Current Genetics
https://doi.org/10.1007/s00294-021-01193-0

MINI-REVIEW

Why have aggregative multicellular organisms stayed simple?

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Received: 2 March 2021 / Revised: 21 May 2021 / Accepted: 24 May 2021

Abstract

Multicellularity has evolved numerous times across the tree of life. One of the most fundamental distinctions among multicellular organisms is their developmental mode: whether they stay together during growth and develop clonally, or form a group through the aggregation of free-living cells. The five eukaryotic lineages to independently evolve complex multicellularity (animals, plants, red algae, brown algae, and fungi) all develop clonally. This fact has largely been explained through social evolutionary theory's lens of cooperation and conflict, where cheating within non-clonal groups has the potential to undermine multicellular adaptation. Multicellular organisms that form groups via aggregation could mitigate the costs of cheating by evolving kin recognition systems that prevent the formation of chimeric groups. However, recent work suggests that selection for the ability to aggregate quickly may constrain the evolution of highly specific kin recognition, sowing the seeds for persistent evolutionary conflict. Importantly, other features of aggregative multicellular life cycles may independently act to constrain the evolution of complex multicellularity. All known aggregative multicellular organisms are facultatively multicellular (as opposed to obligately multicellular), allowing unicellular-level adaptation to environmental selection. Because they primarily exist in a unicellular state, it may be difficult for aggregative multicellular organisms to evolve multicellular traits that carry pleiotropic cell-level fitness costs. Thus, even in the absence of social conflict, aggregative multicellular organisms may have limited potential for the evolution of complex multicellularity.

Keywords Multicellularity · Evolution · Major evolutionary transitions · Social evolution · Complexity

Introduction

Multicellular organisms vary tremendously in their level of complexity, ranging from small, undifferentiated clusters to organisms with trillions of cells and hundreds of dedicated cell types (Bell and Mooers 1997; Bonner 2004; Fisher et al. 2020). Complex multicellularity has evolved in five distinct eukaryotic lineages: animals, plants, red algae, brown algae, and fungi (Knoll 2011). While the definition of what counts as complex will always be somewhat arbitrary, some features are commonly associated with multicellular complexity: cell-cell communication, a genetically encoded developmental program, and the organization of cells in tissues that are not in direct contact with the environment (Knoll 2011). All five of these lineages develop clonally, with cells staying together after division. While there are many organisms that form multicellular groups through aggregation (Sebé-Pedrós et al. 2017; Brown and Silberman 2013; Brown et al. 2012), none have evolved a comparable level of complexity. It is important to note, however, that not all clonally developing organisms have evolved to be complex– indeed, many have remained relatively simple despite multicellularity having evolved hundreds of millions of years ago (e.g., the volvocine green algae, Herron et al. 2009).

Communicated by Michael Polymenis.

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Published online: 10 June 2021

Social evolution and multicellular development

Social evolutionary theory identifies a number of advantages to clonal multicellular development (Brunet and King 2017; Fisher et al. 2013) (see Fig. 1), potentially explaining these macroevolutionary patterns. In clonal groups of cells, there is little potential for among-cell conflict driven by selfish 'cheats' (Fisher et al. 2013; Kuzdzal-Fick et al. 2011), which West et al. (2007) define as "individuals who do not cooperate (or cooperate less than their fair share), but are potentially able to gain the benefit of others cooperating." In chimeric groups, however, cheats can have higher fitness than cooperators, which, if unchecked, can lead to the loss of cooperators from the population (Fletcher and Doebeli 2009). For example, field-collected *Dictyostelium discoideum* isolates readily form chimeric multicellular groups in the lab (Strassmann et al. 2000). In more than half of these strain pairs, one strain cheats by preferentially forming viable spores in the developing stalked sporangium, and contributing less to the construction of the stalk which holds the spores above the soil surface. Clonality is thus a general mechanism for mitigating the potential for cheating, at least until mutational processes generate sufficient within-organism variation to cause cancer (Mathavarajah et al. 2021). In clonal groups, the fitness of cells in the group are aligned with one another, and with the fitness of the group as a whole (Bourrat 2015). Finally, cells interacting with clonemates over many generations, rather than varying members of the population, may facilitate the evolution of cellular differentiation (Yanni et al. 2020).

