

Co-occurrence in nature of different clones of the social amoeba, *Dictyostelium discoideum*

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Abstract

The social amoeba, *Dictyostelium discoideum*, produces a multicellular fruiting body and has become a model system for cell–cell interactions such as signalling, adhesion and development. However, unlike most multicellular organisms, it forms by aggregation of cells and, in the laboratory, forms genetic chimeras where there may be competition among clones. Here we show that chimera formation is also likely in nature, because different clones commonly co-occur on a very small scale. This suggests that *D. discoideum* will likely have evolved strategies for competing in chimeras, and that the function of some developmental genes will be competitive. Natural chimerism also makes *D. discoideum* a good model organism for the investigation of issues relating to coexistence and conflict between cells.

Keywords: chimera, *Dictyostelium*, kin selection, multicellularity, relatedness, slime mould

Received 27 September 2002; revision received 6 January 2003; accepted 6 January 2003

Introduction

Many of the most important transitions in the history of life have involved the assimilation of lower level units into an adaptive higher level unit (Maynard Smith & Szathmáry 1995). Two of these transitions, from single cells to multicellular organisms and from single individuals to super-organismal societies (such as the social insects), share a number of features because both are built on a foundation of kin-selected altruism (Queller 2000). Social insect colonies have long been described as superorganisms wherein the individual is viewed as analogous to a single cell of an organism (Wheeler 1911; Seeley 1989; Moritz & Southwick 1992; Wilson & Sober 1989; Queller & Strassmann 1998).

However, studies of social insects have revealed one crucial difference. Most social insect superorganisms, unlike most multicellular organisms, are not clonal. Colony members are related, and this explains the high degree of cooperation observed (Hamilton 1964a,b, 1972), but these relatives are nevertheless genetically distinct individuals. These genetic differences have led to the evolution

of within-colony conflicts, for example over sex ratios, caste fate, and reproduction (Trivers & Hare 1976; Bourke & Franks 1995; Crozier & Pamilo 1996; Queller & Strassmann 1998; Bourke & Ratnieks 1999).

In contrast, most multicellular organisms are clonal, with all cells derived from a single progenitor cell. Conflicts among cells are therefore not generally expected. Mutations still occur during the numerous mitotic divisions, but the chimerism created is transitory. The single-cell bottleneck when new individuals are formed constantly re-establishes genetic uniformity (Maynard Smith 1989). Models confirm that little conflict is expected in clonal organisms unless the mutation rates are quite high and the number of cell divisions very large (Michod 1996, 1997).

However, there are some multicellular organisms that form by aggregation of separate individuals. Unless they exclude nonclonemates, genetic chimeras can result. Chimeric organisms are rare in nature (Stoner & Weissman 1996) but, like social insects at a higher level, they offer the opportunity to study conflict between different levels in the organization of life (Buss 1999) or 'levels of evolution' (Reeve & Keller 1999). Cells within chimeric organisms can compete for reproduction, potentially reducing the efficiency of the multicellular structure (Buss 1982). Specifically, any clone has the potential to be better represented in future generations if it focuses on reproduction in chimeras, while allowing the other clones to perform

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somatic functions. Thus, chimeras are interesting for understanding both the evolution of multicellularity and the evolution of social organization.

The social amoeba, *Dictyostelium discoideum*, is an organism that can form multicellular chimeras through aggregation (Strassmann *et al.* 2000). It is a haploid amoeba frequently found in the soil of deciduous forests in the temperate zone (Bonner 1967; Raper 1984; Landolt & Stephenson 1990). Molecular biologists have extensively studied *D. discoideum* and employ it as a model organism, for example, in the study of cell signalling and differentiation (Kessin 2001). The life cycle of *D. discoideum* is characterized by a vegetative phase during which unicellular amoebae feed on bacteria and reproduce asexually. When starving, the amoebae initiate aggregation, with the emission of cAMP regulating this process (Konijn *et al.* 1967; Barkley 1969). From these aggregations, there emerges a multicellular pseudoplasmodium or 'slug' capable of movement. During the multicellular phase the amoebae differentiate allowing the pseudoplasmodium to form a fruiting body or sorocarp consisting of two principle cell types: the spore and the stalk cells (Bonner 1967; Raper 1984; Williams 1997; Kessin 2001). The spores are found in a sorus, a globular structure at the apex of a thin stalk that attaches to the substrate by a disc of cells (Raper 1984).

