Update

Research Focus

Resolving the first steps to multicellularity

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Multicellular life has evolved many times, yet each origin requires free cells to integrate unselfishly into a higher-level individual. How can such transitions evolve? In a new paper, Herron and Michod investigate the recent origins of multicellularity in colonial algae. Their phylogenetic reconstructions provide a striking dissection of early steps, and altruistic traits are at the crux of it. Key evolutionary reversals are also revealed, where cellular selfishness might have thwarted multicellular integration.

> "*You can break that big plan into small steps and take the first step right away*." Indira Gandhi

The origins of multicellular life

Early life was single celled and multicellularity has arisen dozens of times in the history of the Earth [1]. However, the transition from unicellular to multicellular life presents two difficult problems. The first is the Darwinian dilemma of complex phenotypes [2]. Darwin struggled with the difficulty that some of the most exquisite results of evolution, such as the origin of the vertebrate eye, are often the hardest to explain. Darwin set forth a challenge for biologists to reduce such evolutionary transitions to a series of plausibly small steps. The second dilemma concerns the evolution of cooperation. To understand how multicellularity has arisen, we must explain how individual cells (each with selfish interests) can evolve to relinquish independence and reproduce cooperatively as part of a multicellular organism. Missing data have long been an impediment to addressing these problems. Most evolutionary transitions to multicellularity occurred anciently, and transitional steps have been erased by eons of divergence and extinction [3].

A recent paper by Herron and Michod sheds light on the origins of multicellularity in the volvocine algae (Volvocales) [4]. This lineage of photosynthetic eukaryotes offers an unparalleled opportunity to look back at multicellular origins because multicellularity in this group evolved relatively recently, and there is a near-continuum of transitional types. Volvocine species range from single-celled green algae (*Chlamydomonas*) to undifferentiated four-celled species (*Basichlamys*) to elegant 50 000-cell colonies with functionally specialized cells (*Volvox*) [3,4] (Figure 1). Recently, Kirk proposed a 'twelve-step program' in which he annotated 12 transitional steps to multicellularity for the volvocine algae and hypothesized their evolutionary order [3] (see legend of Figure 1). Herron and Michod then meticulously dissected Kirk's framework with advanced phylogenetic tools and a large DNA data set [5,6]. They employed ancestral state reconstruction [7,8], which allows researchers to look back in time and test hypotheses about ancestral phenotypes.

The 12-step program and Darwin's dilemma

As in any 12-step program, getting started can be difficult. To evolve multicellularity, independent cells must first find each other or remain connected after dividing [1]. Some volvocine colonies exhibit incomplete cell division (step 1 in Figure 1), which creates bridges among recently divided cells [3]. Similarly, production of extracellular matrix (ECM; step 5) can also bind colonies together [3]. Once colonies have formed, genetic control of cell number (step 6) determines the maximum colony population, and an expanded volume of the ECM (step 8) increases colony size. The next steps involve further colony integration: with the emergence of soma (step 9), many cells give up reproduction and specialize on group-beneficial traits such as motility and, with the evolution of a specialized germline (step 10), the germ–soma divide becomes complete. Based on this abbreviated review of steps, the evolutionary transition to multicellularity appears to advance as a smooth increase in colony size and complexity. This presumption of directional progress toward volvocine multicellularity has been long held [9,10], and is found in widely used texts (e.g. Refs [11,12]).

Herron and Michod's analysis began by reconstructing a well-resolved phylogeny of the volvocine algae based on five chloroplast genes [5,6]. Their phylogenetic reconstruction densely sampled the volvocine algae (single-celled and colonial) as well as related unicellular algae. Herron and Michod mapped Kirk's [3] 12 transitional steps onto the tips of their tree, and reconstructed the ancestral transitions using parsimony and Bayesian methods [7,8]. With specific hypotheses about the ancestral phenotypes in hand, Herron and Michod empirically examined the order of evolutionary steps, the number of times innovations evolved and whether reversals occurred (Figure 1).

Consistent with conventional wisdom about the volvocine algae, Herron and Michod found that many of Kirk's steps [3] evolved once and persist into the present [4]. In particular, tracing the evolutionary lineage that leads to *Volvox carteri* – an apex of volvocine multicellularity – Kirk's steps accumulate in stages of increasing complexity and cellular integration [3]. The *V. carteri* lineage thus represents *par excellence* support for the Darwinian notion that complex traits are reducible to evolution by small steps [2].

