Sociobiology Goes Micro

Long used for studying development, *Dictyostelium* now also provides a model for analyzing social interactions

Joan E. Strassmann and David C. Queller

Sociobiology, the study of social behavior in an evolutionary framework, provides a powerful means for explaining many kinds of social behavior, including mating systems, parental care, and group living. According to sociobiology, genetic relatedness among interactants favors the evolution of cooperation because a gene can further its spread by distributing copies of itself in relatives.

It further predicts that conflict is expected among genetically different individuals, even among relatives or between members of a mated pair. Only genetically identical individuals will not have conflicts. This rule may explain why we see extreme cooperation in the groups of cells we call metazoans; each forms by clonal descent from a single cell, not by aggregation of independent cells that are likely to be genetically different.

Despite its many successes, sociobiology long suffered from a surprising weakness: this consummately genetic theory lacked an experimental system allowing researchers to tie the social behaviors that they study to underlying genes. The search for an organism to serve as a model for analyzing social behavior has proved frustrating because interesting social organisms are difficult to study, while easy-to-study models typically lack interesting social behavior.

For example, lions have complex social interactions, with brothers interacting to take over prides of females, but they do not make good lab animals. The same could be said for other candidates, including ants, meerkats, red deer, and scrub jays. At the other end of the scale, mice are good lab animals, but their sociality is mostly limited to parent-offspring interactions, territoriality, and battles for mates. Honeybees provide one means out of the dilemma. Their intensely studied social behavior is diverse and interesting; it even includes a symbolic dance language. They also have a nearly sequenced genome. But their reproductive cycle takes a year, they come from a small and highly specialized phylogenetic group, their social behaviors may have a complex genetic basis, and tools for genetic manipulation are not well developed.

A better-suited organism for studying sociality would exhibit a broad spectrum of social interactions, including individuals showing the ultimate level of altruism: reproductive death to help others. The model social organism should also be simple enough for interactions to be understood at a molecular level. It should have a sequenced genome as well as a genetic system simple enough to allow investigators to knock out and replace genes of interest. Moreover, the ideal model organism needs to have a very short generation time and should be easily reared in the laboratory. Because the environment can have a large impact on social interactions, it is desirable to study the model organism in a natural setting that can be duplicated in the laboratory. Additionally, it should come from a group whose members show varied social behaviors, enabling us to study the history of social traits and involved genes.

Social Amoebae as a Model for Studying Social Evolution

Surprisingly, the cellular slime mold *Dictyostelium discoideum* is proving well-suited as a model for studying sociality. The choice may seem obvious to microbiologists, but it is surprising to sociobiologists, who usually focus on larger organisms such as mammals, birds, and social insects.

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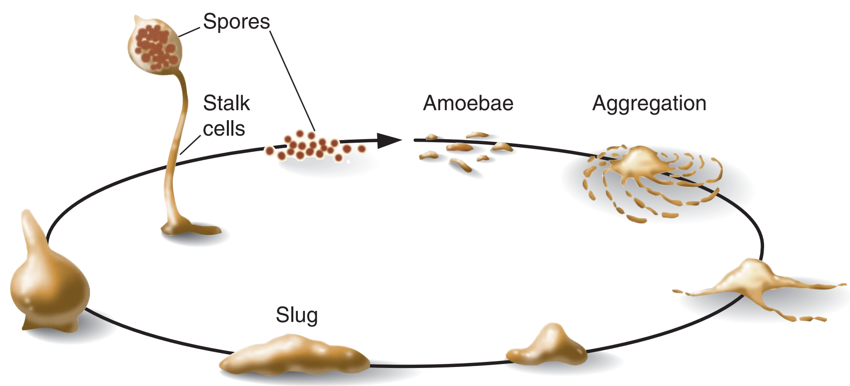
This fascinating species exhibits key social traits necessary for elucidating the mechanisms of the evolution of sociality. It has a social stage in which some individuals die to assist others. It forms groups with non-clonemates in which individuals compete to avoid the fatal altruistic role, thus reducing the overall effectiveness of the social group. Hence, it can be used as a model system for conflict as well as cooperation. Moreover, *D. discoideum* can be easily manipulated in the laboratory, its genome has been sequenced, and there are numerous molecular genetic tools for studying it. Because it meets all the criteria for being a social model system, sociobiologists may have found their long-sought "*Escherichia coli.*"

D. discoideum is a eukaryote in the *Amoebazoa*, which contains cellular and acellular slime molds as well as several other protists. It is at an important place in the tree of life, a sister kingdom to the animals plus fungi, and is more closely related to them than are green plants and red algae.

