Evolution: Constant Change and Common Threads
Lecture Three—Fossils, Genes, and Embryos
David M. Kingsley, Ph.D.

1. Start of Lecture Three (00:18)

[ANNOUNCER:] *From the Howard Hughes Medical Institute. The 2005 Holiday Lectures on Science. This year's lectures— "Evolution, Constant Change "and Common Threads" will be given by Dr. Sean B. Carroll Howard Hughes Medical Institute investigator at the University of Wisconsin, Madison, and Dr. David M. Kingsley, Howard Hughes Medical Institute investigator at Stanford University School of Medicine. The third lecture is titled "Fossils, Genes, and Embryos." And now to introduce our program, the president of the Howard Hughes Medical Institute, Dr. Thomas Cech.*

2. Introduction of HHMI President Dr. Thomas Cech (01:08)

[DR. CECH:] Good morning and welcome back to the Howard Hughes Medical Institute and to the 2005 Holiday Lectures on Science, "Evolution, Constant Change and Common Threads." In his last lecture David Kingsley talked about domestication of wolves into dogs and of teosinte into corn. Today he's going to delve deeper into evolutionary time and show us about how genetic changes in living populations today relate to changes that we see in the fossil record. Now David primarily studies fish, but the rules and mechanisms that he has uncovered have broad implications for the evolution of all kinds of species, including humans. One of the surprising things that David has discovered is that evolution appears to be a great recycler, using the same mechanisms over and over again. The talk is entitled, "Fossils, Genes, and Embryos." And now here's a video of David at work.

3. Introductory Interview with Dr. David Kingsley (02:24)

[DR. KINGSLEY:] For me why I went into science and one of the most rewarding things about it is the rare opportunity to get to spend your life solving problems or studying questions that people have wondered about for thousands of years. I think we live right now at a time when the techniques are available to pick any problem that you're interested in and get to be the one who solves some age-old problem and that's, I think, a fairly unusual opportunity. But for a long time the problems weren't solved because the methods weren't available to work on them. What's different now is that the methods are available to work on them so I think 100 years from now people will look back and say, "That must have been an incredible time to be doing science. The methods were there and all you had to do was pick the problem you were interested in and go apply the methods." And what could be more exciting than to live in that sliver of time when wonderful unsolved problems are solvable by the application of methods that have worked repeatedly in other areas? There's no other job in the world where you could have the privilege of getting to spend your time trying to figure out how things work. I think it's important for students not to decide whether they like science based just on the way science has been presented to them in a few classes. So if you can find some way to get into a lab or to interact with scientists doing science, that it's the best possible way to see the differences between the problem solving that scientists really do and the way it's normally presented in the classroom. There's a big difference between memorizing the words that scientists use and actually doing science and I think that people have to get past that nomenclature aspect and get into lab environments where they can actually do science and that means to be confronted with a problem where the answer isn't known. So don't memorize the answer, try to find the answer.

4. Laws of nature lead to natural selection (04:53)

Welcome back everybody. Yesterday we saw how selection by humans has transformed plants and animals into the modern forms that we see around us today. Darwin and Wallace both realized that the same principles of selection that have so dramatically changed both plants and animals under domestication should also act in nature. So wild plants and animals vary in all directions. The differences are inherited. Many more individuals are produced than can possibly survive and reproduce so that creates a competitive situation. Favorable variants will inevitably leave more offspring and these laws of nature create a process of natural selection that will extensively modify organisms over time. So based on these laws Darwin proposed that modern plants and animals are just the youngest sprouts and twigs of an immense and ancient tree of life and that all organisms that have ever lived may be related by descent with modification extending back to the very earliest stages of life on earth. Now that, I think is obviously a profound intellectual leap. It's always struck me as large as the leap that was made by Newton. Many of you know Newton, he saw an apple falling in his back yard and realized that the same forces of gravity that control objects falling in his backyard might also control the falling of the moon around the earth, the orbiting of the earth around the sun and in fact the principles that construct the entire solar system. So Newton's scope for physics was incredible, from apples to orbits, from his backyard to the entire solar system and I think Darwin's book had a similar breathtaking scope from pigeons in his backyard in the very first chapter of *The Origin of Species* to this immense tree of life that connects all living forms by modification by descent by the final chapter of *The Origin*.

5. Descent with modification explained conundrums (07:01)

Okay, so when in 1859 when Darwin's great theory was proposed, it already explained all sorts of confusing data from different areas, including odd facts from biogeography, the existence of closely related organisms and island groups like the Galapagos. Facts from paleontology like the fact that modern mammals are only found in the most recent fossil strata. Facts from embryology, common structures are seen in embryos of animals that look very different from each other and unusual facts from morphology. So comparative anatomists have known for a long time that some structures existed in animals that were very hard to explain under the prevailing idea that each species had been intelligently designed to optimally match its own environment. For example, lots of animals that live in caves never see light, they have no reason to have eyes, and yet lots of cave animals have vestigial eyes. The eyes begin to form, or they form and they degenerate or they form but they're completely covered by skin. So these are non-functional organs in an environment where eyes aren't needed. Very hard to imagine why they're there if each organism was designed to live in a cave, but easy to explain if current blind animals evolved from precursors that used to see. So descent with modification explains these structures as a vestige, a leftover from their common ancestry.

