Cooperation and conflict in the social amoeba, *Dictyostelium discoideum*

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INTRODUCTION: MAJOR TRANSITIONS, DEVELOPMENT, AND SOCIALITY

Social insects have long provided our best examples of the evolution of cooperation and altruism. For this reason, they have been accorded an important place in behavioral ecology and sociobiology, but at the same time that place has been a limited one. Whereas all organisms must have feeding strategies, and most must have mating strategies, comparatively few seem to have compelling cooperative strategies. Social insect researchers therefore labor in something of a taxonomic ghetto, escaping only occasionally for exchanges with researchers working on something like cooperative breeding in vertebrates.

Recently, however, the interest in sociality has surged for another reason. Cooperation may not be crucial in the lives of all organisms, but cooperation is how those organisms came to be in the first place. This idea was given form by Maynard Smith and Szathmáry's discussions of the major transitions of evolution (1995; Szathmáry and Maynard Smith, 1995). The evolutionary progression through various levels of life, such as cells, eukaryotic cells, and multicellular individuals, often required separate units to merge their interest in a larger unit. The transition to nearly organismal colonies, as occurred in social insects, is the last of these transitions. Many social insects still show the tension between cooperation and conflict that must have characterized the earlier transitions, so the study of social insects may help us to understand some of these earlier transitions.

However, social insects are not ideal study organisms in some respects, particularly for lab studies. One of the chief problems is that they are long-lived. This makes genetic studies slow and tedious and selection studies nearly impossible. Some progress has been made in these areas, for example the study of the Gp-9 polygyny gene in fire ants (Krieger and Ross, 2002) and some genetic dissection and selection of honey bees (Fewell and Page, 2000; Page et al., 2000), but it seems fair to say that social evolution research has yet to find its *Drosophila*.

A social evolution *Drosophila* will need to reproduce rapidly, and in order to reproduce rapidly, it probably needs to be small. Single cells seem like ideal candidates, but when cells cooperate, we often call it development rather than

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sociality. The cells in a multicellular body are indeed highly cooperative and have evolved this cooperation for the same reason as the social insects – kin selection. Queller (2000) has called the transitions to multicellularity and to colonies the "fraternal" major transitions, to distinguish them from the "egalitarian" transitions that involve mutualism more than kin selection. But there is one crucial difference between the transitions to multicellularity and to colonies. Most multicellular organisms are essentially free of internal conflicts among their cells, whereas most social insect colonies retain conflict among their individuals. The reason is that most multicellular organisms develop from a single cell, so they consist of a clone of identical cells. The only cell conflicts possible arise from recurrent mutations but these provide rather limited scope for conflict because each mutation gains at most one generation of within-individual advantage (Slatkin, 1985; Seger, 1988; Maynard Smith, 1989; Queller, 2000). Conflicts of this kind become a potentially serious issue only with large numbers of cell divisions and high mutation rates (Michod, 1996; Michod and Roze, 1997; Michod, 1997) .

However, there are a limited number of organisms that do not necessarily develop from a single cell, but instead form by aggregation. If aggregates contain multiple genotypes, we might well expect them to manifest the same tension between cooperation and conflict that makes social insects so interesting. So perhaps our social evolution *Drosophila* could come from this category of organisms. An obvious candidate is available, *Dictyostelium discoideum*, a well-studied member of the cellular slime molds, also known as social amoebae, a name shift that happens to highlight the reason they are interesting to behavioral ecologists.

THE SOCIAL AMOEBA, *DICTYOSTELIUM DISCOIDEUM*

D. discoideum has long served as a laboratory model system, but for development, not social evolution (Bonner, 1959; Loomis, 1982; Maeda et al. 1997; Kessin, 2001). It is easy to collect from the field (Cavender and Raper, 1965a; Kuserk et al., 1977) and easy to culture and preserve in the lab. A glossary of some common terms is given in Table 1. The cells can be kept separate or made to go through their developmental cycle in just a couple of days. The initial interest in the system came mostly from developmental biologists who were attracted by its manipulability and by the seeming simplicity of its development. There are only two major kinds of differentiated cells, spore and stalk (see Figure 1). In addition, the developmental process involved only differentiation, not increases in cell number.

D. discoideum lives on the forest floor in eastern North America and eastern Asia (Cavender and Raper, 1965b,c; Swanson et al., 1999). It is a predatory amoeba. Single cells migrate through the leaf litter and upper soil in search of bacteria (Kuserk, 1980), which they engulf through phagocytosis. The "development" stage comes when the amoeba run out of food (for review see Kessin, 2001). Figure 2 provides a schematic of this process. Starving amoebae are able to sense each

Table 1. Glossary of terms used in social amoeba literature

Term	Meaning
Aggregation	Stage where cells are coming together, responding to cAMP
Amoeba	The single-cell stage. Migrates using pseudopodia and engulfs bacteria by phagocytosis
Axenic	Liquid medium with nutrients but no bacteria; also the specially selected *D. discoideum* lab strains that can grow in this medium
Basal disc	A supporting disc of cells at the base of the stalk
Cell-autonomous	A mutation that affects that cell and no other; i.e not a mutation in a communicating chemical.
Chimera	The multicellular stage formed from cells of two or more genetically distinct clones
Culmination	Fruiting body formation from the cessation of movement by the slug, through the Mexican hat stage, and the fruiting body formation
Fruiting body	Consists of stalk and spore cells
Grex	slug
Macrocyst	Sexual stage. cAMP calls cells together; first two of different mating types fuse, other joining cells (10^3 to 10^5) are eaten. Recombinants from this are rare in the laboratory for *D. discoideum*
Mexican hat	Stage where the stalk is beginning to be produced from the slug
Microcyst	Single encapsulated cell; stage thought not to occur in *D. discoideum*
Mound	Pile of cells at end of aggregation process before elongation into slug
Null mutants	Mutants with normal function of a specific gene eliminated, often designated with minus sign
Prespore	Cells expressing genes required for differentiation into spores; in slugs located in posterior 80%
Prestalk	Cells expressing genes required for differentiation into stalk; in slugs, most are found in the anterior 20%
REMI mutagenesis	Restriction Enzyme Mediated Insertions to knock out genes, and to identify which gene you have knocked out
Slug	Aggregated mucous-sheathed cells – the multicellular stage of 10^3 to 10^5 cells that can creep around, then form a fruiting body
Sorocarp	Fruiting body consisting of stalk and spore cells
Spores	Dispersal stage of cells, ready to germinate when moistened in an appropriate environment
Sorus	The ball of spores at the top of the stalk
Stalk	Sterile, dead cells with cellulose walls and large vacuoles that lift spore cells above the substrate
Tip	Front of slug, organizes slug movement; cells there become stalk cells

other's presence, and if there are enough of them, some begin to secrete cyclic AMP. Neighbors detect and relay the signal, and amoebae aggregate by following the gradient of cAMP to its source. This results in a mound of up to 10^4 -10^6 cells (Bonner, 2001). Under the control of cells at the tip, the mound elongates in a

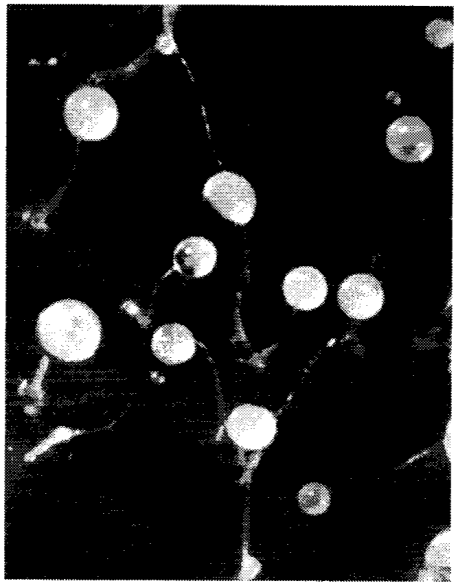

Figure 1. *Dictyostelium discoideum* sorocarps (fruiting bodies) growing on agar in the lab. Each consists of 10^4 -10^6 cells, roughly 80% of which are spores in the spherical sorus, and the remainder of which are dead stalk cells.

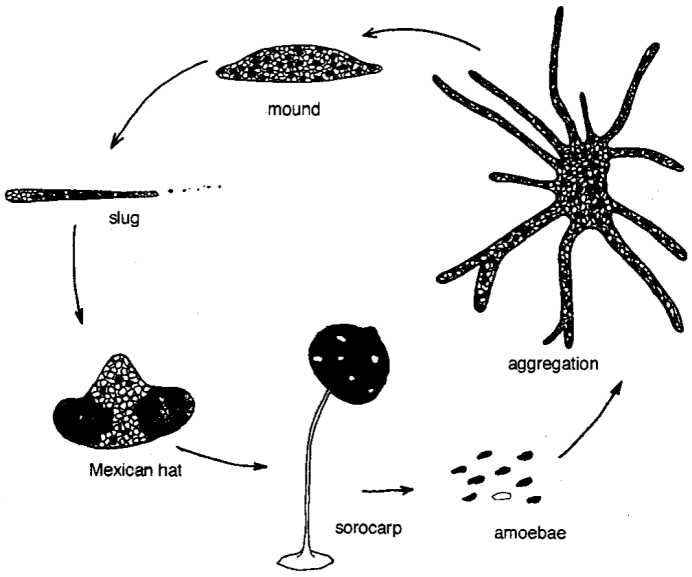

Figure 2. The life cycle of *Dictyostelium discoideum.* Separate amoebae, when they starve, aggregate by means of cyclic AMP signals. The cells stream into an aggregation center, that coalesces into a mound. The mound may then either become a migrating slug, or develop directly into the sorocarp. Actual fruiting aggregations may contain up to a million cells. Here cells are colored dark and light to represent two different clones. The dark clone appears preferentially in the rear of the slug and in the spores of the fruiting body, thus cheating the light clone out of its opportunity to reproduce. Drawing by Christine Mueller.

vertical direction, and then either continues developing directly into the fruiting body, or falls over and become a slug that can migrate to a better location, for example by moving towards light. After migration, or sometimes in the absence of it, the sequence ends in a process called culmination. Cells from the tip migrate down through the center of the aggregate and initiate stalk formation. As the stalk is built of cells that form cellulose walls, vacuolate and die, the remaining cells move up to form a spherical body of spores, called the sorus, at the top of the stalk. Thus, a fruiting body is formed, composed of about 20% dead stalk cells, and 80% spore cells. The stalk is thought to function in lifting the spores above the hazards of the soil (Gadagkar and Bonner, 1994) and in putting them in a position where they can better contact and be dispersed passing invertebrates (Huss, 1989). Dispersal in vertebrate guts is also possible (Suthers, 1985; Stephenson and Landolt, 1992).

