QnAs with Hopi Hoekstra

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> [N]ature cares nothing for appearances, except in so far as they may be useful to any being. She can act on every internal organ, on every shade of constitutional difference, on the whole machinery of life.

Since its publication more than 150 years ago, Charles Darwin's sly observation has earned the status of demonstrable fact. Darwin also held that the stepwise process of natural selection, which sifts through variation and winnows disadvantages, is largely hidden from human view. Yet his followers have found otherwise. Among those who have attempted to chronicle the inexorable march of natural selection with clockwork precision is Harvard University evolutionary geneticist Hopi Hoekstra, whose well-recognized work has revealed how incremental genetic changes allow animals to acquire new traits and adapt to changing environments. Hoekstra's tidy experimental designs have been tested in wide-ranging settings, such as the gypsum sands that ripple through the carnelian-colored New Mexico desert and the sweeping prairie grasslands of the American Great Plains. For her molecular insights into adaptation, Hoekstra won the 2015 Richard Lounsberry Award (www.nasonline.org/programs/awards/richard-lounsbery-award.html) of the National Academy of Sciences. PNAS spoke to Hoekstra to commemorate the honor.

Hopi Hoekstra at Harvard's Museum of Comparative Zoology. Image courtesy of Bear Cierci.

PNAS: What spurred your interest in the genetic basis of adaptation?

Hoekstra: As a graduate student, I entered the field at a time when molecular markers, like microsatellites and mitochondrial DNA sequences, were being used to ask organism-level questions, such as what is a species' migration pattern or phylogeographic history? By design, these markers were neutral, meaning they were used to trace traits of interest but did not directly influence the traits. The latter, to me, seemed more interesting, so as a postdoctoral fellow, I began focusing on the genes that actually mattered for the traits.

PNAS: Among several examples of such traits, you described in a PNAS article how three species of lizards inhabiting White Sands—an awesome terrain of snow-white gypsum dunes that appear to undulate across the Chihuahuan Desert in New Mexico—have each evolved blanched skin to blend into the dunes and avoid predators, whereas the brown-colored ancestral species has colonized the dark soil of the adobe desert around the dunes (1). What did you find about the evolution of skin color in the Eastern fence lizard, the little striped whiptail, and the lesser earless lizard?

Hoekstra: We were interested in understanding how similar traits evolve independently in different species that live in similar environments. In other words, we wanted to know how many genetic solutions there are to a common ecological problem. Among the three species of lizards we studied, we found that a candidate gene, called the melanocortin-1 receptor, which is a signaling receptor in the pigmentation pathway, was implicated in skin color in two species. In each of these species, we found a different mutation in this gene. Although both mutations result in light coloration, each acts through a different mechanism; one mutation reduces the strength of the receptor's signal and the other affects the receptor's integration into the cell membrane, resulting in fewer active

receptors. So you could have a few strong receptors or lots of weak receptors, and the functional outcome is roughly the same.

PNAS: In the same vein, you reported in *Nature* in 2013 that deer mice build small, simple burrows, unlike oldfield mice, a sister species, which build complex burrows with elaborately realized entrance and escape tunnels (2). Your genetic analysis of differences in burrow building revealed surprising insights.

Hoekstra: We became interested in the complex burrow architecture of the oldfield mice when we found that the burrow-building trait in this species represented a gain of functional complexity. First of all, we were surprised that the genetic control of what appeared to be a complex trait was largely simple, tied to four regions of the genome, three of which were associated with the length of the burrow's entrance tunnel. Moreover, each region contributes specifically to a three-centimeter increase in tunnel length. So if you introduced one of these regions from the big burrowing species into the genetic background of the small burrowing one, the small burrowing species will dig a tunnel, on average, three centimeters longer; add another region, and you get a tunnel six centimeters longer, add two, and the tunnel is nine centimeters longer. We also found a different region of the genome that was associated with the presence or absence of an escape tunnel in the burrow. These findings suggest that complex behaviors might be built by piecing together small, simple genetic modules.

PNAS: To go out on a limb, do the findings have any bearing on human behavior?

Hoekstra: To understand how genes can affect specific behaviors at a neurobiological level is an exciting challenge. Even though this trait does not have a direct homolog in humans—we don't build burrows—it is possible that some of the candidate genes we have pinpointed may underlie motivational differences between the two mouse species. Once we nail down the relevant mouse genes, we will of course look for similar variations in the corresponding human genes, if any. But that's beyond the scope of this article.

PNAS: Your work has led you into the molecular thickets of adaptation. In your 2013 *Science* article, for example, you dissected the gene implicated in camouflage in deer mice that scurry across the Nebraska Sandhills, a rolling landscape of sand dune-speckled prairies that seem to recede into the horizon (3). You zeroed in on the mutations that enabled the ancestral dark-brown mice to slowly evolve beige coats and dodge predators that hunt by sight.

Hoekstra: In previous work, we had implicated the *Agouti* gene in deer mice coat color. In this work, we probed the molecular mechanisms underlying the adaptation. Because the light- and dark-colored mice have a lot of pigmentation differences across their bodies, we thought a single mutation in a classic pigmentation gene, namely *Agouti*, may underlie the observed differences in the face, belly, back, and tail of the mice. But, we found something quite surprising: There were multiple mutations in the *Agouti* gene that affected color, and each mutation targeted a different region of the body. And when we looked for evidence of selection acting on these mutations, eight of the nine mutations showed strong signatures of selection, but only on the allele associated with light color (dark-colored mice stand out against the dunes' sun-bleached hues, inviting predators).

PNAS: Why was this finding surprising?

Hoekstra: Many studies have reported on the genetic basis of adaptations, but few have pinpointed the mutations involved, and that is because it is hard work. We started with an observation at the organism level, asking what mutations might underlie the color variations in different regions of the body between the two mouse species. That led us to these eight different regions in the genome, one of which is implicated in belly color, a trait you may not immediately expect to be favored by natural selection as defense against predation (after all, the bellies of mice are normally hidden from predators' view). But, the analysis suggested that belly color may also be independently selected, bringing us full circle to a surprising organism-level insight. It is a case of one gene evolving mutations independently to fine-tune the phenotype.

PNAS: Your findings support Darwin's notion of evolution's step-like progression.

Hoekstra: If we had stopped with our initial finding on the *Agouti* gene, we might have concluded that evolution proceeds in big steps. But, when we dissected the gene to the level of mutations, we found that there are multiple, small-step mutations that underlie adaptation. A lot of population genetics theory is based on these mutations and not on the genes themselves, so identifying the mutations does in fact reinforce Darwin's idea of evolution through small steps.

PNAS: What was your reaction to the prize announcement?

Hoekstra: I was very surprised and am delighted to see that evolutionary genetics is being represented in this way. I am also thankful for all of the creative, dedicated, and hard-working collaborators, postdocs, and students with whom I have had the pleasure to work, both in the laboratory and in the field, and in the past and in the present. I have been very lucky to be part of such a fantastic team.

1 Rosenblum EB, Römpler H, Schöneberg T, Hoekstra HE (2010) Molecular and functional basis of phenotypic convergence in white lizards at White Sands. *Proc Natl Acad Sci USA* 107(5):2113–2117.

2 Weber JN, Peterson BK, Hoekstra HE (2013) Discrete genetic modules are responsible for complex burrow evolution in Peromyscus mice. *Nature* 493(7432):402–405.

3 Linnen CR, et al. (2013) Adaptive evolution of multiple traits through multiple mutations at a single gene. *Science* 339(6125): 1312–1316.