Linking a mutation to survival in wild mice


Adaptive evolution in new or changing environments can be difficult to predict because the functional connections between genotype, phenotype, and fitness are complex. Here, we make these explicit connections by combining field and laboratory experiments in wild mice. We first directly estimate natural selection on pigmentation traits and an underlying pigment locus, Agouti, by using experimental enclosures of mice on different soil colors. Next, we show how a mutation in Agouti associated with survival causes lighter coat color through changes in its protein binding properties. Together, our findings demonstrate how a sequence variant alters phenotype and then reveal the ensuing ecological consequences that drive changes in population allele frequency, thereby illuminating the process of evolution by natural selection.

Although a growing number of genomic studies have pinpointed genes that contribute to phenotypic evolution (1–3), often the ecological mechanisms driving trait evolution remain untested. On the other hand, field studies have documented the action of natural selection on traits (4–6), but the underlying molecular mechanisms are typically unknown. We combine a large-scale manipulative field experiment with laboratory-based genetic and biochemical tests to identify both the ecological and molecular mechanisms underlying trait adaptation in a wild vertebrate. Forging these mechanistic connections will aid in understanding the evolutionary consequences of environmental change in natural populations (7, 8).

We took advantage of recently evolved, cryptically colored populations of deer mice (Peromyscus maniculatus) to investigate the genetic consequences of divergent natural selection. The Sand Hills of Nebraska were formed from light-colored quartz ~8,000 to 10,000 years ago (9). This dune habitat differs from the surrounding habitat in physical properties, most notably the soil color (10) (Fig. 1). Because the Sand Hills are geologically young and ecologically distinct, deer mouse populations inhabiting the area are expected to have recently evolved and strongly selected adaptations to this environment. One example of such an adaptation is pigmentation. The dorsal coats of deer mice are correlated with substrate color, with light mice occupying the light Sand Hills (11). The primary hypothesis for this phenotypic change is selection for crypsis against avian predators (11–13). Pigmentation differences between habitat types are associated with multiple mutations in Agouti (14, 15), a locus that mediates the production of yellow pigment (pheomelanin) in vertebrates (16, 17) and deer mice, specifically (18). Thus, Sand Hills deer mice and the Agouti locus are a useful system to directly test both the ecological and molecular mechanisms by which specific sequence variants alter phenotype and ultimately fitness.

Divergent selection on pigmentation in experimental enclosures

To explicitly test for selection that favors locally adapted pigment phenotypes, we collected 481 wild mice from the ancestral “dark” and derived “light” sites. We then introduced 75 to 100 individuals in equal proportions on the basis of the capture site (i.e., dark versus light) to each of six field enclosures (three in each habitat) that measured 50 m by 50 m and were devoid of native mice and terrestrial predators but open to avian predators (Fig. 1) (19). Among these founding individuals, we identified significant differences in five pigment traits (dorsal brightness, dorsal chroma, ventral brightness, ventral chroma, and tail pattern) between mice captured at dark versus light sites (all traits: \( P < 0.001 \)) (fig. S1). Pigment phenotypes

![Fig. 1. Experimental site and environmental variation in the Sand Hills region of Nebraska.](http://science.sciencemag.org/)

(A) Map of Nebraska showing the Sand Hills region (light color) and location of the enclosure experiment. (B) Map of the replicate enclosures (squares) and sampling locations for mice introduced to the enclosures (stars) used in the experiment (table S8). Light blue asterisks indicate the six enclosures used; we did not introduce mice to the fourth enclosure at either site. (C) Enclosures are shown at the light site in Sand Hills habitat (truck for scale). (D and E) Typical habitat is shown on the Sand Hills (D, light habitat) and off the Sand Hills (E, dark habitat); insets show typical soil substrate.
were largely independent, with weak and mostly insignificant correlations among traits [coefficient of determination ($R^2$) < 0.06 for all traits] (table S1), suggesting that these traits may be subjected to independent selection.