FIGURE 1



The life cycle of *Dictyostelium discoideum.* Solitary amoebae prey on bacteria in the forest soil. When prey become scarce, the amoebae aggregate, first forming a mound of 1,000 to 100,000 cells. Then they form a motile slug which moves up through the leaf litter towards light and away from ammonia. Finally, the slug develops into a fruiting body, with a stalk formed of dead cells that hold up the remaining cells that sporulate, awaiting better times or transport by animals to a better location.

A great deal is known about this organism because it has long been used as a model system for studying development. Developmental biologists prize its simple differentiation into two principal cell types that do not extensively proliferate and its ease of laboratory culture. This eukaryotic, single-celled organism is also being used as a model for studying traits such as motility and phagocytosis.

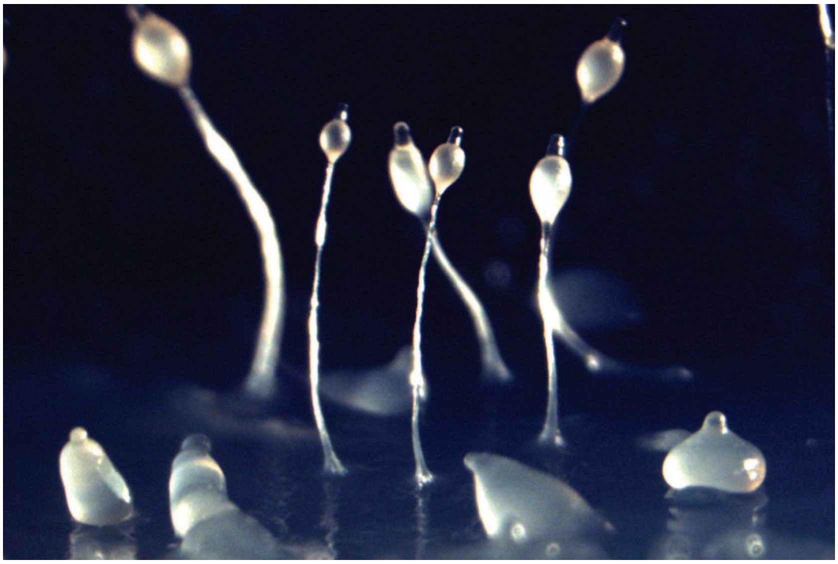
D. discoideum usually lives as a single-celled haploid amoeba, living on the forest floor and preying on bacteria. When bacteria become scarce and *D. discoideum* cells are sufficiently abundant, they secrete cAMP in pulses and follow this signal into aggregates (Fig. 1). Each aggregate of thousands to hundreds of thousands of cells forms a motile slug. The slug may crawl several centimeters through the forest humus before morphing into a fruiting body (Fig. 2).

In the fruiting body about 20% of cells vacuolate, form a cellulose wall, and die, forming a slender pillar about 1–2 mm high. The remaining cells flow up this stalk and sporulate at the top. The stalk places the spores in a more optimal place for dispersal by passing invertebrates. The fruiting body forms through an asexual process that involves little cell division. Instead, it is a differentiation of formerly totipotent individuals into sterile and fertile castes, much as social insects differentiate into sterile workers and fertile queens.

This differentiation into fertile and sterile cells from formerly independent units provides a good model for studying altruism. It also can serve as a model for analyzing conflict, but only if several conditions are met. First, amoebae must group with non-clonemates to form chimeras. If there is a recognition system such that only clonemates aggregate, then the social group is clonal and more like a metazoan that has gone through a single-cell stage. A multicellular body of identical cells will not have genetic conflicts of interest, and so it will not be a good general model system for social interactions.

