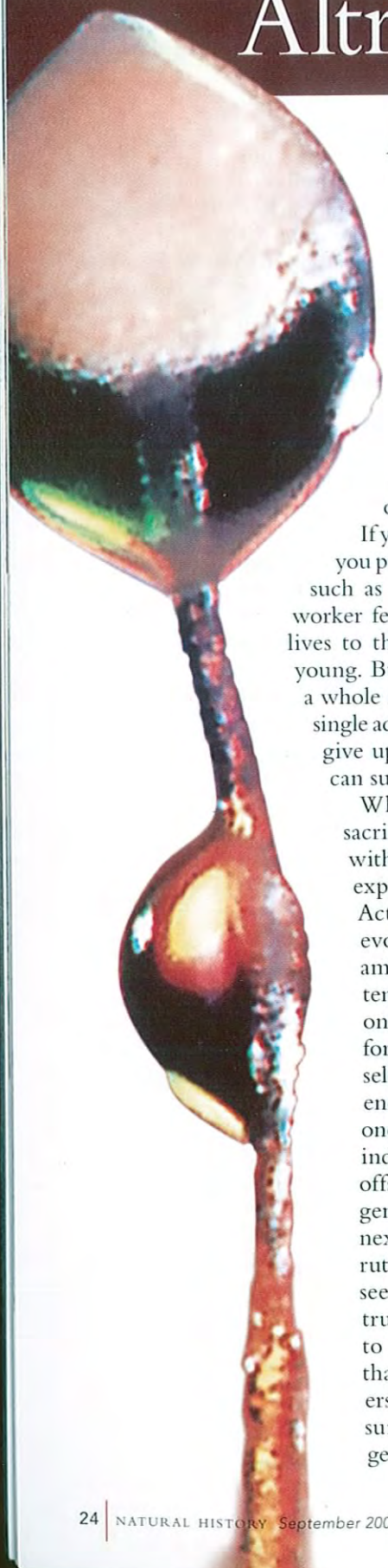
Altruism among Amoebas



A person who dies so that others can escape starvation is a hero. But how can evolution explain the same behavior in a nonhuman organism whose genes are "selfish"?

By Joan E. Strassmann and David C. Queller

Photos by Owen Gilbert

Can you think of a species, other than our own, in which some individuals sacrifice their own interests for the sake of others? If you're like many other nature lovers, you probably thought of the social insects, such as ants and wasps. In those species, worker females devote their long, complex lives to the service of their queen and her young. But another group takes altruism to a whole new level: the social amoebas. In a single act of self-sacrifice, certain individuals give up their lives so that other amoebas can survive and later multiply.

Why should that be puzzling? If self-sacrifice is a characteristic that persists within our own species, wouldn't you expect to find its roots deeper in nature? Actually, all the way up and down the evolutionary scale, from single-celled amoebas to human beings, the persistence of a tendency to help others at one's own expense is a conundrum for natural selection. After all, natural selection normally acts on the genetic endowments of individuals, one by one, not on groups as a whole. If an individual does not pass on its genes to offspring, for whatever reason, those genes will be that much scarcer in the next generation. The process is blind, ruthless, and competitive, and it would seem to shut the door on genes for altruism. In particular, genes that tend to produce freeloaders—individuals that take advantage of altruism in others without sharing the cost—should survive and quickly crowd out any genes for altruism. Such "cheater" genes ought to be favored by natural selection, and spread through any population. So how can self-sacrifice be a successful strategy?

Our curiosity about that question led us to the Appalachian Mountains of Virginia, where social amoebas of the species *Dictyostelium discoideum* had been collected before. *Dictyostelium* amoebas feed on bacteria, so we asked ourselves where bacteria might be most abundant. And sure enough, we discovered a "fruiting body" of social amoebas on the very first pile of not-so-fresh deer pellets we examined under a field microscope. A tiny golden orb, held up by a slender white stalk, seemed to float a millimeter or so above its circular base, glued onto the dung. The light from our microscope made it gleam. The sight was both exotic and commonplace: Hundreds of biologists around the world work on this social amoeba in the laboratory. But we were apparently the first to see a *D. discoideum* fruiting body in its natural habitat [*see photographs on this and opposite pages*].

