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Melanic Coat Color Variation in Rock Pocket Mice (*Chaetodius intermedius*)

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Coat color variation in small mammals is a classic example of phenotypic variation in response to selection in different environments (Hoekstra and Nachman, 2005); many species closely match the color of the substrate in which they live. Examples of intraspecific color variation in rodents include the canyon mouse (*Peromyscus crinitus*), the deer field mouse (*Peromyscus maniculatus*), the old-field mouse (*Peromyscus polionotus*), and the rock pocket mouse (*Chaetodius intermedius*) (Hoekstra and Nachman, 2005). The rock pocket mouse is one of the most studied models for phenotypic color variation.

Many studies have found coat color variation in the rock pocket mouse in deserts of the southwestern states (Nachman, Hoekstra, and D'Agostino, 2005). Authors have showed that *C. intermedius* coat color matched the color of the rocks on which the mice live (Nachman, Hoekstra, and D'Agostino, 2005). Several studies have found that most light-colored mice live on light-colored rocks, but in several geographic regions, they are melanic (black or brown) and live on dark-colored basalt lava (Hoekstra and Nachman, 2003; Hoekstra, Drumm, and Nachman, 2004). Furthermore, the pocket mice on volcanic lava with dark coats do not have banded hairs, whereas mice from nearby light-colored granitic rocks have light coats that do have banded hairs. This color polymorphism is a presumed adaptation to avoid predation from avian and mammalian predators (Nachman, Hoekstra, and D'Agostino, 2005). Rock pocket mice provide a useful system for studying the genetics of adaptation. Genetics seem to play a role in that their coat color typically matches the color of the rocks on which they live. (Hoekstra and Nachman, 2003; Hoekstra, Drumm, and Nachman, 2004).

The main questions that this story brings to focus are: 1) is this coat color variation a consequence of natural selection; and 2) if there is variation in more than one area or region, are these caused by the same gene or not? This paper will explore coat coloration in pocket mice based on the work of evolutionary biologist Hopi Hoekstra. It is suggested that phenotypic variation is due to natural selection and that variation in multiple regions is caused by a few interacting genes.

Hoekstra received her B.A. in Integrative Biology from UC Berkeley. She then completed her Ph.D. in Zoology in 2000 as a Howard Hughes Predoctoral Fellow at the University of Washington. Hoekstra then moved to the University of Arizona as a NIH Postdoctoral Fellow where she studied the genetic basis of adaptive melanism in pocket mice. In 2003, she became an Assistant Professor at the University of California San Diego. Just recently, she moved to Harvard University, where she is the John L. Loeb Associate Professor of Biology in the Department of Organismic and Evolutionary Biology and the Curator of Mammals at the Museum of Comparative Zoology (Hoekstra home page). Pocket mice are the primary model organism for Hoekstra's lab.

Pocket mice inhabit rocky areas and desert scrub

at low elevations in the southwestern deserts of North America. Typically, they range from southern Utah through Arizona, New Mexico, and Texas (Hoekstra and Nachman, 2005). *C. intermedius* are nocturnal mammals and decrease activity from November through February. Young mice are born naked and typically attain their first coat at two to three weeks (Hoekstra and Nachman, 2005); the first coat is generally thinner and duller than the adult coats. Although there are no direct studies of pocket mice predators, it is well established that they are preyed upon by owls, snakes, and mammalian carnivores (Hoekstra and Nachman, 2005). Throughout most of their range, pocket mice have sandy dorsal coats with white underbellies. Hairs on the dorsum are banded, and the ventral hairs are light colored.

When I asked Hoekstra why they use wild rodents as their model organism in the lab in an email interview, these were the answers that she provided: "(1) they are closely related to model organisms like lab mice and rats, which have a plethora of genomic tools, (2) they can be brought into captivity and bred, (3) they can be easily caught in the wild with decent sample sizes, (4) there is a wealth of natural histories dating back to the early 1900's describing geographic variation in *Peromyscus*, and (5) because they are wide-ranging across North America, there are many instances of local adaptation" (2009). Despite the fact that many populations have similar light-colored coats, many populations of rock pocket mice have been described as nearly black or melanic (Hoekstra and Nachman, 2005).

