What do we mean by multicellularity? The Evolutionary Transitions Framework provides answers

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INTRODUCTION

At first glance, the meaning of the word ‘multicellularity’ appears to be unambiguous - it is treated as an “intuitive” concept, something that can be grasped with common sense. On closer inspection, however, it is apparent that there is notable disparity in the recent literature regarding the usage of the term ‘multicellularity’. Whereas traditionally it was mainly attributed to complex organisms (Grosberg and Strathmann, 2007), more recently it has also been used for simple microbial colonies or biofilms (Hengge, 2020). Accordingly, a unifying definition is lacking - whereas some definitions require cells to display an overall coordination of function (Wolpert and Szathmáry, 2002), have physical contact and strong interactions (Kaiser, 2001), others are simply based on the presence of a group-morphology (Schirrmeister et al., 2013).

We think that it is important to be more precise when using the term multicellularity as, for example, a microbial colony differs in important ways from a multicellular organism like us. This distinction has implications for various areas of inquiry such as ‘the sociobiology of microbes’ and ‘the evolutionary transition to multicellularity’. While these research directions have brought together a highly interdisciplinary community of researchers, adequate descriptions of the marginal or nascent cases of multicellularity remain elusive, despite their identification across the entire range of model organisms, such as algae, protozoans, yeast and bacteria (Ratcliff et al., 2012; Claessen et al., 2014; Hammerschmidt et al., 2014; van Gestel and Tarnita, 2017; Brunet et al., 2019; Kapsetaki, and West, 2019). Lack of continuity has also led to vastly different estimates of the number of instances of multicellular emergence in evolutionary history (Niklas and Newman, 2020). Depending on the definition of multicellularity, it is thought to have evolved from unicellular ancestors on 13 to 25 independent occasions. When described simply as a cellular aggregation, multicellular organisms are estimated conservatively to have evolved in at least 25 lineages (Grosberg and Strathmann, 2007), making it a “minor major” evolutionary transition. When more stringent criteria are applied, as for example a requirement for sustained cell-to-cell interconnection, communication, and cooperation, multicellularity has evolved multiple times in bacteria (e.g., Actinobacteria, Myxobacteria, and Cyanobacteria; see Bonner, 2000), but only once in the Animalia, three times in the Fungi (chytrids, ascomycetes, and basidiomycetes), and six times among the algae (twice each in the rhodophytes, stramenopiles, and chlorobionta; Niklas and Newman, 2013).

We argue that we need a better understanding about what multicellularity is to meaningfully discuss factors that determine its evolution. We propose that clarity can be achieved with the realisation that the various definitions of multicellularity are in fact describing different stages that can occur during the course of its evolution. The major evolutionary transition from single cells to multicellular organisms is not an instantaneous shift, but rather a process with multiple transient stages. As such, 'multicellularity' itself is not necessarily a fixed state, but exists as a large range encompassing single cells that are part of multicellular groups, multicellular individuals, and multicellular organisms. We here provide a framework for identifying the various stages of the transition to multicellularity. Importantly, we do not intend to imply that fixed boundaries separate stages of an evolutionary transition from single cells to multicellular organisms. We only demarcate stages here to provide a conceptual link between semantic use and a dynamic evolutionary process.

THE EVOLUTION OF MULTICELLULARITY – A DYNAMIC PROCESS

The transition to multicellularity begins with the evolution of cooperation, where cells unite together and gain an advantage over solitary cells (Stage One; Figure 1). The focus of natural selection remains on cells, albeit in a group-structured context. Stage Two is the true 'transitional stage' of a major evolutionary transition, where the cooperating group also becomes a unit of selection - a 'Darwinian individual'. Crucially, in order to satisfy the conditions of Darwinian individuality, the group itself must become capable of reproduction (Godfrey-Smith, 2009). A high degree of functional organization is an adaptation of groups, *resulting from* selection operating at the higher (group) level (Okasha, 2006). Therefore, complex adaptations of groups accumulate during the third stage of an evolutionary transition. Eventually, group adaptations lead to integration of the cells comprising the group that they can no longer exist independently, and now only survive and replicate as components of the multicellular group – the 'organism' (Stage Three). In contrast to the view of Bourke (2011), who proposes that only complex multicellular organisms possess individuality, in our view individuality occurs at a much earlier phase of the transition. We suggest that initially marginal multicellular individuals that can reproduce (Stage

2) have the potential to accumulate adaptations that result in the evolution of complex multicellularity.