As noted above, the simple nature of this two cell-type system made it an attractive system for developmental biologists, and they have learned much about how the process works. Differentiation begins in the mound stage when certain cells, dispersed through the mound, express certain prestalk proteins and begin to sort out toward the tip. These prestalk cells form the anterior, leading part of the migrating slug, with the prespore cells bringing up the rear. However, exactly what determines cell type has turned out to be rather complex. Cells that differentiate as prestalk cells tend to have poorer nutritional history (Garrod and Ashworth, 1972), to have divided more recently at the time of starvation (Gomer and Firtel, 1987), and to be more susceptible to a secreted morphogen called differentiation inducing factor or DIF (Kay, 1997). The two obvious cell types have multiplied into subtypes (See Figure 3) as patterns of gene expression became known (Williams, 1997). Moreover, differentiation is not entirely explained by initial choice of cell type, because cell types can interconvert for a considerable time. For example, cutting off the anterior part of an early slug causes replacement of lost prestalk cells by conversion of some prespore cells (Raper, 1940).

The surprising complexity of development in *D. discoideum* has perhaps caused it to recede a bit as a model system for development. Other factors have also contributed, particularly the way in which molecular methods have increased the tractability of other systems like fruit files, nematodes and zebrafish. And *D. discoiduem* has the disadvantage of lacking sexual genetics, at least in the lab. They do form macrocysts, which is a sexual stage in other species, but these rarely yields

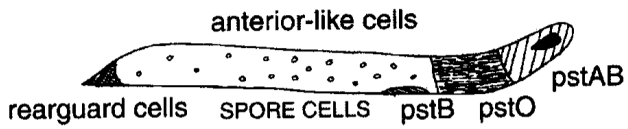

Figure 3. Cell types in a migrating slug. Most of the prestalk (pst) cells are located in the front of the slug, but they consist of several subtypes, expressing different genes, and performing somewhat different roles in stalk formation. A scattering of anterior-like-cells in the prespore region also express prestalk markers and may develop into stalk cells.

segregants in *D. discoideum* (Francis and Eisenberg, 1993; Francis, 1998) But in the meantime, a great deal was learned about *D. discoideum* biology, and the more you know, the more the stage is set for being able to learn more. It has therefore become a more general model system for cell biology (Maeda et al., 1997b; Kessin, 2001), particularly for functions, such as phagocytosis and motility, that cannot be studied in yeast.

We have suggested that *D. discoideum* could also be deployed as a model system for social evolution (Strassmann et al., 2000). Table 2 summarizes some of its desirable features. The simple and unambiguous division into dead stalk cells and reproductive spores mirrors the division into workers and reproductives in social insects. Some cells give up their lives in order to benefit others. Of course, this is true for most differentiated multicellular organisms, but it is fairly uninteresting if all cells belong to the same clone so that altruists automatically benefit copies of themselves. What makes slime molds special is their development via aggregation. *D. discoideum* fruiting bodies may often develop from more than one clone; that is, they may often be chimeric. When this is true, we are likely to see the same kind of tension between cooperation and conflict that we see in social insects. Cells may gain from cooperating, but when possible, they also ought to evolve strategies that allow them to gain the benefits of cooperation without paying the costs. Any clone that manages to make its partners contribute the 20% of cells necessary to build a stalk should be able to produce more offspring via spores. Table 3 translates some commonly used terminology into terms more consonant with a social evolution approach.

Thus, there exists the opportunity to study some of the most interesting and fundamental questions of social insect biology in a social system that is much more amenable to figuring out mechanisms, and to doing selection experiments. This would provide an independent test of major theories developed in game theory and kin selection and initially applied to animals, particularly cooperative mammals and birds and social insects. Our main question overlaps extensively with that studied by the developmental biologists, the division into stalk and spores. We can use the wealth of information that has been gathered on this system, from the classical experiments of Raper and Bonner (reviewed in Bonner 1967), to the fully sequenced genome which will soon be available (Loomis, 1998; Kay and Williams, 1999; Kuspa et al., 2001). We can use the techniques that have been painstakingly worked out, including methods of knocking out genes, transformation, antisense and detection of RNA expression with microchips (Iranfar et al., 2001; Van Driessche et al., 2002). However, we add the evolutionary "why" question. Why do cells differentiate into sterile stalk cells if they may be helping another clone? Adding this dimension may even help with the original problem of understanding how differentiation works in this system. If the system has evolved in the context of competition among clones, the mechanisms are unlikely to be fully understood if we search only for cooperative mechanisms and ignore the possibility of conflict. This is particularly true when all research is done in pure clones, which often differ at only one mutated gene, rather than in chimeras. Indeed, competition could be the

reason that *D. discoideum* development has turned out to be more complicated than had been hoped.

Table 2. Advantages of *Dictyostelium discoideum* as a model system for social evolution

Feature	Advantage
Social behaviour	Clear, separate social and solitary stages
	Social stage can be induced (by starving)
	Clear differentiation between altruists (stalk) and beneficiaries (spore)
	Chimeras of genetically different clones readily form
	Sociality not obligatory; socially defective mutants can be propagated
	Little or no change in group membership during social stage
Life history/ Natural history	Short generation time (hours for cells, days for fruiting cycle)- allows rapid experiments, selection
	Abundant in nature at quantifiable spatial scales
	Easy to collect from field; then use in lab
	Field experiments possible
	Many related species for comparative studies
Lab manipulability	Average relatedness in chimeras can be manipulated
	Domesticated axenic strains grow in liquid culture without bacteria
	Known signals (cAMP, DIF) can be manipulated
	Cells and spores can be preserved, stored, and revived for later use
Genetic techniques	Transformation techniques for introducing foreign DNA
	Mutagenesis techniques, including knock-out, overexpression, insertion of resistance genes, insertion of visual markers like green fluorescent protein
	REMI techniques for mutant screens and gene recovery
	Antisense techniques
	Microarray RNA expression chips
	Selection experiments possible
Knowledge base	Genome being sequenced
	Numerous social (developmental) genes and gene effects characterized
	Large community of researchers
	Many clear, easily used microsatellite markers to identify different clones

Table 3. Term Translator: Development to Social evolution

Development model system	Social evolution model system
cellular slime molds	social amoebae
growth stage or vegetative stage	solitary stage
developmental stage	social stage
differentiation	strategy choice
stalk cells	altruists
spore cells	beneficiaries
signal	signal or manipulation
cell-autonomous	non-social effect
non-cell-autonomous	social effect

We are not the first to see the potential of slime molds for studies of social evolution. Many evolutionary biologists are familiar with them, largely through the writings of John Tyler Bonner, who drew on his experience with slime molds to illustrate ideas in development, evolution, complexity, and even culture (Bonner, 1962. 1965, 1974, 1980, 1988, 1993, 2000). Bonner himself pointed out some analogies with social insects (Gadagkar and Bonner, 1994). In fact, slime molds have been on the radar of sociobiologists from the very beginning; they received multiple-page treatments in both *Adaptation and Natural Selection* (Williams, 1966) and *Sociobiology: the New Synthesis* (Wilson, 1975). They have continued to be featured in discussions of the importance of individual competition (Zahavi and Zahavi, 1997), group selection (Wilson and Sober, 1989), evolution of individuality (Buss 1987), and the major transitions in evolution (Maynard Smith and Szathmáry, 1995). The *Dictyostelium* research community has also begun to note the conflict-of-interest issues (Dao et al., 2000). However, rather little real research on slime molds has been stimulated by sociobiological questions. Several theoreticians have modeled competition in slime molds fruiting bodies, principally showing how the altruism of the spores can be maintained by kin selection (Armstrong, 1984; Matsuda and Harada, 1990; Matapurkar and Watve, 1997; Hudson et al., 2002). There has been even less empirical work (Buss, 1982; DeAngelo et al., 1990; Hilson et al., 1994; Ennis et al., 2000; Strassmann et al., 2000). This will need to change if slime molds are to fulfill their potential as a model system for social evolution.

DO CLONES MIX?

The research program suggested above makes sense only if one important precondition is met: that amoebae from different clones mix to form chimeras. If they do not, then aggregates of *D. discoideum* are no more interesting socially than a metazoan that develops from a single cell. The literature on this point was inconclusive. Buss (1982) reported finding a clone of *Dictyostelium mucoroides* that could not form stalk on its own, but could mix with and parasitize another clone. However, it was reported not to mix with other clones from the area. With respect to *D. discoideum*, reseachers that we queried had varied opinions – some believed clones mixed and some believed they did not – but there seemed to have been no systematic study of the point.

