Research Focus

Mammalian melanism: natural selection in black and white

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Two recent papers on the molecular basis of melanism strengthen the chain of evidence linking genotype and phenotype in nature. Research on coat colour polymorphisms in rock pocket mice from differently coloured rock substrates provides a compelling example of the genetics of adaptation and the serendipitous nature of darwinian selection. Mutations in one gene, melanocortin-1-receptor, are perfectly associated with dark coat colour on black lava. Comparative sequence analysis shows that the same gene is involved in melanic polymorphism in some cats.

Melanism is a ubiquitous phenomenon in the animal kingdom and has long been used to investigate evolutionary change [1]. The melanic forms of peppered moth, *Biston betularia*, and other insects, that have arisen or spread since industrialization, provide some of the best examples of darwinian evolution in the wild. However, the genes responsible for these melanic forms have not been identified. Thus, the crucial step of connecting genotype with phenotype for an adaptive trait has not been accomplished in these classical cases of darwinian evolution in action. Indeed, this link has been made in remarkably few instances, and most of those involve traits under strong selection caused by human influence: pesticide resistance in insects and rats, antibiotic resistance in bacteria and heavy metal tolerance in plants. Now, Michael Nachman, Hopi Hoekstra and Susan D'Agostino, from the University of Arizona, have provided the genotype-phenotype link for an adaptive trait in a natural population of mice in which selection is not influenced by humans [2]: this is the story of the melanic rock pocket mouse.

Melanism in mice

Rock pocket mice, *Chaetodipus intermedius*, vary in coat colour, as do some other desert rodents. Strong correlations have been shown between coat colour and colour of rock substrate in this species (Figure 1) and the deermouse, *Peromyscus maniculatus* [3,4]. The most striking correlations occur where pale rocks adjoin black volcanic lava beds in the south-western USA [5]. The close match between the colour of mouse dorsal coat and substrate is adaptive, giving mice cryptic protection against avian predators, particularly owls [6,7].

Nachman *et al.* [2] sought the molecular genetic basis of melanism in populations of pocket mice from two isolated lava beds and the pale soils between them in Arizona and New Mexico. In a follow-up paper, Hoekstra and Nachman [8] investigated two further melanic populations. Their approach was to sequence candidate genes selected from the large number of known coat colour genes in laboratory mice [9]. They focused on the melanocortin-1-receptor gene (*MC1R*), which plays an essential role in the regulation of melanin synthesis during hair development (Figure 2). Extensive work in domesticated species has identified point mutations in *MC1R* (e.g. in mice [10], cattle [11], pigs [12]) that cause melanic hair colour changes, some of which closely resemble the melanic phenotypes of pocket mice [10].

In one of the four melanic populations from the dark lava beds, found in Pinacate in Arizona, there was an exact association between mutations in *MC1R* and melanic phenotype. Across all populations, 24 single nucleotide polymorphisms (SNPs) were detected in the *MC1R* coding region, producing nine amino acid polymorphisms. Of these, four were found only in dark mice located on or beside the Pinacate bed. These variants were either all present or all absent; of the 17 dark mice sampled, 11 were

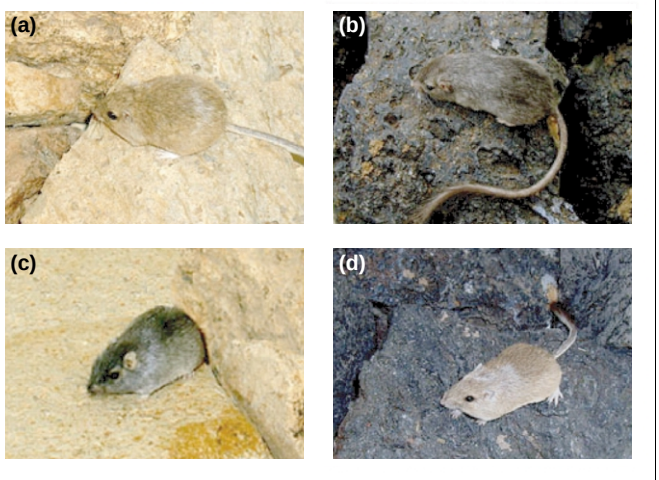

Figure 1. Coat colours of rock pocket mice are adaptive, increasing crypsis on differently coloured rocks. Pale (a) and dark mice (d) with melanic melanocortin-1-receptor (MC1R) alleles (b) and (d) are show the mice on the dark Pinacarte lava rocks, (a) and (c) show the mice on the pale rocks adjoining the lava flow. (Courtesy of Hopi Hoekstra.)