The different color landscape, with ancient volcanic lava flows, created a patchwork of light, sandy habitats and dark lava habitats. The four lava flows studied in most Hoekstra studies include the Pinacate, Armendaris, Kenzin, and Carrizozo lava flows. The Pinacate lava flow is the oldest of the four, approximately 1,700,000 years old, whereas the Carrizozo flow is the youngest, only 1,000 years old (Hoekstra and Nachman, 2005). The Pinacate landscape is the most sampled lava bed because it is assumed to be the oldest; it is situated in northern Sonora, Mexico, and the adjacent Cabeza Prieta national Wildlife Refuge in southern Arizona (Hoekstra and Nachman, 2005). This lava flow has many rocky areas that are disjunct due to the accumulation of intervening deposits of sand (Hoekstra and Nachman, 2005). In this region, and in the other three sites, most of the mice captured on the dark rock (lava beds) are found to be melanic and most of the mice caught on the light rock are light-colored (Hoekstra and Nachman, 2005). Geographically proximate populations tend to be closely related and share morphological similarities, but they can vary significantly in coat color. Correlations between coat color and color of substrate can be demonstrated by quantifying light reflectance of dorsal hairs and the surrounding substrate (Hoekstra and Nachman, 2005).

Furthermore, there appears to be a correlation between lava flow age and the number of mice living on the flow. In 2003, 225 mice were found on the Pinacate flow, whereas only 12 mice were found on the Carrizozo lava flow (Hoekstra and Nachman, 2005). This study suggests that more mice live on the older lava flows, whereas fewer mice live on more recent lava flows (Hoekstra and Nachman, 2005). Perhaps this is because mice have become better adapted to their environment over time and have thus been able to better survive in their habitats. Furthermore, the mice may not have adapted as well to the newer lava beds. Is coat color variation to match substrate an adaptation that rock pocket mice have evolved over time?

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The first question that needs to be addressed in the story of these pocket mice is whether or not the coat color variation is due to natural selection. Natural selection is the process in which favorable heritable traits become more common in successive generations of a population. Furthermore, it is a process of adapting to the environment. Natural selection acts on the phenotype so that individuals with favorable phenotypes are more likely to survive and reproduce than those with less favorable phenotypes; this action occurs through mutations and changes in a species genotype. Pocket mice are presumed to be driven by natural selection for crypsis, or camouflage, because their natural predators are visual hunters (Hoekstra, Hirschmann, Bunday, et al., 2006). Melanism is one form of concealing coloration from on looking predators; melanism is controlled through genetics (Hoekstra, Drumm, and Nachman, 2004). Natural selection on phenotypes leads to evolutionary change at the genetic level via mutation and the spread of favorable alleles.

Hoekstra and her lab did not conduct direct studies of relative survival of pocket mice with different coloration on different substrates. Instead, the introductions of most of her papers just cite the very close match of color of mouse and color of substrate and assume selection is motivated by pressure from owls. She assumes this through literature in which results of phenotypes from natural selection were apparent. Earlier studies by Dice and Blossom (1937; as cited by Hoekstra and Nachman, 2005) clearly demonstrated that owls discriminate between mice that do and do not match the color of their substrate (2005). Mismatched mice experience a higher risk of predation by owls and are therefore less likely to survive and reproduce. Thus, mice that match their substrate are less visible to predators and have a better chance for survival. Because owls presumably eat mismatched mice rather quickly, this leads to a difference in allele and phenotype frequency between light and dark habitats. Thus, owls appear to exert strong selection on coat color in pocket mice, and differences in coat color are an adaptation by pocket mice for crypsis (Nachman, Hoekstra, and D'Agostino, 2003). Adaptive differences in coat color between habitats imply that there must be a genetic difference between the phenotypes. What is responsible for the adaptive color polymorphism observed in *C. intermedius*?

Specific genes are now known to cause the coat coloration changes in rock pocket mice, since phenotype is tied to genotype. There are now available methods to track down what genes are responsible for these phenotypic changes. Since scientists know that a phenotypic change is actually occurring, what are the genes responsible for that change? Hoekstra's lab uses genetic crosses, genetic mapping, and association studies to uncover genomic regions, genes, and mutations involved in phenotypic variation. Furthermore, they use an integrative approach combining molecular genetic techniques, theoretical modeling, experimental tests, breeding studies, and fieldwork to identify the genetic basis of adaptive coat color variation in *C. intermedius* (Hoekstra and Nachman, 2005).