Formation of the fruiting body probably guarantees better survival and dispersion of the spores but requires the death of the cells that differentiate into the stalk and the basal disc. Altogether, approximately 20% of the cells that initially comprise the pseudoplasmodium die in the formation of the stalk and the basal disc (Raper 1984). The dramatic differences in the destinies of the cells that come together in forming a pseudoplasmodium offer the opportunity to study conflict between the cells that aggregate, particularly if amoebae of different genotypes join to form pseudoplasmodia (Buss 1982; Armstrong 1984; DeAngelo *et al.* 1990; Matsuda & Harada 1990; Gadagkar & Bonner 1994; Strassmann *et al.* 2000; Hudson *et al.* 2002; Foster *et al.* 2002; Queller *et al.* in press). In this case the pseudoplasmodium represents not only a multicellular organism, but also a society of unicellular organisms. As in a colony of social insects in which the workers do not reproduce but rather labour on behalf of their colony, the stalk cells of *D. discoideum* altruistically sacrifice their own reproduction in order to aid the success of the cells that become the spores.

Laboratory experiments have shown that cells from different clones can join to form a pseudoplasmodium, making a chimeric multicellular organism (Strassmann *et al.* 2000). Thus there is real potential for conflict within chimeras in *D. discoideum*. However, these studies were conducted in the laboratory and it is not known how often chimeras form in nature. One possibility is that different clones rarely encounter each other in nature. For example,

if all the cells aggregating together typically descend from a single colonizing spore, most fruiting bodies would be uniclinal even though *D. discoideum* will form chimeras in the laboratory. If this is true, it strengthens the case that *D. discoideum* is a good model for multicellular development in general. However, if different clones often occur in close proximity in nature, then conflict between amoebae during the multicellular phase is likely to be a natural phenomenon, and we should expect that selection will have produced strategies to cheat in chimeras. In this case, *D. discoideum* might serve as an excellent model organism for the evolution of social interactions (Strassmann *et al.* 2000; Queller *et al.* in press).

A demonstration of chimerism in nature would ideally look at the genetic composition of slugs and fruiting bodies formed in nature. However, there appear to be no reports in the literature of fruiting bodies in the field. John Bonner, who has worked on *D. discoideum* for over 50 years, reports that Lindsay Olive saw sorocarps in the field, but knew of no other instances (JT Bonner, pers. commun.).

The usual way to collect dictyostelids is to rear populations out from soil samples (Cavender & Raper 1965a). Buss (1982) showed that two clones of *D. mucoroides*, distinguished by their distinct and heritable morphologies, occurred in the same small soil sample. One of these was stalkless, and it cheated the other in chimeras. However, phenotypic markers will distinguish only the most radically distinct clones. Francis & Eisenberg (1993) used molecular markers to demonstrate that there is extensive variation among genotypes collected from nature. However, because their questions did not centre on chimerism, their study did not address the question of how often distinct clones occur close enough to co-aggregate.

The main goal of our study is to determine whether different clones live in close enough proximity to one another in nature that they would be likely to form chimeras. We collected very small soil samples and plated them out completely, in an effort to derive isolates from every cell or spore in the sample. Normally only a few isolates are collected per soil sample, representing too few cells to form a fruiting body. However, under favourable conditions, these few cells would vegetatively divide into the same number of clonal populations of amoebae, which could then mix and join in the same aggregations. Thus, instead of genotyping fruiting bodies directly, we are genotyping cells that co-occur at the small scale required for eventual co-aggregation.

Materials and methods

Sampling

Soil was collected at Bald Knob (37°22'287" N, 80°31'04.2" W) near The Mountain Lake Biological Station in Virginia. It is

a red-oak, red-maple forest on a north facing slope at 1265 m. *Dictyostelium discoideum* is common in Appalachian forest soils (Cavender & Raper 1965a,b,c; Cavender 1980), including our site (Landolt & Stephenson 1986; Stephenson & Landolt 1996). We collected samples at 1-m intervals along a transect on 15 October 2000.

