Evolution of cooperation and control of cheating in a social microbe

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Much of what we know about the evolution of altruism comes from animals. Here, we show that studying a microbe has yielded unique insights, particularly in understanding how social cheaters are controlled. The social stage of *Dictylostelium discoideum* occurs when the amoebae run out of their bacterial prey and aggregate into a multicellular, motile slug. This slug forms a fruiting body in which about a fifth of cells die to form a stalk that supports the remaining cells as they form hardy dispersal-ready spores. Because this social stage forms from aggregation, it is analogous to a social group, or a chimeric multicellular organism, and is vulnerable to internal conflict. Advances in cell labeling, microscopy, single-gene knockouts, and genomics, as well as the results of decades of study of *D. discoideum* as a model for development, allow us to explore the genetic basis of social contests and control of cheaters in unprecedented detail. Cheaters are limited from exploiting other clones by high relatedness, kin discrimination, pleiotropy, noble resistance, and lottery-like role assignment. The active nature of these limits is reflected in the elevated rates of change in social genes compared with nonsocial genes. Despite control of cheaters, some conflict is still expressed in chimeras, with slower movement of slugs, slightly decreased investment in stalk compared with spore cells, and differential contributions to stalk and spores. *D. discoideum* is rapidly becoming a model system of choice for molecular studies of social evolution.

kin selection | kin recognition | multicellularity | major transitions

Natural selection favors cooperation when genes underlying it increase in frequency compared with their noncooperative counterparts (1–3). Evolutionary studies of cooperative interactions have focused on the selective advantages of cooperating, how cooperation is organized, whether cheating a cooperative system can occur, and how cheaters are controlled (4–11). These studies generally, but not always, focus on within-species interactions and have been behaviorally oriented. Social insects have been a major focus (12–14), with cooperative birds and mammals also getting considerable attention (15–17). The past few decades have seen phenomenal progress in understanding cooperation in these organisms by applying the powerful logic of kin selection (1, 3, 18).

Our advances in understanding the evolution of social behavior through kin selection have been very satisfying, but they have been isolated in some respects. This is because most organisms have not been seen to be particularly cooperative. They may come together briefly for mating but, otherwise, go about the business of securing nutrients, avoiding disease and predation, and producing progeny largely on their own.

Cooperation Is Widespread

Behavioral ecologists have begun to study a wider selection of organisms and are finding cooperative interactions to be much more pervasive than previously appreciated. This is particularly true for microbes, wherein the structured environments necessary for cooperation have been discovered to be pervasive (10, 19, 20). Microbes are particularly affected by the actions of their neighbors, because many functions that are internal in multicellular organisms are external in single-celled organisms. Secreted compounds involved in processes like iron sequestration or food digestion are vulnerable to exploitation by neighboring individuals (7, 21, 22). Microorganisms evaluate their numbers with quorum sensing, kill nonclonemates with bacteriocins, hunt in groups, and cooperatively swarm through their environment, to name just a few examples of their social attributes (21, 23–25). Sociality in nontraditional study organisms is only beginning to be understood, however.

Cooperation, Organismality, and Major Transitions in Evolution

The second reason for expanded interest in cooperation is a growing appreciation that it is important for how organisms came to be. Cooperative major transitions in life alter the raw material for natural selection in fundamental ways (26, 27). One of the earliest transitions brought molecules together into cells in which the fates of all were intertwined in a cooperative network. Eukaryotes themselves represent a major transition resulting from the capture of a bacterium that becomes the mitochondrion (28). The level of cooperation between these partners is profound but not complete. Mitochondria are maternally inherited and do not go through meiosis, and thus will favor daughter production and have no interest in son production.

Another major transition resulted in multicellularity (29–32). Multicellularity has evolved multiple times in both bacterial and eukaryote lineages. Animals and plants have elaborated multicellularity into a plethora of diverse types. There are also a number of comparatively simple multicellular forms, like some single-species biofilms, the algal group Volvocales, or *Dictyostelium* (29, 33). The transition to multicellularity is different from the transition to eukaryotes because the former involves an aggregate of like entities, whereas the latter binds different elements. The major transitions can thus be categorized as fraternal, with like cooperating with like, or egalitarian, where the cooperating units bring different things to the collaboration (30). Either kind of collaborative organism will usually retain conflicts, but these conflicts must be controlled if the partnership is to survive. How these controls operate is a major research topic under this view of life.

The selective factors that favored a past transition are not easy to study because they have already completed their work. There are living systems that could be considered to be more representative of transitional stages, however. These, we believe, may be the most productive for investigation into the advantages of cooperation and how conflict is controlled. We have argued elsewhere that organisms themselves can be defined as adapted bundles of cooperative elements, wherein actual conflict is at a

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minimum (33, 34). In a 2D space, with one axis being cooperation and the other being conflict, organisms are those collaborative living units at the high end of cooperation and the low end of conflict. There is variation in the level of organismality, however, and those lacking complete cooperation and retaining conflict represent the best choices for studying the origins of cooperation.