The problem is that rather few biologists work on *Dictyostelium* in the field, or recently derived from the field, and those who did lacked markers to distinguish the clones. Molecular biologists, who had plenty of genetic markers, tended to work on one or a few laboratory clones. The study of Francis and Eisenberg (1993) was an exception; they collected numerous clones from Little Butts Gap, North Carolina, the type location for *D. discoideum*, for a study of population structure using restriction fragment length polymorphisms. We were able to obtain these clones (thanks to Dennis Welker) and perform mixing experiments (Strassmann et al., 2000). These experiments involved growing up clones separately, mixing them in

pairs, and genotyping the resulting slugs. To distinguish the clones, we used microsatellite loci, which vary in the number of repeats of a short DNA motifs (Queller et al., 1993). Using the growing database of the *Dictyostelium* sequencing project, it is easy to find microsatellite loci and design primers from the flanking sequences to use in the polymerase chain reaction. *D. discoideum* is normally haploid, so each clone would show one allele at a locus. After genotyping a number of clones and selecting pairs that possessed different alleles, we mixed the clones and then genotyped the slugs to see if they were chimeric. Each slug always had two bands, one from each of the mixed clones, indicating that both clones were present in a chimeric slug (Strassmann et al., 2000).

WHAT ARE THE BENEFITS AND COSTS OF CHIMERISM?

Mixing of non-relatives to form a chimeric organismal structure is an unusual feature. One expects, if chimerism is at all common in nature, that clones might have evolved strategies to get into the spore rather than the stalk. The resulting conflict could be costly and might end up selecting against chimerism.

We conducted some experiments to test this (Foster et al., 2002). We plated out equal numbers of starving amoebae in clonal and mixed treatments. The basic design involved comparing x cells of clone 1 and x cells of clone 2 with x cells in an equal-parts mixture of clones 1 and 2. The cells aggregated, migrated as slugs, and formed fruiting bodies.

Several functions were tested to see if they had been impaired in chimeras. Most were not. Chimeras did not produce smaller slugs, fewer fruiting bodies, or have lower total spore production. Neither did they display an altered stalk-to-spore ratio. However one function was impaired: slug movement. During development, the Petri dishes were placed under a directional light source, which induces the slugs to move towards the light (see Figure 4). Chimeric slugs moved less far on average than clonal slugs (Figure 5; first two rows). This is presumably costly in the field; chimeric migrating slugs would be less likely to reach optimal places to fruit. It is not known whether this loss of migration represents a cost of competition or some other kind of incompatibility between clones, but this particular cost fits nicely with a competitive interpretation. The slug is led, of course, by its anterior portion, which consists of prestalk cells. Lower slug mobility might result from a reluctance of cells to take this position in chimeras, if it ultimately means sacrificing themselves for non-relatives. Against this interpretation, chimeric fruiting bodies did not end up with smaller stalks, but these functions could be distinct. It may be that the proportion of cells that enter into stalks is fixed by some mechanism, while who ends up in that portion is determined by a variety of other mechanisms. If chimerism is costly, why do clones not evolve to exclude each other? One result of exclusion would be to reduce the number of cells available for joining, which would lead to reduced size of the slug and fruiting body. In the experiment described above, small slugs moved less far than large ones. Thus the decision to join chimeras appears to affect mobility in two ways. The presence of multiple clones affects mobility

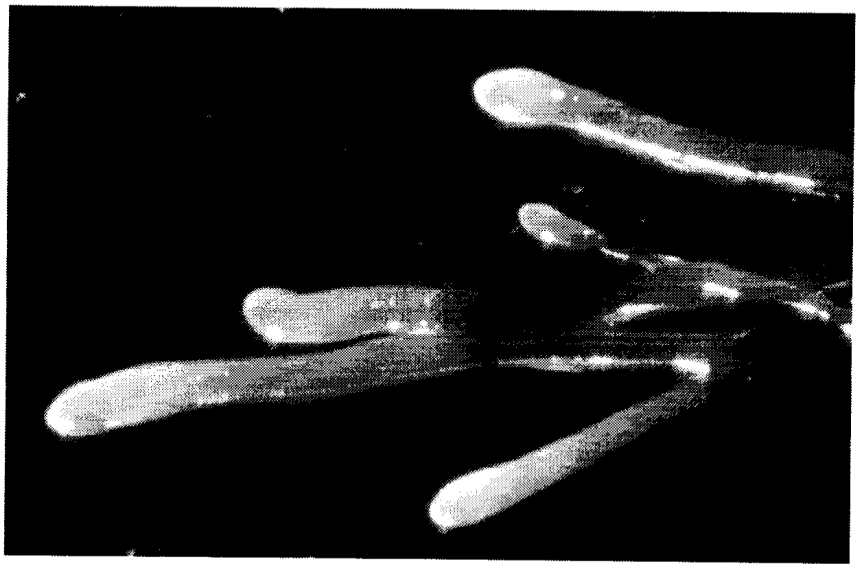

Figure 4. Slugs migrating towards a light source

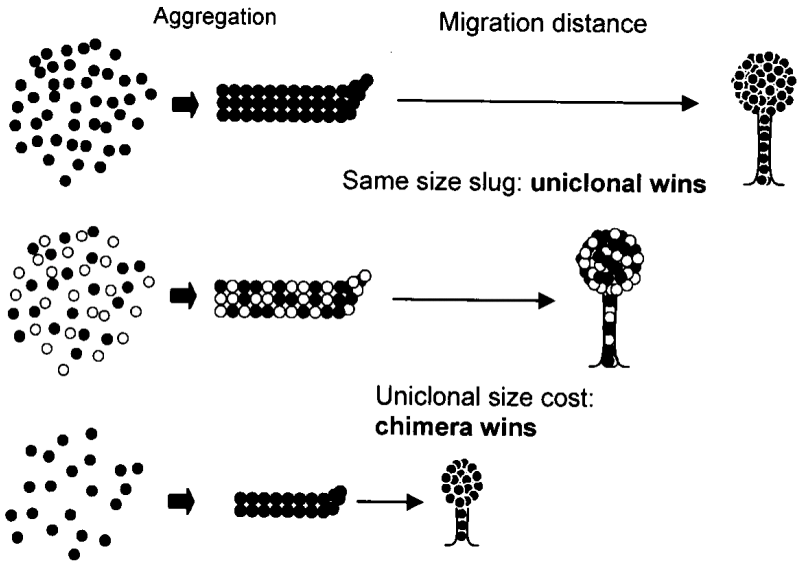

Figure 5. Qualitative results on the costs and benefits of chimerism (Foster et al.,2002). For equal cell numbers, pure clones (line 1) migrate farther than chimeras (line 2) showing an intrinsic cost to chimerism. However, a clone refusing to join with its partner in a 50-50 mixture will have half the density of joinable cells, and a chimera (line 2) migrates farther than uniclonal slug with half as many cells (line 3). Thus a size advantage of chimerism more than compensates for the intrinsic cost.

negatively, but any additional size achieved by chimeras affects mobility positively. A second experiment showed that the balance weighs in favor of chimerism. This time, uniclonal treatments received half the number of cells as the mixtures of pairs of clones. Thus the uniclonal treatment stands as a model for a clone that refused to join with another when paired; both would get the intrinsic uniclonal advantage, both would have only half as many cells available to join. In other words, cells in the mixed treatment experience not just the cost of clonality shown previously, but are also allowed to obtain any benefits of increased size. The mix treatments moved significantly farther than the clonal treatments (Figure 5, last two rows), indicating the benefits of chimerism outweigh the costs.

Other costs of chimerism may exist although, as noted above, other potential costs that we measured did not appear. At least one other benefit seems likely. Besides increasing mobility, larger aggregate size results in taller fruiting bodies, while maintaining stalk/spore proportions. While this has not been demonstrated to increase fitness, it seems highly likely to increase the fitness of spores by making them more available to dispersal. Given that building a stalk is clearly costly – cells must die in the process – some compensating selective benefit such as enhanced dispersal presumably maintains it.

DOES MIXING OCCUR IN NATURE?

Competitive strategies in chimeras are most interesting, at least from an evolutionary standpoint, if chimeras are normal occurrences in nature. Unfortunately, like most well studied lab organisms, *D. discoideum* has hardly been studied in a natural setting. Slime molds are not collected from fruiting bodies in the field. Instead, they are collected by plating out soil samples and growing up clones from single cells. Indeed, we asked a large number of *Dictyostelium* biologists if they had ever seen a wild fruiting body, and none had. Of course, most *Dictyostelium* biologists are bench scientists who spend little time in the field, but at least a few had looked. John Bonner, who has studied *Dictyostelium* for nearly 60 years, believes that Lindsay Olive had seen fruiting bodies but knew of no other instances (John Bonner, pers. comm.). One problem is that *Dictyostelium* lives in the leaf litter, and its fruiting bodies are so fragile that they are likely to break up with any disturbance, such as the removal of the top layer of leaves.

For this reason, our first efforts at studying the distribution of *D. discoideum* in the field (Fortunato et al., 2003a) followed the standard method of rearing clones from cells in soil samples. However, since we were interested in whether different clones were in close enough proximity to potentially form chimeras, we sampled at an unusually small scale. Collecting of multiple clones from the same soil sample has been reported for both *D. discoideum* (Francis and Eisenberg, 1993) and another social amoeba, *Polysphondylium pallidum* (Ketcham and Eisenberg, 1989) but no attempt had been made to collect exhaustively from samples small enough to represent aggregation areas. We sampled with 6 mm diameter drinking straws,

pushed a centimeter or so into the ground, to extract small soil samples averaging less than 0.2 g wet weight. We plated out all the material we collected with the goal of raising a clone from every *D. discoideum* cell in the sample. Thus, instead of looking for chimeric fruiting bodies directly, we looked for cells of different clones that were close enough together that, if provided with sufficient resources to reproduce, would produce a mixed population. Since cells can aggregate from well over 6 mm in the lab, we reasoned that any two clones found within this distance had the potential to form chimeras, even more so if one adds in the spreading out that would occur during the vegetative growth stage preceding fruiting.