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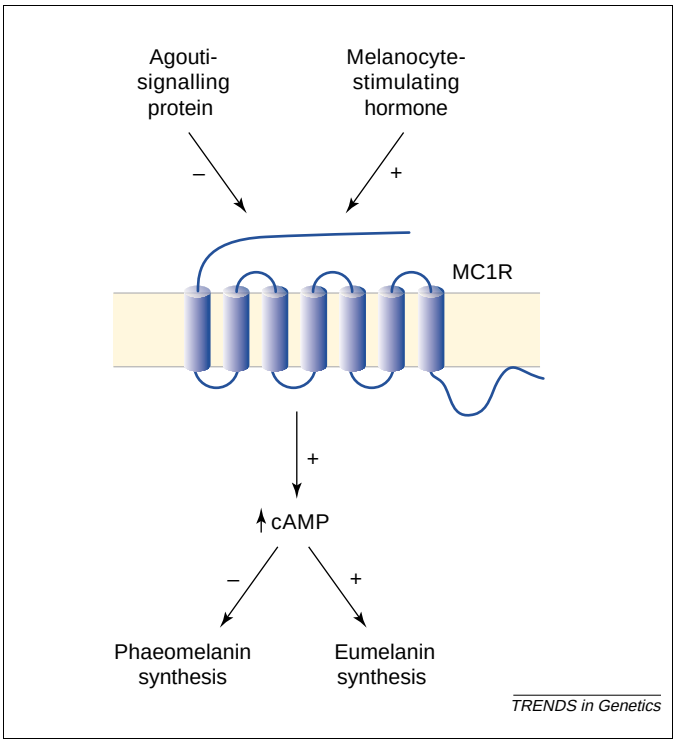

Figure 2. Position of the melanocortin-1-receptor (MC1R) in the melanogenic pathway. MC1R is depicted in the cell membrane of a melanocyte in a developing hair follicle. Agouti-signalling protein is produced by the *Agouti* locus, and melanocyte-stimulating hormone is one of the products of the proopiomelanocortin (*POMC*) locus. The + sign indicates stimulation and the − sign indicates inhibition.

homozygous for all four, and six were heterozygous. This pattern is consistent with the dominant inheritance of melanic *MC1R* alleles seen in other species. All dark mice, with the exception of one heterozygote, were caught on the lava bed, along with just two pale mice, giving a frequency for the melanic allele of 0.75 from the lava bed sample.

That all four mutations in *MC1R* were present together in all the melanic mice on this lava bed, and no mice here or elsewhere were found with one, two or three of the *MC1R* mutations raises several questions. First, could the association between *MC1R* variation and phenotype have occurred simply by population history; the Pinacate population having accumulated substitutions throughout its genome during a period of isolation? Nachman *et al.* [2] refuted this explanation by demonstrating that mtDNA variation is not associated with coat colour. Second, is one of the substitutions responsible for the melanic phenotype, the other substitutions being no more than part of the background haplotype? Or third are all four substitutions necessary to produce the dark melanic phenotype, with individuals bearing only some of the substitutions being 'slightly dark' intermediates? If the third is true, is the lack of 'intermediate' melanic genotypes a result of the spread of such intermediates on dark rocks, followed by their replacement by the more cryptic dark melanic genotype now present, as occurred during the progression of industrial melanism in the willow beauty moth, *Peribatodes rhomboidaria*. Here, a 'slightly dark' form, *perfumaria*, spread in industrial areas in the 19th and early 20th century, only to be completely replaced, in heavily polluted regions, by a very dark form, *rebeli* [13].

Whatever the details of the case, given that the adaptive causes of the differences in allele frequencies in pocket mice are reasonably assumed, this is an unambiguous example of the genetic basis of natural selection, with the stress on the natural.

Serendipitous natural selection

The dark mice from the other three lava beds sampled lacked these *MC1R* mutations, or any other association between *MC1R* mutations and coat colour [2,8]. It is unsurprising that the mutations responsible for melanic coat colour at one location are not present at the others. Natural selection is serendipitous. It acts not by design, but simply on any variant that arises through random mutation if it increases fitness. If darker coloured mice are at an advantage in a habitat, it matters little which gene causes the darkness. The 700 km distance between the Pinacate and other sampled lava beds, coupled with the dispersal range of the mice, mean that the Pinacate bed population is effectively isolated from others on a dark substrate. It is presumed that selection has favoured melanic variants controlled by mutations at other loci on other lava beds, and that strong selective predation against dark mice on the sandy soils between Pinacate and other beds prevent gene flow between these populations. This is particularly the case for a dominant melanic mutation, as discussed here, which is always expressed and so would be exposed to selection on the pale soils between the lava beds.