Laboratory-Friendly, Social Model Organism

Kin selection has been very successful for generating predictions on the impact of queen number, mate number, and caste on sociality in social insects (12, 35). Nevertheless, one would have to say that social insects fall short as an ideal model for studies of social evolution. They are long-lived, often do poorly in the laboratory (except ants), are not amenable to genetic experimentation, and have mostly already crossed the threshold to obligate sociality. Thus, social evolution research has not found its *Drosophila* here.

Another problem with the organisms currently favored for studies of cooperation is that the actual genes underlying cooperative behavior are elusive. This is particularly true for long-lived social insects and vertebrates, although the advances of genomics are slowly mitigating this (14, 36). Still, the twin powers of experimental evolution and single-gene knockouts are beyond the reach of most currently studied social organisms.

A social evolution *Drosophila* would need to address these issues; thus, it would probably be single-celled. In addition to being amenable for experimental evolution and single-gene knockouts, it should have full altruism, with some individuals dying to help others. This makes it easier to interpret the actions of different partners. Other attributes of the ideal social *Drosophila* include feasibility of study in a fairly natural environment, placement in a rich phylogeny with related species that vary in social traits, a sequenced set of genomes, and a collegial community of fellow investigators. Here, we make the case that the ideal model organism for social evolution has been found and is the social amoeba *Dictyostelium discoideum.* This choice is supported by the enormous progress in understanding social evolution that has been made with this organism in the past decade. In addition to *D. discoideum, Volvox* and its relatives are great for studying the origins of multicellularity in a clonal organism (29). *Myxococcus xanthus* offers all the advantages of a bacterial system (37). There are also others, but we focus here on *D. discoideum* (Fig. 1).

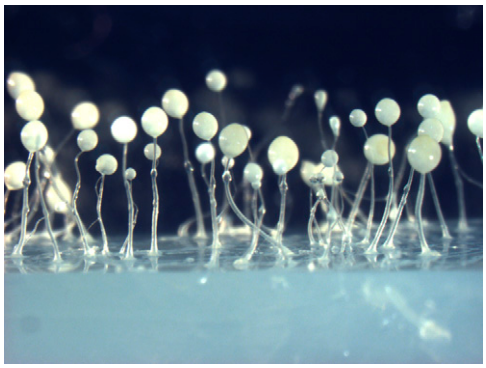

Fig. 1. *D. discoideum* fruiting bodies on an agar plate.

Dictyostelium discoideum as a Model System for Cooperation

What Is a Social Amoeba? Social amoebae are in the eukaryote kingdom Amoebozoa, sister to the Opisthokonts, or animals plus fungi (38). This kingdom is composed of solitary amoebae like *Entamoeba* and *Acathamoeba*, the acellular slime molds like *Physarum*, and the Dictyostelidae. There are over 100 species of *Dictyostelium,* divided into four major taxonomic groups (39, 40). *D. discoideum* is in group four and is the focal species here.

Individual amoebae of *D. discoideum* live in the upper layers of soil and leaf litter in the eastern Northern Hemisphere and in eastern Asia. The most intensely studied clone, NC4, and its derivatives like Ax4, come from a temperate forest near Mount Mitchell in western North Carolina (40). *D. discoideum* amoebae are solitary predators on bacteria, which they consume by engulfment (41). Although this is usually viewed as a solitary stage, they are always able to sense the density of nearby amoebae with a molecule called prestarvation factor (42). Response to this factor is inhibited when bacteria are present (42). When bacteria get scarce, and amoeba density is sufficient, they enter one of two stages, a sexual one, discussed later, or a social one (Fig. 2).

Social Cycle. The social stage, often called the developmental stage, occurs when *D. discoideum* amoebae begin to starve (Fig. 2). Amoebae have a quorum-sensing mechanism; if there are enough other amoebae in the area, they begin to release cAMP and to make receptors to it, products of the CAR genes (42, 43). A signal relay system causes the amoebae to move up the cAMP gradient and form a mound of hundreds of thousands of cells. Differentiation begins in the mound stage, wherein some cells sort out toward the tip and express prestalk genes. The tip becomes the anterior of the slug and organizes forward movement. During movement, cells are lost from the slug posterior. At least some of these are capable of dedifferentiating and consuming any bacteria encountered (44). The slug itself will not fall apart on encountering bacteria. Some shed cells are former sentinel cells, full of toxins and bacteria mopped up as they traversed through the slug (45).

The multicellular slug moves toward heat and light and away from ammonia (42, 46). The cells at the tip then migrate down through the center of the aggregate and initiate stalk formation in a process called culmination. The stalk cells vacuolate and die, forming sturdy cellulose walls in the process that give them the strength to hold up the spherical ball of spores. The final fruiting body consists of about 20% stalk cells and 80% spore cells. Thus, the social stage is triggered by starvation and involves altruism, because the stalk cells die to support the spore cells (42).

