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Selective drivers of simple multicellularity

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In order to understand the evolution of multicellularity, we must understand how and why selection favors the first steps in this process: the evolution of simple multicellular groups. Multicellularity has evolved many times in independent lineages with fundamentally different ecologies, yet no work has yet systematically examined these diverse selective drivers. Here we review recent developments in systematics, comparative biology, paleontology, synthetic biology, theory, and experimental evolution, highlighting ten selective drivers of simple multicellularity. Our survey highlights the many ecological opportunities available for simple multicellularity, and stresses the need for additional work examining how these first steps impact the subsequent evolution of complex multicellularity.

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Introduction

Multicellularity has evolved numerous times in distinct lineages [1]. From simple beginnings of small, simple clusters of cells, multicellular organisms have evolved spectacular, diverse forms, underpinning the evolution of novel ecosystems (e.g. forests, grasslands, and coral reefs) and transforming life on Earth. To understand how multicellularity evolves, we must understand how its initial steps, the evolution of simple multicellular groups, occur.

The vast majority of unicellular linages never evolve to be multicellular, presumably because the potential benefits of multicellularity do not outweigh its costs (i.e. nutrient/oxygen diffusion limitation, etc.). Yet multicellularity has evolved many times in distinct lineages inhabiting markedly different ecologies. In this review, our goal is to describe key selective drivers of simple multicellularity, including both those underpinning the origin of multicellular group formation itself, and traits made possible by a multicellular life cycle (Figure 1).

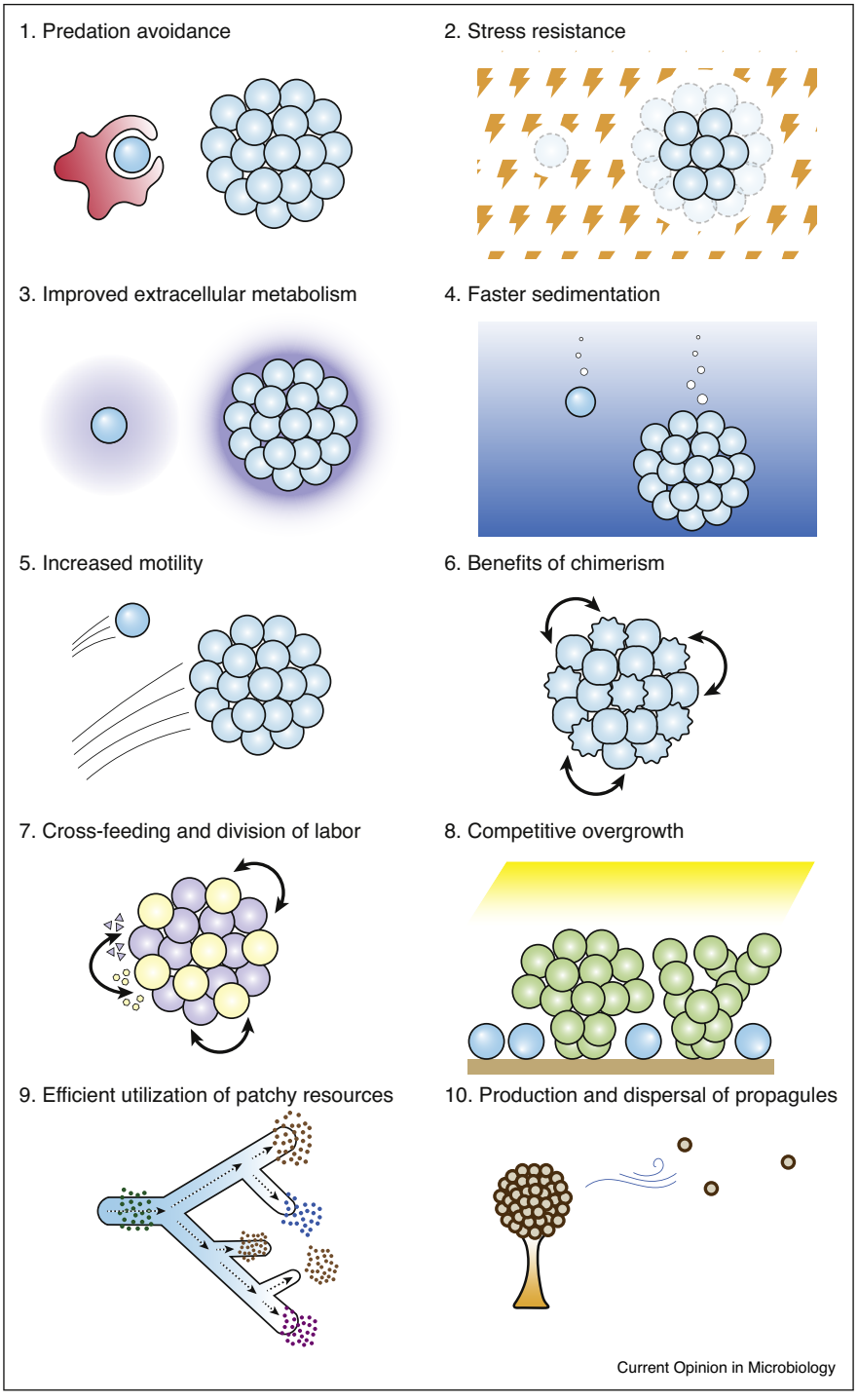
In this review we focus on 'simple' (in quotes, because this is a subjective classification, though here we use the heuristic of few cell types [2]) multicellular groups because it is a crucial stage of multicellular evolution: most multicellular lineages never evolve to be complex, and those that do necessarily started out simple. These multicellular groups are quite diverse: they may form clonally or aggregatively, obligately or facultatively (i.e. as an optional, often environmentally induced life history stage), and they may have little to no cellular differentiation. We have roughly ordered the selective drivers of simple multicellularity from those that may drive the origins of group formation itself, to adaptive multicellular traits made possible by the prior evolution of a multicellular life history stage (e.g. cellular differentiation). How these drivers act in a specific lineage depends on its organismal and ecological context. Some drivers may be quite general (e.g. predation avoidance and stress resistance), while others tend to be more lineage-specific (e.g. production and dispersal of propagules). Importantly, not all the organisms we consider in this review are multicellular. Our focus is on the selective drivers of simple multicellularity themselves, and many of these benefits apply to groups of unicellular organisms as well as those which have made the transition to multicellularity. Finally, this review is not intended to be exhaustive; indeed, this field is rapidly developing, and we anticipate that future discoveries will add to this body of work.

