Selfish cells threaten multicellular life

How far have conflicts at the cell and organism level influenced the evolution of multicellularity? It is often argued that by passing through a single-cell stage during their life cycle, organisms ensure maximal relatedness of their constituent cells. Close genetic relatedness among component cells might be sufficient to preserve the integration of the organism and to reduce selfish trends at the cellular level[1]. However, in some lineages (most notably myxobacteria and the cellular slime mould *Dictyostelium*) the multicellular body develops by aggregation of independent cells. Given that genetically different cells might produce a common fruiting body, mutants that preferentially form spores are expected to have a selective advantage at the cellular level. This possibility is supported by two new experimental findings[2,3].

The life cycles of the prokaryotic myxobacteria and the eukaryotic *Dictyostelium* show astonishing similarities[4]. Under nutrient-rich habitats, these soil organisms are unicellular; however, when food sources become depleted, about 10^5–10^6 cells aggregate to form a common fruiting body. Not all cells become spores: some of them sacrifice their life for improved survival or dispersal of the spores. In slime moulds, these nonspore cells constitute the stalk of the fruiting body; in myxobacteria, these cells autolyse. If spore cells happen to be dispersed to a nutrient-rich habitat, they germinate to give rise to the next generation. Such a system seems vulnerable to exploitation by parasite cells that avoid the stalk fate in mixture with cooperative partners.

This is not only a theoretical possibility. Almost twenty years ago, Leo Buss observed parasite *Dictyostelium mucoroides* variants in nature that avoided the stalk fate in chimeras[5]. However, the molecular mechanism of ‘cheating’ has remained unknown. Two independent research groups working on *Dictyostelium* and on the social bacterium *Myxococcus xanthus*, respectively, gave us a clue about the molecular mechanisms involved in cheating behaviour.

Recently, using insertional mutagenesis, Ennis *et al.*[2] have identified *Dictyostelium discoideum* mutants, which preferentially form spores in chimeras with wild-type cells. From the perspective of the wild type, co-aggregation is an infection with parasitic cells. This research group has elaborated an efficient selection method to enrich the cheater variants in the population. It has been revealed that the rate of increase of the cheater was astonishingly rapid, thus suggesting that the mutant does not simply fail to produce stalks. Rather, the mutant cells seem to change the fate of wild-type cells that would otherwise contribute to spores. Although some details are still obscure, the protein coded by the mutant gene seems to be a crucial regulator of cell fate. The authors speculate that the mutants produce large amounts of a putative signal molecule relative to the wild-type cells. If the signal is an inducer of stalk fate, then cheaters force wild-type cells to convert to stalk cells. Of course, the authors do not claim that the mutant is the only possible cheater or that it would survive in the wild.

The potential for developmental cheaters is not restricted to slime moulds. Velicer *et al.* examined *Myxococcus xanthus* (myxobacteria) mutants that were defective for fruiting-body development[3]. A large number of them showed antisocial behaviour: they produced mainly spores in mixtures with nonmutant cells. It has also been revealed that clones kept under nutrient-rich environments for 1000 generations lose their ability to produce spores in chimeras. Because of the relatively fast loss of social behaviour during the experiment, natural selection might have acted to get rid of functions that were both unnecessary and costly in a nutrient-rich environment.

The ease of finding cheater cells under laboratory conditions and the fact that even a single mutation can lead to this phenotype in both species[2,3] suggests that cheaters can appear in nature. Furthermore, selection procedures used in the experiments indicate that, at least under certain conditions, selection at the cell level favours the spread of cheaters[2,3].

Are cheaters common in nature? To answer this question, we should know how often chimeras form a common fruiting body. If fruiting bodies in nature are clonal, then nonspore cells would be as altruistic as our liver cells would be to our germ cells. Accordingly, kin selection could maintain the evolutionary stability of the observed ratio of spore and nonspore cells. Unfortunately, the data on the population genetic structure of aggregating organisms are still scarce. However, some data suggest that multicellular bodies might at least sometimes develop from mixtures of genetically heterogenous individuals[5–7].

Selection at the cell and aggregate level

If chimeras are frequently formed, then one might wonder how multicellular development could have been maintained during evolution, in spite of the stream of cheaters. One possible solution to this paradox is that although cheaters can invade, they cannot sweep to fixation. This might occur if the advantage at the cellular level is counterbalanced by the reduced performance of the aggregate. This idea is strengthened by the work on *Myxococcus*[3]. First, when mutants were introduced at low frequencies they gained a disproportionate success in becoming spores. Second, when cheaters were present at a sufficiently high frequency, then spore production was remarkably reduced. This suggests that wild-type cells were unable to compensate further for the deficiencies caused by the mutants. Accordingly, wild-type variants might regain their advantage when cheaters become dominant, possibly leading to stable equilibrium of the two types. It would be important to know whether rare, developmentally proficient genotypes are able to invade a population of antisocial bacteria that were previously raised in a nutrient-rich environment.