Because our primary goal was to determine how often different clones could co-aggregate and form chimeric sorocarps, we sampled on a small scale, using 6 mm diameter plastic straws. We plunged each about 5 mm into the decaying leaves and soil, after first removing the layer of largely intact dry leaves. The average weight of these soil samples was 0.2 g (range 0.08–0.47 g). In order to be able to also assess a somewhat larger scale of possible co-aggregation, we collected pairs of adjacent soil samples (two straws inserted simultaneously). Sample pairs were collected at 1-m intervals along the transect. We immediately closed sample-containing straw pieces in plastic tubes. Samples were stored at 4 °C and plated out the next day.

Isolation of dictyostelids

We plated 25 pairs of samples from 25 m of the transect. We added 1.5 mL of distilled water to each tube and shook the contents to suspend the soil in the water. We then equally partitioned the soil–water suspension from each sample among four different Petri dishes, using SM/10 medium (glucose 1 g, Oxoid Bacto peptone 1 g, yeast extract 1 g, MgSO₄·7H₂O 0.2 g, KH₂PO₄ 1.9 g, K₂HPO₄ 1 g, Bacto agar 20 g, H₂O to 1000 mL for 40 Petri dishes). To each plate we added and spread 100 µL of liquid suspension of bacteria (*Klebsiella aerogenes*) as a food source. SM5 is a standard medium for culturing *D. discoideum*, whereas the twofold dilution of nutrients in SM/10 helps suppress growth of nondictyostelids.

Three to five days after plating, we observed distinct areas of dictyostelid growth. Areas of dictyostelid growth are identifiable by decreased density of bacterial film and later by formation of aggregations and fruiting bodies. We

monitored these plates and, as potential *D. discoideum* clones grew, we isolated and transferred each to a separate plate prepared with SM/5 medium to isolate it from other dictyostelids, as well as fungi and other organisms. The process was repeated if necessary. Each isolated area of growth on a plate is assumed to derive from a distinct amoeba or spore present in the soil. Isolates from different plates are certain to be from different amoebae or spores in the soil sample.

Genetic analysis

We obtained a total of 102 *D. discoideum* isolates, deriving from 26 of the 50 straw samples. We archived a frozen sample of spores from each isolate. To obtain DNA for genotyping, we collected the spores contained in the sorus of one fruiting body of each isolate. We extracted the DNA in 150 µL of 5% Bio-Rad Chelex-100 after grinding the spores, running the samples in a PTC-100 programmable thermal controller (step 1: 56.0 °C for 4 h; step 2: 98.0 °C for 30 min). We amplified five microsatellite loci (Table 1) using fluorescently labelled primers in a polymerase chain reaction (PCR) (44 cycles: 95 °C for 30 s, annealing temperature for 30 s, 72 °C for 30 s). We analysed the product using an ABI Prism® 3100 Genetic Analyser.

Most isolates showed clear single peaks for each locus. A few isolates showed indications of multiple peaks, suggesting that more than one clone might be present. These were plated again at low density and sorocarps were re-genotyped. This procedure succeeded at separating the two mixed clones, which were then considered as separate isolates.

Chimeric mixing of co-occurring clones

To confirm that isolated clones from the same small soil sample can form a genetic chimera we mixed spores of two clones (five sori for each clone) from the same soil sample in a suspension of 200 µL of *K. aerogenes* bacteria. We then

Locus	No. of repeats	Size (bp)	Annealing temp. (°C)	Forward and reverse primers (5'–3')
<i>Dict5.AAC</i>	20	240	46	GATAGAGAACTGACACTTGGG GGTGGAGCTTTATTGTCTACC
<i>Dict13.CAT</i>	18	157	49	CCCCTTTTACTTTTTGACAC CCAACAACCTATAACCTCATC
<i>Dict19.AAC</i>	14	173	48	GCTTGATTTGCCAATAGTTC TCAAACCTGATCCATTACC
<i>Dict23.AAC</i>	49	240	49	TCATTCAACACCACCAACATC AGTAACAGATGGGCAGAGTTATTAC
<i>Dict25.AAC</i>	27	210	49	AGAGCCACTCATTATCTATTCC CACAACTATCACTAGAAACTG

Table 1 Microsatellite primers used in genotyping. Number of repeats and product size are for the initial sequence

plated the mixture on a Petri dish prepared with SM/5 medium. We repeated this for 10 different pairs of clones, each pair coming from a different soil sample. Subsequently we picked up and analysed eight pseudoplasmodia from each of the mix experiments. We extracted the DNA in 150 μ L of 5% Bio-Rad Chelex, amplified two different microsatellite loci using PCR, and analysed the products with the ABI Prism® 3100 genetic analyser.