Sticky cooperators

Yet, Herron and Michod's analysis makes it clear that even with incremental steps, forward progress is not guaranteed. In several volvocine lineages, innovations evolved

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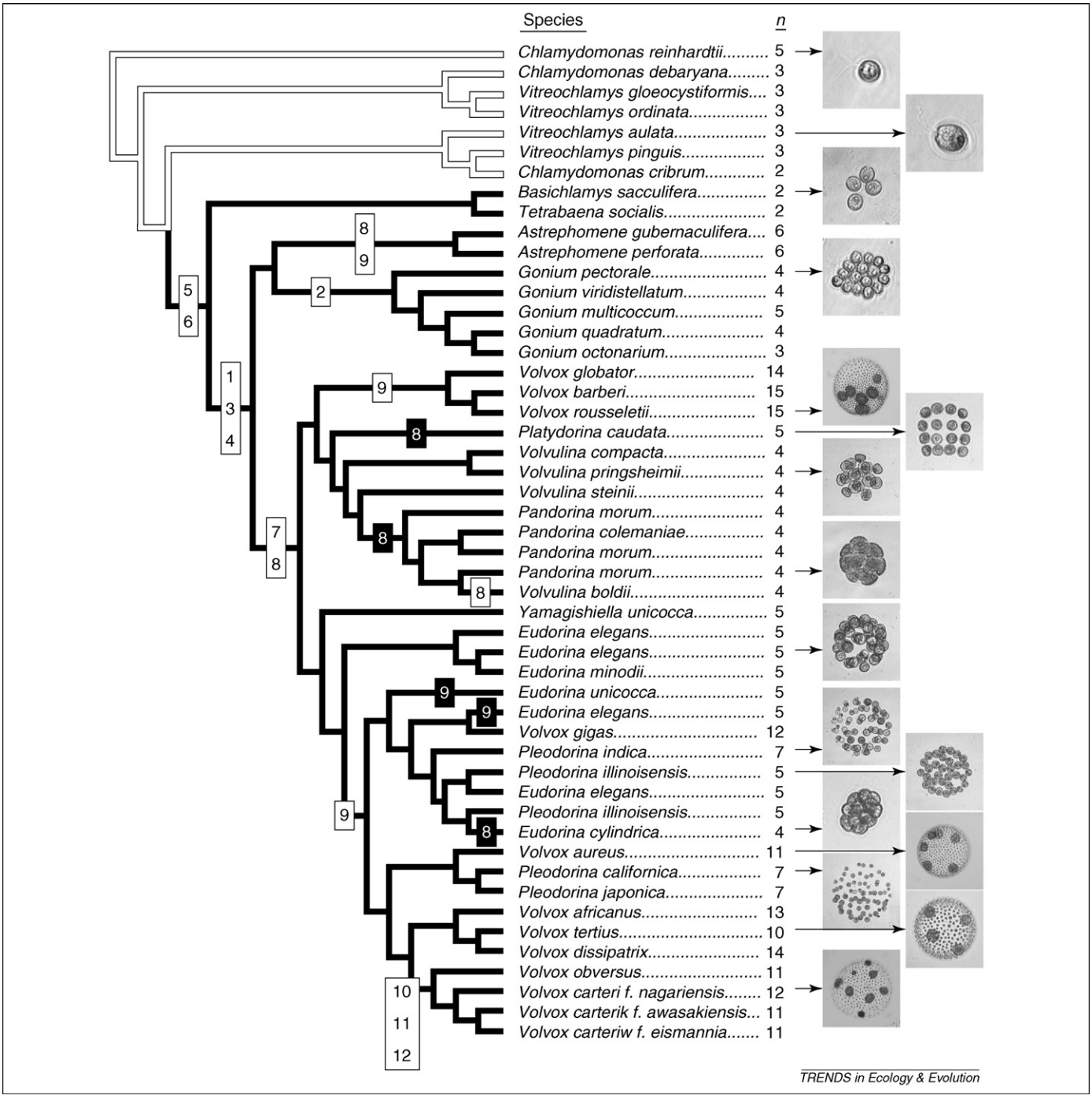

