Evolution's Link to Development Explored

Researchers begin efforts aimed at identifying the developmental control genes that have been altered during evolution

"One of the great ideas in modern biology is that most organismic evolutionary change really reflects changes in developmental regulation," says Eric Davidson of the California Institute of Technology. According to this idea, simple modifications in the genes that control how an organism develops may produce significant alterations in body structure, thereby leading to the emergence of new species. To understand how differences in the forms of organisms evolved, Davidson says, "You have to ask what are the genetic programs that control development and how did they change in time."

A recent symposium* on "The Evolution of Development" explored the current state of research aimed at answering those questions. It showed that researchers are working on two tracks: one reviving the old and venerable approach of comparing the developmental paths followed by different species and the other taking the much newer tack of identifying the genes that control developmental decisions and are thus the raw material for evolutionary change.

The ultimate goal is to merge the two lines of research, to use comparative analysis to pin down the developmental variations that might have resulted from altered control and the molecular approach to pin down the genes responsible for the changes. In this way the questions posed by Davidson might be answered and new insights into evolution gained. But for now, the two approaches are still separate, with only a few early forays into their combination.

Before 1900, comparative analysis was the favored modus operandi of embryologists. However, the approach fell out of favor around the turn of the century as embryologists became more interested in dissecting developmental mechanisms at the cellular level and less interested in analyzing evolutionary relationships. "The whole tradition of thinking about evolution was lost for 70 years," comments Rudolf Raff of Indiana University. "What was interesting about this meeting was that it marked a return to the old way of thinking."

Sometimes comparative studies of organisms and their development yield surprising results. For example, at the development meeting, John Pettigrew of the University of Queensland, Australia, described studies that have led him to conclude that mammalian flight evolved independently at least twice. The findings have also raised questions about how bats should be classified taxonomically.

Pettigrew has been comparing the patterns of the nerve connections between the eye and the brain in members of the two suborders of bats—the Megachiroptera (or "megabats") and the generally smaller Microchiroptera (or "microbats"). Although bats are sometimes grouped in a superorder with the primates, Pettigrew says, "We have a problem. A whole set of features thought to be unique to the primates is in the megabats but not in the microbats."

The investigator finds that the visual nerve pathway of megabats is like that of the primates, in which the optic tectum on each side of the brain receives direct nerve inputs from both eyes in an arrangement that allows stereoscopic vision. In nonprimate vertebrates, however, all the nerve fibers from each eye cross to the tectum on the other side of the brain, and this is the pattern seen in the microbats.

The visual pathways in the two types of bats are consistent with their modes of navigation. The megabats find their way around by sight—stereoscopic vision is a definite plus for them—but the microbats navigate by echolocation, a form of sonar.

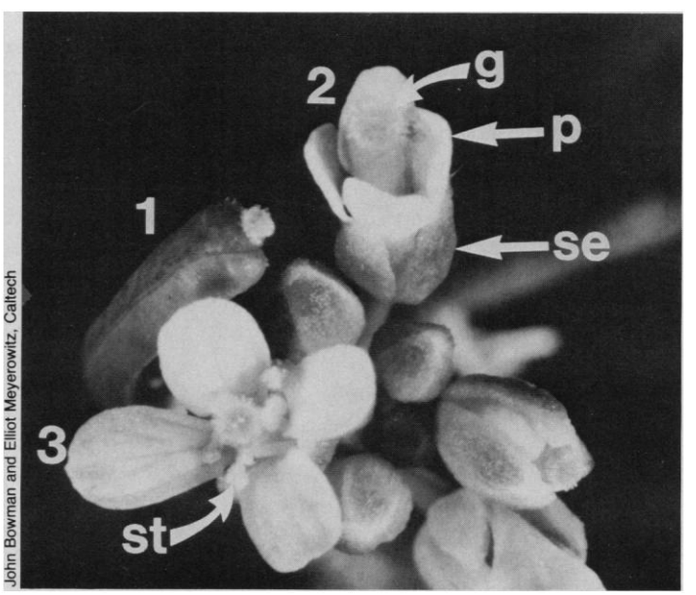
In fact, Pettigrew says the megabats and microbats have very little in common except their wing anatomy and that even this shows some significant differences between the two groups. He suggests that the megabats and primates evolved from the same ancestor with the microbats originating in an independent line that emerged some 25 million years earlier. Other evidence, including the fossil record, is consistent with the earlier evolution of the microbats.

Bernd Fritzsch of the University of Bielefeld in West Germany also reported some unexpected findings, in this case regarding the evolution of the vertebrate auditory system. The general view has been that the brain nuclei that receive signals from the auditory nerves evolved from the lateral line nuclei, which receive nerve signals from receptor cells located along the lateral surfaces of sharks, fish, and amphibians. These cells resemble the sound-detecting hair cells of the inner ear.

