**REVIEW
ARTICLE****Developmental cheating and the evolutionary biology of *Dictyostelium* and *Myxococcus***

Dee N. Dao, Richard H. Kessin and Herbert L. Ennis

Author for correspondence: Richard H. Kessin. Tel: +1 212 305 5653. Fax: +1 212 305 3970.
e-mail: rhk2@columbia.edu

Department of Anatomy and Cell Biology, Columbia University, 630 West 168th St, New York, NY 10032, USA

Keywords: SCF complex, proteolysis, parasitism, slime mould, *Botryllus*

Multi-cellularity by aggregation

Micro-organisms, whether the prokaryotic *Myxococcus* or the eukaryotic amoeba *Dictyostelium*, can achieve an impressive level of multicellular cooperativity and it is revealing to ask how this came to be and how it differs from the cooperativity found in animal development. This is not a sterile exercise that begins and ends with speculation – there is a usefulness, a utility to evolutionary theory, particularly for microbiologists.

Consider the life cycles of *Dictyostelium discoideum* and a developing prokaryote, *Myxococcus xanthus*, both of which live in a similar soil niche. *Dictyostelium* and related species of amoebae feed on many species of bacteria. Once the bacteria are consumed, the amoebae construct a chemotactic and motile machinery that brings them into aggregates of about 100 000 cells. The chemotactic signal, cAMP in the best studied species, is relayed so that amoebae can collect from a fairly large area – perhaps a cubic centimetre of soil. Within the *D. discoideum* aggregate, cell-type differentiation occurs (Fig. 1), giving rise to progenitors of stalk cells and spore cells, in a strict ratio of 1:4 (Raper, 1940; Stenhouse & Williams, 1977; Bonner & Slifkin, 1949). The mound of cells forms a tip, which extends into a motile slug that orients along heat gradients and towards light. At a certain moment, the slug undergoes a process of culmination such that the spores are extended onto a long stalk. The cells that form the stalk die and it is this sacrifice that is important to the argument that follows.

Although difficult to prove, one selective advantage for stalks in *Dictyostelium* and other organisms, including the myxobacteria, may be that by projecting the spores into the crevices of the soil, they will be dispersed by passing micro-arthropods, nematodes or earthworms (Huss, 1989; Kessin *et al.*, 1996). Fruiting bodies tend to orient at right angles to the substratum, even if it is sloped, and this may be an adaptation for dispersal by passing animals (Bonner & Dodd, 1962). Another advantage of the stalk might be to remove the developing spores from the noxious environment of the soil. Thus

the sacrifice of 20% of the cells of *Dictyostelium* increases the fitness of a population so that it would compete successfully with non-stalk-forming variants, even if these formed spores with 100% efficiency. Imagine a clonal population of a primordial species that made no stalk and then acquired a small ability to project itself from the surface and be dispersed, even at the cost of some cell death. This selection occurs on an individual cell because even in a single developmental cycle, each amoeba would have an 80% probability of becoming a spore, surviving to the next generation and being dispersed. In a clonal colony, the advantageous gene would be automatically fixed and because the organism is haploid, evolution could be rapid. It is possible to imagine how stalk-formation programmes could evolve if the advantage in dispersal exceeds the 20% loss due to stalk-cell death (Hamilton, 1964; Maynard Smith, 1964). The evolution of a cellulose-based stalk did not necessarily occur *de novo*: there were pre-existing elements. *Dictyostelium* has a sexual cycle that results in the formation of a macrocyst in which a zygote forms and is surrounded by a thick trilaminar cellulose wall (Raper, 1984). This alternate developmental cycle does not involve problems of cell-type differentiation and proportionality, and because it does provide genetic diversity, we have postulated that it is the evolutionary precursor of the fruiting body. See Kessin (2000) for a more complete discussion of the stages of *Dictyostelium* evolution. The stalk of the fruiting body is also formed of cellulose and thus the complex act of assembling extracellular cellulose fibrils may have existed in an ancestor of the fruiting species we know today. The precursors of stalk-forming cells in myxobacteria are not known, nor is there any certainty about genetic exchange in the myxobacteria.