6. Major questions resulting from Darwin's theories (08:36)

Although Darwin's theory explained lots of peculiar facts that existed in 1859 it also set off a whole series of raging debates about all sorts of other issues. Many scientists believed the earth was too young to be compatible with Darwin's theory of evolution when it was published in 1859. There was an absence of transitional forms that Darwin predicted should be all over the place in the fossil record. Many people thought natural selection couldn't work the way Darwin proposed because any rare variants that occurred would get swamped out by a form of blending inheritance that we'll come back to in a second. And finally many people just fundamentally thought that animals looked too different from each other to have possibly come from common ancestors. So all of these issues were extensively debated during Darwin's lifetime. He tried to address them in subsequent issues of *The Origin of Species* but in fact many of them weren't resolved for decades. Let's come back and talk about each of these key problems.

7. Is the earth too young for descent from a single ancestor? (09:41)

How about the age of the earth? The age of the earth has been calculated in lots of different ways. There was a famous calculation by Archbishop Ussher in the 1600s that creation had occurred on October 23rd, 4004 B.C. That estimate was based on genealogy in the Bible. So in the 1800s there was already accumulating geological evidence that the earth must be much older than that. One of Darwin's contemporaries was a famous physicist named Lord Kelvin. How many of you guys have ever heard of Kelvin? Yeah. This is the Kelvin of the second law of thermodynamics. This is also the Kelvin of the absolute temperature scale. So degrees are named after Lord Kelvin, one of the most famous physicists of the 1800s. So Kelvin calculated that the earth couldn't be older than—the earth and sun had a maximum age of 40 million years. That was based on the physics of the cooling of the earth and sun. That estimate bothered Darwin a lot because Darwin had estimated in *The Origin* that it might take hundreds of millions of years for his process of descent with modification to generate this immense tree of life. Darwin wanted 400 million years, ten-fold more than Lord Kelvin thought was physically possible.

8. Modern physics shows that the earth is 4.6 billion years old (11:01)

So this debate was actually resolved by an amazing series of discoveries in 20th century physics that Darwin didn't live to see. Einstein realized in the earliest 20th century that energy and mass are actually interconvertible. His famous equation E=mc squared, E for energy and m for mass. Detailed measurements of the actual mass of atoms shows that four hydrogen atoms actually weigh slightly more than one helium atom and the way the sun actually shines is a process of fusion energy. So four hydrogen atoms are fused together to make one helium and a small bit of excess mass is converted into energy. So the sun is a nuclear furnace, not the kind of conventional furnace that Kelvin had modeled in the 1800s. You can actually measure the amount of hydrogen and helium in the sun and calculate how old the sun must be based on that nuclear furnace. Similarly around the turn of the century radioactivity was discovered for the first time. That actually provided a new source of heat within rocks and it also provided a brand new way to calculate the absolute age of rocks. Detailed measurements from both the physics of stars and the half life of radioactive decay show that the earth and the solar system are billions of years old not millions of years old. So 100 times longer than Lord Kelvin had estimated during the debates with Darwin. So when Darwin said an infinite number of generations which the mind can't grasp must have succeeded each other in the long roll of years, he was even more right than he thought at the time.

9. Where are the transitional forms in the fossil record? (12:42)

How about the fossil record? Well the fossil record has always been imperfect. Darwin worried about this in *The Origin*. As soon as *The Origin* was published lots of interesting fossils started to be published and that's been true ever since. In 1861, two years after *The Origin* appeared, a spectacular fossil was found. This was the first of several archaeopteryx fossils. Remarkable preservation of feather structures in an ancient reptile. So archaeopteryx has traits of both birds and reptiles. You can see feathers but it's on an ancient reptile that still has teeth, structures that are normally never found in modern birds. This kind of fossil with a mixture of traits of different organisms is exactly the kind of fossil that Darwin's theory of descent with modification predicted must exist. Well, key fossil discoveries have kept being made ever since *The Origin*. One of the biggest gaps in the fossil record that Darwin worried a lot about was the complete absence of any known fossils in pre-Cambrian strata. So the fossil record as Darwin knew it, the first appearance of fossil life in the record at that time, by the time the fossils appeared they were already complex and diverse. No record of simple forms that Darwin proposed must exist at the base of his immense tree of life.

10. Billion-year-old fossils of early lifeforms (14:10)

Since *The Origin*, the fossil evidence of life on earth has been pushed back in spectacular ways. Fossils of simple single-celled organisms have now been confirmed in rocks that are billions of years old shown there in the figure at the top. Just as Darwin predicted, the early forms of life are much simpler than the complex life that's seen later. When the early fossil record looks like mats of bacteria cyanobacteria and single-celled microorganisms. Similarly since 1859 the earliest forms of multi-cellular life have also been found in the fossil record. These also occur in rocks that predate the explosion of more complex forms in the Cambrian. So unicellular life and billions of years old rocks. The earliest forms of multi-cellular life in pre-Cambrian rock and in addition many transitional fossils have now been found for higher animal groups.