Species that have auditory nuclei usually do not have lateral line systems, and the supposition was that the lateral line nuclei became adapted to receiving similar input from the auditory hair cells. Fritzsch's analysis of frog brain development suggests otherwise, however. Although the tadpole larvae of frogs have lateral line systems, these are generally lost during metamorphosis when the auditory nuclei develop.

Arabidopsis thaliana

The left view shows normal flowers (2 and 3) and a mature fruit (1). The labels indicate the female organ (g for gynoecium), the petals (p), sepals (se), and stamen (st). The right view (facing page) shows the apetala-3 *mutant, in which the petals have been converted to sepals and the stamen to carpels (c). An ovule (o) is also visible.*



John Bowman and Elliot Meyerowitz, Caltech

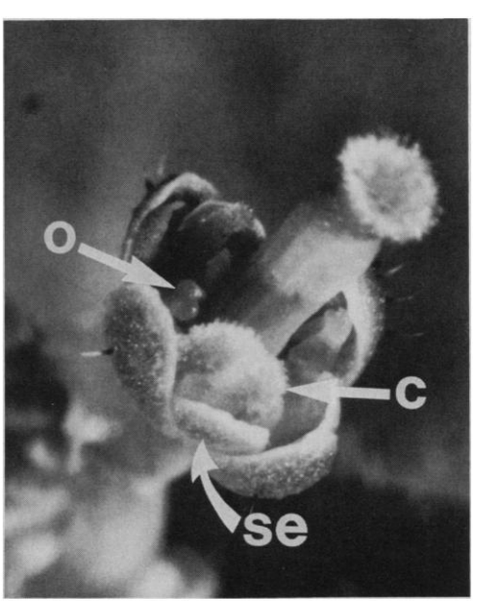
*The Second Lucille P. Markey Charitable Trust Symposium in Developmental Biology was held at Caltech on 4 to 6 April.

But not always, Fritzsch finds. The frog *Xenopus laevis* is a case in point. "*Xenopus* has both lateral line and auditory nuclei side by side," Fritzsch says. "How then could the lateral line nuclei have evolved into auditory nuclei?" He proposes instead that the lateral line nuclei degenerate in frogs that do not have the system and are replaced by a new set of nuclei for the auditory system.

Despite the information being gleaned about evolution from the studies of these relatively advanced species, for now at least, frogs and bats—whether mega or micro—are not amenable to the kinds of genetic and developmental analysis needed for identifying developmental control genes. For these types of studies researchers have turned to simpler organisms, the sea urchins, fruit flies, and roundworms that received the lion's share of attention at the development meeting.

For example, Raff and his colleagues are comparing the development of two sea urchin species, *Heliocidaris tuberculata* and *H. erythrogramma*. Although these sea urchins are closely related and their adult forms are very similar, the two species develop in very different ways. *Heliocidaris tuberculata* displays the typical developmental pattern for sea urchins in which the embryo first produces a feeding larva, known as a pluteus, that then metamorphoses into the adult form. In contrast, *H. erythrogramma* is a "direct developer" that produces adults without going through a typical feeding larval stage.

"The differences are so great at first sight that you're just flabbergasted," Raff says. This is something of a surprise in view of the great similarity of the adult forms of the two species. The expectation was that an alteration in early development would be magnified later on, but for these sea urchins that is not the case.



Although Raff notes that it seems paradoxical to focus on direct development, a kind of evolution that has little effect on adult morphology, he points out that the system has the great advantage of experimental accessibility. "The sea urchin larva is simple, with not very many cells," Raff explains. "We ought to be able to define what the system is doing."

Moreover, direct development is the result of heterochronies, that is, of alterations in the timing of developmental events. Many researchers consider heterochronies to be a general means of effecting evolution and would like to understand the molecular events that cause them. *Heliocidaris erythrogramma* embryos do not make a larval skeleton, for example, but skip ahead to production of the adult type. Moreover, the skeleton-producing cells also show an adult pattern of gene expression. Meanwhile, however, other aspects of *H. erythrogramma* development and gene expression progress much as they do in *H. tuberculata*.

Raff and his colleagues are now using dye injection methods to trace cell lineages in *H. erythrogramma* embryos. This will enable them to determine which cells give rise to which specialized tissues and at what points *H. erythrogramma* development diverges from that of typical sea urchins. Ultimately they may be able to identify the genes that control the developmental decisions in the sea urchin embryos.

Investigators have already identified some developmental control genes in the fruit fly (*Drosophila melanogaster*) and the roundworm *Caenorhabditis elegans*. The roundworm is especially useful for analyzing the activities of such genes because the complete lineages of all the approximately 1000 somatic cells that make up the adult body are known. Consequently, researchers can determine how particular genes affect the fates of specific cells.

