From Darwin to DNA: The Genetic Basis of Color Adaptations

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In 2009, we celebrated Darwin's 200th birthday and the 150th anniversary of his magnum opus, *On The Origin of Species*. The celebrations took varied form. There were the usual, but far more numerous, scientific meetings, symposia, and festivals held in Darwin's honor at universities worldwide. Darwin's complete works—50,000 pages of text and 40,000 images—were released online and available to all. There were also more creative celebrations. A German shipwright built a replica of the *Beagle*, which retraced Darwin's famous voyage around the world, but this time carrying his great great granddaughter, Sarah Darwin (and modern engines, radar, GPS navigation, satellite communications, and a large film crew). Another descendant of Darwin, his great great grandson, and heir to Darwin's Y chromosome, had his genome sequenced, providing a glimpse into Darwin's genetic ancestry.

So why all the fuss about Darwin's birthday? What did Darwin really do? Arguably, more than any other scientist, Darwin changed our worldview. Whereas Newton and Einstein unquestionably had profound effects in their respective fields, one might argue that they each revolutionized physics. But Darwin, not only revolutionized biology, he also forced us to fundamentally change the way we think about ourselves, and our position among all living creatures. Simply put, Darwin was able to explain biological diversity without invoking a deity. That is, the apparent "design" we observe in nature—the close fit of organisms to their environment—could be explained largely by the combination of random mutations (genetic variability) and the nonrandom sorting of that variation by the process of natural selection. Humans included.

The idea may seem simple now, but back in 1859, the source of variation (i.e., mutations) and, more precisely, the mechanism by which this variation was inherited from parent to offspring, was a black box. Although Gregor Mendel's famous genetic work showing simple inheritance patterns of smooth and wrinkly peas, for example, was published in 1866, it wasn't rediscovered (or fully appreciated) until 1900. There was no notion of a chromosome, a gene, or even DNA. This, in many respects, makes Darwin's accomplishments all the more remarkable—he observed that offspring resembled their parents and thus

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knew traits could be passed on from generation to generation, but he had no idea *how*.

In a historical twist of fate (unearthed by Matt Ridley in 2004), Darwin has been linked inextricably to a second great discovery—that is, the double-helical structure of DNA. Let me explain. In 1882, just two weeks before he died, Darwin published a paper in the prestigious journal *Nature*. He reported the discovery of a small clam found clamped to the leg of a beetle found in a pond in the English Midlands. This was to be his last publication. For its time, this was an important discovery. Why? Naturalists (particularly those interested in freshwater bivalves) were in a heated debate about why clams, living in isolated ponds, were so uniform in size and shape, when if they were truly isolated one would expect them to differentiate with time. There were two main hypotheses to explain this apparent paradox. The first, and leading hypothesis, was that all the small lakes were only recently isolated, so that there has not been enough time for populations to diverge. The second was that clams could move from one lake to the next, but this explanation seemed unlikely—how could aquatic clams traverse terrestrial habitat? Darwin's beetle, and its hitchhiking clam, provided a way by which molluscs could move from pond to pond—migration (via hitchhiking on mobile beetles) could homogenize populations. The puzzle had thus been solved. But more to the point (for this essay): the man who sent Darwin this important beetle was a young British shoemaker and amateur naturalist by the name of Walter Drawbridge Crick.

Almost a century after the publication of *The Origin*, that same shoemaker's grandson, Francis Crick, with another young colleague by the name of James Watson, modeled the three-dimensional structure of DNA (for which they would later win the Nobel Prize along with their mentor and colleague Maurice Wilkins). And the discovery of its double-stranded helical structure provided a way in which DNA could replicate (sometimes with a few errors) and thus was the "secret to life"—the fundamental unit of hereditable information and the source of new variants. This discovery provided the missing link to Darwin's theory about descent with modification—evolution by the process of natural selection. And further, it is in this DNA text which we can find support for almost all of Darwin's ideas, including the genetic nuts and bolts of *how* this biological diversity evolved. It is this point that will be the basis for this essay.

THE QUESTIONS

Many aspects of modern evolutionary research are motivated by the desire to understand how diversity arises and is maintained in nature. How and why do organisms look and act so differently, and in some cases, so strangely? In fact, these are the same questions that inspired Darwin, but thanks to Watson and

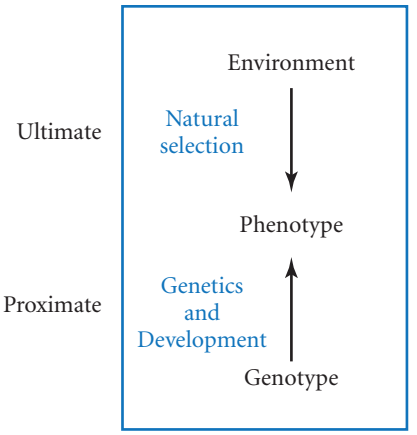

FIGURE 1 *The genetic basis of adaptive traits.* To gain a complete understanding of how variation is generated and maintained within and between natural populations, we must dissect both its ultimate and proximate causes. First, we need to understand how variation in a particular trait (i.e., phenotype) affects the fitness of individuals in their local environment. For example, what is the role of natural selection, if any, in generating trait variation? Second, we want to know the proximate basis of variation: what are the genes and mutations encoding differences in phenotype, and how do those genetic variants function through development to produce different traits? Making the links between environment, phenotype, and genotype will provide unique insight into the evolutionary process.

Crick, we now can look for the answers in the language of DNA. Specifically, we can ask questions about the genetic basis of evolutionary change—that is, what are the precise DNA changes that allow organisms to adapt to their environment (FIGURE 1)?

