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The genetic basis of adaptation: lessons from concealing coloration in pocket mice

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Abstract

Recent studies on the genetics of adaptive coat-color variation in pocket mice (*Chaetodipus intermedius*) are reviewed in the context of several on-going debates about the genetics of adaptation. Association mapping with candidate genes was used to identify mutations responsible for melanism in four different populations of *C. intermedius*. Here, I review four main results (i) a single gene, the melanocortin-1-receptor (*Mc1r*), appears to be responsible for most of the phenotypic variation in color in one population, the Pinacate site; (ii) four or fewer nucleotide changes at *Mc1r* appear to be responsible for the difference in receptor function; (iii) studies of migration-selection balance suggest that the selection coefficient associated with the dark *Mc1r* allele at the Pinacate site is large; and (iv) different (unknown) genes underlie the evolution of melanism on three other lava flows. These findings are discussed in light of the evolution of convergent phenotypes, the average size of phenotypic effects underlying adaptation, the evolution of dominance, and the distinction between adaptations caused by changes in gene dosage versus gene structure.

Introduction

More than a century after the publication of 'The Origin of Species' many questions about the genetics of adaptation remain unanswered. Darwin (1859) provided a mechanism for evolution, but he was unaware of Mendel, and thus early evolutionary theory was developed without an accurate understanding of the nature of inheritance. The integration of Mendelian inheritance with evolutionary theory was provided by the work of Haldane, Fisher, and Wright, who, among many other things, developed the first models of the dynamics of allele frequency change under various forms of selection (Fisher, 1930; Wright, 1931; Haldane, 1932). In these models, fitness is typically summarized by a single parameter, the selection coefficient, which is usually associated with a particular allele at a single locus. Early empirical studies of adaptation proceeded somewhat independently of the theoretical studies of Fisher, Wright and Haldane. Empiricists such as Dobzhansky (1937, 1970), Dice (1940), Mayr (1942, 1963), Lack (1947), Stebbins (1950) and others began to describe geographic and temporal patterns of phenotypic variation, and many of these patterns provided convincing, though indirect, evidence for selection.

Natural selection acts on the phenotype, but it is the genotype that is passed from one generation to the next. Nonetheless, even today, relatively few studies have been able to make links between genotype and phenotype for traits under selection. To a considerable extent, theoretical studies (often dealing mostly with genotypes) and empirical studies (often dealing mainly with phenotypes) have remained divorced from each other. In principle, finding the genes underlying adaptation might allow us to bring these two approaches together; that is, to study the ecology of adaptation

in the context of explicit population genetic models.

Some of the best examples of the genetic basis of phenotypic responses to selection involve anthropogenic influences, either intentionally through artificial selection, or accidentally through human-induced changes to the environment. It is well known that the first chapter of The Origin of Species (Darwin, 1859) describes extensive changes in phenotype caused by selective breeding. There is now an enormous literature on both plant and animal breeding, and in some cases, the specific genes underlying response to artificial selection have been identified (e.g., Doebley, Stec & Hubbard, 1997; Wang et al., 1999; Newton et al., 2000). Examples of responses to human disturbance include insecticide, herbicide, and drug resistance (Palumbi, 2001; Reznick & Ghalambor, 2001), and in many cases, the genes underlying theses traits have also been identified (e.g., Fidock et al., 2000; Raymond et al., 2001; Walsh, 2000; Cowen, Anderson & Kohn, 2002; Daborn et al., 2002; Wootton et al., 2002; Hughes, 2003). One potential limitation of both kinds of studies for developing a more general understanding of the genetic basis of adaptation is that selection caused by anthropogenic influence is likely to be unusually strong (Darwin, 1859; Reznick & Ghalambor, 2001). Ideally we would like to be able to make links between genotype and phenotype for fitness-related traits in a more natural setting.

Many general questions about the genetics of adaptation remain, and in principle, might be answered by identifying the genes underlying adaptive phenotypes. For example, do adaptations result from the fixation of many mutations individually of small effect (Fisher, 1932), or do they involve single mutations of large effect, as documented for insecticide resistance (e.g. Daborn et al., 2002)? Are most adaptive mutants dominant as suggested by Haldane (1924), and do they correspond to gain-of-function mutations at the molecular level (Wright, 1934)? What kinds of molecular changes result in adaptation; are most adaptations the result of changes in protein structure or changes in gene regulation (Britten & Davidson, 1969)? How common are pleiotropy and epistasis? Do epistatic interactions typically involve other mutations in the same gene or mutations in different genes (Kondrashov, Sunyaev & Kondrashov, 2002)? With the ultimate goal of addressing these and related questions, we have taken a candidate-gene approach to understand the genetic basis of adaptive melanism in the rock pocket mouse, *Chaetodipus intermedius*. While some of these questions can be addressed without identifying the specific mutations underlying a trait, others cannot. Using a candidate-gene approach also has some serious limitations, as discussed below. First, I describe the relevant natural history of pocket mice, including variation in pigmentation. Second, I describe the genetics and biochemistry of mammalian pigmentation and the power and limitations of a candidate-gene approach in this system. Finally, I describe some of our chief findings and their implications for addressing the questions above.

