A Conversation with David Kingsley

INTERVIEWER: JAN WITKOWSKI

Executive Director, Banbury Center at Cold Spring Harbor Laboratory

David Kingsley is a Professor in the Department of Developmental Biology at the Stanford University School of Medicine.

Jan Witkowski: Could you give us a brief rundown on your work?

Dr. Kingsley: We're trying to use Mendelian principles to study evolution. Darwin didn't have a hereditary mechanism, and a lot of the biggest holes in *On the Origin of Species* were because of a lack of information about where variants came from, how they segregated in families, how traits could disappear for a while and then reappear. Mendel fixed a lot of that in papers published only 5 or 6 years after Darwin's work, but Darwin never really knew about it. Research today can revisit the same questions that Darwin and other people worried about but using Mendelian principles to figure out where the key chromosome regions are that control evolutionary change. What are the actual genes that underlie the interesting traits that we see between natural species? About 15 years ago, we decided that to answer a lot of questions in evolutionary biology, we would have to take an unbiased genetic approach to figure out what had happened in naturally occurring animals. We've been looking for systems that would make it possible to study naturally occurring species by genetics.

Jan Witkowski: I presume it's very important what species you choose to study.

Dr. Kingsley: That's the paradox. You have to look for loopholes in the definition of "species," which is that they're "reproductively isolated." You would think that puts the origin of species outside the realm of traditional genetics. We looked for organisms that had evolved recently enough that you could still cross them. That's the loophole. You can be reproductively isolated for lots of reasons; nonviability or sterility are just the most extreme examples. We went looking for natural species that were so recently evolved that it was only behavioral and mechanical incompatibilities that kept them apart.

We found our model in these young fish called the three-spined stickleback, which radiated just in the last 10,000 years. They're all over the place, and they show remarkable differences: morphological, physiological,

behavioral. There are tens of thousands of different stickleback populations that were established when their marine ancestors colonized and gave rise to new freshwater populations when the glaciers melted. If you look across the whole range of populations, there's a spectrum of biological incompatibility. There are pairs that are sterile if you put them together, but a lot of them just don't want to mate with each other, and that can be overcome by setting up fertilization in the lab. You can squeeze out eggs and sperm from these very different looking fish and set up crosses to try to map the genetic basis of evolutionary change.

Jan Witkowski: And these various forms in the wild, presumably the ones that don't breed, are considered different species?

Dr. Kingsley: Absolutely. There are species pairs in the same lake where one form has specialized for shallow water and one is specialized for the open water. Those forms have different sizes, different colors, different teeth, different armor. They eat different food, they encounter different predators. If you follow genetic markers in the lake, they are by and large reproductively isolated. They would meet the formal definition of "species," yet if you compared their genomes, they're very similar. There's maybe one base pair difference per every 300 bases in the genome.

Jan Witkowski: What sort of traits have you been looking at?

Dr. Kingsley: Despite them only having evolved for 10,000 years, they show huge differences, like a 30-fold difference in the number of armored plates along the side of the fish or a doubling of the number of teeth. Some of the populations had adapted to different food sources, so they completely transformed their jaw and their chewing apparatus to take advantage of different things. Entire fins are present or absent between species. In some ways, fish are like land animals; they're supposed to have two sets of paired appendages. In many stickleback populations, the

hind fin has completely disappeared. It's the same kind of trait you see in whale evolution, only in a fish that just evolved that trait within the last 10,000 years and that you can still cross to study their genetics.

Jan Witkowski: Sticking with the evolutionary side, are these sticklebacks peculiarly variable as compared with other organisms?

Dr. Kingsley: Because they have a migratory life cycle that set things off just when the glaciers melted, we have an opportunity to see dramatic evolutionary change that occurred very recently. I think the kinds of things they do are a microcosm of what's played out over and over again in the history of life on Earth.

Jan Witkowski: Back to the genetic side of the equation.

Dr. Kingsley: I had interesting discussions when we started this project, because a lot of people felt that evolution was so complicated that if we tried to set up genetic crosses between very different naturally occurring species, the differences we see today would be due to thousands of tiny changes that had accumulated over time. Darwin actually believed that. He said over and over again that Nature doesn't make leaps, that evolution is going to be based on infinitesimal changes that are accumulated. When Mendel's laws were rediscovered, the Neo-Darwinian synthesis used Mendelian principles, but took the effect size, again, almost infinitesimally small. Several colleagues said we would not be able to find chromosome regions that had very large genetic effects, because all of the effects that underlie evolution are these tiny things that have been accumulated.

That's not what we found. We found that if you simply cross naturally occurring species, that very often when we did the genetic mapping, we could find chromosome regions that controlled a lot of the trait. It wasn't Mendelian. We didn't find chromosome regions that could explain 100% of the variance in a trait. We would find chromosome regions that explained half, or two-thirds, or three-quarters of the variation in armor, or teeth, or the presence or absence of a hind fin. That meant as a geneticist, there was a handle that you could chase down to find the molecular mechanisms that had produced these dramatic morphological differences. For example, we found a gene that accounts for a large proportion of the loss of the hind fin.