So what will finding the genes and mutations that give rise to changes in "phenotype" [i.e., the way organisms look (morphology), metabolize (physiology), or act (behavior)] tell us about the evolutionary process? There are several long-standing questions about adaptation—the evolutionary process whereby a population becomes better suited to its local environment—that can only be addressed when we know the chromosomal regions, genes, and mutations responsible for adaptive variation (BOX 1). For example, can adaptation occur through large leaps (i.e., a small number of mutations each having a large effect on phenotype) or does adaptation require many small steps? How often does natural selection rely on the same genes and/or mutations to drive the evolution of similar but independent phenotypes (i.e., convergent evolution)? Answers to these questions are starting to emerge as we are uncovering the genetic under-

Box 1:

MAJOR QUESTIONS ABOUT THE GENETIC BASIS OF ADATATION

Many questions about how adaptation occurs date back to the early 1900s when they were hotly debated among the founders of population genetics. At that time, these questions were approached largely through mathematical models and statistics. Now, we are able to return to these same questions, armed with molecular tools. Thus, the field can move beyond statistical associations and find the precise genes and DNA mutations that contribute to adaptive traits. While the questions are largely the same, the tools to answer them are remarkably different, and therefore we are better equipped to understand the nature of adaptation than ever before.

- Does evolutionary change proceed gradually through many small mutational steps or can adaptation occur via a few large leaps?
- Does adaptation generally proceed through dominant or recessive mutations?
- Do genes involved in adaptation act independently or do they interact to produce adaptive traits?
- Do beneficial mutations tend to affect protein function (i.e., mutations in the protein itself) or its spatial or temporal expression (i.e., mutations outside the protein that control its regulation)?
- Are the same genes and mutations responsible for similar traits in different populations or species?

pinnings of a number of different traits in a variety of species. In this essay, we will take a journey—into the laboratory and the field—to understand the genetic basis of adaptation in natural populations of mice, and the phenotype we will focus on is the color of their fur.

THE APPROACH

There are many different approaches to find genes contributing to traits that vary within and between species. Human geneticists have pioneered this field in their quest to find deleterious genetic changes that cause disease. For evolution-

ary biologists, the search is focused on beneficial gene variants, those that contribute to adaptive traits that increase fitness. In both human and evolutionary biology, finding the "genes that matter" represents a Holy Grail of sorts, and is no easy task.

Here, we will focus on the study of trait variation when it first arises—that is, variation *within* species. This approach has its limitations because it relies on comparisons between new populations (rather than species separated by millions of years), so the trait differences are often not extreme. However, comparisons among populations allow us to (1) better understand the evolutionary forces responsible for the trait differences, such as natural selection, because the evolutionary changes are more recent and we know something about the ecological context (e.g., the organism's habitat) in which the variation occurs, and (2) take advantage of the power of genetics and bring organisms into the lab and examine the inheritance of traits in a controlled environment (see below). To take this approach, we must first identify both a trait to study and a species in which that trait varies in a way that impacts fitness—the ability of individuals to survive and reproduce in the wild.

THE SYSTEM

If we think about diversity among vertebrates, differences in the shape or number of skeletal elements (e.g., the number of vertebrae in snakes versus mammals), altered morphology of appendages (i.e., fins, wings, and arms), and variation in color and color pattern combine to produce the majority of what distinguishes one species from another. Among these characteristics, color represents an excellent trait to study how morphological differences arise. This is because color is one of the most diverse traits among organisms and often varies between closely related species, or even within a species. Color is also one of the primary ways in which organisms interact with their environment—it is used in a variety of biological processes including mate choice, warning coloration, mimicry, and crypsis (i.e., camouflage)—and thus can clearly have dramatic effects on fitness.

Not only is color important for fitness, but we already know a lot about the genes necessary to produce pigments in vertebrates, which give rise to their color and pattern. Why? Because pigmentation has served as a model system in the field of genetics and development. Starting in the early 1900s, right after Mendel's work was rediscovered but before the term "genetics" was coined, geneticists tracked coat-color mutations that spontaneously appeared in colonies of lab mice (and thus are immediately obvious to a research technician). Today the genetics community has identified more than 200 genes necessary to produce "normal" coat color. Importantly, many of these genes are conserved

Box 2:

FRANCIS SUMNER AND NATURAL HISTORY

Francis Bertody Sumner was a naturalist. He spent almost 20 years of his career describing variation among populations of deer mice (genus *Peromyscus*) in North America with the goal of understanding how variation was maintained in nature. To this end, he both documented the relationship between coat-color variation and environment in wild populations and also brought back mice to the lab to do experiments on inheritance under different environmental conditions. Based on measurements of thousands of mice, he concluded that the variation in coat color among mouse populations was likely driven by natural selection for camouflage—he showed a strong correlation between pelage color and soil color among populations in the field and showed these traits were inherited genetically in the lab. It is natural history research like that of Sumner that has provided the foundation for the research we are doing today. Moreover, most of Sumner's specimens are preserved in UC Berkeley's Museum of Vertebrate Zoology, where they are available for study to all researchers. Thus, it is important to remember that past results, as old-fashioned as they may seem, provide the foundation for current research.

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(i.e., similar in DNA sequence) across vertebrates. Therefore, we can take advantage of this wealth of genetic information discovered in lab populations as we start to ask which genes contribute to variation in nature.