Second, even if *D. discoideum* does not have a recognition system, it might be so sparsely distributed in nature that different clones seldom or never encounter each other. In this case, it would be unlikely that genes for conflict would be selected. Hence, it is essential that groups of non-clonemates of *D. discoideum* be found together in nature. Third, if *D. discoideum* does form chimeras, clones must compete, for example, to avoid the stalk and get into spores.

FIGURE 2



The social stage of *D. discoideum*. In the foreground are aggregated cells. In the background are mature fruiting bodies showing the stalks made up of dead cells and the clumps of living spores on top. Photo by Owen Gilbert.

Chimerism and Cheating

To answer such questions we needed to study naturally variable clones collected from native habitats, instead of well-characterized lab clones. We sampled a locale where *D. discoideum* is common, namely the University of Virginia Mountain Lake Biological Station in the southern Appalachians. We collected wild clones from samples of decaying leaves taken from the forest floor, suspended those samples in water, and spread them on Petri plates with a weakly nutrient agar substrate and *Klebsiella aerogenes* bacteria as prey for the amoebae.

Genetically different clones are easily identified using polymorphisms at DNA microsatellite loci (tandem repeats of short motifs, such as AAC, that are scattered throughout most eukaryote genomes). Cells from genetically distinct clones can be placed together on a Petri plate and can then be genotyped individually to see if distinct clones separate in fruiting bodies. In fact, they do not because fruiting body genotypes contain alleles of both clones. Thus, the first condition necessary for *D. discoideum* to serve as a model system for social evolution is met.

Genetically different clones co-aggregate in the same cooperating group, but how likely is what happens in the Petri plate to happen in the wild? To answer this, we need to discover whether genetically distinct clones encounter each other in nature. Scale is important for answering this question. How far are amoebae likely to move along the forest floor? Although they can move centimeters across the slick surface of agar in the laboratory, we chose to sample at a smaller scale of 6 mm because soil is a more difficult substrate. Isolates from the same 6-mm soil plugs revealed considerable genetic diversity (Fig. 3). Thus, the mixing seen in the lab probably also occurs in nature.

These chimeric fruiting bodies could have a fair allocation to spore and stalk tissues, or one clone may have evolved the means to exploit others. Because the front 20% of the slug normally becomes stalk cells and the remainder becomes spores, genotyping the front and back portions of chimeric slugs can reveal exploitation. In 6 of 12 mixtures of two different clones, one clone significantly undercontributed to prestalk and overcontributed to prespore, thus exploiting its partner clone.

Such findings indicated that *D. discoideum* both cooperates and is involved in complex conflicts that are comparable to those of social insects and vertebrates. This finding opens the door to two research strategies. The first is conventional for sociobiology because it entails studying the selective value of social strategies at the level of phenotypes. The second approach is more novel for sociobiology, but standard for molecular biologists because it involves identifying and studying genes underlying those phenotypes. An example of each strategy follows.

Social Conflict Has a Cost, but Grouping Has a Benefit

Social conflict among nonrelatives or distant relatives usually has a cost. If *D. discoideum* chimeras experience conflict, then we expect there to be a cost of chimerism. Cells finding themselves in chimeras might respond by being less cooperative. One possibility is that chimeras will produce shorter, less-effective stalks because there is less payoff to being altruistic when

From Social Insects to Social Amoebae

Joan Strassmann grew up with a forest behind her house. Beyond the forest lay second-growth fields, gravel pits, temporary ponds—"a biologist's dream," she says. By age 11 she was running her first laboratory in a small furniture crate behind her family home in East Lansing, Mich. "I identified or collected insects, reptiles, amphibians, and whatever I could catch," she recalls. "I kept live animals in terraria and aquaria and jars of formalin-preserved animals that I had found dead."

Her father, a professor at Michigan State University, kept the family traveling, however, because of his research in economics. Thus, she soon was forced to abandon her lab when her family moved to London and eventually to Geneva, Switzerland. She spent part of first grade and all of second grade in Mexico. In eighth grade, while a student in London, she won second place for her spring flora collection.