Evolutionary drivers of simple multicellularity

Predation avoidance

Predation of unicellular microbes by protozoan or metazoan predators creates a strong selective pressure for traits that enhance their survival. A simple but effective defensive strategy is to form cellular groups that are too large to be eaten by the predator. Indeed, many unicellular microbes (e.g. algae and bacteria) facultatively form predation-resistant multicellular groups when predators are present (often by sensing predator-secreted chemicals), while others constitutively express simple multicellular forms [3–5]. These multicellular structures vary in morphology (e.g. filaments, sheets, spheroids, irregular clumps) and mode of group formation (i.e. incomplete cell separation, aggregation, or both) [3,4,6,7].

Figure 1



Ten selective drivers of simple multicellularity.

Continuously co-culturing unicellular algae [8–10] or fungi [11] with a predator has been shown to drive the evolution of simple multicellularity within weeks to months, which persisted even when the predators were removed. Paleontological records and phylogenetic analyses suggest that the evolution of unicellular (and later animal) predators in the Neoproterozoic may be a powerful driver of the origin and/or subsequent evolution of multicellularity in animals, green algae and red algae [12,13]. However, larger predators [6,14•] and raptorial

protozoan predators [15••] may in fact prefer colonial prey, thus the effect of group formation against predation may depend highly on the predator composition in the surrounding environment.

Stress resistance

Group formation is a widely evolved strategy for dealing with environmental stress, as groups provide shelter for internal cells. In the yeast *Saccharomyces cerevisiae*, flocs (formed aggregatively) [16], lab-evolved snowflake-like clusters (formed clonally) [17] and small globose clumps in a wild strain (formed clonally) [18] all survived better under chemical stresses than single cells, and multicellular clusters also evolved under lab adaptation to low pH (in lactic acid but not in hydrochloric acid) [19]. This benefit may stem from physical shielding via diffusion limitation, and/or physiological changes of constituent (especially internal) cells that increase stress resistance [16]. Similarly, the unicellular green alga *Chlamydomonas reinhardtii* forms small palmelloid colonies or large aggregates when exposed to nutrient deficiency, salt stress, low or high pH, oxidative stress, heat shock, and so on, with larger groups being more stress-resistant [20–22]. Moreover, forming biofilms (surface-attached aggregates of microbes) is a widespread multicellular solution across the unicellular tree of life to withstand various physical, chemical and biotic stressors, often mediated by concerted actions of multiple underlying mechanisms [23,24]. In aggregative multicellular organisms (e.g. the cellular slime mould *Dictyostelium discoideum* and the myxobacterium *Myxococcus xanthus*), cells typically feed and proliferate in the unicellular stage, and actively aggregate when stressed to form multicellular fruiting bodies that contain aerially lifted spores or cysts [25,26]. It is hypothesized that active aggregation into simple cell mounds may evolve before fruiting bodies, likely driven by its rapidly conferred sheltering benefits against stresses [27•].