Myxobacteria are Gram-negative gliding bacteria and comprise a number of genera of the *Proteobacteria* (Dworkin, 1996). They are common in the soil, where they feed on other bacteria. Most of this feeding is done in packs and the rationale for this behaviour has been that the secretion of large quantities of digestive enzymes

Fig. 1. The life cycle of *Dictyostelium*. A scanning electron micrograph of *Dictyostelium* development is shown on the left and the sorting pattern of prestalk and prespore cells on the right. Blue represents prestalk cells, which initially differentiate interspersed among the prespore cells and then sort out to form an apical tip. The prior aggregation stage employs chemotaxis to assemble 100 000 amoebae into the mound shown here (Parent & Devreotes, 1999; Kessin, 2000). The panel on the left is reproduced courtesy of R.L. Blanton & M. Grimson, Texas Technical University. The panels on the right are reproduced with permission from Kessin & van Lookeren Campagne (1992).

Fig. 2. The developmental cycle of *M. xanthus*. Development requires two colour variants – tan and yellow, indicated here by white and hatched developing myxospores. In the presence of nutrients, growth continues. Under certain inductive regimes, individual cells can form myxospores. Modified from Dworkin (1996), with permission.

Fig. 3. The fruiting structures of *M. xanthus* (left) and *C. crocatus* (right). Note that the stalk structure of *C. crocatus* is acellular. Bars, 100 μ m. Reproduced courtesy of Professor Hans Reichenbach.

is most effectively done in groups. This is in contrast to *Dictyostelium*, which feeds by phagocytosis, a process that is not aided by sociality. The cooperativity of the myxobacteria extends to their development – when their nutrients are consumed, they glide toward aggregation centres and produce a mound of cells (Fig. 2), although the role of chemotaxis in this process is controversial (Kearns & Shimkets, 1998). The complexity of the fruiting structures that result is highly variable, depending on which of the families of myxobacteria is being studied, but some, such as *Chondromyces crocatus*,

have elaborate stalk structures, while the more thoroughly studied *M. xanthus* has a simpler fruiting body (Fig. 3). As in the case of the dictyostelids, many cells, even a majority, die in the process of forming a fruiting body in *M. xanthus* (Dworkin, 1996; Wireman & Dworkin, 1977). Other species have not been as thoroughly examined. Whether the death of the majority of cells benefits those that form the myxospores is not as clearly established as is the case of stalk formation in *Dictyostelium*.

The problem of parasitism

There have been previous comparisons between the myxobacteria and the cellular slime moulds like *Dictyostelium* (Kaiser, 1986, 1993). The parallelisms of their evolution are striking and include the process of aggregation, the use of sensor histidine kinases to regulate development, and the use of mechanisms of quorum sensing to count the number of cells before undertaking multicellular development. To the evolutionary biologist there is an additional similarity that involves the challenges posed to creating a multicellular organism by aggregation of genetically distinct individuals. This problem has been ignored, except by a few authors (Zahavi & Ralt, 1984; Buss, 1982, 1999; Michod, 1999). There are also great differences – the advantage of aggregation to the myxobacteria may be that it could allow germination of many sticky myxospores in one spot so that their pack-based feeding can occur. In *Dictyostelium*, the spores are effectively dispersed to form colonies from single cells.

Dictyostelium and myxobacteria collect cells from a small area to form a collection that has a much larger mass than an individual cell. There are two major limitations to this means of creating multicellular structures. The first is size. The fruiting bodies of *Dictyostelium* and myxobacteria are limited by the number of cells in a particular volume of soil and the distances that the cells can migrate to enter an aggregate. Animals depend on a great evolutionary innovation – they can retain their differentiated state while they feed, grow and develop, and do not suffer the size limitation of aggregative organisms. The second limitation of the collective life stems from the fact that increasing size by aggregation is a rather open procedure. *Dictyostelium* sacrifices 20% of its cells to form a dead supporting stalk and 90% of *M. xanthus* cells die during development of myxospores (Dworkin, 1996; Wireman & Dworkin, 1977). Over evolutionary time spans, and given genetic diversity in the wild, we expect that strains would evolve that make no contribution to the stalk of *Dictyostelium* or the fruiting structures of the various myxobacteria. We call such strains cheaters because of their partial or complete evasion of the stalk-cell fate. The formation of chimeric colonies in which cheater mutants can function requires that isolated populations be brought into contact. We have known since Darwin studied earthworms that the soil is constantly churned and that therefore social amoebae with different genotypes would be brought into contact. Cheaters, we