Our discovery marked both a departure and a continuity in our careers as biologists. Early on, we each developed a deep interest in biological altruism, inspired by the work of the English evolutionary biologist William D. Hamilton. In the 1960s Hamilton argued that altruistic behavior could evolve if the genes responsible for that behavior benefited relatives that shared copies of the same altruistic genes. (Relatives are more or less likely to share a gene depending on how closely or distantly the individuals are related.)

Hamilton pointed out that an individual can pass on altruism genes even if it has no offspring—by helping a relative pass on copies of genes they share. If that helping, or altruistic, behavior is more effective at passing on the individual's genes than some alternate behavior, Hamilton reasoned, the genes for altruism are likely to propagate, through a process

called kin selection. He argued, for instance, that the unusual three-quarters relatedness among ant sisters could help explain their altruism. Richard Dawkins, an evolutionary biologist at the University of Oxford, later popularized Hamilton's idea in his best seller, *The Selfish Gene*.

With our mutual interest in altruism, it was natural for us to collaborate. We spent a quarter century studying the social behavior of wasps in places such as the olive groves of Tuscany and the rain forests of Venezuela. Yet having become experts in the habits and habitats of wasps, we decided to switch to social amoebas, a group of organisms we knew little about. But what was so compelling about these tiny creatures that, in midcareers, we veered onto an entirely new path that soon had us genuflecting before dung in the Appalachians?

What we saw was the chance to remedy a major deficit in the study of selfishness and altruism. The selfish-gene account of altruism has been pursued largely without knowing anything about the actual genes that underlie social behavior. The social amoeba *D. discoideum* had the advantage of being a model laboratory organism, cultured in great numbers and studied by a large community of biologists. The organism's genome has been sequenced. Investigators have developed a superb toolkit for manipulating its DNA. Experimenters can selectively knock out, or inactivate, any genes of interest, or even replace them. In social amoebas we could study real selfish genes.

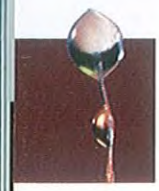
Social amoebas are also known as "cellular slime molds," but the name is a misnomer. The creatures are not slimy, and they are not molds. They comprise a hundred or so species belonging to the Amoebozoa, an ancient taxon which arose perhaps a billion years ago when it split off from the evolutionary branch that later gave rise to animals and fungi. Thus the cellular slime molds are no more closely related to any fungal mold than they are to your Aunt Alice.

Most of the time social amoebas do what most people think amoebas do: they move through soil by extending their pseudopods, or amorphous "feet" of protoplasm, and engulf prey along the way. We think of them as slow-motion cheetahs on the microbial equivalent of the African plains, feasting on bacteria, the even slower equivalent of gazelles. Each unicellular amoeba eats, grows, and then, as every schoolchild knows, splits down the middle to make two genetically identical cells.

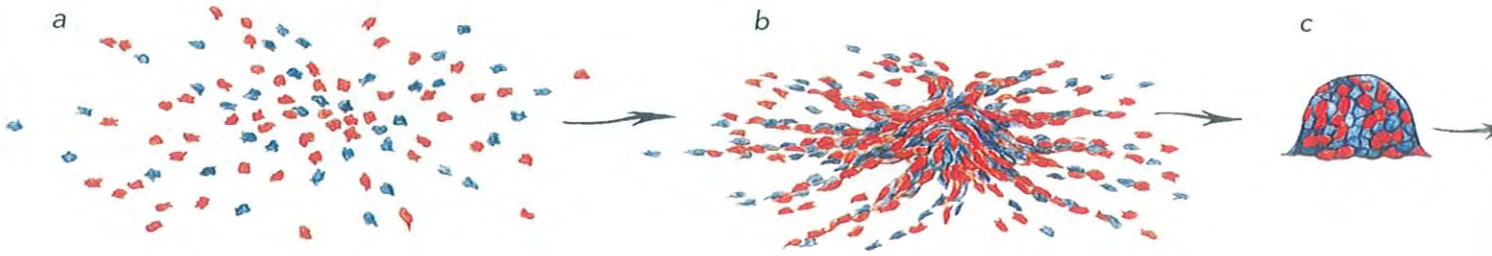
Tens of thousands of individual social amoebas of the species Dictyostelium discoideum *join together to form a fruiting body that is just visible to the naked eye. The fruiting body is the hairlike structure with what appears to be a shiny droplet at its top, in the center of the photograph above, magnified 26×; a closeup of the "droplet" is shown on the opposite page, magnified 1,100×. The droplet is made up of many fertile spores. The "hair," or stalk, is made up of individual amoebas that died after producing strong cellulose walls. The altruistic self-sacrifice of the amoebas in the stalk raises the spores to a more prominent position, increasing the likelihood that the spores will be dispersed and survive.*

Social amoebas live nearly everywhere there is soil, decaying vegetable matter, and a little bit of moisture. On the abundant bacteria in a deer pellet, social amoebas can persist through many generations of eating and dividing. In that stage of their lives they are not even particularly social; still, they constantly send out and receive signals that keep them informed about the presence and abundance of others of their kind, as well as about any nearby herds of bacteria.