Improved extracellular metabolism

Group formation may increase the efficiency of extracellular metabolism by both increasing the local concentration of extracellular metabolites and reducing their loss due to diffusion. Cells in a group may often be closely related (i.e. if the group develops clonally, or if aggregation occurs in a structured environment), limiting exploitation by non-productive cheats. Koschwanez *et al.* examined this using the yeast invertase system [28•], a well-studied model of microbial cooperation [29]. In yeast, the cell-wall localized invertase enzyme breaks down extracellular sucrose into glucose and fructose. However, invertase-producing cells cannot capture a significant fraction of the glucose and fructose as they diffuse away from the focal cell [30], inhibiting growth on low sucrose in diffusely populated unicellular populations [28•]. When Koschwanez *et al.* [31•] experimentally evolved unicellular yeast in low sucrose for 35 days, all replicate populations evolved into undifferentiated clonal multicellular groups that were able to grow in this low-resource environment by increasing the extracellular glucose concentration to sufficiently high levels. Furthermore, multicellular yeast efficiently privatized these extracellular common goods, preventing the invasion of non-producing cheats, whereas unicellular yeast performed poorly against cheats. Since extracellular enzymes predate multicellularity in fungi [32] and are widespread across microbes [33], including aggregative and filamentous species of multicellular bacteria [34,35], selection for efficient extracellular metabolism may be a common route to multicellularity.

Faster sedimentation

By Stokes' law, organisms sink faster with increased size and density. Group formation increases size and thus sedimentation rate, allowing aquatic organisms to reach deeper positions in the water column to access different nutrients or evade predators. A recent study showed that in the ichthyosporean genus *Sphaeroforma* (close relatives of animals, with a transient multicellular stage), sedimentation rate is a variable and evolvable trait affected by multicellular size and density, which may be used to control the position of these immotile organisms in the water column [36•]. In particular, continuously selecting *Sphaeroforma arctica* in the lab for fast settling through liquid media led to the evolution of fast-settling clonal clumps [36•]. Similar settling selection has also been used to evolve multicellularity and increased size in the unicellular yeast *S. cerevisiae* [37,38] (even macroscopic size [39•]) and *Kluyveromyces lactis* [40] and the unicellular alga *C. reinhardtii* [41]. Fast-sedimenting phenotypes also rapidly evolve in human-altered ecosystems, like water-treatment plants [42]. However, faster sedimentation due to group formation is often considered as a cost to phytoplankton, as it reduces their access to light [3,6]. This cost is countered in some species by mobility or buoyancy control, and is alleviated in shallow or mixed water bodies [3,6,43].

Increased motility

Forming cooperative groups is a powerful mechanism to increase organismal motility, which allows organisms to move more rapidly towards favorable environments and away from dangers. Multicellular *D. discoideum* slugs migrate faster than solitary cells, with larger slugs capable of achieving higher speeds, and are able to cross soil barriers that solitary cells cannot [44,45]. In the volvocine algae, colonial species (e.g. *Volvox carteri*) swim faster than their unicellular relative *C. reinhardtii* (also in a size-dependent manner), enabling more sustained access to light and nutrients [43,46]. Interestingly, the sessile stalked cells of the bacterium *Caulobacter crescentus* can become motile by forming aggregates where flagellated cells are intermittently produced through asymmetric cell division and propel the aggregate to roll on the surface

[47]. However, group formation alone may not be sufficient to confer increased motility. Predation/stress-induced and lab-evolved colonial forms of *C. reinhardtii* have impaired motility due to lost, excised, or internally oriented flagella [48–50]. While *V. carteri* beats flagella in a coordinated direction [46], facultative rosette colonies in the choanoflagellate *Salpingoeca rosetta* exhibit uncoordinated flagellar motion [51,52], which leads to flagellar forces largely cancelling out each other and thus slower swimming speed than single cells [15••]. Moreover, the configuration of multicellular groups may be used to modify organismal motility. In the choanoflagellate *Choanoeca flexa*, the cup-shaped colonies can respond to light-to-dark transition by rapidly inverting their curvature so that flagella change from pointing inwards to outwards, which results in enhanced swimming and reduced feeding [53•]. Finally, recent theoretical work has shown that enhanced motility is not always adaptive, and here multicellularity can be an efficient mechanism to exploit scarce nutrients in a patchy landscape [54].