11. The reinvasion of water by land mammals (15:11)

Let's go much later in the fossil record and talk about the reinvasion of water by mammals. We're used to thinking of vertebrates as land animals with skeletal structures that are appropriate for walking around on two or four legs on land. Most mammals that's true, but in fact some mammals are found in the ocean as aquatic organisms. They have streamlined bodies such as seen here in the manatee. The manatee have a streamlined body, they still have two forefins, or flippers, but they no longer have hindlimbs. So they also have a series of other aquatic adaptations. Nostrils that are located high up on the head for breathing at the water/air interface. Very unusual downturned jaws that are only seen in manatees and a closely related animal called dugongs. Unusual ribs. So most animals have ribs that are hollow, filled with bone marrow. In manatees and dugongs the ribs are actually solid they're filled with bone. That's thought to serve as a form of ballast for these marine creatures.

12. Traits suggest manatees evolved from land mammals (16:16)

And finally in addition to those features, manatees show some characteristics that suggest they may have evolved from land animals. That includes both toenails on their flippers, and vestigial hindlimbs. So this shows a blow up of a manatee flipper. You see out at the ends these toenails. That's very unusual since toenails are usually only found on land animals. This was recognized a long time ago. The manatee's toenails actually look very much like elephant toenails. DNA studies show that the DNA of manatees and DNA of elephants is more closely related than most other mammals. So it looks like these may have evolved from four legged creatures. In addition, although manatees don't have hindlimbs if you look at their skeleton, they do have tiny rudimentary pelvic bones where a hindlimb would normally be found. These aren't attached to the vertebral column, they can't support weight. There's no leg that comes off of the pelvis. Surprisingly, those rudimentary pelvic bones do still have a tiny hip socket, even though the femur isn't there. We actually have a manatee bone, I'll put it up here at the front and invite you to come up during the break. This is a small rudimentary pelvis of the manatee. You see a little circle here. This is a vestigial hip socket for a ghost femur that's no longer there. Again that's just like the vestigial eyes in cave organisms, the example of a kind of structure that's very hard to explain if manatees had been designed from scratch to live in the ocean. On the other hand those kind of structures are easy to explain if manatees actually evolved from four-legged precursors.

13. Transitional manatee ancestors (18:05)

Well, if manatees evolved from four-legged land animals where are the supposed fossils of intermediate forms? Well, the fossils are actually in Jamaica. Very interesting paper that was published in 2001 by Dr. Domning from the Howard University here in the Washington, DC area. He described this spectacular fossil that was found in 50 million year old deposits in Jamaica. This nearly complete skeleton shows all sorts of characteristic diagnostic manatee features including the nostrils up on the top of the head. Characteristic solid ballast-like ribs. The unusual downturned jaws that are only found in manatees and dugongs. Despite having all these features, it has another very interesting feature, it still has hindlimbs. So the hindlimbs are present and they look just as robust as the forelimbs. Exactly the kind of organisms for evolving manatees from a previous four legged ancestor. Other intermediate stages in pelvic reduction have been found for this group, manatees and dugongs belong to a group called the sirenia and other proto-sirenia fossils have been shown that show intermediate stages of hindlimb reduction. Here is a fossil, you can see the hindlimbs are present but they're now much smaller than the forelimbs. They still have a pelvis, a femur, and lower leg bones. So we have now a fossil record that goes all the way from a complete four-legged looking manatee to things that have intermediate pelvic reduction, and then the simple vestigial pelvis that's still found in the modern forms.

14. The dolphin and its transitional forms (19:41)

The other group of marine mammals that's known are the cetaceans, the whales and the dolphins. We actually have a dolphin skeleton here at the front of the auditorium. So dolphins also have characteristic streamlined marine body form. They have four flippers but they don't have hindlimbs. You can see here in the skeleton, the obvious flipper or forelimbs of the dolphin, up here at the front of the body. There are no hindlimbs in this skeleton, but hanging beneath the vertebral column, at the spot where a hindlimb would normally be, you can again see these two rudimentary bones, unattached, no legs coming out, but these represent what's left of a pelvis in this form of marine mammal. That kind of rudimentary pelvis suggests that dolphins and whales also evolved from four-legged land animals. If that's true, where are the transitional fossils that show those precursors? Well, for manatee and whale evolution, the spectacular fossils have also been found in the last 20 years. In this case the key fossils are located in Pakistan and Egypt, 47 million year old skeletons have heads that look like whales, but still have four legs with the hindlimbs as robust as the forelimbs. The Egyptian fossils from 36 million years again show skulls that are clearly whale-like. You can now see that the hindlimb has been extensively reduced, very small, still has a pelvis and femur in the lower leg bone, but it's detached from the vertebral column, could no longer support body weight. Again, a beautiful set of transitional fossils from a four-legged animal with the skull of a whale, to partial hindlimb reduction, to the kind of tiny rudimentary pelvises that you see today in a modern dolphin or whale.

15. Fossils of transitional forms in stickleback evolution (21:37)

Finally large and complete mammalian fossils are rare but an amazingly detailed fossil record exists for the process of pelvic reduction in sticklebacks. We introduced sticklebacks yesterday. I want to show you a short video that summarizes extensive paleontological studies of the fossil record of pelvic reduction that have been carried out by Mike Bell.

