Group formation: hypotheses for the evolution of clonal multicellularity

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Abstract

In this chapter we focus specifically on clonal multicellularity, where multicellular groups form through daughter cells remaining attached to mother cells after division. This type of group formation has evolved in several prominent multicellular taxa, including the animals, plants and fungi, and is unique in that it guarantees clonal relatedness between cells. This is in contrast to aggregative group formation, found in the cellular slime moulds, some algae, ciliates, and many species of bacteria, where genetically distinct cells come together to form the multicellular body (see Chapters 5-8). Clonal relatedness allows highly cooperative behaviour to evolve, including altruism, and is the only type of group formation that has led to the evolution of obligate multicellularity. We give an introduction to clonal multicellularity and set the scene with a short primer in social evolution theory. We then review several examples of species with clonal group formation and assess the benefits and costs of multicellular cooperation in clonal groups. We conclude by discussing the impact of the environment on multicellular group formation and asking whether it is possible to evolve obligate multicellularity *without* clonality.

10.1 Introduction

Multicellularity occurs all over the tree of life in myriad different forms and, depending on the estimate, has evolved independently between 8 and 25 times (Fisher et al., 2013; Grosberg & Strathmann, 2007; Knoll, 2011; Lyons & Kolter, 2015; Niklas, 2014; Niklas & Newman, 2013). Multicellularity underpins much of the complex life that we can see, but increasingly we are becoming aware of the plethora of microbial species that are multicellular or have cooperative multicellular behaviours. In Chapters 5-8 we heard about the myxobacteria and about *Dictyostelium* and the cellular slime moulds. However, regardless of the taxa in which multicellularity has evolved, the way in which multicellular groups form has important ramifications for cooperative behaviour, multicellular complexity, and the potential to evolve obligate multicellularity (which we will come to later). In this chapter we focus on clonal multicellularity, where multicellular groups form through daughter cells sticking to mother cells after cell division. This type of multicellularity has evolved in at least 12 different lineages and has led to some of the most complex and diverse multicellular species (Fisher et al., 2013).

First, we briefly review the different lineages where multicellularity is found and ways in which multicellular groups can form, before focusing on clonal multicellularity. Next, we give a primer in social evolution theory to allow us to explore the reasons why clonality has allowed the evolution of extreme cooperative behaviour, such as altruistic somatic cells. We then give some examples of clonal multicellular taxa to explore the advantages this type of group formation can pose in the natural world. Finally, we ask whether clonality is the only route to complex, obligate

multicellularity, like we see in animals and plants, and finish by reflecting on the major evolutionary transitions in individuality.

10.2 What do we mean by multicellularity?

What do we mean by multicellularity? On a basic level, we define multicellularity as when cells stick together (hence 'multicell') and have been selected to do so. This definition captures a huge variety of multicellular phenotypes, including Bacteria and Archaea that form cooperative groups (e.g. Bonner, 1998, 2000; LaPaglia & Hartzell, 1997; Mayerhofer et al., 1992), fungal hyphae, plant multicellularity, fruiting bodies in ciliates, a range of algal phenotypes, and of course, metazoans. However, it is clear that multicellularity can either be facultative, where cells are able to survive and reproduce independently or be part of a group, or obligate, where cells are permanently part of a group and cannot survive and reproduce independently of that group. As an example, humans are obligately multicellular. Our somatic cells (e.g. skin cells, neurons, muscle cells) cannot separate themselves from our multicellular bodies and live an independent existence. They exist as part of a multicellular whole and are terminally differentiated into their respective somatic phenotypes so that they have no (sexually) reproductive function. Our gametes may exist transiently as unicells, but this is an existence which either ends in death or a new, obligately multicellular individual. This is in stark contrast to species with facultative multicellularity, where individual cells need not join a multicellular group and may do so only under specific conditions. An example of this is the cellular slime mould *Dictyostelium.* In this species, single cells can survive and proliferate in the environment quite happily (Hashimura et al., 2019; Strassmann & Queller, 2011), only coming together to form a multicellular fruiting body during times of hardship where food supplies run low (Bonner, 2009; Strassmann et al., 2000). The fruiting body phase is in no way required for *Dictyostelium* amoebae to feed, move, communicate and divide (Kessin, 2001; Strassmann, 2019).

Multicellular species also form multicellular groups in distinctly different ways. These can be broadly classified as non-clonal (aggregative) multicellularity and clonal multicellularity. A possible third category, hyphal multicellularity as described below, displays similarities to both types of group formation but seems (at least in practice) to more closely resemble clonal multicellularity.

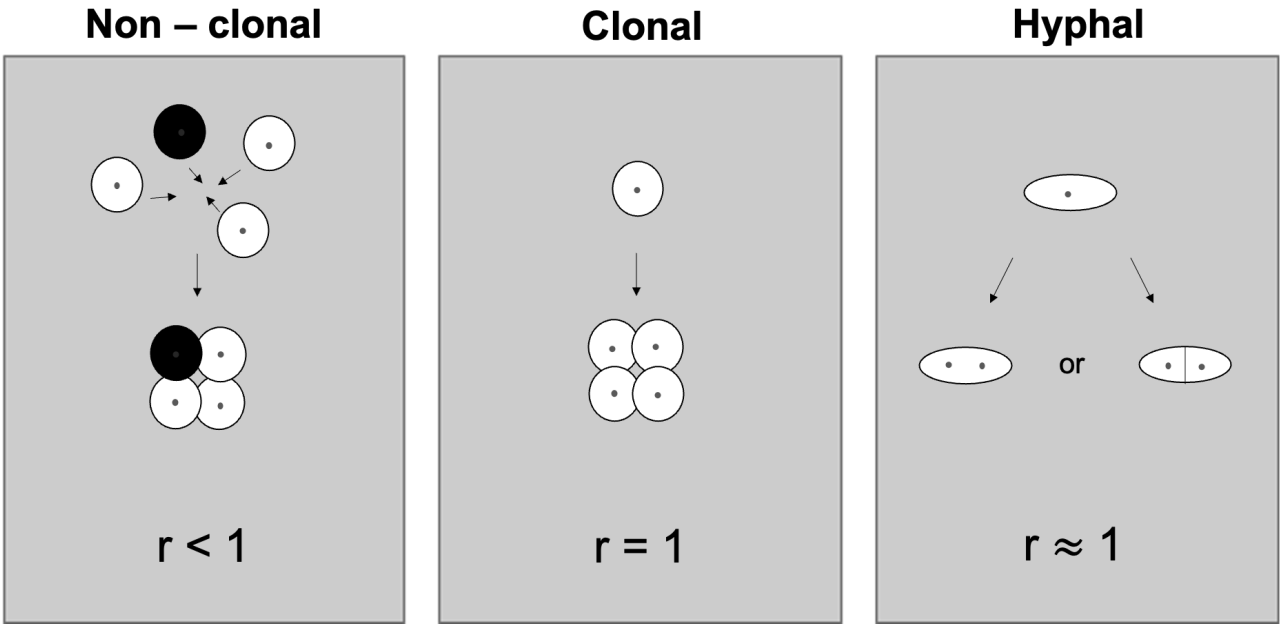

Figure 10.1: Multicellular group formation. Sketches showing non-clonal, clonal and hyphal multicellular group formation and the consequences of each for relatedness between cells.

10.2.1 Non-clonal (aggregative) multicellularity

We will only describe aggregative multicellularity very briefly here, as it has been covered extensively in Chapters 5-8. However, it is worth stating that non-clonal aggregative multicellularity has evolved independently in 5 different lineages and is therefore more common than clonal aggregative multicellularity, that has evolved independently in 3 different lineages, as a way of forming multicellular groups (Fisher et al., 2013). Succinctly put, aggregative multicellularity occurs when many individual cells in the environment come together to form a multicellular group. They do this not through mitotic cell division, but through either active movement or chance. This results in a group made of often genetically distinct cells (which is why it is also referred to as 'non-clonal' multicellularity). Some classic examples of species with non-clonal group formation include *Dictyostelium* (mentioned here before, and in detail in Chapter 7), *Myxobacteria* (Chapter 6), and many species of green algae in the Chlorophyta.

10.2.2 Hyphal multicellularity

Hyphal multicellularity is specific to Fungi, a lineage where multicellularity is somewhat distinct from all others. Multicellular fungi are composed of many hyphae (tubular structures that form by apical extension), and these hyphae form a mycelium that spreads out through a substrate to forage for nutrients (Olsson et al., 2002). This is a unique setup for a few reasons. Firstly, hyphae are not compartmentalized so do not need to solve conflicts between cells in the same way as non-clonal species (Scott et al., 2019). Secondly, there can also be multiple nuclei in one hypha. This makes hyphal multicellularity similar to the non-clonality that occurs in aggregative multicellularity. However, the way hyphae form is through cell division and does often result in an alignment of fitness interests across hyphae, which more closely resembles clonal group formation (Kuhn et al., 2001; Marleau et al., 2011). Hyphal multicellularity in the fungi probably didn't evolve in response to predators, like we think might have happened in other lineages, but instead helped with foraging for nutrients (Heaton et al., 2020; Olsson et al., 2002; Richards et al., 2017).