Strassmann detoured from biology while back in Michigan as a teenager. "I had a turbulent time in East Lansing High School in the late sixties," she says. "There I lost my early love of biology and turned to foreign languages and literature—so I was happy to go to Geneva, where I graduated from high school." Armed with a working knowledge of Spanish, French, and German, she enrolled at the University of Michigan as linguistics major. But she took a freshman biology course to satisfy an academic requirement, and "my love of the field came flooding back," she says.

Today Strassmann, 51, is professor and chair of ecology and evolutionary biology at Rice University. Her research involves analyzing "cooperative alliances" in social amoebae, especially *Dictyostelium discoideum*, and in social insects, wasps in particular.

"I worked for more than 25 years on social insects," she says. "Why are they social? Why does a mated female that could be queen instead help someone else? How do conflicts of interest play out in a colony?" She concentrated on social wasps with open nests, so everything could be watched. "It was a real soap opera, with wasps fighting, switching nests, eating each other's eggs, and I was entranced," she says "This system was amenable to experimentation—and I did some of the early experiments showing how wasps used relatedness in their choices."

She received a B.S. degree in zoology in 1974 from the University of Michigan and her Ph.D. in 1979, also in zoology, from the University of Texas at Austin. A few years after moving to Rice University in 1980, she began working with David Queller—a collaboration that includes both science (in the same lab) and life—they are married. She and her husband developed techniques to use DNA microsatellites to measure relatedness within colonies and to ask very specific questions about the social choices of wasps. They studied wasps in Venezuela and Italy, and stingless bees in Brazil.

"It seems that in small colonies queens get their way, while in species with large multiqueen colonies, the majority interests of the most numerous group, the workers, prevail," she says.

In 1998, Strassmann and Queller began studying social amoebae. It "was a huge, difficult change for us," she admits. "We left a system we knew well, one that took us to field sites in Tuscany and Venezuela, for one that took us to forests and cow pastures right down the road in rural Texas. We made the change to take advantage of the molecular techniques that '*Dicty*' developmental biologists had made over the last few decades." Moreover, the switch "offered us a social system with sterile castes, as we had with wasps, but now we also had a sequenced genome, knockout techniques, and abilities to get at the mechanisms of sociality," she says. "One thing about working on social systems is that we appreciate how wonderful it is when cooperation trumps conflict, and we know advantages must accrue to all interacting parties."

Strassmann is the oldest of three girls. One sister, Diana Strassmann, founded and edits the *Journal of Feminist Economics*. The other sister, Beverly Strassmann, is a human evolutionary biologist who studies the Dogon in Africa and is well known for using rigorous evolutionary methods to understand human social behavior. Their mother, who stayed home when Joan and her sisters were young, later taught college-level political science until she retired. "My father always said that growing up as a Christian half-Jew in Nazi Germany did not foster a love or respect for authority—he and his family got out of Berlin in 1937 and came to Houston," she says. "This attitude encouraged scientific adventurousness and creativity in me and my two sisters."

Strassmann has three children: The oldest, Anna, is a graduate student in sociology; middle son Danny is taking time off from college to write a novel; and Philip, the youngest, is in 9th grade. Outside the lab, Strassmann likes to cook (vegetarian, Italian, Venezuelan), read novels, and raise vegetables such as arugula, lettuce, and peppers. She also likes to hike, bird watch, snorkel, and watch her youngest play soccer. "He's a keeper," she says.

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much of the benefit goes to non-relatives instead of clonemates. But this is not the case. Fruiting bodies made up of a given number of cells have the same stalk proportions whether they are uniclonal or chimeric.

But there is another cost. Even if the same number of cells become altruistic stalk cells, they might delay assuming that role. For example, they might resist going into the prestalk zone in the anterior region of the slug, waiting for the slots to be taken by other cells. Chimeras might therefore migrate shorter distances in the slug stage. Experiments confirm this prediction. Given equal numbers of cells, chimeras migrate less far towards light than do clones (Fig. 4), suggesting that their ability to reach an ideal fruiting location is impaired.

Some clones are thus paying a dual cost in chimeras: migrating less far as a slug and being overrepresented in sterile stalk cells.

Why have amoebae not evolved to discriminate against non-clonemates in grouping? The answer is likely to lie with an important benefit that in natural circumstances can be obtained only by forming chimeras: large group size. Larger groups form larger slugs and larger slugs can move farther. A clone refusing to join in chimeras would avoid the cost of chimerism, but would lose by giving up the benefit of large size. A chimeric slug will move farther than a smaller slug containing only one clone (Fig. 4).

