The Making of the Fittest: Evolving Switches, Evolving Bodies

[NARRATOR:] For tens of thousands of years, much of North America lay buried under ice up to a mile thick. Then, the massive ice sheets receded. In what is now Alaska, ocean bound streams and rivers emerged... ...opening up new possibilities for countless species. One of the animals that came calling was the three-spined stickleback. Common to the northern ocean, this little fish spawns in fresh water. There were now lots of new spawning grounds to explore. But as the ice-free land began to rise, streams, and the fish in them, were cut off from the sea. Isolated populations of sticklebacks faced a survival challenge. Could they adapt to full-time life in a freshwater lake? 10,000 years later, they're still there. But they have been transformed.

[CARROLL:] Stickleback bodies changed in many ways as they adapted to life in post-glacial lakes. They got smaller, their coloring changed, and most strikingly, even their skeletons changed.

[NARRATOR:] As we begin to learn exactly how stickleback bones evolved, we're learning about a lot more than just fish. We're learning about how all animal bodies evolved.

[BELL:] Hope I caught some fish.

[NARRATOR:] This is Bear Paw Lake, one of many lakes created in Alaska by the glaciers' retreat 8 to 10 thousand years ago. The sticklebacks one can catch here are especially intriguing to biologists interested in evolution such as Mike Bell. Like all freshwater sticklebacks, their ancestors lived in the sea.

[BELL:] In the ocean there are lots of large, predatory fish, and there's no place to hide.

[NARRATOR:] So, sticklebacks evolved body armor, bony plates on their side, and long sharp spines coming off their pelvis and back.

[BELL:] They're generally easy for predatory fish to catch, but they're not easy to swallow.

[NARRATOR:] In Bear Paw Lake, however, spines are a liability. There are no large mouth predators here. But there are hungry dragonfly larvae that grab sticklebacks by their spines. So, pelvic spines actually reduce fitness and lessen a fish's chances of surviving and reproducing. In this lake, natural selection has been at work.

[BELL:] If you look at the pelvis of this fish, there's practically nothing there.

[NARRATOR:] In just a few thousand years, these fish underwent a dramatic skeletal change, completely losing their pelvic spines. As pelvic spines are homologous to the hind limbs of four-legged vertebrates, the change we see in sticklebacks is the equivalent of losing legs. How does such a dramatic change in form occur? For Stanford molecular geneticist David Kingsley, the transformation of the stickleback pelvis opened a door on an evolutionary puzzle.

[KINGSLEY:] What happened at the genetic level, at these early stages where the body plan is first being laid out, that makes the difference that we now see?

[NARRATOR:] The physical forms of all animals are products of development-- that process in which a fertilized egg grows and is shaped into an adult. Changes in form, therefore, arise from changes in development. And since genes control development, changes in form are ultimately due to changes in genes.

[CARROLL:] David, these two fish look different. But they have thousands of genes. How do you pinpoint which genes make the difference?

[KINGSLEY:] We started like any geneticist starts. You gotta have two things that are different and you gotta cross them.

[NARRATOR:] Geneticists use crosses to map the location of genes that make the difference. Ocean and freshwater varieties of stickleback can be crossed by collecting sperm-filled testes from males and eggs from females and mixing them together. In a matter of days, the beating hearts of stickleback embryos are visible through a microscope. When mature, this first generation is bred again. Each cross re-shuffles the genetic material and traits that are passed on from one generation to the next. Traveling with the genes are stretches of DNA geneticists use as markers.

[KINGSLEY:] And that gives you the chance then to try to figure out which of the pieces at the genetic level go together with the traits that you see visually at the whole organism level. That's done using the DNA markers to link the trait-- in this case the presence or absence of a pelvic spine-- to general locations on specific chromosomes. This hunt eventually pointed a finger at a well-known and powerful developmental control gene called *Pitx1*. So, naturally, they compared the *Pitx1* protein coding sequence in fish with and without pelvises.

[CARROLL:] And what'd you find?

[KINGSLEY:] Well, actually we didn't find anything at all. At the coding region of the *Pitx1* gene, the actual part that makes the protein, there isn't any difference between marine and freshwater fish.

[CARROLL:] Well, that's fairly puzzling, I mean, we for years were used to the idea that if there's an evolutionary change, that would be a change in the protein made by a gene.

[KINGSLEY:] Yup.

[CARROLL:] So you see no differences in the sequence of the *Pitx1* protein between the two fish. I mean, isn't that a paradox? Isn't that a surprise?

[KINGSLEY:] Well, it's still possible that there's something about the expression or the regulation of the gene that's changed. So the structure's fine but maybe the timing or the place that it's normally expressed is different.

[NARRATOR:] To find out, Kingsley's team soaked embryos with a chemical dye that turns blue any tissue where the *Pitx1* messenger RNA is produced.

[KINGSLEY:] If you look at a marine embryo, you see the *Pitx1* gene is expressed in multiple places. It turns on in the head region, in the lips, inside it would be on in the pituitary, but it also turns on along the side of the body, this very strong blue patch here...