We found a surprising amount of diversity at this very local scale. More often than not, if one clone appears in a small soil sample, one or more other clones appear as well (Fortunato et al., 2003a). These results strongly suggest that chimera formation is common in nature. The best data would be from fruiting bodies obtained from the field. We have discovered at least two ways to obtain such fruiting bodies. First, reasoning from the fact that *D. discoideum* need ample bacteria to grow to large population sizes, we decided to search deer feces at Mountain Lake Biological Station in Virginia. We did find a few *D. discoideum* fruiting bodies in this habitat and more extensive searching may yield sample sizes large enough to be informative. Second, we can go one step beyond growing up cells from soil in the lab. Instead, we have inoculated small patches of soil in the field with concentrated bacterial cultures, and allowed the resident *Dictyostelium* cells to grow up *in situ*. Genotype data from these sources will eventually allow us to confirm (or refute) our current conclusion that chimerism is common.

DO CLONES COMPETE IN CHIMERAS?

When clones join together in chimeras, the opportunity arises for one clone to gain at the expense of another. Any clone would transmit more genes to future generations if it could induce its partner to make most of the sterile stalk, thus leaving more of its own cells to become reproductive spores. Clones might pay some cost to obtain this benefit, and such costs of competition may be the basis of the whole-organism cost of chimerism shown above.

A clone that exploits other clones when they form chimeras, avoiding contribution to the stalk, is most likely to be the most successful if it is able to form a normal stalk when alone. However detecting such clones was not possible until recently. Early reports are of morphological variants that were either formed by mutation (Sussman and Sussman, 1953), or found in nature (Filosa, 1962). One of the earliest cases reported was that of Filosa (1962) who found several morphological variants of *D. mucoroides* DM-11 isolated from a single fruiting body of a clone collected at the Bronx zoo from giraffe dung. The types were TYP, or the wild type, MV, a clone that has prespore and prestalk cells confused in the slug, AV, a clone without fruiting bodies, BV which has more fruiting bodies/plate, and GV which has no migration of slugs, and fruiting bodies where nearly all cells

turn into spores (Filosa, 1962). Other single fruiting bodies collected also had variants, though not so many as DM-11. Buss (1982) also found a stalkless mutant in one of his samples. This mutant would aggregate with the stalked clone it was found with but not with other clones of the species from the same general area, perhaps indicating that it was a recent mutation. Buss also showed that when it began as rare, the stalkless mutant increased in frequency over generations in competition with the stalked clone.

The tendency of a cheater clone to increase in frequency was used by Kessin's group to search for single-gene cheater mutations (Ennis et al., 2000). They began with a large random collection of clones created by REMI (restriction enzyme-mediated integration), an insertional mutagenesis procedure which allows knock-out and recovery of single genes (Kuspa and Loomis, 1992). They put the clone mixture through 20 generations of selection for spores and isolated a clone that had increased in frequency. This mutant has a mutation in a gene, *ChtA* (also called *FbxA*), that is an F box protein functioning in the breakdown of a protein regulating development (Mohanty et al., 2001). *ChtA* contributes only to spores in chimeras. The clone carrying the mutation has an abnormal phenotype when not mixed with wild-type cells, and so is probably not a mutation found in natural cheating clones.

Molecular tools make it fairly simple to look for cheating in chimeras. We used a procedure involving mixing of two clones with different alleles at a microsatellite locus (Strassmann et al., 2000). The two clones were grown up in mixture, and then starved to induce fruiting body development. Slugs were then harvested and portions were taken from the anterior (prestalk) region and the posterior (prespore) region. Our question was whether one clone cheated by being over-represented in the pre-spore region, compared to its representation in the pre-stalk region. This was assessed by quantifying the relative amounts of the two microsatellite alleles in the prespore and prestalk regions. We extracted DNA from each region, and amplified the microsatellite marker by the polymerase chain reaction (PCR) with incorporation of ^{35}S labelled dATP. The product was run out on a gel to separate the two alleles, and then we measured the radiation emanating from each band using a phosphorimager. The relative amounts of radiation from the two bands represent the relative amounts of the two parental alleles in the sample. We measured seven slugs in each experiment and conducted 12 experiments involving different pairs of clones.

We found that the two parental clones were typically not randomly distributed in mixtures. In 9 of the 12 experiments taken individually, one of the two clones was significantly over-represented in the prespore region compared to prestalk (Figure 6) Six remained significantly different when corrected for multiple comparisons (Strassmann et al., 2000). In contrast to Buss's stalkless mutant, and Kessin's *cheater A* mutant, our cheaters showed no obvious defect when grown alone. All formed normal fruiting bodies. In particular, they did not show any deficit in spore/stalk ratio; staying out of the stalk in mixture does not require deficient stalks in uniclonal fruiting bodies. This kind of cheating may be much more effective in

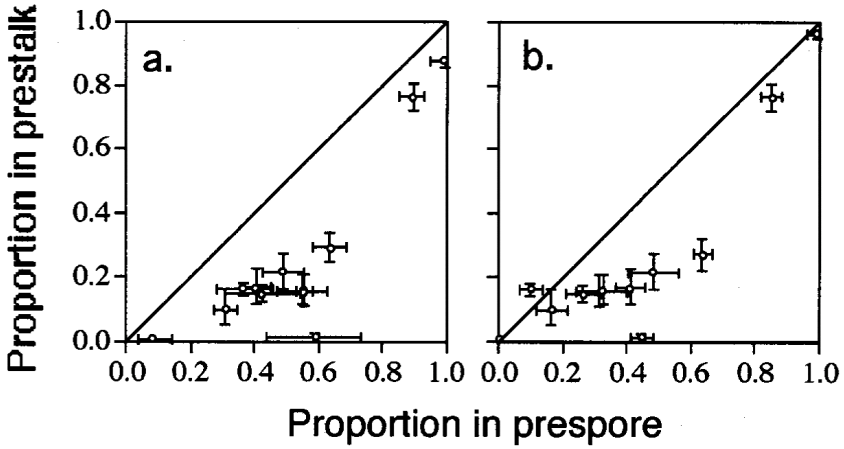

Figure 6. Representation of mixed clones in prespore and prestalk regions. Points on the diagonal would represent mixtures in which both clones contributed proportionately to stalk and spores. For 12 mixtures of clone pairs, the plotted points show the means and standard errors (over seven slugs) of the clone that was relatively more represented in the stalk (its partner clone's proportions would be represented by a point reflected about the diagonal). In part **a** the anterior 10% of the slug was genotyped for the prestalk region and the posterior 10% of the slug for the prespore region. Though generally in the prespore area, the posterior includes some cells with prestalk characteristics that may form the sterile basal disk. Part **b** shows the same slugs, but using the middle10% of the slug for the prespore sample. Using the average of the two prespore regions, 6 of the 12 pairs showed significantly disproportionate representation in stalk and spores (Strassmann et al., 2000).

nature because it does not entail any obvious cost when there is no other clone to parasitize. These experiments included an initial vegetative growth phase, during which one of the two clones may out-reproduce the other. This imposed a constraint on how we could test for cheating. We did not test for absolute representation in spores, which would include differences in vegetative growth. Instead we needed a relative measure. Representation in spores versus stalks would be the obvious one, but getting good DNA out of dead stalk cells seemed problematic. Therefore we measured the difference in representation in prestalk and prespore regions of the slug. Thus we had some pairs is which the cheater (the clone relatively over-represented in the pre-spore region) was actually the minority clone in that region, presumably because it had been outreproduced in the vegetative stage. In other words, although it contributed fewer cells to the fruiting bodies in general, it was more effective at getting into the prespore region.

We have since done experiments in which the vegetative growth is more controlled (Fortunato et al., 2003b), and these confirm that cheating effects persist to the final spore stage. In these experiments, each clone was grown up separately, and then they were starved, counted, and mixed in equal numbers. The representation of the two clones was assessed as before, but this time using only spore cells in mature fruiting bodies. Cheating was inferred when significantly more than 50% of the spores were of one type. Again, cheating was common. These experiments showed one additional feature of cheating. For seven clones tested in

all pairwise combinations, cheating was transitive. That is, if clone A beat clone B and clone B beat clone C, then clone A would beat clone C. This shows that there are consistent cheaters and losers. The result is what one might expect if cheating were due to a single mechanism, though it does not prove that this is so.

WHAT IS CHEATING?

One might wonder about exactly what cheating consists of when discussing the behavior of single cells in an organism that is multicellular only briefly in its life cycle. Obviously, conscious motivation to exploit another is not part of any reasonable definition of cheating in *D. discoideum*. In fact, there need not be any motivation to exploit or any recognition of the presence of victims for there to be effective cheating. One definition of cheating is that cheating of one clone by another has occurred when the cheating clone is represented by a lower proportion of cells in the stalk than in the spores. This means it has done something that allows it to undercontribute to the altruistic part of the fruiting body.

The most sophisticated cheating would involve recognizing the presence of another clone and changing one's behavior accordingly. By analogy, consider a child sharing a bowl of popcorn with another. He may recognize that he will get more if he eats faster, and change his behavior accordingly. This kind of cheating is more sophisticated because it requires detecting when one is in a chimera and altering the developmental program to contribute less to the stalk than they might otherwise. Such a strategy could have refinements such as frequency dependent strategies. A clone might contribute to the stalk when it was most common in the chimera and not when it was rare.