16. Video: Fossils show 25,000 years of stickleback evolution (21:59)

These are all based on studies of a fossil lake site in Nevada. At this site diatoms die each year, float to the bottom of the lake and establish these thin rocky layers that are like growth rings in a tree. You can actually walk through time by walking your way up and down the slopes of this quarry. What we're going to show you is detailed studies of the forms of stickleback that are present in one section of this quarry, it represents about 25,000 years of evolution that Mike's looked at in detail. At each stage you can pull out rocks, split them from the quarry at lots of different areas to calculate which fish were present at a given time. They come in different forms, including the complete stickleback with a pelvis and a spine. Reduced stickleback with a tiny rudiment like you would find in a manatee or whale. Or an intermediate form where there's two bones left instead of just one. At each stage in the fossil record you can count the fish that are seen as a similar layer across rocks in the quarry and calculate what's the percentage of the fish that are reduced or intermediate or complete. And you can do that for successive stages as you walk through time. So this is a summary of actual data from Mike's lab calculating and counting lots of different fish and rocks they all start out reduced and then in this series of about 10,000 years a new stickleback invades the environment, replaces the previous form. This stickleback has a complete pelvis and a very interesting thing happens over the next 10 or 15,000 years, that complete form re-evolves pelvic reduction, with intermediate fossils seen along the way. Okay, we'll show you that time series again. Starts out with a population that's 100% pelvic-reduced. Rapid replacement around 10,000 years, new form, all with a complete pelvis and then the re-evolution of a pelvic-reduced phenotype, intermediate forms seen along the way. You can also see that in line graph form. A blue line for the reduced form that predominates in the early stage, a red line for the complete form that rapidly replaces at 10,000 years and then the re-evolution of pelvic reduction with an intermediate form summarized by the green line shown during the time series.

17. Transitional fossils are everywhere (24:32)

So it's remarkable to be able to watch evolutionary change with this level of detail. You can actually watch the morphological changes happen as centuries roll by and catch the intermediate forms as a complete stickleback evolves to a pelvic-reduced state. Okay so lots of people ask, all the time, where are the fossils that are predicted by evolution? Darwin asked that question himself in 1859. Maybe because Darwin was so honest about pointing out the problems with his theory, there is still a strange urban legend or myth that transitional fossils have never been found and that this is somehow a major problem with evolutionary theory. So after 150 years of discoveries in paleontology that simply isn't true. Where are the key fossils? Well for early forms of unicellular and multi-cellular life, the fossils are in ancient pre-Cambrian rocks of Australia. For feathers they're in Germany, for manatees they're in Jamaica, for whales they're in Pakistan and Egypt. For sticklebacks they're in Nevada; they're actually now in many of your own backpacks for those students who participated in the fossil activity yesterday afternoon. So the fossil record actually provides incredibly strong support for Darwin's key idea of descent with modification and I hope anyone who's seriously interested in this issue will look at the data as it exists in 2005, not as it existed in 1859, including all kinds of beautiful fossils, just a small segment of which we've been able to describe in the Holiday Lectures.

18. Q&A: What caused rapid replacement in the fossil record? (26:12)

So I'll stop there, it's a good time for a break and we can take a few questions. Yeah.

[STUDENT:] What was the cause of the rapid development of the stickleback?

[DR. KINGSLEY:] So at the point when the complete form arises. Well sticklebacks can migrate. There could have been an environmental change, it's not really clear where that stickleback came from. But it's very clear that the complete form arrives in the lake and all at once the structures now have the form of a new stickleback without intermediates along the way so that does look like a migration or a replacement event instead of an evolutionary event. In contrast when pelvic reduction re-evolves at the site, you clearly see the intermediates along the way.

19. Q&A: What were the first common ancestors? (27:06)

Yeah?

[STUDENT:] You keep speaking of common ancestors to support evolution, and, like, how many common ancestors do you think there are or, like, what types were they? What were the kind of the first ancestors of all the different types of organisms we see today?

[DR. KINGSLEY:] Final paragraph of Darwin's book says "life originally "breathed by the Creator into a few simple forms or one." Okay, so he thought that things must have gotten much simpler. He was, he allowed the possibility of not just of one, of maybe a few simple forms. What the fossil record actually shows is very simple forms at the beginning of the fossil record of life on earth. Bacteria, the unicellular animal, the unicellular organism, then the single- celled, simple prokaryotic organisms begin to be found also with eukaryotic organisms, then multi-cellular organisms then more complex forms. Again I think the key idea of a tree of life is that things are related by descent with modification. It's not clear if the tree has to be very broad or very narrow. It should be simpler at the beginning and more complex later and that's exactly what the fossil records show. Yeah.

20. Q&A: Does evolution address how life started? (28:30)

[STUDENT:] You say you've found fossils of single-celled microorganisms and I was wondering if those helped you figure out how the origin of the earth began, how earth was created, if it helps explain it.

[DR. KINGSLEY:] I think it's also important to remember that Darwin's theory is a theory of how life changes. So there aren't whole chapters of Darwin's book that explain the creation, where did the universe come from? That's not what Darwin's book is about. Darwin's book is about how life is changed and how organisms are related by descent with modification. I think that's a frequent confusion that you see a lot in debates about religion and evolution that somehow evolution proves that the universe was created by some random process. Evolution describes how life changes once life is here. It's not a theory about how life originally arose or why the whole universe exists.

21. Q&A: Can the evolution rate change over time? (29:32)

Yeah.

[STUDENT:] I was wondering, does the entire process move at one steady rate, or can it sometimes accelerate with different environments and during evolutionary time.