Clonal identity and relatedness estimation

Our choice of the 6-mm scale was based on the fact that mixing of clones could occur not only during aggregation itself, but also during the vegetative growth stage preceding it. If the soil sample had been left in place, fruiting would require a considerable growth in population, for example after a flush of resources. Each of the cells present would have multiplied and spread out, mixing in the process. Amoebae move at $\approx 5 \mu\text{m}/\text{min}$ on agar (RH Gomer, pers. commun.) and thus could travel about 7 mm in one day, which is more than the diameter of our soil samples, though presumably movement on soil is slower.

Relatedness was estimated (Queller & Goodnight 1989) using the Macintosh program RELATEDNESS Version 5.0.5 (<http://gsoft.smu.edu/GSoft.html>). Relatedness estimates were averaged by weighting each isolate equally. Because clones are sometimes represented by multiple isolates, this is equivalent to weighting each clone according to its abundance in the soil sample. We estimated standard errors and 95% confidence intervals by jackknifing over loci. Because of the large number of samples, no correction for small sample size bias was used.

In addition to estimating standard relatedness averaged over all five loci, we also calculated a quantity we call haplotypic relatedness (abbreviated by h to distinguish it from the standard r). Haplotypic relatedness measures identity due to clonal identity only. It was estimated using

RELATEDNESS 5.05 in the same manner, but with the five-locus genotypes replaced by the single haplotypic locus. Thus, just as standard relatedness measures identity above chance levels at a given locus, haplotypic relatedness measures identity above chance levels of the entire haplotype. Thus two isolates with the same genotypes at all five loci would have haplotypic identity of 1, and haplotypic identity would be 0 for two isolates with mismatched alleles at any of the five loci.

Results

Genetic diversity of isolates

We isolated numerous *Dictyostelium discoideum* as well as other dictyostelid species from the soil samples. We analysed all 102 isolates of *D. discoideum*, which had a somewhat patchy distribution along the transect (Fig. 1).

We identified a total of 46 different haplotypes. Some haplotypes were found in multiple soil samples (Fig. 1) with the most abundant found in 6 of the 26 samples having *D. discoideum*. However, most haplotypes were found in only one soil sample. For each soil sample with isolates of *D. discoideum*, we have identified between 1 and 6 different haplotypes (Fig. 1). Nineteen of the 26 (63%) soil samples with isolates had more than one haplotype (Fig. 1).

Different isolates from the same plate sometimes showed the same multilocus genotype, so we performed a check to confirm that these were truly different isolates rather than duplicate collections from a single colony, i.e. descended from one founder cell on the plate. We compared haplotypic relatedness within plates ($h = 0.139 \pm 0.070$, $n = 1$ soil samples) to haplotypic relatedness between plates from the same sample ($h = 0.195 \pm 0.067$, $n = 23$), where isolates must be descended from different cells. As these estimates did not differ (t -test, $P = 0.57$), we concluded that our collections within plates, like those between plates, are from different colonies.