Social life gets interesting only when food gets scarce. When *D. discoideum* amoebas begin to starve, they release a small molecule known as cAMP, which attracts other amoebas. Chains of hundreds of amoebas move up the cAMP concentration



Solitary, unicellular D. discoideum *amoebas of two genetically distinct strains, or clones (red, blue), begin to aggregate when food is scarce (a). Forming long chains (b), the two clones move toward a common, central area, where a visible mound arises (c). The amoebas then elongate into a "slug" (d) that lifts its "head" and crawls toward heat and light (e). Amoebas in the front 20 percent of the slug later form a stalk and die; the blue clone is cheating by not sending its fair share to the front. When the slug reaches a suitable place for producing*



gradient and merge into a mound made up of tens of thousands of individuals [*see illustration above*]. The minute but now visible mound elongates into a "slug," which crawls as one multicellular body across the forest floor toward heat and light, and away from ammonia, a common waste product.

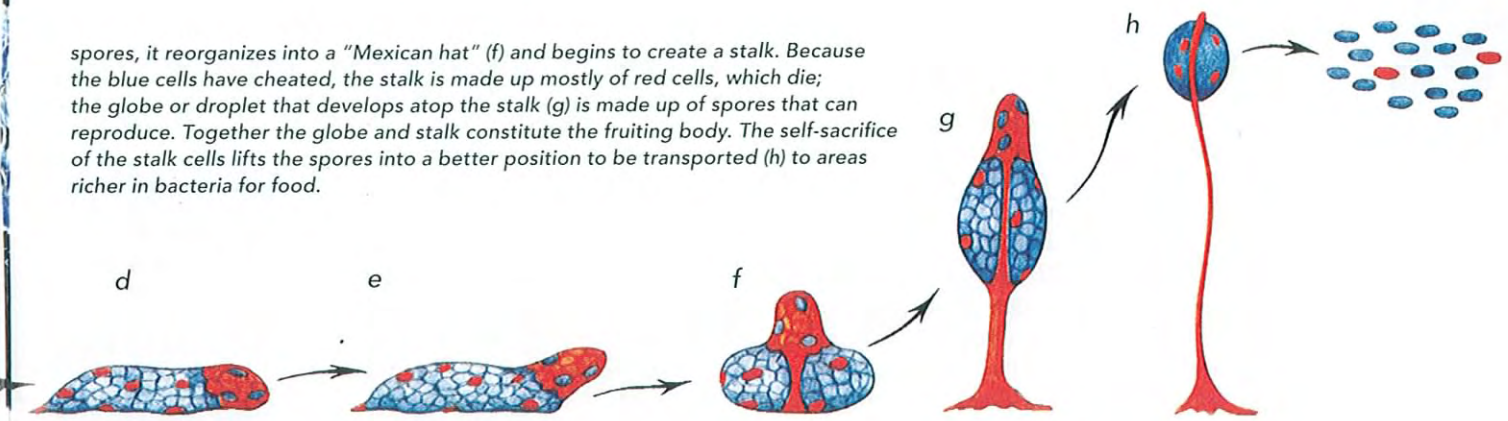
When the slug finds a suitable place, it stops and reorganizes. The individual amoebas that formed the front 20 percent of the slug arrange themselves into a stalk, laying down tough cell walls of cellulose, just as plants do. Individuals from the back 80 percent flow up the stalk, then reorganize at the top into a ball of hardy spores—the orb we spotted with our field microscope in Virginia. The amoebas that form the stalk die, but the spores, elevated by the self-sacrificing stalk amoebas, are thereby put in a good position to stick to passing insects or other organisms that can carry them to "greener pastures," richer in bacterial food.