[DR. KINGSLEY:] We're going to come back to that point during the second part here because we actually can see jitters in the fossil record of evolutionary change. It does happen with different rates at different times sometimes fast, sometimes slow. Again, there's nothing in the theory of evolution that says it has to be particularly fast or particularly slow. In fact, we know genetic mechanisms where major genes can have big effects or other genes can have small effects that could create rapid evolution or slower evolution. There's no particular rate that evolution requires and in fact a range of rates are seen in the fossil record and in experimental systems. We better come back to the second section. I'm happy to take more questions here in a minute.

22. Can rare forms be swamped out? (30:38)

Okay, so let's come back to some of these scientific debates about the way selection could or couldn't work. One of the major debates was whether natural selection would be able to let an interesting new variant affect traits within a population. So as I said, people believed in blending inheritance at the time *The Origin* was first published. The problem that was pointed out was, suppose a little tiny drop of black ink appeared and was dropped into a giant vat of white liquid, like milk. Okay, a tiny drop of black ink in a giant vat of white milk has no effect on the overall color of the vat. It's still just blended out and the population would stay predominately milky white. Sorry, that's even confusing to try to explain because we now know so much more about the genetic mechanisms that actually exist that blending inheritance clearly doesn't happen.

23. Mendelian genetics: Variants are not lost by blending (31:39)

The Origin was written before any detailed knowledge of genetics or those kinds of ideas were out there, but when Mendel's laws were rediscovered in 1900 it was clear that variants aren't just lost by blending, in fact variants exist as stable genetic changes that exist on chromosomes. Those stable variants are sometimes hard to see in populations, variants can be recessive. But once a mutation arises, it can increase in frequency simply by being transmitted to more and more offspring. We made extensive use of Mendel's laws yesterday in talking about the genetic architecture of corn and teosinte evolution, stickleback evolution, and the evolution of color forms in rock pocket mice.

24. Pocket mouse simulation and real stickleback data (32:29)

Sean actually showed you a simulation based on Mendel's laws and population genetics of how a rare variant, when it appears, doesn't just get blended in, in fact a small selective advantage, even a 5% increase in the number of offspring that survive and reproduce from a black variant will lead to a rapid increase in the frequency of that variant over time. So at the population level the variant doesn't blend it appears and if it generates more offspring it will actually increase in frequency as the white form decreases. Now this data was based on a simulation, but I'd like to compare the results of that simulation with the results of the actual frequency of pelvic forms that are seen in the fossil record that we just described for pelvic reduction in sticklebacks. So here the line graphs represent the reduced form or pelvis, originally as a rare variant that sweeps to fixation in the successive layers that can be dug up out of this Miocene lake bed. I hope you can see that although this is simulated data, and this is actual data that the form of the curves are strikingly similar so variants don't blend when they appear, in fact they can spread through populations using laws of both Mendel and population genetics that are now well understood and that are also confirmed by observations in real systems.

25. Are animals too different to share an ancestor? (34:05)

Okay, finally how about this last problem, do animals simply look too different to come from common ancestors? This is again an area that Darwin tried to address in *The Origin*. He's got this passage: "All living things have much in "common, similar germinal vesicles, we see this also in "that the same poison can similarly affect both plants "and animals or that the poison secreted by the gall "fly will produce growths both on wild roses or "on oak trees." Darwin was trying here, but you can tell that at this stage the knowledge of biochemistry and molecular genetics was so rudimentary that the arguments for the inter-relationship of all living forms was still very circumstantial. Since *The Origin of Species,* pathways for the synthesis of DNA and RNA and proteins and lipids and carbohydrates have now been worked out in detail. Turns out most bacterial plants and animals share fundamental

26. Organisms share molecular pathways and enzymes (35:06)

metabolic pathways, they all use ATP for energy, they have lots of other biological characteristics in common. You can actually take enzymes out of one organism like a mammal, mix them together with bacterial extracts and the enzymes in bits and pieces from different organisms are compatible and can catalyze these reactions.

27. Organisms share DNA as the basis for heredity (35:24)

Most importantly and one of the triumphs of 20th century biology, the molecular basis of heredity has been worked out in detail. So the structure of DNA was discovered in 1953. That led to the identification of a universal code for converting DNA sequence into amino acid sequence in most animals. Same sorts of DNA, same sorts of genetic code, whether you're a virus or a bacteria, or a plant, or an insect, or a mouse or a human. Genes from one organism can actually replicate and function in another. A striking confirmation of the common biochemical and genetic heritage of all living things.

28. Different animals share developmental pathways (36:04)

Finally, very different looking animals are actually built using similar genes and pathways. One of the most striking examples of this was originally found in research in *Drosophila,* the fruit fly. *Drosophila* has a whole set of key developmental control genes called *Hox* genes. These genes occur in clusters along the chromosome and the expression pattern of the genes in the embryos is related to their position along the cluster. So the blue genes here at one end of the cluster are expressed at the head end of the embryo that's shown there in the bottom right. The colored genes in the middle are expressed in the middle of the embryo and the green genes at the end of the cluster are expressed in the tail region of the embryo. So those genes aren't just expressed at different anterior/posterior head-to-tail positions, they're also required for the formation of the particular tissues that you find from the head to the tail of the adult *Drosophila*. So if you make mutations in those genes, you change the development of the characteristic tissues that should form along the anterior/posterior body axis.

