

THE SELECTIVE ADVANTAGE OF CRYPSIS IN MICE

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The light color of mice that inhabit the sandy dunes of Florida's coast have served as a textbook example of adaptation for nearly a century, despite the fact that the selective advantage of crypsis has never been directly tested or quantified in nature. Using plasticine mouse models of light and dark color, we demonstrate a strong selective advantage for mice that match their local background substrate. Further our data suggest that stabilizing selection maintains color matching within a single habitat, as models that are both lighter and darker than their local environment are selected against. These results provide empirical evidence in support of the hypothesis that visual hunting predators shape color patterning in *Peromyscus* mice and suggest a mechanism by which selection drives the pronounced color variation among populations.

KEY WORDS: Adaptation, camouflage, natural selection, *Peromyscus*, plasticine model, stabilizing selection.

One-hundred fifty years ago in *The Origin of Species*, Charles Darwin famously proposed his theory of evolution by natural selection. To build the argument for natural selection, Darwin often relied on intuitive examples: “when we see. . .the alpine ptarmigan white in winter, the red grouse the color of heather, and the black-grouse of peaty earth, we must believe that these tints are of service . . . in preserving them from danger” (Darwin 1859). Perhaps inspired by Darwin's intuition that camouflage confers a survival advantage, color variation became the focus of many of the early studies of adaptation. In particular, studies on color matching in *Peromyscus* mice were instrumental in documenting natural selection in the wild—strong correlations between local soil color and dorsal coat color were repeatedly found among populations (e.g., Dice 1940). Further, JBS Haldane (1948) demonstrated theoretically that spatially varying selection could lead to and maintain locally adapted phenotypes, again using *Peromyscus* color matching as the prime empirical example. These few early studies were broadly seen as evidence that selection could drive color matching in a variety of taxa.

Despite nearly a century of work on this system, however, a direct and empirical quantification of the selective advantage

for color matching in a natural setting is still lacking. Enclosure experiments have been suggestive, albeit contrived—these studies often use unnatural densities of mice, extreme color variants, and/or largely depend on the personalities of one or two individual predators (“the long horned owl was very shy and erratic in his behavior,” whereas “the barn owl was a much more consistent worker”; Dice 1949). Moreover, even with enclosure experiments and more traditional common-garden approaches, it is difficult to disentangle direct selection on color (due to crypsis) from selection on other traits (e.g., odor, activity level, or escape behavior) that may be correlated with color. To address these concerns, recent research has taken a novel experimental approach—using photographs (Webster et al. 2009), manipulated prey (Cuthill et al. 2005; Ioannou and Krause 2009), and computer-generated images (Kiltie and Laine 1992; Chlao et al. 2007)—to empirically test hypotheses about the adaptive significance and function of animal color (Stevens and Merilaita 2009). Similar to these studies, here we expose predators to prey models to directly estimate the selective advantage of camouflage in nature.

The use of plasticine models to address questions in evolutionary biology was pioneered by Brodie (1993) and since has

been used to document predation in reptile and amphibian species. These studies have demonstrated how variation in aposematism (e.g., Kuchta 2005; Saporito et al. 2007; Noonan and Comeault 2009), Batesian mimicry (e.g., Brodie and Janzen 1995; Pfennig et al. 2001), body shape (e.g., Shepard 2007), and sexual signaling (e.g., Husak et al. 2006) affect predation rates. Although simple, this method has several advantages. First, because plasticine preserves evidence of predation attempts (i.e., tooth, beak or claw imprints), it is possible to quantify both predation rate and predator type. Moreover, using models, we can deploy a large, and biologically realistic, number of individuals within a given environment. Finally, this experimental approach allows us to focus on variation in a single trait of interest (and thereby control for indirect selection on correlated traits) because models can be made otherwise identical. Here, we use plasticine models of mice, which differ in only color phenotype, to test for and quantify the selective advantage of camouflaging color in realistic models by exposing them to natural predators in the wild.