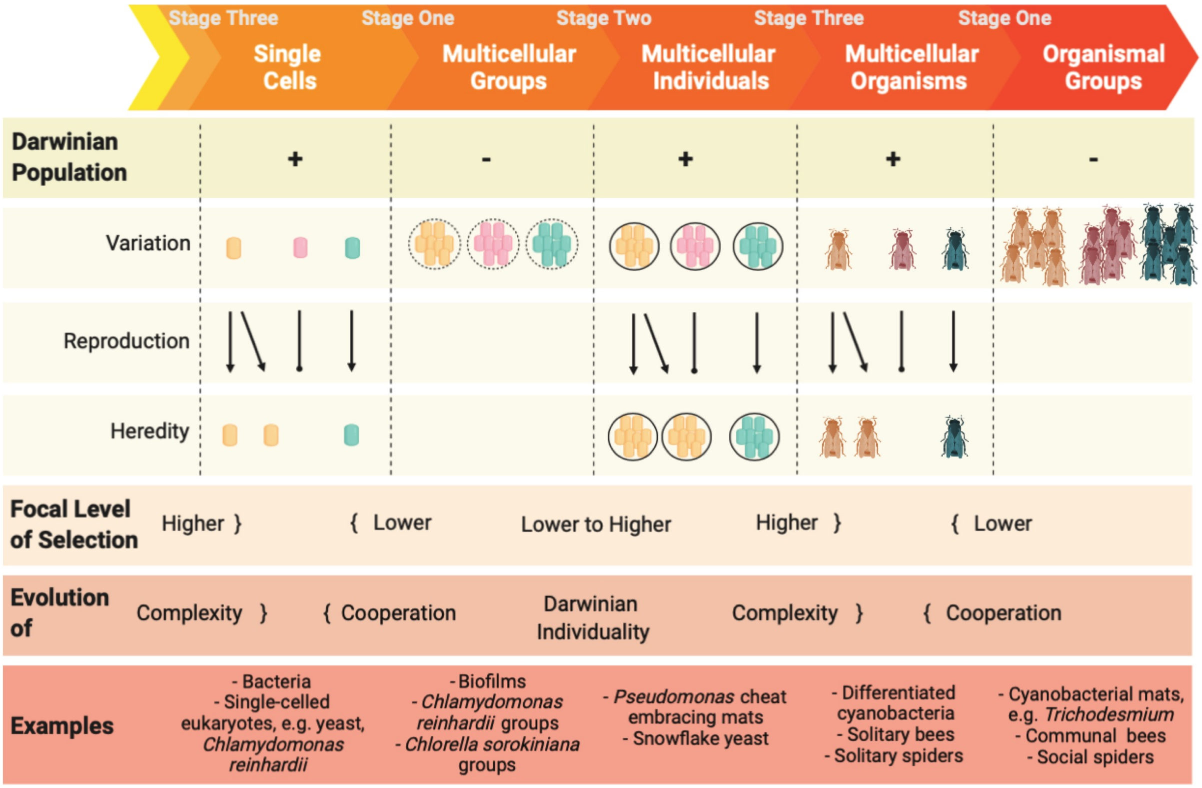

Figure 1. Multicellular entities are defined as either Multicellular Groups, Multicellular Individuals, or Multicellular Organisms, reflecting the stages of the evolutionary transition from single cells to multicellularity imbedded within the hierarchical structure of life. 'Darwinian populations' are populations of 'Darwinian individuals' or 'units of selection', which are entities that can evolve by natural selection by virtue of possessing these essential characteristics: variation between entities within the population, which is heritable and causally associated with their differential reproduction (Lewontin, 1970).