In mammals, coat color arguably varies the most among rodents. Some of the most extreme variation occurs in deer mice (genus *Peromyscus*), which have served as the subject of many classic studies in mammalogy starting almost a century ago (see Box 2). In particular, one species of deer mouse, *Peromyscus polionotus*, shows a tremendous amount of variation in coat color over short geographic distances. These mice occur throughout the southeastern United

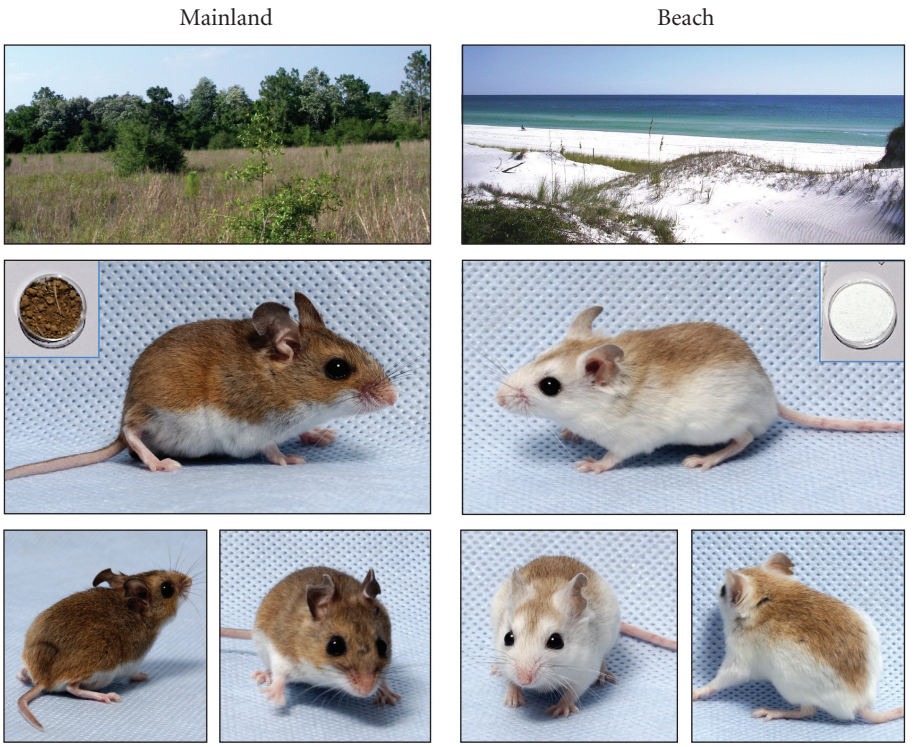

FIGURE 2 *Camouflaging color patterns of mice from different habitats.* Oldfield mice (*Peromyscus polionotus*) can be found in two distinct habitats in Florida—oldfields which are vegetated and have dark loamy soil, and coastal sand dunes which have little vegetation and brilliant white sand. Mice that occupy these different habitats have distinct coat-color phenotypes: mainland mice have a typical dark brown coat, whereas beach mice largely lack pigmentation on their face, flanks and tail. Typical habitat, soil samples and mice are shown. [Sacha Vignieri (habitat), Clint Cook (mice)]

States (i.e., Alabama, Georgia, South Carolina and northern Florida), where they are commonly referred to as "oldfield mice" because they inhabit abandoned agricultural fields with dark loamy soils. In these habitats, mice have dark brown dorsal coats, a light grey belly, and a sharply bi-colored tail (FIGURE 2), which is typical for many wild rodents. These oldfield mice also have colonized the sand dunes and barrier islands off the Gulf Coast of Alabama and northern Florida and independently the coastal habitat on the Atlantic seaboard (almost 300 km away). These sand-dwelling populations are commonly referred to as "beach mice."

Unlike their mainland counterparts, beach mice inhabit a very different environment (FIGURE 2). The beach mice are most abundant in the primary coastal sand dunes, where the sand is lighter in color than the inland soil (in fact, it is almost like walking on hills of granulated sugar). There is also much less vegetative cover—the dominant vegetation is sea oats, a sparsely distributed lanky, tall grass—and thus beach mice are more visible to predators. Thus, it may not be surprising that beach mice, relative to the mainland forms, have reduced pigmentation and unique patterning that gives them an overall lighter color which blends into the light-colored sand. Interestingly, the barrier islands on which these mice reside are thought to be quite young (approx. 4–6,000 years old), so this difference in color and pattern may have evolved recently and rapidly.

THE ADAPTIVE SIGNIFICANCE OF COLOR VARIATION

At first glance, it seems intuitive that being a light-colored mouse living on light-colored soil affords mice a survival advantage. Yet, as scientists, we wanted to empirically demonstrate that these color differences are beneficial as well as measure how much it matters for survival (i.e., the strength of selection) and know precisely who was doing the selecting (i.e., the selective agent). But how could we do this? One "ideal" experiment would be to tag and release an equal number of light and dark mice in both light and dark habitat, and return several weeks later to measure which survived—the prediction being we would recapture more light mice in light habitat and dark mice in dark habitat. Of course, this is a difficult experiment, requiring hundreds of mice and permission to relocate mice into non-native habitats. So, instead, we thought of another approach—we could make mice!

If we could produce hundreds of mouse models that resembled the light beach and dark mainland forms, we could conduct a "survival" experiment. The upshot of this approach is that the only trait that differed among model mice was color (whereas the live mice may differ in odor, escape behavior, or activity level). Therefore, any differences in survival would be due only to color differences. The downside, however, was that we didn't know if we could fool the predators. Would a hawk, owl, or coyote attempt to capture a fake mouse? We would find out only by trying.