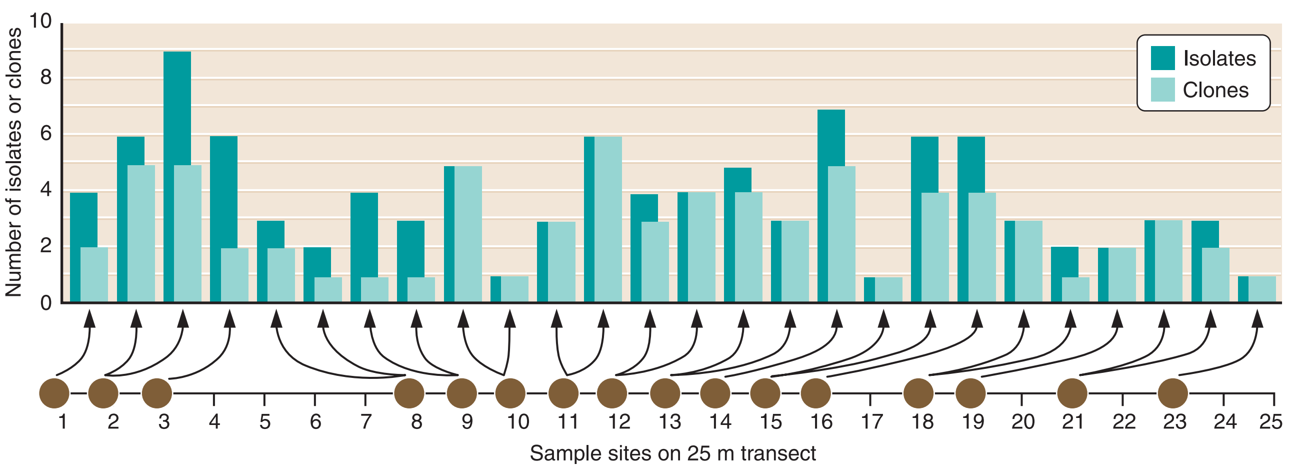
The First Greenbeard Gene

The real potential for studying *D. discoideum* as a social system lies in our ability to look at genes that affect a clone's fate in a group. Consider the "greenbeard" gene that sociobiologists predicted but could not observe. Such a gene, which uses an especially direct mechanism to aid copies of itself in others, is postulated to do three things: code for a trait, recognize that trait in others, and perform an altruistic act directed only to other carriers of that trait.

If all three capabilities were not features of a single gene, then the system could be vulnerable to cheating by a mutant that had the signal but failed to perform the altruistic act. Yet having a single gene encode all three effects has widely been regarded as implausible.

The *D. discoideum* greenbeard gene is *csA*, which codes for a homophilic cell adhesion protein, gp80. The homophilic binding site of this protein binds to copies of itself on the membranes of adjoining cells. In this case, the greenbeard gene is fixed in the population, and we see the effects of its absence only in artificially constructed knockouts. On the natural substrate of soil the adhering cells pull each other into aggregates, while knockout cells lacking gp80 are left behind. However, on the smoother surface of agar, the *csA* knockout is not impeded from aggregating, and once in the aggregate performs as a cheater, preferentially becoming a spore,