Limitations to highly effective assortment

Aggregative multicellular organisms could, in principle, avoid the potential pitfalls of within-group genetic diversity by evolving effective partner choice mechanisms (Gilbert et al. 2007; Inglis et al. 2017). Indeed, aggregative multicellular organisms have evolved various forms of kin recognition systems, often using 'receptor-like' pairs of proteins. For example, the slime mold *Dictylostelium* uses TgrB1/C1 (Ho et al. 2013) and *Myxococcus* bacteria use TraA/B (Sah and Wall 2020). Yet these kin recognition systems are not particularly effective at kin discrimination (Gruenheit et al. 2017)- both *Dictylostelium discoideum* and

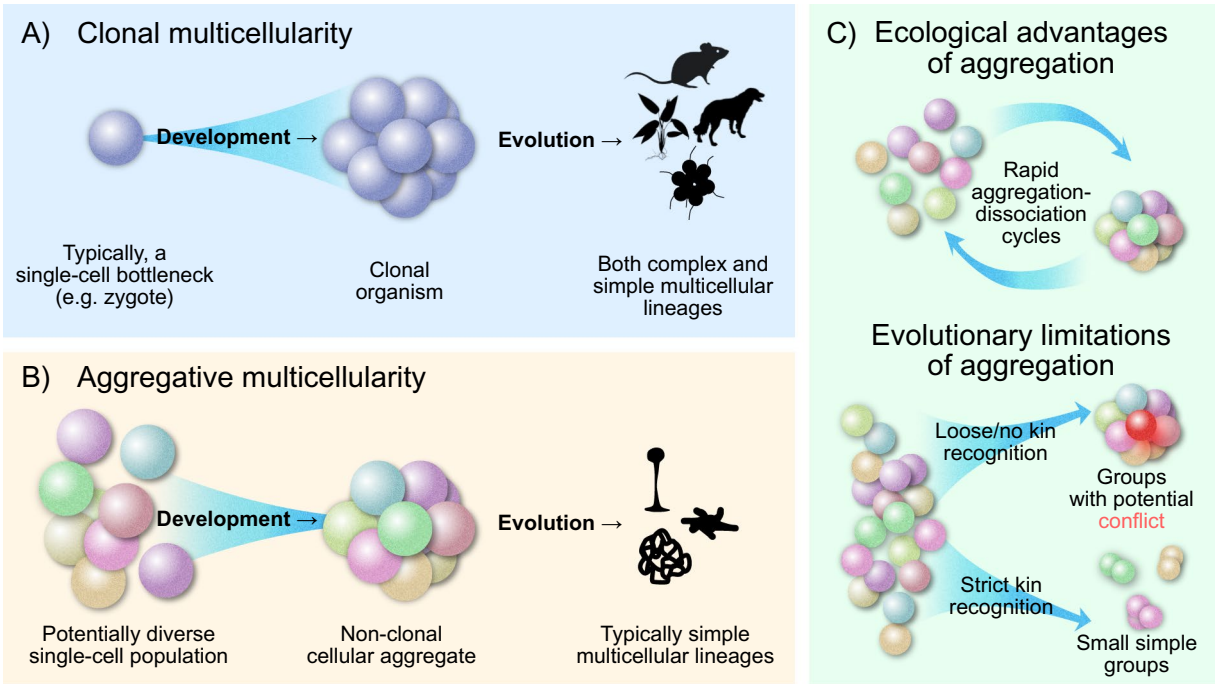

Fig. 1 Key differences between clonal and aggregative multicellularity. **A** Multicellular organisms with a single cell bottleneck during development grow clonally. **B** In contrast, aggregative multicellularity involves the association of potentially diverse genotypes into a single group. **C** While aggregation provides an ecological advantage of rapid group formation/dissociation, it may be difficult to avoid genetic conflict (particularly in large groups) over evolutionary timescales. Selection for rapid group formation may undermine selection for stringent kin discrimination

Myxococcus xanthus readily form chimeric groups under laboratory conditions, and fruiting bodies found in nature can be chimeric (Ostrowski 2019; Ostrowski et al. 2008; Vos and Velicer 2009). While relatively little work has examined group assembly in the wild, within-group relatedness in *D. discoideum* appears to be relatively high (Gilbert et al. 2007), while chimerism seems to be quite common in the closely related multicellular species *D. giganteum* and *D. purpureum* (Sathe et al. 2010), as well as the multicellular bacterium *M. xanthus* (Vos and Velicer 2006, 2009; Kraemer and Velicer 2011).