Thus began the production of hundreds of model mice. The first step was to make a mold of a crouching *Peromyscus* mouse, which could then be used to replicate hundreds of identical models using a non-hardening clay. Once the models were produced, we spray-painted half to resemble a beach form and half the mainland form (with the same type of paint and the

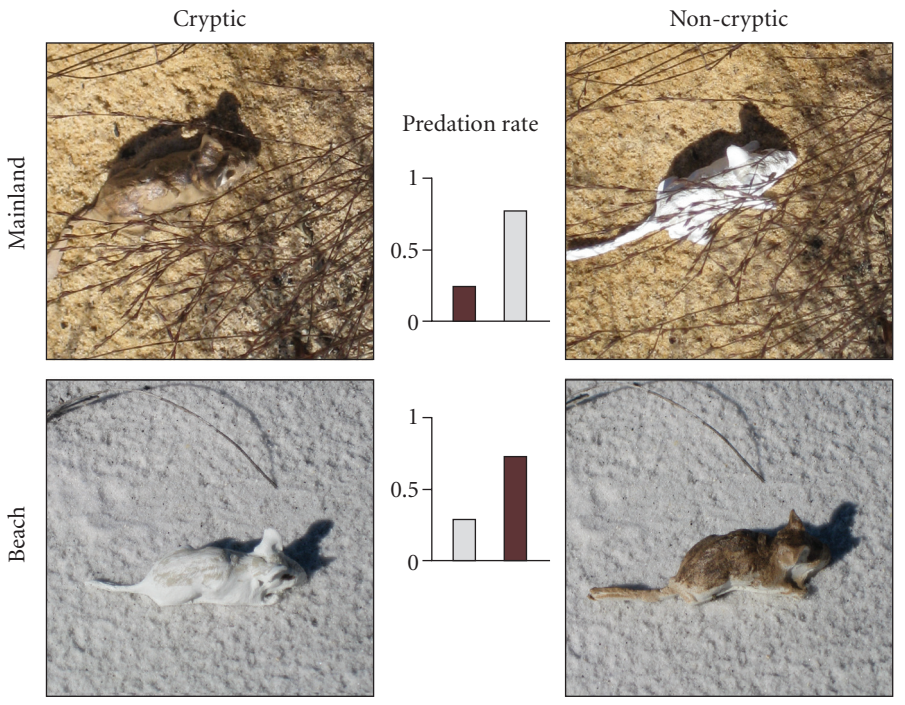

FIGURE 3 *Linking environment to phenotype: cryptic coloration matters for survival in the wild.* Typical clay models of mice, painted to resemble beach and mainland forms, laid out in both mainland (Lafayette Creek Wildlife Area) and beach habitat (Topsail Hill State Park) in Florida. Relative predation rates of dark and light models in mainland (top) and beach (bottom) habitats are shown. Crypsis reduces predation rate by approximately one-half in both the mainland and beach habitat. (Sacha Vignieri)

same number of paint coats). Each afternoon, we set out the light and dark models in a straight line and in random order about 10 m apart in habitat known to be occupied by either beach or mainland mice (and hence their natural predators). When we returned the next day, we recorded which models showed evidence of predation. Some had missing ears, others large gouges on their back, and still others were completely missing. The shape of the predatory imprint (bill or tooth) and the surrounding tracks gave clues as to the type of predator—bird or mammal. By documenting predation events in both habitats, we could ask *if* color matters and *how much* it matters for survival.

What did we find? There are three striking patterns (FIGURE 3). First, camouflaging color decreases predation, and this survival advantage is symmetrical—dark models on light soil experience the same relative increase in preda-

tion as do light models on dark soil. (Remember: both beach and mainland mice have evolved camouflage in their local environments, even if beach mice have done so more recently.) Second, the advantage that camouflaging color affords mice is large—camouflaged mice have a 50% higher probability of "survival" compared to mismatched mice. Thus, color matters *a lot* for survival. Finally, about half of the predation events could be attributed to a mammalian predator (e.g., foxes and coyotes) and half to an avian predator (e.g., owls, herons, and hawks). Thus, both mammals and birds were the agents of selection. In sum, this very simple experiment nicely demonstrates that color, and camouflaging color specifically, has a large effect on fitness in these mice.

THE GENETIC BASIS OF COLOR VARIATION

Now that we have a better understanding of why color varies among populations of mice, next we would like to know *how* it varies. Specifically, what are the precise genetic changes responsible for these color differences? To address this question, we have taken a forward-genetics approach (FIGURE 4)—that is, we start by focusing on phenotypic differences and working down to genes (in contrast to "reverse genetics," which seeks to find what phenotypes arise as a result of mutations to a particular gene). First, we allowed the beach and mainland mice to mate in the lab (i.e., a genetic cross). The mixed pairs gave birth to pups (i.e., first-generation hybrids), each of which had one chromosome from the dark mainland parent and one from the light beach parent and were thus intermediate in color relative to the two parents. We next mated these hybrids to each other, and their pups (second-generation hybrids) have genomes with shuffled regions of beach and mainland genes because recombination, or chromosomal crossovers, occur between the light and dark chromosomes. Therefore, each of the second generation hybrids has a unique combination of alleles (i.e., gene variants) from the light and dark parents, and are thus variable in color. By measuring pigmentation and determining the origin of chromosomal regions (from either the dark or light parent) using molecular techniques for each hybrid, we can determine which regions of the genome are statistically associated with the differences in color. For example, if all the hybrids that have a completely white rump also all share a region on chromosome 8 from the beach mouse parent in their shuffled genomes, then chromosome 8 likely harbors a gene that contributes to rump color. Ultimately, these are the genes we are after—those that cause the adaptive color differences between mainland and beach mice.