Pigmentation variation in rock pocket mice

The rock pocket mouse, *Chaetodipus intermedius*, is a small rodent that inhabits rocky areas and desert scrub at low elevations principally in the Sonoran and Chihuahuan deserts. Its range includes southern Arizona, southern New Mexico, western Texas, and adjacent areas in northern Mexico. Pocket mice are in the family Heteromyidae, a New World family of rodents that includes six genera (*Chaetodipus*, *Perognathus*, *Dipodomys*, *Microdipodops*, *Liomys*, and *Heteromys*) and has its center of diversification in xeric habitats of Central and North America. Heteromyid rodents are distantly related to murid rodents, such as laboratory mice (*Mus domesticus*). Like many species of heteromyids, rock pocket mice are well adapted for deserts: they are strictly nocturnal and remain in underground burrows during the heat of the day. Pocket mice are so named because of external cheek pouches which are used to carry seeds during bouts of foraging. Pocket mice can subsist entirely on a dry diet and do not require free water. *C. intermedius* is restricted to rocky habitats, and is broadly sympatric with *C. penicillatus*, its sister species, which is found in more sandy habitats.

In most parts of its range, *C. intermedius* has a light, sandy-colored dorsal pelage and lives on light-colored rocks. In several different regions throughout its range, however, *C. intermedius* is found on lava flows which are typically dark in color. The mice on these lava flows typically have a melanic dorsal pelage. Examples of typical habitat

are shown in Figure 1, and variation in coat color is shown in Figure 2. The lava flows on which the mice are found tend to be geographically isolated from one another and vary in size from a few km^2 to over 1500 km^2, and they vary in age from less than 1000 years old to nearly 2 million years old (Hoekstra & Nachman, 2003). Lava flows are typically separated from one another by intervening habitat consisting either of light-colored rocks, which is suitable habitat for *C. intermedius*, or sand, which is unsuitable habitat for *C. intermedius*. This system was first described in detail in the 1930's by Benson (1933) and Dice and Blossom (1937) who documented a strong positive association between the color of the mice and the color of the substrate on which the mice live. Dice and Blossom noted that owls are major predators of these mice, and suggested that the variation in mouse coat color served as concealing coloration from predators.

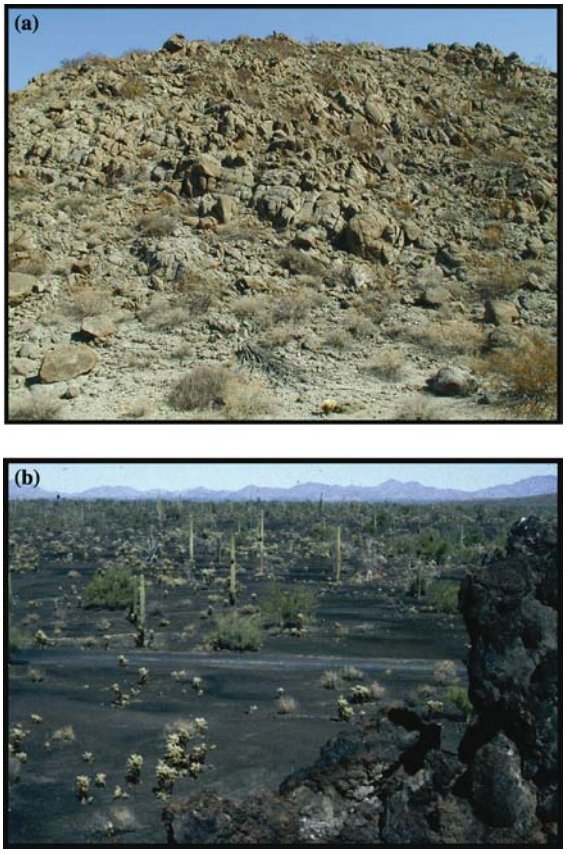

Figure 1. Typical habitats for *C. intermedius* showing light rocks (a) and dark lava (b).