From melanic mice to black cats

The variable genetic basis of similar melanic phenotypes is endorsed by work on the molecular genetics of melanism in cats. Melanic polymorphism is common in felines, occurring at high frequency in 11 out of 37 species. Eduardo Eizirik *et al*. show that different deletions in the *MC1R* are associated with melanism in jaguars, *Panthera onca*, and jaguarundis, *Herpailurus yaguarondi* [14]. Conversely, the recessive black coat colour of domestic cats is associated with a 2 bp deletion in exon two of the *agouti* gene, another locus known to affect coat colour in mammals. These mutations are lacking from five other cat species with melanic polymorphism, emphasizing the independent origins of melanism in different species [14]. Unlike the completely dominant pattern seen in rock pocket mice and jaguars, the association with *MC1R* in the jaguarundis shows incomplete dominance, with homozygotes being darker than heterozygotes. Remarkably, the same eight amino acid deletion in the *MC1R* of jaguarundis is fixed in a primate, the golden-headed lion tamarin, *Leontopithecus chrysomelas*, and therefore might contribute to the relatively melanic phenotype of that species [15]. The derived nature of the *MC1R* deletion mutant shows that coat colour evolution was from red to black, arguing against the previously held view that melanism in jaguarundis was an ancestor of the less common red forms.

In contrast to pocket mice, the adaptive benefits of melanism in cats are obscure. Eizirik *et al.* [14] suggest that high melanic frequency in jaguarundis is probably

due to selection, but drift cannot be ruled out. As in pocket mice, population genetic analysis might be fruitful in obtaining evidence of selection acting on *MC1R* in cats.

Natural engineering of *MC1R*

Together, the results of Nachman *et al.* [2,8] and Eizirik *et al.* [14] double the number of vertebrate species having *MC1R* mutations associated with coat colour changes occurring in wild populations. Other examples are the association of an *E92K* mutation in *MC1R* with melanism in a passerine bird, the bananaquit, *Coereba flaveola* [16], and two cases of *MC1R* loss of function mutations: pale coat in black bears, *Ursus americanus* (*Y298C*) [17], and red hair and pale skin in humans, in which there is a more complex association with several point substitutions in *MC1R* (*R151C, R160W, D294H* and possibly others) [18] (Figure 3).

Comparison of these *MC1R* colour mutations to those from studies of domestic animals reveals some interesting patterns (Figure 3). The most striking is that three out of four melanic mutations (in jaguars, jaguarundis and bananaquits) are clustered at the boundary of the second transmembrane domain and second extracellular loop. This is a region where several melanism-causing point mutations in *MC1R* in domestic animals are due to MC1R constitutive activation. One of the mutations in pocket mice (*R109W*) is near to this region. Loss-of-function mutations are more widely scattered, as might be predicted. Intriguingly, the same *R160W* mutation is associated with melanism in rock pocket mice and with red hair in humans, where the mutation causes reduction of MC1R signalling [19]. It is worth stressing that, except in humans, there is no direct evidence that these naturally occurring *MC1R* mutations are causative. An important avenue for future research will be to obtain direct information about the function of *MC1R* variants from *in vitro* expression studies. Nachman *et al.* [2] report preliminary findings that the MC1R receptor in melanic pocket mice is hyperactive. It is surprising that more examples of *MC1R* association with pale skin and/or red hair in wild mammals have yet to emerge. One possibility is that loss of function *MC1R* mutations have deleterious pleiotropic effects. It is therefore interesting that the *MC1R* has recently been shown to affect analgesia in mice and humans [20].

From mice to moths

In rock pocket mice and felines, variation in coat colour appears to be at equilibrium, i.e. morph frequencies are not currently changing under selection. Similar work on a system with phenotype frequencies known to be changing under selection would be timely, allowing declines in adaptive mutations to be tracked. The obvious candidate species is the peppered moth, with allelic frequencies of industrial melanic forms declining on both sides of the Atlantic [13,21]. A similar association analysis, using candidate genes in which melanic mutations are known from *Drosophila* or the commercial silk moth, *Bombyx mori*, should prove fruitful. This could provide the link between genotype and phenotype for the best-known