The multicellular fruiting body is not unusual in being cooperative. After all, the cells in your own body cooperate as well, altruistically doing their jobs and dying without getting into the next generation. But that altruism is easy to understand because your body is one big clone of genetically identical cells, derived from the division of a single fertilized egg cell. A gene that causes a liver cell to cooperate dies when the liver cell dies, but identical copies of the gene are passed on through sperm and eggs. The genes in liver cells destined to die would gain no evolutionary advantage by, say, sneaking into the gonads and getting into the next generation.

What is unusual about the *D. discoideum* slug and fruiting body is that they form from dispersed cells that aggregate even though not all of them are genetically identical. Such an aggregate is called a chimera, and in a chimera, one genetic type can gain an evolutionary advantage by outcompeting the others. For example, a clone of genetically identical *D. discoideum* cells can leave more descendants if it cheats and makes more than its share of spores, forcing cells of other clones into the doomed stalk. We wanted to understand how altruism can be a successful strategy in the face of such cheating.

Our switch from wasp studies to social-amoeba research paralleled, in a curious way, the behavior of the amoebas themselves. We were accustomed to the rather solitary mode of field biology, but to get to greener research pastures, we had to work more cooperatively with the larger *Dictyostelium* community. Not only were we switching research organisms, but we were also switching scale, from macroscopic to microscopic, and switching to work that would involve the unfamiliar areas of cell biology and molecular genetics. Would we find the "dicty" community welcoming and cooperative, or skeptical and distrustful of admittedly ignorant outsiders like us?

We made our first efforts to find out via the Internet. Most dicty investigators are signed up for a listserv, and so we began to "send out signals" by posting elementary questions there, which were patiently answered by leaders in *Dictyostelium* molecular biology. When we first made contact, we did not yet know whether genetically distinct clones grouped together. But unfortunately, our new colleagues could shed no real light on that question or some of the others we were keenest to answer: If genetically distinct clones group together, do individuals in each clone get an equal chance to become fertile



spores, it reorganizes into a "Mexican hat" (f) and begins to create a stalk. Because the blue cells have cheated, the stalk is made up mostly of red cells, which die; the globe or droplet that develops atop the stalk (g) is made up of spores that can reproduce. Together the globe and stalk constitute the fruiting body. The self-sacrifice of the stalk cells lifts the spores into a better position to be transported (h) to areas richer in bacteria for food.

spores, or is one clone unfairly consigned to serving primarily as altruistic stalk? Can social interactions among amoebas be studied in the wild?

Instead of answers, one of the dicty biologists, Dennis Welker of Utah State University in Logan, gave us something far more valuable: a genetically diverse collection of wild-caught clones. Such a collection might not seem special, since hundreds of molecular biologists work on *D. discoideum*. But a molecular biologist almost always works with the descendants of a single clone, which has been bred to behave well in the laboratory. To us a single clone was of little use, because one would expect a clone to behave purely cooperatively, for the same reasons the cells in the human body do.

The wild clones enabled us to run some simple tests to see whether cooperation among the amoebas was vulnerable to cheating. We mixed cells of two clones together, then examined the resulting fruiting bodies for the presence of both. Sure enough, each fruiting body included cells from both clones. Yet in some pairs of clones, one of the clones cheated by contributing disproportionately to the spores.

If this earliest work had indicated that *D. discoideum* sorted by clone—as we later found to be true of its relative *D. purpureum* [*see photograph at right*], we might not have pursued the study of *Dictyostelium* further. But the mixing and cheating confirmed that the aggregate is a complex social system rather than just another genetically uniform multicellular organism.

The very existence of cheating suggests that individuals can distinguish their clone-mates from unrelated clones. Members of our laboratory, led by Kevin Foster, a postdoctoral investigator, corroborated that hunch experimentally by showing that chimeras behave differently from pure clones. Foster's team mixed amoebas from two, five, or ten distinct clones, and compared them to pure clones in their ability to cross a Petri plate. Taking advantage of their attraction to light, we covered the plates with dark paper, leaving a pinhole at the opposite end from where we put down the cells. The amoebas formed slugs and moved across the plate toward the light from the pinholes. The pure clonal slugs traveled farther than chimeric ones did before stopping and forming fruiting bodies.