Benefits of chimerism

While clonal multicellular organisms typically develop from a single cell and thereby minimize within-group genetic heterogeneity, aggregative multicellular organisms form via the coming together of potentially unrelated cells. Indeed, genetically chimeric fruiting bodies of the aggregative organisms *D. discoideum* [55] and *M. xanthus* [56,57,58•] are frequently found in the wild and readily formed in the lab. Chimerism has long been known to impose costs on group fitness (chimeric load), often in the form of genetic conflict (e.g. cheating) [1,59]. However, a recent study found that wild *M. xanthus* clones isolated from the same fruiting body often produce more spores in chimeric groups than each alone, suggesting synergistic interactions among genotypes that improve group performance (chimeric synergy) [57]. Genome sequencing indicated that such chimeric synergy may be the result of group-level selection promoting synergistic coevolution of diversified kin lineages that remain together and repeatedly interact over long periods [58•]. Another benefit of chimerism may be ecological: low barriers to group formation should, in principle, increase the rate at which groups form and/or their final size, potentially providing size-related advantages. For example, given the same cell number per clone, chimeric slugs of *D. discoideum* are larger than monoclonal ones and thus can migrate farther, despite the presence of chimeric load [60].

Cross-feeding and division of labor

Interactions among single-species or multi-species communities provide ample opportunity for metabolic complementarity and differentiation, and can drive the evolution of novel higher-level organisms [61,62]. For example, cross-feeding in bacterial multicellular communities can increase fitness by raising resource utilization efficiency and growth rate [63,64,65•]. This benefit arises because uni-directional or bi-directional resource sharing allows communities to increase their metabolic efficiency.

Cross-feeding readily evolves in single-species bacterial colonies or biofilms because direct physical contact between cells enables more efficient metabolite sharing [66]. Furthermore, compact, three-dimensional growth creates diffusion gradients, such as oxic and anoxic zones [67], regulating the formation of distinct cell populations running complementary biochemical pathways [68]. For instance, in single-species communities of *Escherichia coli* or *Pseudomonas aeruginosa*, metabolite sharing can be advantageous due to an increased carbon consumption efficiency [64,69,70]. Recent work has also showed that cooperative cross-feeding can readily evolve in multi-species communities [65•].

Following the formation of simple multicellular groups, the evolution of division of labor can increase the efficiency of resource utilization and production [71,72] without incurring task-switching costs [73]. While unicellular organisms may perform two vital yet incompatible processes by switching between them in time, multicellularity allows tasks to be segregated across cells in space [1]. Two classical examples are the division of labor between photosynthetic cells and nitrogen-fixing heterocysts in filamentous cyanobacteria [74], and differentiation into flagellated somatic cells and non-flagellated reproductive germ cells in colonial volvocine algae [43,72,75]. There are trade-offs in both cases that limit individual cells from performing both tasks simultaneously, in the former example nitrogen-fixing enzymes must work in an oxygen-depleted condition not compatible with oxygenic photosynthesis, and in the latter the microtubule-organizing center cannot be used simultaneously for flagellar motility and mitosis. Interestingly, a similar motility-mitosis trade-off may have played a role in the origin of animals [76,77]. Reconstructing the order of trait emergence indeed suggests that nitrogen fixation may be a prime driver of cyanobacterial multicellularity [78•]. More broadly, stress-induced differentiation into spores and lineage-specific supportive cell types is widespread in microorganisms (discussed further below). Moreover, some constituent cells can even be sacrificed through programmed cell death to benefit the group by providing extra nutrients [79–81], forming multicellular structures (e.g. stalk for raising spores [82]), or fragmenting the group to release propagules [37,74].