The deposition of pigment in hair and skin include complex processes that involve the coordinated action of many genes and cell types. Melanocytes, pigment-producing cells, are responsible for the dark-colored coats on many rock pocket mice through a process called melanogenesis. Furthermore, approximately 80 genes have been identified that affect coat color, and subsequently melanogenesis, in the lab mouse *Mus musculus*; approximately one-quarter of these genes have been cloned, sequenced, and characterized at the molecular level (Hoekstra and Nachman, 2005). Although many genes are believed to be involved in melanogenesis, melanocortin-1

receptor (*Mc1r*) is believed to play a primary role in phenotypic change in a variety of species (Hoekstra and Nachman, 2005).

In mammals, coat color is determined by amounts of two pigments: eumelanin, which is responsible for brown to black color, and pheomelanin, which is responsible for yellow to red color (Hoekstra and Nachman, 2003). The switch between the productions of these two pigments is controlled by the interaction of *Mc1r*, a transmembrane receptor found on melanocytes, and the Agouti signaling protein (Hoekstra and Nachman, 2003). *Mc1r*, which is a transmembrane protein that spans the entire biological membrane, is found on melanocytes and is one of the key proteins involved in melanogenesis. Agouti, the signaling protein that communicates with and coordinates a cell's (and *Mc1r*'s) action, either inhibits or allows melanogenesis. When *Mc1r* is activated by a melanocyte-signaling protein, the intracellular messenger cAMP shows elevated levels through a G-protein signaling pathway, and eumelanin is produced. A G-protein signaling pathway comprises a large protein family of transmembrane receptors that sense molecules outside the cell and activate inside signal transduction pathways and, ultimately, cellular responses (Hoekstra and Nachman, 2005). Agouti is an antagonist of this signaling pathway, and in the presence of Agouti, cAMP levels are reduced and pheomelanin is produced, which is responsible for light coloration (Hoekstra and Nachman, 2003). The Agouti antagonist negates the action of *Mc1r*. Once full of melanin, eumelanosomes or pheomelanosomes are secreted from the melanocyte as pigment granules (Hoekstra and Nachman, 2005). Thus, the interaction of these two proteins plays a major role in determining the pigmentation patterns on coat hairs of pocket mice.

Mutations at the *Agouti* and *Mc1r* loci produce a range of phenotypes from dark to light color (Hoekstra and Nachman, 2005). These loci are fixed positions on a chromosome that are occupied by specific genes; they are the genetic underpinnings that contain the complex code and pathways involved in pigment production. Dominant *Agouti* mutations result in increased Agouti expression and largely light phenotypes. On the other hand, the recessive mutation results in black phenotypes because *Mc1r* is the dominant allele (Hoekstra and Nachman, 2005). The opposite relationship is true with the *Mc1r* gene. The recessive mutation leads to light or yellow phenotypes, and the dominant mutation leads to black phenotypes. Thus, the rock pocket mice that are dark must be a result of a recessive mutation at the *Agouti* loci and a dominant mutation at the *Mc1r* loci (Hoekstra and Nachman, 2005). Deposition of pigment occurs during the hair growth cycle (Hoekstra and Nachman, 2005).

Hoekstra's lab has previously used a candidate-gene approach to identify the genes underlying color variation in the rock pocket mouse. The strategy behind this method is to develop single-nucleotide-polymorphism (SNP) markers for each candidate gene. A SNP is a single base difference in the sequence of a gene which alters the structure and function of the gene product (Hoekstra and Nachman, 2005). The SNPs are then surveyed in populations of light and dark mice. If a strong association between SNP variants and coat color phenotype was found, the lab sequenced the entire gene in light and dark individuals and tested for other markers. The Hoekstra lab used the SNP method in one particular population, the Pinacate population. These methods resulted in a strong correlation between substrate color and coat color for the four mutations in the *Mc1r* SNPs gene, but they showed no correlation with the *Agouti* SNPs in the Pinacate population (Hoekstra and Nachman, 2005). This finding led to the

characterization of the entire *Mc1r* gene in pocket mice. Four charge-changing amino acid polymorphisms in *Mc1r* are perfectly associated with coat color (Hoekstra and Nachman, 2003). The four mutations are at amino acid numbers 18, 109, 160, and 233 (Nachman, Hoekstra, and D'Agostino, 2003). Mice with one or two copies of the melanic allele are melanic, while mice without this allele are light; dark alleles are dominant over light alleles (Hoekstra and Nachman, 2005). This led to the question of whether or not similar melanic phenotypes arise independently in rock pocket populations on different lava flows and if these phenotypic changes occur through changes in the same or different genes and at the same or different mutations.