Using a mark-recapture approach, we tracked survival of these introduced individuals during five 2-week sampling periods over 14 months, by which time mortality reached 100% in most enclosures (Fig. 2, A and B), similar to mortality rates in the wild (19). Because sampling error is inversely proportional to the number of survivors, we focused our analyses on a comparison between the colonizing populations (time point 0) and survivors present at time point 1 (~3 months after the start of the experiment), when average survival rates were 45%. Regardless of sampling origin or phenotype, the survival rates were twice as high in dark enclosures relative to light enclosures (60% versus 30% at time point 1).

Mice introduced to enclosures that matched the habitat type in which they were originally caught had greater survival than nonlocal mice (Fig. 2, A and B, and table S2) (18), suggesting local adaptation of populations from each environment type.

To explicitly test if pigmentation may be contributing to local adaptation, we tested for shifts in the distribution of pigment traits over time. We documented significant selection on pigmentation, primarily manifested through higher survival of mice with locally cryptic dorsal pigmentation (85% Bayesian credible intervals for the effect of the interaction between dorsal brightness and experimental treatment on survival do not contain zero (18)) (Fig. 2, C to F, and tables S2 and S3). In light enclosures, the surviving mice were, on average, 1.44 times lighter in dorsal color than the average mouse in the founding populations, whereas in dark enclosures, the average mouse was 1.98 times darker. Linear selection gradients for dorsal brightness were positive in all single light site enclosures and negative in all three dark site enclosures (one-sided t test of linear selection gradients in light versus dark enclosures: $t = -6.079$, df = 2.518, $P = 0.015$ (table S3). With the exception of ventral chroma in a single enclosure, no significant directional selection was detected on any other trait but dorsal brightness (tables S2 and S3). There was also no evidence for quadratic or correlational selection in the data (tables S4 and S5). Thus, divergent natural selection was likely acting on dorsal brightness between the two environment types.

Previous work with plasticine model mice suggests that avian predation is high in this region (18). Moreover, owls are highly effective predators of mice and can discriminate between different color morphs even under moonlight conditions (22). During our field experiment, we observed owls hunting at the experimental sites (eight observations over 70 nights). Because the enclosures largely exclude other predators, we suggest that the significant association between dorsal brightness and survival

**Fig. 2.** Mortality and phenotypic change in the experimental populations. (A and B) Mortality in pooled enclosures at light (A) and dark (B) sites over five sequential episodes of selection (18). Bars represent the number of surviving individuals (independent of coat color) at each time point. Black lines represent the proportion of surviving individuals that were originally caught on the opposite habitat type of the enclosure type they were placed in (mice from dark habitat in light enclosures and mice from light habitat in dark enclosures). Conspicuously colored mice are shown on typical substrate at each experimental site. Dashed boxes denote the time period used in selection analyses. (C and D) Distributions of dorsal brightness at time point 0 (blue) and time point 1 (red) at the light (C) and dark (D) sites. (E and F) Visualizations of selection on dorsal brightness at the light (E) and dark (F) sites between time point 0 and 1. Cubic spline plots are generated from predicted values. The solid lines represent the fitted spline, and the dotted lines represent ±1 Bayesian SE.
is likely driven by higher rates of avian predation on mice with conspicuous pigmentation.

The genetic consequences of selection on pigmentation
To investigate how selection on dorsal brightness impacts allele frequencies at the Agouti locus, we generated polymorphism data with enriched sequencing of (i) a 185-kb region that encompasses Agouti and all known regulatory elements and (ii) ~2100 unlinked genome-wide regions, each averaging 1.5 kb in length (following (20)), to control for demographic effects. In brief, we sequenced all 481 individuals and, after filtering, identified 2442 and 53,507 variable, high-quality sites in or near the Agouti gene and genome-wide, respectively. From these data, we observed greater changes in allele frequency at Agouti over time in the light than in the dark enclosures, consistent with higher mortality in light enclosures (Wilcoxon rank sum test: \(W = 3,497,200, P < 0.001\)) (fig. S2A).