predict, would form only viable spores and upon co-aggregation with normal cells, might ignore signals to become supporting structures. If they were really good cheaters they might actively drive the wild-type to be stalk cells, perhaps by suborning a normal regulatory feature of development. Some evolutionary biologists muse that *Dictyostelium* should never have evolved because of its susceptibility to parasitism by non-cooperative mutants of its own species. Indeed, non-cooperating variants are a major difficulty when considering selection theories that would lead to co-operating structures like those of the dictyostelids or of the myxobacteria (Michod, 1999; Zahavi & Ralt, 1984).

There are several ways out of this dilemma. If new mutations leading to, or sustaining, cooperative behaviour appeared more frequently than non-cooperative cheater mutations, the sort of selection we see in the evolution of the dictyostelids could occur (Zahavi & Ralt, 1984). Since the elaboration of a *Dictyostelium* fruiting body requires hundreds of genes acting in concert and the construction of a non-cooperating strain requires only one mutation, this is not likely. Since *Dictyostelium* spores are often dispersed to give rise to clonal colonies (Kessin *et al.*, 1996), a non-cooperative mutant would have to be able to survive on its own and could not, in the process of becoming a parasite, sacrifice its own developmental capacity. The frequent imposition of clonal growth would select against a large class of potential parasites – those that require a partner wild-type cell – at least in the wild. The third way to avoid non-cooperating cells is to evolve incompatibility or policing mechanisms, or to change chemotactic molecules so that cheaters could not enter aggregates (Armstrong, 1984; Buss, 1987; Frank, 1995). Finally, there is a species called *Acytostelium leptomosum* (Raper & Quinlan, 1958) that produces a non-cellular stalk and therefore does not expend the normal 20% of cells in forming a stalk. *Acytostelium*, although perhaps still subject to cheating, indicates that there is more than one strategy for forming a stalked structure, as in the case of *C. crocatus* (Fig. 3).

There have been two reports indicating that in freshly isolated uncloned populations of *Dictyostelium* amoebae, variants preferentially make spores in chimeras with normal fruiting cells. The first report was by Filosa (1962), working with *Dictyostelium mucoroides*. Starting with an uncloned population, Filosa found individual colonies with variant morphologies. One, which formed slugs but no fruiting bodies, preferentially made spores when mixed with wild-type cells. The second observation of parasitism was by Buss (1982), also with *D. mucoroides*. Buss found a single very small soil sample which, when plated clonally, gave rise to normal colonies and colonies which only formed spores. The latter, in chimeras with the fruiting strain, could suppress the wild-type. It was Buss who first pointed out the potentially disruptive role of parasites on multi-cellularity by aggregation. One might imagine that some cells have mutated to secrete greater amounts of a factor that causes other cells to differentiate into

Fig. 4. In the competition to form spores rather than stalk, some mutants can cheat. The increase in frequency of the *chtA* mutant in the population is shown in (b). In this experiment a mixture of one *chtA* mutant to 1000 wild-type cells was allowed to co-aggregate and develop. The resulting spores were plated again and allowed to develop. The frequency of increase of the mutant over 12 growth and development cycles is shown. Since the mutant also expresses β -galactosidase in spore cells, the increase in mutant abundance can be seen in the increasing blue staining of the sorocarps shown in (a). If a β -galactosidase-expressing plasmid is inserted in a spot other than the *chtA* gene, the frequency of blue cells in the sorocarp does not increase with cycles of development.

stalk. A compound called differentiation-inducing factor (DIF), which has properties that could cause such an effect, has been described (Traynor & Kay, 1991; Williams *et al.*, 1989). It is possible that DIF evolved not to mediate cell-type differentiation, but as an instrument of the competition among genetically diverse populations of *D. discoideum*. By this logic, combining wild-type isolates of *Dictyostelium* might reveal that one strain is overrepresented in the spore population. Such studies have been undertaken by Strassmann and colleagues, who made chimeras of genetically diverse amoebae that were isolated from the same locale. Microsatellite analysis revealed that these chimeric mixtures are often not fair and that often one clone contributes less than its proportional share to the stalk and more to the spores (J. Strassmann, personal communication). Whether the ability to achieve extra representation in the spore population is based on the actions of DIF is not known.