Melanic phenotypes are believed to evolve independently. In a 2003 study, the same four mutations in *Mc1r* seemed to be responsible for the adaptive melanism in the dark phenotype of Pinacate pocket mice (Nachman, Hoekstra, and D'Agostino, 2003). However, interestingly enough, the melanic Armendaris population showed no association with *Mc1r* mutations, indicating that adaptive dark color has evolved independently in this species through changes at different genes. The genes causing the melanic differentiation in the Armendaris population have yet to be identified. This is a big finding in the Hoekstra lab because they now know that phenotypes evolve independently and they can now look for different primary genes causing melanic coat colors besides *Mc1r*, if there are any.

Let's add a twist to the story. Pocket mice are not the only mice to show color polymorphisms; old-field mice and beach mice show similar adaptations. The mainland subspecies, called an old-field mouse, has a cryptic dark brown dorsal coat. The younger beach-dwelling subspecies, called the beach mouse, has a lighter coat produced by natural selection for camouflage on pale coastal sand dunes (Steiner, Weber, and Hoekstra, 2007). In beach mice and old-field mice, are coat color differences dependent on the same genes as pocket mice?

Beach mice comprise eight subspecies of the old-field mouse. The eight subspecies include five on the Gulf Coast and three (historically) on the Atlantic Coast (Steiner, et al., 2008). In a recent study (2007), Hoekstra and her lab found results indicating that coat color differences are dependent on the same genes as pocket mice. They used genome-wide linkage mapping in order to find the gene in which a mutation is located; in order to do this, they needed to use SNP analysis. Using genome-wide linkage mapping, they identified three regions associated with differences in pigmentation traits in beach mice and old-field mice (Steiner, Weber, and Hoekstra, 2007). *Mc1r* and its antagonist, *Agouti*, map two independent regions that together are responsible for most of the pigment differentiations between the subspecies.

But are those the only genes causing the phenotypes? In another recent study, Hoekstra analyzed mechanisms underlying convergent pigment pattern among subspecies of beach mouse inhabiting the Gulf and Atlantic coasts of Florida (Steiner, Rompler, Boettger, Schoneberg, and Hoekstra, 2009). After measuring color pattern in eight beach mouse subspecies, Steiner, et al. showed that three of the Gulf Coast subspecies are more phenotypically similar to an Atlantic coast subspecies than to their Gulf Coast neighbors (2009). However, light-colored beach mice do not form a monophyletic group, a group of organisms descended from a common ancestor (Steiner, Rompler, et al., 2009). Previous studies indicated that a mutation in *Mc1r* was the major contributor to pigment pattern in the Gulf Coast mice. However, despite their color similarities, the *Mc1r* allele was not found in the Atlantic coast mouse populations. Atlantic coast mice have high levels of *Mc1r* but lack unique alleles (Steiner, Rompler, et al., 2009). Different populations of pale

mice appear to be pale because of differing genetic mechanisms. *Mc1r* and *Agouti* are together responsible for most, but not all, of the pigment differentiations between subspecies (Steiner, Weber, and Hoekstra, 2007). This is the same result that occurs in completely different groups of mice. This suggests that mouse subspecies have evolved through many different genetic mechanisms, not just one. Melanism has evolved independently on different lava flows through changes at different genes.