Materials and Methods

The most extreme case of color adaptation in *Peromyscus* occurs among subspecies of *Peromyscus polionotus*—the dark-brown dorsal coats of inland subspecies occupying abandoned agricultural fields (oldfields) contrast strikingly with the pale-colored beach mice that inhabit Florida's coastal sand dunes and barrier islands (Sumner 1929a,b). Using nonhardening plasticine, we constructed 250 models of *P. polionotus*, half of which were painted to mimic the coat color and pattern of the dark oldfield mouse (*Peromyscus polionotus subgriseus*) and half the light Santa Rosa Island beach mouse (*P. p. leucocephalus*). We then deployed these models in both light and dark habitat, recorded predation events, and estimated the selective advantage of camouflage.

MOUSE MODEL CONSTRUCTION

To produce the most realistic models, we created silicone molds of *P. polionotus* specimens preserved in a crouched position and poured fully liquefied Van Aken Plastilina into these molds. When cooled, we removed models and painted them using Rustoleum (Rust-Oleum, Vernon Hills, IL) textured spray paint. All models were first spray-coated with white, the dark models received an additional coat of gray then tan paint, and both types were finalized with hand painting of eyes and dorsal pelage in either brown (dark models) or tan (light models).

MODEL DEPLOYMENT

Models were simultaneously deployed in eight linear transects, four set in beach habitat, at Topsail Hill Preserve State Park (N30°21'50.831"W86°17'18.368") and four in inland fields,

29.2 kilometers northeast at Lafayette Creek Wildlife Management Area in Florida (N30°32'22.999" W86°3'26.568"; Fig. 1A). At both locations, we had recently captured live *P. polionotus*. Over the course of the experiment, we moved each of the eight transects four times, for a total of 32 transects, each left in place for 72 h. Within each habitat (beach or inland), simultaneous transects were set a minimum of 100 m apart or end to end, and consecutive sets of transects were set a minimum of 500 m apart. Transects consisted of 14 light and 14 dark models set out in a random order, spaced 10 m apart, and placed in the most open soil patch available. Models were checked every 24 h, and attacked models were replaced with an identical type to maintain light:dark ratios. This design resulted in a total of 2688 "model-nights."

PREDATION SCORES

Predation events were identified as models that had been obviously attacked (based on the presence of tooth marks, bill marks or other imprints) or clearly picked up and carried in the presence of predator tracks. We were unable to find 12 models, which we identified as missing. These data were not included in selection estimates, but could be included in survival analyses for which they were considered "censored data." Five models had imprints that we could not assign to a predator class (i.e., mammalian or avian); however, the results of the analyses did not differ when these data were omitted, so they were included (and labeled "ambiguous"). We also excluded five models that showed evidence of nonpredatory rodent gnawing.

MODEL AND SOIL BRIGHTNESS

We collected soil samples from around each deployed model and measured soil brightness. Brightness was measured similarly for models and four representative museum specimens of each subspecies (Fig. S1). Specifically, we measured reflectance across the light spectrum visible to most predators (300–700 nm; Bennett and Cuthill 1994), using a USB2000 spectrophotometer (Ocean Optics, Dunedin, FL). We calculated overall brightness as the average reflectance across these wavelengths (following Mullen and Hoekstra 2008). Because the models were exposed to a natural community of predators, we did not take into account the visual sensitivity of any particular receiver, which can provide a more accurate measure of camouflage (see Stevens 2007). To account for heterogeneity within a sample, we averaged across multiple independent measurements; 10 for soil and 22 for mouse models and specimens (14 from the shoulder to the rump laterally on the left [1–7] and right [8–14], and 8 dorsally from the forehead to the base of the tail [15–22]). The average brightness for each soil and model sample was used in subsequent analyses. The measurements obtained for real mice fall within the range of those obtained for model mice (Fig. S2).