In general, single locus kin recognition systems are a poor choice for efficient genome-wide kin recognition, as they can be decoupled from the majority of the genome with just a few generations of sexual recombination. In principle, there is no reason that aggregative multicellular organisms would need to rely on a single-locus recognition system, as multilocus recognition systems are both taxonomically widespread and more effective at kin recognition (e.g., Lyons et al. 2016; Takayama and Isogai 2005; Manning et al. 1992; Wenren et al. 2013). Given the clear costs of cheating (Ho et al. 2013) and hundreds of millions of years of evolution (Du et al. 2015; Kawabe et al. 2019), why are many kin recognition systems still so inefficient?

Pentz et al. (2020) provide some insight into this conundrum: weak kin recognition may be an adaptive feature of aggregative multicellular life histories, not a bug that has yet to be improved. A central ecological advantage of an aggregative multicellular strategy, relative to clonal development, is that it allows for rapid group formation (see Fig. 1A, B). In contrast, a clonal multicellular organism that forms by cells 'staying together' takes many cellular generations to form (Fig. 1C). Hence, aggregative multicellular organisms tend to live in environments where selection favors a rapid change between unicellular and multicellular life history strategies, like the formation of a multicellular fruiting body upon starvation (Du et al. 2015).

Consider a free-living cell that is part of a genetically-diverse population. In response to an environmental trigger (e.g., starvation), it attempts to join a multicellular group. If its genotype is at all uncommon (and many genotypes will be if the population is even somewhat diverse), then strict kin recognition (restricting association to clonemates) may limit its ability to find clonemates and form a group (Pentz et al. 2020). All else equal, strict kin recognition can only delay the time of group formation, and/or decrease the size of the group that a focal cell finds itself in, undermining the ecological advantages of this developmental mode. Thus, the ecology of aggregative multicellularity may preclude the evolution of strict kin recognition, sowing the seeds for persistent evolutionary conflict and limiting the potential for aggregative multicellular organisms to evolve a high degree of complexity (Fig. 1C, see also Pentz et al. 2020).

Facultative vs. obligate multicellularity

Yet we are still describing aggregative multicellular organisms as if the only salient difference between them and clonal multicellular organisms is their developmental mode, which, in our view, misses a central point: all known aggregative multicellular organisms form groups facultatively (Fig. 2A, Fisher et al. 2013), spending most of their time (and performing most of the cellular division) in a unicellular phase. In contrast, the majority of clonal multicellular organisms form groups obligately, spending most of their time and doing all of their cellular division in a multicellular state. This makes for qualitatively distinct evolutionary dynamics at the cell and group levels, when comparing typical clonal and aggregative life cycles. And this distinction can have profound implications for the evolution of multicellular complexity.

Within a multicellular group, mutations can be considered to have different effects at the cell and group level—a mutation that encodes an altruistic trait may come at a cost to the cells expressing that trait but provide a benefit to their group (Shelton and Michod 2020). The same is true across phases of a multicellular life cycle – mutations can have differential fitness effects during the free-living unicellular phase (cell-level) and multicellular group phase (group-level) that affect their fixation probabilities. In aggregative multicellular organisms that spend most of their life cycle in a unicellular phase, there is ample opportunity for selection to act on free-living unicellular life history traits (Fig. 2B, C). This may make it difficult for selection to favor group-beneficial traits that come with a cell-level fitness cost (Ratcliff et al. 2017). These pleiotropic costs may be avoided, however, if the trait is developmentally regulated such that it is expressed only in the multicellular life history stage. Indeed, this appears to be common within extant aggregative multicellular taxa. For example, the behaviors underlying cellular differentiation and multicellular morphogenesis only occur in a multicellular context within the social dictyostelid amoebae (Romeralo et al. 2013; Schilde et al. 2014) and *Myxococcus* bacteria (Arias Del Angel et al. 2017). Further work will be required to determine the extent to which the extended unicellular life history phase of aggregative multicellular taxa constrains multicellular adaptation.