To determine whether the changes in allele frequency at Agouti are best explained by selection or neutrality (i.e., random mortality), we calculated, for every Agouti variant site independently, the probability that the distribution of genotype frequencies observed in the survivors represents a random sample from the initial population (18). After 3 months, the surviving mice showed nonrandom genotype frequencies at 353 and 549 single-nucleotide polymorphisms (SNPs) in the light and dark enclosures, respectively (fig. 3A and B). To account for the large number of tests involved, we used a resampling procedure to determine how many SNPs would be expected to show significant changes by chance alone. In the light enclosures, the patterns of allele frequency change at Agouti SNPs could not be distinguished from neutrality (fig. 3C), likely because of reduced statistical power caused by the low number of survivors. By contrast, in the dark enclosures, our results reject the null hypothesis, suggesting that the number of significant changes in allele frequency is incompatible with a strictly neutral model (fig. 3D). Therefore, in the dark enclosures, we find allele frequency changes at the Agouti locus consistent with selection, and thus, patterns at the genetic level parallel the change observed at the phenotypic level.

Because there is no recombination between loci in a single generation, we further tested whether the large number of sites with significant allele frequency changes in the dark enclosures could be explained by correlated responses at loci linked to a limited number of SNPs under selection (18). From our phenotypic selection results, we a priori hypothesized that SNPs associated with dorsal brightness should be experiencing direct selection. Thus, for each of 31 Agouti SNPs associated with dorsal brightness (15), we compared genotype frequencies under a model with and without selection (18). Of these, seven SNPs, including six SNPs in or near regulatory regions of Agouti and a single amino acid deletion of serine at amino acid position 48 in exon 2 (ASer), had an allele frequency change that could not be explained solely by random sampling (fig. 4A and table S6). Four of these seven SNPs also exhibited high levels of differentiation between mice originally captured from light and dark habitats (fig. 4B and table S6). In addition, one regulatory SNP and the ASer have been associated with historical signals of positive selection in Sand Hills populations (14, 15).

To test whether selection on each of these candidate variants could account for the observed number of SNPs with biased genotype frequencies in the survivors, we recalculated null distributions by assigning each candidate individually as our single selected target site. After correction for multiple testing, each of the seven could account for the observed change in genotype frequencies in the survivors (fig. 4C). By contrast, a model using the SNP from the genome-wide control dataset with the most significant allele frequency change cannot explain the observed patterns (fig. S2B). Linkage disequilibrium (LD) analyses of the seven candidate variants identified three linkage blocks (fig. S2C): two sets of three physically proximate regulatory SNPs and the ASer, the latter displaying low LD with all other candidate SNPs (fig. 4D). These data suggest that each of these three linkage blocks harbors variants directly responding to selection on dorsal brightness. Thus, selection on a limited number of genetic targets in the Agouti locus is likely sufficient to drive shifts in allele frequency and rapid change in phenotype.

The functional and ecological effects of a deletion mutation in Agouti
To test the functional link between one of the variants in Agouti associated with survival and pigmentation, as well as uncover the causal molecular mechanism, we focused on the amino acid mutation ASer in Agouti. We chose this variant because the ASer was strongly associated with dorsal brightness (\(R^2 = 0.11, P < 0.001\)) (fig. 5A), showed a signature of selection in the enclosure populations (fig. 4A) as well as in an admixed natural population (15), and showed the highest level of genetic differentiation across the Agouti locus between mice that were originally captured from light and dark habitat (\(F_{ST} = 0.34\)) (fig. 4B and table S6). To determine whether the ASer mutation alone has an effect on hair color in vivo, we generated matching lines of transgenic lab mice (C57BL/6 mice, a strain with no endogenous Agouti expression) carrying the wild-type (WT) or the ASer Peromyscus Agouti cDNA, constitutively driven by the Hsp68 promoter (fig. 5B). We used the eCas9 integrase system, which produces single-copy integrants at the H1HP3 locus on mouse chromosome 11 to directly measure the effect of the Agouti ASer while avoiding variation caused by copy number, insertion site, or orientation of the construct (21) (fig. S3, A and B). Using a spectrophotometer to quantify differences in coat color, we found that ASer mice had significantly lighter coats than mice carrying the WT Peromyscus Agouti cDNA (ASer versus WT, two-tailed t test; \(n = 5, P = 0.001\)) (fig. 5C). Thus, the Agouti ASer mutation alone has a measurable effect on pigmentation and in the direction expected on the basis of the genotype-phenotype association data in natural Peromyscus populations.