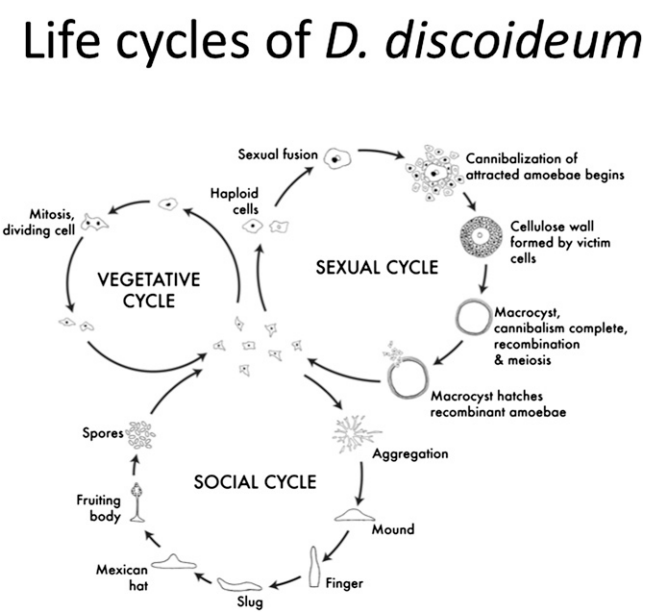

Fig. 2. Colony cycles of *D. discoideum*. This study focuses on the social cycle, but the sexual cycle is a promising area for future study.

D. discoideum arrives at multicellularity not through development from a single cell but through aggregation of dispersed cells. Therefore, the social stage of *D. discoideum* is vulnerable to cheaters. This makes it fundamentally different from a metazoan that has gone through a single-cell bottleneck and had the interests of all cells in the organism reset to complete cooperation every generation (47). This conflict, its control, and the resulting cooperation are what make *D. discoideum* such a great model for social evolution.

Why Have a Social Stage? During the social process, three things happen, and we predict that all three are adaptive. First, spores are made. The adaptive value of a hardy spore is clear and has been demonstrated; it is not easily digested by predators and can withstand long periods of cold, heat, or drought (40). Second, the spores are only made atop a relatively long stalk composed of dead cells. These stalks can be anywhere from 1 to about 4 mm long, and their construction is the most vital part of the altruism story of *D. discoideum.* Why are spores made only atop stalks? It could be that cells are vulnerable during the transformation to spore, and doing so atop a stalk protects them from hazards in the soil. Another possibility is that dispersal is facilitated when the spores are lifted above the soil and that this is the main purpose of the stalk. In *D. discoideum*, spores are likely to be actively transported on small invertebrates, although the guts of vertebrates and stalks could increase the chance that they are contacted. The third advantage to grouping is slug movement; slugs move farther than amoebae, which could position them into a better place for dispersal. The complex orchestration of fruiting body formation could only have arisen through natural selection, but more work on the actual advantages is needed. In this review, we focus on the interactions of genetically different clones in this social process and not on the reasons why it is adaptive.

Chimerism and Cheating the Social Contract. Mixing of two or more genetically distinct clones is likely for social groups that form by aggregation. To see if this actually occurs, we collected tiny soil samples of 0.2 g at Mountain Lake Biological Station (48). We reasoned that this was a reasonable scale over which social aggregation might occur. We found that our 0.2-g samples contained zero to five clones and that relatedness within the samples was about 0.52. These data support the view that chimerism is possible, at least in this population.

Later, we were able to find and genotype individual wild-fruiting bodies collected from the very rich resource of deer dung and nearby soil. This approach gave much higher relatednesses, between 0.86 and 0.98 depending on the sample and technique (49). Thus, relatedness is clearly high enough for kin selection under reasonable values of costs and benefits, and chimerism is common enough for social competition to be favored evolutionarily. Nevertheless, for cooperation to occur, there must be control of cheating. Here, we discuss what cheating is and then move on to evidence for it and its control in *D. discoideum*.

Complications with Defining Cheating. Cheating can only happen when one organism takes advantage of another; however, it is more than that. We would not say the lion cheated the gazelle out of its life with the lion's pounce and suffocating bite. This is because there is no expectation that the lion would behave in any other way. So, for an exploitative behavior to be considered cheating, there must be some expectation of cooperation that is not met. Cheating, therefore, is a fundamentally social action that takes place in the context of ordinarily cooperative acts, which the cheater somehow violates.

In *D. discoideum*, we talk of cheating in the context of cell allocation to the somatic, dead stalk and the living spores. The expected social contract is that the frequency of each clone among the spores will be the same as it was in the original mixture of aggregated cells. The same should be true in the stalk tissue. If this is not the case, we can say that the dominant clone cheated the minority clone by getting more than its fair share into spores, and cooperation can be put at risk when cheaters gain an advantage.

In many kinds of interactions, the starting and ending frequency may be viewed as enough information to determine if one partner is cheating the other. The formation of a fruiting body from an initial population of spores is a process that could vary for reasons other than social competition, however. Some clones may make longer or more robust stalks than others when they are entirely on their own. Some clones may migrate farther than others, losing cells in the process. Some clones may lose more cells from the slug than others even if they migrate the same distance. Variation is particularly expected in the highly variable environment of the soil. For example, a loose-grained soil may favor longer stalks for a given number of cells than a tighter grained soil if the adapted trait is to rise above the surface. Selection on these traits can occur independent of cheating but then have consequences in chimeras. If one clone in isolation allocates more to spore and continues to do so in the chimera with another clone that allocates less to spore, the first clone may then be viewed as a cheater, although it has behaved no differently in the chimera.