Mutations that are beneficial at the group level but deleterious at the cell level are significant because they may act to promote the evolution of group cohesion and increase the evolutionary stability of multicellularity through time by constraining reversion to unicellularity (Shelton and Michod 2020; Hammerschmidt et al. 2014; Libby et al. 2016). Thus, the inability to fix such mutations could serve as a barrier to evolving complex multicellularity for

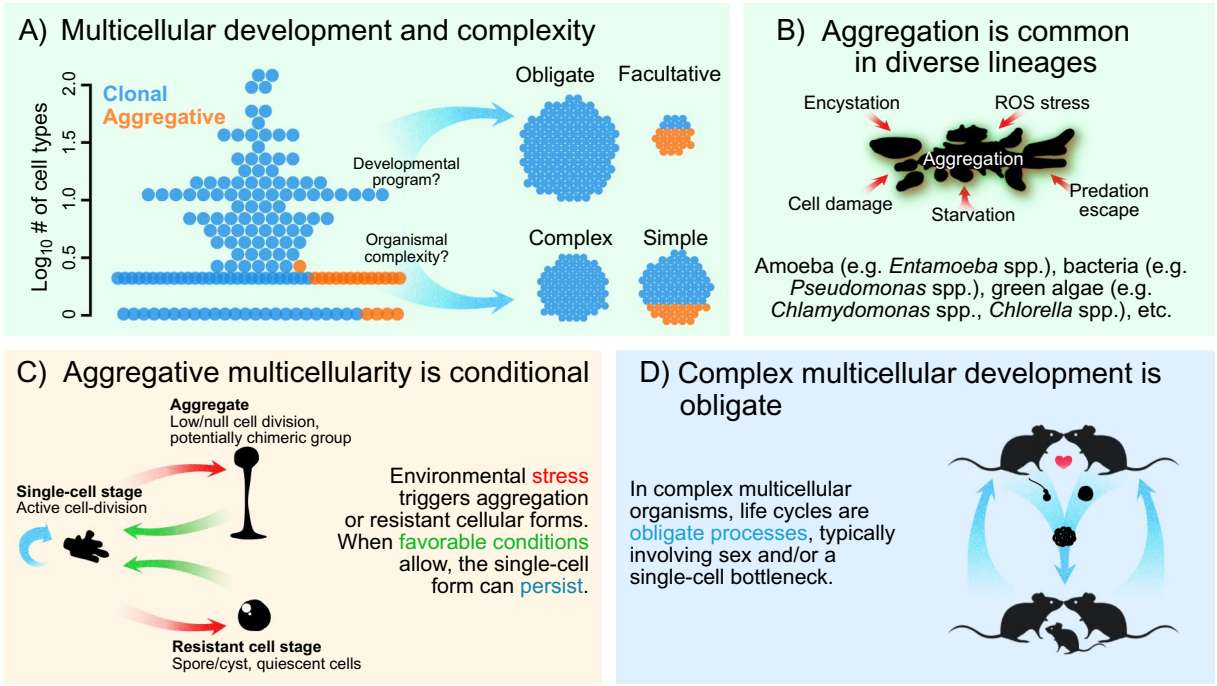

Fig. 2 Multicellular complexity has only evolved in clonally developing lineages. **A** Clonal development can be facultative or obligate, but only the obligate forms have evolved to be complex (each dot represents a lineage, color coded by the developmental mode); these same lineages can be classified into obligate/facultative and complex/simple categories (data set and classification from Fisher et al. 2013). **B** Aggregation is prevalent in various unicellular lineages, usually in response to stress (see Nagaraja and Ankri 2018; Manna et al. 2020; Kapsetaki et al. 2017; Klebensberger et al. 2006; Secor et al. 2018; Schriber et al. 2019). **C** In all known aggregative multicellular organisms, the formation of a multicellular stage is a facultative process. **D** In contrast, complex multicellular organisms always develop a multicellular stage during their life cycles

aggregative multicellular organisms. This is not an obstacle for clonal multicellular organisms.