10.2.3 Clonal multicellularity

Clonal multicellularity is defined as when multicellular groups form through daughter cells remaining attached to their mother cells after division (Figure 10.1). This can be very simple, as displayed in *Chlamydomonas reinhardtii* (see later section), where several rounds of divisions take place leading to multiple cells attached with an extracellular matrix. This simple clonal group formation is found in, for example, *Scenedesmus*, *Candida albicans* and *Physarum polycephalum* (Baldauf & Doolittle, 1997; Berman, 2006; Engelberg et al., 1998; Everhart & Keller, 2008; Kapsetaki et al., 2017; Lürling, 2001; Whiteway & Bachewich, 2007). However, in the metazoan and plant lineages, large and complex multicellular bodies are formed through much the same process - a zygote divides many thousands of times to form a body with perhaps up to 100 quadrillion individual cells (Zhang et al., 2005). The fundamental similarity between all multicellular species that form this way is that (given everything else being equal) all cells in the multicellular body are clonally related. In other words, they are all copies of each other formed through mitosis (hence, the name). This mode of group formation results in special relatedness conditions between cells, which we deal with in more depth in our 'Social evolution primer' (below). This is in stark contrast to non-clonal group formation, where cells with different genetic backgrounds form a multicellular group.

Whilst aggregative and hyphal multicellularity can result in myriad of multicellular forms and, in some cases, impressively complex and coordinated behaviour (as described in Chapters 5 - 8), only clonal multicellularity has led to the levels of complexity demonstrated in the lineages of animals and plants. Only clonal multicellularity, where all cells in the body result from one (or very few) initial cells, has led to permanent division of labour and >100 different cell types (Fisher et al., 2013; Nagy et al., 2020). And whilst multicellularity has evolved >24 times, obligate multicellular organisms have in fact only evolved 9 times, and only in species with clonal multicellularity (Fisher et al., 2013). It is clear there is something unique about clonal multicellularity. To understand what, it is necessary to go back to social evolution theory.

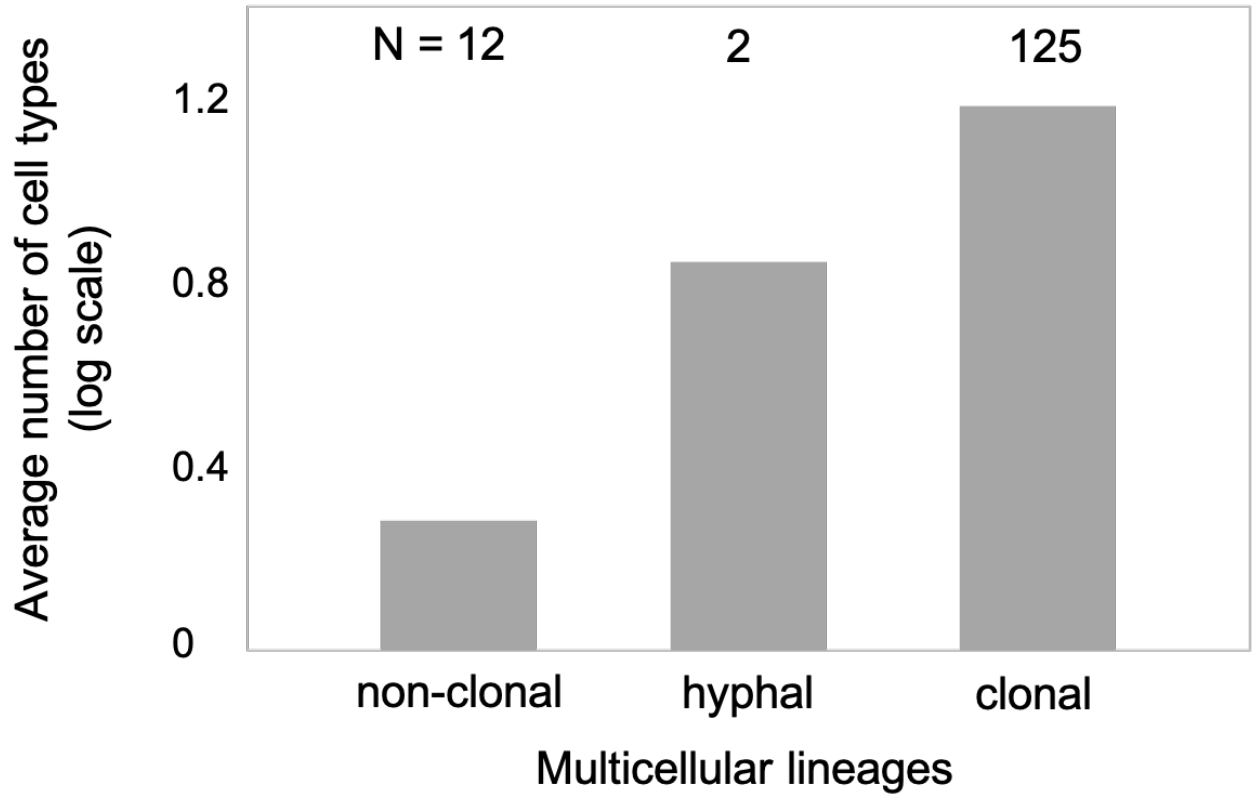

Figure 2: Cell-type complexity in non-clonal, hyphal and clonal multicellular lineages. N indicates the number of lineages in each category. Reproduced from Fisher et al., 2020.

10.3 Social evolution primer

At a fundamental level, multicellularity is a cooperative behaviour between cells. Here, we will just briefly touch on how cooperative behaviours can evolve between cells, but for an in-depth explanation of the evolution of cooperative behaviour, we direct the reader to Bourke (2011), Davies et al. (2012) and West et al. (2007). Simply put, in order for cooperation to evolve the cooperative behaviour needs to be beneficial and the benefits of cooperating need to be felt by the individual cells themselves or the relatives of those cells. For example, if clumping together in a group means that each individual cell has a lower risk of being eaten by a predator (Kapsetaki & West, 2019; Lürling, 1999b, 2020; Lürling et al., 1996; Van Donk et al., 1993), then cooperation can be favoured - there is a clear survival benefit to cooperating (not being eaten) and that benefit is felt by the individual cells in the group. In the above example, the cells experience *direct* benefits - the cooperating cells are the cells that receive the benefit of cooperation. However, cooperation can also evolve when cooperating cells experience *indirect* benefits - where it is the *relatives* of the cooperating cells that receive the benefits of cooperation. This requires that the cells are genetically related, so that the benefits of cooperation are directed towards other individuals that carry the same genes as the cooperator (for a more thorough explanation, see Fisher et al., 2013 and West et al., 2007).

When cells are clonally related, i.e. when relatedness (r) = 1, this creates specific conditions that allow altruism to evolve. This is because altruistic behaviours (Foster et al., 2006; Hamilton, 1964a,

1964b; West et al., 2007a) do not provide direct benefits to the cooperating cell, but instead provide indirect benefits to related cells. When the condition of $r = 1$ is consistent in time (throughout the lifespan of the multicellular body) and space (between all cells in the body), unconditionally altruistic cell types can evolve, that will always cooperate despite receiving no direct benefits. If $r < 1$ between cells, this opens up the possibility for natural selection to favour cells not cooperating.

Clonal group formation guarantees that all cells in the group will be genetically identical ($r = 1$), as they have all arisen through mitotic divisions from a single (or very few) cell(s). It is for this reason that multicellular species that develop through clonal group formation have been able to evolve an unconditional division of labour leading to many different cell types in obligately multicellular bodies. This has not been achieved by species developing through non-clonal group formation because relatedness between cells has not been high enough (and/or consistently high enough in time and space) to allow altruistic cooperation to evolve between cells (Fisher et al., 2013). For these species, there has always been the (at least theoretical) possibility that cells could 'do better' by not cooperating, and hence natural selection has not favoured the evolution of unconditional altruistic behaviour, such as permanent division of labour (for a detailed explanation, see Cooper & West, 2018).

As an interesting side note, this observation is reflected in other cooperative systems, most strikingly in animal societies. Just like multicellularity, animals from a variety of taxonomic groups live in cooperative groups (to varying degrees), including spiders, aphids, fish, meerkats, lions, bees, birds, monkeys - the list goes on. However, consistent with social evolution theory, the only lineages where unconditionally altruistic behaviours have evolved and cooperative groups have developed into obligate associations, is where groups begin with a strictly monogamous sexual pair (e.g. in ants) or where groups develop clonally (e.g. aphids) (Boomsma, 2007, 2009; Boomsma & Gawne, 2018; West et al., 2015).

It is worth noting that not all clonally developing species have become obligately multicellular. Clonal group formation does not guarantee that a species will evolve obligate multicellularity. This is because relatedness alone does not determine whether cooperation can evolve. It is a crucial point that clonal genetic relatedness is a necessary condition of obligate multicellularity, but it is not sufficient. The benefits (and costs) of multicellular cooperation still need to be high (and low) enough over time and through space to allow the evolution of unconditional altruism and obligate multicellularity (Foster et al., 2006; West et al., 2015). That is why there are many examples (see below) of multicellular species that develop clonally, but that have remained facultatively multicellular.

