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ECOLOGICAL GENETICS OF ADAPTIVE COLOR POLYMORPHISM IN POCKET MICE: GEOGRAPHIC VARIATION IN SELECTED AND NEUTRAL GENES

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Abstract.—Patterns of geographic variation in phenotype or genotype may provide evidence for natural selection. Here, we compare phenotypic variation in color, allele frequencies of a pigmentation gene (the melanocortin-1 receptor, *Mc1r*), and patterns of neutral mitochondrial DNA (mtDNA) variation in rock pocket mice (*Chaetodipus intermedius*) across a habitat gradient in southern Arizona. Pocket mice inhabiting volcanic lava have dark coats with unbanded, uniformly melanic hairs, whereas mice from nearby light-colored granitic rocks have light coats with banded hairs. This color polymorphism is a presumed adaptation to avoid predation. Previous work has demonstrated that two *Mc1r* alleles, *D* and *d*, differ by four amino acids, and are responsible for the color polymorphism: *DD* and *Dd* genotypes are melanic whereas *dd* genotypes are light colored. To determine the frequency of the two *Mc1r* allelic classes across the dark-colored lava and neighboring light-colored granite, we sequenced the *Mc1r* gene in 175 individuals from a 35-km transect in the Pinacate lava region. We also sequenced two neutral mtDNA genes, COIII and ND3, in the same individuals. We found a strong correlation between *Mc1r* allele frequency and habitat color and no correlation between mtDNA markers and habitat color. Using estimates of migration from mtDNA haplotypes between dark- and light-colored sampling sites and *Mc1r* allele frequencies at each site, we estimated selection coefficients against mismatched *Mc1r* alleles, assuming a simple model of migration-selection balance. Habitat-dependent selection appears strong but asymmetric: selection is stronger against light mice on dark rock than against melanic mice on light rock. Together these results suggest that natural selection acts to match pocket mouse coat color to substrate color, despite high levels of gene flow between light and melanic populations.

Key words.—Adaptation, *Chaetodipus*, cline, *Mc1r*, melanocortin-1 receptor, migration, selection coefficient.

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Patterns of geographic variation in phenotype or genotype may provide evidence for natural selection (Endler 1977). Two common patterns of geographic variation that may be caused by selection are clines along a habitat gradient and ecotypic distributions in a heterogeneous environment. For example, several metabolic enzymes in animals vary with latitude, and are thought to be under positive selection (reviewed in Eanes 1999), and several plant species have evolved adaptive physiological traits based on local soil conditions, which can be sporadically distributed (reviewed in Linhart and Grant 1996). In both cases, local adaptation depends on spatially varying selection that is sufficiently strong to overcome the effects of migration.

The relative effects of gene flow (migration) and natural selection on phenotypic differentiation have been widely discussed (see Lenormand 2002). In many cases, gene flow is thought to impede local adaptation by homogenizing the genetic composition of populations (Haldane 1930; Slatkin 1985). However, if natural selection is strong, local gene frequencies may be maintained in the face of countervailing gene flow (e.g., Camin and Ehrlich 1958; Cook and Mani 1980; Sandoval 1994; Chevillon et al. 1995; Ross and Keller 1995). In such cases, clinal or ecotypic variation in gene frequencies can occur across heterogenous environments (Nagylaki 1975). However, demographic history or population structure (e.g., isolation by distance) can also generate patterns of clinal and ecotypic variation. Thus, it is important to rule out these effects by comparing neutral markers, which track demography, to either adaptive morphological variation or genetic loci presumed to be under selection (Prout and Barker 1993).

A classic example of local adaptation is cryptic coloration (Young 1916; Benson 1933; Cott 1940; Kettlewell 1956; Endler 1984). Melanism is one form of concealing coloration that has appeared repeatedly in many organisms (reviewed in Majerus 1998); however, the selective agent or the genes that are responsible for melanism are often unknown. In the rock pocket mouse, *Chaeotodipus intermedius*, both the selective agent and the genetic basis of melanism have been identified (Nachman et al. 2003).

Throughout most of its range, *C. intermedius* inhabit light-colored granitic rocks and have correspondingly light-colored pelage with banded hairs, characterized by a light-colored subterminal band and a dark-colored base and tip. However, there are also several basalt lava flows on which dark (melanic) mice are found at high frequencies (Sumner 1921, 1935; Benson 1933; Dice and Blossom 1937; Hoekstra and Nachman 2003). These melanic mice have uniformly dark hairs with no subterminal band.

Similar dark or melanic coloration has evolved in several lava-dwelling lizards, snakes, and mammals, including the deer mouse, *Peromyscus maniculatus* (Sumner 1921; Dice and Blossom 1937; Blair 1947; Norris 1965). Previous experiments have demonstrated that dark coloration in *P. maniculatus* decreases risk of predation by owls on dark substrate relative to light-colored counterparts (Dice 1947). Moreover, these experiments suggest that dark-colored *P. maniculatus* have reduced fitness on light-colored substrate. Although comparable experiments have not been conducted with *C. intermedius*, we expect cryptic coloration in *C. intermedius*

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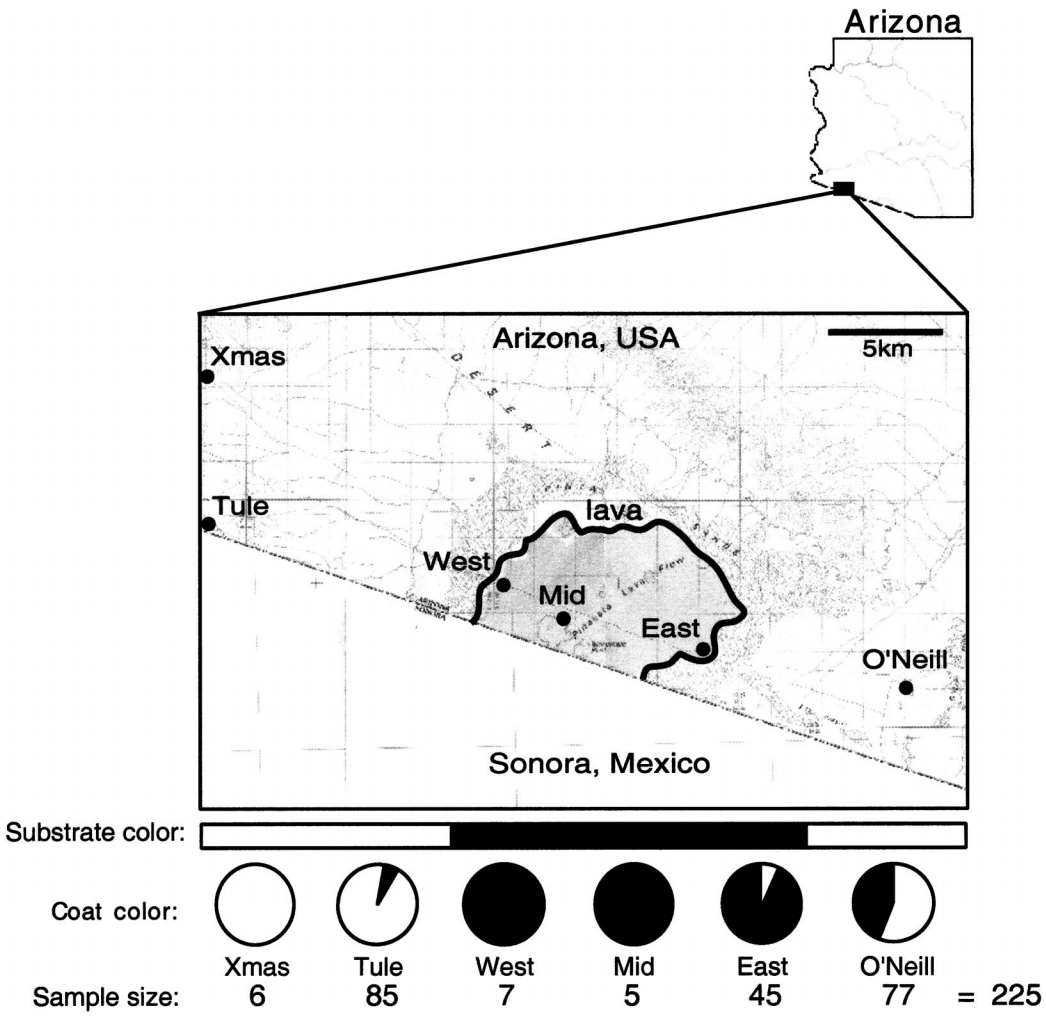


FIG. 1. Collecting sites, substrate color, and coat color frequencies on and neighboring the Pinacate lava flow in south central Arizona. Six sites were sampled: three on dark volcanic rock and three on light-colored substrate. The lava flow is surrounded by approximately 1 km of the Pinta Sands. Substrate color is indicated schematically below. Pie diagrams refer to the frequencies of light and melanic mice at each collecting site. Sample sizes are given.

to also be an important antipredator adaptation, since the degree of substrate matching is more extreme in *C. intermedius* than in *P. maniculatus*. In addition, it is well established that desert heteromyids, like *C. intermedius*, are preyed upon by visual predators including owls and mammalian carnivores (Brown and Harney 1993).