Using this approach, we identified three regions of the genome, each of which contained a known pigmentation gene that is statistically associated with color differences. Here, we will focus on one region which codes for the gene,

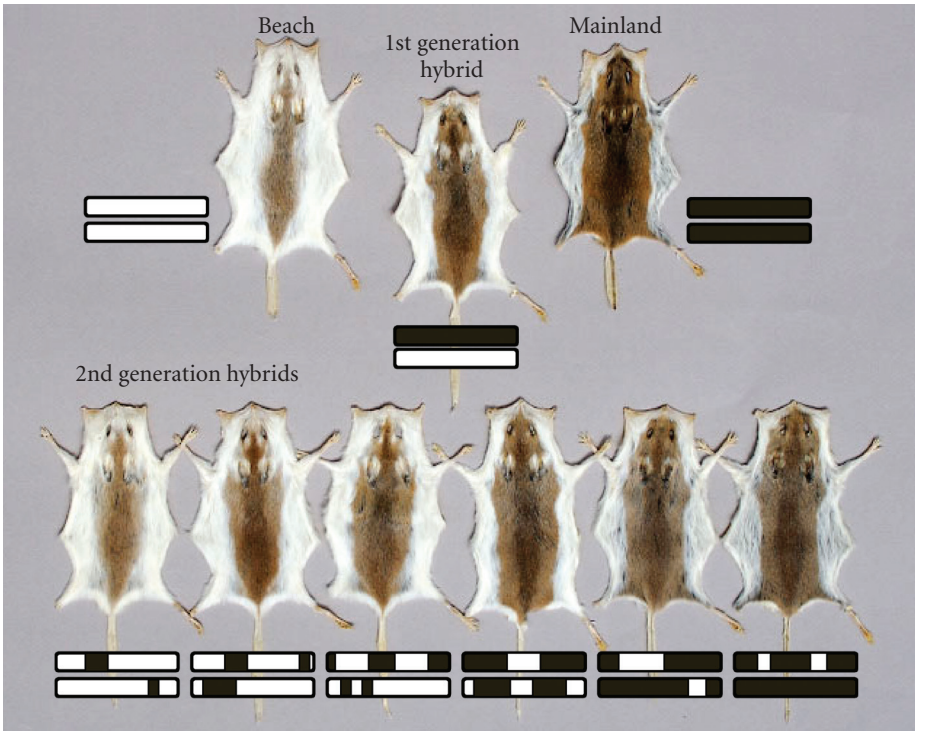

FIGURE 4 *Linking phenotype to genotype: cryptic coloration is encoded by a few regions of the genome.* By crossing mainland and beach mice in controlled laboratory conditions, we can dissect the genetic basis of color pattern differences (seen here in these museum specimens). Offspring of a "mainland × beach" cross (first generation hybrids) have one chromosome from each parent and an intermediate color phenotype. Offspring that result from an intercross between these hybrids represent the second generation hybrids. Each second generation mouse has a different combination of genetic regions (and pigmentation genes) inherited from the dark and light parents, and therefore exhibits a range of phenotypes. These hybrid mice can be used then to pinpoint the genetic regions (and ultimately genes and mutations) that cause differences in coat color between mainland and beach mice. Photos represent typical color patterns of mice and bars below represent cartoon chromosomes, with dark genomic regions inherited from mainland mice and white from beach mice.

the *melanocortin-1 receptor* (*Mc1r*), because we know a lot about its structure and function. As a first step, we decided to sequence the gene (it only has about 1,000 DNA nucleotide positions, or base pairs) in a beach and mainland mouse. In the entire gene, we found only a single mutation—a nucleotide that differs between the mainland and the beach mice. At nucleotide position 293, beach mice have thymine (T) and mainland mice (as well as all other *Peromyscus* spe-

cies) have a cytosine (C). The C-to-T mutation is unique to light-colored beach mice. This single nucleotide mutation causes an amino acid change in the protein (remember that unique combinations of three DNA nucleotides code for each amino acid, which in turn make up proteins). The amino acid change observed—arginine to cysteine at amino acid position 65 ($Arg^{65}Cys$ is the scientific shorthand for this mutation)—is from a large charged amino acid to a small uncharged one. This radical change in the Mc1r protein's chemistry is predicted to affect the structure of the protein, and hence its activity.

While finding such a mutation in a known pigmentation gene is quite exciting, the relationship between genotype and phenotype was still largely a statistical association—all light mice had one version of the gene and all dark mice had another. To prove, that this mutation affects coat color, we needed to demonstrate that it alters protein function. But how? A typical initial step is to test its function in a petri dish. For example, we could engineer (using standard molecular biology techniques) both versions of the protein, one with arginine and one with cysteine at position 65, and then determine if the two proteins have different properties.

First, however, it is useful to know a bit more about Mc1r and its role in determining pigmentation. The Mc1r receptor is associated primarily with melanocytes, our pigment-producing cells. In mammals, we produce only two types of pigments: eumelanin (brown to black pigment) and phaeomelanin (blonde to red pigment). You can look at your own hair and determine which of the two pigment types you have. Mc1r acts as a switch in determining the type of pigment a melanocyte produces (FIGURE 5A). When Mc1r is "turned on," it signals through a cascade of effects in the melanocyte cell for dark eumelanin to be produced; when Mc1r is "turned off" and signaling is reduced, the default state is restored and light phaeomelanin is produced. Based on this knowledge, we can make a prediction about the expected effect of the $Arg^{65}Cys$ mutation on Mc1r function: the Mc1r variant from beach mice (hereto referred to as the "light Mc1r protein or variant") will show decreased activity and therefore lower signaling, leading to light pigmentation.

To test this prediction, we measured receptor activity in a simple assay. Specifically, we produced the two proteins (which differ by only the $Arg^{65}Cys$ mutation) in cells and measured signaling as an indicator of the level of receptor activity. Under the same cellular conditions, we found that the dark Mc1r protein always acts more strongly than the light Mc1r protein (FIGURE 5B). These results suggest that the light version of the Mc1r gene therefore can signal the production of only light pheomelanin. Importantly, not only have we demonstrated that the $Arg^{65}Cys$ mutation affects receptor function, but it also changes receptor activity in the direction we predicted—the light Mc1r variant causes light coat color.