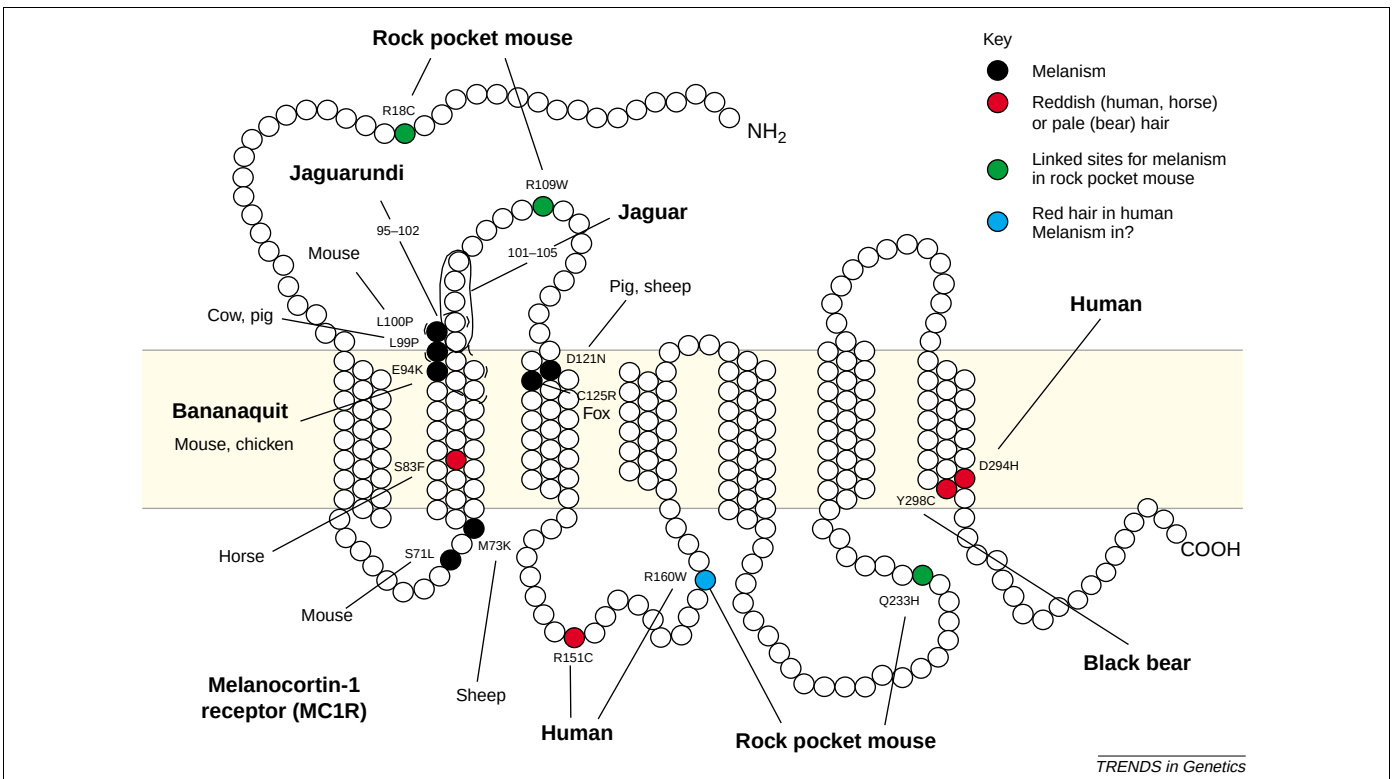

Figure 3. Melanocortin-1 receptor (*MC1R*) variants associated with coat colour changes in mammals and birds. The *MC1R* gene is depicted in the cell membrane of a melanocyte, with the extracellular surface facing up. The six species for which naturally occurring *MC1R* associations have been described are shown in bold. Residue numbering follows human *MC1R*. The broken black loop shows the eight amino acid deletion in jaguarundis and the solid black loop shows the five amino acid deletion in jaguar. Three further amino acid changes are possibly associated with melanism in jaguarundis: *P22L, 163V, Q310R* [14]. See Ref. [14] for references to other studies.

example of darwinian evolution in which the selective factors causing allele frequency changes have been identified and observed. The question of whether industrial melanic forms in different parts of the world – the *carbonaria* form in Britain and the *swettaria* form in the USA – are caused by the same mutation or just look the same could also be answered. Moreover, this would put to bed suggestions that industrial melanism in moths result from the mutagenic action of pollutants, which has recently been receiving renewed attention [22]. But time is running out for work on the melanic moths; the *carbonaria* and *swettaria* forms of the peppered moth are predicted to disappear within the next couple of decades [23].

