Precarious development: The uncertain social life of cellular slime molds

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Cellular slime molds are enigmatic and fascinating organisms whose life cycles of both single and multicellular stages have long charmed and perplexed biologists (1). Increasingly, cellular slime molds—of which the most widely studied is *Dictyostelium discoideum*—have been used as models to study the maintenance of cooperation, but viewed from this perspective, there are puzzles to resolve. Two recent papers (2, 3) reveal a hitherto unrecognized aspect of *D. discoideum* life history that prompts a rethink of commonly held views concerning the sociobiology of this and related slime molds.

D. discoideum lives in soil, decaying leaves, and on animal scat, where it exists for the most part as individual amoeboid cells that feed on bacteria. When starved, the single cells—of sometimes diverse genotypes—aggregate to form an often chimeric multicellular slug that undergoes morphogenesis and differentiation culminating in the production of a mass of spores suspended at the tip of a stalk. Spores presented in this way are readily dispersed by soil invertebrates. Given appropriate conditions, spores germinate, produce amoebae, and thus complete the life cycle. No such possibility for regeneration is provided to stalk cells: cells fated to become stalk die in the process of fruit body formation (1).

The seemingly self-sacrificial nature of stalk cells is interesting from both developmental and evolutionary perspectives. In the context of multicellular organisms more generally, self-sacrificial behavior is nothing new. In metazoans, for example, cells that comprise soma are, like the stalk cells of slime molds, an evolutionary dead end. The primary purpose of soma is to ensure perpetuation of the germ line; indeed, development of soma is crucially dependent on apoptosis. However, there is a big difference between multicellularity in metazoans and multicellularity in slime molds that makes cell death understandable in metazoans but less so in slime molds. This difference concerns the mode of formation of the multicellular entity

(4). In metazoans, the multicellular stage typically arises from a single cell (a fertilized egg). In slime molds, the multicellular stage is an aggregate of sometimes unrelated genotypes.

From an evolutionary perspective, development of a multicellular entity from a single cell ensures that cells of the organism are derived from a single genotype (thus ensuring perfect relatedness) and limits the possibility that conflicts arising during the life time of the individual are passed to future generations (5). In slime molds, formation of the multicellular entity by aggregation of different genotypes is a sure-fire recipe for conflict (6): in any given chimera, selection is expected to favor those types that take unfair advantage of altruists (those genotypes that invest more in stalk cells). Such “cheater” types threaten persistence of altruists. How self-sacrificial behavior is thus maintained in the face of conditions that would seem to select for its elimination has motivated numerous studies (7).

The likelihood of conflict is more than just a theoretical notion. Studies going back several decades provide experimental evidence that cheats exist in nature (8), and linear hierarchies of competitors have been reported (9). Such interactions should limit genetic diversity in natural populations, but this is at odds with reports of high diversity and coexistence of genotypes (10).

Recognizing Loners

Motivated by the paradox of diversity, Tarnita et al. draw attention to a hitherto overlooked aspect of *D. discoideum* life history: nonaggregating “loner” cells. Loners are amoeba that do not aggregate with the mass of starving cells and therefore do not become part of the developing slug. Although the existence of such loners has long been recognized (9) [they were even incorporated into an early model of cheater avoidance (11) and more generally into models of cooperation (12)], their existence in the context of

D. discoideum has been ignored because it was assumed that failure to join the slug meant death. This is not so. Dubravcic et al. (2)—confirmed by Tarnita et al. (3)—show that not only are loners viable, but when provided with a source of nutrients, they begin to multiply. Moreover, when further starved, the majority of amoeba aggregate and produce slugs.

Uncertainty and Bet Hedging

Formation of slugs and subsequent fruit bodies occurs under starvation conditions and results in spores that survive harsh times. Although this makes sense, the decision to follow such a developmental path—a largely irreversible commitment that takes ~18 h to complete—is not without risk. The commitment is necessarily made on the basis of knowledge of the present state of the environment, but the present state does not always accurately predict the future. The cost of making a wrong decision (commitment to sporulation when in fact minutes later the nutrient status of the environment is replenished or vice versa) is likely to be high. Given the risk involved, it would make sense for *D. discoideum* to hedge its evolutionary bets (13)—for commitment to sporulation to be underpinned by a stochastic switch that resulted in generation of both aggregating and nonaggregating cells. Such a behavior would mean that some cells commit to a strategy that is at odds with the current environment on the off chance that in some future state of the environment that strategy is optimal.

A compelling case exists for loners being a component of *D. discoideum* life history and that together loners and aggregating cells constitute a bet hedging strategy (2, 3). Recognized as such, Tarnita et al. argue that variation in the rate at which different genotypes generate loners can explain both chimeric slugs and the paradox of diversity.