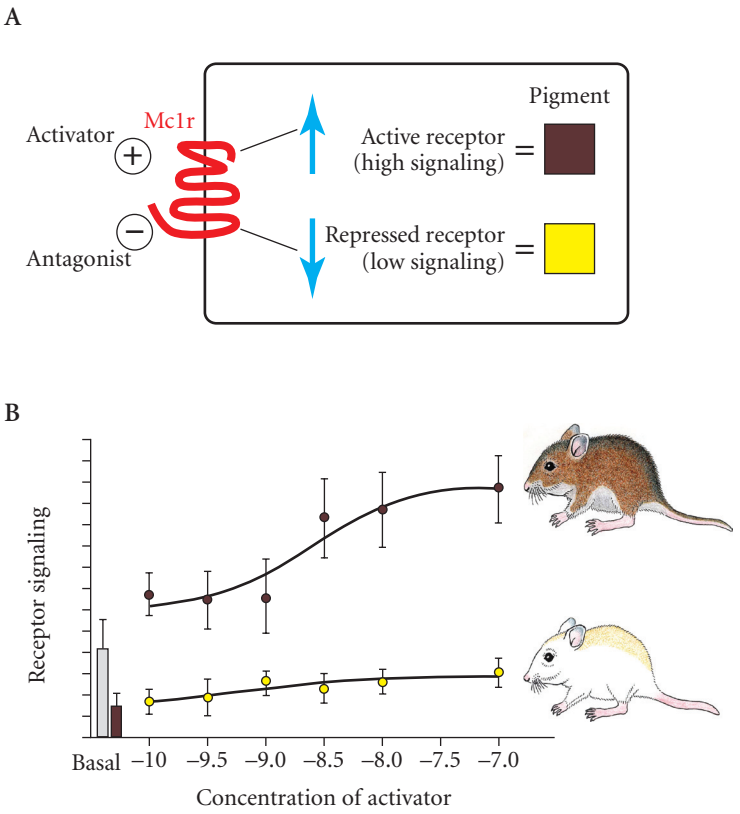

FIGURE 5 *The role of the melanocortin-1 receptor (Mc1r) in producing pigment variation.* (a) Mc1r is a receptor (shown in red) located on the membrane of melanocytes, pigment-producing cells, which in mammals produce two types of pigment, black to brown eumelanin and red to blonde phaeomelanin. Mc1r is the switch that controls whether dark or light pigments are produced. When an "activator" binds to Mc1r, it is "turned on" and generates a signal inside the cell which leads to the production of dark pigmentation. By contrast, if Mc1r is repressed, such as by the binding of an antagonist, its intracellular signal is reduced and light pigments are produced. (b) Different forms of the Mc1r protein may differ in their ability to produce a signal, and therefore will result in the production of a different pigment. We can quantify Mc1r activity (i.e., signaling potential) by expressing the protein in cells, adding increasing amounts of activator (to turn Mc1r on) and measuring signaling activity. The dark mainland Mc1r protein shows a typical pattern—adding increasing amounts of activator results in increasing amounts of signaling until it reaches its maximum. By contrast, the light Mc1r protein from beach mice (which differs by only one amino acid, ^{65}Cys) has much lower activity for the same amount of activator compared to the mainland protein (^{65}Arg), and thus is more likely to produce light pigments than dark pigments under the same conditions.

LINKING ENVIRONMENT TO PHENOTYPE TO GENOTYPE

Now let's take a step back at look at the bigger picture. What do these results mean? First, we identified a single DNA change that codes for an amino acid difference in the Mc1r protein, which in turn affects its function. This change in receptor activity leads to the production of light-colored pigments and, hence, light-colored mice. Finally, light coloration in mice inhabiting light-colored beach dunes provides a survival advantage. Thus, we have successfully made the link between a single DNA base-pair change and fitness in wild populations! But, this is only half the story. What is most exciting about this system is that we can ask questions about when and where these alleles evolved by going back to natural populations.

HOW ORGANISMS ADAPT IN NATURE

Once the genes, and even the mutations, responsible for adaptive traits are identified, we have a unique opportunity to learn how these traits evolve in nature. To illustrate this point, we will explore an example of how knowing the molecular basis of an adaptation can teach us something more general about the evolutionary process. To do so, we must move from the lab into the field. With a 4×4 truck filled with live traps, bait, and flagging for catching mice, and computers, tubes and a field kit for recording data and taking DNA samples, we headed south to the Gulf Coast of Florida and from there, 300 km east to Florida's Atlantic coast. It was time to trap some mice and survey natural variation in color among populations.

By way of reminder, all of the genetic work (described above) focused on a single subspecies of beach mouse, the Santa Rosa Island beach mouse. On the Gulf Coast, however, there are a total of five beach subspecies, each with a pale but unique coat-color pattern (FIGURE 6A), and each of which is uniquely matched to their local habitat—the darkest beach mouse subspecies occurs in the darkest sands and with the thickest vegetation, whereas the lightest subspecies occurs in the lightest sand dune with little vegetation. On the Atlantic coast of Florida there are more beach mice. While there were once three subspecies, now only two exist. The Pallid beach mouse is thought to have gone extinct some 50 years ago, likely due to habitat loss as more and more beach homes were constructed. (Because specimens of the Pallid mouse are part of natural history collections, we can still study their pelage, as well as their genetics, by extracting DNA from snippets of museum skins). The other two subspecies now occur only in protected areas. Nonetheless, all three of these beach mouse subspecies, like their Gulf Coast counterparts, are pale in color. In fact,

