A New Look at Maternal Guidance

Parents pass more than genes on to their offspring, and these nongenetic contributions can have profound ecological and evolutionary implications

Few things frustrate biologists more than watching the animals they are studying die for no apparent reason. Charles Fox, an evolutionary biologist at Fordham University's Louis Calder Center in Armonk, New York, experienced that frustration in 1994. Fox was doing what should have been a straightforward experiment on the seed beetle *Stator limbatus*. Homeowners in Phoenix, Arizona, had noticed these 3-millimeter insects living on Texas ebony, an ornamental tree the beetles first encountered in the 1970s, when it was introduced to that city. But when Fox tried to raise beetles in the lab on Texas ebony seeds, the newly hatched larvae all died. "I couldn't understand what was going on," he recalls.

PHOTOS BY UDO M. SAVALLI AND CHARLES W. FOX

Mothers decide. The seed beetle makes large eggs *(above, left)* on palo verde seeds and small eggs *(above, right)* on acacia seeds.

Like dozens of other experimental ecologists and evolutionary biologists before him, Fox had been tripped up by a phenomenon that has often been observed but—until recently—rarely seriously considered: a so-called "maternal effect," which occurs when something about the mother's environment alters how her offspring look, act, and function. As Fox and his colleagues will report in a paper in press in *Evolutionary Ecology*, the researchers ultimately found that the seed beetle larvae thrived on Texas ebony, a new host for the species, only when their mothers had been raised on the palo verde tree, one of their indigenous hosts.

Maternal effects such as these are proving to be much more than obstacles that occasionally confound experiments. In June, at an annual meeting* of evolutionary biologists and systematists, some two dozen reports testified to their widespread nature. "They popped up in all sorts of plants and animals," says Timothy Mousseau, an evolutionary biologist at the University of South Carolina, Columbia, who helped organize the symposium. Maternal effects can enhance an offspring's chances of survival, skew sex ratios, and drive fluctuations in population size, making them something to reckon with in efforts to protect endangered species and control insect infestations.

What's more, recent evidence shows that the effects can influence a species' ability to adapt to its environment. "Parents are typically thought to only give genetic information to offspring. But maternal effects provide a powerful avenue for [altering] the course of the future by adding information content and material content," says Barry Sinervo, an evolutionary biologist at Indiana University, Bloomington. That content can be in the form of gene-regulating proteins in a gamete or an extra dollop of yolk in a egg—anything that can influence how an embryo develops.

And because parents vary in the degree to which they supplement the genetic inheritance, the additional information will make some individuals better able to thrive and reproduce in a population than others. Over time, then, their genetic repertoires become more common, and in this way, maternal effects "influence the very short-term evolutionary dynamics," explains evolutionary biologist Denise Thiede of the University of Pittsburgh in Pennsylvania.

Researchers had been aware of maternal effects for decades, primarily through studies of egg-laying and provisioning behaviors in animals and seed production in plants. Animal breeders observed, for example, that better fed mothers often produced bigger, healthier offspring. Likewise, plant seeds were found to vary in size, depending on conditions.

But for the most part, these early workers viewed these effects as "random noise that tended to obscure the genetic variation that we were interested in," says Mousseau. Thus animal breeders and evolutionary biologists would first grow several generations of the organism they wanted to study in controlled conditions so as to eliminate this "noise."

That began to change in the late 1980s and early 1990s, partly as a result of a mathematical model proposed by Mark Kirkpatrick of the University of Texas, Austin, and Russell Lande, then at the University of Chicago. Earlier theoretical work by other researchers had indicated that there was a genetic component to maternal effects and that the condition of the mother sometimes led to unexpected traits in the offspring, observations that had previously gone unexplained. In their model, Kirkpatrick and Lande expanded on that theme, suggesting that maternal effects affected many traits and could create "some rather interesting and counterintuitive evolutionary dynamics," Mousseau says.

Among other things, their model, as well as another model proposed a few years earlier by Bruce Riska, then at the University of California, Davis, predicted that maternal effects retard or speed up evolution depending on whether they delay or accelerate an organism's response to selection pressures. For example, bigger mice should do better in general than smaller ones. But the evolution of ever larger body size is constrained in part because large females may have more young that compete for her limited milk supply, and thus end up smaller and less fit than their mothers. Thus the increase in body size is retarded over time by the mothers' reproductive habits.