Exploiting parasitism

If *Dictyostelium* or *Myxococcus* aggregates form from competing, genetically distinct cells, we might take advantage of this property to select for mutant cells that have an advantage in the formation of a fruiting body. This is interesting because one way to gain an advantage over wild-type cells would be to suborn a natural signalling mechanism that cells use to coordinate cell-type proportion. Despite many years of effort with both organisms, such signalling mechanisms remain elusive. In the laboratory, mutants that do not contribute to the stalk population would be relieved of the burden of having to survive in the wild and thus even cheaters that are defective in some final stage of development, but do well in a chimera, can be selected. If we can then isolate

the affected genes and assign them to a particular developmental pathway, we could be on our way to understanding the mechanism of cheating and also of normal development.

In our laboratory we have isolated a cheater mutant of *D. discoideum* (denoted *chtA*) by a selection procedure that was based on the idea that a cheater mutant should increase its frequency in a chimeric population. A population of 14000 amoebae, each with a different insertional mutation, was allowed to form spores and then the spores were harvested and treated with detergent to kill any cell that was not a spore. The spores were then plated on lawns of bacteria, where they were allowed to feed by phagocytosis, aggregate and form spores again. The process was repeated a number of times and at the end, a single mutant made up an increasing portion of the population. This procedure only recovers mutants that have a distinct morphological phenotype. The recovered mutant was marked with a *lacZ* gene that is only expressed in cells destined to be spores. The marked strain was then mixed in a ratio of 1:1000 with unlabelled wild-type cells and the fruiting bodies were monitored for an increase in the number of mutant spores. This can be done by plating the spores clonally and observing the phenotypes of the colonies, or by staining the fruiting bodies for β -galactosidase activity, as shown in Fig. 4.

The mutant makes no spores unless it is mixed with wild-type cells, which form the stalk while the *chtA* mutant preferentially forms viable spores. Very few wild-type cells – only a few percent – are necessary. The final signal for spore encapsulation comes from the stalk cells, so it is not surprising that a mutant that is incapable of forming a stalk cannot form spores unless there are wild-type stalk cells present (Wang *et al.*,

Fig. 5. *ChtA* drives wild-type cells into the prestalk region of the sorocarp. (a), (c) and (e) show a 20% wild-type labelled with a prespore-specific promoter fused to the *lacZ* gene: 80% unlabelled wild-type mixture. Cells in the prespore zone of the slugs are labelled, as are the spore masses of culminants. When 20% wild-type cells labelled with a prespore-specific promoter driving β -galactosidase were mixed with 80% *chtA* cells, the wild-type cells moved from the rear of the slug to the prestalk zone and ultimately to the upper cup, a prestalk zone of the fruiting body [panels (b), (d) and (f)]. Reproduced from Ennis *et al.* (2000) with permission. Copyright (2000) National Academy of Sciences, USA.

1999). Perhaps it is significant that the final signal for spore encapsulation comes from the cells whose genotypes will not be preserved. The *chtA* mutant has an altered ratio of prespore to prestalk cells, with very few of the latter in slugs, when developed without a wild-type partner.

In slugs formed from chimeras, the mutant cells always populate the zones that are fated to give rise to spores. The wild-type cells always end up in the prestalk zone, even if they were originally in the rear of the slug – a region normally fated to produce spores. The mutant causes wild-type cells that have begun to express spore-specific genes in the rear of the slug to migrate to the prestalk compartment of the developing organism. This conversion can be seen if the wild-type cells are marked with a prespore promoter expressing β -galactosidase (Fig. 5). Trans-differentiation of the wild-type into a