A few years ago, for example, Paul Sternberg, Iva Greenwald, and H. Robert Horvitz of Massachusetts Institute of Technology identified the gene *lin-12*, which acts as a sort of binary switch in *C. elegans*, determining which of two fates will be adopted by the members of certain homologous cell pairs. In one such decision, appropriate *lin-12* expression is required for formation of the anchor cell, which induces the subsequent formation of the *C. elegans* vulva.

Moreover, according to Sternberg, who is now at Caltech, analysis of cell fate specification during vulva formation in the roundworm suggests that even complex developmental processes can be broken down into a series of binary decisions that are amenable to molecular analysis. So far, nearly 30 genes that participate in vulval development have been identified, although many of them are in the cellular response path rather than in the control path.

Perhaps the best studied developmental control genes are those of the bithorax complex of the fruit fly, which help to specify the identities of the various segments of the insect's body. Researchers have made a great deal of progress in understanding the molecular biology of the bithorax complex since its cloning several years ago, but they have just begun to explore how changes in bithorax gene regulation might affect insect evolution. Michael Akam of the University of Cambridge, England, is beginning to approach this issue by examining the expression of bithorax gene counterparts in the locust, an insect with a morphology very different from that of the fruit fly. The work is still at an early stage, however.

Although animal evolution received the bulk of the attention at the development meeting, the plant world was not neglected. Elliot Meyerowitz of Caltech described his group's identification of mutations that affect flower development in one of plant geneticists' current favorites, *Arabidopsis thaliana*. "There is a natural laboratory in flowers for studying how different morphologies have arisen in evolution," Meyerowitz says, "but the developmental questions—which genes specify structures in flowers—have to be answered before the evolutionary questions can be asked."

The mutations that Meyerowitz and his colleagues have identified so far indicate that two types of genes control flower development in *Arabidopsis*; some specify the kind of flower organ—sepal, petal, pistil, or stamen—to be made and others determine the number.

Meyerowitz is beginning efforts aimed at isolating the genes that control flower formation in *Arabidopsis*. This should be relatively easy as these things go. The plant has a small genome, only 70,000 kilobases, with little repetitive DNA. Meyerowitz already has identified 100 DNA markers located throughout the *Arabidopsis* genome that will help him zero in on the desired genes. (He also suggested that Leroy Hood of Caltech might want to sequence the *Arabidopsis* genome as a warm-up for the human genome project.)

Direct studies of genes, although they may not shed any light on developmental control in specific organisms, have nevertheless provided information about how gene structure or regulation may have changed during evolution, or not changed, as the case may be. For several years now, Fotis Kafatos of Harvard University has been

studying the genes that encode the proteins of the insect chorion (eggshell) and in particular comparing those of the fruit fly with those of moths. "Moths and flies are very different, about as far apart—240 million years—as birds and mammals," Kafatos points out.

During that long course of evolution there have been major changes in the structure of both the chorion itself and the genes encoding the chorion proteins. "The gene structures are so varied, you might not recognize them as being homologous if you didn't know they serve the same function," Kafatos explains.

Moreover, the two types of insects used very different strategies to solve the problem of how to make large quantities of chorion proteins very rapidly during the late stages of egg formation. The moths opted for a great expansion of the chorion gene superfamily, which encompasses more than 100 genes in those insects. In contrast, fruit flies have many fewer genes, but amplify their number during development.

Nevertheless, despite all these differences, the DNA sequences that regulate the expression of the genes have been conserved. Moth chorion genes that are transferred into in fruit-fly embryos are expressed in the normal way. Kafatos and his colleagues have identified a six-nucleotide consensus sequence, associated with both the moth and fruit-fly chorion genes, that specifies the tissue where genes are to be expressed. They also have evidence for the existence of control sequences that regulate when the genes are to be active, but have not yet pinpointed these regulatory elements.

Gene studies have also shown that duplication is one of the major ways in which genes have evolved to carry out new functions. The immunoglobulin gene superfamily serves as the ultimate example of this evolutionary mechanism.

According to Hood, this superfamily now includes no fewer than 32 distinct structurally related genes or gene families, all of them presumably descended from the same original gene segment that underwent a series of duplications over time. The extra copies might then change in structure with the emergence of genes with new functions. A partial list of the genes in the immunoglobulin superfamily include those encoding antibodies and other immune system molecules needed for recognizing and destroying foreign antigens; molecules that mediate cell-to-cell interactions, including some occurring during development; and some growth factor receptors.