Other examples of microbial division of labor have been reviewed elsewhere [71,83–85,86•] and novel types of division of labor are frequently discovered (see for instance Refs. [87,88]). Notably, recent work has shown that division of labor can arise via a single mutation or gene [89,90], and that the topology of simple multicellular bodies strongly favors germ-soma cellular differentiation

[91]. Taken together, this work suggests that division of labor may either be a driver of group formation (in the case of cross-feeding), or evolve shortly after group formation, providing selective benefits even in the simplest multicellular groups.

Competitive overgrowth

Multicellularity can allow surface-attached organisms to overgrow competitors and thus gain preferential access to exogenous resources. For example, crust-forming algae are the first to colonize bare rock, but are subsequently overgrown by filamentous, turf-forming multicellular taxa [92]. The arms race for access to light above ground, and nutrients and water below ground, have long been thought to be a major driver of land plant evolution (though other drivers, like increased propagule dispersal with height, may have played an important role in tree evolution as well [93]). Similarly, microbes growing on a solid substrate may gain an advantage by overgrowing competitors [94], resulting in structural similarities between millimeter-scale biofilms and hundreds-of-meters-scale forests [95].

Efficient utilization of patchy resources

Multicellularity can provide an advantage in patchy resource landscapes. The most striking example of this is the hyphal multicellular fungi, which can transport nutrients from resource-poor to resource-rich environments through their cytoplasmic networks [96•]. Filamentous multicellularity provides an advantage because it allows for nutritional arbitrage in heterogeneous, unmixed landscapes. Immotile unicellular heterotrophs will stop growing when they run out of a locally available limiting resource, even if they have a surplus of all other essential resources. But a multicellular organism capable of transporting resources among its cells can leverage the power of cell–cell trade to continue growing, even when locally available resources would not support it [97•]. While this has been taken to an extreme in modern fungi, the benefits of nutrient transport doubtless act over the smaller scales of nascent multicellular organisms as well. Filamentous growth is not solely the domain of fungi. For example, primitive embryophytes (early multicellular forms in plants) also formed branched, filamentous bodies, which have been hypothesized to be important for exploring local resource environments [98,99]. Even without nutrient exchange, filamentous organisms may be able to explore novel environments more effectively by growing in relatively straight lines, rather than forming a dense hemispherical colony. More generally, patchy resources are thought to be a key driver of evolutionary transitions in individuality across scales, including the evolution of 'super organismality' in both insects and mammals [100,101].

Within poorly mixed liquid environments, multicellularity can also facilitate the capture of patchy resources. For example, collective flagellar motion can stir and mix the surrounding fluid, improving the efficiency of nutrient uptake in colonial volvocine algae [46,102] and prey capture in some colonial choanoflagellates [15••].

Production and dispersal of propagules

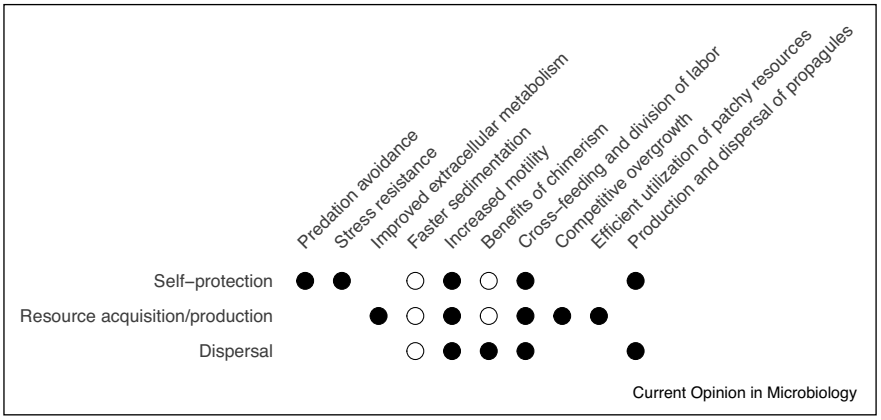
In the lineages known to aggregate and form spore-laden fruiting bodies, multicellular development may also aid in the production and dispersal of stress-resistant propagules (also see spore formation in the bacterium *Bacillus subtilis* and the actinomycete *Streptomyces coelicolor* [103]). Spores can withstand harsh environmental conditions [103,104], in part because some components released by dying cells during fruiting body development may be incorporated to build resilient spore coats [27•,81,105•]. Indeed, frost resistance was recently proposed as a potential cause of multicellular sporulation in *Dictyostelia*, as an adaptation to global cooling in the Neoproterozoic [105•]. Moreover, fruiting bodies can further protect spores by shielding them (e.g. in a protective extracellular matrix [106]) and lifting them above hazards of the soil [82,107].