FIGURE 3

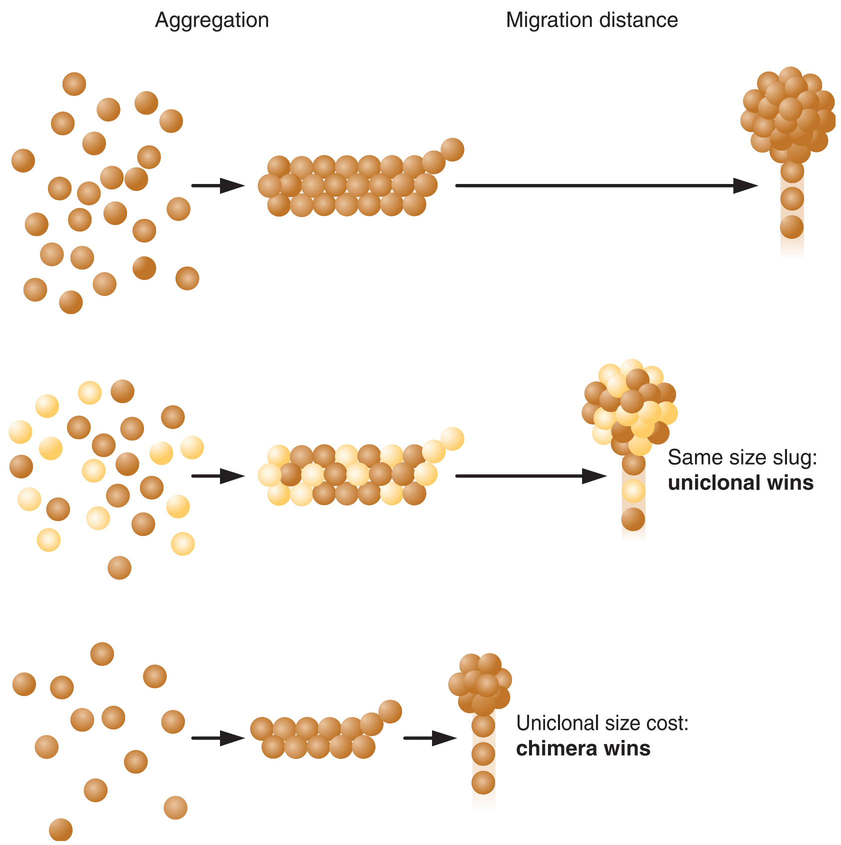


The number of *D. discoideum* clones and isolates from 0.2-g soil samples along a 25-m transect. The main panel shows the number of isolates and distinct clones in each sample. From Fortunato et al. 2003.

not part of a stalk. This behavior is also partly due to its weakened intercellular binding, because less-adhesive cells will slice to the back of the slug where they are more likely to become spores. Thus, the greater adhesion of wild-type cells makes them more altruistic, but the adhesion also insures that the benefit does not go to less-adhesive cheaters.

Other knockouts function as cheaters. *ChtA*, also called *fbxA*, is a gene whose knockout preferentially occupies spore tissues, not stalk. This gene was identified by subjecting mutants in a 14,000-mutant library to 20 rounds of selection for getting into the spores. By the end of these seletive rounds, the library should be preferentially enriched for cheaters that contribute more to spore than to stalk. Other cheater genes are being recovered by this kind of procedure.

FIGURE 4



Qualitative results on the costs and benefits of multiple clone groups. For equal cell numbers, groups of a single clone (line 1) migrate farther than multiple clone groups (line 2) showing an intrinsic cost to grouping with non-relatives. However, a clone refusing to join with a non-relative in a 50–50 mixture will have half the density of joinable cells, and a multiple clone group (line 2) migrates farther than uniclonal slug with half as many cells (line 3). Thus a size advantage of grouping with non-relatives more than compensates for the intrinsic cost. From Foster et al. 2003. The number of *D. discoideum* clones and isolates from 0.2-g soil samples along a 25-m transect. The main panel shows the number of isolates and distinct clones in each sample. From Fortunato et al. 2003. (a) Slugs on a Petri plate. (b) Fruiting bodies.

Other Social Microbes

Dictyostelium shines in having several social behaviors that are easily studied and manipulated. It groups, migrates, and forms sterile altruistic castes, and also bickers about which genotype wins. It is an organism that is easily cultured, yields single-gene knockouts, and has a known genome sequence. Its numerous congeners, some with very different social behavior, will allow the evolutionary history of genes to be traced. In some respects, this small soil protist may be more revealing for sociobiology than are lions or honeybees.

But *D. discoideum* is not the only microbial system with interesting social interactions. For instance, the bacterium *Myxococcus xanthus* has a number of similar social traits. Yet other bacteria produce bacteriocins to kill nonproducers and therefore favor their own type. In biofilms, there is an abundance of within- and between-species interactions. Studies of *D. discoideum* and other microbial systems will tell us much about social behavior in the years to come.

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