10.4 Benefits and costs of clonal multicellularity

In order to understand what the benefits and costs of clonal multicellularity might be, we cannot just focus on obligate, complex multicellular species. This is because it can be difficult to disentangle the benefits and costs of multicellularity in species with hundreds of cell types, and even more so in species that are obligately multicellular where we cannot compare the free-living cells with their multicellular equivalents. It is also difficult to disentangle the current benefits of multicellularity (e.g. the benefits of having specialized organs) with the initial benefits of clonality. For clonal group formation to have been favoured, there must have been consistent benefits of clonal group formation

versus unicellularity, and this can be especially hard to evaluate in highly complex species such as metazoans.

Ideally, we want to be able to 'look back in time' to see what the initial benefits may have been for forming multicellular groups through clonal group formation. Here, we focus on four examples of multicellular species that develop clonally and allow us to explore what the benefits and costs of clonal multicellularity might be.

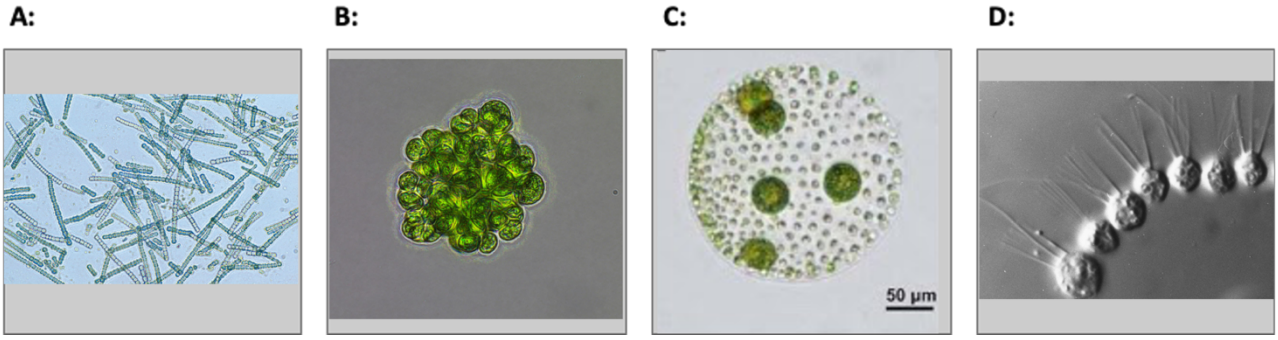

Figure 3. Examples of clonal multicellularity. A: Cyanobacteria (*Cylindrospermum* sp). Image courtesy of Adelaide laboratories of CSIRO Land and Water (1993). B: *Chlamydomonas reinhardtii.* Image courtesy of Josh Ming Borin (Herron et al., 2019). C: *Volvox carteri*. Image courtesy of Shelton & Michod, 2010. D: Choanoflagellate (*Desmarella moniliformis*). Image courtesy of CC BY-SA 3.0.

10.5 Cyanobacteria

Cyanobacteria are a large and diverse group of bacteria with an estimated 2698 species (Nabout et al., 2013). They include species such as *Microcystis aeruginosa* and *Nostoc thermotolerans*. For our purposes, what is particularly interesting about the cyanobacteria is that they include some of the simplest but oldest examples of clonal multicellularity (and, in fact, multicellularity in general). Evidence of multicellular filaments of cyanobacteria have been found in rocks from 3 billion years ago (Schirrmeister et al., 2011, 2013).

Certain species, notably *Oscillatoria sancta, Anabaena sp*., and *Cylindrospermum sp*., form long filaments of individual cells that originate from one precursor cell through clonal group formation (see Figure 10.3) (Schirrmeister et al., 2011). These filaments are obligately multicellular in the sense that the individual cells cannot survive and reproduce on their own (i.e. distinct from the group). Several species also have a division of labour between cells in the filament. For example, *Nostoc* species have cells called 'heterocysts' which fix nitrogen at night and vegetative cells that photosynthesise during the day (Bonner, 2003; Golden & Yoon, 2003; Kaiser, 2001; Schirrmeister et al., 2011). Nitrogen from the atmosphere is converted into ammonia via the enzyme nitrogenase, and this enzyme is inactivated by oxygen (Gallon, 1981; Rossetti et al., 2010). Such division of labour evolves when different partners (vegetative and heterocyst cells) receive accelerating fitness benefits from their environment when their tasks do not mix well or become more efficient (Cooper & West, 2018; Rossetti et al., 2010; West & Cooper, 2016; West et al., 2015). Accelerating fitness benefits means that the fitness of the individual performing the tasks increases over time (West et al., 2015). The fixation of nitrogen and photosynthesis are both beneficial to the cyanobacteria.

Cyanobacteria provide an example of how clonal group formation has allowed extreme morphological, functional, and spatiotemporal division of labour even in a relatively simple species (Cooper & West, 2018; Flores & Herrero, 2010; Schirrmeister et al., 2011). In fact, Rossetti et al. 2010 show that spatial structure, specifically the organisation of cells in compartments, is important in preventing the spread of cheating cells, allowing the evolution of cooperation and division of labour in this species.

10.6 *Chlamydomonas reinhardtii*

Chlamydomonas reinhardtii is a single-celled eukaryotic alga. When these cells are exposed to predators, they form multicellular groups through clonal group formation (Lurling & Beekman, 2006). One cell divides to gradually form a palmelloid cluster of many cells - a behaviour that increases their survival (Lurling & Beekman, 2006). Cellular clumping in response to predation is selectively advantageous for the prey, and has been observed in many more species of green algae (Kapsetaki & West, 2019; Lüring et al., 1997; Lürling, 1999a; Lürling et al., 1996; Van Donk et al., 1993).

However, these groups are not obligately multicellular (Figure 10.3). When removing the predators, algal groups break apart into single cells (Fisher et al., 2016; Kapsetaki et al., 2016). One of the explanations for this phenomenon is that being in a group can be costly for individual cells (Kapsetaki & West, 2019; J. T. O. Kirk, 1994; Lürling, 1999a; Ploug et al., 1999; Reynolds, 1984; Tollrian & Dodson, 1999; Trainor, 1998) because cells in the group may have less access to light due to higher sinking rates, and may pay a cost of producing extracellular adhesive molecules (Kirk, 1994; Lürling, 1999a; Ploug et al., 1999; Reynolds, 1984; Tollrian & Dodson, 1999; Francis Rice Trainor, 1998). *Chlamydomonas* is therefore a good example of a species that can form multicellular groups through clonal group formation, but does so facultatively.

An interesting consideration is the experimental evolution of multicellularity in *C. reinhardtii* by Herron et al. (2019) in response to predation pressure by *Paramecium tetraurelia*. Multicellular life cycles had a fitness advantage over the unicellular ancestors, showing that multicellularity conferred a significant benefit to *C. reinhardtii* cells. They were also able to show that multicellular groups of *C. reinhardtii* were stable over multiple generations, suggesting that obligate multicellularity could possibly evolve in the lab given the right conditions (Herron et al., 2019).

10.7 *Volvox carteri*

C. reinhardtii is a species in the order Chlamydomonadales also known as Volvocales (Buss, 1987; Grosberg & Strathmann, 2007; Koufopanou, 1994). This group contains more than 1760 species, many of which display multicellular phenotypes to varying degrees (Fritsch & West, 1927; Herron, 2009; Umen, 2020). Here, we focus on *Volvox carteri* (Fig. 3), arguably one of the most extensively studied multicellular species in the group (Herron et al., 2009), but a detailed account of multicellularity in the Volvocine algae is given in Chapter 11.

Volvox carteri forms spherical colonies composed of up to 6000 cells (Kochert, 1968; Smith, 1944), each of which bears a striking resemblance to *Chlamydomonas*. *V. carteri* is obligately multicellular, and its cells are functionally and reproductively differentiated (Buss, 1987; Grosberg & Strathmann,

2007; Koufopanou, 1994). Every colony contains around 16 larger reproductive cells that are enclosed by an outer layer of many more somatic cells that are terminally differentiated and contribute only to motility and photosynthesis (Matt & Umen, 2016; Shelton et al., 2012).

Division of labour between reproductive and somatic cells is achieved because some cells, during the first five rounds of cell division, express the gene regA whose protein inhibits cell growth (Herron, 2016; Meissner et al., 1999). These cells become smaller than 8μm, they keep their flagella, and become somatic non-reproductive cells. Other cells do not express regA and grow much larger, lose their flagella, and become reproductive germ cells (Hallmann, 2011; Kirk et al., 1993; Koufopanou, 1994).

Clonality plays a major role in *V. carteri*. The somatic and germ cells of *V. carteri* are clonal. In other words, their fitness interests are aligned. If a cell was to 'choose' to maximise its inclusive fitness by dividing (direct fitness) or sacrificing its reproduction to help a neighbouring cell divide (indirect fitness), either 'choice' would be similar in terms of fitness, since the neighbouring cell here is a clone (Foster et al., 2006). This is the case in *V. carteri*. Somatic cells have altruistically sacrificed their ability to reproduce, they are sterile, and only the germ cells reproduce. In this case of clonality, the maximal fitness of an individual cell is equal to the maximum fitness of the group (Davies et al., 2012; Michod et al., 2003, 2006).