Although this new view challenged textbook theories of evolution, which said that only the genetic contributions of parents were important, it quickly gained favor with some experimental biologists because the ideas proposed "have the ability to explain unexpected results," says Allen Moore, an evolutionary biologist at the University of Kentucky.

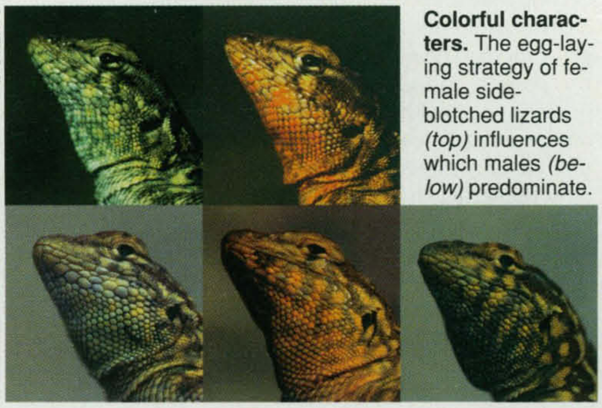
The unexpected deaths of Fox's seed beetles in the Texas ebony experiments provide one example. Had the beetles only required the right genes to be able to use this tree as a host, his lab beetles should have survived just fine. But instead, the beetles could expand onto this new food source only when the right maternal effect had been set in motion long before the newly hatched larvae encountered the Texas ebony. And as Fox and his colleagues, including Mousseau, describe in upcoming issues of *Oecologia* and *American Naturalist*, their seed-beetle studies not only revealed why lab beetles failed to thrive on Texas ebony, but also convinced them that maternal effects help drive the beetle's evolution.

The team has demonstrated that this insect varies its egg-laying behavior depending on where it lives. Its other native host tree besides the palo verde is the acacia. Female

* The Annual Meeting of the Society for the Study of Evolution and the Society of Systematic Biologists, 19 to 23 June, St. Louis.

seed beetles living on that species make lots of small eggs. But they produce just a few large eggs when they live on palo verde. Fox says this change is "absolutely nongenetic. I can make [large-egg laying] disappear and reappear just by changing the environment." He suspects that some chemical from the palo verde tree alters the expression of the genes that control the beetle's egg-laying.

B. SINERVO



Colorful characters. The egg-laying strategy of female side-blotched lizards *(top)* influences which males *(below)* predominate.

But whatever the cause of the change, it is adaptive. The palo verde seed has a tough coat, which may also be slightly toxic, and the larger egg provides enough sustenance for the beetle larvae to finally gnaw through to the seed. This ability to switch egg-laying behavior is beneficial for beetles living on the native hosts, because the female beefs up her eggs only when necessary, a circumstance that helps her conserve her resources. Also, the larger eggs allowed the beetle to colonize a new host—Texas ebony—whose seed coats are also quite tough. "Maternal effects [provide] a mechanism for adapting to variable environments," Fox proposes.

The same also seems to be true for plants. Brown University plant evolutionary ecologist Johanna Schmitt, working with postdoctoral fellow Kathleen Donohue, has found that the weed English plantain (*Plantago lanceolata*), like the seed beetle, produces fewer, better equipped offspring in adverse conditions. As Schmitt reported in June at the evolution meeting, *Plantago* planted in sunny, open areas make lots of small seeds that thrive only in sunshine. In contrast, plants from shaded areas produce relatively few large seeds, but those big seeds grow well in both sunny and shaded environments. This maternal effect maximizes the number of the seeds' progeny, particularly if the offspring experience the same environment as the maternal plant, Schmitt says.

Traditionally, an organism's success correlates with the number of offspring produced. But the seed beetles and the plantain "are going a step beyond fitness according to the standard definition," notes evolutionary biologist Lev Ginzburg from the State University of New York (SUNY), Stony Brook. For them, quality takes precedence over quantity in some circumstances because it helps ensure that their progeny will themselves reproduce successfully. "It's a higher level of selection," Ginzburg adds.

This strategy for boosting the fitness of offspring can influence the size and makeup of whole populations. Take the side-blotched lizards that Indiana's Sinervo is studying. He and his colleagues have documented that the males come in three varieties, or morphs, each with a different, genetically determined throat color and mating strategy. Very aggressive ones with orange throats corner large territories for a harem of females. Blue-throated males guard smaller territories and usually keep just one closely guarded mate. And males with yellow-striped throats that make them look like some females defend no territories of their own, but use their deceptive coloration to "sneak" around mating with orange males' females.