Fig. 6. F-box WD40 domain proteins bind phosphorylated substrates and bring them into contact with the ubiquitination machinery, here shown as a ubiquitin E2-conjugating enzyme. These SCF (Skp1, Cullin, F-box) complexes are present in many eukaryotic cells. The F-box proteins come in many forms and have specificities for different substrates (Bai *et al.*, 1996; Skowrya *et al.*, 1997). The figure is based on information from Tyers & Willems (1999). Binding a phosphorylated target to the SCF complex results in its ubiquitination and then its destruction by the proteasome, a multi-subunit proteolytic enzyme.

non-viable stalk-cell population allows the cheater cells to increase in the population. Under certain conditions of development, which are more controlled than those used in the original selection, they increase five- to sixfold in one cycle of development (Ennis *et al.*, 2000). An alternative explanation could be extra cell divisions of the *chtA* mutant cells. Our preliminary experiments have not detected a gross increase in *chtA* cell number (H. L. Ennis, unpublished). It should be remembered that these cells are starving, and this would mitigate against DNA synthesis and cell division.

Recovering the *chtA* gene

The molecular genetic techniques available in *D. discoideum* allow us to isolate the mutated genes that result from selection. The *chtA* gene encodes a protein with an F-box and WD40 domains. This class of protein usually forms part of a complex that targets specific protein substrates for ubiquitination and degradation. Phosphorylated target proteins bind to the WD40 domains at the carboxy terminus of the F-box protein, while the F-box binds to a protein in the complex called Skp1. Other proteins provide scaffolding functions and an E2 class ubiquitin-conjugating enzyme is also a member. The conjugating enzyme is brought into contact with the target, transfers its ubiquitin, and the tagged target protein is then recognized and destroyed by a large protease complex, the proteasome. A prototypical F-box complex is shown in Fig. 6. F-box proteins are essentially matchmakers that provide exquisite specificity to protein degradation. We speculate that among the targets of the *Dictyostelium* F-box protein encoded by *chtA* are the regulatory proteins that control important events during the final stages of development

when a stalk forms and spores encapsulate. Although the F-box protein is several steps removed from cell–cell communication mechanisms, we predict that these phenomena will be linked. *Dictyostelium* presents a number of experimental advantages to make it possible to find the nature of these targets, including suppressor mutations and the ability to select mutants that are impervious to the killing effects of *chtA*. None of the targets of the ChtA protein product is known yet.

Do equivalent phenomena occur in the myxobacteria?

Recently, Velicer *et al.* (2000) have isolated mutants of *M. xanthus* whose behaviour resembles *chtA* mutants of *Dictyostelium*. These authors have used several approaches to show that social cheating also occurs among the prokaryotes. In one set of experiments, Velicer and colleagues grew the bacteria for a thousand generations in the absence of social development and then asked if cheaters had evolved that would have an advantage in chimeras with their progenitor. Several such mutants were recovered. In a more specific approach, a number of mutants with defects in known communications pathways were analysed, and two of these also exhibited cheating during development – that is in chimeras with an isogenic parent, the mutants were overrepresented among the myxospores. As in the case of the *chtA* mutant of *Dictyostelium*, pure populations of the mutants do very poorly at making myxospores by themselves, but are efficient when paired with a normal partner. The authors make another important point that is applicable to the *Dictyostelium* case. If a cheater is going to be defective when growing clonally, it is an advantage if the myxospores are sticky and that when dispersed, no mono-cultures occur. Apparently *M. xanthus* myxospores are sticky (Velicer *et al.*, 2000). *Dictyostelium* spores are not obviously sticky, but whether they are completely dispersed in nature is not known.

Phase variation in *M. xanthus* could also lead to cheating. One classical example of phase variation is the alteration of flagellar antigens in *Salmonella typhimurium*. In the case of *S. typhimurium*, a site-specific recombination system causes the inversion of a genomic fragment, leading to expression of different flagellar structural proteins (Scott & Simon, 1982). The molecular basis of phase variation in *M. xanthus* is not known. *M. xanthus* has yellow and tan variants that appear by phase variation in all colonies, with the yellow form predominating and the tan making up 1–5% in the standard strains. Yellow colonies have a rough appearance and swarm, while tan colonies have a smooth, mucoid appearance. A freshly isolated tan colony has about 25% yellow variants, indicating that the variation occurs at high frequency and in both directions, and that all colonies are mixed. However, the frequency of the tan variants rises six- to sevenfold over one cycle of fruiting-body production and sporulation. Starting at 5% tan variants in the vegetative population, fruiting bodies end up with about 35% tan variants.

