recent range expansion and phenotypic differentiation. J Mammal. 91:1112–1123.

Mountjoy KG, Robbins LS, Mortrud MT, Cone RD. 1992. The cloning of a family of genes that encode the melanocortin receptors. Science. 254:1248–1251.

Mundy NI. 2005. A window on the genetics of evolution: MC1R and plumage colouration in birds. Proc R Soc Lond B Biol Sci. 272:1633–1640.

Nachman MW, Hoekstra HE, D'Agostino SL. 2003. The genetic basis of adaptive melanism in pocket mice. Proc Natl Acad Sci USA. 100:5268–5273.

Robbins LS, Nadeau JH, Johnson KR, Kelly MA, Roselli-Rehfuss L, Baack E, Mountjoy KG, Cone RD. 1993. Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. Cell. 72:827–834.

Rosenblum EB, Rompler H, Schoneberg T, Hoekstra HE. 2010. Molecular and functional basis of phenotypic convergence in white lizards at White Sands. Proc Natl Acad Sci USA. 107:2113–2117.

Takeuchi S, Suzuki H, Hirose S, Yabuuchi M, Sato C, Yamamoto H, Takahashi S. 1996. Molecular cloning and sequence analysis of the chick melanocortin 1 receptor gene. Biochim Biophys Acta. 1306:122–126.

Theron E, Hawkins K, Bermingham E, Ricklefs RE, Mundy NI. 2001. The molecular basis of avian plumage polymorphism in the wild: a melanocortin-1-receptor point mutation is perfectly associated with the melanic plumage morph of the bananaquit (*Coereba flaveola*). Curr Biol. 11:550–557.

Vage DI, Lu D, Klungland H, Lien S, Adalsteinsson S, Cone RD. 1997. A non-epistatic interaction of agouti and extension in the fox, *Vulpes vulpes*. Nat Genet. 15:311–315.

Wlasiuk G, Nachman MW. 2007. The genetics of adaptive coat color in gophers: coding variation at Mc1r is not responsible for dorsal color differences. J Hered. 98:567–574.

Received October 16, 2013; First decision November 12, 2013; Accepted January 9, 2014

Corresponding Editor: William Modi