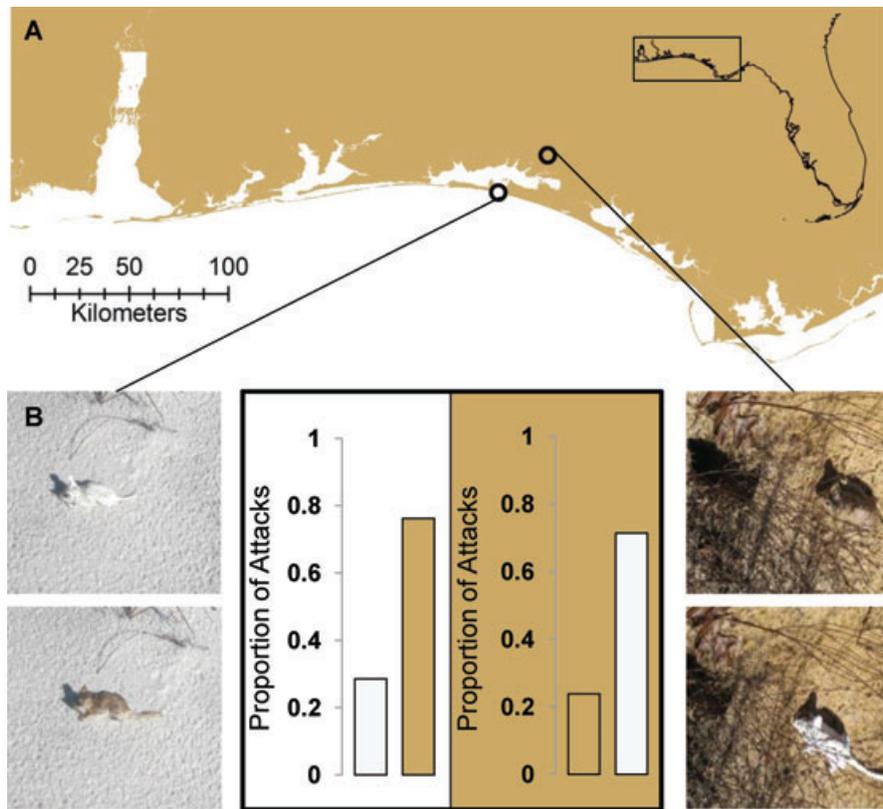


Figure 1. Predation on dark and light models on dark and light soil. (A) Location of experiments in dark inland habitat at Lafayette Creek Wildlife Area and light beach habitat at Topsail Hill State Park in Florida. (B) Typical light and dark models on soil at dark and light locations and the corresponding proportion of predation events occurring in each category.

SELECTION ESTIMATES

We calculated the proportion of attacked mice that mismatched their habitat as a percentage (number of noncryptic attacked mice relative to total number of mice attacked; Table 1) and measured significance using G-tests with 1:1 expected proportions. We next estimated the strength of this selection against conspicuous mice in both habitats by calculating a selection index (SI; Dice 1947, 1949). Specifically, the selection index quantifies the relative survival of two phenotypes, which are initially in equal abundance—simply $(a - b)/(a + b)$, in which “*a*” and “*b*” are the numbers of attacked individuals for each phenotypic class. The SI estimate is particularly appropriate here because we deployed equal numbers of light and dark models, the phenotype is discrete (cryptic vs. noncryptic), and we are interested in relative survival based on color differences. Finally, we used survival analysis to measure the cumulative survival probabilities of cryptic and noncryptic models overall and then separately in each habitat. Specifically, we used a Kaplan–Meier estimator (1958) to describe the survival functions, a log rank test (Mantel and Haenszel 1959; Cox 1972) to determine differences between survival functions, and Cox regression (Cox 1972; Cuthill et al. 2005) to test for a significant effect of habitat. This approach allowed

us to estimate the comparative risk of a predation event in both habitats over time.

To explore the pattern of selection within a habitat, we measured the difference in brightness between each model and its local soil environment (except cases in which the models were not recovered). To specifically test the hypothesis that selection was highest against the most conspicuous models, we treated the difference in model-soil brightness as a single trait, and estimated selection using relative survival of all models as an estimate of fitness. Attacked models had a fitness of 0, nonattacked models a fitness of 1, and relative fitness was estimated by dividing these estimates by mean survival of the population. Following the approach of Lande and Arnold (1983), we calculated the selection gradient as a quadratic regression of the standardized model-soil difference values on relative “survival” and doubled the quadratic coefficient to more accurately estimate the selection coefficient (Stinchcombe et al. 2008). We determined significance using logistic regression (Fairbairn and Preziosi 1996) and a one-tailed test. To visualize the fitness surface, we binned model-soil differences into categories of five and fit a cubic spline through these values (using a $\lambda = 1.3$; Schluter 1988; Schluter and Nychka 1994).