Why are those results consistent with the idea

Social amoebas of the species Dictyostelium purpureum *form fruiting bodies in the laboratory primarily with clone-mates. Although two clones of* D. purpureum *may aggregate during a collective migration, they later separate into two fruiting bodies. The image is magnified 32×.*

that amoebas can distinguish clone-mates from unrelated clones in chimeras? We suspect that as the clones in a mixed slug compete to stay at the rear, where the spores will develop, the mixed slug as a whole moves forward more slowly than a slug made up of amoebas from a single clone.

We have also identified a different and unusual kind of recognition among *D. discoideum*. Hamilton noted that, in principle, recognition could be based not on overall kinship, but on the sharing of a single gene. Such a gene, he argued, would have to code for three things: a trait, the recognition of that trait in another individual, and altruism toward others with that trait. Dawkins whimsically compared such hypothetical genes to men with green beards who recognized other men with green beards and behaved altruistically toward them; he called them greenbeard genes. Most biologists, however, thought that such recognition was probably too complex to exist in nature: how could a single gene code for all three things?

Yet in the literature on *D. discoideum* we found a gene called *csaA*, for "contact site a," that seemed to qualify. The gene codes for a cell-adhesion protein that sticks out of the cell membrane and binds to identical cell-adhesion proteins protruding from other cells. The binding of like to like satisfies the first two requirements of a greenbeard gene: *csaA* codes for a trait as well as the recognition of that trait in others. But what about the altruism part?

To find out, we contacted Salvatore Bozzaro, a molecular biologist at the University of Turin in Italy, who had studied the gene. Bozzaro's group worked with a strain of *D. discoideum* in which the *csaA* gene was knocked out, and so his strain of amoebas lacked the adhesion protein. What would happen, we wondered, if we mixed the two otherwise identical strains, with and without *csaA*, fifty-fifty in a Petri plate? Would one of them act altruistically? Would the other one cheat?

It turned out that the knockout is a cheater—it contributes more than its share to spore tissues. The strain with intact *csaA*, known as the wild-type strain (so called because it is the typical form), ended up in the stalk. Hence in that mixture the wild-type amoebas are the more altruistic ones.

But there is more to the story. When we placed the knockout and the wild type together on the rough natural surface of soil, the weaker adhesion of the knockouts caused them to get left behind when other amoebas began to aggregate. In contrast, the wild types tended to bind to each other and pull each other into the aggregation. Thus the greenbeard recognition by the *csaA* gene ensures that the subsequent altruism of the amoebas that carry the gene only benefits other amoebas that also carry the gene. The *csaA* gene is the only known example so far of a single greenbeard gene that can control altruistic behavior toward other genes of the same kind.

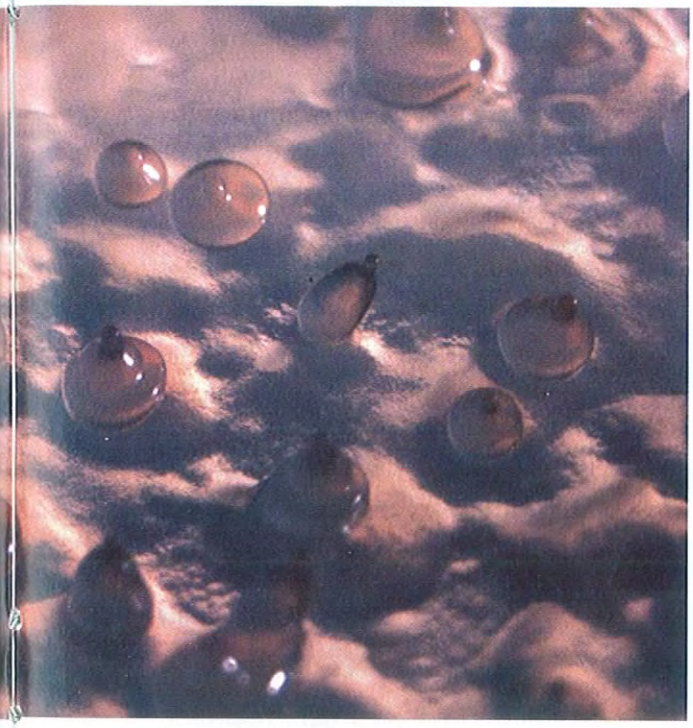
As it happens, the *csaA* gene is carried by all *D. discoideum* individuals we have examined so far. Hence it does not currently fully function as a greenbeard gene, because it is useless as a way to discriminate degrees of kinship within the species. But when it first arose, it could have survived as a minority gene for several generations, before it swept through the species as a result of the way it recognized and benefited itself. (It can, of course, discriminate against the rare mutants that lose the gene.) We suspect that there are other genes that do function as recognition genes. Such a gene would have to be highly variable in the species and would probably code for a molecule that protrudes from the cell membrane. The search is on for such molecules.