29. *Hox* "toolkit" genes guide development in mice and flies (37:13)

Well, surprisingly although animals look very different mice look very different from fruit flies, their anterior/posterior body axis is built from the very same sets of genes. So mice and humans, other vertebrates, have sequences that are closely related to these *Hox* genes of flies. The *Hox* genes in mammals also occur in clusters. The clusters are also expressed in patterns that are related to interior/posterior development. Blue genes at one end of the cluster, they've actually duplicated so there's more copies in mammals, but blue genes at the end of the clusters are expressed in the head regions of the embryos shown at the lower left. The middle genes are expressed in the middle part of the embryo. The green genes at the end of the cluster are expressed in the tail region of the embryo and just as in fruit flies if you make mutations in these key *Hox* genes, those mutations alter the formation of the characteristic tissues that would normally form at each position along the anterior/posterior body axis of the mouse. So it looks like the head-to- tail axis of animals, even that look completely different are in fact built by an ancient tool kit of these key developmental regulators called *Hox* genes. Similar tool kit genes have been discovered that control a range of other structures in diverse organisms, so the *Hox* genes for the anterior/posterior axis other sets of genes for the dorsal/ventral axis, other tool kit genes for the left/right axis, and other took kit genes for the formation of particular body tissues.

30. Eye development in humans, flies, and mice uses *pax6* gene (38:47)

Give an example of one particular tissue, eye development. So eyes, very interesting structures found in lots of different animals. They look quite different in different animals. That's a human eye and a *Drosophila* eye. Human eyes have single light-gathering organ and lens. In contrast insects have these compound eyes thousands of little light-gathering organs all in a little crystalline array. The histology of the eye is quite different, single light-gathering lens for the mammalian eye and this array of independent little light-gathering units in the compound eye of insects. Although those eyes look very different, it turns out that the same tool kit gene is required for eye development in many different organisms. So this gene is called *Pax6.* If you make a mutation in one chromosomes, one of the two copies of *Pax6* during human development you begin to lose particular parts of the eye, in particular the colored iris muscle that normally surrounds the pupil. You can see there the eye in a patient with a defect in one copy of his *Pax6* gene and that produces the absence of an iris, a disease called aniridia, so the pupil now nearly fills the eye. Similarly in the mouse, mutation in one of the two copies of the *Pax6* gene partially reduces the size of the eye, mutations in both copies completely eliminate the eye, the mouse embryo head shown at the upper right. Although *Drosophila* eyes look very different than mammalian eyes, there's a similar *Pax6* gene in fruit flies and if you make a mutation in that gene, you eliminate the compound eye of the fruit fly.

31. Overexpressing *pax6* in flies creates eyes in wrong places (40:29)

Even more remarkably, if you overexpress this key developmental regulator of eye formation, you can generate new eye tissue in completely different body parts. This is an experiment that was done by Walter Gehring's lab, taking this *Pax6* gene and engineering it to be expressed during leg development in a developing fruit fly. You can see when you over express the eye regulator gene, you induce on the leg of the fruit fly a tiny little patch of red compound eye tissue. If you do a scanning electron micrograph, that structure has exactly the kind of independent repeated eye unit that you would find in the normal fly eye and remarkably you can get that result whether you do the experiment with the fly *Pax6* gene or with a *Pax6* gene from mice.

32. *Pax6* is a toolkit gene that turns other genes on or off (41:21)

So we think that genes like *Pax6* are part of an ancient tool kit that's been inherited from a common ancestor and can be put to work to build related structures even in very different looking animals. So how do these sorts of master regulators work? Many of the ancient tool kit genes that control the development of the A/P body axis or the formation of a particular tissue turn out to encode gene products that act by switching other genes on and off. Okay, so for example, to build an eye you have to express lens proteins and photo receptors. What the *Pax6* gene does, is it acts as a regulatory molecule that flips switches on target genes and causes things like lens proteins and photo receptors to be expressed at the site where the *Pax6* gene is expressed. So we have a short video to show that kind of regulatory structure.

33. Animation: How regulatory switches work (42:20)

So this is a gene with the yellow part of the DNA, the coding part colored yellow. That might encode something like a lens protein. It would normally only be expressed if an RNA polymerase lands on the gene's promoter and makes a messenger RNA from the gene. The coding region of the gene is surrounded by a series of regulatory switches. So these are parts of DNA that don't code for any protein, instead they act as switches that determine where and when the gene turns on. Those switches are the landing sites for regulatory molecules that bind to the switches, recruit RNA polymerase to the gene's promoter and cause an increase in the total number of messenger RNA transcripts coming from the gene. Typically a gene will be surrounded by multiple switches that allows the gene to be turned on at different times and places under the control of different signals and regulatory molecules. A lens protein in the mouse is also expressed in the liver for example. It might have a switch where a regulatory molecule turns it on in the eye lens. The *Pax6* gene is an example of a regulatory molecule that would bind to one of those switches. There would be a different switch for turning the gene on in the liver.

34. Forelimb vs. hindlimb development in vertebrates (43:35)

Okay with that background on master regulators, let's come back to the problem of forelimb and hindlimb development in different animals. Forelimbs or hindlimbs are a good example of those structures that vary in different ways along the anterior/posterior body axis. So forelimbs and hindlimbs can become wings or legs short legs in the hopping legs, spines and fins in fish.