STAGE ONE – EVOLUTION OF MUTICELLULAR GROUPS

The evolution of cooperation encapsulates Stage One of the evolution of multicellularity. A cooperative behaviour is generally described as a costly investment in resources that benefits an individual (the recipient) other than the actor (Chase, 1980), regardless of whether the recipient adopts the same behavioural strategy. Cooperative interactions are central to an evolutionary transition because the necessary fitness cost associated with cooperation is offset by a group-level benefit.

During the transition to multicellularity, cooperation between cells resulted from the advantages gained by adhering to each other. This occurred through two mechanisms: clonality and aggregation (Grosberg and Strathmann, 2007; Tarnita et al., 2013). During a transition to clonal multicellularity, cells fail to adequately separate after cell division and ergo remain attached. Consequently, clonal forms of multicellularity, such as plants and animals (Stage 3), developed from a small number of cells (Stage 1/ 2) – an evolutionary ‘bottleneck’. The aggregative mode of multicellularity usually results from motile single cells (Stage 1/ 2) clustering together to form fruiting bodies for sporulation and dispersal, often in response to environmental starvation (Gross, 1994). Aggregative forms of multicellularity have arisen independently in eubacteria, several cellular slime moulds, and in ciliates (Bonner, 1998). While aggregative forms of multicellularity are numerous and widespread, particularly in terrestrial environments, clonal multicellularity has led to greater diversity and complexity (Fisher et al., 2013).

The challenge for understanding the evolution of cooperation is explaining how cooperation generates a benefit (Calcott, 2011). Multicellular cooperation in many lineages may have originally obtained the advantage of increased size afforded by the ever-present open niche at the top of the size scale (Bonner, 1988, 2000). Proposed advantages of increased size are that larger assemblages of cells avoid predation by filter feeders or that increased size enhances feeding efficiency (Dworkin, 1972; Bell, 1985; Bonner, 1998; Boraas et al., 1998; Pfeiffer et al., 2001; Alegado et al., 2012; Koschwanez et al., 2013; Herron et al. 2019; Kapsetaki and West, 2019). Other advantages of cellular cooperation include benefits associated with both fixed surface attachment and enhanced dispersal. Single cells located in an ideal position for growth may be swept away by currents or wind, whereas an increased ability to adhere to surfaces by cell clusters might be selectively advantageous (Gross, 1994; Bonner, 1998). Tradeoffs between two incompatible processes that cannot be performed in one cell at the same time have also been proposed as important drivers of multicellular cooperation. Examples of such tradeoffs include motility and mitosis in metazoans (Margulis, 1981; Buss, 1987; King 2004), reproduction and motility in the volvocene green algae (Koufopanou 1994), and N_2 and CO_2 fixation in cyanobacteria (Rossetti et al., 2010; Herrero et al., 2016; Hammerschmidt et al., 2021).

STAGE TWO – EVOLUTION OF MULTICELLULAR INDIVIDUALS

Stage Two is the true ‘transitional’ phase of a major evolutionary transition because during this stage, natural selection operates between groups, rendering them ‘Darwinian individuals’. In order to be a ‘unit of selection’ (Lewontin, 1970), a group itself must become capable of reproduction (Figure 1). The particular question of relevance to major evolutionary transitions is the puzzle of group reproducers (Godfrey-Smith, 2009) – reproducing units comprised of particles which themselves have the capacity to reproduce. Multicellular groups, for example biofilms or *Chlamydomonas reinhardtii* groups (Herron et al., 2019), multicellular individuals, such as snowflake yeast (Ratcliff et al., 2012) or *Pseudomonas* cheat embracing mats (Hammerschmidt et al., 2014), and multicellular organisms (filamentous cyanobacteria, solitary bees) are all examples of group reproducers. The challenge is to identify which are cases of reproduction of groups, and which are cases of growth of groups resulting from reproduction and structural organization of their particles (see Figure 1 for details). A second challenge during Stage Two of a major evolutionary transition is to explain how groups acquired the fundamental requirement for reproduction – a life cycle.