Cheating could also occur even if the cheater does not change its behavior in the presence of another. For example, of our two children sharing the bowl of popcorn, one may simply be a faster eater, whether he is sharing or not. He will get more than his fair share of the popcorn even if there was no such intent. The analogy for *D. discoideum* might hold if one clone develops more slowly than the other, and the result is that it contributes fewer cells to the stalk. This sort of behavior can be viewed as a consistent behavior that leads to exploitation, or cheating, when in chimeras.

We have not yet determined which of these two kinds of cheating appears to be happening in chimeras of wild *D. discoideum*. We think it appropriate to call both kinds cheating. After all, the slower eater is likely to cry "no fair!" in both popcorn examples.

DO CLONES RECOGNIZE CLONE-MATES?

Social insects have well-developed kin recognition abilities, and they deploy these abilities in their strategies for maximizing inclusive fitness. We have established

that *D. discoideum* do not exclude non-clonemates, which might suggest an inability to recognize them. On the other hand, we have shown that chimerism has advantages, so that non-exclusion makes sense even if they can recognize.

Recognition could still be advantageous for behavior within chimeras. We have shown that some clones obtain advantages against others in chimeras, contributing less than their share to the stalk, while they form normal stalks on their own (Strassmann et al., 2000). This kind of result would seem to suggest that these clones deploy clever strategies of making fewer stalk cells when they detect the presence of non-kin. However, one can get this kind of result without detection or facultative behavior. For example, it could be that cells from one clone cease the wandering stage of slugs earlier than the other in a given chimera, making the former clone more represented in stalk cells and the latter commoner in spore cells whose cell fate appears to be committed somewhat later.

If clones do recognize each other, one might expect them to allocate less to stalk when they find themselves in chimera (Matsuda and Harada, 1990; Hudson et al., 2002). In terms of kin selection, each stalk cell experiences the same cost (death) whether it is in a uniclonal or chimeric aggregation, but the benefits differ. In the chimeric aggregation, the benefit will be devalued by the decrease in relatedness to spores caused by the presence of other clones. DeAngelo et al. (1990) tested two clones and found that they did decrease allocation to stalk in mixture, but later experiments with the same two clones showed that under different conditions the effect disappeared (Hilson et al., 1994). We tested for such an effect in the course of our experiment, noted above, on the costs and benefits of chimerism. Across all clone pairs tested, we found no overall decrease in allocation to stalk in chimeras (Foster et al., 2002).

Recognition is not a prerequisite for cell competition being important. If cells do not know when their partners are clonemates and when they are not, they will be selected to behave according to the average condition historically experienced in the population. Low average relatedness within aggregations should select for lower altruism, that is, for a smaller percentage of stalk cells. We are therefore currently testing the prediction that sparse populations, presumably with little mixing between clones, are more altruistic than dense populations with more extensive mixing.

MECHANISMS AND CELL COMPETITION

There is another method to examine whether competition among clones has been historically important: reverse engineering of the mechanisms. If cell competition is important in chimeras then, even in the absence of recognition, we might still expect the mechanisms of stalk-spore differentiation to reflect a history of competition, rather than the pure cooperation expected of organisms that develop uniclonally. If we were studying larger organisms, we would look for evidence of fighting or aggressive behavior, or at least for evidence that the proposed disputes are settled according to relative strength. For the most part, fighting will not be obvious in

Dictyostelium. We can however look for evidence that the strongest cells win places in the spores, an outcome that is expected under competition, but not necessarily expected in a purely clonal, purely cooperative, society (Atzmony et al., 1997). We therefore return, with a little more detail, to what is known of the mechanisms of spore-stalk differentiation.

There has long been controversy over the first events leading to differentiation. A standard developmental model based on other organisms is that cells differentiate according to their position in the developing organism, and specifically with respect to a gradient in some differentiating signal. A candidate for such a morphogen in *D. discoideum* is a chlorinated alkyl phenone called DIF, or differentiation inducing factor (Kay and Jermyn, 1983; Kay, 1997; Thompson and Kay, 2000b). DIF has been isolated and application of it to developing cells been shown to induce stalk cell differentiation, by way of inducing many genes characteristic of stalk cells and repressing those characteristic of spore cells. A recent knockout of the last gene in the DIF synthesis pathway shows that DIF actually induces one of the major sub-populations of stalk cells, the pstO cells (Thompson and Kay, 2000a) but that other stalk cells may be induced by some other pathway.

Others have argued that *D. discoideum* differentiation is influenced by pre-existing intrinsic cell properties, and that these influence differentiation before there is any morphogen gradient. Chief among the influences studied are glucose and cell cycle stage. Cells grown in a medium with glucose tend to become spores when mixed with cells grown without glucose (Leach et al., 1973; Inouye and Takeuchi, 1982). These results are consistent with the view that cells in better condition (with glucose) use their advantage to become spores (Atzmony et al., 1997). But the influence of glucose is not completely determining. Cells grown with glucose, when developed with each other, have somewhat higher spore-stalk ratios than cells grown without glucose, but the difference is modest (Garrod and Ashworth, 1972; Forman and Garrod, 1977). Clearly there is some regulation overlaid on the difference in condition so that populations in poorer conditions do not turn out sporeless fruiting bodies and populations in good conditions do not turn out stalkless ones.

Roughly parallel results have been obtained for cell cycle stage (Weijer et al., 1984; McDonald and Durston, 1984; Gomer and Firtel, 1987). Development begins when the cells are starved. Cells that are in the S stage of the cell cycle (synthesizing DNA) at the time of starvation, or early in the following G2 (growth) stage, tend to become stalk cells. Cells in the later G2 stage tend to become spores (there is little or no G1 stage in *D. discoideum*). Again, this phenomenon is most pronounced when the two types of cells are mixed together, but persists to some degree when synchronized-stage populations are developed (S stage populations being more stalky). This may be consistent with the more fit cells taking advantage of their better condition to become spores, because the S stage cells will be engaged in the expensive process of DNA replication. They are also smaller because of having divided recently.

These kinds of predispositions appear to determine the initial differentiation prior to any spatial information based on DIF gradients. Prestalk markers (genes that are

expressed in cells that normally become stalk) begin to be expressed in the mound stage, not in any particular spatial position, but in a minority of cells dispersed throughout the mound. Subsequently, they sort themselves out, with prestalk cells moving to the periphery and eventually to the tip of the mound (Datta et al., 1986; Williams et al., 1989). This kind of differentiation, with cells differentiating in a dispersed salt-and-pepper fashion and then sorting, is not generally viewed as common in other organisms. If this mode of development truly is unusual, it may be because *Dictyostelium* is unusual in having competitive development. Cells in good condition, initially dispersed at random throughout the mound, should be reluctant to give up their advantage and become dead stalk cells just because they happen to occupy a particular position in a morphogen gradient. Instead, the most competitive cells should seek to move from their initially random positions to more favorable ones. Such competitive movements are pointless in the purely clonal development of most other organisms.

DIF gradients begin to appear after the cells have sorted out to some degree, but this does not mean they are unimportant. Indeed, DIF appears to reinforce the initial differences: cells grown in the absence of glucose and cells starved in the S-stage both are more susceptible to the stalk-inducing properties of DIF (Thompson and Kay, 2000a).

The manner in which DIF is deployed also seems consistent with competition between cells. Indeed, a reasonable hypothesis to consider is that DIF producing cells are poisoning other cells to induce them to be stalks (Atzmony et al., 1997; Zahavi and Zahavi, 1997). An initially puzzling feature was that DIF, though it induces stalk cells, was found at higher concentrations in the prespore region. The explanation is that the prespore cells are the main source of DIF (Kay and Thompson, 2001), while the prestalk cells break it down into a much less active form (Kay et al., 1993). This certainly has the appearance of the stronger cells attempting to force the weaker ones to do their duty.

More information on how DIF works could shed light on the question. Some evidence suggests that DIF interferes with mitochondrial function in one assay (Shaulsky and Loomis, 1995) but it not clear that such effects occur at the physiological concentrations experienced *in vivo* (Kay et al., 1999). It would also be useful to know the identity of the receptor, if any, on the cells that respond to DIF. The poison hypothesis would predict that DIF would exploit some pre-existing receptor that serves some other function. The chemical characteristics of DIF suggest that it may not need a membrane receptor to enter cells, that it can pass directly through the plasma membrane (Kay et al., 1999). That would certainly be consistent with a poisoning mechanism though we still need to understand better how they keep from poisoning themselves.

Several observations may argue against the differentiation mechanisms being primarily competitive. First, the prestalk cells in the anterior portion of the slug seem to take a leading role in the organization and movement of the slug, something one might expect of cells that were committed to their eventual altruistic fate rather than cells that are trying to shirk that fate if possible. Second, the argument that DIF

is a poison is somewhat weakened by the observation that prespore cells seem to thrive in higher concentrations of DIF, without even deploying the DIF dechlorinase mechanism for getting rid of it. This kind of system does not prove competition because it can also be argued that it works well in regulating cell proportioning (Kay et al., 1999). When one cell type produces an inducer and the induced cell type breaks it down, regulation results from the frequency dependence. If there are too few stalk cells, then lots of DIF is produced and little is broken down, raising the number of stalk cells. If there are too many stalk cells, there is little DIF produced and lots of cells breaking it down, with the opposite effect of reducing the number of stalk cells.