On the Pinacate lava beds in southern Arizona, a single gene, the melanocortin-1 receptor (*Mc1r*), is responsible for the adaptive color polymorphism observed in *C. intermedius* (Nachman et al. 2003). Melanic mice carry one or two copies of the *Mc1r D* allele, which is distinguished by four amino acid variants relative to the wild-type *Mc1r d* allele. All four mutations cause a change in amino acid charge and are in complete linkage disequilibrium with each other.

Here, we provide evidence that strong natural selection is acting to match coat color of *C. intermedius* to substrate color through changes in *Mc1r* allele frequencies in the Pinacate lava region. First, we quantified phenotypic differences using a spectrophotometer to measure reflectance from the pelage of melanic and light mice across six sites on dark volcanic basalt and the neighboring light-colored rocks. Second, we compared the distribution of alleles at *Mc1r* to the distribution of alleles at two neutral mitochondrial DNA (mtDNA) loci at each of the six sites. Finally, we used estimates of *Mc1r* allele frequencies and migration rates between sites to estimate the strength of selection acting on coat color in this system.

MATERIALS AND METHODS

Sampling

The majority of the Pinacate lava flow is located in northern Sonora Mexico. The northernmost extension of the lava reaches southern Arizona and is found within the Cabeza Prieta National Wildlife Refuge. We sampled a 35-km east-west transect across this extension of Pinacate lava at six sites (Fig. 1, Table 1): three sites were on rocky outcrops of lava (West, Mid, and East), and three sites were in the light-colored granite of the O'Neill Hills (O'Neill Pass) to the East, and the Tule Mountains (Tule Well) and Cabeza Prieta Moun-

TABLE 1. Collection sites (west to east) for *Chaetodipus intermedius* showing substrate color, sample size (*N*), number of each color phenotype sampled, distance to neighboring sites, and geographic location.

Collecting site	Substrate color[1]	*N*	Phenotype (L/M)[2]	Distance (km)[3]	Latitude (N)/longitude (W)
Christmas Pass	L	6	6/0	7.6	32°14.8′/113°41.4′
Tule Well	L	85 (38)[4]	80/5	12.6	32°10.5′/113°40.2′
Lava (West)	D	7	0/7	4.1	32°07.5′/113°32.8′
Lava (Mid)	D	5	0/5	4.8	32°06.9′/113°31.6′
Lava (East)	D	45	3/42	9.6	32°05.9′/113°28.3′
O'Neill Pass	L	77	34/43	—	32°06.5′/113°22.5′

[1] L, light-colored granitic rock; D, dark-colored volcanic basalt.
[2] L, light-colored, banded hair; M, melanic, unbanded hair.
[3] Distance to next site toward the east.
[4] Number of individuals from which tissue was collected.

tains (Christmas Pass) to the west. Each site was separated by approximately 8 km from neighboring sites, but the average distance between dark sites was approximately half (4.5 km) of the average distance between light sites (9.9 km). The largest distance between adjacent sites was 12.6 km separating the western edge of the lava and the Tule Mountains. Rocky habitat is not continuous between sites. The lava flow is separated from the nearby Tule Mountains and O'Neill Hills sites by the light-colored Pinta Sands. The lava flow itself is made up of discontinuous but geographically proximate outcrops of dark-colored volcanic rock. We sampled the largest outcrops that were closest to the neighboring light-colored granite hills. Because *C. intermedius* are restricted to rocky habitats, their distribution is not continuous across the transect.

A total of 225 mice were trapped at the six collecting sites (Fig. 1; Table 1; Appendix). Although there is some variation in color within light and melanic classes, the presence or absence of a subterminal band of phaeomelanin is a discrete character, and mice could be easily classified as light (banded hairs) or melanic (unbanded hairs). We recorded age class, (adult, subadult, or juvenile) based on size, pelage, and reproductive status. Standard measurements, including sex; weight; and body, tail, hind foot, and ear length; were also recorded.

Of the total sample, tissues were taken from 178 mice, which were prepared as museum specimens and deposited in the Zoological Collections of the Department of Ecology and Evolutionary Biology at the University of Arizona, Tucson. The difference in sample size was due to the release of 47 light-colored mice at the Tule Well site. Based on the previously documented dominance of the *Mc1r D* allele and the data presented here, these light-colored mice were presumed to be homozygous at the *Mc1r* locus (*dd*).

Spectrophotometry Measurements

The reflectance spectra of individual mouse coats were measured using a USB2000 spectrometer (Ocean Optics, Dunedin, FL) with dual deuterium and halogen light source (Analytical Instruments Systems, Inc., Flemington, NJ). We used the program OOIBase32 (Ocean Optics) to capture reflectance readings over the ultraviolet and visible (250–800 nm) spectrum following Hoekstra and Nachman (2003).

All non-molting adults ($N = 86$) from the six collecting sites were included. These individuals represented a subset of the individuals used in the molecular analysis. Reflectance measurements at 10 spots across the dorsal surface of each museum specimen were taken. Measurements were averaged across the dorsum, to provide a representative description of the dorsal surface brightness. To quantify differences in pelage color between melanic *Mc1r DD* homozygotes, melanic *Mc1r Dd* heterozygotes and light *Mc1r dd* individuals, we calculated the absolute reflectance or brightness of each sample relative to a white standard. We measured the percent reflectance at 1656 increments across 250–800 nm wavelengths and quantified the total area under the resulting spectrum. Statistical analyses were done in JMP (SAS Institute, Cary, NC). Comparisons of reflectance intensity across the three *Mc1r* genotypes allowed us to estimate the dominance coefficient (*h*) of the *Mc1r D* and *d* alleles in relation to reflectance.

Melanocortin-1 Receptor Locus Genotypes

Previous work has demonstrated that the *Mc1r D* and *Mc1r d* alleles are responsible for coat color polymorphism in *C. intermedius* from the Pinacates: *Mc1r DD* and *Mc1r Dd* mice are melanic, whereas *Mc1r dd* mice are light colored (Nachman et al. 2003). The *Mc1r D* allele differs from the *Mc1r d* allele by four amino acid mutations: Arg18Cys, Arg109Trp, Arg160Trp and Gln233His. In this study we identified *Mc1r DD, Dd,* and *dd* genotypes by direct sequencing of PCR-amplified products.

Whole genomic DNA was extracted from frozen (−80°) liver samples using DNeasy tissue kits (Qiagen, Valencia, CA). The *Mc1r* gene was amplified using *Chaetodipus*-specific primers described in Nachman et al. (2003). PCR products were purified using spin columns (Qiagen). Diploid PCR products were sequenced on an ABI (Applied Biosystems, Foster City, CA) 3700 using a single sequencing primer (F11b 5′-ACTGGGTCCTTTCAACTCCAC-3′), which captured all four nucleotide sites that distinguish the *Mc1r D* and *Mc1r d* alleles. Individual alleles from *Mc1r Dd* heterozygotes were cloned and sequenced. The four polymorphisms that distinguish *Mc1r D* and *Mc1r d* alleles were in complete linkage disequilibrium, as reported previously based on a much smaller sample (Nachman et al. 2003). *Mc1r D* and *Mc1r d* allele frequencies were calculated for the total sample ($N = 225$) and for each of the collecting sites individually.