There is speculation that the yellow cells lyse to provide essential inducers or nutrients for the encapsulation of the spores of the tan cells (Dworkin, 1996; Wireman & Dworkin, 1977).

Laue & Gill (1995) have asked what the developmental significance of these mixed colonies is by creating a variant that is locked almost completely in the tan phase. These cells form prespores, but do not make the final resistant spore cells unless some of the yellow variants are present. Recall that the prespores of the *D. discoideum chtA* mutant do not form encapsulated spores unless some of the wild-type are present. This is perhaps the best solution that the bacterial genome can provide to establish two essential cell types (Laue & Gill, 1995). How is it that sporulation of one cell type (the tan or the cheater) is dependent on the presence of the second sacrificial cell type (the yellow or the stalk)? Given that mixed colonies are required for successful sporulation, high-frequency phase variation, whether genetic or epigenetic, assures that even after dispersal, a single yellow or tan cell would not suffer an inability to sporulate. A *Dictyostelium* cheater mutant would be counter-selected if dispersed into a single clonal colony, as would a phase-locked tan or yellow variant, such as that described by Laue & Gill (1995). There is one way in which phase variation could be exploited to achieve an advantage. If there are competing populations that form chimeric fruiting bodies, the cells that have a reduced frequency of variation in the direction tan to yellow would be selected. A phase-locked variant such as the one described by Laue & Gill would have an advantage as long as it sticks to wild-type myxospores. There could be other genes, having nothing to do with phase variation, which would give certain strains of myxobacteria an advantage in chimeric colonies. To our knowledge, the types of selection experiment performed in *Dictyostelium* have not yet been done with the myxobacteria.

Does the cheating phenomenon extend to other multicellular organisms?

One great defence against parasitism of an organism by variant members of the same species is the sequestration of a germ line. Plants are another story, but animals have almost solved the problem of cheaters within an individual because all cells in a body are genetically identical and because they have evolved a germ-line, such that any somatic mutation has no future, no matter what benefit it gives a somatic cell. Thus, for animals with a sequestered germ line, the question of cheaters is reduced to germ cells, and post-meiotic ones at that. These are the only cells in the body that are not genetically identical and, as theory would predict, cheaters evolve among germ cells, as seen in the phenomenon of meiotic drive, particularly in mice and *Drosophila*. In the mouse case, sperm carrying a particular allele at the *t* locus contribute to progeny more than wild-type alleles in a non-Mendelian inheritance pattern (Ardlie, 1998). In *Drosophila*, the best characterized system is Segregation Distorter, which has

long been known to skew the normal segregation ratio in flies. This gene, which encodes a RanGAP protein, is widespread in wild populations of *Drosophila* (for a recent review see Genetzky, 2000). Numerous other meiotic-drive systems are known (Lyttle, 1993).

If we make the prediction that the germ line is important as an evolutionary innovation because it blocks the invasion of somatic-cell variants, there should be complex organisms that do not produce completely sequestered germ lines and suffer the consequences of competition. The phenomenon of cheating is seen in certain complex metazoans that do not set aside a germ line. These include the colonial protochordates, as most recently shown by Stoner *et al.* (1999). This large class of organisms reproduce asexually, but with an occasional sexual generation. The zygotes of *Botryllus schosseri* mature into a tadpole larval stage with the body plan of a chordate. As Stoner and colleagues point out, metamorphosis results in the loss of the chordate body plan, including the notochord and the musculature, and results in a sessile oozoid with its own tissues and circulation. Individual oozoids fuse at their borders so that they have a common circulation. Eventually, ovaries and testes form and sexual reproduction takes place. The fusion of individuals is the interest here because sometimes there are colonies of different genotypes on the same substratum. Because these individuals have not yet constructed gonads, the primordial germ cells are not sequestered, but are free to move. When fusion occurs, an incompatibility mechanism is engaged and two genetically distinct individuals usually remain separate. The fusion and rejection mechanism is controlled by a single polymorphic locus. In certain cases, the incompatibility mechanism is not engaged and individuals with two different genotypes fuse. Using microsatellite markers, Stoner and colleagues showed that there is migration of cells with one genotype into somatic tissues with another to form a true chimera. Most important, the germ cells of one individual (there is a hierarchy) populate the gonads of a genetically distinct individual – a situation similar to the cheater phenomenon described for *Dictyostelium*. For a further comparison, see the commentary by Buss (1999). Buss (1987) and others cited by Stoner *et al.* (1999) have postulated that the great variety of incompatibility mechanisms that we see in nature are an adaptation designed to limit cell-lineage competition.