There could be other factors limiting the spread of cheaters. Previously, it has been revealed that mixed cultures of *Dictyostelium* possess a significantly higher spore to stalk ratio[8]. One might speculate that aggregates with more cheater cells possess shorter stalks, thus their dispersal efficiencies remain limited. This might influence the evolutionary dynamics of cheaters in two different ways: first, aggregates crammed with cheaters cannot escape their depleted habitats and are more likely to go extinct; and second, the limited dispersal of cheaters also reduces the chance that mixed cultures will be formed.

If organisms with aggregating development have to face the danger of selfish variants, they are expected to invent special policing mechanisms to mediate cellular conflicts. Two lines of evidence seem to support this idea. First, in *Dictyostelium mucoroides*, genetically different amoebae frequently fail to coaggregate[3,9]; however, the genetic basis of this system is unfortunately unknown. Second, some mutant *Dictyostelium* strains seem to be resistant against cheaters in chimeras (R.H. Kessin, pers. commun.).

If cheaters do not pose serious costs on aggregate performance, then they are expected to be fixed in the population. After being fixed, no action of cheating is found because all cells are cheaters. Thus, it is possible that the act of selfish

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behaviour remains unnoticed, as long as cheater lines are not mixed with wild-type variants. Perhaps this reasoning can explain the finding that mixed strains of slime mould possess higher spore to stalk ratio[8], as mentioned previously.

The two groups working on *Dictyostelium*[2] and *Myxococcus*[3], respectively, took advantage of experimental selection procedures and molecular tools to isolate cheater variants. Accordingly, they not only established the fact of cheating, but also tried to uncover the underlying developmental mechanisms. It should be emphasized that, in principle, many different mechanisms could result in selfish phenotypes in a single species. Indeed, in the case of *Myxococcus*, five distinct selfish forms were isolated. Moreover, Velicer *et al.*[3] have also measured frequency-dependent fitness of the cheater lines.

What are the implications of these findings on multicellular evolution in general? One common feature of the major transitions in life is the danger that selection at the lower level will disrupt integration at the higher level[1]. Many theoretical and experimental papers deal with conflicts at the genetic or individual level. However, the idea that selfish cells might have influenced the evolution of multicellularity has remained controversial. Theoretical works on the subject largely concentrate on organisms developing from a single cell[10]. However, it is obvious that the chance for selfish variants is much more pronounced when genetically heterogenous cells aggregate to give a common body. Further research on the developmental biology and ecology of aggregating organisms will probably shed light on particular solutions to this challenge.

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Achievement and challenge

What are the key challenges in ecology for the next millennium and what have been our main achievements? At a joint symposium of the British Ecological Society (BES) and the Ecological Society of America (ESA)*, extended talks by nineteen prominent ecologists, together with many outstanding contributed posters, addressed these two questions.

The range of scales and approaches discussed was huge, although marine systems were barely touched upon. Richard Lenski (Michigan State University, East Lansing, USA) described predator–prey interactions between a phage and a bacterium in small flasks, which he has run for 20 000 generations. His experiments elegantly allow the rates of adaptation and constraints on adaptation to be measured, in ways that would be quite impossible in most other systems. By contrast, Jim Ehleringer (University of Utah, Salt Lake City, USA), John Grace (University of Edinburgh, UK) and Ian Woodward (University of Sheffield, UK) considered problems of global carbon (C) balance at the scale of the biosphere. Four central questions emerged:

- What is the impact of global climate change on ecological systems, how will the systems respond and how should we respond?
- What is the probable impact of loss of biodiversity and complexity on ecosystem function, and how can biodiversity losses be minimized?
- How should we build on the links between ecology and other areas of science?
- Following from the above, how do we ensure that our scientific knowledge translates into appropriate policy actions?

All presenters showed that our achievements as ecologists are substantial, but John Lawton (Imperial College at Silwood Park, Ascot, UK) made it clear that communication with decision makers is a major challenge. Whilst we have unequivocal evidence that there will be major global changes in whole ecosystem function in the next 50 years, it remains difficult to make politicians take the problem seriously.

Global warming, CO_2 and biodiversity

John Grace used evidence from ice-cores to show that CO_2 levels in the earth's atmosphere have increased from about 280 ppm in preindustrial times to about 350 ppm at present. The puzzle is that the problem should be worse: fossil fuel burning and deforestation add about 8.0 gigatonnes (gT) of C to the atmosphere annually, but the atmospheric stock increases by only 2.0–3.0 gT annually. What the C sinks are and whether (and at what level) their capacity is limited are key questions (see also pp. 332–337 of the August issue of *TREE*). Grace showed, using ingenious automated devices that measure vertical air movement and CO_2 levels above forests, that even mature rainforests accumulate C at rates of perhaps 5.0 $\mathrm{T\ ha^{-1}\ y^{-1}}$, although a forest in steady state should be neutral in C accumulation. Soils probably do accumulate C, but forests eventually burn or are otherwise destroyed. On a sufficiently large scale, the steady-state assumption for undisturbed forest is probably reasonably accurate. More worryingly, it seems that the temperature rate constant (Q_{10}) for decomposition exceeds that for photosynthesis: minor global warming might turn C sinks in forests into sources. Furthermore, there is a huge stock of C in northern hemisphere peat and the

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