While the phenotypic variation in color would seem to be a good example of crypsis to avoid predation, an obvious question, given that pocket mice are nocturnal, is whether owls discriminate between light and dark mice (on either light or dark backgrounds) while foraging at night. Dice (1947) conducted such experiments with two species of owls (Barn owl and Long-eared owl) in enclosures using varying degrees of illumination. Dice showed that owls capture approximately twice as many conspicuously colored mice as concealingly colored mice, even in near total darkness. Interestingly, this difference was seen only in enclosures containing a complex substrate with places for the mice to hide. When the experiment was done in an enclosure with a bare substrate, owls did not discriminate between conspicuously colored and concealingly colored mice. Moreover, on bare substrate, owls captured equal numbers of mice in low-light and in total darkness, suggesting that in this simplified situation owls hunt effectively using only hearing (Dice, 1947). These experiments were conducted using dark-colored and light-colored deer mice (*Peromyscus maniculatus*), rather than pocket mice, and comparable experiments have not been conducted with rock pocket mice. Nonetheless, the difference between light and dark *C. intermedius* is greater than the difference between light and dark *P. maniculatus*, so it seems likely that similar or more extreme results would be obtained with pocket mice. The close match between mouse color and substrate color across a wide range of populations (Dice & Blossom, 1937), the fact that owls are known to be major predators of pocket mice, and the fact that owls can effectively discriminate between light and dark mice even in low light conditions all suggest that the variation in coat color of *C. intermedius* is an adaptation to avoid predation. It is unlikely that variation in coat-color plays a significant role in thermoregulation since these mice are nocturnal and typically do not emerge from their burrows until ambient temperatures are below body temperature.

Candidate genes: the pigmentation process in mammals

This system is amenable to genetic analysis because of the wealth of information on the

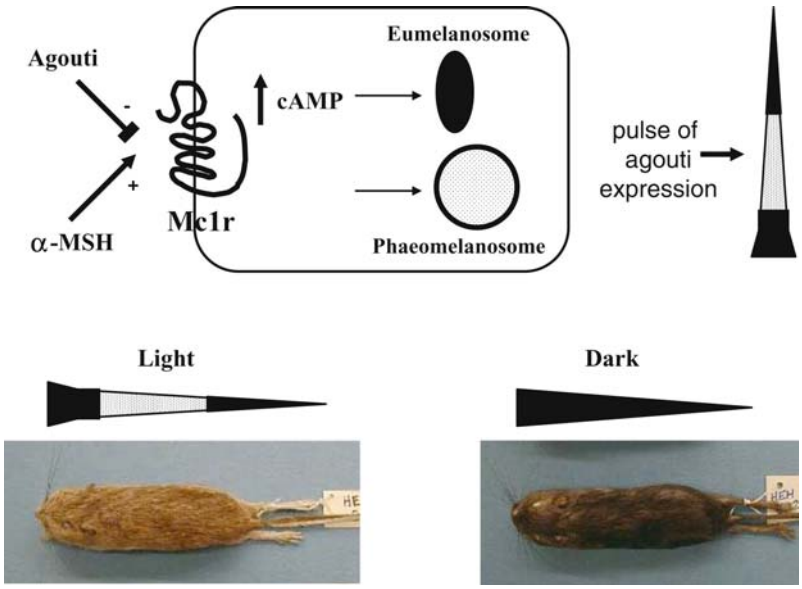

Figure 2. Regulatory control of melanogenesis (top) and typical light and dark *C. intermedius* (bottom). Alpha-MSH signals MC1R, resulting in higher levels of cAMP and production of eumelanin. Agouti is an antagonist that increases production of phaeomelanin. Agouti expression during the haircycle results in a banding pattern on individual hairs, a phenotype known as the 'agouti' hair (shown at right). Light *C. intermedius*, typically found on light-colored rocks, have agouti hairs on their dorsum, while dark *C. intermedius*, typically found on lava, have unbanded, uniformly melanic hairs on their dorsum. See text for further details.

genetics, development, and biochemistry of pigmentation, largely from studies on laboratory mice (reviewed in Silvers, 1979; Jackson, 1994, 1997; Barsh, 1996).

The deposition of pigment in hair and skin is the end-point of a process that involves the coordinated action of many genes and cell types. Melanocytes, the pigment-producing cells, originate in the neural crest and migrate during development throughout the dermis. The melanoblast cell lineage that gives rise to melanocytes is committed early in development and subsequent expression of many gene products is regulated in a cell-specific manner (Steel et al., 1992; Erickson, 1993; Bronner-Fraser, 1995). Within melanocytes are specialized organelles known as melanosomes (reviewed in Prota, 1992); they are the site of melanogenesis. There are two primary types of melanosomes and they differ both structurally and biochemically: eumelanosomes are ellipsoidal and are the site of synthesis of black or brown eumelanin whereas phaeomelanosomes are spherical and are the site of synthesis of yellow or red phaeomelanin (Figure 2). Once full of melanin, melanosomes are secreted from the melanocyte as pigment granules. Several lines of evidence suggest a close relationship between melanosomes and lysosomes and it is possible that melanosomes are modified lysosomes (Jackson, 1994, 1997). For example, many mouse mutations which affect melanosome function also disrupt lysosome function (e.g. Barbosa et al., 1996; Feng et al., 1997), raising the possibility that evolution of some pigmentation genes will be constrained by pleiotropic effects. Finally, synthesis of melanin within melanosomes involves the interactions of many loci, and some aspects of melanogenesis are under hormonal regulation.