10.8 Choanoflagellates

In marine and freshwater environments we find some single-celled eukaryotes, the choanoflagellates. They share a common ancestor with multicellular animals (e.g. Brunet & King, 2017; Fairclough et al., 2010; King, 2005; Koehl, 2020; Leadbeater, 2015; Mikhailov et al., 2009; Salvini-Plawen, 1978; Valentine & Marshall, 2015), and are therefore widely used as model organisms in the study of multicellular origins (Brunet et al., 2019; Brunet & King, 2017; Fairclough et al., 2013; King, 2004; King et al., 2008; Richter & King, 2013), including the benefits and costs of group formation. Groups of choanoflagellates have an evolutionary advantage over single cells in terms of their ability to forage (Kirkegaard & Goldstein, 2016; Nichols et al., 2009; Roper et al., 2013) and avoid predation (Koehl, 2020). Prey bacteria *Algoriphagus machipongonensis* release a sulfonolipid, RIF-1, in the extracellular environment. This localized concentration of RIF-1 can trigger facultative multicellular group formation in their predator *Salpingoeca rosetta* (Alegado et al., 2012). Group formation can provide a selective advantage to the choanoflagellate cells since they are better able to capture their prey than single cells (Kreft, 2010). The groups are formed by cells remaining attached after division (Dayel et al., 2011; Fairclough et al., 2010; Koehl, 2020; Laundon et al., 2019). Such attachment allows high relatedness that minimises conflict between cells (Buss, 1987; Grosberg & Strathmann, 2007), despite the potential costs that come with living in a multicellular group such as production and secretion of an extracellular matrix (Cavalier-Smith, 2017).

10.9 The role of the environment

All species are shaped by their environment, and multicellular species are of course no exception to this (Bonner, 1998; Darwin, 1859). In the section above, we have considered how the ecological benefits and costs of cooperation influence multicellularity in clonal species, and these can vary depending on the species and ecological conditions. However, it is becoming clear that the environment could play a larger role in determining the course of multicellular evolution and that the environment may impact non-clonal and clonal multicellular species in different ways.

A recent comparative study across 14 independent transitions to multicellularity has shown that in aquatic environments a higher proportion of lineages that originated there form groups clonally than non-clonally (Fisher et al., 2020). This includes multicellular lineages such as animals, plants, green algae, red algae and brown algae, who all originated in the sea and develop through clonal group formation. This result suggests (although is by no means definitive) that aquatic environment in general has an impact on which mode of group formation works best. One hypothesis, first suggested by Bonner (1998), is that in order to reap the benefits of being in a group, cells in the water need to stick together directly after division to avoid being dispersed by water currents. The study also showed that there have been more transitions to obligate multicellularity in aquatic environments compared to on land, which supports the previous observation that obligate multicellularity has only ever evolved in groups with clonal group formation (Fisher et al., 2013).

Temperature, calcium concentration, resource availability, and artificial selection can also affect multicellular group formation. At low temperatures, the facultatively multicellular algae *Scenedesmus* form multicellular groups (Egan & Trainor, 1989; Trainor, 1993; Trainor, 1992). High calcium concentrations induce aggregation in the cyanobacteria *Microcystis*, but when calcium concentrations decrease, they form colonies clonally by cell division (Chen & Lürling, 2020). In terms of resources, when they are abundant, we see monoclonal multicellular groups being prevalent, whereas when resources are scarce, polyclonal groups are more common (Hamant et al., 2019). In experimental conditions of artificial selection for fast-settling multicellular yeast, unicellular yeast can evolve into clonal multicellular clusters with division of labour (Ratcliff et al., 2012).

10.10 Is clonal development the only way?

In this chapter we have focused on the observation that all instances of obligate multicellularity have evolved in species with clonal group formation. But is clonality always necessary in the formation of an obligate multicellular organism? Here, we give several examples of how obligate multicellular organisms can 'break the rules' of clonality and challenge our idea that clonality is essential for obligateness.

10.11 Placozoa

Placozoa are an obligately multicellular phylum of animals consisting of three species: *Trichoplax adhaerens*, *Hoilungia hongkongensis*, and *Polyplacotoma mediterranea* (Eitel et al., 2013; Schierwater & Eitel, 2019; Bernd Schierwater & DeSalle, 2018). These microscopic marine organisms (Eitel et al., 2013; Grell & Ludwig, 1971; Pearse & Voigt, 2007; Signorovitch et al., 2006; Voigt et al., 2004) break up into single cells upon exposure to certain chemicals (colchicine, vinblastine, and sea water without divalent ions). Removal of these chemicals makes the cells reaggregate (Ruthmann & Terwelp, 1979). This phenomenon raises several questions. First, can cells of the disaggregated individual grow independently, as in a facultatively multicellular organism? Second, are non-clonal cells able to join the group during reaggregation? Third, do the reaggregated cells form an obligate multicellular organism (West et al., 2015)? Future experiments would need to confirm Ruthmann & Terwelp's (1979) findings of disaggregation and reaggregation in Placozoa, and further assess whether addition of non-clonal cells in the disaggregated clonal cellular culture leads to chimeric obligately multicellular individuals. This will help us answer whether obligate multicellularity can arise non-clonally in placozoa.

10.12 Microchimerism

Microchimerism refers to the presence of non-clonal cells inside the brain, thyroid, breast tissue, and bloodstream of mammals, including cattle, dogs, Rhesus monkeys, and even humans (Axiak-Bechtel et al., 2013; Bakkour et al., 2014; Barinaga, 2002; Boddy et al., 2015; Muehlenbachs et al., 2015; Owen, 1945; Turin et al., 2007; Van Dijk et al., 1996; Youssoufian & Pyeritz, 2002). These cells are exchanged between mother and fetus during pregnancy for many generations and can survive for over 27 years inside the mother as seen in humans (Barinaga, 2002; Boddy et al., 2015; Chan et al., 2012; Kinder et al., 2017; O'Donoghue et al., 2004; Yan et al., 2005). According to social evolution theory, non-clonal cells can be a source of conflict inside the multicellular organism, as the genetic fitness interests of all cells in the multicellular group are not aligned (Burt & Trivers, 2006; Gardner & Grafen, 2009; Queller & Strassmann, 2009; Roze & Michod, 2001; West & Gardner, 2013). Even though the presence of microchimerism cells is a fact, they do not outnumber the majority of the cells in our body which are clonal. Furthermore, centuries of transplantation experiments have shown that despite some observations of donor transplant cells moving to the semen of a recipient patient (Long & Chilton, 2019), multicellular organisms are known to reject foreign tissue via rapid immune responses (Brent, 1996; Medawar, 1948). The immune system tightly regulates such sources of conflict within the multicellular organism after obligateness has evolved.

10.13 Cancer

Another source of conflict within the obligate multicellular organism that challenges the notion of clonality being necessary in obligate multicellularity, are nucleotide substitutions among clonal cells. If these substitutions lead to cellular overproliferation and movement to other tissues, these non-clonal cells now can cause disease inside the multicellular body in the form of cancer. Cancer is found within the body of multicellular organisms across the tree of life (Aktipis et al., 2015), even between different bodies as in transmissible cancers (Metzger et al., 2016; Murchison, 2009; Murchison et al., 2014). However, this does not mean that the multicellular organism is non-clonal and obligate at the same time. The first steps in its development are clonal, with single-cell bottlenecks minimising the potential for conflict among cells (Buss, 1987; Fisher et al., 2013; Grosberg & Strathmann, 2007; Hurst et al., 1996; Michod, 2003; Michod et al., 1997; Michod, 1999; Niklas, 2014). If the first few divisions of multicellular life are non-clonal, with substitutions accumulating among cells, the embryo usually dies and is naturally aborted (Pandey et al., 2005; Tur-Torres et al., 2017).

Slime molds reveal that even if cells in a multicellular group are highly related, they are not necessarily on the 'road towards obligate multicellularity'. Slime molds form multicellular aggregates upon starvation (Bourke, 2011; Fisher et al., 2013; Smith et al., 2014). High relatedness ($r = 0.97$) prevents cheating cells from spreading in the population and allows high levels of altruism (Gilbert et al., 2007; Strassmann et al., 2000). Cells in the stalk altruistically sacrifice their ability to reproduce, and can only pass their genes to the next generation by helping the spores that reproduce. Still, these multicellular groups are facultatively multicellular (Gilbert et al., 2007). There is conflict among cells, as there has been selection for within-group kin discrimination (Mehdiabadi et al., 2006; Strassmann et al., 2000). For altruistic behaviour to become unconditional (permanent), any deviation from $r = 1$ means that ($rb < c$) the cost of cooperating will be larger, even if only slightly larger, than the benefit of cooperating and this will lead to a breakdown of the evolution of unconditional altruism (Bourke, 2011; Hamilton, 1964a, 1964b; West et al., 2007b, 2015).

10.14 Conclusion

In this chapter, we have tried to describe the importance and consequences of clonal group formation in multicellular evolution. Our purpose has been to provide an overview of the topic and we have tried to direct the reader to more detailed accounts where necessary.