References

1 Tutt, J.W. (1891) *Melanism and Melanochroism in British Lepidoptera*, Swan Sonnenschein
2 Nachman, M.W. *et al.* (2003) The genetic basis of adaptive melanism in pocket mice. *Proc. Natl. Acad. Sci. U. S. A.* 100, 5268–5273
3 Dice, L.R. (1930) Mammal distribution in the Alanogordo region, New Mexico. Occasional papers of the Museum of Zoology. *Univ. Michigan* 213, 1–32
4 Blair, W.F. (1943) Ecological distribution of mammals of the Tularosa Basin, New Mexico. *Contr. Lab. Vert. Biol. Univ. Michigan* 20, 20–24
5 Benson, S.B. (1933) Concealing coloration among some desert rodents of the Southwestern United States. *Univ. Calif. Publ. Zool.* 40, 1–70
6 Sumner, F.B. (1934) Does 'protective coloration' protect? Results of some experiments with fishes and birds. *Proc. Natl. Acad. Sci. U. S. A.* 20, 559–564
7 Dice, L.R. (1947) Effectiveness of selection by owls on Deermice (*Permycus maniculatus*) which contrast in colour with their background. *Contr. Lab. Vert. Biol. Univ. Michigan* 34, 1–20
8 Hoekstra, H.E. and Nachman, M.W. (2003) Different genes underlie adaptive melanism in different populations of rock pocket mice. *Mol. Ecol.* 12, 1185–1194
9 Jackson, I.J. (1997) Homologous pigmentation mutations in human, mouse, and other model organisms. *Hum. Mol. Genet.* 6, 1613–1624
10 Robbins, L.S. *et al.* (1993) Pigmentation phenotypes of variant extension locus alleles results from point mutations that alter MSH receptor function. *Cell* 72, 827–834
11 Klungland, H. *et al.* (1995) The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. *Mamm. Genome* 6, 636–639
12 Kijas, J.M.H. *et al.* (1998) Melanocortin receptor 1 (*MC1R*) mutations and coat color in pigs. *Genetics* 150, 1177–1185
13 Majerus, M.E.N. (1998) *Melanism: Evolution in Action*, Oxford University Press
14 Eizirik, E. *et al.* (2003) Molecular genetics and evolution of melanism in the cat family. *Curr. Biol.* 13, 448–453
15 Mundy, N.I. and Kelly, J. (2003) Evolution of a pigmentation gene, the melanocortin-1 receptor, in primates. *Am. J. Phys. Anthropol.* 121, 67–80
16 Theron, E. *et al.* (2001) The molecular basis of an avian plumage polymorphism in the wild: a point mutation in the melanocortin-1 receptor is perfectly associated with melanism in the bananaquit (*Coereba flaveola*). *Curr. Biol.* 11, 550–557
17 Ritland, K. *et al.* (2001) Inheritance and population structure of the white-phased 'Kermode' black bear. *Curr. Biol.* 11, 1468–1472
18 Valverde, P. *et al.* (1995) Variants of the melanoctye-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat. Genet.* 11, 328–330
19 Schiöth, H.B. *et al.* (1999) Loss of function mutations of the human melanocortin 1 receptor are common and are associated with red hair. *Biochem. Biophys. Res. Commun.* 260, 488–491
20 Mogil, J.S. *et al.* (2003) The melanocortin-1 receptor gene mediates female-specific mechanism of analgesia in mice and humans. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4867–4872
21 Grant, B.S. *et al.* (1996) Parallel rise and fall of melanic peppered moths in America and Britain. *J. Hered.* 87, 351–357
22 Hooper, J. (2002) *Of Moths and Men*, Fourth Estate
23 Mani, G.S. and Majerus, M.E.N. (1993) Peppered moth revisited: analysis of recent decreases in melanic frequency and predictions for the future. *Biol. J. Linn. Soc.* 48, 157–165

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Generating and modifying DiGeorge syndrome-like phenotypes in model organisms: is there a common genetic pathway?

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Most DiGeorge syndrome (DGS) patients have a similar chromosomal 22q11.2 deletion (*del22q11*) but show great clinical variability, suggesting the presence of genetic modifiers. We review recent mouse studies describing DGS-like phenotypes associated with mutations in genes not included in *del22q11*. It is reasonable to predict that mutations at these loci in humans might cause DGS in patients without *del22q11*, or could modify the *del22q11* phenotype. We discuss how these loci might interact with the leading DGS candidate gene, the transcription factor *Tbx1*.

DiGeorge or Velocardiofacial syndrome (DGS; OMIM#188400) is a clinically heterogenous disease characterized by cardiovascular defects, thymic, parathyroid and craniofacial anomalies. Approximately 80% of DGS cases are caused by a chromosomal deletion generally referred to as *del22q11* [1,2]. Among the patients with chromosomal deletions, 90% have virtually an identical three megabase (Mb) heterozygous deletion of 22q11.2

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