Fruiting bodies have also long been hypothesized to enhance spore dispersal by lifting spores in the air to better contact passing animals, air currents, or water [27•,107,108]. Lab studies found that fruit flies can acquire more spores from intact fruiting bodies of *D. discoideum* than from disrupted ones [109]. However, this dispersal hypothesis lacks direct tests of whether fruiting bodies can indeed facilitate dispersing spores to favorable environments, and it does not explain the morphological diversity of fruiting bodies [27•]. Notably, in a subgroup of *Dictyostelia* (including *D. discoideum*), aggregation initially forms motile multicellular slugs [26], which may also improve spore dispersal by migrating to suitable environments before transforming into fruiting bodies [44,110]. Finally, it has also been hypothesized that concentrating spores in fruiting bodies may be beneficial in *M. xanthus* because high spore density facilitates germination and cooperative feeding when encountering favorable environments [111].

Conclusion

Simple multicellularity has evolved numerous times across the tree of life and takes a diverse array of forms. In this mini review we described ten key selective drivers of simple multicellularity, all of which stem from the benefits of increased size or novel cell–cell interactions, and serve as a collective solution to self-protection, resource acquisition/production, or dispersal (Figure 2). While multicellularity has evolved numerous times, given the geological time scales at play, it is still a relatively rare innovation and the vast majority of unicellular lineages have stayed unicellular. Our best estimates for the number of independent origins of multicellularity is almost certainly an undercount, due to an intrinsic bias in phylogenetic reconstruction: if a lineage goes extinct before

Figure 2



The selective drivers described above can be placed under three broad ecological categories: self-protection, resource acquisition/production, and dispersal. Each driver may fall under multiple categories. Filled circles denote strong support for the specific ecological benefit, while open dots denote plausible benefits that currently lack direct experimental or theoretical support.

we can sample it, we're unlikely to know it existed. Yet even if the current estimates are low, the fact remains that the vast majority of lineages on Earth remain unicellular. This may be because multicellular growth imposes significant costs (e.g. nutrient and oxygen diffusion limitation, impaired motility, reduced passive dispersal in air or water currents, metabolic costs required for group formation and maintenance, the potential for social exploitation, vulnerability to predators or pathogens, etc.) which must be overcome by a multicellular-specific benefit. Yet it is clear from the examples described above that there are many routes to multicellularity, with each route being highly dependent on the nature of the organism (e.g. feeding mode, motility, mode of group formation, multicellular configuration, constraints of cell biology) and the spatiotemporal dynamics of its environment.

We chose to examine the selective drivers of simple multicellularity because it is both important and relatively understudied. Indeed, it remains challenging to determine the selective benefit of nascent multicellularity in extant multicellular lineages, in part because the early evolution of multicellularity is often difficult to resolve. While we may never have certainty about how specific lineages first evolved multicellularity, advances in phylogenetics/comparative biology (e.g. Refs. [78•,86•]), paleontology (e.g. Refs. [12,105•]), synthetic biology (e.g. Refs. [39•,112]), theory (e.g. Ref. [97•]), experimental evolution (e.g. Refs. [39•,113]), and experiments with non-model organisms (e.g. Refs. [36•,53•]) will continue to refine our hypotheses. We see great value in these approaches for understanding the evolution of multicellularity, helping address a number of outstanding questions: ***i)*** How, when, and why do simple multicellular organisms evolve from unicellular ancestors, and what were their ecological contexts? ***ii)*** How do the dynamics (traits, mechanisms, etc.) of a nascent multicellular lineage influence the subsequent evolution of multicellular complexity? ***iii)*** Which ecological drivers of multicellularity stabilize with relatively small, simple organisms, and which result in an open-ended arms race for increased size and complexity? ***iv)*** Under what conditions do evolutionary priority effects constrain subsequent innovation, and how does the breadth of multicellular niches affect the number of independent transitions to multicellularity within different clades? Both recent developments in technology (i.e. synthetic biology, genome editing, genome and single-cell sequencing, etc.) and extensive collaboration among researchers with diverse backgrounds make this a particularly exciting time to work on the evolution of multicellularity.

Conflict of interest statement

Nothing declared.

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