In sum, much of what is known of the mechanisms of initial differentiation is consistent with a history of cell competition, but the evidence is not conclusive. Zahavi and his colleagues tried to make the case for stalk-spore differentiation being completely competitive, because they considered group and kin selected cooperation to be vulnerable to cheaters and therefore explanations of last resort (Atzmon et al., 1997; Zahavi and Zahavi, 1997). Few other researchers accept that premise. Kin selection is well supported both theoretically and empirically and need not be viewed as an explanation of last resort. If individuals affect relatives who bear their genes, then that will be part of the process by which genes make it to the next generation. Moreover, Zahavi notes that the individual model itself is also vulnerable to cheaters. Why should a cell not let others take on the cost of producing DIF to subdue the weaker ones? Kin selection can help prevent such cheating. More seriously, pure individual competition has trouble explaining the final result of a stalk. A stalk is not just a random jumble of cells that have lost out in competition. It is a highly ordered structure that required specific and coordinated actions on the part of its constituent cells. Those cells may indeed be losers to some degree, but their final actions cannot be understood unless they are benefiting relatives. It is important to remember that competition and kin selection are not mutually exclusive. Social insects manage to cooperate and compete at the same time (Queller and Strassmann, 1998), and there is every reason to believe that slime molds may do the same.

SOME GENES OF INTEREST

The slightly expanded account of *D. discoideum* mechanisms given above is still woefully oversimplified. Hundreds of genes are involved in development/sociality. Many have been isolated from cDNA libraries (Morio et al., 1998) and characterized by the effect of REMI knockouts (Kuspa and Loomis, 1992). Microarrays are now being used to show the time-course of expression (Iranfar et al., 2001; van Driessche et al., 2002). The genes involved in DIF synthesis and breakdown are clearly of special interest to students of social evolution, but many others also offer some promise. The knockouts include forms deficient in stalk or spore production (Figure

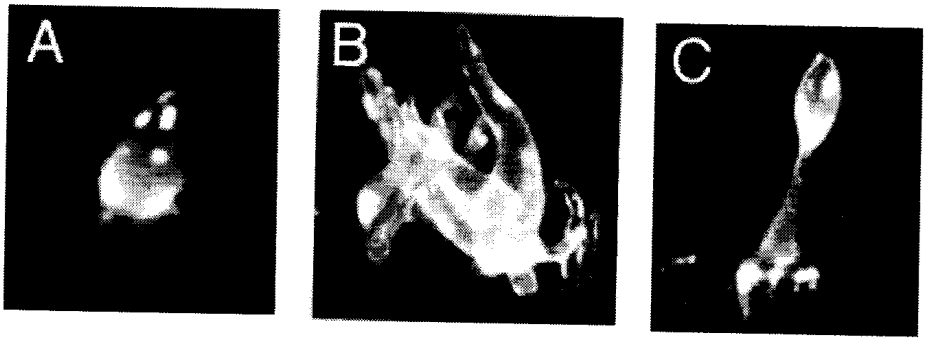

Figure 7. Altered development in selected single-gene knockout mutants, including a stalkless clone (A). These are mutants DG1099, DG1035, and DG1003, selected from a set on view at http://www.biology.ucsd.edu/labs/loomis/REMI/index.html. Photos courtesy of William F. Loomis.

7). Undoubtedly some of these, like cheater A (Ennis et al., 2000), can form normal fruiting bodies in chimeras with wild type, with some of them acting as cheaters or victims according to whether they end up preferentially in stalk or spores.

However, these are not good models for the kind of cheating we observed in chimeras from natural isolates (Strassmann et al., 2000), because those formed normal fruiting bodies on their own. So in the following discussion, we will focus primarily on some indications of these more cryptic types of mutants.

Cyclic AMP is a major signal in *D. discoideum* development, controlling aggregation and continuing to play a role in later stages. Much work has therefore focused on the genes involved in producing cAMP, membrane receptors for cAMP, and signal transducers (Verkerke-van Wijk and Schaap, 1997; van Haastert, 1997; Rogers et al., 1997). The *cAR3* gene offers one interesting example. One of its effects is to regulate a gene called *gskA* which codes for glycogen synthase kinase-3 (Harwood et al., 1995). This is one of numerous *Dictyostelium* genes that affect cell fate. In this case, the *gskA* null mutant makes fewer spores and more prestalk B cells that end up in an unusually large basal disc at the foot of the stalk. Interestingly, *gskA* regulates cell fate not only in *D. discoideum*, but also in animals like *Drosophila* and *Xenopus*. Since multicellularity is thought to have arisen independently in animals and slime molds, this and other similar genes (Brown and Firtel, 1999) suggest that evolution has seized upon some of the same pre-existing pathways. CAR3, one of four cyclic AMP membrane receptors, is thought to regulate GSKA because the *cAR3* null decreases activation of GSKA kinase, and causes similar effects in the slug as the *gskA* null, notably an expanded area of prestalk B cells (Plyte et al., 1999). Curiously, however, the *cAR3* null forms a normally proportioned fruiting body, without the large basal disc. Two factors appear to cause this (Plyte et al., 1999). First, the mutant slug migrates, unlike its parental strain, and some of the excess prestalk B cells are left behind. Second, as the slug migrates, some of the prestalk B cells apparently redifferentiated, restoring a normal proportion of spores. What this suggests is that significant reorganization can sometimes take place in the mound or the slug without compromising the final

fruiting body. This is a property required by cryptic cheaters, which must be able to avoid the stalk in chimeras, without giving up stalk production on their own.

This principle is shown more completely in a study of the *modB* mutant, which causes a defect in glycosylation of various proteins (Houle et al., 1989). On its own, the *modB* mutant forms a normal fruiting body. Yet, when it is mixed with normal cells in chimeras, it sorts preferentially to the stalk. In fact, when the fraction of *modB* cells is less than 30%, they are virtually unrepresented in the spores, suggesting that *modB* cells essentially fill up the stalk before any differentiate into spores. This provides a real example of what a single-gene cheater gene might look like. Note that in this case the mutant is the victim and the normal type is the cheater.

One of the proteins affected by *modB*, gp80, has extremely interesting effects in its own right. gp80 is coded by the *csA* gene (for contact site A). It is a homophilic cell adhesion protein; it is anchored in the plasma membrane and causes binding to the same protein on other cells. Such binding is important in both aggregation and in cell sorting, as has been shown by studies of a knockout of the *csA* gene. When knockout cells are mixed with wildtype under starvation conditions on agar, the wildtype sorts preferentially into the stalk (Queller et al., in press). Why then do null mutants not spread in the population? When the two cell types are mixed on soil, the natural substrate, few null cells make it into the aggregations (Ponte et al., 1998). Their lack of binding causes most of them to be lost from the aggregation streams so their advantage in aggregations is rarely manifested. In effect, wildtype *csA* is a greenbeard gene (Queller et al., in press), a gene that recognizes copies of itself in others and directs altruism preferentially toward those individuals (Dawkins, 1976). Thus, this gene aids copies of itself by a mechanism different from the general recognition of relatives commonly employed in social insects and other organisms (Alexander, 1979). The fire ant *gp9* locus (Keller and Ross, 1998) provides one of the other rare examples of a greenbeard effect. But the *csA* gene is so far unique in having its effects due to well understood mechanism (homophilic adhesion) of a single gene and protein (Queller et al., 2003).

The study of *csA* on soil (Ponte et al., 1998) represents an unusual instance of molecular biologists using knowledge of *D. discoideum*'s evolutionarily relevant environment to advance understanding of gene function. We suggest that similar progress may be made by recognizing that the evolutionary environment has included other clones, so that function may be revealed by behavior in chimeras.

CONCLUSION

Clearly, *D. discoideum* is potentially a powerful new model system for social evolution. However, the language of cell and molecular biology which defines conflict and cooperation in this system will be foreign to most behavioral ecologists. Interactions that are behavioral in traditional organisms like fighting, grooming, guarding, and alarm calling, are molecular in *D. discoideum,* and involve things like

promoters, receptors, signal transducers, and cell adhesion. This difference is a conceptual barrier, but it derives from strength of the system: mechanisms are much better known for communication in *D. discoideum,* and the tie to genes is direct. Sociobiological predictions for costs and benefits associated with altruism, for conditions under which cooperation will fall apart, and for the role of relatedness and kin selection can all be tested very directly. These theories were developed hand-in-hand with investigations of social Hymenoptera. Now is the time to see how powerfully they apply to a phylogenetically distinct organism that has not contributed to the theory development.

Not only can tests of social evolution theories in *D. discoideum* enrich our understanding of the theory, but exactly how sociality works can be explored in detail. The machinery assembled so painstakingly by the developmental and cell biologists can be put to use in understanding exactly what costs and benefits are, and how dominance rank, exploitation, cooperation, or altruism are achieved. Just as studies of social conflict in *D. discoideum* are likely to distil our understanding of the generality and mechanisms of these general processes, evolutionary theory is likely to crystallize our understanding of *D. discoideum.* To date, molecular studies have been conducted mostly on pure clones. A systematic investigation of the impact of social genes in chimeras may clarify some puzzling aspects of *D. discoideum* multicellularity. The study of *D. discoideum* as a model system for social evolution has a rich future.

SUMMARY

The social amoeba, *Dictyostelium discoideum*, has long been used as a model system for development, with the focus on differentiation into two parts of the multicellular fruiting body: spores and the stalk cells that hold them aloft. Because stalk cells die in order to further the success of the spores, *Dictyostelium discoideum* could also provide a model system for social biologists interested in cooperation and conflict. The fruiting bodies from by aggregation of amoebae, unlike most multicellular organisms, which develop clonally from a single cell. Collections from the field suggest that multiple, genetically distinct clones often occur in close enough proximity to mix. Experiments show that amoebae from different clones will readily join together to form chimeric fruiting bodies. Chimeras suffer a cost in terms of mobility of the multicellular slug which migrates before fruiting; however, this cost is more than compensated by an increased mobility advantage of having more cells in the slug. Within the chimeras, some clones act as cheaters, gaining over-representation in the fertile spores compared to sterile stalk. We discuss several genes that could be involved in this kind of cheating. The prospects for further understanding the mechanism of cooperation and cheating are enhanced by a number of features, including short generation time, lab domestication, a wide range of molecular genetic tools, and a soon-to-be-completed genome sequence. These advantages make *D. discoideum* a very promising new model system for studying

social evolution.