Figure 1. Transitional steps to multicellularity [3] mapped onto a phylogeny of volvocine algae [4]. The 12 proposed steps follow Kirk's original numbering scheme [3]: (1) incomplete cell division, (2) partial inversion of the embryo, (3) rotation of basal bodies, (4) establishment of polarity, (5) production of ECM, (6) genetic control of cell number, (7) complete inversion of the embryo, (8) increased ECM volume, (9) partial germ/soma division of labor, (10) full germ/soma division of labor, (11) asymmetric cell division and (12) bifurcated cell division program. Unicellular algae are on white branches and multicellular species are on black branches. Gains of derived characters (steps) are indicated with black numbers and losses with white numbers. Herron and Michod's analysis of character-state changes estimated that step 8 evolved twice, was lost three times and reevolved once, and step 9 evolved independently in three different lineages and was lost twice. Representative volvocine algae are shown in photos (not to scale): a. *Chlamydomonas reinhardtii*, b. *Vitreochlamys aulata*, c. *Basichlamys sacculifera*, d. *Gonium pectorale*, e. *Volvox rousseletii*, f. *Platydorina caudata*, g. *Volvulina pringsheimii*, h. *Pandorina morum*, i. *Eudorina elegans*, j. *Pleodorina indica*, k. *Pleodorina illinoisensis*, l. *Eudorina cylindrical*, m. *Volvox aureus*, n. *Pleodorina californica*, o. *Volvox tertius*, p. *Volvox carteri*. Adapted from Herron and Michod 2008, Figure 4 [4].

and were subsequently lost over evolutionary time. The expansion of the ECM evolved twice, was lost three times and thence reevolved once. The emergence of somatic cells evolved independently in three different lineages and was also lost twice over time. Hence, certain parts of Herron and Michod's results refute the conventional wisdom that volvocine multicellularity emerges as a simple progression toward increased complexity (e.g. Refs [9–12]). Instead, the evolutionary sequence that they uncovered exhibits both repeated transitions and interesting reversals. What might explain this pattern?

The answer to this question might lie in the second aspect of the evolution of complexity: the dilemma of cooperation. Cooperation among counterpart cells appears

essential for the evolution of multicellularity, as cheaters (that do not contribute to the group) could potentially thwart the benefits of multicellular life [13–15]. Accordingly, Herron and Michod hypothesize that cycles of cooperation, conflict and conflict mediation are ongoing in some volvocine lineages [4].

Early in volvocine evolution, independent cells first united, at which time the unit of selection could shift from individual cells to the nascent group [13]. Herron and Michod's reconstruction suggests that this seminal step involved two innovations: the transformation of the cell wall into the ECM, and genetic control of cell number. Hence, their data suggest that Kirk's first step (incomplete cell division) is not necessarily required to unite cells [4]. Experiments by Hallmann and Kirk are also consistent with the idea that the ECM (and not the cellular bridges created by incomplete cell division) is needed to bind independent cells [16]. Interestingly, recent work has revealed that sticking together is more than just a physical prerequisite to multicellularity: it can be a selective force for cooperation [13–15,17]. For instance, myxococcal bacteria can produce extracellular pili that stick cells together. Velicer and Yu showed that although costly to individuals, this trait greatly enhances the growth of cooperative myxococcal swarms [14]. In fact, when Velicer and Yu created gene-deletion mutants without pili, they found that stickiness reevolved via an independent pathway and cooperative swarming was restored [14]. Experiments by Rainey and Rainey revealed benefits to stickiness in experimental populations of *Pseudomonas*. When grown in heterogeneous cultures, these bacteria mutate and diversify into a range of niche specialists. One mutant type, the 'wrinkly spreader,' produces an adhesive polymer that allows cooperative groups of bound cells to invade the resource-rich air–liquid interface and dominate the population [13]. However, in the case of the wrinkly spreaders, defector mutants (that do not stick together) ultimately take over and the cooperating population collapses. In both bacterial examples, mechanisms that stick related partners together align their fitness interests, and can select against cheaters [15]. However, as the Raineys' work shows, sticking together is not always sufficient, and further conflict-mediation mechanisms might be necessary to keep cheaters at bay [4].

Two steps forward, one step back

How do the patterns of reversal in the evolution of volvocine multicellularity fit the idea of cooperation and conflict? Interestingly, both types of reversal show evidence of breakdown of cooperative structures. The ECM is produced by the individual cells and consists of costly glycoproteins [4] that can provide group benefits such as increased colony size, group cohesion [1] and nutrients [18]. It might be that the contraction of the ECM in three independent volvocine lineages represents successful invasions of cheater mutants that produce little or no ECM. On the other hand, somatic cells have evolved that sacrifice their own reproduction to provide other functions for the colony. The subsequent evolutionary loss of soma in two independent lineages is akin to an orderly bee colony (with queens and sterile workers) experiencing social breakdown resulting in workers taking over reproduction. However, the relatedness among algal colony members is clonal, so unlike the bees the cheaters presumably arise via novel mutations.