We will argue that even this case should be called cheating, because one clone does take advantage of the other. It might even have evolved for that purpose: Selection in chimeras could have favored variants that do suboptimal things on their own. We call this type of cheating "fixed," following Buttery et al. (50). Cheating that results from behavior different from what they would do when clonal, in recognition that there is a partner to cheat, we then call "facultative" (Fig. 3). If the only information we have is how they behave in a chimera compared with starting frequencies, we cannot distinguish between these two and just call it "cheating."

It is probably worth pointing out that we are not implying any sort of conscious awareness to cheating in *D. discoideum.* In humans, cheating is value-based and assumes a certain awareness of the moral grounds of an act. This, of course, is impossible in an organism lacking a nervous system.

Evidence for Cheating in *D. discoideum*

Do Wild Clones Cheat? When wild clones are mixed together, one clone often prevails over the other (51). Furthermore, there is a transitive hierarchy of cheaters (50, 52). In all these cases, the clones are perfectly able to produce fruiting bodies with normal, although variable, spore/stalk ratios as pure clones. Buttery et al. (50) found both fixed and facultative cheating among the clones.

Other evidence for social conflict among wild clones in the social stage comes from comparing chimeras with pure clones in

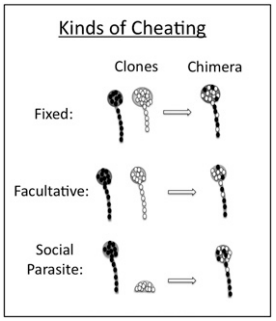

Fig. 3. In the social stage, clones may take advantage of their partner in three different ways. They may allocate cells to spore and stalk in the same proportions as alone but allocate less to stalk than their partner, fixed cheating. They may modify their behavior in chimera to take advantage of their partner, facultative cheating. Third, a social parasite can only make fruiting bodies in chimera with a victim.

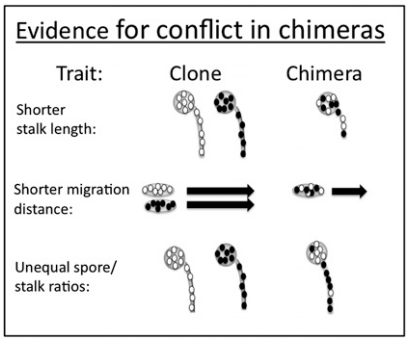

Fig. 4. Conflict is manifested in chimeras in the form of shorter stalk lengths, shorter migration distances, and unequal spore/stalk ratios.

their ability to migrate as slugs and to form tall fruiting bodies (Fig. 4). Chimeric slugs move less far than clonal slugs when cell number is controlled (53). This may be the result of increased competition to stay out of the front control region that becomes the sterile stalk. The other effect is that there are more spore cells in chimeric mixtures, presumably because there is less selective benefit to becoming a stalk to lift nonrelatives (50).

Cheating by Single-Gene Knockouts. Nearly all the research by cell, developmental, and molecular biologists on *D. discoideum* has used a single clone, or descendants of that clone. This means that these studies could not reveal cheating even if it were common. The exception is that they could reveal circumstances under which a clone with a single gene that was knocked out cheated its immediate ancestor. Kessin and colleagues (54) did just such a study. They made a large random collection of clones that each had a single gene disrupted by restriction enzyme-mediated integration (REMI), a process that randomly inserts a known sequence containing both restriction cut sites, and an antibiotic resistance gene (55). Kessin and colleagues (54) put a pool of REMI knockouts through 20 generations of selection in a well-mixed (low-relatedness) environment. At each round, they harvested the spores and began the next round from them; thus, any clone that cheated the others increased in frequency over these rounds. They then characterized one mutant, *fbxA*. The *fbxA* knockout cheats its ancestor but cannot make spores on its own.

Pools of REMI mutants can also be screened to obtain cheaters that are able to make normal fruiting bodies on their own but cheat their ancestor in a chimera. A large study of this type used pools of REMI mutants and required that every mutant be able to fruit on its own (56). This approach identified over 100 different knockout mutants that cheated their ancestor. If knockout cheaters are so easy to generate and cheating is advantageous, one has to ask why these genes have not lost function in the wild. We discuss the answer to this question below in the section on the control of cheating.

Control of Cheating

When wild clones come together in the social stage, cheating occurs between pairs of co-occurring wild clones. This could be the result of genetic or environmental factors. The work on single-gene knockouts suggests that at least some of the differences are genetic. Why are genes underlying victim status not eliminated from the population? We think the answer lies in the ways cheating is controlled. It can be controlled by high relatedness within social groups, which could result from kin discrimination. It can be controlled by positive pleiotropy, wherein a cooperation gene also has another essential function. Cheating can also be controlled if spore vs. stalk fate is the result of environmental rather than genetic factors. For example, spore fate could be the result of position in the mitotic cell cycle or it could be dependent on who starved first. Here, we take up these issues (Fig. 5).