Mitochondrial DNA

The mitochondrial ND3 and COIII genes were amplified for all individuals from which DNA was collected ($N = 175$). A 600 bp fragment containing the ND3 gene (321 bp) was amplified using degenerate primers designed from *Mus* sequence (ND3.L 5′-CGTYTCYATYTATTGATGAGG-3′ and ND3.H 5′-CATAATCTAATGAGTCGAAATC-3′). ND3 amplification conditions were: 95° for 3 min followed by 35 cycles of 94° for 30 sec, 40° for 30 sec, and 72° for 1 min, followed by a 8 min extension at 72°. An 850-bp fragment containing the entire COIII gene (790 bp) was amplified using degenerate primers (CO3.L 5′-GCWGTMGCMWTWATY-CAWGC-3′ and CO3.H 5′-YCARAAYTWRYTRATTGGA-AGTCA-3′). COIII amplification conditions were: 95° for 3 min followed by 35 cycles of 94° for 30 sec, 48° for 1 min, and 72° for 1 min, followed by a 8 min extension at 72°. PCR purification and sequencing followed the protocol described for the *Mc1r* locus. Sequences have been deposited in GenBank.

Phylogenetic Reconstruction

To document population structure across the Pinacate transect, neighbor-joining (NJ) trees were generated in PAUP*4.01b (Swofford 1999) with the combined ND3 and COIII sequence data. Homologous sequences were used from *C. penicillatus*, the sister species, and *C. baileyi* to root the tree. Bootstrap support for each branch in the phylogeny was generated with both NJ and maximum likelihood (ML) algorithms. To determine the geographic distribution of mtDNA haplotypes, we determined the presence or absence of each haplotype at each of the six collecting sites. A single clade with the strongest bootstrap support was identified, and the frequency of mtDNA haplotypes from this clade was recorded at each collecting site. We repeated this analysis with additional clades and the findings were unaffected.

Statistical Analyses of DNA Sequence Variation

In the analyses below, we grouped mice into three samples: (1) Tule Mountains, (2) Pinacate lava (West, Mid, and East sites), and (3) O'Neill Hills. This grouping reflects the ecology of the organisms. The Pinacate lava flow is separated from the Tule Mountains in the west and O'Neill Hills in the east by the Pinta Sands, which serve as a barrier to dispersal. Importantly, there was no evidence for population structure among the three collecting sites on the lava (see below).

Estimating population parameters.—Two tests were used to assess the effect of demographic processes on patterns of mtDNA sequence variation. Tajima's *D* (1989) summarizes the frequency distribution of polymorphic sites compared to the expected distribution at mutation-drift equilibrium. The Rogers and Harpending (1992) test is based on the distribution of pairwise comparisons among all alleles in a sample (the mismatch distribution). Both tests were performed in DnaSP (Rozas and Rozas 1999) on the global sample and on each of the three samples separately.

Estimating gene flow.—We estimated gene flow (historical migration rates between sites) using synonymous mtDNA nucleotide data. We used two main approaches. First, we calculated F_{ST} (Wright 1969) and a related estimator, γ_{ST} (Nei 1982), which describe the amount of the total genetic variation that is attributable to differences among populations. At equilibrium, with respect to migration and drift, both estimates are approximately equal to $1/(1 + 2N_f m_f)$, where N_f is the female effective population size and m_f is the proportion of female migrants each generation. We used this relationship to solve for $N_f m_f$. For clarity, we will refer to $N_f m_f$ as Nm throughout the text with the understanding that mtDNA markers track female demography. These methods assumed symmetrical migration between sites at migration-drift balance.

Second, we used the program Migrate to generate maximum likelihood (ML) estimates of Nm (Beerli and Felsenstein 1999, 2001). We initially used default settings to estimate Nm and $N\mu$ (θ), where μ is the neutral mutation rate per nucleotide site per generation. We optimized the likelihood by using initial estimates as starting parameters in additional searches until the ln(L) peaked and stabilized. Migrate allows for asymmetrical estimates of migration between populations (for discussion see Beerli and Felsenstein 1999).

To estimate m (the proportion of female migrants per generation) from Nm, we first estimated N from observed levels of mtDNA variability ($N\mu$), assuming a neutral mtDNA mutation rate of $\mu = 10^{-6}$ to 10^{-7} per site per generation (Brown et al. 1979, 1982).

Estimating selection coefficients.—A model of migration-selection balance (Haldane 1930; Wright 1931) was used to estimate selection coefficients associated with *Mc1r D* and *Mc1r d* alleles on light and dark rocks, respectively. This model assumes that the equilibrium frequency of mismatched alleles is determined by the balance between natural selection removing deleterious alleles and migration introducing deleterious alleles. The change in the deleterious allele frequency in a population is given by:

$$\Delta q = \frac{-spq[q + h(p - q)]}{1 - sq(2hp + q)} + mQ - Mq, \qquad (1)$$

where s is the selection coefficient against the deleterious allele, q is the frequency of the deleterious allele in the population, p is the frequency of nondeleterious alleles (or $1 - q$), h is the dominance coefficient, m is the migration rate of individuals into the population, Q is the frequency of the deleterious allele outside the population, and M is the emigration rate of individuals from the population. In this model, there are two components driving allele frequency change. The first is selection, and its efficiency is influenced by the relative allele frequencies and the dominance coefficient. The second is migration, which introduces deleterious alleles based on the immigration rate and the deleterious allele frequency outside the population ($m \times Q$). Emigration of deleterious alleles can also occur and is based on the migration rate out of the population and the deleterious allele frequency in the population ($M \times q$). Using observed values of q and Q and estimates of m, M, and h, we set Δq equal to 0 and solved for s. In our calculations below (see Results), we used this exact solution, although approximate solutions exist under the following assumptions; (1) migration only occurs into the population, (2) the frequency of deleterious alleles in the source population is 1, and (3) $s >> m$. Under these con-

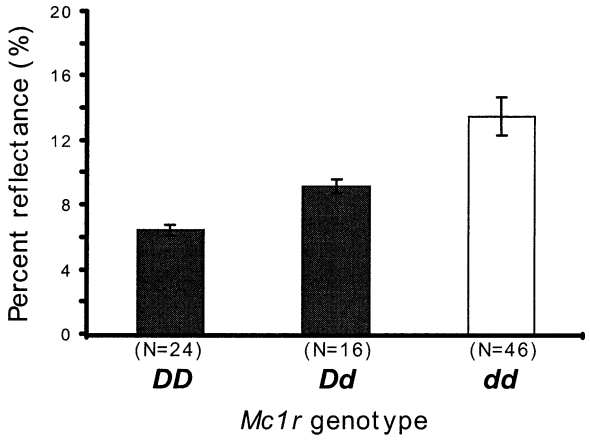


FIG. 2. Graph of percent reflectance for the three *Mc1r* genotypes: *Mc1r DD*, *Mc1r Dd*, and *Mc1r dd*. Error bars represent SE. Sample sizes are shown below the bars.

ditions, the equilibrium frequency of a dominant deleterious allele is approximated by $p = m/s$, and the equilibrium frequency of a recessive deleterious allele is approximated by $q = (m/s)^{1/2}$ (Wright 1931).

RESULTS

Spectral Analysis of Coat Color Variation

The average reflectance was measured for 86 nonmolting adults across six collecting sites as a function of wavelength from 250 nm to 800 nm. Consistent with earlier results (Hoekstra and Nachman 2003), we found a highly significant difference in reflectance between mice with light (banded) dorsal hairs and mice with uniformly melanic (unbanded) dorsal hairs (Fig. 2; $t_{84} = 11.18$, $P < 0.0001$). Surprisingly, however, and in contrast to our earlier study based on a smaller sample, we found differences in reflectance among mice with all three genotypes: *Mc1r DD*, *Mc1r Dd*, and *Mc1r dd* (Fig. 2). *Mc1r DD* mice and *Mc1r Dd* mice differed significantly in percent reflectance (Fig. 2; $t_{36} = 3.70$, $P = 0.0007$). However, there was some overlap in the distribution of reflectance measurements across genotypes, that is, some *Mc1r Dd* mice were darker (lower reflectance) than some *Mc1r DD* mice. The mean total and percent reflectance for *Mc1r Dd* individuals (5560 counts and 9.8%, respectively) is intermediate between the reflectance for *Mc1r dd* mice (8178 counts and 14.2%) and the reflectance for *Mc1r DD* mice (3918 counts and 6.3%). Using these mean values of total reflectance for each genotype, the coefficient of dominance (h) for the *d* allele is approximately 0.4.