Though *Dictyostelium*, *Myxococcus* and *Botryllus* have no direct relationship in phylogeny, they all confront a problem of parasitism by cells of their own species and this problem is probably at the root of the evolution of a variety of incompatibility mechanisms. In the case of the genetically tractable *Dictyostelium*, the tendency to parasitism can be exploited to investigate the normal regulation of development.

Acknowledgements

We would like to thank Professors Martin Dworkin, Joan Strassmann, Gregory J. Velicer and John Tyler Bonner for helpful comments. We are grateful to Gregory Velicer, Lee

Kroos and Richard Lenski for a preprint of their paper on myxobacterial cheaters. We would also like to thank Mary Wu and Palma Volino for excellent technical support. Supported by NIH grant GM33136 to R.H.K and NSF grant IBN 9904645 to H.L.E.

References

- Ardlie, K. G. (1998). Putting the brake on drive: meiotic drive of *t* haplotypes in natural populations of mice. *Trends Genet* **14**, 189–193.
- Armstrong, D. P. (1984). Why don't cellular slime molds cheat. *J Theor Biol* **109**, 271–283.
- Bai, C., Sen, P., Hofmann, K., Ma, L., Goebel, M., Harper, J. W. & Elledge, S. J. (1996). SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell* **86**, 263–274.
- Bonner, J. T. & Dodd, M. R. (1962). Evidence for gas-induced orientation in the cellular slime molds. *Dev Biol* **5**, 344–361.
- Bonner, J. T. & Slifkin, M. K. (1949). A study of the control of differentiation: the proportions of stalk and spore cells in the slime mold *Dictyostelium discoideum*. *Am J Bot* **36**, 727–734.
- Buss, L. W. (1982). Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc Natl Acad Sci USA* **79**, 5337–5341.
- Buss, L. W. (1987). *The Evolution of Individuality*. Princeton, NJ: Princeton University Press.
- Buss, L. W. (1999). Slime molds, ascidians, and the utility of evolutionary theory. *Proc Natl Acad Sci USA* **96**, 8801–8803.
- Dworkin, M. (1996). Recent advances in the social and developmental biology of the myxobacteria. *Microbiol Rev* **60**, 70–102.
- Ennis, H. L., Dao, D. N., Pukatzki, S. U. & Kessin, R. H. (2000). *Dictyostelium* amoebae lacking an F-box protein form spores rather than stalk in chimeras with wild type. *Proc Natl Acad Sci USA* **97**, 3292–3297.
- Filosa, M. F. (1962). Heterocytosis in cellular slime molds. *Am Nat* **XCVI**, 79–92.
- Frank, S. A. (1995). Mutual policing and repression of competition in the evolution of cooperative groups. *Nature* **377**, 520–522.
- Genetzky, B. (2000). Tracking down a cheating gene. *Am Sci* **88**, 128–135.
- Hamilton, W. D. (1964). The genetical evolution of social behaviour. *J Theor Biol* **7**, 1–16.
- Huss, M. J. (1989). Dispersal of cellular slime moulds by two soil invertebrates. *Mycologia* **81**, 677–682.
- Kaiser, D. (1986). Control of multicellular development: *Dictyostelium* and *Myxococcus*. *Annu Rev Genet* **20**, 539–566.
- Kaiser, D. (1993). Roland Thaxter's legacy and the origins of multicellular development. *Genetics* **135**, 249–254.
- Kearns, D. B. & Shinkets, L. J. (1998). Chemotaxis in a gliding bacterium. *Proc Natl Acad Sci USA* **95**, 11957–11962.
- Kessin, R. H. (2000). *Dictyostelium: the Evolution, Cell Biology and Development of a Social Organism*. Cambridge: Cambridge University Press (in press).
- Kessin, R. H. & van Lookeren Campagne, M. M. (1992). The development of a social amoeba. *Am Sci* **80**, 556–565.
- Kessin, R. H., Gundersen, G. G., Zaydfudim, V., Grimson, M. & Blanton, R. L. (1996). How cellular slime molds evade nematodes. *Proc Natl Acad Sci USA* **93**, 4857–4861.
- Laue, B. E. & Gill, R. E. (1995). Using a phase-locked mutant of