In a field study conducted in Merced County, California, from 1989 through 1996, Sinervo and his colleagues found that the predominant morph changed over time, with one type of male seeming to outcompete another in a perpetual cycle. When the researchers started, the blue males outnumbered orange or yellow ones. But over the first 2 years, the more aggressive orange males took over the blue males' territories and the number of blue males decreased. After 1992, however, the number of orange males declined, and the number of yellow ones increased. By 1995, the blue males again predominated, setting the stage for another repeat of the cycle. (These results were reported in the 21 March issue of *Nature*.) It seemed that each morph created certain social conditions that actually made it possible for another morph to gradually take over, with no single one having the upper hand for very long.

PHOTOS BY J. SCHMITT

Plentiful plantains. Brown University researchers track maternal effects in plantain *(upper right)*.

Further analysis revealed that more than male-male interactions are behind the shifts in the relative populations of each morph. The researchers calculated that if male behavior alone determined which morph predominated, then the cycle should have taken 16 years to complete, rather than six. "Maternal effects accelerate the cycle," Sinervo reported at the June meeting.

This happens, the researchers found, because the female lizards, which themselves come in two varieties, with throats that are either yellow-striped or orange, alter their egg-laying patterns depending on their social environment. "Females are [doing] things that are altering the development of her offspring," Sinervo says.

For example, an orange female surrounded by orange females, which tend to be more aggressive than yellowish females, makes the eggs of her future female offspring larger, providing them with an added boost for dealing with harsh orange neighbors, at the expense of her male eggs. But yellow females in the same environment might do just the reverse, boosting the size of their male eggs at the expense of the female ones, presumably because the "sneaky" yellow-throated males would be more successful than subordinate females when surrounded by orange-throated lizards. Together those two trends could pave the way for yellow-striped males to replace the orange ones.

Maternal effects can also bias the composition of a population by tipping the balance between the sexes—a factor that could undermine efforts to save endangered populations. In the April issue of *American Zoologist*, for example, evolutionary biologist Willem Roosenburg of Ohio University in Athens reports that by changing where she lays her eggs, the female diamondback terrapin alters their incubation temperature and, consequently, the sex of the young that hatch. He has also observed that the larger females deposit their eggs in open nest sites that receive lots of sunlight, and the smaller, younger ones lay theirs in cooler sites.

Those warmer eggs become females. And because the eggs themselves are larger and have more yolk, those females mature more rapidly and can start laying their own eggs earlier. In contrast, males mature at about the same rate, regardless of their egg size. Thus larger females with their ability to produce large eggs can generate the most descendants by heading for sunny nest sites, while smaller, younger females fare better by producing males. This means that if this turtle were to become endangered, conservationists might need to take into account not only the number of turtles in a population, but also the sizes of the females so as to guarantee a mixed population.

Maternal effects can even influence the dynamics of entire populations—and thus may be a key to the spread of some insect pests. In studies of the gypsy moth, *Lymantria dispar*, a serious pest that is spreading across the United States defoliating forests each summer, MaryCarol Rossiter of the University of Georgia, Athens, has found that in good times, the female moth doesn't just produce more eggs; she produces larger ones as well. Her ever more healthy, faster growing young do likewise, and the population explodes. But eventually it gets so large, the moth "overshoots its resources," says Ginzburg. With food then in short supply, the undernourished females do not provision their eggs well and therefore create young that are not very capable reproducers themselves. The population crashes, setting the stage for the cycle to begin anew.

By incorporating such maternal effects into their model of population growth, Ginzburg and Dale Taneyhill of SUNY Stony Brook have been able to predict the cycling time for population increases and decreases in six moth species, including the gypsy moth. The model also seems to predict the growth and crashes of small mammal populations. While including information on the parents' quality when predicting population growth seems like common sense, most theoretical models have not done that, Ginzburg notes.

All these observations and predictions highlight that maternal effects do play an adaptive role. The next step will be understanding just how a mother's experiences are linked to the future of her progeny—and that means unraveling the molecular genetic mechanisms that mediate maternal effects, says Sinervo. Lots of developmental biologists study the role of maternal environments, but usually only in the context of the very early stages of an embryo. They examine, for example, how proteins contributed by a parent influence the timing of gene activation in the fertilized egg and, consequently, affect patterns of development. These, too, are maternal effects but are rarely considered in long-term ecological or evolutionary contexts. But Sinervo and others expect that to change as the importance of maternal effects in these other contexts sinks in. Says Fox: "[The field] is certainly not mainstream at the moment. But I think it will be."