However, the presence or absence of a subterminal band of phaeomelanin is a discrete character: all *DD* and *Dd* individuals have unbanded (melanic) hairs whereas *dd* individuals have banded (wild-type) hairs. With respect to this aspect of the phenotype, the coefficient of dominance for the *d* allele is 0.

Distribution of Color Phenotypes across the Transect

At six sites across the Pinacate lava flow and neighboring granite rocky habitat, 57 mice were trapped on the lava and 168 mice were caught on light rock (Fig. 1). In total, 102 mice were melanic (unbanded dorsal hairs) and 123 mice were light (banded dorsal hairs). Melanic mice were most abundant on dark substrate: 94.7% of the mice caught on dark rock were melanic. There was a highly significant association between substrate color and mouse color (Table 2; Fig. 3A; Fisher's exact test; $P < 10^{-6}$), driven primarily by a deficiency of light mice caught on dark rock. The frequency of mice that matched their habitat was less on light rock; 71.4% of the mice caught on light rock were light. However, the frequency of melanic mice on light rock is dependent on the distance from the light rock to the nearest dark rock. For example, at O'Neill Hills, the closest light-colored site to the lava, 55.8% of the mice were melanic. Both the Tule Mountains and Xmas Pass sites are farther away from the Pinacate lava (12.6 and 20.2 km, respectively) and have a much lower frequency of melanic mice (5% and 0%, respectively).

TABLE 2. Distribution of color phenotype and *Mc1r* genotype on light and dark substrate.

Substrate	Phenotype		*Mc1r* alleles	
	melanic (unbanded)	light (banded)	*Mc1r D*	*Mc1r d*
Dark (lava)	54	3	98	16
Light (granite)	48	120	57	279
Fisher's exact test:	$P < 10^{-6}$		$P < 10^{-6}$	

Geographic Distribution of Mc1r Alleles

There is a significant nonrandom distribution of *Mc1r* alleles across different-colored substrate; the *Mc1r D* allele is at high frequency on the lava (Table 2, Fig. 3B; Fisher's exact test, $P < 10^{-6}$). The correlation between *Mc1r* allele frequency and habitat color was more pronounced than the correlation between phenotype and habitat color. This results from the fact that most melanic individuals caught on dark rock were *Mc1r DD* homozygotes (83%), whereas most melanic mice caught on light rock were *Mc1r Dd* heterozygotes (89%; Fig. 3C).

Patterns of Mitochondrial DNA Variability

To estimate population structure, we combined sequence data from two mtDNA genes, ND3 (321 bp) and COIII (790 bp). There were 78 mtDNA haplotypes present in 175 individuals (Fig. 4). In the combined 1111 bp, there were 125 polymorphic sites, of which 38 were singletons. The level of overall nucleotide diversity was similar to that seen for other species of rodents for mtDNA ($\pi = 0.0102$, $\Theta = 0.0203$; Nachman et al. 1994). Moreover, levels of nucleotide diversity were similar among all three populations: Tule Mountains ($\pi = 0.0101$), Pinacate lava ($\pi = 0.0098$), and O'Neill Hills ($\pi = 0.0096$).

Phylogenetic analysis of the 78 haplotypes using the neighbor-joining algorithm showed no correlation between mtDNA haplotype and either geography or color phenotype (Fig. 5). In other words, mtDNA haplotypes are not clustered by collecting locale, suggesting these populations are not highly structured. Additionally, mtDNA haplotypes were not clustered by phenotype; light and melanic mice were scattered

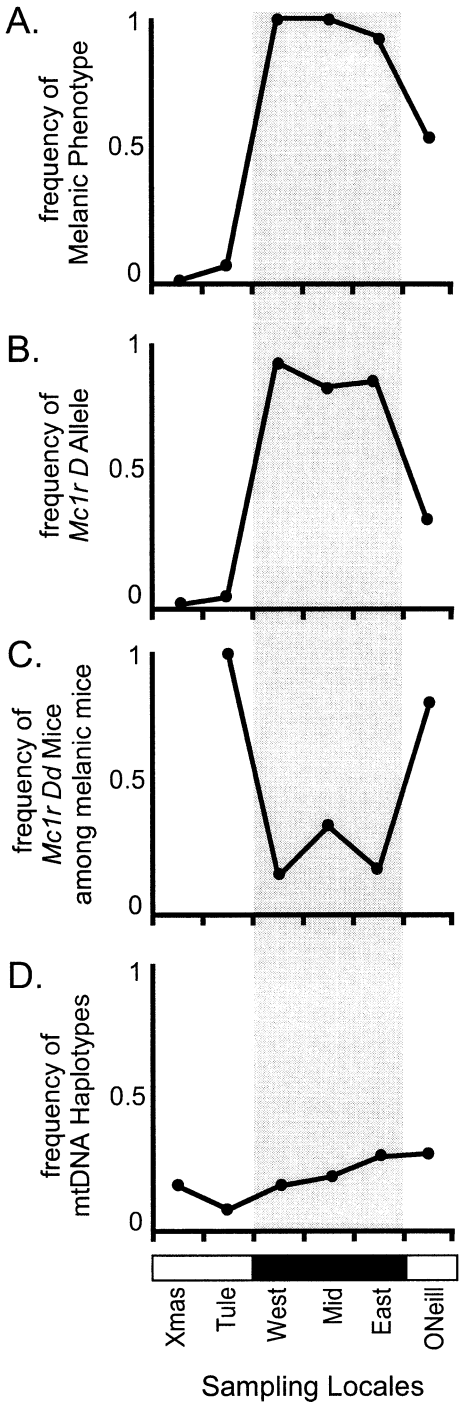


FIG. 3. Change in frequencies of four traits across six collecting sites in a west-to-east transect. (A) Frequency of melanic mice, (B) frequency of the *Mc1r D* allele, (C) frequency of *Mc1r Dd* individuals among melanic mice, (D) frequency of the most common, well-supported mtDNA haplogroup (from Fig. 5).

across the tips of the mtDNA phylogeny. In fact, 15 mtDNA haplotypes (of 36 haplotypes found in more than a single individual) were found in both light and melanic individuals.

Tajima's *D* calculated from all sites in COIII and ND3 together was not significantly different from the neutral equilibrium expectation of 0 ($D = -1.58$, $P > 0.05$). However, nonsynonymous and synonymous sites differed in the frequency distribution of polymorphic sites. Tajima's *D* was significantly negative for nonsynonymous sites ($D = -2.43$, $P < 0.01$), but not for synonymous sites ($D = -1.36$, $P > 0.1$), consistent with the idea that many mitochondrial nonsynonymous polymorphisms are weakly deleterious (Nachman et al. 1996; Nielsen and Weinreich 1999). For estimates of gene flow (below) we restricted our analysis to synonymous sites. The mismatch distribution (the distribution of differences between all pairs of sequences in the sample) provided no evidence for population expansion (raggedness statistic, $r = 0.003$).

Population Structure and Gene Flow

Estimates of F_{ST} and γ_{ST} were generated from mtDNA sequence data between Tule Mountains and the Pinacate lava, as well as between O'Neill Hills and the Pinacate lava. These estimates of F_{ST} and γ_{ST} were used to calculate *Nm* (Table 3). We also generated maximum likelihood estimates of *Nm*, using the program Migrate (Table 4). Migration rates were high between O'Neill Hills and the Pinacate lava, consistent with the F_{ST} calculations. However, in each of the pairwise comparisons between populations, migration was asymmetric. Migration rates were higher from the Tule Mountains to the Pinacate lava than in the reverse direction, and similarly, migration rates were higher from the O'Neill Hills to the Pinacate lava than in the reverse direction. In both cases, the Pinacate lava population appears to be a sink, receiving more migrants from neighboring regions. The average of these two estimates of migration (one in each direction) for each pair of populations was similar to the value obtained from F_{ST} (above).