Mouse pigmentation mutations have been identified in all steps of this process (Prota, 1992; Jackson,1994). For example, there are mutant phenotypes such as *piebald*, *steel*, and *white spotting* that result from improper development or migration of melanocytes, leaving portions of the body without pigment-producing cells. Other mutations, such as *beige* and *pale ear*, interfere with the proper structure and function of melanosomes. Some mutations, such as *albino*, *brown*, or *slaty*, interfere directly with proteins involved in synthesis of melanin. Finally, mutations at the *agouti*, *extension*, and *mahogany* loci disrupt the control and regulation of melanogenesis.

Approximately 80 genes have been identified that affect coat-color in the mouse (Jackson, 1997), and a large and growing number of these have now been characterized at the molecular level.

When employing a candidate-gene approach to finding the genes underlying a particular trait, it is typical to look for laboratory mutants that mimic naturally occurring variation (Palopoli & Patel, 1996; Haag & True, 2001). In this regard, there are several mouse coat-color mutants that suggest themselves as particularly relevant for understanding coat-color variation in *Chaetodipus*. In mammals, there are two basic kinds of melanin: eumelanin, which produces a dark brown or black color, and phaeomelanin, which produces a cream, yellow, or red color. The switch between production of eumelanin and phaeomelanin is controlled largely by the interaction of two key proteins, the melanocortin-1 receptor (MC1R) and the agouti signaling protein (Figure 2). MC1R is a transmembrane G-protein-coupled receptor that is highly expressed in melanocytes. Alpha-melanocyte-stimulating-hormone (α-MSH) activates MC1R, resulting in elevated levels of cAMP and increased production of eumelanin. The agouti protein is an antagonist of MC1R; local expression of agouti results in suppression of synthesis of eumelanin and increased production of phaeomelanin. Many dominant agouti mutations result in increased agouti expression and largely yellow phenotypes. In contrast, recessive, loss-of-function agouti mutations result in nonagouti, all black phenotypes. Dominance relationships among *Mc1r* alleles are opposite in order to those at agouti: recessive, loss-of-function Mc1r mutations typically result in yellow phenotypes (although slightly different phenotypically from the dominant yellow of agouti).

Wild mice have light bellies as a result of constitutive ventral agouti expression and associated production of phaeomelanin. In contrast, hairs on the dorsum of wild mice have a banded pattern, with a black tip, a middle yellow band, and a black base (the agouti hair). This banding is due to a pulse of agouti expression during the mid-phase of the hair cycle, resulting in deposition of phaeomelanin during the middle of hair growth and deposition of eumelanin at the beginning and end of hair growth (Figure 2). Mutations at both *agouti* (Vrieling et al., 1994; Bultman et al., 1994) and at *Mc1r* (Robbins et al., 1993) have been identified that produce black, unbanded dorsal hairs in the laboratory mouse but light hairs on the belly. Importantly, we observed a very similar phenotype in *C. intermedius* from lava flows; we found unbanded, uniformly melanic hairs in all dark *C. intermedius,* and banded dorsal hairs in all light *C. intermedius* (Figure 2), suggesting a possible role for either *agouti* or *Mc1r*.

A candidate-gene approach has both advantages and limitations. One clear advantage is that it may be possible to find the genes underlying a trait rather easily. Moreover, studies on laboratory mutants can provide important clues to the development, biochemistry, or cell biology that will help explain the mechanism by which a given genetic change produces a particular phenotype in nature. An obvious but important limitation of this approach is that, by itself, it will only lead to genes for which candidates are available. In the absence of a comprehensive mapping study, it is difficult to know how many undiscovered loci may contribute to the phenotypic variation of interest. Another limitation of a candidate-gene approach is that most laboratory mutants are changes of relatively large effect. If most of adaptive evolution typically occurs through many changes of small effect, we might expect that in most circumstances developmental mutants from the laboratory will not be useful mimics of naturally occurring variation (Haag & True, 2001). This is a question open to validation empirically by studies such as those described here. Perhaps the most powerful approach to study the genetic architecture of phenotypic variation in nature is to use a combination of mapping and candidate genes.

The genetic basis of adaptive melanism in pocket mice

We have sequenced portions of several genes known to produce coat-color mutants in the laboratory mouse and conducted association studies between polymorphisms in these genes and phenotypic variation in natural populations of *C. intermedius* (Nachman, Hoekstra & D'Agostino, 2003; Hoekstra & Nachman, 2003; Hoekstra, Drumm & Nachman, 2004). The general strategy has been to compare melanic mice collected on lava flows with light-colored mice collected on adjacent light-colored rocks (usually within a few

kilometers of the lava). We have explored genetic and phenotypic variation in this way at four paired sites, representing four different lava flows in Arizona and New Mexico (Figure 3). Several key results have emerged: (i) a single gene, *Mc1r*, appears to be responsible for most of the phenotypic variation in color in one population, the Pinacate site; (ii) four or fewer nucleotide changes at *Mc1r* appear to be responsible for the difference in receptor function; (iii) studies of migration-selection balance suggest that the selection coefficient associated with the dark *Mc1r* allele at the Pinacate site is large; and (iv) different (unknown) genes underlie the evolution of melanism on three other lava flows. These are briefly described below.