–Elizabeth Pennisi

X-RAY CRYSTALLOGRAPHY

Structure of Gene-Tag Protein Solved

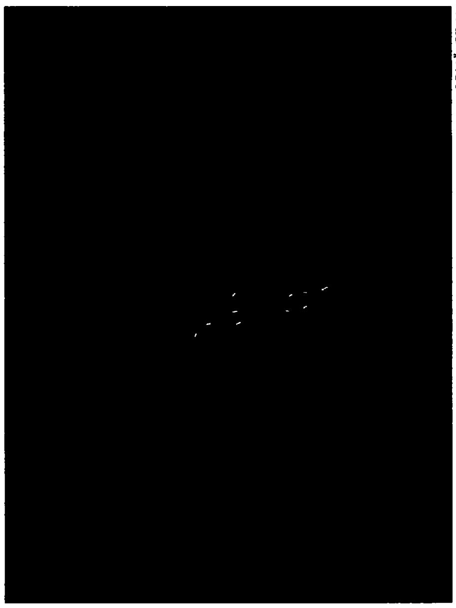
When the Roman natural philosopher Pliny the Elder wrote about a glowing marine creature nearly 2 millennia ago, he could never have imagined that future scientists would turn this marvel of nature into an everyday tool. But within the past several years, a green fluorescent protein (GFP) that lights up one sea creature, the Pacific Northwest jellyfish, has become a powerful marker for modern molecular and cell biologists. By linking the GFP gene to those encoding other proteins, they can track when and where the genes are expressed and also trace the workings of the protein products in living cells and tissues. Now GFP is poised to become even more useful. Instead of taking what nature provides, researchers may be able to tailor the protein for new purposes.

That is the prospect raised by scientists' first look at the three-dimensional (3D) structures of GFP molecules. On page 1392, a team led by S. James Remington of the University of Oregon, Eugene, reports the structure of a mutated form of the protein. And in work that will be published in the October issue of *Nature Biotechnology*, George Phillips and his colleagues at Rice University in Houston have determined the structure of the normal version of GFP.

Molecular geneticist Martin Chalfie, whose team at Columbia University pioneered GFP's application to cellular studies, has seen both structures and says they are very similar and "absolutely beautiful." They resemble a covered barrel with the glow-producing dye sealed inside. But the structures' beauty is a lot more than skin-deep. By helping to explain the properties of the fluorescent protein, the 3D map provides a guide to modifying it.

In particular, researchers hope to produce versions with new colors—say red or orange—that would allow them to track two or more proteins at the same time in the same cell. "We need two color labels to check whether proteins are near each other," says molecular biologist Roger Tsien of the University of California, San Diego, who co-authored the *Science* paper. "Most of molecular and protein biology involves proteins that are, in essence, kissing each other."

Remington says his team decided to work with the mutant protein because its properties are somewhat superior to those of the unmutated protein, and it has but a single amino acid change. In the normal protein, the green glow emanates from a ring formed by three amino acids—serine-65, tyrosine-66, and glycine-67. In the mutant the serine in this chromophore is replaced by a threonine. As a result of this structural change, the mutant protein glows more brightly and at a measurably—but not visibly—yellower color than normal GFP.



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Barrels of fun. The new GFP structures should help design more colorful gene tags.

Just tinkering with the three amino acids in the chromophore is unlikely to produce dramatic changes in the color of light it emits. But now that the protein's 3D structure is known, researchers can expand their range of options by changing other amino acids known to be in contact with the chromophore. Indeed, the Remington-Tsien team has already made a start at that. They replaced threonine-203, an amino acid that resides near the light-producing coil, with tyrosine, a larger amino acid. They expected that the replacement would distort the structure of the coil and thus alter the wavelength of light it emits. It did. The new mutant radiates an even yellower shade of green than the serine-to-threonine mutant does.

For his part, Phillips declines to comment on the details of the normal GFP structure before the results are published. He does say that it, too, resembles a barrel. He also says he plans to work with Remington's group. "Together they [mutant and normal GFP structures] may well explain the behavior that the mutant has," Phillips says. "And they may help us to generate more mutants."

Besides expanding GFP's color palette, the structures may allow investigators to apply the protein in new ways. Tsien suggests, for example, that finding out how mutations alter the GFP's color might help understand what mutations do to other color proteins, such as eye pigments. "Now that we know the structure, there is no end to what we can do," says Tsien. "The jellyfish has handed us a gift that keeps on glowing."

–Trisha Gura

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