Estimating Selection Coefficients

We used the estimates of *Nm* to estimate the strength of selection against *Mc1r D* alleles on light rocks and against *Mc1r d* alleles on dark rocks, assuming a simple model of migration-selection balance at equilibrium. Estimates of *Nm* were used to provide estimates of *m* by first calculating *N*. An estimate of *N* was obtained from the mitochondrial mutation rate, $\mu = 10^{-6} - 10^{-7}$, and the observed nucleotide diversity, $\theta = N\mu = 0.01$, suggesting that $N = 10^4 - 10^5$. In the migration-selection model, *N* refers to local population size, and $N = 10^4$ does not seem unreasonable for these rodents, which are often found at high density. Using estimates of $N = 10^4 - 10^5$, we solved for migration rate (*m*) between the Pinacate lava and the two light sites, O'Neill Hills to the east and Tule Mountains to the west.

Using both F_{ST} and Migrate estimates of *Nm*, we calculated *m* from the lava to neighboring light-colored rocks (Table 5). Using migration rates estimated with Migrate and two estimates of $N(10^4$ and $10^5)$, $m = 7.0 \times 10^{-5}$ to 7.0×10^{-6} from the Pinacate lava to the Tule Mountains and $m = 7.3 \times 10^{-4}$ to 7.3×10^{-5} from the Pinacate lava to O'Neill Hills. In both cases, estimates from F_{ST} were approximately three to six times higher than the Migrate estimates. We also estimated migration to the Pinacate lava by combining migration from both the O'Neill Hills and the Tule Mountains sites to get the total immigration of alleles into the lava population.

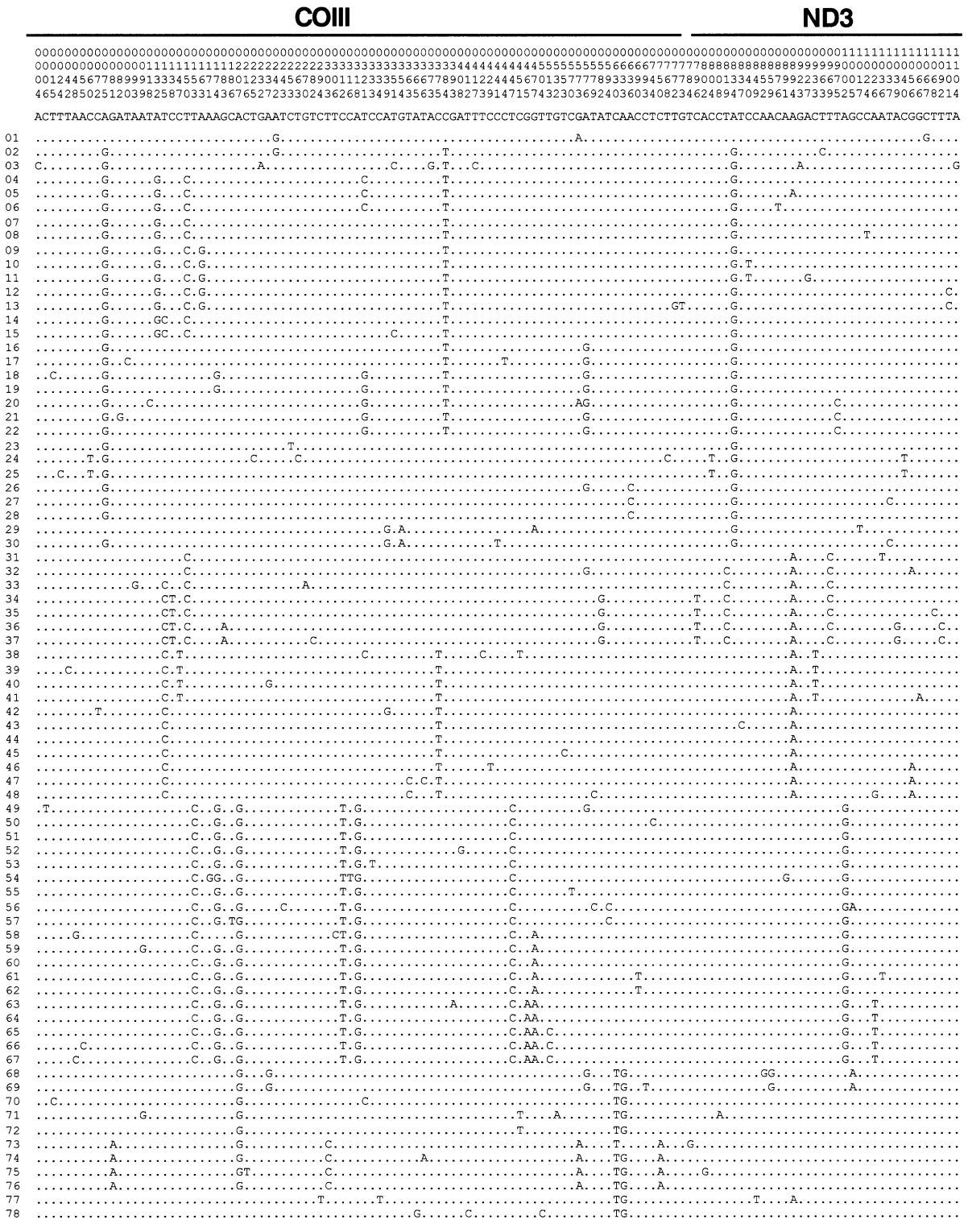
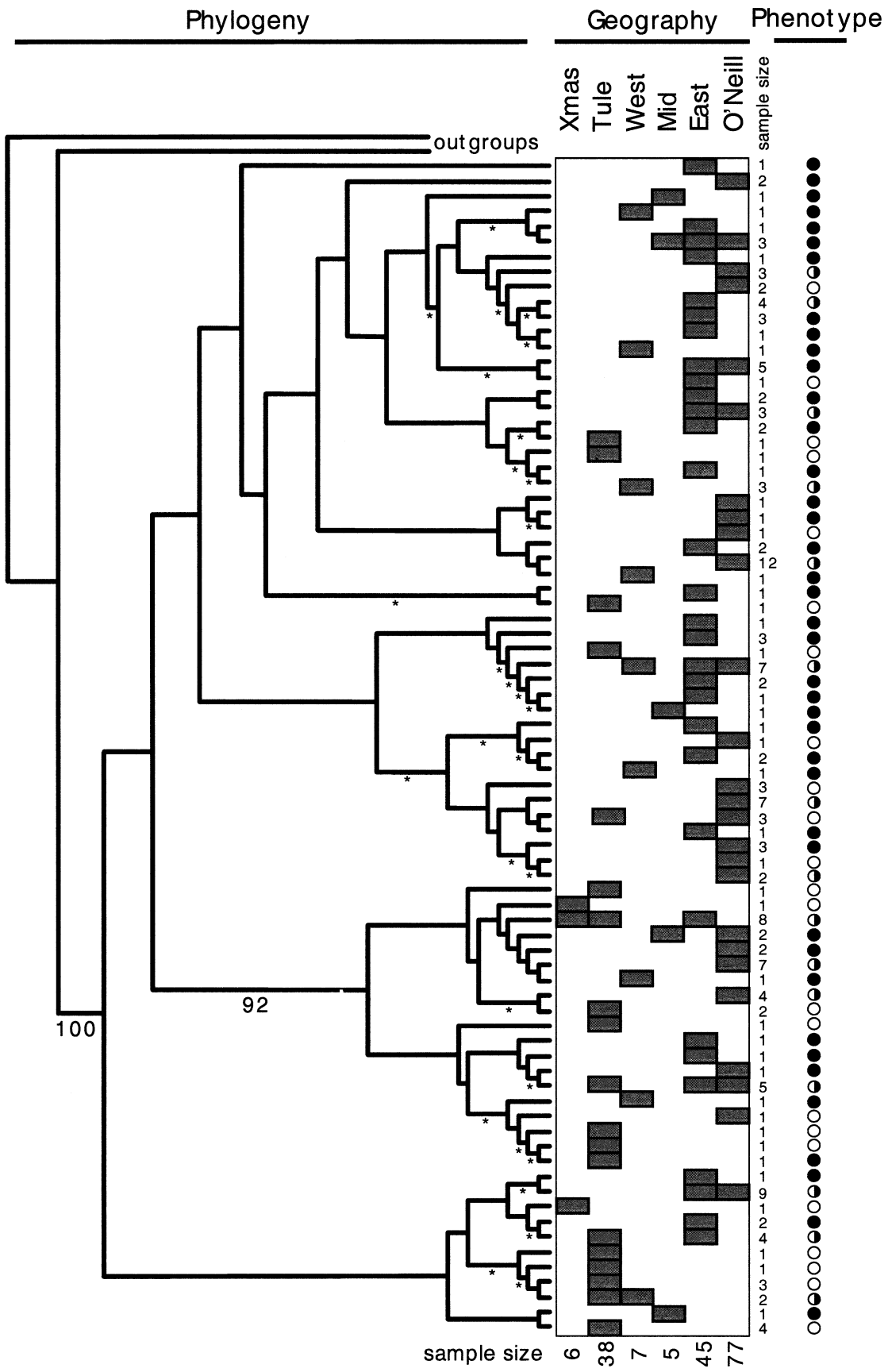