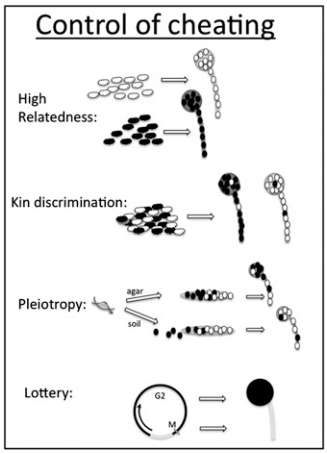

Fig. 5. Cheating can be controlled in the social stage if fruiting bodies are clonal, as might happen if they arise from different patches. They may mix but then sort into nearly clonal fruiting bodies through kin discrimination. Pleiotropic effects may prevent cheating genes from spreading. Caste fate may be determined through a lottery, with cells in the M or S stage of the cell cycle becoming stalk and those in the G2 stage becoming spore. *D. discoideum* apparently has no G1 stage, although this is controversial.

Control of Cheating by High Relatedness. Cheaters can be controlled if relatedness within social groups is high enough. This is because the benefits of the sacrifice that stalk cells make will mostly go to relatives, and thus could be favored under kin selection. The importance of high relatedness can be seen in an experiment that used the knockout cheater *fbxA* (49). In this study, we showed that at low relatedness, the *fbxA* cheater knockout wins within groups at all mixture frequencies. This means that it should increase in frequency in the population. There is a tradeoff, however. The higher the frequency of the cheater in a group, the lower the spore production becomes, hurting the *fbxA* knockout and WT alike within that group. This means that the cheater knockout can only flourish at low relatedness because at high relatedness, it is selected against by its own compromised spore production.

We expect social parasites like this one to fail in nature because of the high relatedness within fruiting bodies found in the wild. If this is true, we should not find any clones within wild fruiting bodies that are unable to form fruiting bodies on their own. We tested this by plating cells from wild fruiting bodies clonally. Of 3,316 clonal isolates from 95 wild fruiting bodies, all were able to make completely normal fruiting bodies on their own. There was not a single social parasite like *fbxA*. Clearly, high relatedness within fruiting bodies is a powerful evolutionary deterrent to cheating. This does not mean cheater mutants that are competent on their own are equally controlled, however (56).

Control of Cheating by Kin Discrimination. One way of achieving high relatedness is to exclude nonkin from the group. This behavior could explain the difference in relatedness between small soil samples and fruiting bodies. Different clones might aggregate together to cAMP and then sort into genetically homogeneous slugs. Even different species coaggregate to cAMP and then separate (57); thus, it is not unreasonable to postulate a similar process within species.

Studies of chimerism between two clones of *D. discoideum* have generally found fairly homogeneous mixing, however (50–52). A couple of studies found some evidence for sorting, particularly between clones collected far apart (58) or, in another study, particularly between clones found close together (59).

Neither approached the levels of sorting found in another species, *Dictylostelium purpureum* (60).

At this point, we have a puzzle. Tiny soil samples have multiple clones of *D. discoideum*, but fruiting bodies are nearly clonal. Kin discrimination is weak as far as we can tell in laboratory mixtures of equal numbers of cells from two clones. The finding of an apparently selected molecular mechanism for sorting deepens the puzzle. Our supposition that sorting will occur in the aggregation stage or later means that cells are likely to discriminate when they are in direct contact with each other. This suggests that adhesion genes are likely candidates for recognition. To function as recognition genes, adhesion genes would have to be highly variable. The variability would provide an opportunity for discrimination that favors others carrying the same adhesion protein variant over others carrying different forms of the molecule. They should recognize self, with a homophilic binding site, or they should recognize a highly variable receptor.

There is excellent evidence that two cell adhesion genes, initially called *lagC* and *lagB* but now called *tgrC* and *tgrB*, are the kin discrimination genes in *D. discoideum* (61). These two genes are extremely variable and are part of a large gene family of generally much less variable genes. The protein produced by *tgrC* is hypothesized to adhere to the protein produced by *tgrB.* If one is knocked out, it causes development to fail at the aggregation stage. In that case, the amoebae aggregate begins to make a mound but then falls apart, as if a crucial component of recognition necessary for the subsequent altruistic steps were missing. The temporal coexpression, knockout behavior, high variability, and impact on sorting make these likely kin recognition genes. More work is clearly needed on this system to see if there are consequences of recognition other than sorting. It could be that it is advantageous to remain in the group but that the chimeric nature is recognized and responded to, causing reduced migration distances and shorter stalks, for example.

Control of Cheating by Pleiotropy. Pleiotropy means that a single gene has an impact on multiple phenotypic traits. It is therefore usually viewed as something that impedes selection on a specific trait, because any changes in the underlying genes will affect other traits as well. This conservative force in pleiotropic genes can have interesting consequences for social genes. If an altruistic trait is piggy-backed on an essential gene, a mutation that causes selfish behavior is unlikely to proliferate, because the essential function would also be lost.