35. Master regulators are expressed in one limb or other (43:59)

All vertebrates share another set of tool kit master regulatory genes that are involved in controlling the formation of hindlimbs or forelimbs. So these slides show a series of chick embryos that have been stained with a blue dye, this is a method that allows you to look at where an individual gene is being expressed so you see sites of blue at the sites where the gene is normally turning on. There's a gene called *Tbx5* that's expressed in the wing bud of a chick but not the leg. There's another gene called *Tbx4* that's expressed in the hind leg, but not the wing. Another gene called *Pitx1* that turns on in the leg, but not the wing. So all vertebrates have these master regulatory genes that are expressed in one limb or the other and help determine how the limbs normally form, doesn't matter whether you're a fish or a frog or a chick, or a mouse. These genes are turning on in one limb structure or the other.

36. *Pitx1*: master regulator for stickleback hindfin reduction (44:58)

So what's happened in organisms where the development of one limb has been drastically altered? So we went through yesterday the example of completely losing the hindlimb in natural populations of sticklebacks, and I summarized a series of genetic experiments that showed that a major gene located at the distal end of linkage group 7 controls the presence or the absence of the pelvis in these natural populations. So it's possible to actually also determine the location of these tool kit master regulators that are known to be involved in hindlimb or forelimb development and when you do that it turns out that one of the hindlimb master regulators maps exactly to the locus that controls the presence or absence of the hindfin in the sticklebacks. That gene is called the *Pitx1* gene, the one showed you on the earlier expression slides as well.

37. *Pitx1* plays multiple roles in development (45:50)

This gene actually plays several different roles during normal development. You may wonder why it's called *Pitx1*. Well, in addition to normally turning on in hindlimbs but not forelimbs, it also turns on in some other body tissues. It turns on in the pituitary and plays an important role in controlling pituitary gene expression. It also turns on in jaws and mouth parts. If you completely eliminate the *Pitx1* gene in a mouse you shrink the hindlimbs, but the mouse dies at birth with pituitary abnormalities, and jaw abnormalities craniofacial defects.

38. Variants have changes in switch regions, not in *Pitx1* (46:25)

In some ways it doesn't look very promising for trying to use this gene to evolve a new structure in populations that are subject to a full range of fitness constraints in the wild. On the other hand if you look at what's happened to the *Pitx1* gene in stickleback, the protein coding region of the gene has not changed at all in marine and pelvic reduced sticklebacks. In contrast if you look at where this tool kit gene is normally expressed in embryos, there is an obvious difference in *Pitx1* expression. Now we're seeing blue at those sites where the *Pitx1* gene is expressed in either a marine or a pelvic reduced population. You can see in the marine population, expression in the blue mouth and jaw region. It also turns on at a spot at the site of the body where the hindfin would normally grow out. You also see that from the belly of the fish, here in the lower left with two spots where the hindlimbs would normally form. In the pelvic-reduced population you still have normal expression in the developing head region, the mouth and the jaw parts, but at the site in the body where the hindlimb would normally form, the *Pitx1* gene normally no longer turns on in that location. So what do we think is happening? That's summarized in the final brief animation.

39. Animation: *Pitx1* switching in two types of sticklebacks (47:41)

Both the marine and the pelvic reduced population have a *Pitx1* locus. The coding region of the gene is still intact in both populations. This gene we think is surrounded by a series of these regulatory switches that cause it to turn on in specific body parts like the jaw or the pituitary or the hindlimb. In both populations some of those switches are still present and function normally so the gene's expressed normally in the mouth parts, you still build normal mouths and jaws in both populations. Still expressed in the pituitary. You still form a normal pituitary in both organisms. In the marine population it turns on in the hindlimb and you build the pelvis. In the reduced population the hindlimb control switch has been inactivated. You no longer express the *Pitx1* gene at that location and as a result the hindlimb fails to form in the fish.

40. Genetic basis of evolutionary change in species (48:38)

Okay, you'll hear from Sean that similar tweaking with these switches that surround genes provides a very flexible method for evolution to use to create major changes, morphological changes, in particular body regions but still preserve the overall viability, the coding region and the expression of the genes in other tissues. Okay, I've gone through limb reduction in some detail, I think it's a great sort of trait, it's evolved repeatedly in lots of different animals. This is an example of sort of a macro-evolutionary change that's seen in whales and manatees and it's a kind of trait now where evolutionary studies have made it possible to document major morphological change that occurs in naturally occurring species, to trace those changes through the fossil record, to track down the number and the location of the chromosome regions that control the morphological differences, to identify specific genes that normally build the corresponding morphological structure, and to study how those genes have changed in natural populations.

41. Darwin's predictions supported by multiple sciences (49:43)

So we now know a lot more about the detailed molecular mechanisms that control these sorts of traits than we did at the time that Darwin wrote, but the details and the additional data that come from many fields all support the key original idea of descent with modification. That's exactly what's supposed to happen with a good scientific theory. Science should fundamentally both explain existing data and make predictions. As we've seen Darwin predicted that the earth must be old, that transitional fossils must have existed in the past, that variants must be able to arise and spread through populations and that very different organisms must be related to one another. Every one of those predictions has been resoundingly confirmed by all kinds of independent data from physics, from geology, from paleontology, from genetics, from biochemistry, from molecular biology and from embryology. That's why evolution is regarded as a well-established fact by scientists and that's why we consider it a key organizing principle for understanding all of biology and the inter-relationships between living forms. So I'll stop there and I'll be happy to answer questions.