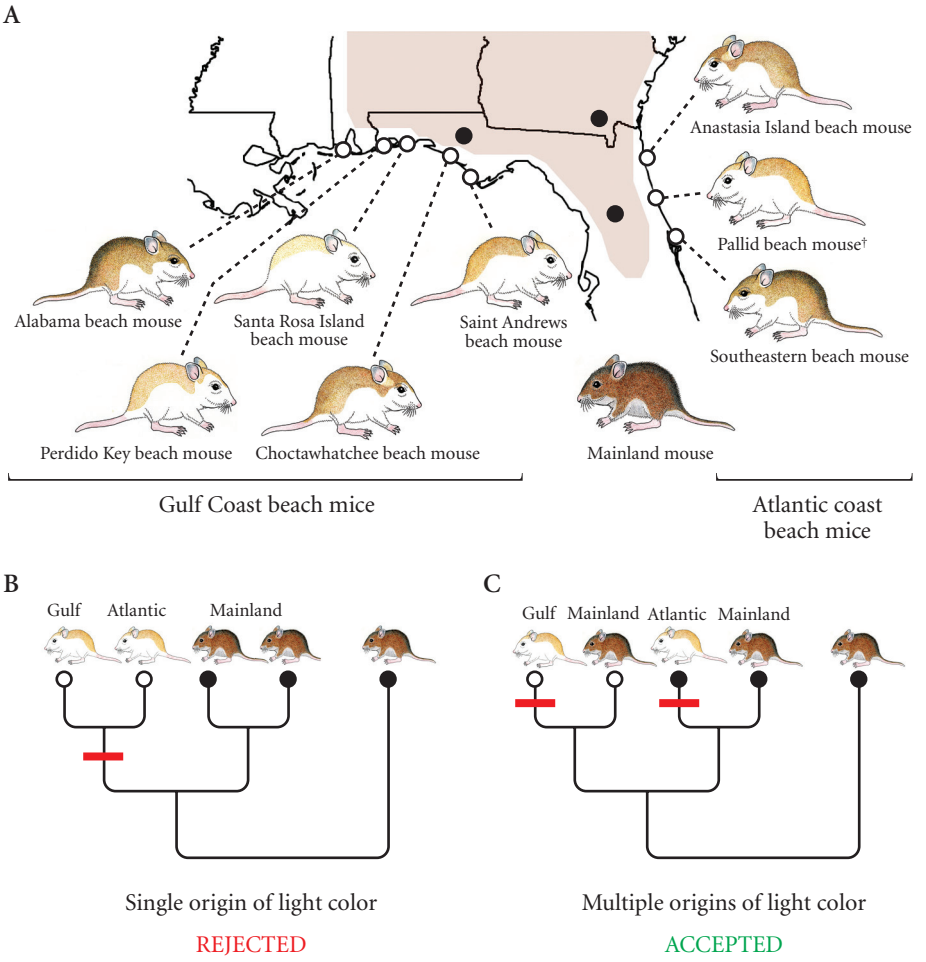

FIGURE 6 *Pale beach mouse subspecies occur along both the Gulf Coast and the Atlantic coast of Florida.* (a) Cartoons represent the typical color pattern of each of the eight beach mouse subspecies. Circles represent the location of each subspecies, whereas the range of mainland mice is shown in tan, with black dots representing collecting sites in the panhandle and central Florida as well as Georgia. (b) Possible relationship among subspecies if light pigmentation has evolved a single time. (c) Actual relationship of populations based on DNA data showing that light coloration in beach mice likely evolved twice independently—once in the Gulf Coast and a second time in Atlantic populations. Red bars indicate the evolution of light pigmentation.

the color patterns of mice on the Atlantic coast are more similar to those on the Gulf Coast than they are to each other—a remarkable case of convergent evolution.

Given the striking similarity in color patterns, a first question to ask is: are the Gulf and Atlantic beach mice closely related? One may predict that all the pale beach mice are each other's closest relatives to the exclusion of all the dark mainland mice (FIGURE 6B). However, this is not the case. Using DNA to trace the ancestry of populations, we find that while the five pale Gulf Coast subspecies are very closely related, they are more genetically similar to the dark mainland mice from Florida's panhandle than to the pale Atlantic beach mice (FIGURE 6C). Similarly, Atlantic coast beach mice are most similar to mainland mice from central Florida. These data suggest that light coloration has evolved independently on the Gulf and Atlantic coasts.

If such similar coat-color patterns evolved twice, must the same genetic changes have evolved twice, too, or can different genetic changes lead to similar traits? To address this question, we can focus once again on Mc1r. Recall that a single mutation in Mc1r changes the receptor activity and thus contributes to the light pigmentation of the Gulf Coast subspecies, the Santa Rosa Island beach mouse. Thus, we can survey the Atlantic coast mice for the presence or absence of the particular $Arg^{65}Cys$ genetic change—do the pale Atlantic beach mice have the same mutation? Quite surprisingly, none of the Atlantic coast beach mice has the $Arg^{65}Cys$ mutation! Despite their remarkable similarity in coat color, the same Mc1r mutation does not contribute to light pigmentation on the Atlantic coast.

A second possibility is that different mutations in the same Mc1r gene evolved in the Atlantic coast mice. These alternative mutation(s) could have a similar effect on Mc1r receptor signaling and thus produce a similar color pattern. To test this hypothesis, we compared the entire DNA sequence for Mc1r in Atlantic coast mice to the sequence from mainland mice to see if there were any new mutations unique to the Atlantic beach mice. We identified four new nucleotide mutations, each of which altered the amino acid sequence of the protein (although not as radically as $Arg^{65}Cys$). To determine if any of these mutations affected Mc1r activity, we once again engineered proteins, four of them, each with one new mutation, and tested their signaling compared to the dark mainland protein. All four of the new proteins behaved just as the mainland protein (and were all more active than the light Mc1r protein). These results suggest that there are no new mutations in Mc1r that lead to convergent light pigmentation on the Atlantic coast. In other words, even within a single species, there appear sometimes to be multiple genetic solutions to a common ecological problem of blending into the local environment.

