Profile of David M. Kingsley

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When talking about his career as a developmental and evolutionary biologist, Stanford University's David M. Kingsley likes to say that "genetics works." He means that genetics can solve biological problems that have long been mysterious. He has repeatedly put that theory to the test throughout his research career, initially using genetics to study a cellular process called receptor-mediated endocytosis. Kingsley subsequently discovered several genes controlling bone formation and repair in vertebrates and harnessed genetic tools to identify genes that underlie major evolutionary differences in natural species. In his joint Inaugural Article with long-time collaborator Dolph Schluter, Kingsley pinpoints a genetic region that appears to affect overall species fitness in stickleback fish (1). Kingsley is a Howard Hughes Medical Institute investigator and was elected to the National Academy of Sciences in 2011.

David M. Kingsley. Image credit: Cynthea Kingsley (photographer).

Research that Makes a Difference

Kingsley grew up in Des Moines, Iowa with his parents and siblings. When Kingsley was 4 years old, a rare form of cancer killed his father at age 34. "I started thinking about wanting to use whatever time that I had in my own life to study problems bigger than myself and come up with answers that might last longer than myself," Kingsley says. "The feeling of progress that I could see from asking and answering scientific questions seemed like an island of stability in an otherwise uncertain world."

As a child, the questions he loved most involved dinosaurs and skeletons, a passion encouraged by his mother with museum trips to Chicago and Washington, DC. An advanced biology teacher in high school, Jack Koch, challenged and excited Kingsley's interest in biology. He chose to attend Yale University as an undergraduate because the brochure they sent him highlighted its new Kline Biology Tower.

For graduate school, Kingsley moved to Harvard University, intent on studying cell biology. He quickly learned that the best classes were across the Charles River at the Massachusetts Institute of Technology (MIT). Kingsley took courses at MIT and attended a seminar by MIT professor Monty Krieger, who described how he was combining genetics and cell biology to study receptor-mediated endocytosis and low-density lipoprotein cholesterol metabolism. Kingsley transferred to MIT and joined Krieger's laboratory.

At MIT, Kingsley met David Botstein, who touted the ability of genetics to solve biological mysteries. "I found his message incredibly appealing. If you could figure out a way to turn your problem into a genetics problem you had a pretty good chance of being able to figure things out eventually," Kingsley said.

In Krieger's laboratory, Kingsley used Chinese hamster ovary cells to study the cellular machinery that internalizes endocytic vesicles and sends them to particular destinations inside cells. He isolated many cell lines with endocytosis mutations. One was a glycosylation mutant that caused an enzyme deficiency (2). He found that the mammalian form of the enzyme was different from the forms in yeast and bacteria. Two children, identified through a newborn metabolism test, had defects in the same gene, and Kingsley's research helped reveal what sugars those children should avoid to prevent medical problems (3).

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Kingsley's newfound appreciation for the genetic distinction between humans and other organisms sent him in search of a problem in vertebrate genetics distinct from what yeast, bacteria, and fly researchers were doing. He settled on the skeleton. A seminar by Stanford geneticist David Hogness on chromosome walking in *Drosophila* convinced him that it would be possible to map organismal traits to genes and molecules. "I thought if he's doing that for flies, it should also be possible for vertebrates, particularly in mice," says Kingsley. He spent his last year at MIT looking for a postdoctoral stint that would train him in both classic mouse genetics and molecular methods to trace traits to genes.

Kingsley decided to join Neal Copland's and Nancy Jenkins' mouse genetics laboratory at the National Institutes of Health. They had isolated a gene called *dilute* that was close to a classic skeletal trait termed *short-ear*. Kingsley figured it was close enough to walk from *dilute* to *short-ear*. He estimated it would take him about six walking steps to get there; instead, it took 60 steps and 5 years. Kingsley finally found the gene in May 1992 (4) after accepting a faculty position at Stanford.

In the end, Kingsley's *short-ear* gene was worth the wait. The classic morphological trait was due to mutations in a bone morphogenetic protein (BMP), a secreted signal that biochemists had also found in ground-up bones and that stimulated cartilage and bone formation. *Short-ear* was the first known vertebrate BMP mutation and demonstrated that the proteins were required for normal skeletal formation (5). Two years later, Kingsley found another classic skeletal trait controlled by a different BMP (6). "These mutants provided a really compelling case that BMPs are the molecules that the body uses to control when cartilage and bone forms and what they're going to look like. For someone who had stared at skeletons, marveling at the morphology for years, to actually have your hands on the key signals that embryos use to make and lay out the formation of bones was incredibly satisfying," says Kingsley.