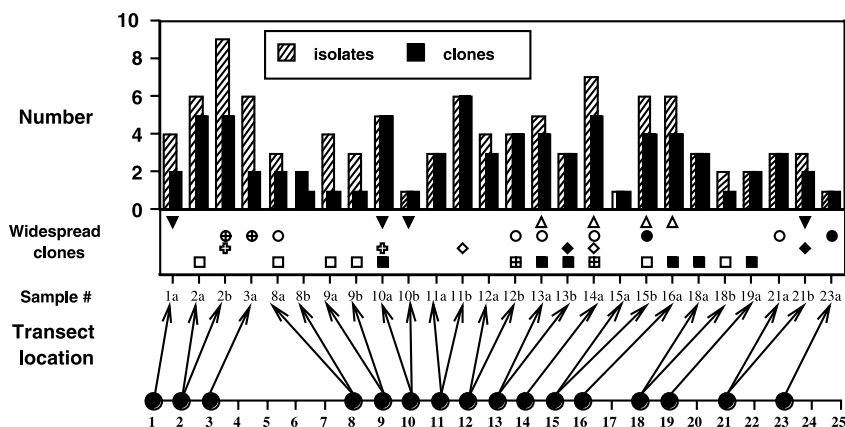


Fig. 1 The number of *Dictyostelium discoideum* clones and isolates from 6-mm diameter soil samples along a 25-m transect. The main panel shows the number of isolates and distinct clones in each sample. The panel beneath it shows which samples shared identical clones; each distinct symbol represents a different clone. The many clones found at only one location in the transect are not shown. The bottom line shows location of samples on the transect.

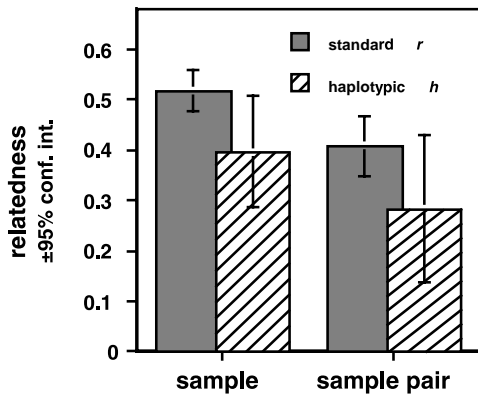


Fig. 2 Genetic relatedness within samples (\pm SE). Relatedness is estimated at two different scales: within 6-mm samples (left) and within two adjacent 6-mm samples spanning 12 mm (right). The dark bars show standard relatedness estimated across five microsatellite loci. The hatched bars show haplotypic relatedness based on treating the entire five-locus genotype as a unit, with identity between any two isolates being scored as all-or-none.

Relatedness

Figure 2 shows standard relatedness and haplotypic relatedness for two small scales, a single 6 mm straw sample, and a combined pair of adjacent straw samples spanning 12 mm. These estimates include each isolate's relatedness to itself. This is appropriate when we want to estimate relatedness within fruiting bodies that would develop from these samples; each of the n cells in a sample will have multiplied many times over, so that for each cell in the resulting chimeric population, $1/n$ of the other cells will be descended clonally from the same ancestral cell.

The estimates of relatedness were intermediate, well below the maximum of one for clones, but well above the random association value of zero (Fig. 2). Standard relatedness was just over half for 6 mm single-straw samples (\pm SE) ($r = 0.519 \pm 0.014$) and slightly lower for the 12 mm paired samples ($r = 0.409 \pm 0.021$). Estimates of haplotypic relatedness were somewhat lower than the standard relatedness for both the single samples ($h = 0.398 \pm 0.054$) and the pairs ($h = 0.284 \pm 0.069$). This suggests that the overall relatedness is not entirely due to clonal identity. This conclusion is also supported by a significant relatedness to nonclonemates in same soil sample ($r = 0.1508 \pm 0.0249$, 95% CI does not include zero). The corresponding estimate for the paired soil samples was also significant ($r = 0.130 \pm 0.023$; 0249, 95% CI does not include zero).

D. discoideum are not highly related at larger scales. We defined three large patches separated by intervening gaps: metres 1–3, 8–16, and 18–23 (Fig. 1). Relatedness to isolates in the same patch, but not in the same sample pair, is not significant ($r = 0.035 \pm 0.023$; 95% CI includes zero). Because *D. discoideum* is haploid, this relatedness estimate

is equivalent to an F_{ST} estimates at the patch level. Relatedness to patchmates is significant if we include the already-noted relatedness within sample pairs ($r = 0.076 \pm 0.018$).

Chimeric mixing of co-occurring clones

Microsatellite genotyping showed that both parental genotypes are present in the pseudoplasmodium when clones from the same soil sample are mixed. For all 10 clone pairs tested, microsatellite genotypes showed both parental alleles.