![Fig. 3. Allele frequency change at the Agouti locus. (A and B) Allele frequency change from mortality during the experiment in the pooled light (A) and dark (B) enclosures. The x axis represents the change in allele frequency between initial colonizing populations and survivors sampled after 3 months. The y axis represents the probability of the distribution of genotype frequencies observed in the survivors, assuming a neutral model. All red points are significant at the 1% level: Light red points are significant because of a bias in the observed proportion of heterozygotes, whereas dark red points exhibit a bias in the observed number of homozygotes. (C and D) Null distribution of the number of sites expected to show significant allele frequency change at the 1% level in the pooled light (C) and dark (D) enclosures. Vertical red lines represent the observed number of sites with significant allele frequency change.](http://science.sciencemag.org/content/sci/363/6426/499.full)
To further characterize the phenotypic effects of the ΔSer variant, we examined and quantified pigment in dorsal hair. Microscopic examination of individual hairs revealed that the hair of ΔSer mice contained a qualitatively lighter pigment than that of WT mice (Fig. 5B). We then analyzed pheomelanin content in the hair by using chemical degradation products followed by high-performance liquid chromatography (HPLC) (22–25). ΔSer mice had significantly lower amounts of pheomelanin (both benzothiazine and benzothiazole types) than hair from WT mice (ΔSer versus WT, two-tailed t test; \( n = 5, P = 0.002 \)) (Fig. 5C and fig. S3C). These results indicate that the Peromyscus ΔSer causes a decrease in production of pheomelanin, which in turn causes hair to appear brighter overall.

The ΔSer mutation is found in a highly conserved region of the N-terminal domain of the agouti protein, a region that directly binds to attractin, a transmembrane receptor expressed in melanocyte membranes and required for agouti function (26). To understand the mechanism by which ΔSer decreases pheomelanin production, we measured real-time binding interactions between the agouti and attractin proteins by using surface plasmon resonance (SPR). In SPR, one molecule (ligand) is immobilized on a sensor surface while a potential interacting partner (analyte) is injected; the reflection angle of polarized light from the sensor then serves as a proxy for the strength of the interaction between the molecules. For a ligand, we used the secreted isoform of natural human Attractin (ATRNEc), and for the analyte, we used a synthetic version of the Peromyscus agouti WT or ΔSer N-terminal domain, a region known to retain full biochemical activity and bind attractin (26). Application of the WT or ΔSer agouti N-terminal domain to an attractin-coated chip produced sensorgrams characteristic of a biological interaction, approaching equilibrium over several minutes and declining during washout to levels above baseline (Fig. 5D). However, we found that the WT N-terminal domain showed a stronger
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Competing interests: The authors declare no competing financial interests.

Data and materials availability: We have deposited sampling, phenotype, and survival data in the Dryad Digital Repository (44) and sequence data in the NCBI Short Read Archive with the primary accession code SUB411476. The R code implementing capture-recapture analyses is available from https://doi.org/10.5281/zenodo.1758243 (45). The R code implementing analyses of genotype distributions is available from https://doi.org/10.5281/zenodo.1758243 (46).

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/363/6426/499/suppl/DC1

Materials and Methods

Supplementary Text

Figs. S1 to S3

Tables S1 to S9

References (47–64)

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How natural selection affects mouse coat color
Evolution, at its core, involves changes in the frequency of alleles subject to natural selection. But identifying the target of selection can be difficult. Barrett et al. investigated how allele frequencies affecting pigmentation change over time (see the Perspective by Pelletier). Wild-caught mice (Peromyscus maniculatus) were exposed to avian predators against naturally occurring dark or light backgrounds. Natural selection yielded shifts in coloration owing to genetic variants in the mouse coat color Agouti gene.

Science, this issue p. 499; see also p. 452

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