- Myxococcus xanthus* to study the role of phase variation in development. *J Bacteriol* **177**, 4089–4096.
- Lyttle, T. W. (1993).** Cheaters sometimes prosper: distortion of Mendelian segregation by meiotic drive. *Trends Genet* **9**, 205–210.
- Maynard Smith, J. (1964).** Group selection and kin selection. *Nature* **201**, 1145–1147.
- Michod, R. E. (1999).** *Darwinian Dynamics: Evolutionary Transitions in Fitness and Individuality*. Princeton, NJ: Princeton University Press.
- Parent, C. A. & Devreotes, P. N. (1999).** A cell's sense of direction. *Science* **284**, 765–770.
- Raper, K. B. (1940).** The communal nature of the fruiting process in the *Acrasieae*. *Am J Bot* **27**, 436–448.
- Raper, K. B. (1984).** *The Dictyostelids*. Princeton, NJ: Princeton University Press.
- Raper, K. B. & Quinlan, M. S. (1958).** *Acytostelium leptosomum*: a unique cellular slime mould with an acellular stalk. *J Gen Microbiol* **18**, 16–32.
- Scott, T. N. & Simon, M. I. (1982).** Genetic analysis of the mechanism of the *Salmonella* phase variation site specific recombination system. *Mol Gen Genet* **188**, 313–321.
- Skowrya, D., Craig, K. L., Tyers, M., Elledge, S. J. & Harper, J. W. (1997).** F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* **91**, 209–219.
- Stenhouse, F. O. & Williams, K. L. (1977).** Patterning in *Dictyostelium discoideum*: the proportions of the three differentiated cell types (spore, stalk, and basal disk) in the fruiting body. *Dev Biol* **59**, 140–152.
- Stoner, D. S., Rinkevich, B. & Weissman, I. L. (1999).** Heritable germ and somatic cell lineage competitions in chimeric colonial protochordates. *Proc Natl Acad Sci USA* **96**, 9148–9153.
- Traynor, D. & Kay, R. R. (1991).** The DIF-1 signalling system in *Dictyostelium* – metabolism of the signal. *J Biol Chem* **266**, 5291–5297.
- Tyers, M. & Willems, A. R. (1999).** One ring to rule a superfamily of E3 ubiquitin ligases. *Science* **284**, 601, 603–604.
- Velicer, G., Kroos, L. & Lenski, R. E. (2000).** Developmental cheating in the social bacterium *Myxococcus xanthus*. *Nature*, **404**, 598–601.
- Wang, N., Soderbom, F., Anjard, C., Shaulsky, G. & Loomis, W. F. (1999).** SDF-2 induction of terminal differentiation in *Dictyostelium discoideum* is mediated by the membrane-spanning sensor kinase DhkA. *Mol Cell Biol* **19**, 4750–4756.
- Williams, J. G., Duffy, K. T., Lane, D. P., McRobbie, S. J., Harwood, A. J., Traynor, D., Kay, R. R. & Jermyn, K. A. (1989).** Origins of the prestalk-prespore pattern in *Dictyostelium* development. *Cell* **59**, 1157–1163.
- Wireman, J. W. & Dworkin, M. (1977).** Developmentally induced autolysis during fruiting body formation by *Myxococcus xanthus*. *J Bacteriol* **129**, 798–802.
- Zahavi, A. & Ralt, D. (1984).** Social adaptations in myxobacteria. In *Myxobacteria: Development and Cell Interactions*, pp. 215–220. Edited by E. Rosenberg. New York: Springer.