REFERENCES

Alexander, R.D., 1979. *Darwinism and Human Affairs*. University of Washington Press, Seattle, WA.

Armstrong, D. P., 1984. Why don't cellular slime molds cheat. *J. Theor. Biol. 109* : 271-283.

Atzmony, D., A. Zahavi and V. Nanjundiah, 1997. Altruistic behaviour in *Dictyostelium discoideum* explained on the basis of individual selection. *Curr. Sci. 72*: 142-145.

Bonner, J. T., 1959. *The Cellular Slime Molds.* Princeton University Press, Princeton, NJ.

Bonner, J. T., 1962. *The Ideas of Biology*. Harper & Row, New York.

Bonner, J. T., 1965. *Size and Cycle. An Essay on the Structure of Biology.* Princeton University Press, Princeton, NJ.

Bonner, J.T., 1967. *The Cellular Slime Molds, 2nd ed.* Princeton University Press, Princeton, NJ.

Bonner, J.T., 1974. *On Development: the Biology of Form*. Harvard University Press, Cambridge, MA.

Bonner, J.T., 1980. *The Evolution of Culture in Animals.* Princeton University Press, Princeton, NJ.

Bonner, J.T., 1988. *The Evolution of Complexity by Means of Natural Selection.* Princeton University Press, Princeton, NJ.

Bonner, J.T., 1993. *Life Cycles: reflections of an evolutionary biologist.* Princeton University Press, Princeton, NJ.

Bonner, J.T., 2000. *First Signals - The Evolution of Multicellular Development.* Princeton University Press, Princeton, NJ.

Bonner, J.T., 2001 A note on the number of cells in a slug of *Dictyostelium discoideum*. http://dictybase.org/bonner%20paper.pdf

Brown, J.M. and R.A. Firtel, 1999. Regulation of cell-fate determination in *Dictyostelium. Dev. Biol. 216*: 426-441.

Buss, L.W., 1982. Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc. Nat. Acad. Sci. 79*: 5337-5341.

Buss, L.W., 1987. *The Evolution of Individuality*. Princeton University Press, Princeton, NJ.

Cavender, J.C. and K.B. Raper, 1965a. The Acrasieae in nature. I. Isolation. *Am. J. Bot. 52*: 294-296.

Cavender, J.C. and K.B. Raper, 1965b. The Acrasieae in nature. II. Forest soil as primary habitat. *Am. J. Bot. 52*: 297-301.

Cavender, J.C. and K.B. Raper, 1965c. The Acrasieae in nature. III. Occurrence and distribution in forests of Eastern, North America. *Am. J. Bot. 52*: 302-308.

Dao, D.N., R.H. Kessin and H.L. Ennis, 2000. Developmental cheating and the

evolutionary biology of *Dictyostelium* and *Myxococcus*. *Microbiol. UK 146*: 1505-1512.

Datta, S., R.H. Gomer and R.A. Firtel, 1986. Spatial and temporal regulation of a foreign gene by a prestalk-specific promotor in transformed *Dictyostelium discoideum*. *Mol. Cell. Biol. 6*: 811-820.

Dawkins, R., 1976. *The Selfish Gene*. Oxford University Press, Oxford.

DeAngelo, M.J., V.M. Kish and S.A. Kolmes, 1990. Altruism, selfishness, and heterocytosis in cellular slime molds. *Ethol. Ecol. Evol. 2*: 439-443.

Ennis, H.L., D.N. Dao, S.U. Pukatzki and R.H. Kessin, 2000. *Dictyostelium* amoebae lacking an F-box protein form spores rather than stalk in chimeras with wild type. *Proc. Nat. Acad. Sci. 97*: 3292-3297.

Fewell, J.H. and R.E. Page, 2000. Colony-level selection effects on individual and colony foraging task performance in honeybees, *Apis mellifera* L. *Behav. Ecol. Sociobiol. 48*: 173-181.

Filosa, M.F., 1962. Heterocytosis in cellular slime molds. *Am. Nat. 96*: 79-92.

Forman, D. and D. R. Garrod, 1977. Pattern formation in *Dictyostelium discoideum*. I. Development of prespore cells ant its relationship to the pattern of the fruiting body. *J. A. Embryol. Exp. Morphol. 40*: 215-228.

Fortunato, A., J.E. Strassmann, L. Santorelli and D.C. Queller, 2003a. Co-occurrence in nature of different clones of the social amoeba *Dictyostelium discoideum*. *Mol. Ecol. 12*: 1031-1038.

Fortunato, A., D.C. Queller and J.E. Strassmann, 2003b. A linear dominance hierarchy among clones in chimeras of the social amoeba *Dictyostelium discoideum*. *J. Evol. Biol. 16:* 438-445.

Foster, K.R., Fortunato, J.E. Strassmann and D.C. Queller, 2002. The costs and benefits of being a chimera. *Phil. Trans. Royal Soc. B. 269*: 2357-2362.

Francis, D., 1998, High frequency recombination during the sexual cycle of *Dictyostelium discoideum*. *Genetics 148*: 1829-1832.

Francis, D. and R. Eisenberg, 1993. Genetic structure of a natural population of *Dictyostelium discoideum*, a cellular slime mould. *Mol. Ecol. 2*: 385-391.

Gadagkar, R. and J.T. Bonner, 1994. Social insects and social amoebae. *J. Biosci. 19*: 219-245.

Garrod, D.R. and J.M. Ashworth, 1972. Effect of growth conditions on development of the cellular slime mould *Dictyostelium discoideum*. *J. Embryol. Exp. Morphol. 28*: 463-479.

Gomer, R.H. and R.A. Firtel, 1987. Cell-autonomous determination of cell-type choice in *Dictyostelium* development by cell-cycle phase. *Science 237*: 758-762.

Harwood, A.J., S.E. Plyte, J. Woodgett, H. Strutt and R.R. Kay, 1995. Glycogen synthase kinase 3 regulates cell fate in *Dictyostelium*. *Cell 80*: 139-148.

Hilson, J.A., S.A. Kolmes and L.F. Nellis, 1994. Fruiting body architecture, spore capsule contents, selfishness, and heterocytosis in the cellular slime mold, *Dictyostelium discoideum*. *Ethol. Ecol. Evol. 6*: 529-535.

Houle, J., J. Balthazar and C.M. West, 1989. A glycosylation mutation affects cell

fate in chimeras of *Dictyostelium discoideum*. *PNAS. 86*: 3679-3683.

Hudson, R.E., J.E. Aukema, C. Rispe and D. Roze, 2002. Altruism, cheating, and anticheater adaptations in cellular slime molds. *Am. Nat. 160*: 31-43.

Huss, M.J., 1989. Dispersal of cellular slime moulds by two soil invertebrates. *Mycologia 81*: 677-682.

Inouye, K. and I. Takeuchi, 1982. Correlations between prestalk-prespore tendencies and cAMP-related activities in *Dictyostelium discoideum*. *Exp. Cell Res. 138*: 311-318.

Iranfar, N., D. Fuller, R. Sasik, T. Hwa, M. Laub and W.F. Loomis, 2001. Expression patterns of cell-type-specific genes in *Dictyostelium*. *Mol. Biol. Cell 12*: 2590-2600.

Kay, R.R., 1997. DIF signalling. In: *Dictyostelium: a model system for cell and developmental biology* (eds. Maeda, Y., K. Inouye and I. Takeuchi), pp. 279-292. Universal Academy Press, Tokyo.

Kay, R.R., P. Flatman and C.R.L. Thompson, 1999. DIF signalling and cell fate. *Sem. Cell Dev. Biol. 10*: 577-585.

Kay, R.R. and K.A. Jermyn, 1983. A possible morphogen controlling differentiation in *Dictyostelium*. *Nature 303*: 242-244.

Kay, R.R., S. Large, D. Traynor and O. Nayler, 1993. A localized differentiation-inducing-factor sink in the front of the *Dictyostelium* slug. *PNAS. 90*: 487-491.

Kay, R.R. and C.R.L. Thompson, 2001. Cross-induction of cell types in *Dictyostelium discoideum*: evidence that DIF-1 is made by prespore cells. *Development 128*: 4959-4966.

Kay, R.R. and J.G. Williams, 1999. The *Dictyostelium* genome project - an invitation to species hopping. *Trends Genet. 15*: 294-297.

Keller, L. and K.R. Ross, 1998. Selfish genes: green beard in the red fire ant. *Nature 394*: 573-575.

Kessin, R.H., 2001. *Dictyostelium: evolution, cell biology, and the development of multicellularity.* Cambridge University Press, Cambridge, UK.

Ketcham, R.B. and R.M. Eisenberg, 1989. Clonal diversity in populations of *Polysphondylium pallidum*, a cellular slime mold. *Ecology 70*: 1425-1433.

Krieger, M.J.B. and K.G. Ross, 2002. Identification of a major gene regulating complex social behavior. *Science 295*: 328-332.

Kuserk, F.T., 1980. The relationship between cellular slime molds and bacteria in forest soil. *Ecology 61*: 1474-1485.