Herron and Michod argue that cheating is especially likely to evolve in the large volvocine colonies. Many cells in large colonies bear high costs for cooperation such as loss of reproduction (in soma) and the metabolism of ECM. Hence, the benefits of cheating might be great. Furthermore, the large cell populations of these colonies provide more opportunity for selfish mutations, and also lower the initial impact of defectors [4]. By contrast, Graham Bell has argued that the evolution of soma and greater size (increased ECM) both provide automatic benefits to colonies, because growth efficiency is increased and predation risk is decreased, respectively [18]. If Bell's hypothesis is correct, and these cooperative traits provide automatic benefits (known as byproducts) to their bearers, then cheating is not predicted [13].

Whereas the Darwinian dilemma appears mostly resolved for volvocine multicellularity, the evolutionary roles of cooperation and conflict remain unclear. Future research should focus on the selective forces and molecular mechanisms behind the reversals in volvocine multicellularity. One approach would be to study algal cheaters and cooperators in laboratory populations. For instance, *in vitro* evolution with mutagens could be used to lower relatedness among colony members and promote the evolution of cheater mutants. Competition experiments using different volvocine lineages would also be interesting, as such studies might resolve the benefits of gain or loss of cooperative traits. Finally, genetic studies could attempt to unravel the molecular pathways responsible for the evolutionary reversals in the volvocine lineages. Herron and Michod's work has illuminated a fascinating transition in evolutionary history and has set the stage for many more interesting studies.

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Research Focus

Fight or learn to live with the consequences?

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Individuals can fight their infectious diseases by reducing the growth of a pathogen (resistance), but they can also ameliorate the disease it causes (tolerance). A recent paper shows that there is variation between mouse strains in tolerance to a rodent malaria and that this was negatively correlated with resistance. This is important, because tolerance has major implications for the epidemiology and coevolution of host–parasite interactions, but has been neglected in the animal literature.

Defence against parasites: tolerance and resistance

We tend to think about hosts 'fighting' their parasites by either preventing infection in the first place, or trying to control them and potentially recover if they infect. All of these defence mechanisms – avoidance, control or recovery – are classified as forms of resistance [1,2] because they all lead to a reduction in the fitness of the parasite. However, of course, the host can also simply fight the 'disease' the parasite causes rather than the parasite itself. In other words, selection can act on mechanisms that reduce the damage that the parasite causes, allowing hosts to tolerate the infection. Researchers working on plant defences to natural enemies have shown the importance of such tolerance mechanisms, particularly in response to herbivores [3,4] but also to parasitic infection [5]. However, there has been very little empirical work in animals on the role of tolerance in the response to infectious disease. Råberg and colleagues [6] have recently reported significant variation in tolerance to malaria in a rodent model system and furthermore shown that this tolerance is negatively correlated with resistance. The study is important because tolerance has very different ecological and evolutionary implications for resistance, but has not received much attention in the animal infectious disease literature.

Why is tolerance important? What theory tells us

So why does it matter whether hosts tolerate rather than resist their parasites? The reason is that there is a fundamental difference in the ecological feedbacks that occur as tolerance evolves. Tolerant individuals if they live longer when infected increase the infectious period of the parasite. This means that, as a gene that reduces the death rate of infected individuals spreads through a population, it will tend to increase the prevalence of the disease. This is in stark contrast to any form of resistance that, by definition, reduces the fitness of the parasite, meaning that as resistance genes spread they reduce the prevalence of the parasite. Theory tells us that there are important evolutionary implications for these patterns, not least that tolerance is likely to become fixed in populations once it starts to spread because of the positive frequency-dependent selection outlined above [7]. There are also complex coevolutionary implications for tolerance, such as the parasite evolving in response to the fixation of the parasite, and potentially causing more deaths [8]. The difference between resistance and tolerance is therefore a fundamental one, and the study by Råberg and colleagues suggests that it is one that we need to take into account also when studying animals.

How do you measure tolerance?

A key issue that Råberg and colleagues address is how to reliably measure tolerance. A reduction in virulence in the host is not necessarily tolerance, because it might be the result of a mechanism that reduces the growth rate of the parasite. By contrast, tolerance can be measured as the amount of damage caused to the host for a particular parasite burden. Råberg and colleagues show that by plotting parasite burden against host health, we can demonstrate when genotypes vary in tolerance, resistance, or both at the same time (Box 1). This simple analysis, originally developed in the plant–pathogen world [9], can be applied to many other systems, and indeed many workers may well have such data available. Råberg and colleagues have presented the framework in which to analyse these data for the animal pathogen world. If you have, or can acquire, data on parasite burden versus a host health measure, you can look for variation in tolerance.

There is significant variation in tolerance, and it correlates with resistance

Råberg and colleagues used the approach of plotting parasite burden against host fitness to look for significant

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