[NARRATOR:] In that tissue, it's telling cells to start growing a full pelvis and spines.

[CARROLL:] And what about in fish that aren't going to make a pelvis?

[KINGSLEY:] Right, key moment in the lab was the same experiment in the lake fish. In the head region, you still see blue on the lips, you still see blue inside the head. You don't see that little key blue spot along the side or on the ventral surface of the fish.

[CARROLL:] So the structure of the protein's the same between the two populations, and the expression of the gene is the same between the two populations except for just in the pelvis.

[NARRATOR:] How can the expression of a gene change in one part of the body but not another? This is possible because the coding regions of most genes that control development are surrounded by a number of regulatory switches, each of which controls gene expression in a different tissue. Like all DNA, the sequences of switches can acquire mutations. Kingsley had a hunch that the switch regulating *Pitx1* expression in the pelvic tissue of freshwater sticklebacks was broken. But to find out, he had to first find that switch. Geneticists find switches by tracking the expression of a reporter protein that glows green where a switch is active. After cutting the DNA around the *Pitx1* coding sequence into many different fragments, they attached the green reporter gene to each of them. Then, they injected those fragments into stickleback eggs.

[KINGSLEY:] We wait a week or two and then we ask "Are our sticklebacks glowing in the pelvis?" After five years of testing different fragments, they had fish with glowing pelvic tissue. They'd found the stretch of DNA that contained the *Pitx1* switch. Sequencing that region revealed a dramatic mutation.

[KINGSLEY:] Fish that have lost their pelvis have deleted the pelvic switch. It's gone.

[NARRATOR:] But because this mutation only crippled one specific switch, the *Pitx1* gene remained fully functional in the rest of the body.

[KINGSLEY:] If you do that, you can have a huge effect on the development of that structure but the fish is fine. Actually, the fish is better than fine. When that deletion occurred it conferred an advantage on the fish and that mutation spread throughout the entire population.

[CARROLL:] So the obliteration of that switch actually makes these fish better adapted to the new environment they're in than their ancestors.

[KINGSLEY:] That's right.

[NARRATOR:] With the switch identified, he was ready for a final test.

[KINGSLEY:] If you've got the right switch you ought to be able to put it back and reverse the evolutionary trait. So, they joined the working switch to the *Pitx1* coding region and injected the combination into eggs from a freshwater stickleback that would normally never form pelvic spines.

[CARROLL:] And?

[KINGSLEY:] That was a good day in the lab. It worked. There is a fish now swimming around in the tank, hasn't formed a pelvis for maybe thousands of years, it does-- if you put back in the key sequence. [

[NARRATOR:] Kingsley's team had found the broken switch that caused fish from one lake to be without spines. But that isn't the only place one can find spineless sticklebacks. When he looked at fish from other lakes, he found something remarkable.

[KINGSLEY:] If you look at a fish that's lost its pelvis in Scotland, or Iceland, or Alaska, or British Columbia, the same switch has been thrown away over and over and over again whenever the fish have evolved a loss of a pelvis.

[NARRATOR:] Given the same selective conditions, evolution can and does repeat itself, right down to the level of the same gene and genetic switch. And amazingly, it appears that the same adaptation has also occurred in the much deeper past. Ten million years ago, this Nevada desert was a lake, full of sticklebacks. Their fossil remains have long fascinated Mike Bell.

[BELL:] Every year many stickleback would die and their bodies would drift to the bottom and be covered with sediment. The flesh would rot off the bones and very often leave a beautiful, intact skeleton.

[NARRATOR:] Early on, Bell realized that there were two distinct types of sticklebacks preserved here.

[BELL:] Some of the fish had a really big pelvic bone behind the head. And other fish didn't have that bone but a little tiny pelvic bone.

[NARRATOR:] One might expect one or the other to be favored by natural selection. So why were they both here? This quarry has a thousand sediment layers in every foot of rock, a thousand years of annual deposits. A record of change like that is an evolutionary biologist's dream. To move from one end to the other is to move through time. By painstakingly checking fossil pelvis size over a 20,000 year period, Bell made a surprising discovery. Fish with a full pelvis had arrived suddenly, perhaps when some geological event briefly opened the lake to the sea. Yet within a few thousand years, almost all sticklebacks here lacked pelvic spines.

[BELL:] And in Alaska we're seeing exactly the same phenomenon taking place but it's 10 million years later.

[NARRATOR:] The same animal at two distant moments in time undergoing the same transformation, in both cases pretty quickly, and in all likelihood, via the same evolving switch.

[BELL:] This is a really exciting time to be a biologist. Only ten years ago we couldn't get at the DNA of stickleback in a detailed way. And now you can combine that kind of information with natural history. We can link up genetics to development, development to phenotypes, phenotypes to environments; we can look at change through time in the fossil record. We can put together the whole package.

[NARRATOR:] As biologists do just that, they're finding that the most common mechanism driving the seemingly endless diversity of animal bodies is mutations in the switches that regulate developmental control genes.