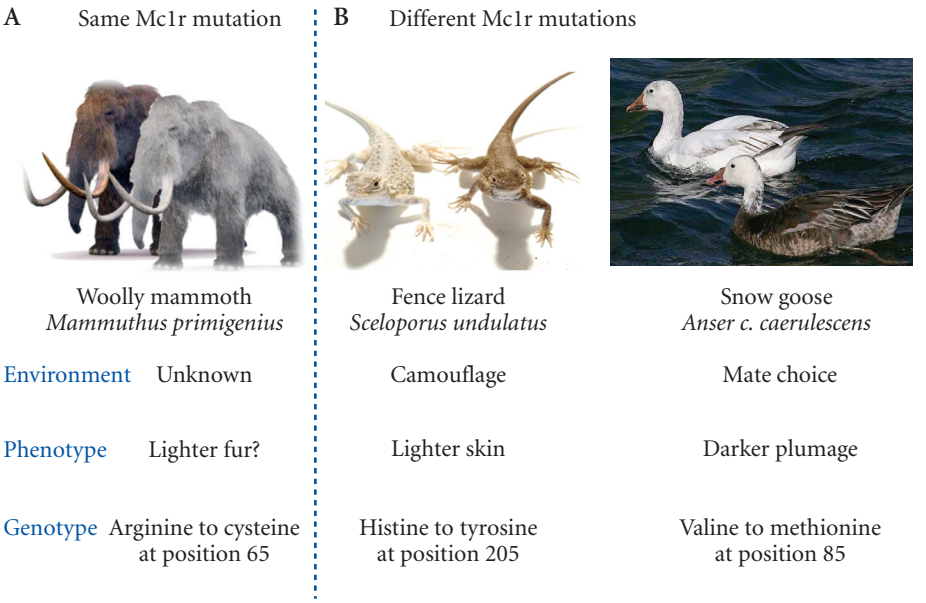

FIGURE 7 . *Mutations in Mc1r contribute to color variation in several vertebrate species.* (a) The same mutation ($Arg^{65}Cys$) that occurs in beach mice is also found in a population of mammoths (surveyed using ancient DNA techniques), suggesting they, too, may be variable in color. (b) Different mutations in Mc1r contribute to similar color differences in lizards and birds. [Simone Des Roches (lizards) and Terry Sohl (snow geese)]

However, other times, the same mutation or gene can contribute to similar traits in very divergent species. In 2006, a team of scientists based in Leipzig, Germany, was able to successfully extract DNA from 14,000-year-old mammoth bones, excavated from the permafrost of Siberia. Their goal was to get the sequence of an entire nuclear gene from an extinct organism (which at the time had never been done). They chose Mc1r as the target gene because of its simple structure. When all the technical challenges had been overcome (e.g., avoiding contamination and working with degraded DNA), they were able to compare the two Mc1r sequences from a single individual and they found a mutation—at amino acid position 65 (FIGURE 7A). Even more surprising, it coded for an $Arg^{65}Cys$ amino acid change; whereas most mammoths (and most mammals) have arginine amino acid in this position, one mammoth, just like beach mice, had a cysteine. Mammoths and mice both evolved the exact same mutation! This convergent change then raises the possibility that mammoths, like mice, were variable in coat color. While we don't know the color phenotype of this particular individual, because only bones were available, mammoth hairs red-

dish in color have been found previously. However, the environmental forces, if any, driving color variation in mammoths are unknown.

Mutations in Mc1r are also associated with color changes in still other species. There are a growing number of studies that have identified an association between Mc1r mutations and color variation in a diversity of vertebrates (FIGURE 7B). From these studies it is clear that Mc1r mutations can produce pale or dark coloration, even in species without fur. For example, lizards that inhabit White Sands, New Mexico, have evolved camouflaging light-colored skin and scales relative to nearby desert-dwelling populations. Like beach mice, this light color helps them blend into their local environment and reduces their risk of predation. These blanched lizards have a unique mutation that affects Mc1r signaling, like the mutations in beach mice. Alternatively, mutations in other species, like snow geese, lead to dark coloration (in this case feathers), by altering Mc1r so that it is permanently active and thus exclusively produces eumelanin. In birds, color is often important in attracting mates (i.e., sexual selection), and in the case of snow geese, females prefer males with plumage of similar coloration to their own. These are only a few examples, but serve to illustrate that a multitude of Mc1r mutations contribute to color variation in a variety of species and for a variety of purposes. Thus, in some cases, mutations in the same gene can contribute to convergent evolution in distinct species.

LESSONS ABOUT THE EVOLUTIONARY PROCESS

The goal of this essay was first to depict the connections we can now make between environment and phenotype (i.e., the ultimate mechanisms) and phenotype and genotype (i.e., the proximate mechanisms) for variation in a single trait—in this case, color variation in natural populations of mice. The second goal was to show how identification of the precise molecular mechanism underlying an adaptive trait can shed light on the evolutionary process more generally. Here, we have seen that even single mutations in the Mc1r gene can cause large changes in fur color, which in turn has a large effect on the fitness of mice in the wild. However, Mc1r mutations do not cause light pigmentation in all beach mice subspecies; there are multiple possible genetic paths to the same phenotypic end. On the other hand, Mc1r mutations do cause changes in color in a wide diversity of other vertebrates, suggesting that sometimes the same gene can be a repeated target of adaptive change. Thus, using a variety of approaches—some, like fieldwork, rooted firmly in Darwin's own tradition of natural history, and others, like molecular genetics enabled by Watson and Crick's discovery of DNA—we are unraveling the molecular basis of what Darwin called "that perfection of structure and co-adaptation which most justly

excites our admiration", and thereby learning how the spectacular diversity of traits within and between species evolves in the wild. And this is just the start.

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