Several lines of evidence implicate *Mc1r* in coat-color variation at the Pinacate site (Nachman, Hoekstra & D'Agostino, 2003). First, there is a perfect association between *Mc1r* genotype and coat-color phenotype among all mice in this population. The *Mc1r* D allele is distinguished from the *Mc1r* d allele by four amino acid substitutions and one synonymous substitution, and mice with DD or Dd genotypes have melanic, unbanded dorsal hairs while mice with dd genotypes are light-colored, with agouti hairs on their dorsum. Second, the darkening *Mc1r* D allele is dominant over the *Mc1r* d allele, consistent with dominance relationships seen among *Mc1r* alleles in the laboratory mouse. Third, all four amino acid substitutions that distinguish the D and d alleles are charge-changing substitutions and are found in regions of the receptor that may be important for ligand binding or for interactions with other proteins. Fourth, the four amino acid sites at which substitutions distinguish *Mc1r* D and *Mc1r* d alleles are otherwise invariant across all other species of pocket mice (unpublished results), suggesting that these sites are functionally important. Fifth, the pattern of nucleotide variation seen at *Mc1r* is consistent with the recent action of natural selection; *Mc1r* D chromosomes have approximately one tenth as much variation as *Mc1r* d chromosomes. Sixth, genotype–phenotype associations decay immediately upstream and downstream of *Mc1r*, indicating that the observed association between *Mc1r* alleles and coat-color is not a consequence of linkage to some other, nearby locus. Finally, cAMP assays of receptor function *in vitro* show that the *Mc1r* D allele encodes a hyperactive receptor relative to the *Mc1r* d allele (Nachman, Hoekstra & D'Agostino, 2003). All of these observations strongly support the involvement of *Mc1r* in coat-color variation at the Pinacate site.

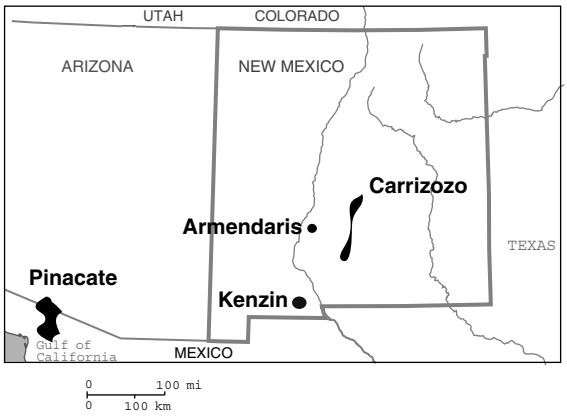

Figure 3. Four lava flows on which *C. intermedius* were studied. In each case, mice were collected on lava and on nearby light-colored rocks.

It is noteworthy that the differences in coat color are associated with a relatively small number of amino acid changes. At present, it is unknown whether each of the four *Mc1r* amino acid substitutions contributes to the difference in phenotype, or whether a subset of these four mutations is responsible for the difference in coat color. It does seem likely, however, that most of the coat-color variation can be explained by *Mc1r* genotype without a significant contribution from other genes. Most of the phenotypic variance correlates with *Mc1r* genotypic differences; there is little variation in coat-color within each of the three *Mc1r* genotypic classes (DD, Dd, dd). In principle, a gene linked to *Mc1r* could also contribute to the variation in phenotype, but this seems unlikely because of the rapid decay of linkage disequilibrium immediately upstream and downstream of *Mc1r*.

To estimate the strength of selection on *Mc1r* D and d alleles, we conducted a transect across the Pinacate site, collecting animals on light-colored rock as well on the lava flow (Hoekstra, Drumm & Nachman, 2004). At this site, the light rocks are separated from the lava by ~5 km of sand, which is not suitable habitat for *C. intermedius*. In general, most of the mice trapped on the lava were dark, and most of the mice trapped on the light-colored rocks were light. However, a small number of mis-matched mice were found, both on the lava and on the light rocks, suggesting that migration

between the two substrates occurs. We estimated migration rates from the degree of mitochondrial DNA differentiation between mice on light rocks and on lava. We assumed that the frequencies of mis-matched *Mc1r* alleles (D on light rock, and d on lava) were determined by the balance between the input of new alleles due to migration and their elimination by selection (migration-selection balance). Selection coefficients estimated this way were large (~2–40%) for light alleles (*Mc1r* d) on dark rock, but were considerably smaller (< 1%) for dark alleles (*Mc1r* D) on light rock.