The question, however, remains: why do some species form multicellular groups clonally, rather than by other means? We have described the consequences and advantages of clonal group formation *once it has evolved*, but what about the initial benefit? This is a much more difficult question to answer. We would argue that a combination of the physical environment and a species' biology imposes constraints on what type of group formation is possible - as always with evolution, there is some luck and chance at its core! It is possible that the physical environment of living in water created conditions that made clonal group formation much more likely than non-clonal group formation. It is also possible that certain species were predisposed to form groups clonally due to almost random facts of their biology, e.g. those with cell walls or particular proteins in their extracellular matrix. Whatever the reason, it is a fact that clonal group formation, under the influence of many ecological, intercellular, and intracellular, known and unknown factors, has led to some of the most impressive and complex multicellular radiations in the living world.

A crucial point that we have only briefly touched upon is the necessity of clonal group formation for the major evolutionary transition to multicellularity. In other words - the evolution of a multicellular individual. This is not a chapter devoted to the major evolutionary transitions in individuality, but it is worth noting that multicellularity has been included amongst the 8 or so transitions from the outset. Maynard-Smith & Szathmary recognised that the transition from unicells to multicells represented a 'key event in the history of life' (Maynard Smith & Szathmary, 1995) but it has only been more recently (Bourke, 2011; Michod, 2007; West et al., 2015) that the underlying evolutionary theory behind the major evolutionary transitions in individuality has been refined.

It is now clear that in order to transition from one level of individuality, single cells, to a new level of individuality, the obligate multicellular organism, isn't just made more likely by clonal group formation, but *requires* it. To use Andrew Bourke's terminology, clonal group formation allows not only group formation and group maintenance, but allows the final step in the evolution of multicellularity, group transformation (which could also be called the major evolutionary transition to individuality). This point leads us nicely to the next chapter in this book, which deals with multicellular group maintenance (Chapter 11).

References

Aktipis, C. A., Boddy, A. M., Jansen, G., Hibner, U., Hochberg, M. E., Maley, C. C., & Wilkinson, G. S. (2015). Cancer across the tree of life: cooperation and cheating in multicellularity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1673), 20140219–20140219. https://doi.org/10.1098/rstb.2014.0219

Alegado, R. A., Brown, L. W., Cao, S., Dermenjian, R. K., Zuzow, R., Fairclough, S. R., Clardy, J., & King, N. (2012). *A bacterial sulfonolipid triggers multicellular development in the closest living relatives of animals*. *1*, e00013. https://doi.org/10.7554/eLife.00013

Axiak-Bechtel, S. M., Kumar, S. R., Hansen, S. A., & Bryan, J. N. (2013). Y-chromosome DNA Is Present in the Blood of Female Dogs Suggesting the Presence of Fetal Microchimerism. *PLoS ONE*, *8*(7), e68114. https://doi.org/10.1371/journal.pone.0068114

Bakkour, S., Baker, C. A., Tarantal, A. F., Wen, L., Busch, M. P., Lee, T.-H., & McCune, J. M. (2014). Analysis of maternal microchimerism in rhesus monkeys (Macaca mulatta) using real-time quantitative PCR amplification of MHC polymorphisms. *Chimerism*, *5*(1), 6–15. https://doi.org/10.4161/chim.27778

Baldauf, S. L., & Doolittle, W. F. (1997). Origin and evolution of the slime molds (Mycetozoa). *Proceedings of the National Academy of Sciences*, *94*(22), 12007–12012.

Barinaga, M. (2002). *Cells exchanged during pregnancy live on*. American Association for the Advancement of Science.

Berman, J. (2006). Morphogenesis and cell cycle progression in Candida albicans. In *Current Opinion in Microbiology* (Vol. 9, Issue 6, pp. 595–601). https://doi.org/10.1016/j.mib.2006.10.007

Boddy, A. M., Fortunato, A., Wilson Sayres, M., & Aktipis, A. (2015). Fetal microchimerism and maternal health: A review and evolutionary analysis of cooperation and conflict beyond the womb. *BioEssays*, *37*(10), 1106–1118. https://doi.org/10.1002/bies.201500059

Bonner, J. (2009). *First signals: the evolution of multicellular development*. http://books.google.com/books?hl=en&lr=&id=RgB-NxcpTrEC&oi=fnd&pg=PP10&dq=evolution+of+multicellularity&ots=mDZMffuL2w&sig=vSgKAoowA_CS4U8738D0MXqs_oQ

Bonner, J. T. (1998). The origins of multicellularity. *Integrative Biology: Issues, News, and Reviews*, *1*(1), 27–36. https://doi.org/10.1002/(SICI)1520-6602(1998)1:1<27::AID-INBI4>3.3.CO;2-Y

Bonner, J. T. (2000). *First signals: the evolution of multicellular development*. Princeton University Press.

Bonner, J. T. (2003). Evolution of development in the cellular slime molds. *Evolution & Development*, *5*(3), 305–313.

Boomsma, J. J. (2007). Kin selection versus sexual selection: why the ends do not meet. *Current Biology*, *17*(16), R673–R683.

Boomsma, J. J. (2009). Lifetime monogamy and the evolution of eusociality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1533), 3191–3207. https://doi.org/10.1098/rstb.2009.0101

Boomsma, J. J., & Gawne, R. (2018). Superorganismality and caste differentiation as points of no

return: how the major evolutionary transitions were lost in translation. *Biological Reviews*, *93*(1), 28–54.

Bourke, A. F. G. (2011). *Principles of social evolution*. Oxford University Press Oxford.

Brent, L. (1996). *A history of transplantation immunology*. Academic Press.

Brunet, T., & King, N. (2017). The Origin of Animal Multicellularity and Cell Differentiation. In *Developmental Cell* (Vol. 43, Issue 2, pp. 124–140). Cell Press. https://doi.org/10.1016/j.devcel.2017.09.016

Brunet, T., Larson, B. T., Linden, T. A., Vermeij, M. J. A., McDonald, K., & King, N. (2019). Light-regulated collective contractility in a multicellular choanoflagellate. *Science*, *366*(6463), 326–334. https://doi.org/10.1126/science.aay2346

Burt, A., & Trivers, R. L. (2006). *Genes in Conflict (Belknap, Cambridge, MA)*.

Buss, L. W. (1987). The evolution of individuality. *Princeton Univ. Press, Princeton, NJ.*

Cavalier-Smith, T. (2017). Origin of animal multicellularity: precursors, causes, consequences—the choanoflagellate/sponge transition, neurogenesis and the Cambrian explosion. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1713), 20150476.

Chan, W. F. N., Gurnot, C., Montine, T. J., Sonnen, J. A., Guthrie, K. A., & Nelson, J. L. (2012). Male microchimerism in the human female brain. *PLoS One*, *7*(9), e45592.

Chen, H., & Lürling, M. (2020). Calcium promotes formation of large colonies of the cyanobacterium Microcystis by enhancing cell-adhesion. *Harmful Algae*, *92*, 101768.

Cooper, G. A., & West, S. A. (2018). Division of labour and the evolution of extreme specialization. *Nature Ecology & Evolution*, *2*(7), 1161–1167.

Darwin, C. (1859). *The Origin of Species by Means of Natural Election, Or the Preservation of Favored Races in the Struggle for Life*. AL Burt.

Davies, N. B., Krebs, J. R., & West, S. A. (2012). *An introduction to behavioural ecology*. John Wiley & Sons.

Dayel, M. J., Alegado, R. A., Fairclough, S. R., Levin, T. C., Nichols, S. A., McDonald, K., & King, N. (2011). Cell differentiation and morphogenesis in the colony-forming choanoflagellate Salpingoeca rosetta. *Developmental Biology*, *357*(1), 73–82. https://doi.org/10.1016/j.ydbio.2011.06.003

Egan, P. F., & Trainor, F. R. (1989). Low cell density: the unifying principle for unicell development in Scenedesmus (Chlorophyceae). *British Phycological Journal*, *24*(3), 271–283. https://doi.org/10.1080/00071618900650291

Eitel, M., Osigus, H.-J., DeSalle, R., & Schierwater, B. (2013). Global diversity of the Placozoa. *PloS One*, *8*(4), e57131.

Engelberg, D., Mimran, A., Martinetto, H., Otto, J., Simchen, G., Karin, M., & Fink, G. R. (1998). Multicellular Stalk-Like Structures inSaccharomyces cerevisiae. *Journal of Bacteriology*, *180*(15), 3992–3996.

Everhart, S. E., & Keller, H. W. (2008). Life history strategies of corticolous myxomycetes: the life cycle, plasmodial types, fruiting bodies, and taxonomic orders. *Fungal Diversity*, *29*, 1–16.

Fairclough, S. R., Chen, Z., Kramer, E., Zeng, Q., Young, S., Robertson, H. M., Begovic, E.,

Richter, D. J., Russ, C., Westbrook, M. J., Manning, G., Lang, B. F., Haas, B., Nusbaum, C., & King, N. (2013). Premetazoan genome evolution and the regulation of cell differentiation in the choanoflagellate Salpingoeca rosetta. *Genome Biology*, *14*(2), 1–15. https://doi.org/10.1186/gb-2013-14-2-r15

Fairclough, S. R., Dayel, M. J., & King, N. (2010). Multicellular development in a choanoflagellate. *Current Biology*, *20*(20), R875–R876.