Kuserk, F.T., R.M. Eisenberg and A.M. Olsen, 1977. An examination of the methods for isolating cellular slime molds (Dictyosteliida) from soil samples. *J. Protozool. 24*: 297-299.

Kuspa, A. and W. F. Loomis, 1992. Tagging developmental genes in *Dictyostelium* by restriction enzyme-mediated integration of plasmid DNA. *Proc. Nat. Acad. Sci. 89*: 8803-8807.

Kuspa, A., R. Sucgang and G. Shaulsky, 2001. The promise of a protist: the *Dictyostelium* genome project. *Funct. Integr. Genomics 1*: 279-293.

Leach, C.K., J.M. Ashworth and D.R. Garrod, 1973. Cell sorting out during the differentiation of mixtures of metabolically distinct populations of *Dictyostelium discoideum*. *J. Embryol. Exp. Morphol. 29*: 647-661.

Loomis, W.F., 1982. *The Development of Dictyostelium discoideum.* Academic Press, New York.

Loomis, W.F., 1998. The *Dictyostelium* genome sequencing project. *Protist 149*: 209-212.

Maeda, Y., K. Inouye and I. Takeuchi, 1997. *Dictyostelium: a model system for cell and developmental biology.* Universal Academy Press, Tokyo.

Matapurkar, A.K. and M.G. Watve, 1997. Altruist-cheater dynamics in *Dictyostelium*: aggregated distribution gives stable oscillations. *Am. Nat. 150*: 790-797.

Matsuda, H. and Y. Harada, 1990. Evolutionarily stable stalk to spore ratio in cellular slime molds and the law of equalization in net incomes. *J. Theor. Biol. 147*: 329-344.

Maynard Smith, J., 1989. Evolutionary progress and the levels of selection. In: *Evolutionary Progress* (ed. Nitecki, M. H.), pp. 219-230. University of Chicago Press, Chicago.

Maynard Smith, J. and E. Szathmáry, 1995. *The Major Transitions in Evolution.* W. H. Freeman, Oxford.

McDonald, S.A. and A.J. Durston, 1984. The cell cycle and sorting behaviour in *Dictyostelium discoideum*. *J. Cell Sci. 66*: 195-204.

Michod, R.E., 1996. Cooperation and conflict in the evolution of individuality. II. Conflict mediation. *P. Roy. Soc. Lond. B Bio. 263*: 813-822.

Michod, R.E., 1997. Cooperation and conflict in the evolution of individuality: I. Multi-level selection of the organism. *Am. Nat. 149*: 607-645.

Michod, R.E. and D. Roze, 1997. Transitions in individuality. *P. Roy. Soc. Lond. B. Bio 264*: 853-857.

Mohanty, S., S. Lee, N. Yadava, M.J. Dealy, R.S. Johnson and R.A. Firtel, 2001. Regulated protein degradation controls PKA function and cell-type differentiation in *Dictyostelium*. *Genes Devel. 15*: 1435-1448.

Morio, T., H. Urushihara, T. Saito, Y. Ugawa, H. Mizuno, M. Yoshida, R. Yoshino, B. N. Mitra, M. Pi, T. Sato, K. Takemoto, H. Yasukawa, J. Williams, M. Maeda, I. Takeuchi, H. Ochiai and Y. Tanaka, 1998. The *Dictyostelium* developmental cDNA project: generation and analysis of expressed sequence tags from the first-finger stage of development. *DNA Res. 5*: 335-340.

Page R.E., M.K. Fondrk, G.J. Hunt, E. Guzman-Novoa, M.A. Humphries, 2000. Genetic dissection of honeybee (*Apis mellifera* L.) foraging behavior. *J. Heredity 91*: 474-479.

Plyte, S.E., E. O'Donovan, J.R. Woodgett and A.J. Harwood, 1999. Glycogen synthase kinase-3 (GSK-3) is regulated during *Dictyostelium* development via the serpentine receptor cAR3. *Development.126*: 325-333.

Ponte, E., E. Bracco, J. Faix and S. Bozzaro, 1998. Detection of subtle phenotypes: the case of the cell adhesion molecule csA in *Dictyostelium*. *Proc. Nat. Acad.*

Sci. 95: 9360-9365.

Queller, D.C., 2000. Relatedness and the fraternal major transitions. *Phil. Trans. Royal Soc. B 355*: 1647-1655.

Queller, D.C. and J.E. Strassmann, 1998. Kin selection and social insects. *Bioscience 48*: 165-175.

Queller, D.C., J.E. Strassmann and C.R. Hughes, 1993. Microsatellites and kinship. *Trends Ecol. Evol. 8*, 285-288.

Queller, D.C., E. Ponte, S. Bozzaro and J.E. Strassmann, 2003. Single-gene greenbeard effects in the social amoeba, *Dictyostelium discoideum. Science 299*: 105-106.

Raper, K.B., 1940. Pseudoplasmodium formation and organization in *Dictyostelium* discoideum. *J. Elisha Mitchell Sci. Soc. 56*: 241-282.

Rogers, K.C., G.T. Ginsburg, X. Mu, R. Gollop, P. Balint-Kurti, J.M. Louis and A. R. Kimmel. 1997. The cAMP receptor gene family of *Dictyostelium discoideum*: expression, regulation, function. In: *Dictyostelium: a model system for cell and developmental biology* (eds. Maeda, Y., K. Inouye and I. Takeuchi), pp. 163-172.Universal Academy Press, Tokyo.

Seger, J., 1988. Review of L. W. Buss, *The Evolution of Individuality. Q. Rev. Biol. 66*: 336-337.

Shaulsky, G. and W. F. Loomis, 1995. Mitochondrial DNA replication but no nuclear DNA replication during development of *Dictyostelium. PNAS 92*: 5660-5663.

Slatkin, M., 1985. Somatic mutations as an evolutionary force. In: *Evolution: essays in honour of John Maynard Smith* (eds. Greenwood, P. J., P. H. Harvey and M. Slatkin,), pp.17-30. Cambridge University Press, Cambridge.

Stephenson, S.L. and J.C. Landolt, 1992. Vertebrates as vectors of cellular slime molds in temperate forests. *Mycol. Res. 96*: 670-672.

Strassmann, J.E., Y. Zhu and D.C. Queller, 2000, Altruism and social cheating in the social amoeba *Dictyostelium discoideum. Nature 408*: 965-967.

Sussman, R.R. and M. Sussman, 1953. Cellular differentiation in Dictyosteliaceae: heritable modifications of the developmental pattern. *Ann. N. Y. Acad. Sci. 56*: 949-960.

Suthers, H.B., 1985. Ground-feeding migratory songbirds as cellular slime mold distribution vectors. *Oecologia 65*: 526-530.

Swanson, A.R., E.M. Vadell and J.C. Cavender, 1999. Global distribution of forest soil dictyostelids. *J. Biogeography 26*: 133-148.

Szathmáry, E. and J. Maynard Smith, 1995. The major evolutionary transitions. *Nature 374*: 227-232.

Thompson, C.R.L. and R.R. Kay, 2000a. Cell-fate choice in *Dictyostelium*: intrinsic biases modulate sensitivity to DIF signaling. *Dev. Biol. 227*: 56-64.

Thompson, C.R.L. and R.R. Kay, 2000b. The role of DIF-1 signaling in *Dictyostelium* development. *Mol. Cell 6*: 1509-1514.

van Driessche, N., C. Shaw, M. Katoh, T. Morio, R. Sucgang, M. Ibarra, H. Kuwayama, T. Saito, H. Urushikawa, M. Maeda, I. Takeuchi, H. Ochiai, W.

Eaton, J. Tollett, J. Halter, A. Kuspa, Y. Tanaka and G. Shaulsky, 2002. A transcriptional profile of multicellular development in Dictyostelium discoideum. *Development 129*: 1543-1552.

van Haastert, P.J.M., 1997. Transduction of the chemotactic cAMP signal across the plasma membrane. In: *Dictyostelium: a model system for cell and developmental biology* (eds. Maeda, Y., K. Inouye and I. Takeuchi), pp.173-191. Universal Academy Press, Tokyo.

Verkerke-van Wijk, I. and P. Schaap, 1997. cAMP, a signal for survival. In: *Dictyostelium: a model system for cell and developmental biology* (eds. Maeda, Y., K. Inouye and I. Takeuchi), pp. 145-162. Universal Academy Press, Tokyo.

Weijer, C.J., G. Duschl and C.N. David, 1984. Dependence of cell-type proportioning and sorting on cell cycle phase in *Dictyostelium discoideum. J. Cell Sci. 70*: 133-145.

Williams, G.C. 1966. *Adaptation and Natural Selection: a critique of some current evolutionary thought*. Princeton University Press, Princeton, NJ.

Williams, J. 1997. Prestalk and stalk heterogeneity in *Dictyostelium*. In: *Dictyostelium: a model system for cell and developmental biology* (eds. Maeda, Y., K. Inouye and I. Takeuchi), pp. 293-304. Universal Academy Press, Tokyo.

Williams, J.G., K.T. Duffy, D.P. Lane, S.J. McRobbie, A.J. Harwood, D. Traynor, R. R. Kay and K.A. Jermyn, 1989. Origins of the prestalk-prespore pattern in *Dictyostelium* development. *Cell 59*: 1157-1163.

Wilson, D.S. and E. Sober, 1989. Reviving the superorganism. *J. theor. Biol. 136*: 337-356.

Wilson, E.O., 1975. *Sociobiology: the new synthesis*. Harvard University Press, Cambridge, MA.

Zahavi, A. and A. Zahavi, 1997. *The Handicap Principle: a missing piece of Darwin's puzzle*. Oxford University Press, Oxford.