FIG. 4. Polymorphic sites for 78 mtDNA haplotypes based on 1111 bp of the COIII and ND3 genes from 175 individuals. The number of individuals sharing each haplotype is shown in parentheses and *Mc1r* genotypes are shown on the right. Alleles are ordered phylogenetically as shown in Figure 5.


Phylogeny
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sample size
outgroups
100
92
sample size
6
38
7
5
45
77

Migrate estimates of the combined migration were $m = 9.6 \times 10^{-3}$ to 9.6×10^{-4}. Here, migration estimates derived from F_{ST} calculations were approximately four times lower than those estimated using Migrate.

Using the rate of migration between paired sites (above), we estimated the strength of selection necessary to maintain the observed *Mc1r* allele frequencies at each site (Table 5). First, we estimated selection coefficients against the *Mc1r D* allele on light-colored rock at both the Tule Mountains and O'Neill Hills. The observed frequency of melanic *Mc1r* alleles (*D*) at Tule Mountains was $q = 0.029$ and at O'Neill Hills, $q = 0.338$. Following equation 1, selection coefficients were similar at Tule Mountains and O'Neill Hills when the trait was completely dominant (for example, if $N = 10^4$, $s = 0.01$ approximately). Under a model of partial dominance ($h = 0.6$), selection estimates increased slightly. In these populations on light rocks, selection coefficient estimates were based on F_{ST} estimates of migration because the asymmetric migration rates calculated from Migrate suggest that emigration of deleterious alleles ($M \times q$) was similar in magnitude to immigration of deleterious alleles ($m \times Q$) and thus, the selection coefficient is effectively zero. To estimate the strength of selection against light mice on the lava, we used the combined migration from the Tule Mountains and the O'Neill Hills sites to get the total immigration of alleles into the Pinacate lava population. We estimated selection coefficients using both Migrate and F_{ST}-based estimates of migration. We calculated the observed allele frequency of light alleles (*d*) on the lava ($q = 0.140$). Under a model of complete dominance, selection against the *Mc1r d* allele was 0.039–0.389, and under a model of partial dominance ($h = 0.4$) selection against the *Mc1r d* allele was 0.013–0.126. Selection estimates using F_{ST}-based migration rates were approximately four times lower. In all cases, selection against light mice (the *Mc1r d* allele) on the lava was stronger than selection against dark mice (the *Mc1r D* allele) on light rock.

Discussion

In the Pinacate lava region, a strong correlation between *Mc1r* allele distribution and substrate color exists in the face of high levels of gene flow. These results suggest that strong environment-specific selection on mouse color is acting to maintain habitat-specific *Mc1r* allele frequencies. In this system, identification of the genetic basis of an ecologically relevant trait (coat color) allowed us to explore geographic patterns of allele frequencies in the context of known selection pressures.

Phenotypic Variation

Phenotypes differed qualitatively, based on banding patterns of individual hairs, and quantitatively, in reflectance measured using a spectrophotometer. All individuals that were homozygous or heterozygous for the *Mc1r D* allele had uniformly melanic, unbanded hairs, suggesting that the *Mc1r D* allele is completely dominant for unbanded melanic hairs. Spectrophotometry results showed that banded (light) and unbanded (melanic) individuals varied significantly in their total and percent reflectance. However, reflectance intensities differed between unbanded individuals that were homozygous and heterozygous for the *Mc1r D* allele. Thus, the *Mc1r D* allele appears partially dominant in relation to reflectance intensity, raising the possibility that *Mc1r* homozygotes (*DD*) and heterozygotes (*Dd*) may also experience differential selection. However, at present, it is not clear whether predators respond to reflectance intensity or to banding pattern on individual hairs.

We found a strong concordance between substrate color and mouse coat color across all six collecting sites. In addition, we found a strong concordance between *Mc1r* allele frequency and substrate color. These two patterns are correlated because amino acid variation in the *Mc1r* coding region is thought to be largely responsible for differences in coat color. However, the distribution of *Mc1r* alleles was more strongly associated with substrate than was color because most melanic individuals inhabiting light rock were heterozygous, and almost all melanic individuals found on dark rock were homozygous for the *Mc1r D* allele.

TABLE 3. Symmetrical estimates of gene flow between the three sampling sites based on synonymous sites of COIII and ND3 genes.

Site	Distance (km)	Hudson et al. (1992)		Nei (1982)	
		F_{ST}	*Nm*	γ_{ST}	*Nm*
Tule–lava	12.6	0.102	4.40	0.060	7.77
Lava–O'Neill	9.6	0.024	20.59	0.020	24.97

Migration Patterns and Rates

Although the distribution of *Mc1r* alleles was highly structured, analysis of nucleotide polymorphism in the ND3 and COIII mtDNA genes showed no evidence for restricted gene flow between light and dark habitats. First, phylogenetic analyses of mtDNA haplotypes showed no concordance between mtDNA variation and geography. The F_{ST} estimate based on synonymous mtDNA diversity was 0.10 between the Pinacate lava and Tule Mountains and 0.02 between the Pinacate lava and O'Neill Hills. In addition, effective migration rates between paired sites were relatively high ($Nm > 5$), greater than levels that typically lead to population differentiation (Slatkin 1987).

Because of its maternal inheritance, mtDNA only tracks female migration. In mammals, male-biased dispersal is common (Greenwood 1980). Although movement patterns have

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FIG. 5. Neighbor-joining phylogeny of 78 mtDNA haplotypes. Shaded boxes indicate presence of each mtDNA haplotype at each of six collecting localities (shown west to east). Number at the right indicates the number of individuals sharing a given haplotype. Circles indicate light, melanic, or both color phenotypes. Number of individuals collected at each site is shown at the bottom. Asterisks on tree branches represent bootstrap support greater than 60% by both neighbor-joining and maximum-likelihood algorithms. The mtDNA clade used in Figure 3 is denoted by the 92% bootstrap support.

TABLE 4. Asymmetrical maximum likelihood estimates of *Nm* between the three populations, Tule Mountains, Pinacate lava, and O'Neill Hills, using Migrate.

Source population	*Nm*		
	Tule	Lava	O'Neill
Tule	—	20.88	
Lava	0.70	—	7.32
O'Neill		75.03	—

not been studied specifically for *Chaetodipus intermedius*, in heteromyid rodents, migration occurs in both males and females (with slight trend toward female bias in long-distance movements), depending on the species (Jones 1993). In two pocket mice species studied in detail, there was no apparent sex-biased dispersal (Jones 1993). Therefore, in pocket mice, mtDNA may in fact provide an accurate measure of average gene flow for both sexes. If most dispersal is by males, our estimates of *Nm* will be underestimated and our estimates of selection on *Mc1r* will be underestimated. If most dispersal is by females, in contrast, we will have overestimated selection coefficients on *Mc1r*.