Exactly how important this might be in social traits is unknown, because we know the genetic underpinnings for comparatively few traits. There are a couple of genes having an impact on altruism in *D. discoideum* that could be maintained through pleiotropy, however. They are from very different parts of the genetic landscape underlying altruism in *D. discoideum.* One is a cell adhesion gene, and the other is involved in the differentiation-inducing factor (DIF-1) signaling system.

Cell adhesion is an essential part of the social process because it is how the multicellular group stays together (42). Variation in adhesion can have an impact on cell fate, because the cells at the front of the slug become stalk and the cells in the back three-quarters or so become spore (62). One way of increasing the likelihood of becoming spore could therefore be to have reduced adhesion to the other cells and to slip back in the slug (63, 64). The knockout of the cell adhesion gene *csaA* has just this effect. When *csaA* is knocked out, adhesion is reduced. On agar, this has the impact of increasing the knockout's frequency in the spores, presumably because reduced adhesion allows it to slip out of the stalk-forming tip (63, 64). On the more natural substrate of soil, however, *csaA* knockouts apparently do not hold together enough to get into aggregations. It is therefore no surprise that the *csaA* gene continues to be expressed normally and that cheater knockouts have not prospered.

Another gene that could be a cheater were it not for pleiotropic effects is *dimA* (65). This gene was isolated in a screen of REMI mutants that are unresponsive to DIF-1, a small molecule that forces some cells to become stalk (more on this later). In chimeras with WT, *dimA* knockouts predominate in the prespore zone, presumably because they are insensitive to DIF (66). Ultimately, however, they are in a minority in the actual spores. This could be true if they transdifferentiate from prespore cells to prestalk cells later in development, and this was shown to be the case (65). We interpreted this to be the result of another unknown function of *dimA*, an essential function that made the knockouts worse spore cells. This is another case in which pleiotropy inhibits the spread of a cheater.

Control of Cheating by Lottery. When two or more individuals take unequal roles in a social interaction, with one being the recipient and the other being the beneficiary, conflict can result. One way of controlling this conflict is if the partners do not know which role they will assume on entering the interaction. A human equivalent is called the veil of ignorance (67), and it calls for resource allocation between partners by someone who does not know which lot he or she will get. A familiar example is the common family situation of dividing up a cake. If the child cutting the pieces does not get to decide which piece he or she gets, under the veil of ignorance model, he or she will be more likely to make the pieces equally sized. Cheating could be controlled in *D. discoideum* if there were a lottery to become spore based on the cell cycle.

The *D. discoideum* cell cycle has a very short G1 phase; thus, immediately after the mitosis (M) phase, cells enter the synthesis (S) phase and cytokinesis occurs during the S phase (68). Therefore, in a population, the cells in S and early growth after synthesis (G2) phases tend to be the smallest cells with the fewest nutrient reserves. An experiment on a thin layer of cells not touching other cells, followed with videography, indicated that stalk cells were most likely to arise from cells that happened to be in the S or early G2 phase of the cell cycle at the time of starvation, whereas cells that happened to be in the late G2 phase became prespore (69). A variety of other experiments have also shown this (70, 71). If weaker cells are more likely to become stalk, this makes sense, because recently divided cells would have fewer nutrients. This cell cycle lottery system fits the veil of ignorance model. As cells encounter less and less food, however, it could be that those dividing earlier than others are selected against because these cells will be in the stage that sends them to stalk.

Another interesting result of this paper (69) suggests that delaying cell division may not be necessary for a cell to avoid becoming a stalk cell. This result was derived from careful observation of the fate of sister cells through videography. Every time a cell divided, one sister cell became prestalk or prespore according to the above musical chairs lottery mechanism, whereas the sister cell became a null cell, a third cell type that stained with neither prespore nor prestalk markers (69). The fate of these null cells is unclear. These null cells could become pstO, because that region of the slug also did not stain with prespore or prestalk markers. This region can be viewed as the most flexible area, with cells in that region remaining pstO on exposure to DIF, and perhaps becoming prespore otherwise. These interesting results remain controversial, however, and should be followed up on carefully (72, 73).

If a recently divided cell becomes stalk because it is smaller and weaker, cell division could be disfavored as starvation approached for this social reason. Under normal circumstances, however, amoebae will be selected to eat and proliferate as rapidly as possible. These two counterforces might achieve a compromise that could support altruism under a wide variety of conditions in *D. discoideum*, if one of two recently divided cells becomes stalk and the other becomes spore. This scenario is consistent with the data.