When asked what her next steps in the lab would be, Hoekstra answered, "[they] have focused mostly on genetics of morphological variation, and now [they] are starting to become interested in the genetics of behavior." She argues that this interest in behavior is the next frontier in biology. More specifically, Hoekstra would like to look at burrowing, burrow design, mate choice, and mating systems in pocket mice; she is interested in what genes contribute to these adaptations. Hoekstra's lab is also working on projects with new rodents such as chipmunks, squirrels, and zebra mice. Furthermore, Hoekstra's lab should continue to search for other genes that play a major role in melanic phenotypic changes besides *Mc1r* and *Agouti*. The role of this epistatic relationship in adaptation would be useful to figure out. Epistasis is a phenomenon where genes that evolve in one group produce reproductive isolation by interacting with genes evolving in another group (Coyne and Orr, 56). Epistasis is almost always involved in intrinsic postzygotic isolation where hybrids are sterile or infertile because of incompatible developmental systems or inappropriate mating behavior (Coyne and Orr, 56). Thus, insights into epistasis would give us insight into reproductive isolation and adaptation of pocket mice.

It would also be useful to determine if one or more of the *Mc1r* mutations are responsible for phenotypic differences. It is possible that each mutation contributes a small amount to the observed coat color or that two or more mutations interact epistatically to produce the phenotype. Furthermore, some mutations may be neutral, having hitchhiked along with selected mutations (Hoekstra and Nachman, 2005). Furthermore, what are the genes involved in coat color variation in populations where *Mc1r* is not playing a role? Finally, it would be useful to explore other reasons why this adaptive phenotypic change is occurring, other than crypsis. For instance, perhaps thermoregulation or the matching of coat color with the color of substrate are plausible mechanisms for keeping warm.

In a few short years of research, Hoekstra and her lab have already discovered a classic study system about adaptation and polymorphism destined for textbooks. Hoekstra and her lab have made extreme strides in evolutionary biology in recent years, especially with the coat coloration story of rock pocket mice. They are only a few strides away from linking the exact genotype and phenotype for coat color in many pocket mice populations. Hoekstra supports the idea that phenotypic variation is due to natural selection driven by crypsis. Furthermore, variation in the phenotype of pocket mice is not always a result of the same allele. Many mutations on the *Mc1r* and *Agouti* loci affect phenotypic color patterns of pocket mice. When asked what impact Hoekstra hopes to have on evolutionary biology, she responded by saying, "I hope to help us understand how adaptive traits evolve in natural populations" (2009). Hoekstra and her lab are making history.

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References

- Coyne, J.A., & Orr, H.A. (2004). *Speciation*. Massachusetts: Sinauer Associates, Inc.
- Hoekstra, H. E., Drumm, K. E., & Nachman, M. W. (2004). Ecological genetics of adaptive color polymorphism in pocket mice: Geographic variation in selected and neutral genes. *Evolution; International Journal of Organic Evolution*, 58(6), 1329-1341.
- Hoekstra, H. E., Hirschmann, R. J., Bunday, R. A., Insel, P. A., & Crossland, J. P. (2006). A single amino acid mutation contributes to adaptive beach mouse color pattern. *Science (New York, N.Y.)*, 313(5783), 101-104.
- Hoekstra, H. E., & Nachman, M. W. (2005). Coat color variation in rock pocket mice (*Chaetodipus intermedius*): From genotype to phenotype. *Zoology*, 133, 79-99.
- Hoekstra, H. E., & Nachman, M. W. (2003). Different genes underlie adaptive melanism in different populations of rock pocket mice. *Molecular Ecology*, 12(5), 1185-1194.
- Nachman, M. W., Hoekstra, H. E., & D'Agostino, S. L. (2003). The genetic basis of adaptive melanism in pocket mice. *Proceedings of the National Academy of Sciences of the United States of America*, 100(9), 5268-5273.
- Steiner, C. C., Rompler, H., Boettger, L. M., Schoneberg, T., & Hoekstra, H. E. (2009). The genetic basis of phenotypic convergence in beach mice: Similar pigment patterns but different genes. *Molecular Biology and Evolution*, 26(1), 35-45.
- Steiner, C. C., Weber, J. N., & Hoekstra, H. E. (2007). Adaptive variation in beach mice produced by two interacting pigmentation genes. *PLoS Biology*, 5(9), e219.
- "The Hoekstra Laboratory: Mammalian Evolutionary Genetics." (2009). Hoekstra Home Page. Harvard University. Viewed Feb. 22, 2009 at www.oeb.harvard.edu/faculty/hoekstra.