Central to the claim that chimeric slugs are replete with social interactions—in which cheats take advantage of altruists—are experiments in which equal ratios of starving

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See companion article on page 2776.

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amoeba of two different types are allowed to aggregate to form slugs. After spores are formed, the ratio of the two genotypes is determined by counting spores. A bias in favor of one genotype indicates a reproductive skew (10, 14) that is typically assumed to arise as a consequence of interactions within the slug: one type gains advantage by avoiding placement within the stalk. However, Tarnita et al. suggest that such a skew can be explained by differences in the relative investment in loner vs. aggregating cells (see figure 2 from Tarnita et al.). Their conjecture depends on the assumption that ecological interactions among competing types are neutral: an assumption they argue is not unrealistic given experimental difficulties associated with tracking cells within the slug stage.

In terms of a solution to the diversity problem, Tarnita et al. (3) draw on long-established theoretical and experimental studies in ecology that show how fitness tradeoffs—in this case between investment in spores and nonaggregating cells—can result in the coexistence of strategies in spatially and or temporally varying environments. The variation relevant to the ecology of *D. discoideum* is considered as differences in the rate of resource replenishment. Crucially for the maintenance of diversity, Tarnita et al. show that environmental patches need to be connected by weak-to-moderate dispersal.

Are Tarnita et al. correct? Evidence that loners are part of the *D. discoideum* life history is compelling. That loners and aggregating types together constitute a bet-hedging strategy parallels similar strategies in other organisms (15). What remains unclear, however, is the extent to which incorporation of loners is an exclusive explanation for diversity. A central assumption is lack of interaction among genotypes within slugs. Such an assumption may seem presumptive and even provocative, but to some extent it doesn't matter: the value of this theoretical analysis shows that it is not necessary to invoke social interactions within chimeric slugs to explain differences in reproductive output among *D. discoideum* genotypes. Tarnita et al. present a valid and plausible null model: a model that will undoubtedly fuel new experimental studies.

Herein there lies an additional important and general point: ecology matters and its incorporation can significantly alter the understanding of interactions (16, 17). Interactions studied exclusively in the laboratory—as is typical of microbes—can fall short of

capturing the richness that exists in the wild. In this regard, the field of slime mold biology has long held, and continues to foster, work that traverses boundaries between laboratory and field (1, 7, 10).

Loser Cells as Stalk?

However, what of the broader question posed by chimeric *D. discoideum* slugs and the maintenance of altruism in the face of selective conditions that would appear to favor its demise? Tarnita et al. provide few answers, but nonetheless contribute to a sense that there is something yet to explain.

There is no escaping the fact that amoeba fated as stalk cells die and that their death underpins formation of an ecologically significant dispersal structure. Seen from the perspective of the stalk cell, it seems reasonable to ask why certain amoeba would give up their right to autonomous replication. However, perhaps this is the wrong way of posing the question. Too often, development is seen as the cause of its own evolution. A division of labor enables an organism to achieve more than it could were it comprised of a single cell type, but the end does not necessarily justify the means.

What if the distinction between stalk and spore was a consequence of selection for dispersal (18), with the driver being outright competition? Within such a framework, amoeba fated to become stalk cells might be viewed as losers (and not altruists), but losers that nonetheless had equal opportunity to win: life as lottery (19). Formation of spores provides a means of surviving unfavorable

conditions, but spores held above a substrate have an elevated chance of dispersal and thus the likelihood of discovering favorable conditions elsewhere. According to a dispersal-driven scenario, participation is necessary to win, but participation carries the risk of losing. Such a scenario could operate free of the sociobiological dilemmas posed by cooperate and defect, if differentiation of cell types within the slug was underpinned by a significant element of chance such that it were nigh impossible for cheating genotypes to evolve via selection-informed interactions within chimeric slugs.

Interestingly, studies of cell differentiation in *D. discoideum* support the notion that cell fate within slugs is determined to a large extent by the cell cycle stage that each amoeba finds itself in, on receipt of the signal marking onset of the aggregation phase. Cells in early stages of the cell cycle—with low nutrient reserves—are likely to become stalk cells, whereas cells that have completed the cell cycle and carry greater nutrient reserves are more likely to become prespore cells (20). Provided that cell fate is determined before formation of the slug, as studies of cell biology indicate, then opportunities for the evolution of cheating genotypes are likely to be severely restricted.

Irrespective of the appropriateness of this model, recognition that loners are part of the life history of slime molds and that they, in combination with aggregating cells, may constitute a bet hedging strategy is an important advance in understanding the biology of a most distinctive group of organisms.

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