Discussion

Dictyostelium discoideum fruits cooperatively. Dispersed cells come together, and some altruistically differentiate into dead stalk cells. The main purpose of this cooperative venture is dispersal. The low relatedness estimate at the patch level suggests that dispersal is quite effective over a scale of metres.

Our study also demonstrates that different clones often coexist in the same small soil sample collected in nature. The amount of soil taken is so little in weight and surface area that we expect that these clones would mix when the conditions for fruiting occurred. Such conditions would involve a flush of bacteria allowing population growth, followed by starvation. Mixing of clones would result from both the dispersion of cells during population growth and migration of cells into aggregations after starvation.

If each of the cells isolated from a sample had multiplied and contributed equally to a fruiting body, the relevant relatedness for kin selection is the relatedness among these multiplied cells. This equals the relatedness among the original isolates, including each isolate's relatedness to itself. The average relatedness within the small soil samples was $r = 0.52$ (Fig. 2), which is approximately half the theoretical maximum of 1 for purely clonal samples. If this value were due entirely to clonal identity and nonidentity, it would mean that half of a typical cell's interactants are clonemates. In fact, the haplotypic identity is a bit lower, and there is some relatedness, $r = 0.15$, among different clones in the sample. This relatedness among different clones could be due to the presence of other degrees of relatives such as brothers. However, it is unknown how often *D. discoideum* undergoes sexual meiosis to produce such relatives. Alternatively, this relatedness could derive from clonal descent coupled with some mutations at the microsatellite markers, so that some members of a lineage no longer share alleles at every locus. As microsatellites mutate at high rates, the discrepancy between relatedness and haplotypic relatedness may be higher than it is for the genes affecting cooperation.

This result can be viewed in two ways. First, relatedness among isolates found in the same soil sample is reasonably

high. Thus it is not difficult to understand that cooperation could evolve by kin selection. A cell sacrificing itself in the stalk has a fairly high probability that the cells it helps share the same genes. Indeed, a relatedness of about 0.5 matches the relatedness of altruistic social insect workers to the offspring they benefit, when there is a single unmated queen. However, the two numbers are not exactly comparable. Social insect workers help rear their siblings (average = 0.5) instead of rearing their own offspring ($r = 0.5$). Social amoebae that become stalk cells help others ($r = 0.5$) at the cost of their own clonal offspring ($r = 1$), so it is a bigger genetic sacrifice for them. Still, there are some social insects that are comparable because worker relatedness to brood is lower owing to multiple mating of the queen or to multiple queens (Crozier & Pamilo 1996).

Looking at the results from the other direction, a relatedness of about 0.5 implies considerable potential for conflict within chimeras. Despite the overall cooperation and harmony of social insects, reproductive conflicts are now known to be common and important for understanding the functioning of colonies (Trivers & Hare 1976; Bourke & Franks 1995; Crozier & Pamilo 1996; Queller & Strassmann 1998; Bourke & Ratnieks 1999). We expect the same to be true for social amoebae. Because clone mixing is frequent, any clone that can manage to get preferentially into spores, while leaving its partners to form the stalk, would have a selective advantage. In the laboratory, unequal contribution to spores and stalk is common in chimeras (Strassmann *et al.* 2000) and such competition can lead to a substantial selective advantage (Buss 1982; Ennis *et al.* 2000).

Our estimates should not be taken as an absolute measure of relatedness within fruiting bodies, as several factors might modify them. First, we used five microsatellite loci and it is possible that by using more we could have detected more clones (although this would not be expected to lower standard relatedness). Second, we could also be underestimating the diversity within soil samples if we missed some cells in the samples. We attempted to minimize this problem in two ways: we plated out the entire soil sample rather than just a portion of it, and we suspended the soil in water by gentle shaking, which is less destructive of amoebae than more vigorous methods (Kuserk *et al.* 1977).