Table 1. Location (dark or light habitat), number and type (dark or light) of models attacked, and class of predator (mammalian, avian or ambiguous).

Transect	Dark model-light habitat Noncryptic	Dark model-dark habitat Cryptic	Light model-dark habitat Noncryptic	Light model-light habitat Cryptic
L-1	2 (mamm/avian)			
D-1		1 (mammalian)	1 (mammalian)	
D-2		1 (avian)		
L-4	1 (ambiguous)			
D-4			1 (ambiguous)	
D-5			1 (mammalian)	
D-7			1 (avian)	
D-8		1 (mammalian)	1 (mammalian)	
L-9	1 (avian)			1 (mammalian)
D-9			1 (avian)	
D-10			2 (mamm/ambig)	
L-11	1 (avian)			
D-11			1 (ambiguous)	
D-12			1 (ambiguous)	
D-14			4 (3 mamm/1 avian)	
L-15				1 (avian)
D-15		1 (mammalian)	1 (mammalian)	
D-16		1 (mammalian)	1 (mammalian)	
Total	5	5	16	2

Results

Survival was lower in conspicuous models (Fig. 2A; $P = 0.056$). Specifically, 75% of all predation events occurred on mice that did not match their substrate (Fig. 1B), representing a significantly higher rate of predation ($G = 6.27$, $P = 0.01$) and a large selective disadvantage ($SI = 0.50$, χ^2 , $P = 0.01$; SI varies from 0 to 1). In other words, conspicuous models are three times more likely

to experience predation. Overall predation rate was lower in the beach habitat (Fig. 2B; $P = 0.01$), where active predator control is practiced to protect threatened/endangered beach populations. Yet, we still found a significant difference in the survival functions among all four comparisons (Fig. 2B; $P = 0.01$)—cryptic models had higher survival than noncryptic models both overall and in each habitat. Within a habitat, we found evidence that nonlinear

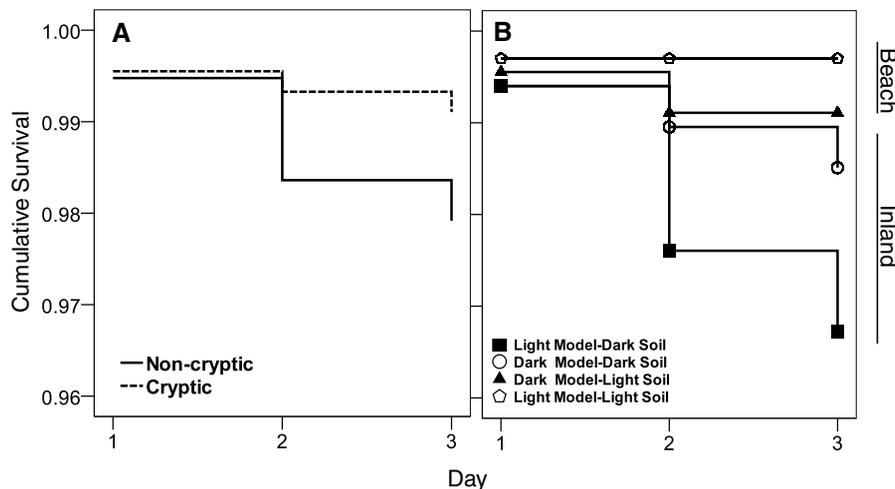


Figure 2. Survival functions for cryptic and noncryptic models (A) across both dark inland and light beach soils and (B) in each habitat separately, in which open symbols indicate cryptic models, and solid symbols represent noncryptic models. “Day” is the number of days a model was exposed to predators. “Cumulative survival” was as calculated using the Kaplan–Meier estimator.