Control of Conflict by Power

We began the section on control of cheating with a discussion of social contracts and defined cheating as the violation of those contracts. In this case, we mean evolved contracts that favor the evolution of cooperation. One form of contract may be that the stronger individuals take the best roles. Here, we explore the evidence for this idea in *D. discoideum.*

First-Strike Power. One of the most common determinants of whether an individual in a social interaction becomes the altruist or the beneficiary is that individual's relative strength, or ability to prevail in a contest. Such contests under social and cooperative circumstances may look very similar to contests between nonsocial organisms for scarce resources like good territories. The difference is that if the contest is between relatives, or mutually dependent individuals, after the contest is decided, the loser may acquiesce and go to work for the winner. Such contests can be valuable for all concerned, particularly if weaker individuals that lose contests are more effective in taking on the helping role than they would be with the winning, reproductive role.

How do we evaluate power in *D. discoideum* interactions? In some ways, all predictors of fate also involve power. The lottery system has a power element, because cells that recently divided may be weaker and go to stalk. If becoming a spore cell is competitive, the first amoebae to depart from growth and binary fission and enter the social stage may get a head start on preparing their weapons. Under this hypothesis, the first to starve would become spore. That this is the case has been very nicely demonstrated in both an experiment that manipulates timing of starvation in genetically identical cells and an experiment that uses an aggregation-initiation knockout. In the first experiment, cells were put into nutrient-free medium 4 h apart. Those with the 4-h head-start in the social stage preferentially became spores (74). The other experiment used a knockout that was incapable of initiating aggregation but was capable of responding to the initial signal from others and relaying it (75). In this case, the single cell initiating aggregation became a spore.

Glucose Feeding, Condition, and Power. Power based on condition has also been studied directly by making chimeras of cells that were well-fed with cells that were poorly fed. This was done by varying the amount of glucose in the medium of axenically grown cells. The cells fed with glucose were more likely to become spore than the glucose-starved cells (76). This effect holds with other metabolizable sugars and is absent with other sugars (77). This is strong support for the hypothesis, but there could be something special about sugars; thus, we repeated this experiment with a glucose treatment and added another treatment to separate cells. In this treatment, we stressed the cells by growing them in a more acid pH than usual (78). We affirmed the weakening effects of both treatments by documenting that they increased doubling times in the solitary stage. As expected, both acid-stressed and glucose-starved cells ended up preferentially in the stalk. Both treatments also made fewer spores when grown alone, however; thus, the chimera results are not attributable to competition alone (78).

DIF-1 and Power. One of the delights in working with a microbial system is the accessibility of mechanisms. Whether a cell becomes spore or stalk is mediated by DIF-1, a small, secreted, chlorinated alkyl phenone (79). Stronger cells that are immune to its effects at biological levels produce DIF-1. Weaker cells can break it down but become stalk cells from its impact, mostly ending up in the lower cup or the basal disk, both of which are dead parts of the stalk (80, 81). DIF-1 is unlikely simply to be a signal rather than a mediator of competition for several reasons. Signals are unlikely to include chlorine, something that is common for poisons. Levels of DIF-1 in the slug are about 62 nm (79), which is high, given that it can be lethal at concentrations as low as 200 nm (82). Signals have receptors and poisons do not, and no receptor has ever been found for DIF-1. Its small, toxic nature is just what might be expected of a poison (83). Unlike most morphogens, it is distributed evenly through the social stage and varies on its cell-specific impact (84–86). In some respects, it is a tame poison, incorporated into social life to mediate condition in a homogeneous mixture into different cell fates.

The condition variants resulting from position in the cell cycle or glucose feeding are tied to DIF-1 levels with weaker, more recently divided cells more vulnerable to DIF-1. There are single-gene knockouts with an impact on cell cycle and nutritional responses that further support the involvement of DIF-1, in a story nicely summarized by Chattwood and Thompson (86). Cells that have *rtoA* knocked out lose the specificity toward stalk of the M and/or S cell cycle phase, producing fruiting bodies that are mostly stalk with tiny spore heads (87). This has been shown to be the result of high intracellular calcium, which has independently been shown to bias cell fate toward stalk (71, 86, 88). Cells with high intracellular calcium are far more sensitive to DIF-1 (88, 89). A similar story can be told with a gene that links nutritional status to cell fate, a *D. discoideum* homolog of the human retinoblastoma gene, *rblA* (86, 90). Knockouts of *rblA* are hypersensitive to DIF-1 and preferentially become stalk.

Other work by Thompson and colleagues (84) has shown that the patterns linking DIF-1, or more generally stalk-inducing factors (StIFs), are also important in spore-stalk hierarchies of natural clones. These hierarchies are based on whether clones become spore or stalk when mixed pairwise with other clones (50, 52). They separately evaluated response to and production of StIFs and found a threefold difference in production and a 15-fold difference in response; the latter was most powerful in explaining the hierarchy observed in natural clones (84). Thus, we know a satisfying amount about how power affects cell fate through DIF-1. There is more to learn, however, particularly because cheating can result from knocking out so many different genes (56). This led to another general approach to identifying resistance genes.