Third, to estimate relatedness, we had to assume that each isolate would have contributed equally to the fruiting body. Unequal contribution would tend to increase relatedness. Although we have no way to correct for this bias, we note that it causes an opposite effect to the previous one. Finally, our soil samples may not represent exactly the correct scale. If mixing occurs over a smaller scale, relatedness will increase somewhat. If mixing occurs over a larger scale, it will decrease. Ultimately the question will need to be addressed by finding fruiting bodies in the field and

genotyping them to see how often they are chimeric mixtures. We have found that *D. discoideum* sorocarps can indeed be located in the field, at least in one specialized location: deer faeces. However, even if our estimates are inexact, our more qualitative conclusions are likely to be robust. For both our 6- and 12-mm scales, relatedness is reasonably high, promoting the evolution of cooperation, but it is also low enough to promote the evolution of competitive strategies.

Proximity of different clones in soil samples is relevant only if the clones actually mix to form chimeras rather than exclude each other. A laboratory study (Strassmann *et al.* 2000) of *D. discoideum* clones from North Carolina has previously shown that cells of different clones can join in the same multicellular structure, forming genetic chimeras. Now we have confirmed this finding for clones collected from the same 6 mm soil sample, that is, for clones that are in a position to join in the same multicellular organism in nature.

Our results suggest that *D. discoideum* is truly unusual among multicellular organisms. Not only does it form by aggregation, but such aggregations are likely to be chimeric. Conflict occurs among clones of the chimeric tunicate, *Botryllus schlosseri*, but chimerism is limited because fusion occurs only among clones sharing an allele at a polymorphic recognition locus (Stoner & Weissman 1996). Formally, the *D. discoideum* case is similar to that of clonal aphids that mix across clones and cheat (Abbot *et al.* 2001).

Intense conflicts within chimeras may explain why most multicellular organisms are not chimeric, but instead develop clonally from a single cell (Maynard Smith 1989; Grosberg & Strathmann 1998). But why, then, does *D. discoideum* form chimeras? One possibility is that, under starvation conditions, joining with other cells is the only way to survive. *D. discoideum*, in contrast with some other dictyostelids, has apparently lost the capacity to produce microcysts (Kessin 2001). In natural conditions where the amoebae do not have enough clonemate cells to make a functional slug and fruiting body, joining with genetically different amoebae could compensate for any costs of conflict. Even if there are enough amoebae to form a fruiting body, it may pay to have more cells.

A recent study explored some of the costs and benefits for *D. discoideum* (Foster *et al.* 2002). Laboratory experiments show that chimeric *D. discoideum* slugs migrate shorter distances than do same-sized uniclonal slugs, so they presumably are less able to reach favourable sites. However, there is an advantage that more than compensates. Clones that join in chimeras will have more potential partners than clones that refuse to join, and larger slugs travel farther. Those wild fruiting bodies that we have observed are much smaller than is usual in the laboratory (unpublished data), confirming that finding enough cells may be a problem.

On the basis of these data from the field, it seems clear that the laboratory studies of the formation of chimeras and competition within chimeras (Strassmann *et al.* 2000) are not evolutionarily irrelevant products of the laboratory setting, but rather real components of the natural history of this organism. Our results thus provide additional support for the use of *D. discoideum* as a model organism for the evolution of social behaviour (Strassmann *et al.* 2000; Queller *et al.* in press). This may also be true of other microorganisms with similar life cycles, such as the bacterium *Myxococcus* (Velicer 1998). As a well-studied social organism that is readily investigated using molecular techniques, *D. discoideum* is well suited to the investigation of the mechanisms that underlie cooperation and conflict. In addition, if chimerism occurs in nature, as our results suggest, we expect that many genes will have evolved in the context of competition. As a practical consequence, the functions of some of these genes may prove difficult to fathom when tested under standard clonal conditions, but may be clarified by testing in chimeras.

Acknowledgements

We thank Mountain Lake Biological Station for access to facilities, SL Stephenson for sharing his field site and expertise, and RH Kessin for advice and for help in the field. We thank JT Bonner and RH Gomer for sharing unpublished information, and N Mehdiabadi and D Rozen for comments on the manuscript. Supported by NSF grants DEB-0108478 and DEB-0075581.

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Joan Strassmann and David Queller run a programme investigating the evolution of social interactions. All of the authors have backgrounds in social insect biology, but have begun studying social amoebae as a model system to understand how social evolution works at the molecular level. This work was part of Angelo Fortunato's PhD thesis.