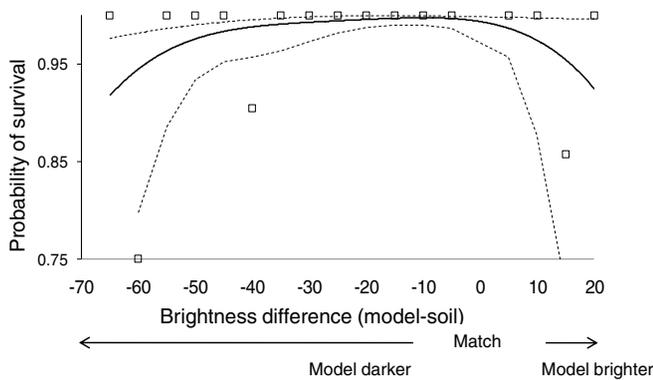


Figure 3. The fitness surface, as calculated using a cubic spline, suggesting reduced survival when models are either much darker or much lighter than their local substrate. Dashed lines indicate bootstrap-calculated standard errors. To best visualize the data, we binned the model-soil difference (squares) in multiples of 5, thus each square may represent more than one model. Bins in which there were no attacks have a “probability of survival” of one, whereas those in which attacks occurred fall below one, proportional to the frequency of attacked models within that bin.

selection favors crypsis. Because the degree of matching between the brightness of the models and their local substrate varied, we could measure predation within the light habitat. Models that were both lighter and darker than their local environment experienced a lower rate of survival than models that were better matched (stabilizing selection gradient $\gamma = -0.034$, $P = 0.04$; Fig. 3). Finally, we used tooth or bill imprints to determine the natural predators and their relative predation rates (Table 1): 54% of attacks were by mammalian carnivores (i.e., coyotes, foxes, or domestic cats) and 26% by avian predators (i.e., owls, raptors, or herons). The remaining 18% had ambiguous marks (scratches or tears to the ears or tail). Although sample sizes are small, these data suggest that natural predators may be adept at detecting even slight variations in mouse color relative to their substrate and may explain why beach mice retain some pigment on their dorsum.

Discussion

We found that cryptic individuals had a clear and strong survival advantage over conspicuous individuals, who were three times more likely to be attacked. This result was consistent across both the light, sparsely vegetated beach environment and the darker, more densely vegetated, inland habitat. Although the overall rate of predation was higher in the dark inland habitats, in relative terms, it was equally disadvantageous to be a light mouse in dark habitat as a dark mouse in light habitat. Further, mismatched individuals were selected against by both visually hunting avian predators and mammalian predators.

We also were able to document the selective advantage of substrate matching at a finer scale by examining predation in a single environment. Here, we focused on the evolution of light coloration in the beach environment because: (1) we expected selection for color matching to be stronger than in mainland habitat as there is less vegetative cover, (2) the light phenotype is derived and may have evolved recently (McNeil 1950), and (3) the genetic changes responsible for the evolution of light color have recently been identified (Hoekstra et al. 2006; Steiner et al. 2007; Mullen and Hoekstra 2008). In the light-substrate beach environment, most attacked mice were dark, but some light models also were attacked. We found that these light-colored models were all much lighter than their local substrate. In other words, selection acts against mice that are either too dark or too light relative to their background. This result not only demonstrates that there is selection on subtle color phenotypes within a habitat, but also suggests a mechanism for the substantial and rapid phenotypic divergence among beach mouse subspecies (Mullen et al. 2009).

Together our data demonstrate that cryptic coloration in *Peromyscus* confers a selective advantage in nature and show that color matching is driven by visually hunting predators. These results not only provide field-collected empirical evidence for a century-old assumption, but also provide the ecological underpinning for recent molecular-genetic studies that have identified the genes, and in some cases mutations, responsible for the evolution of cryptic coloration in these mice (Hoekstra et al. 2006; Steiner et al. 2007). The results of this study provide the ecological context that makes color matching in *Peromyscus* one of the most complete examples—from genes to ecology—of natural selection in the wild.

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Supporting Information

The following supporting information is available for this article:

Figure S1: Spectrographs (300–700 nm wavelengths) for (A) light (above 50% reflectance) and dark (below 50% reflectance) models, (B) representative *P. p. leucocephalus* (above 50%) and *P. p. subgriseus* (below 50%), (C) soil samples from light transects, and (D) soil samples from dark transects.

Figure S2: Average brightness calculated for light and dark models relative to representative specimens of each subspecies (*P. p. subgriseus* = dark, and *P. polionotus leucocephalus* = light).

Supporting Information may be found in the online version of this article.

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