Duplicating genes is not the only way of achieving new functions, however. Joram Piatigorsky of the National Eye Institute in Bethesda, Maryland, described some new results from his laboratory that point to a surprising conclusion. Piatigorsky and his colleagues have been studying the crystallins, the structural proteins that form the clear lens of the eye. Nearly ten different crystallins or crystallin families have been identified so far in various species. For the most part, the different crystallins are structurally unrelated. What is surprising is that some of the crystallins have the same structures as well-known enzymes.

Epsilon crystallin from birds and reptiles is apparently identical to argininosuccinate lyase, an enzyme from the urea cycle. Tau crystallin from turtles is the same as alpha enolase, an enzyme in the glycolytic pathway for sugar breakdown. And epsilon crystallin, from ducks and crocodiles, appears to be the same as the B form of lactate dehydrogenase. "A single gene can perform two different functions without changing a single base pair. Gene duplication is not needed for the origin of new functions," Piatigorsky says.

What apparently changes is the control of the genes. For example, the tau crystallin product is made in small amounts in the heart, where it serves as lactate dehydrogenase, but in large amounts in the lens of the eye, where it performs its structural role. The next step then is to determine what accounts for this differential control. The answer might lie in the sequences that regulate gene transcription into messenger RNA or it might be in later steps in the pathway from gene to protein.

Finally, Jeffrey Palmer of the University of Michigan has been focusing on the gene migrations that occurred during the evolution of the chloroplast, the photosynthesizing organelle of plant cells. Chloroplasts are apparently derived from blue-green algae (cyanobacteria) that took up residence in nucleated cells early in evolution.

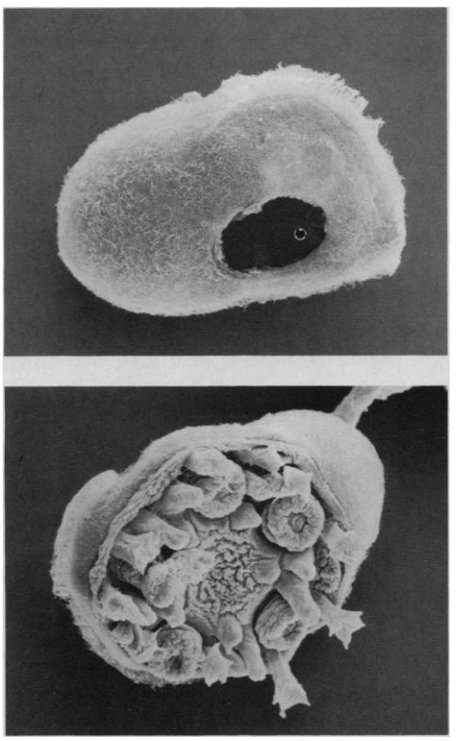
"Chloroplast DNA has many fewer genes than the cyanobacteria genome," Palmer says. "Many of the missing genes were just lost, but about 80% of those that remained were transferred to the nucleus." Most of the transfer occurred before the divergence, some 400 million years ago, of water and land plants, but Palmer's work indicates that some transfers occurred much more recently.

A gene encoding one of the proteins of chloroplast ribosomes, for example, is absent from the chloroplast genome of legumes and only legumes. This gene must have left the chloroplast genome of the common legume ancestor about 75 million years ago, Palmer suggests. In a similar vein, the chloroplasts of all 40 members of the *Pelargonium* genus, which includes geraniums, lack the gene for the alpha subunit of chloroplast DNA polymerase, a finding that suggests the gene departed from the chloroplasts of the geranium ancestor just 1 million to 10 million years ago. Palmer and his colleagues have evidence that the genes have moved to the nuclei of the plants.

He notes that the physical movement of the genes is the easy part of the transfer. Becoming functional once in the nucleus would be much more difficult. The genes would have to acquire the correct regulatory elements to be expressed there and also coding sequences for the transit peptides needed to direct the proteins back to the chloroplast where they are required.

According to Palmer, genes may not just move from chloroplast to nucleus but may travel in the reverse direction as well. He has detected repeated DNA segments, possibly transposable elements of nuclear origin, in the chloroplast genome of subclover where they may be promoting rearrangements. Transposable elements are prominent causes of mutations in the plant nuclear genome, and therefore contributors to plant evolution, and may play a similar role in chloroplast genomes, if they gain entry there. ■

JEAN L. MARX



Louis Hetlands and Rudolf Raff, Indiana University

A direct-devloping sea urchin larvae *(top) is shaped something like a cucumber with a belt of cilia at one end, to the right here. Adult body parts are developing internally. The hole marks the vestibule on the lower face of the juvenile sea urchin. (bottom) During metamorphoses, the spines and tube feet emerge from the vestibule, in which they were hidden in the larva.*