Within clonal groups there is limited scope for among-cell selection due to the limited time spent as free-living single cells (Fig. 2D). In contrast, aggregative multicellular organisms are likely to experience significant among-cell selection during the unicellular phase of their life cycles, where unicellular populations are rarely, if ever clonal (Sathe et al. 2010; Kraemer and Velicer 2011). This is true even in the absence of social conflicts arising due to chimerism.

Facultative multicellular life cycles can thus constrain the evolution of multicellular complexity, as most of their physiological functions and evolutionary time are spent as unicellular individuals. This might explain why some aggregative multicellular organisms, which form multicellular groups in response to an environmental trigger, are relatively simple, having only a few cell types (see Fig. 2A, based on Kapsetaki et al. 2017; Klebensberger et al. 2006; Secor et al. 2018; Schriber et al. 2019; de Carpentier et al. 2019).

Facultative multicellular organisms have the ability to transition between single celled and multicellular states (e.g. Fig. 2C). As a result, they have the ability to adapt via either unicellular or multicellular strategies. This flexibility may be particularly useful when partitioned between growth (unicellular stage) and survival (multicellular stage), but it may also limit multicellular complexity. Obligate multicellular organisms, in contrast, have no choice but to find multicellular solutions to selective challenges. This may drive evolutionary innovation: sustained multicellular adaptation requires the evolution of genetically encoded development, which typically requires (among other factors) the evolution of complex mechanisms of cellular signaling as a means of driving differentiation, maintenance, and repair of the multicellular state (Brunet and King 2017; Sebé-Pedrós et al. 2018; Márquez-Zacarías et al. 2020). Solving novel challenges posed by obligate multicellular life (e.g., the evolution of a circulatory system to circumvent diffusion limitation for cells growing in a large multicellular group) is thought to be a key driver of complex multicellularity (Knoll 2011).

Obligate aggregative multicellularity would alleviate some of the issues raised above, yet no obligate aggregative multicellular organisms have been described (see Fig. 2, based on data from Fisher et al. 2013). Instead, all known aggregative multicellular organisms have facultative multicellular life history stages, forming groups in response to

environmental cues (typically stress). In principle, obligate aggregative multicellularity should be easy to evolve—all it requires is that the multicellular life history phase be constitutive, not conditional on an environmental stimulus. Indeed, such a strategy can easily be generated in the lab by constitutively expressing the adhesive glycoprotein *FLO1* in yeast (Pentz et al. 2020; Smukalla et al. 2008)). The fact that species with obligate aggregative life cycles have yet to be found in nature suggests that this mode of development might be evolutionarily constrained.

Conclusion

Evolutionary theory has successfully explained how social conflict plays an important role in multicellular evolution and adaptation. But it is equally important to recognize factors outside this traditional framework, such as the role of ecological and evolutionary constraints that result from having a particular developmental mode (Pentz et al. 2020). The paradigmatic aggregative multicellular organisms (e.g., *Dictylostelium* and *Myxococcus*) are qualitatively different than paradigmatic clonal multicellular organisms (e.g., animals, plants, red algae, brown algae, and fungi), spending the majority of their time in a unicellular state. As a result, there is relatively little reason to expect that selection would dramatically increase multicellular complexity. To attribute all of the variation that we see in nature to social evolutionary causes alone places too much weight on this one important—but probably not dominant—factor.

Acknowledgements The authors would like to thank the members of the Ratcliff Lab for useful discussions and comments leading to this paper, as well as Matt Herron for reading a previous version and providing feedback. We also thank two anonymous reviewers for useful comments that significantly improved our manuscript. This work was supported by NSF DEB-1845363 to W.C.R, NSF grant no. IOS-1656549 to W.C.R., and a Packard Foundation Fellowship for Science and Engineering to W.C.R.

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