Fisher, R. M., Shik, J. Z., & Boomsma, J. J. (2020). *The evolution of multicellular complexity: the role of relatedness and environmental constraints*. *287*(1931), 20192963. https://doi.org/10.1098/rspb.2019.2963

Fisher, Roberta M., Cornwallis, C. K., & West, S. A. (2013). Group Formation, Relatedness, and the Evolution of Multicellularity. *Current Biology*, *23*(12), 1120–1125. https://doi.org/10.1016/j.cub.2013.05.004

Fisher, Roberta May, Bell, T., & West, S. A. (2016). Multicellular group formation in response to predators in the alga Chlorella vulgaris. *Journal of Evolutionary Biology*, *29*(3), 551–559.

Flores, E., & Herrero, A. (2010). Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nature Reviews. Microbiology*, *8*(1), 39–50. https://doi.org/10.1038/nrmicro2242

Foster, K. R., Wenseleers, T., & Ratnieks, F. L. W. (2006). Kin selection is the key to altruism. In *Trends in Ecology and Evolution*. https://doi.org/10.1016/j.tree.2005.11.020

Fritsch, F. E., & West, G. S. (1927). A treatise on the British Freshwater Algae. *By the Late GS West. New and Revised Ed*.

Gallon, J. R. (1981). The oxygen sensitivity of nitrogenase: a problem for biochemists and micro-organisms. *Trends in Biochemical Sciences*, *6*, 19–23.

Gardner, A., & Grafen, A. (2009). Capturing the superorganism: a formal theory of group adaptation. *Journal of Evolutionary Biology*, *22*(4), 659–671.

Gilbert, O. M., Foster, K. R., Mehdiabadi, N. J., Strassmann, J. E., & Queller, D. C. (2007). *High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants*. www.pnas.org/cgi/content/full/

Golden, J. W., & Yoon, H.-S. (2003). Heterocyst development in Anabaena. *Current Opinion in Microbiology*, *6*(6), 557–563. https://doi.org/10.1016/j.mib.2003.10.004

Grell, K. G., & Ludwig, C. (1971). *Trichoplax adhaerens (Placozoa): Bewegung und Organisation*. Inst. fd wiss. Film.

Grosberg, R. K., & Strathmann, R. R. (2007). The Evolution of Multicellularity: A Minor Major Transition? *Annual Review of Ecology, Evolution, and Systematics*, *38*(1), 621–654.

Hallmann, A. (2011). Evolution of reproductive development in the volvocine algae. In *Sexual Plant Reproduction* (Vol. 24, Issue 2, pp. 97–112). https://doi.org/10.1007/s00497-010-0158-4

Hamant, O., Bhat, R., Nanjundiah, V., & Newman, S. A. (2019). Does resource availability help determine the evolutionary route to multicellularity? *Evolution and Development*, *21*(3), 115–119. https://doi.org/10.1111/ede.12287

Hamilton. (1964a). *The genetical evolution of social behaviour. Part I. J. Theor. Biol.*

Hamilton, W. D. D. (1964b). The genetical evolution of social behaviour. II. *Journal of Theoretical*

Biology, *7*(1), 17–52. https://doi.org/10.1016/0022-5193(64)90039-6

Hashimura, H., Morimoto, Y. V, Yasui, M., & Ueda, M. (2019). Collective cell migration of Dictyostelium without cAMP oscillations at multicellular stages. *Communications Biology*, *2*(1), 1–15.

Heaton, L. L. M., Jones, N. S., & Fricker, M. D. (2020). A mechanistic explanation of the transition to simple multicellularity in fungi. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-16072-4

Herron, M. (2009). Many from one: lessons from the volvocine algae on the evolution of multicellularity. *Communicative & Integrative Biology*, *2*(4), 368–370.

Herron, M. D. (2016). Origins of Multicellular Complexity: Volvox and the Volvocine Algae. *Molecular Ecology*, n/a-n/a. https://doi.org/10.1111/mec.13551

Herron, Matthew D, Borin, J. M., Boswell, J. C., Walker, J., Chen, I.-C. K., Knox, C. A., Boyd, M., Rosenzweig, F., & Ratcliff, W. C. (2019). De novo origins of multicellularity in response to predation. *Scientific Reports*, *9*(1), 1–9.

Herron, Matthew D, Hackett, J. D., Aylward, F. O., & Michod, R. E. (2009). Triassic origin and early radiation of multicellular volvocine algae. *Proceedings of the National Academy of Sciences*, *106*(9), 3254–3258.

Hurst, L. D., Atlan, A., & Bengtsson, B. O. (1996). Genetic Conflicts. *The Quarterly Review of Biology*, *71*(3), 317–364. https://doi.org/10.1086/419442

Kaiser, D. (2001). Building a multicellular organism. *Annual Review of Genetics*, *35*(1), 103–123.

Kapsetaki, S. E., Fisher, R. M., & West, S. A. (2016). Predation and the formation of multicellular groups in algae. *Evolutionary Ecology Research*, *17*(5), 651–669.

Kapsetaki, S. E., Tep, A., & West, S. A. (2017). How do algae form multicellular groups? *Evolutionary Ecology Research*, *18*, 663–675.

Kapsetaki, S. E., & West, S. A. S. A. (2019). The costs and benefits of multicellular group formation in algae*. *Evolution*, *73*(6), 1296–1308. https://doi.org/10.1111/evo.13712

Kessin, R. H. (2001). *Dictyostelium: evolution, cell biology, and the development of multicellularity.*

Kinder, J. M., Stelzer, I. A., Arck, P. C., & Way, S. S. (2017). Immunological implications of pregnancy-induced microchimerism. *Nature Reviews Immunology*, *17*(8), 483.

King, N. (2004). Review The Unicellular Ancestry of Animal Development emergence of comparative genomics have paved the way for new insights. Long-standing hypotheses regarding the identity of our protozoan relatives and the cellular. In *Developmental Cell* (Vol. 7).

King, N. (2005). Choanoflagellates. *Current Biology*, *15*(4), R113–R114.

King, N., Westbrook, M. J., Young, S. L., Kuo, A., Abedin, M., Chapman, J., Fairclough, S., Hellsten, U., Isogai, Y., Letunic, I., Marr, M., Pincus, D., Putnam, N., Rokas, A., Wright, K. J., Zuzow, R., Dirks, W., Good, M., Goodstein, D., … Rokhsar, D. (2008). The genome of the choanoflagellate Monosiga brevicollis and the origin of metazoans. *Nature*, *451*(7180), 783–788. https://doi.org/10.1038/nature06617

Kirk, J. T. O. (1994). *Light and photosynthesis in aquatic ecosystems*. Cambridge university press.

Kirk, M. M., Ransick, A., McRae, S. E., & Kirk, D. L. (1993). The relationship between cell size and cell fate in Volvox carteri. *The Journal of Cell Biology*, *123*(1), 191–208. https://doi.org/10.1083/jcb.123.1.191

Kirkegaard, J. B., & Goldstein, R. E. (2016). Filter-feeding, near-field flows, and the morphologies of colonial choanoflagellates. *Physical Review E*, *94*(5), 052401.

Knoll, A. H. (2011). The multiple origins of complex multicellularity. *Annual Review of Earth and Planetary Sciences*, *39*, 217–239.

KOCHERT, G. (1968). Differentiation of reproductive cells in Volvox carteri. *The Journal of Protozoology*, *15*(3), 438–452.

Koehl, M. A. R. (2020). Selective factors in the evolution of multicellularity in choanoflagellates. In *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*. John Wiley and Sons Inc. https://doi.org/10.1002/jez.b.22941

Koufopanou, V. (1994). The Evolution of Soma in the Volvocales. In *Source: The American Naturalist* (Vol. 143, Issue 5).

Kreft, J. M. (2010). *Effects of forming multicellular colonies or attaching to surfaces on feeding rates of the choanoflagellate Salpingoeca rosetta*. University of California, Berkeley.

Kuhn, G., Hijri, M., & Sanders, I. R. (2001). Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature*, *414*(6865), 745–748.

LaPaglia, C., & Hartzell, P. L. (1997). Stress-induced production of biofilm in thehyperthermophile Archaeoglobus fulgidus. *Applied and Environmental Microbiology*, *63*(8), 3158–3163. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1389226&tool=pmcentrez&rendertype=abstract

Laundon, D., Larson, B. T., McDonald, K., King, N., & Burkhardt, P. (2019). The architecture of cell differentiation in choanoflagellates and sponge choanocytes. *PLoS Biology*, *17*(4). https://doi.org/10.1371/journal.pbio.3000226

Leadbeater, B. S. C. (2015). *The choanoflagellates*. Cambridge University Press.