42. Q&A: How do major changes in gene structure occur? (50:57)

Yeah.

[STUDENT:] How do major changes in the gene structure, like the addition of new chromosomes, or reduction of those that you see in different animals that have different numbers of chromosomes, how did those take place?

[DR. KINGSLEY:] There's lots of different mechanisms that can alter DNA sequence. Sean mentioned single base pair changes that can occur by errors of replication during cell replication. That occurs randomly and will just alter individual base pairs. There's also transposable elements that will hop around. There's also gene duplication events that take place, in fact we now have genome sequences of lots of different animals and you can see how whole gene sections have been duplicated creating new regions. We think that those kinds of mechanisms provide a flexible basis of generating the kinds of genetic variation that underlies the things that evolution can do to produce new alterations in morphology.

43. Q&A: Are the extra eyes on the *pax6* flies functional? (52:00)

Shouldn't just go to one side of the room. Yeah.

[STUDENT:] In the example of the *Drosophila* with an extra eye on its leg, did the eye work and did it affect the working of the two regular eyes on its head?

[DR. KINGSLEY:] Remarkably, that extra eye spot is actually innervated and if you shine light on the leg of the fly and record from the neurons that project to that ectopic eye patch they respond to the light. So it's a remarkably semi-functional eye that not only has the right lens proteins and photo receptors but actually is innervated, will respond to light and stimulate electrical activity. Getting down to the last of the t-shirts here.

44. Q&A: Why are some religions and evolution in conflict? (52:43)

Yeah?

[STUDENT:] I want to go back to Darwin for a second. You said before that in the last paragraph of his book Darwin mentioned a creator and I was just wondering why so many religious figures and groups of people even today react so negatively to Darwin's theories even though he clearly, or at least pretended to clearly, still believe in a creator?

[DR. KINGSLEY:] So we'll talk more about that this afternoon. The point I'd like to make is that in fact most major religious groups in the world don't see a conflict between evolution and religion, including the Pope himself who looks at the same data that I summarized here and says this is independent data that overwhelmingly supports the theory of evolution. There's not a conflict between the idea that life has been modified, generated by a process of evolution and descent with modification, that's a separate issue from where the universe originally came from and what set everything off at the beginning. The Bible also doesn't say how God created things. It says it all came from dust. That's not so different than an evolutionary model where from very simple beginnings life forms are generated by descent with modification.

45. Q&A: How do you look for single-celled fossils? (54:05)

Yeah.

[STUDENT:] You talked about the fossil record, earlier in the fossil record that you found, like, the single-celled microorganisms. But how do you go about looking for single-celled microorganisms in fossils when there's so many rocks on earth and being that they're so tiny?

[DR. KINGSLEY:] That's actually why that gap in the fossil record existed for a long time because it wasn't clear how to recognize the early forms of life on earth. One of the real breakthroughs came from a realization that sometimes these single-celled microorganisms will accumulate in large mats, so you can actually see this today off the western coast of Australia, there's these structures called stromatolite, stromatolite-like structures which are these big mat-like forms of cyanobacteria that form and they're big enough that they actually make a giant kind of mushroom that's just full of bacteria. So those forms can be seen today and those are the structures that were first recognized in the fossil record. Very similar sorts of accumulations, incredibly high density all laid out in little chains and mats just like you would see today in a modern structure off of Australia, these bacteria still exist in the world today and they can be recognized in the fossil record originally because of that unusual grouping of them and then once it was clear that those things existed you could begin looking for more and more rock that had the characteristics that they would have been laid down in the kind of near-shore environments where those structures are formed.

46. Q&A: Has the evolution of viruses been traced? (55:38)

This is my last t-shirt but I hope that doesn't mean the end of the questions. Yeah.

[STUDENT:] I was wondering if they've been able to see evolution of viruses as well, and possibly when viruses first came into existence from specific tissues?

[DR. KINGSLEY:] So there's lots of work when new diseases break out there's lots of questions about where the disease organism came from. That's looked at by sequencing the virus and trying to see how it's related to other known viruses. As patients are treated with drugs, viral-resistant... drug-resistant forms of the virus will emerge within the body, a dramatic example of evolution occurring in medicine. Again if you sequence the genome of the viruses that have emerged in a patient that's being treated with drugs you can see individual base pair changes that have occurred within the virus that are part of the evolution of drug resistance. So in fact the strategies that doctors use to try to suppress viral infections are heavily based on the idea of trying to minimize the chance that viral-resistant forms will pop out and spread. The best way to do that is to treat patients with multiple drugs simultaneously so that even if a single base pair change occurs, there is some other drug that is inhibiting multiple parts of the virus independently and keeps the infection squashed down. Okay, thanks very much, those were great questions and I'll turn things back over to Tom.

47. Closing remarks from HHMI President Dr. Thomas Cech (57:07)

[DR. CECH:] Thank you David Kingsley for an outstanding talk. We're going to break now for half an hour. When we return Sean Carroll will conclude our journey of evolution by talking about human fossils and also human genetics.