Finding these genes helped frame the next questions. If bones were made by BMPs, what lays out the shapes and patterns of the key inducing molecules? Kingsley has spent two decades identifying the underlying DNA sequences. Mouse mutations pointed the way to huge arrays of special regulatory sequences surrounding the BMPs (7). Separate regulatory sequences control specific bones (8), particular joints (9), and reinduction of BMPs after injury (10). Changes in BMP regulatory sequences underlie classic morphological traits in humans as well as mice, including common height and arthritis variations in modern populations (11).

Genetics of Vertebrate Evolution

Kingsley next set out to study the genetics of vertebrate evolution. He wanted to cross-breed natural species that had evolved major skeletal differences and find the genes that control the appearance of new traits in nature. Many colleagues were skeptical. The general consensus at the time was that evolution mostly occurs via countless small and diverse genetic changes that add up over time. Kingsley and postdoctoral scientist Katie Peichel nonetheless took up the challenge. They spent the summer of 1998 looking for species that showed dramatic skeletal differences but could still be mated to produce viable hybrids. They found a rich literature on threespine sticklebacks, a small fish found in oceans, freshwater lakes, and streams throughout the northern hemisphere (12). Many stickleback species have independently evolved similar traits, for example, shedding armor plating and changing color. Kingsley and Peichel also found the University of British Columbia's Schluter, who was already cross-breeding sticklebacks to examine the properties of pure and hybrid forms in different environments.

"It's been a great collaboration because our backgrounds are complementary," says Kingsley. "My [laboratory] had years of experience starting with traits and getting them all the way down to chromosomes and the genes and the mutations that controlled them. And Dolph had synergistic expertise spanning traits, organisms, populations, ecology, speciation, and mathematical modeling."

Together, Kingsley and Schluter have identified several key genes that control evolutionary changes in stickleback morphology. For example, changes to the key regulatory gene *PITX1* causes the loss of the entire pelvic girdle in some populations (13, 14). Changes to the developmental signaling gene *EDA* underlie large differences in armor plating (15). Regulatory changes in BMPs alter bone dimensions in freshwater sticklebacks (16), and changes in a stem cell signal (*KITLG*) control changes in body pigmentation (17). In every case, genetic crosses showed evolution occurred through particular loci with big effects. The key loci turned out to be essential developmental control genes, and for each trait, nature had side-stepped deleterious consequences by making changes in the regulatory part of the genes, not the part that encodes a protein. This trick allows a gene's expression to change in particular body parts without knocking the gene out completely, which could be deadly. Finally, and most interestingly, evolution was using the same genes and mechanisms each time similar traits evolved in different populations (13–17). Strikingly, this reuse of evolutionary mechanisms extended far beyond just the sticklebacks. For example, Kingsley's laboratory showed that *KITLG* had been selected both in fish and humans adjusting pigmentation in different environments (17). They identified a specific regulatory change underlying blond hair in northern Europeans, a classic trait in humans evolving through the same principles found in sticklebacks (18). Subsequent genome-wide studies show that 85% or more of loci positively selected during recent stickleback (19) and human evolution (20) have evolved through regulatory rather than coding changes in genes.

In their Inaugural Article (1), Kingsley and Schluter bring the same genetics approach they have used for morphological traits to study evolutionary fitness

itself. They crossed two species of stickleback, put the hybrid stickleback in a pond, and asked whether any specific chromosome regions affected the number of offspring a fish produced in the next generation. The results identified a now familiar locus: the same *EDA* region controlling armor plating and other traits in freshwater fish. Females with two freshwater copies of *EDA* leave twice as many offspring as females with two marine copies of *EDA*. By teaming up with stickleback biologist Mike Bell, they also found that this strong fitness effect is maintained over subsequent generations. Bell has collected annual population samples from a lake in Alaska that was colonized by marine stickleback in the early 1990s (21). "You can see in these fish that the stickleback will make the morphological transition from fully plated marine to low-plated freshwater fish in 10 years," said Kingsley, and *EDA* and other key genomic loci can now be followed throughout this process.

Kingsley thinks the Inaugural Article (1) will lead to future work to pin down the basic mechanisms controlling rapid evolution as organisms adapt to new environments. "Sticklebacks have been a system where it's possible to combine many different levels of analysis, from basic genetics, to development, whole-organism phenotypes, environmental interactions, and now dynamic evolution happening before our eyes." One of his great satisfactions has been uncovering general principles that cut across species and have wider relevance. "We've already found mechanisms that also contribute to classic traits and health conditions in billions of people around the world," Kingsley says. "There's no doubt there are many more to find, as we study nature's own recurrent solutions to a constantly changing world."

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