Long, & Chilton. (2019). *CHIMERIC FLUIDITY: A case of a male stem cell/bone marrow transplant patient.*

Lüring, M., Van Donk, E., Lürling, M., & Van Donk, E. (1997). Morphological changes in Scenedesmus induced by infochemicals released in situ from zooplankton grazers. *Limnology and Oceanography*, *42*(4), 783–788. https://doi.org/10.4319/lo.1997.42.4.0783

Lürling, M. (1999a). Grazer-induced coenobial formation in clonal cultures of Scenedesmus obliquus (Chlorococcales, Chlorophyceae). *Journal of Plankton Research*, *23*, 19–23. https://doi.org/10.1046/j.1529-8817.1999.3510019.x

Lürling, M. (1999b). Grazer-induced coenobial formation in clonal cultures of Scenendesmus obliquus (Chlorococcales, Chlorophyceae). *Journal of Phycology*, *35*(1), 19–23.

Lürling, M. (2001). Grazing-associated infochemicals induce colony formation in the green alga Scenedesmus. In *Protist* (Vol. 152, Issue 1, pp. 7–16). https://doi.org/10.1078/1434-4610-00038

Lürling, M. (2020). Grazing resistance in phytoplankton. *Hydrobiologia*, 1–13.

Lurling, M., & Beekman, W. (2006). Palmelloids formation in Chlamydomonas reinhardtii: defence

against rotifer predators? *Annales de Limnologie-International Journal of Limnology*, *42*(2), 65–72.

Lürling, M., Van Donk, E., Donk, E., & Van Donk, E. (1996). Zooplankton-induced unicell-colony transformation in Scenedesmus acutus and its effect on growth of herbivore Daphnia. *Oecologia*, *108*(3), 432–437. https://doi.org/10.1007/BF00333718

Lyons, N. A., & Kolter, R. (2015). On the evolution of bacterial multicellularity. *Current Opinion in Microbiology*, *24*, 21–28. https://doi.org/10.1016/j.mib.2014.12.007

Marleau, J., Dalpé, Y., St-Arnaud, M., & Hijri, M. (2011). Spore development and nuclear inheritance in arbuscular mycorrhizal fungi. *BMC Evolutionary Biology*, *11*(1), 1–11.

Matt, G., & Umen, J. (2016). Volvox: A simple algal model for embryogenesis, morphogenesis and cellular differentiation. *Developmental Biology*, *419*(1), 99–113.

Mayerhofer, L. E., Macario, A. J. L., Conway, E., & Macario, D. E. (1992). NOTES Lamina, a Novel Multicellular Form of Methanosarcina mazei S-6 Downloaded from. In *JOURNAL OF BACTERIOLOGY* (Vol. 174, Issue 1). http://jb.asm.org/

Maynard Smith, J., & Szathmary, E. (1995). *The major transitions in evolution*. W.H. Freeman Spektrum.

Medawar, P. B. (1948). Immunity to homologous grafted skin. III. The fate of skin homographs transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *British Journal of Experimental Pathology*, *29*(1), 58.

Mehdiabadi, N. J., Jack, C. N., Farnham, T. T., Platt, T. G., Kalla, S. E., Shaulsky, G., Queller, D. C., & Strassmann, J. E. (2006). Social evolution: kin preference in a social microbe. *Nature*, *442*(7105), 881–882.

Meissner, M., Stark, K., Cresnar, B., Kirk, D. L., & Schmitt, R. (1999). Volvox germline-specific genes that are putative targets of RegA repression encode chloroplast proteins. *Current Genetics*, *36*(6), 363–370. https://doi.org/10.1007/s002940050511

Metzger, M. J., Villalba, A., Carballal, M. J. M. J., Iglesias, D., Sherry, J., Reinisch, C., Muttray, A. F., Baldwin, S. A., & Goff, S. P. (2016). Widespread transmission of independent cancer lineages within multiple bivalve species. *Nature*, *534*(7609), 705–709. https://doi.org/10.1038/nature18599

Michod, R E. (2003). Cooperation and conflict mediation in the evolution of multicellularity. *Genetic and Cultural Evolution of Cooperation. MIT Press, Cambridge, Massachusetts*, 291–308.

Michod, Richard E. (2007). Evolution of individuality during the transition from unicellular to multicellular life. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 8613–8618. https://doi.org/10.1073/pnas.0701489104

Michod, Richard E., Roze, D., & Biolog, E. (1997). Transitions in individuality. *Proceedings. Biological Sciences / The Royal Society*, *264*(1383), 853–857. https://doi.org/10.1098/rspb.1997.0119

Michod, Richard E. (1999). Individuality, immortality, and sex. *Levels of Selection in Evolution*, *66*, 53.

Michod, Richard E, Nedelcu, A. M., & Roze, D. (2003). Cooperation and conflict in the evolution of individuality: IV. Conflict mediation and evolvability in Volvox carteri. *BioSystems*, *69*(2–

3), 95–114.

Michod, Richard E, Viossat, Y., Solari, C. A., Hurand, M., & Nedelcu, A. M. (2006). Life-history evolution and the origin of multicellularity. *Journal of Theoretical Biology*, *239*(2), 257–272.

Mikhailov, K. V., Konstantinova, A. V., Nikitin, M. A., Troshin, P. V., Rusin, L. Y., Lyubetsky, V. A., Panchin, Y. V., Mylnikov, A. P., Moroz, L. L., Kumar, S., & Aleoshin, V. V. (2009). The origin of Metazoa: A transition from temporal to spatial cell differentiation. *BioEssays*, *31*(7), 758–768. https://doi.org/10.1002/bies.200800214

Muehlenbachs, A., Bhatnagar, J., Agudelo, C. A., Hidron, A., Eberhard, M. L., Mathison, B. A., Frace, M. A., Ito, A., Metcalfe, M. G., Rollin, D. C., Visvesvara, G. S., Pham, C. D., Jones, T. L., Greer, P. W., Hoyos, A. V., Olson, P. D., Diazgranados, L. R., & Zaki, S. R. (2015). Malignant transformation of Hymenolepis nana in a human host. *New England Journal of Medicine*, *373*(19), 1845–1852. https://doi.org/10.1056/NEJMoa1505892

Murchison, E P. (2009). Clonally transmissible cancers in dogs and Tasmanian devils. *Oncogene*, *27*(S2), S19–S30. https://doi.org/10.1038/onc.2009.350

Murchison, Elizabeth P., Wedge, D. C., Alexandrov, L. B., Fu, B., Martincorena, I., Ning, Z., Tubio, J. M. C. C., Werner, E. I., Allen, J., De Nardi, A. B., Donelan, E. M., Marino, G., Fassati, A., Campbell, P. J., Yang, F., Burt, A., Weiss, R. A., & Stratton, M. R. (2014). Transmissible dog cancer genome reveals the origin and history of an ancient cell lineage. *Science*, *343*(6169), 437–440. https://doi.org/10.1126/science.1247167

Nabout, J. C., da Silva Rocha, B., Carneiro, F. M., & Sant'Anna, C. L. (2013). How many species of Cyanobacteria are there? Using a discovery curve to predict the species number. *Biodiversity and Conservation*, *22*(12), 2907–2918.

Nagy, L. G., Varga, T., Csernetics, Á., & Virágh, M. (2020). Fungi took a unique evolutionary route to multicellularity: Seven key challenges for fungal multicellular life. In *Fungal Biology Reviews*. Elsevier Ltd. https://doi.org/10.1016/j.fbr.2020.07.002

Nichols, S. A., Dayel, M. J., & King, N. (2009). Genomic, phylogenetic, and cell biological insights into metazoan origins. *Animal Evolution: Genomes, Fossils and Trees*, 24–32.

Niklas, K. J. (2014). The evolutionary-developmental origins of multicellularity. *American Journal of Botany*, *101*(1), 6–25. https://doi.org/10.3732/ajb.1300314

Niklas, K. J., & Newman, S. A. (2013). The origins of multicellular organisms. *Evolution & Development*, *15*(1), 41–52.

O'Donoghue, K., Chan, J., de la Fuente, J., Kennea, N., Sandison, A., Anderson, J. R., Roberts, I. A. G., & Fisk, N. M. (2004). Microchimerism in female bone marrow and bone decades after fetal mesenchymal stem-cell trafficking in pregnancy. *The Lancet*, *364*(9429), 179–182.

Olsson, P. A., Jakobsen, I., & Wallander, H. (2002). Foraging and resource allocation strategies of mycorrhizal fungi in a patchy environment. In *Mycorrhizal ecology* (pp. 93–115). Springer.

Owen, R. D. (1945). Immunogenetic consequences of vascular anastomoses between bovine twins. *Science*, *102*(2651), 400–401.

Pandey, M. K., Rani, R., & Agrawal, S. (2005). An update in recurrent spontaneous abortion. In *Archives of Gynecology and Obstetrics* (Vol. 272, Issue 2, pp. 95–108). https://doi.org/10.1007/s00404-004-0706-y

Pearse, V. B., & Voigt, O. (2007). Field biology of placozoans (Trichoplax): distribution, diversity,

biotic interactions. *Integrative and Comparative Biology*, *47*(5), 677–692.

Ploug, H., Stolte, W., & Jørgensen, B. B. (1999). Diffusive boundary layers of the colony-forming plankton alga, Phaeocystis sp. - implications for nutrient uptake and cellular growth. *Limnology and Oceanography*, *44*(8), 1959–1967.