The particular mode by which the earliest multicellular groups reproduce, for example through a dedicated (germ) cell or by fragmentation, has implications for their ability to transition in individuality and participate in natural selection (Ratcliff et al., 2012; Hammerschmidt et al., 2014). Furthermore, during this transitional phase, ecological conditions are of critical importance (Pichugin et al., 2019; Staps et al., 2019), such as structured environments that maintain the discreteness of groups, and crucially, their reproductive cells (Rose et al., 2020). Such conditions provide the ecological scaffold for selection to act on less-integrated groups until they complete the transition to 'multicellular individuals' (Black et al., 2020).

STAGE THREE – EVOLUTION OF MULTICELLULAR ORGANISMS

After a multicellular group acquires the capacity to reproduce, is it possible for natural selection to operate on traits that enhance the fitness of the group as a collective unit. The accumulation of such traits leads to the evolution of progressively higher complexity. Hence, the term 'complexity' does not refer to a specific state reached by a multicellular organism, but it is a relative term used to describe a wide spectrum of collective functions. Multicellular complexity is often represented by the number of different cell types coexisting in the collective, although epigenetic control of this cellular differentiation is clearly an important innovation resulting from group-level selection (Buss, 1987; Arnellos et al., 2013). Epigenetic regulation of development itself evolves as increasingly more complex genetic networks. The accumulation of group adaptations may eventually lead to such a degree of integration of parts that the cells no longer exist independently – their survival and reproduction depends entirely on the survival of the group. We suggest that this loss of lower level autonomy be the defining feature of the term ‘organism’, ultimately rendering an organism indivisible. In the level above multicellular organisms, eusocial insect colonies are sometimes referred to as ‘superorgansims’ when the lower level units no longer exist autonomously and instead subsist as sterile workers. This has also been shown to involve an increase in complexity of gene networks (Kapheim et al., 2015).

The evolution of developmental regulation is mechanistically unproblematic because the genetic machinery for coordination of differentiated cell types existed in primitive ‘multicellular’ prokaryotes and close eukaryotic unicellular relatives of metazoans (Gombar et al., 2014; Glöckner et al., 2016; Sebé-Pedrós et al., 2016; Brunet and King, 2017). It is therefore surmised that few mutational steps should be required in a regulatory pathway to produce additional cellular differentiation. Indeed, thousands of differences in gene expression between cell types in multicellular organisms are often controlled by a small set of regulatory proteins. This is supported by the fact that the presence of most genes underlying multicellular development and function has been inferred in the unicellular ancestors of metazoans, algae, and fungi, providing strong indications that regulatory changes indeed led to the co-option of the ancestral genes (Hanschen et al., 2016; Sebé-Pedrós et al., 2016; Kiss et al., 2019). Nevertheless, important metazoan developmental gene families, notably the homeobox genes, are not present in unicellular ancestors (Ruiz-Trillo et al., 2007), indicating that these gene regulatory pathways evolved later as a consequence of multicellular individuality.

CONCLUSION

The transition to multicellularity is of seminal biological significance as it led to the vast biological complexity and diversity we see on our planet today. Reconstructing the stages that occurred during the process of evolutionary transitions that took place in the distant past is a major challenge. While most research has focused on theoretical and philosophical aspects of these events, several recent developments and novel techniques have transformed this research area and brought together a highly interdisciplinary community of researchers who are rapidly advancing the field.

One novel approach is the utilization of unicellular model organisms, such as yeast, algae, protozoans, and bacteria in experimental evolution studies to mimic the evolution of early stages of the transition to multicellularity.

This new research direction has already contributed many exciting results that feed back into theory. However, these studies have also led to confusion regarding the definition of the term ‘multicellular’, because they focus on marginal or nascent cases of multicellularity. In addition, the utility of the various definitions of multicellularity remains vague for extant organisms. We advocate that clarity can be achieved by considering the diverse use of the term ‘multicellularity’ as sequential stages of a dynamic evolutionary process, from multicellular groups, to multicellular individuals, and finally to multicellular organisms. Semantic continuity among researchers will lead to more productive communication between evolutionary biologists and ecologists, microbiologists, philosophers, physicists and theoreticians, further advancing this exciting field.

CONFLICT OF INTEREST

There are no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Both authors have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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