To study the genetic basis of melanism in different geographic regions, we captured *C. intermedius* on four different lava flows (Figure 3) and found dark mice on all of them (Hoekstra & Nachman, 2003). The Pinacate site is in Arizona and is separated from the three lava flows in New Mexico by over 700 km. We sequenced *Mc1r* in dark mice from each lava flow and in light mice from light-colored rocks adjacent to each lava flow; we found that *Mc1r* does not seem to be involved in pigmentation variation at any of the three New Mexico sites. The four amino acid substitutions that define the *Mc1r* D allele were not observed in any dark mice from New Mexico. Moreover, no other associations between *Mc1r* polymorphisms and color variation were observed. Dark mice from all four lava flows are similar phenotypically in having unbanded, entirely melanic hairs on the dorsum, but they differ somewhat in the amount of reflectance off the dorsum as measured with a spectrophotometer: in general, melanic mice from the New Mexico sites are darker than melanic mice from the Pinacate site.

Implications for the understanding the genetics of adaptation

These results help us understand the genetic details of adaptive melanism in mice and provide a good example of evolution by natural selection. Beyond serving as an example, can these findings shed light more generally on the evolutionary process? Below I discuss several evolutionary principles in the context of these observations. In some cases, knowing the specific genetic changes underlying a trait of interest allows us to address issues that would be otherwise intractable; in other cases, a candidate-gene approach is one of several methods that can be used to address a particular problem.

Constraint and convergence

A key issue in evolution is the extent to which adaptive change is constrained by developmental pathways. If there are many ways to arrive at a given phenotype we might expect convergent evolution to be common. If, on the other hand, pathways are highly constrained, we might expect a similar ''genetic solution'' in different instances of the same 'evolutionary problem'. The observation that *Mc1r* is responsible for dark color in *C. intermedius* on one lava flow but not in three others has two immediate implications. First, it shows conclusively that dark color has evolved multiple times in this species. The alternative hypothesis, that dark color evolved once and spread through long-distance migration among lava flows, is clearly ruled out. Second, it provides evidence for convergence: nearly identical phenotypes have evolved through changes in different genes. We still have not identified the genes responsible for dark color in *C. intermedius* from the three New Mexico sites, but the candidate-gene approach may continue to prove useful in finding them.

In some respects, we knew *a priori*, that different genes might underlie similar color variation in different populations. In the laboratory mouse, mutations at different pigmentation genes can produce similar phenotypes. For example, some gain-of-function *Mc1r* mutations resemble, at least superficially, some loss-of-function *agouti* mutations. But laboratory studies are typically unable to reveal small or even modest fitness differences, and consequently the full range of pleiotropic effects is difficult to assess in the laboratory. If different mutants produce similar coat-color but affect fitness in other ways, their probability of fixation in natural populations may be dramatically different. Our data show that in rock pocket mice, not only are there different genes that may contribute to dark color, but there are different solutions that are evolutionarily viable.

Fisher's microscope

A long-standing debate in evolution concerns the average amount of phenotypic change caused by

adaptive mutations. Darwin (1859) argued that most adaptations result from numerous small changes. This view was given theoretical support from Fisher (1930) who showed that mutations of large effect had a higher probability of being deleterious than mutations of small effect, and that mutations of very small effect had an equal chance of being advantageous or deleterious. To illustrate this point, Fisher used the analogy of a microscope that is slightly out of focus: a large change will almost certainly make the situation worse, but a small change may improve the focus. Fisher's model contains many simplifying assumptions; for example, it considers a phenotype consisting of n orthogonal characters, whereas real characters are often correlated. It also assumes that organisms are evolving in an adaptive landscape that contains a single, fixed optimum. Importantly, Fisher only considered the probability that an individual mutation will be advantageous or deleterious, and as Kimura (1983) pointed out, this is different from the *rate* of adaptive substitution, which includes both the number of mutations and their probabilities of fixation. Kimura (1983) showed that while mutations of large effect have a lower probability of being beneficial, they have a higher probability of being fixed than mutations of small effect. Assuming that the 'size' of a mutation (i.e. the magnitude of its phenotypic effect) is proportional to its effect on fitness (s), Kimura (1983, p. 155) derived the distribution of substitution rates for mutations of different sizes and argued that adaptation might consist mainly of mutations of intermediate effect. This literature has been nicely summarized by Orr (1998) who expanded on the results of Fisher and Kimura to show that the distribution of mutational effects fixed during an 'adaptive walk' is typically exponential and can include one or more mutations of fairly large effect.