Indirect estimates of gene flow inferred from genetic data, as described here, have been criticized for several reasons; one major concern is that most migration models are not biologically realistic (Bossart and Pashley-Prowell 1998). However, the system described here may be a reasonable fit to an island model of gene flow. *Chaetodipus intermedius* are found exclusively in rocky habitats, and are replaced by *C. penicillatus* in sandy areas (Hoffmeister 1986). Thus, the lava flows and rocky outcrops sampled here represent habitat islands. One concern in our system is the assumption that new alleles were introduced exclusively from the sites we sampled. In the Pinacate region, there are several rocky outcrops that we did not sample, and it is possible that migration was occurring between these patches. Although we attempted to minimize this error by sampling in habitat islands that were geographically close, we may have underestimated levels of migration.

Our estimates of migration rate from different methods yielded roughly consistent results. Specifically, measures based on the distribution of genetic variation within and between sites (e.g., F_{ST} and γ_{ST}) were very similar. Although estimating *Nm* from F_{ST} relies on a number of assumptions that may not be biologically realistic (e.g., Whitlock and McCauley 1999), these estimates serve as a reference for *Nm* estimates from Migrate. Both F_{ST} and γ_{ST} produced similar results (within an order of magnitude) to average ML estimates of gene flow (*Nm*) from Migrate, which allowed for asymmetrical gene flow. Estimates from these methods support two observations about migration patterns of pocket mice across this transect. First, levels of migration were limited by distance. Gene flow between the Tule Mountains and the Pinacate lava was ten times lower than gene flow between the O'Neill Hills and the lava. Correspondingly, the distance between the Tule Mountains and the Pinacate lava was greater than the distance between O'Neill Hills and the lava. The lowest levels of migration occurred between Tule Mountains and O'Neill Hills (data not shown, see Fig. 5), consistent

TABLE 5. Estimates of selection coefficients against *Mc1r D* alleles on light rocks and against *Mc1r d* alleles on dark rocks. *N*, sample size; *f*, observed frequency of deleterious alleles (*q*); N_e, effective population size; *s*, selection coefficient; and *h*, dominance coefficient (complete dominance: $h = 0$ when *Mc1r D* is favored and $h = 1$ when *Mc1r d* is favored; partial dominance: $h = 0.4$ when *Mc1r D* is favored and $h = 0.6$ when *Mc1r d* is favored). Estimates of migration, *m*, are derived from Migrate (Table 4), and estimates shown in parentheses are derived from F_{ST} calculations assuming symmetrical migration (Table 3). Migration estimates, to and from the lava site, are combined from the estimates of migration from the Tule and O'Neill sites. Similarly, the average deleterious allele frequency for immigrants to the lava site was taken as the weighted average of the observed allele frequency of *Mc1r d* at the Tule and O'Neill Hills localities ($Q = 0.70$). The average deleterious allele frequency for immigrants to the Tule and O'Neill sites was equal to the observed *Mc1r D* allele frequency on the lava ($Q = 0.86$).

Site	Substrate color	*N*	*f* (*Mc1r* alleles)	N_e	*m*	*M*	*s*, when $h = 0.0/1.0$	*s*, when $h = 0.4/0.6$
Tule	light	85	$q = 0.029$	10^4	7.0×10^{-5} (4.4×10^{-4})	2.1×10^{-3} (4.4×10^{-4})	—[1]	(0.0136)	—	(0.0218)
				10^5	7.0×10^{-6} (4.4×10^{-5})	2.1×10^{-4} (4.4×10^{-5})	—	(0.0014)	—	(0.0022)
Lava	dark	55	$q = 0.140$	10^4	9.6×10^{-3} (2.5×10^{-3})	8.0×10^{-4} (2.5×10^{-3})	0.3890	(0.0829)	0.1264	(0.0271)
				10^5	9.6×10^{-4} (2.5×10^{-4})	8.0×10^{-5} (2.5×10^{-4})	0.0389	(0.0083)	0.0126	(0.0027)
O'Neill	light	77	$q = 0.338$	10^4	7.3×10^{-4} (2.1×10^{-3})	7.5×10^{-3} (2.1×10^{-3})	—[1]	(0.0074)	—	(0.0092)
				10^5	7.3×10^{-5} (2.1×10^{-4})	7.5×10^{-4} (2.1×10^{-4})	—	(0.0007)	—	(0.0009)

[1] Emigration of deleterious alleles ($M \times q$) is equal to or greater than immigration of deleterious alleles ($m \times Q$) when using the Migrate estimates of migration rate. This result is likely due to variance associated with migration estimates and suggests that selection against *Mc1r D* may be quite weak (effectively zero) in the Tule and O'Neill populations.

TABLE 6. Some published estimates of selection coefficients, *s*, acting on color polymorphism. A question mark indicates unidentified gene.

Species	Gene	Selective agent	*s*	Direction of selection	Reference
Pocket mice (*Chaetodipus intermedius*)	*Mc1r*	predation	0.39–0.013	against light mice on dark rock	this study
	Mc1r		0.020–0.0002	against melanic mice on light rock	this study
Peppered moths (*Biston betularia*)	?	predation	0.33–0.19	against light moths on dark trees	Haldane 1924; Kettlewell 1956
	?		0.12	against melanic moths on light trees	Cook and Mani 1980
Ladybirds (*Adalia bipunctata*)	?	predation/physiology[1]	0.67–0.24	against melanics in winter	Creed 1975; Muggleton 1978
	?		0.43–0.09	against nonmelanic (red) in summer	Creed 1975
	?		0.10	against melanics	Brakefield and Lees 1987
Land snails (*Cepaea nemoralis*	?	predation/physiology[1]	0.062	against dark brown	Clarke and Murray 1962
	?		0.052	against unmodified banding	Clarke and Murray 1962

[1] Probable selective agent.

with a signature of isolation by distance. Together these results also suggested that sandy habitats represent biologically significant partial barriers to dispersal for *C. intermedius*. Second, our results suggest that mice from O'Neill Hills and Tule Mountains migrate more often and/or more successfully to the Pinacate lava than vice versa. This pattern of asymmetrical migration may suggest that light-colored mice migrate more successfully over the light-colored Pinta Sands than do melanic mice, perhaps due to cryptic coloration.

It is important to note that our estimates of migration rates are based on data from a single nonrecombining region of the genome (mtDNA) and that single-locus estimates of F_{ST} have a notoriously large variance. Measurements of population structure from additional loci would help refine our estimates. Direct measurements of dispersal through mark-and-recapture studies, while difficult, would also refine our estimates and might distinguish differential migration between melanic and wild-type mice.

Estimating the Strength of Selection on Color Matching

Although we have made many assumptions and our estimates of selection coefficients are necessarily rough, several interesting patterns emerge. First, it appears likely that natural selection may be relatively strong (e.g., estimates of *s* equal 0.389–0.039 against light mice [the *Mc1r d* allele] on dark rock assuming $h = 0$). This value is three times lower under partial dominance ($h = 0.4$). Dice (1947) also found evidence of strong selection against light-colored mice on dark backgrounds in predation experiments using deer mice (*Peromyscus maniculatus*), although his ''selection index'' cannot be translated easily into values of the selection coefficient (*s*).

Evidence of strong habitat-specific selection has also been documented in other systems in which color polymorphism affects survival probabilities, particularly where the selective agent is avian predation (Endler 1986). For example, selection coefficients against light-colored peppered moths (*Biston bechuleria*) on dark-colored trees have been estimated independently by Haldane (1924) to be approximately 0.33 and by Kettlewell (1958) to be 0.19–0.26. Large selection coefficients have been documented on color polymorphisms in other systems as well (Table 6). Selection coefficients on two color morphs of two-spot ladybirds (*Adalia bipunctata*) are exceptionally large ($s = 0.67$–0.10), but selection appears to vary seasonally and the selective agent remains uncertain (Creed 1975; Muggleton 1978). Selection on melanic and banded land snails (*Cepaea nemoralis*) clearly varies with habitat and is thought to be driven by a combination of visual and physiological selection (Clarke and Murray 1962).