If you map pelvic reduction, you get a major locus that does about two-thirds of the variation in the trait, and four or five modifier regions that each might control a few percent of the variants in the trait. The major chromosome region is a very well known developmental regulator, a master transcription factor called PITX1 that's a homeodomain transcription factor that binds to and regulates all sorts of genes during normal development. It doesn't sound like it has anything to do with hind limb or pelvic development at all, because like many of these key developmental regulators, it does a lot of other things during normal development; for example, it's required for normal pituitary formation. This relates directly to a classic

debate in evolutionary biology, which is the idea of monsters.

Jan Witkowski: I was going to come to that. What is a "hopeful monster"?

Dr. Kingsley: Richard Goldschmidt proposed "hopeful monsters," and the leaders of the Neo-Darwinian synthesis absolutely hated that, because Goldschmidt was saying that interesting differences might have emerged all at once in a hopeful monster. The counterargument was that genetic changes that do big things to animals usually are deleterious. They're "hopeless monsters," in the words of Ernst Mayr.

Jan Witkowski: I was going to mention Bateson's homeotic mutants. They would follow the "hopeless monsters" theory.

Dr. Kingsley: Goldschmidt pointed to the existence of homeotic mutations, and gave those as examples of the remarkable things that can happen to an animal all at once. Mayr's response was that homeotic mutations are sterile and have reduced viability. For me, it was a real pleasure to find a homeodomain transcription factor underlying pelvic reduction in sticklebacks because if you knock that gene out, you get a hopeless monster. It has small hind limbs, but it dies at birth. It's got craniofacial abnormalities, cleft palate, pituitary defects, and it doesn't look like a very promising basis to evolve traits that have been under positive selection in natural populations, but nonetheless that's exactly the gene that Nature has used.

Jan Witkowski: So when you knock out the gene, you get the hopeless monster because you've lost everything. Then how does PITX1 have such a dramatic effect on just the pelvis without having deleterious effects elsewhere?

Dr. Kingsley: The way Nature makes an advantageous change instead of a hopeless monster depends on powerful developmental control genes that are themselves regulated by highly tissue-specific enhancers that recruit their expression at different times and places during development, independently of each other. When we looked at the difference between a natural species that had lost its pelvis through PITX1 or at the marine ancestor, most of what PITX1 does is completely intact in both natural species. The protein region's fine, the expression in the pituitary's fine, the expression in the head's fine. The pelvic-reduced fish have lost a noncoding bit of DNA, far away from the transcript itself that causes that gene to turn on specifically in the developing pelvis. If you lose that information, you can get a huge hopeful monster effect at that place in the body, and preserve everything else the gene does. Nature essentially makes regulatory changes instead of coding changes, and that allows it to tweak specific aspects of gene function instead of everything the gene does globally.

Jan Witkowski: Is PITX1 involved in the loss of rear limbs in mammals?

Dr. Kingsley: There's a morphological signature, an odd directional asymmetry, for losing the pelvis through PITX1 that we see in sticklebacks and also in knockout mice. PITX1 is closely related to a cousin gene named PITX2, and PITX2 is involved in left-right axis formation. If you lose the pelvis by the PITX1 gene, the morphological effect is usually more severe on the right side than on the left side, because the PITX2 cousin is still present on the left side. When PITX1 was knocked out in mice, researchers shrank the pelvis, but there was more reduction on the right than the left. In sticklebacks, if you do the crosses between the naturally occurring forms, and map the asymmetry, you again identify the PITX1 locus, and you find exactly the same directional effects; the pelvis is bigger on the left than the right.

We've looked at the pelvic rudiments of whales and the other mammalian example of pelvic reduction, manatees. Manatees show exactly the same kind of bigger-on-the-left-than-the-right bias that we see in the sticklebacks and mice, so at least the morphological signature strongly suggests that the same mechanism might be involved. We are actively trying to track down the regulatory regions in the mammalian gene that control pelvic expression, but unfortunately the sequence for the pelvic enhancer in the stickleback gene does not align to mammals. We've had to re-search through the mammalian gene to find the relevant regulatory switches.

Jan Witkowski: A quick aside, the first proper director of Cold Spring Harbor, Charles Davenport, died after getting pneumonia dissecting a whale out of East End.

Dr. Kingsley: I will be careful during these studies!

Jan Witkowski: It seems then there's only a handful of major, more functional, genes involved in making these sticklebacks so different?

Dr. Kingsley: When I say it's possible to find the chromosome regions that control evolutionary traits, I don't want to minimize the complexity of the problem, because we've probably mapped over 200 chromosome regions that contribute to some aspect of the difference between forms. Those are just the ones that are big enough for us to see, and just the things we've decided are major. The global issue of evolving a new form involves a whole bunch of genetic changes, but if you focus on a particular trait at a time, if you atomize the problem and look at the components of the phenotypes, you can track down the genetic basis of traits much more easily than would have been predicted by the old debates about infinitesimal change.

We are now trying to take the lessons we've learned from how evolution works in sticklebacks and apply it to a search for the molecular basis of becoming human. That's an ambitious problem that faces a lot of the same objections we heard when we started working on sticklebacks. You can, in fact, find signatures that we think contributed to limb modifications, brain changes, and other morphological traits, and I think we'll be able to study the problems of cumulative evolution of humans as well.

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