Queller, D. C., & Strassmann, J. E. (2009). Beyond society: the evolution of organismality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1533), 3143–3155.

Ratcliff, W. C., Denison, R. F., Borrello, M., & Travisano, M. (2012). Experimental evolution of multicellularity. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(5), 1595–1600. https://doi.org/10.1073/pnas.1115323109

Reynolds, C. S. (1984). *The ecology of freshwater phytoplankton*. Cambridge University Press.

Richards, T. A., Leonard, G., & Wideman, J. G. (2017). What defines the "kingdom" fungi? *The Fungal Kingdom*, 57–77.

Richter, D. J., & King, N. (2013). The genomic and cellular foundations of animal origins. In *Annual Review of Genetics* (Vol. 47, pp. 509–537). Annual Reviews Inc. https://doi.org/10.1146/annurev-genet-111212-133456

Roper, M., Dayel, M. J., Pepper, R. E., & Koehl, M. A. R. (2013). Cooperatively generated stresslet flows supply fresh fluid to multicellular choanoflagellate colonies. *Physical Review Letters*, *110*(22), 1–5. https://doi.org/10.1103/PhysRevLett.110.228104

Rossetti, V., Schirrmeister, B. E., Bernasconi, M. V., & Bagheri, H. C. (2010). The evolutionary path to terminal differentiation and division of labor in cyanobacteria. *Journal of Theoretical Biology*, *262*(1), 23–34. https://doi.org/10.1016/j.jtbi.2009.09.009

Roze, D., & Michod, R. E. (2001). Mutation , Multilevel Selection , and the Evolution of Propagule Size during the Origin of Multicellularity. *The American Naturalist*, *158*(6), 638–654.

Ruthmann, A., & Terwelp, U. (1979). Disaggregation and reaggregation of cells of the primitive metazoon Trichoplax adhaerens. *Differentiation*, *13*(3), 185–198.

Salvini-Plawen, L. V. (1978). On the origin and evolution of the lower Metazoa. *Journal of Zoological Systematics and Evolutionary Research*, *16*(1), 40–87.

Schierwater, B, & Eitel, M. (2019). *World Placozoa Database in the Catalogue of Life*.

Schierwater, Bernd, & DeSalle, R. (2018). Placozoa. *Current Biology*, *28*(3), R97–R98.

Schirrmeister, B. E., Antonelli, A., & Bagheri, H. C. (2011). The origin of multicellularity in cyanobacteria. *BMC Evolutionary Biology*, *11*(1), 45.

Schirrmeister, B. E., de Vos, J. M., Antonelli, A., & Bagheri, H. C. (2013). Evolution of multicellularity coincided with increased diversification of cyanobacteria and the Great Oxidation Event. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(5), 1791–1796. https://doi.org/10.1073/pnas.1209927110

Scott, T. W., Kiers, E. T., Cooper, G. A., dos Santos, M., & West, S. A. (2019). Evolutionary maintenance of genomic diversity within arbuscular mycorrhizal fungi. *Ecology and Evolution*, *9*(5), 2425–2435. https://doi.org/10.1002/ece3.4834

Shelton, D. E., Desnitskiy, A. G., & Michod, R. E. (2012). Distributions of reproductive and somatic cell numbers in diverse Volvox (Chlorophyta) species. *Evolutionary Ecology*

Research, *14*, 707.

Shelton, D. E., & Michod, R. E. (2010). Philosophical foundations for the hierarchy of life. *Biology & Philosophy*, *25*(3), 391–403.

Signorovitch, A. Y., Dellaporta, S. L., & Buss, L. W. (2006). Caribbean placozoan phylogeography. *The Biological Bulletin*, *211*(2), 149–156.

Smith, G. M. (1944). A comparative study of the species of Volvox. *Transactions of the American Microscopical Society*, *63*(4), 265–310.

Smith, J., Queller, D. C., & Strassmann, J. E. (2014). Fruiting bodies of the social amoeba Dictyostelium discoideum increase spore transport by Drosophila. *BMC Evolutionary Biology*, *14*(1), 105.

Strassmann, J. E. (2019). Dictyostelium, the social amoeba. *Elsevier*.

Strassmann, J. E., & Queller, D. C. (2011). How social evolution theory impacts our understanding of development in the social amoeba Dictyostelium. *Development, Growth & Differentiation*, *53*(4), 597–607.

Strassmann, J. E., Zhu, Y., & Queller, D. C. (2000). *Altruism and social cheating in the social amoeba Dictyostelium discoideum*. 965–967.

Tollrian, R., & Dodson, S. I. (1999). Inducible defences in cladocera: constraints, costs, and multipredator environments. *The Ecology and Evolution of Inducible Defenses (Ed TRHCD)*, 177–202.

Trainor, F R. (1993). Cyclomorphosis in Scenedesmus subspicatus (Chlorococcales, Chlorophyta): stimulation of colony development at low temperature. *Phycologia*, *32*(6), 429–433.

Trainor, Francis R. (1992). CYCLOMORPHOSIS IN SCENEDESMUS ARMATUS (CHLOROPHYTA): AN ORDERED SEQUENCE OF ECOMORPH DEVELOPMENT 1. *Journal of Phycology*, *28*(4), 553–558.

Trainor, Francis Rice. (1998). *Biological aspects of Scenedesmus (Chlorophyceae)-phenotypic plasticity* (Vol. 117). J. Cramer.

Tur-Torres, M. H., Garrido-Gimenez, C., & Alijotas-Reig, J. (2017). Genetics of recurrent miscarriage and fetal loss. In *Best Practice and Research: Clinical Obstetrics and Gynaecology* (Vol. 42, pp. 11–25). Bailliere Tindall Ltd. https://doi.org/10.1016/j.bpobgyn.2017.03.007

Turin, L., Invernizzi, P., Woodcock, M., Grati, F. R., Riva, F., Tribbioli, G., & Laible, G. (2007). Bovine fetal microchimerism in normal and embryo transfer pregnancies and its implications for biotechnology applications in cattle. *Biotechnology Journal: Healthcare Nutrition Technology*, *2*(4), 486–491.

Umen, J. G. (2020). Volvox and volvocine green algae. *EvoDevo*, *11*(1), 1–9.

Valentine, J. W., & Marshall, C. R. (2015). Fossil and transcriptomic perspectives on the origins and success of metazoan multicellularity. In *Evolutionary transitions to multicellular life* (pp. 31–46). Springer.

Van Dijk, B. A., Boomsma, D. I., & De Man, A. J. M. (1996). Blood Group Chimerism in Human Multiple Births Is Not Rare. In *American Journal of Medical Genetics* (Vol. 61).

Van Donk, E., Hessen, D. O., & Van Donk, E. (1993). Morphological changes in Scenedesmus

induced by substances released from Daphnia. *Archiv Für Hydrobiologie*, *127*, 129–140.

Voigt, O., Collins, A. G., Pearse, V. B., Pearse, J. S., Ender, A., Hadrys, H., & Schierwater, B. (2004). Placozoa–no longer a phylum of one. *Current Biology*, *14*(22), R944–R945.

West, S. A. A., & Gardner, A. (2013). Adaptation and Inclusive Fitness. *Current Biology*, *23*(13), R577–R584. https://doi.org/10.1016/j.cub.2013.05.031

West, Stuart A, & Cooper, G. A. (2016). Division of labour in microorganisms: an evolutionary perspective. *Nature Reviews Microbiology*, *14*(11), 716.

West, Stuart Andrew, Fisher, R. M., Gardner, A., & Kiers, E. T. (2015). Major evolutionary transitions in individuality. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(33), 10112–10119. https://doi.org/10.1073/pnas.1421402112

West, Stuart Andrew, Griffin, a. S., & Gardner, a. (2007a). Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. *Journal of Evolutionary Biology*, *20*(2), 415–432. https://doi.org/10.1111/j.1420-9101.2006.01258.x

West, Stuart Andrew, Griffin, A. S., & Gardner, A. (2007b). Evolutionary explanations for cooperation. *Current Biology : CB*, *17*(16), R661-72. https://doi.org/10.1016/j.cub.2007.06.004

Whiteway, M., & Bachewich, C. (2007). Morphogenesis in Candida albicans. *Annu. Rev. Microbiol.*, *61*, 529–553.

Yan, Z., Lambert, N. C., Guthrie, K. A., Porter, A. J., Loubiere, L. S., Madeleine, M. M., Stevens, A. M., Hermes, H. M., & Nelson, J. L. (2005). Male microchimerism in women without sons: quantitative assessment and correlation with pregnancy history. *The American Journal of Medicine*, *118*(8), 899–906.

Youssoufian, H., & Pyeritz, R. E. (2002). Mechanisms and consequences of somatic mosaicism in humans. *Nature Reviews Genetics*, *3*(10), 748–758. https://doi.org/10.1038/nrg906

Zhang, J., Del Aguila, R., Schneider, C., & Schneider, B. L. (2005). The importance of being big. *Journal of Investigative Dermatology Symposium Proceedings*, *10*(2), 131–141.