A second general observation is that selection coefficients on light mice and melanic mice inhabiting mismatched substrate are not equal. It appears that selection is stronger against light mice inhabiting dark rock than against melanic mice inhabiting light rock. Interestingly, the larger estimate of *s* is associated with the evolution of the novel phenotype: initially light mice encountered and colonized dark rock, and strong selection led to the proliferation of the melanic adaptive phenotype. These asymmetrical selection coefficients also parallel observations from peppered moths, in which there was a rapid increase in the frequency of melanic moths, but a comparatively slow decrease in the frequency of melanic moths when habitat became lighter as pollution levels declined (Kettlewell 1955). It is also important to note, however, that the change in background color from dark polluted habitat to lighter unpolluted habitat was a relatively slow process and also may have contributed to the slower decline of melanic moths. Kettlewell's (1958) observations based on changes in melanic frequencies suggest that selection was stronger against light moths on dark background than against melanic moths on light background. Using mathematical models, Cook and Mani (1980) also found higher selection coefficients for light moths on dark background ($s = 0.19$–0.33) than for melanic moths on light backgrounds. This pattern is also supported by observations that melanic moths are particularly difficult to see when in flight during poor light conditions (Majerus 1998). Thus, these patterns in pocket

mice and moths may be driven by a bias in avian visual systems in which light objects on dark backgrounds are more conspicuous than the reverse.

Our estimates of *s* serve as a first approximation of the strength of selection acting on color matching in lava-dwelling pocket mice. Although these estimates rely on several assumptions and are indeed rough, they help us understand several aspects of the biology of this system. For example, for small values of *s*, the probability of fixation of a new mutation is approximately 2*s*. The large value of *s* associated with the *Mc1r D* allele may explain the evolution of melanism over relatively short evolutionary time scales (less than one million years). Moreover, if *s* is large, we expect selection to maintain locally adapted alleles in the face of gene flow, and we might predict that even small, isolated patches of lava will harbor melanic mice.

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Appendix

Individuals are listed by collecting locale. Collection number is given followed by a code describing phenotype, sex, and age: L, light phenotype; D, melanic phenotype; M, male; F, female; A, adult; J, juvenile. For each individual, the *Mc1r* genotype is provided.

Xmas Pass (N = 6): HEH528 LMA *dd*; HEH529 LMA *dd*; HEH530 LMA *dd*; HEH531 LMA *dd*; HEH532 LMA *dd*; HEH533 LMA *dd*. Tule Mountains (N = 38): HEH544 LMA *dd*; HEH545 LMA *dd*; HEH546 LMA *dd*; HEH547 LMA *dd*; HEH548 LMA *dd*; HEH549 L?A *dd*; HEH550 LFJ *dd*; HEH551 LMA *dd*; HEH552 LMA *dd*; HEH553 LMA *dd*; HEH587 LFA *dd*; HEH588 LFA *dd*; HEH589 LFA *dd*; HEH590 LFA *dd*; HEH591 DMJ *Dd*; HEH592 LFA *dd*; HEH593 LFJ *dd*; HEH594 LFA *dd*; HEH595 DMA *Dd*; HEH596 LFA *dd*; HEH597 LFA *dd*; HEH598 DMA *Dd*; HEH599 LFA *dd*; HEH600 LMJ *dd*; HEH601 LMJ *dd*; HEH602 DFJ *Dd*; HEH603 LFA *dd*; HEH604 LFA *dd*; HEH605 LFA *dd*; HEH606 LMA *dd*; HEH607 LFA *dd*; HEH608 DMJ *Dd*; HEH609 LMA *dd*; HEH610 LMA *dd*; HEH611 LFJ *dd*; HEH612 LFA *dd*; HEH613 LFA *dd*; HEH614 LFA *dd*. Pinacate West (N = 7): HEH534 DMA *DD*; HEH535 DMA *DD*; HEH536 DMA *DD*; HEH537 DMA *DD*; HEH538 DMJ *DD*; HEH539 DFA *Dd*; HEH DMA *DD*. Pinacate Mid (N = 5): HEH541 DMA *Dd*; HEH542 DMA *Dd*; HEH512 DMA *DD*; HEH513 DFA *DD*; HEH514 DFA *DD*. Pinacate East (N = 45): MWN1371 DFA *DD*; MWN1372 DFA *Dd*; MWN1373 DMA *DD*; MWN1374 DMA *DD*; MWN1375 DMA *DD*; MWN1376 DMA *DD*; MWN1377 DMA *DD*; MWN1378 DFA *DD*; MWN1379 DMA *Dd*; MWN1381 DFA *DD*; MWN1382 DFA *Dd*; MWN1383 DMA *DD*; MWN1384 DFA *Dd*; MWN1385 DMA *DD*; MWN1386 DMA *DD*; MWN1387 LFA *dd*; MWN1414 LMA *dd*; MWN1416 DFJ *Dd*; MWN1428 DFJ *DD*; MWN1429 DMJ *Dd*; MWN1430 DFJ *DD*; MWN1431 DMJ *DD*; MWN1432 DMJ *DD*; MWN1433 DFA *DD*; MWN1434 DMA *DD*; MWN1435 DMJ *DD*; MWN1436 DMA *DD*; MWN1437 DFA *DD*; MWN1438 DFJ *DD*; MWN1439 DMJ *DD*; MWN1440 DMJ *Dd*; MWN1441 DFA *DD*; MWN1442 LFA *dd*; MWN1443 DMJ *DD*; MWN1444 DMA *DD*; MWN1445 DFA *DD*; MWN1446 DFA *DD*; MWN1447 DFA *DD*; MWN1448 DFJ *DD*; MWN1449 DFJ *DD*; MWN1450 DFJ *DD*; MWN1451 DFJ *DD*; MWN1452 DMJ *DD*; MWN1453 DFJ *DD*; MWN1454 DMJ *DD*. O'Neill Hills (N = 77): MWN1402 LFJ *dd*; MWN1403 LMJ *dd*; MWN1404 LFA *dd*; MWN1405 LMA *dd*; MWN1406 LFA *dd*; MWN1407 LFA *dd*; MWN1408 LMA *dd*; MWN1409 LFA *dd*; MWN1410 LFA *dd*; MWN1411 LFA *dd*; MWN1412 DMA *Dd*; MWN1455 LFA *dd*; MWN1456 DFJ *Dd*; MWN1457 DMJ *Dd*; MWN1458 DFJ *Dd*; MWN1459 DFA *Dd*; MWN1460 DFJ *Dd*; MWN1461 LMJ *dd*; MWN1462 LFJ *dd*; MWN1463 DMJ *Dd*; MWN1464 DMJ *Dd*; MWN1465 LFJ *dd*; MWN1466 LFA *dd*; MWN1467 LMJ *dd*; MWN1468 DFA *Dd*; MWN1469 DFA *Dd*; MWN1470 LFJ *dd*; MWN1471 DFJ *DD*; MWN1472 DFA *Dd*; MWN1473 DFA *Dd*; MWN1474 DFA *Dd*; MWN1475 DFJ *DD*; MWN1476 LFJ *dd*; MWN1477 DMJ *Dd*; MWN1478 DFJ *Dd*; MWN1479 LFA *dd*; MWN1480 DFJ *Dd*; MWN1481 LMJ *dd*; MWN1482 DMA *DD*; MWN1483 LFJ *dd*; MWN1484 DMJ *DD*; MWN1485 LFJ *dd*; MWN1486 LMJ *dd*; MWN1487 LFJ *dd*; MWN1488 LMJ *dd*; MWN1489 LMJ *dd*; MWN1490 LFJ *dd*; MWN1491 DMJ *Dd*; MWN1492 LMJ *dd*; MWN1493 DMJ *DD*; MWN1494 DFJ *Dd*; MWN1495 DFJ *Dd*; MWN1496 DFJ *Dd*; MWN1497 LMJ *dd*; MWN1498 DMJ *Dd*; MWN1499 LFJ *dd*; MWN1500 DFA *DD*; MWN1501 LFJ *dd*; MWN1502 DMJ *Dd*; MWN1503 LMA *dd*; MWN1504 DMJ *Dd*; MWN1505 LFA *dd*; MWN1506 DFA *Dd*; MWN1507 DFA *Dd*; MWN1508 LFJ *dd*; MWN1509 DFA *Dd*; MWN1510 DFA *Dd*; MWN1511 DFJ *DD*; MWN1512 LMA *dd*; MWN1513 LFJ *dd*; MWN1514 DFJ *Dd*; MWN1515 DFA *Dd*; MWN1516 DFJ *Dd*; MWN1517 DMJ *Dd*; MWN1518 DFJ *DD*; MWN1519 DMJ *Dd*; MWN1520 DFJ *Dd*.