We had another good reason to suspect that social amoebas can identify and thereby help their close kin. The spores that aggregated on any given fruiting body we collected in the wild usually belonged to the same clone. Some mixing took place, but on average the amoebas in wild fruiting bodies were very close kin, closer than the workers in colonies of social insects.

We do not know precisely why such close kinship is the rule, but it is important for controlling cheaters. In the laboratory of Richard Kessin, a cell biologist at Columbia University, workers isolated a single-gene mutant that cheats. The cheater was highly effective in our experimental mixtures. It contributed hardly any cells to the stalk at all and instead ended up almost entirely in the spores. But the cheating, from a wider perspective, came at a high price. The cheater, on its own, cannot assemble into a viable fruiting body, and so it cannot propagate its spores. It reproduces only by mixing and forming fruiting bodies with a non-mutant strain of amoebas; in those mixed fruiting bodies, however, the presence of the mutant also lowers the total spore production. Yet despite the low spore production, the mutant can still spread in populations of mixed fruiting bodies because it cheats.

In the wild, however, relatedness is high; most

fruiting bodies form from a single clone. But for the mutant, being excluded from mixing would be disastrous; it would simply fail to produce spores. We therefore predicted that cheaters whose reproduction depends on forming mixed aggregates with other amoeba strains would not be present in the wild. Our



Aggregations of D. discoideum *are pictured in the Mexican-hat stage, though some of them are beginning to elongate. The image is magnified 25×.*

subsequent searches confirmed that prediction, suggesting that high relatedness does play an important role in limiting cheaters in nature.

Another important collaboration for us began in 2000, when we first met Adam Kuspa and Gad Shaulsky of the Baylor College of Medicine in Houston, Texas. Their expertise in genomics and cell and molecular biology seemed the perfect complement to ours in evolutionary biology and behavior. Some of the first results of our collaboration were initiated by Christopher R. L. Thompson and Foster, then postdoctoral investigators in our laboratories.

One way aggregating cells become altruistic stalk cells is by responding to a small molecule called DIF, or differentiation inducing factor. (Intriguingly, DIF seems to be produced by better-fed cells, and it induces weaker cells to become stalk. So perhaps the stalk cells are coerced into becoming stalk and are less altruistic than we thought.)

In any case, Thompson had identified a gene he called *dimA,* which, when knocked out, causes its bearer to ignore the DIF signal. We reasoned that the *dimA* knockout would be a cheater. Sure enough, when we mixed the knockout with wild-type cells, the knockout was overrepresented among the spore-forming cells at the slug stage. To our great surprise, though, when the spores developed, the knockout was underrepresented compared to the wild type. That told us the *dimA* gene must have a second function, besides recognizing DIF signals, that is important to slug-stage amoebas for transforming them into spores. That second unknown function probably evolved first, whereas receiving DIF signals, a cooperative social function, likely evolved later.

Notice that piggybacking the cooperative social function on a gene that controls another essential function is a good way to defeat cheating. A cheater that simply dropped the gene for responding to a signal such as DIF would also lose control of the second, essential function. The cheater would not survive. Building cooperative functions out of otherwise essential pathways may turn out to be a general way that many organisms control cheating.

Our stories about *csaA* and *dimA,* two genes important to cooperative social functions in *D. discoideum,* offer a glimpse of what might be learned via the genetic approach about the evolutionary benefits, and costs, of cooperation and conflict. Of course human cooperation is more complex; it often depends on reciprocation. Our own scientific experience is instructive. Our molecular biology colleagues from the dicty community have helped us learn more about evolution. But we also think our experience as evolutionary biologists brings a fresh perspective to genetics, and we hope to repay our new colleagues in their preferred currency, by illuminating gene function.

In conventional genetic studies, all the genes involved in multicellularity are studied in a single clone. Given the potential for conflict between clones, we think that is akin to studying the function of an army by watching it in peacetime. Parades and pushups give little idea of an army's actual purpose. Likewise, if social amoebas have cheater genes, the function of such genes may be impossible to discern until we examine them in the context in which they evolved: in competition with other clones. □

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