How do empirical observations conform with theory? Orr and Coyne (1992, p. 725) summarized the data available 10 years ago and argued that while 'some adaptations are apparently based on many genes of small effect, others clearly involve major genes'. QTL studies, especially in plants (Mauricio, 2001), often find a mixture of minor and major genes contributing to phenotypic variation, but it is not uncommon to find a few genes that account for a substantial amount of the phenotypic variation. Other evidence comes from organisms in disturbed environments, where single mutations of large effect seem to be the rule for explaining traits such as industrial melanism, insecticide resistance, and antibiotic resistance (e.g. Fidock et al., 2000; Walsh, 2000; Raymond et al., 2001; Cowen, Anderson & Kohn, 2002; Daborn et al., 2002; Wootton et al., 2002; Hughes, 2003) Clearly in this situation, selection is very strong, so that negative pleiotropic effects, like the physiological cost of resistance, may be easily outweighed by the benefits of resistance. The extent to which mutations of large effect are also seen in more natural situations is still unclear (Orr & Coyne, 1992; Charlesworth, 1994; Orr, 1999).

Pocket mice provide several important lessons here. First, the phenotypic difference between light and dark mice is striking and large, and the fit of mice to their environment seems to be quite good. Spectrophotometry measurements of reflectance from mice and from the rocks on which they are found show a strong positive correlation (Dice & Blossom, 1937; Hoekstra & Nachman, 2003). In the Pinacate site, this close fit seems to be due almost entirely to a single locus, *Mc1r*; the presence or absence of banded 'agouti' hairs on the dorsum appears to be a discrete rather than a quantitative trait, and is perfectly associated with *Mc1r* genotype. The situation is slightly more complicated than this, however, since, mice with different *Mc1r* genotypes (DD, Dd, dd) also differ in total reflectance, and Dd mice are roughly intermediate in reflectance between DD and dd mice. Thus, there appears to be some quantitative variation in reflectance among mice with uniformly melanic, unbanded hairs. Nonetheless, the amount of this variation is much greater between *Mc1r* genotypic classes than within genotypic classes, again suggesting a major role for *Mc1r*. The difficulty of breeding pocket mice has precluded a mapping study to identify QTL, and thus we do not know how many other loci (of presumably minor effect) may be contributing to the observed variation. Nonetheless, it is clear that *Mc1r* is a major gene, and therefore that major genes are not restricted to phenotypes associated with artificial selection or human disturbance (see also Haag & True, 2001).

The second lesson is that while *Mc1r* is a major gene, the dark allele (D) differs from the light allele (d) by four amino acid substitutions and one silent substitution. We do not know the relative contributions of each of these mutations (the

synonymous substitution may, of course, have no effect). At one extreme, a single mutation may be responsible for the phenotypic variation, and at the other extreme, each of four mutations may contribute to the phenotypic variation, and they may be either additive or epistatic. This distinction is instructive: conventional mapping studies typically identify chromosomal regions of importance but do not identify the number of mutations within those regions that contribute to the phenotype of interest. Thus the support for genes of major effect from QTL studies must be tempered with the caveat that these genes may, in fact, contain multiple mutations of smaller effect. We hope to disentangle the relative contribution of each mutation in *Mc1r* using site-directed mutagenesis and an *in vitro* cAMP assay for receptor function. These studies should also enable us to ask whether these mutations act together in an additive or epistatic manner. In this regard, knowing the identity of the gene enables us to address questions that would be impossible otherwise.

Haldane's sieve

Haldane (1924) showed that selection on rare, autosomal recessive mutations is ineffective because they are most often found in heterozygotes where they are hidden from selection. This stands in contrast to autosomal dominant mutations, which, when present in heterozygotes, are visible to selection. From this result, Haldane argued 'it seems therefore very doubtful whether natural selection in random mating organisms can cause the spread of autosomal recessive characters unless they are extraordinarily valuable to their possessors' (Haldane 1924, p. 38). This notion, later termed Haldane's sieve by Turner (1981), was supported by the observation that many known adaptations resulted from dominant mutations, despite the fact that many laboratory mutants were recessive (Haldane, 1924). Haldane also pointed out that the situation is quite different for sex-linked genes and for high levels of selfing, where recessive mutations may spread under selection, and both of these ideas have been explored in greater detail by Charlesworth, Coyne and Barton (1987) and Charlesworth (1992). Much was written on the evolution of dominance during the first 50 years of population genetics (reviewed in Merrell, 1969) but the following observation now seems well supported: many mutations in the laboratory with large phenotypic effects are recessive while many adaptations in animal populations that result from genes of major effect are usually dominant or semi-dominant. This result appears consistent with the preferential fixation of beneficial dominant mutations. An alternative possibility, however, is that most favorable mutations are dominant rather than recessive, and thus the large number of dominant mutations underlying adaptation would simply reflect their greater occurrence rather than their higher probability of fixation. Beneficial mutations may often result from gain-of-function, and dominance may simply correspond to gain of function at the biochemical level (Wright, 1929, 1934). Finally, Orr and Betancourt (2001) have recently shown that the situation is quite different if one considers adaptive fixations resulting from standing variation rather than from new mutations; when positive selection favors a previously deleterious allele at mutation-selection balance, the probability of fixation is largely independent of the degree of dominance.