Genetic Control of Cheating by Noble Resistors. The evolution of resistance to cheater genes may limit their spread. To test this idea, we selected for resistors of cheater genes. We took one cheater, *chtC*, and exposed a pool of REMI mutants to it over successive rounds (91). We allowed selection of the REMI pool but not of the *chtC* knockout. We did this by removing the G418 resistance from the *chtC* clone so that we could kill it at each round, leaving the mutants we were selecting intact. We then simply added back in the naive *chtC* clone for the next round. This process resulted in a number of mutants that were resistant to *chtC* knockouts and could not be cheated by it. Interestingly, they were not cheaters of their ancestor; thus, we called them noble resistors (91).

Social Genes, Arms Races, and the Red Queen

Cheating and countering cheating are social processes that we predict will result in rapid evolution in the underlying genes. Our test of this hypothesis used the newly sequenced species *D. purpureum* and compared it with *D. discoideum* (92). Unfortunately, this is not an ideal pair of species because their proteins are as diverged as those of humans and fish. This means that silent amino acid changes (ds) are saturated, and thus are not useful in comparisons. Instead, we compared homologs; rates of amino acid change; and conservation scores, a measure of similarity that includes indels. We used two sets of social genes for comparisons. The first set was the 100 or so REMI mutants that cheated their ancestors when mixed equally with them (56). These genes did not show more rapid evolution, and thus failed to support our hypothesis that social genes evolve more rapidly.

The second set of genes we used was based on a social index, which was higher when a gene was more expressed in the social stage compared with the nonsocial stage. In this analysis, the more social genes had a lower probability of having homologs, an elevated rate of amino acid change, and a lower conservation score, supporting our hypothesis (92). The result could also be attributable to weaker purifying selection on social genes, however, and a better analysis would be between more closely related species.

Other Arenas for Cooperation: Mutualisms and Sex

No review of social behavior of *D. discoideum* would be complete without mentioning two very exciting areas for future study. The sexual cycle is also a social cycle but has been studied very little. The other area is the discovery of a farming mutualism between *D. discoideum* and bacteria. This opens up the opportunity for studies of between-species symbioses.

Sexual Cycle Has Social Elements That Involve the Ultimate Sacrifice. The sexual cycle is triggered by starvation in the presence of sufficient numbers of other amoebae under wet, phosphate-poor conditions and begins with aggregation to cAMP (41, 42). Two cells of different mating types fuse, forming a diploid zygote. The amoeba stage is ordinarily haploid and divides by mitosis; thus, no reduction division is necessary before sexual fusion. Aggregation does not cease with the formation of a diploid zygote (93, 94). Other amoebae continue to swarm in by the thousands, up the cAMP gradient. The zygote proceeds to consume the other cells by phagocytosis. The pace of consumption is slowed to a level that allows the waiting victim amoebae to construct an envelope around the aggregation, and this slowing is also regulated by cAMP. After a time, there is a firm wall around the zygote and its victims, and the latter are consumed and digested. Recombination and crossing over then happen, the zygote undergoes meiosis, and many recombinants are formed.

In a major recent advance, the sex-determining locus was identified and the presence of three mating types was confirmed, clearly establishing the genetic basis of sex (95). The sexual cycle is somewhat of an enigma because it rarely leads to recombinant progeny under laboratory conditions (42), but estimates of recombination rates of natural clones indicate they are very high, with a population ρ of 37.75 and baseline linkage disequilibrium achieved between 10 and 25 kb (59). Getting the system to work in the laboratory would open up many interesting social questions to investigation. For example, we could select for social traits in sexually recombined pools and look for quantitative trait loci associated with social traits.

***D. discoideum* Farms Bacteria.** There is another reason why *D. discoideum* is particularly good for studies of cooperation: mutualism. The standard view of the social stage of development is that all bacteria are purged from the aggregate (42). There are known mechanisms for this that function at different stages, from mound, to slug, to final fruiting body. The sorus is considered to be sterile apart from the *D. discoideum* spores. Very recently, we discovered that this is not the case for about one-third of all clones (96). These clones carry bacteria with them through the social stage like a farmer might bring a flock of sheep to a different pasture. These bacteria are found within the fruiting body. When the spores hatch after favorable growing conditions have been encountered, they can feed on the proliferating population of the bacteria they brought. These farmed bacteria are better food than most wild bacteria. This farming mutualism is highly amenable for study, because all partners are microbial, advantages are clear, and the relationship is not obligate, at least at the species level. This discovery adds between-species cooperation to the things that can be studied about *D. discoideum.*

Conclusion

The ultimate advantage to an ideal model organism is what you can learn from it. In *D. discoideum*, we have shown that conflict exists in the form of shorter stalk lengths, reduced migration distances, and cheating to avoid the sterile caste. We have delineated cheating into fixed, facultative, and social parasite forms. We have shown that cheating can be controlled by high relatedness, kin discrimination, pleiotropy, or lotteries. We have shown that conflict can be controlled by conventions and power. The first cells to starve become spore, as do stronger cells. A small, toxic molecule called DIF-1 mediates social interactions. We and others have backed up much of this work with specific genes and knockouts. Further whole-genome outcomes are on the horizon, as is a much more detailed understanding of kin discrimination. Frontiers include the farming symbiosis and exploration of the sexual cycle. Clearly, this is a system that has yielded many important secrets about the cooperative side of major transitions.

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