How do our observations in pocket mice fit with these theoretical considerations? It is worth pointing out that *Mc1r* is autosomal rather than X linked in all mammals where it has been mapped, so it seems likely that it is autosomal in pocket mice as well; thus, the special considerations for dominance in sex-linked genes do not need to be considered. First, adaptive melanism at the Pinacate site appears to be caused by a dominant or semi-dominant allele at a single major gene. This observation is entirely consistent with the observation of dominance for genes underlying adaptations to human disturbance (e.g. Haldane, 1924; Jasieniuk, Brule-Babel & Morrison, 1996). The studies on pocket mice also underscore the difficulty of correctly ascertaining the degree of dominance. The presence or absence of a sub-terminal band of phaeomelanin on individual hairs is a Mendelian trait, with the melanic hair (*Mc1r* D) fully dominant over the agouti hair (*Mc1r* d). To the human eye, this difference appears to be the most significant aspect of color variation in these mice; all observers easily group mice into 'light' and 'dark' categories based on the presence or absence of agouti hairs on the dorsum (Figure 2). However, spectrophotometry measurements indicate that *Mc1r* Dd mice are intermediate in total reflectance

between *Mc1r* DD and *Mc1r* dd mice, an attribute that is not easily detected by the human eye (Hoekstra & Nachman 2003, Figure 2C). It remains unclear whether *Mc1r* DD and *Mc1r* Dd genotypes have the same fitness. Knowing the gene underlying adaptive melanism also makes it possible to relate dominance to biochemical function. Our studies measuring *Mc1r* function *in vitro* show that the *Mc1r* D allele encodes a hyperactive receptor relative to the *Mc1r* d allele, and thus dominance in this case corresponds to the gain of biochemical function (Wright, 1934). However, as described above, darkening alleles are known from both dominant, gain-of-function mutations at *Mc1r* and recessive, loss-of-function mutations at *agouti* in the laboratory mouse. In principle, we might expect that either could serve as a substrate for adaptive evolution in natural populations, and thus there is *no a priori* reason for thinking that most adaptive pigmentation mutations arise from gain-of-function mutants. So far, however, we have only been able to identify gain-of-function (dominant) mutants in the wild; it will be interesting to see whether recessive alleles are responsible for melanic phenotypes in other populations. Finally, can we say anything about the likelihood that melanic mice arise from new mutations rather than from standing variation? In several species of mammals, occasional melanic individuals are observed, raising the possibility that melanic forms are present at low frequency in mutation-selection balance. Although we have never observed melanic *C. intermedius* at sites that are far from dark rocks (based on approximately 1000 mice), the possibility that selection acted on pre-existing variation cannot be excluded.

Gene regulation and gene structure

A question of considerable recent interest concerns the degree to which adaptive evolution derives from changes in gene dosage versus changes in gene product. Britten and Davidson (1969) argued that much of evolution may be caused by modifications to regulatory networks, and current microarray technology has allowed investigators to explore large-scale changes in gene expression between closely related species (e.g. Enard et al., 2002). Knowing the identify of the gene underlying a trait allows us to address this question directly. Adaptive melanism in the Pinacate mice is caused by changes in the amino acid sequence of *Mc1r*, and these changes alone produce a receptor that functions differently. Importantly, however, these changes have many downstream effects. In mice with *Mc1r* DD genotypes, there appears to be no production of phaeomelanin in dorsal melanocytes. Thus while changes at *Mc1r* are clearly structural, they cause changes in the expression pattern of many downstream genes. This highlights a potential difficulty with using differences in expression to identify causative mutations.

Linking phenotype to genotype

The candidate-gene approach has been useful here for making several connections between genotype and phenotype. In addition to the description of phenotypic differences associated with different *Mc1r* genotypes, we have made some preliminary estimates of the strength of selection on *Mc1r* D and *Mc1r* d alleles. In principle, this should allow us to compare both the magnitude of phenotypic effect and the value of s for different alleles. However, because the *Mc1r* D and d alleles differ by four amino acid substitutions and each of these may have been a separate step in the 'adaptive walk', we may not be able to link the effect size with s for individual mutations. Nonetheless, the approach used here has allowed us to shed light on the biochemistry, population genetics, and ecological genetics associated with the evolution of melanism and it serves as an example of the utility (and limitations) of this method. This approach will clearly not work in all situations; when adaptive differences are quantitative and caused by many genes of small effect, a mapping study may prove more useful. But for traits where good candidate genes are available and phenotypic differences are relatively simple, studies of candidate genes may be quite useful for understanding the evolutionary process.

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