

Multi-species multicellular life cycles

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Abstract

Textbook examples of multicellular organisms vary in their scale and complexity but are typically composed of a single species. The prevalence of entities such as lichens, however, suggest that two different species may be capable of forming a type of multi-species multicellularity—though it may appear or act dissimilar from its clonal counterparts. In this chapter, we consider the possibility of multi-species multicellularity and in particular its origins. Drawing upon previous studies of the evolutionary origins of clonal multicellularity, we focus on the emergence of simple reproducing groups that have the capacity to gain adaptations. We present a framework for organizing these initial multi-species group life cycles based on whether the constituent species are unicellular or multicellular and whether the groups reproduce via fragmentation or cycles of dissociation and re-association. We discuss characteristics of each type of multi-species multicellularity and representative examples to assess their likely evolutionary trajectories. Ultimately, we conclude that the multi-species groups that most resemble textbook multicellular organisms are composed of a unicellular and multicellular species and use cycles of dissociation and re-association to reproduce.

17.1 Grafting, chimeras, and Frankenstein creations

In order to boost productivity, commercial agriculture embraces something unnatural (Lee et al., 2010). Like some creation of Dr. Frankenstein or a creature from Greek mythology, different plant species are fused together in a process called grafting. For example, the fruiting part of a watermelon may be fused to the root system of a pumpkin. The resulting chimeric plant produces watermelon fruit but is more resistant to disease and stress than a typical watermelon (Davis et al., 2008). Grafting allows farmers to harness beneficial aspects from different species but in a way that does not naturally occur. Watermelons and pumpkins do not normally detach parts in

order to fuse with each other, even though grafting shows there could be potential gains from doing so. So why do such multi-species chimeras need human intervention to come into existence—why are they more associated with mythology than biology?

At first glance, there seem to be significant potential benefits to forming multi-species chimeras. The extensive number and diversity of mutualisms within the biological world demonstrate that cooperation among different species can produce synergies either through division of labor or novel combinations of functionalities (Bronstein et al., 2006; Callaway, 1995; Gestel et al., 2015; Hay et al., 2004; Janzen, 1985; Knowlton and Rohwer, 2003). Moreover, combining different genomes into a single, fitter entity is also widespread within biology; it is the basis of hybrid vigor (Birchler et al., 2003) and a well-recognized benefit of sexual reproduction (Colegrave, 2002; Michod et al., 2008). Yet, unlike hybridization and sex, multi-species chimeras keep their different genomes distinct, which means they likely face fewer issues of genetic incompatibilities. Since multi-species chimeras are not limited by traditional species boundaries, they have many possible combinations and opportunities available to uncover potential synergistic benefits.

Of course, even if there are significant possible benefits of forming multi-species chimeras, there may be barriers that prevent their formation or persistence. If we return to the example of the grafted watermelon-pumpkin combination, one obvious barrier to them forming naturally is reproduction. The pumpkin roots have no way of producing offspring so there is no direct way for the watermelon-pumpkin chimera to produce watermelon-pumpkin offspring. However, unlike mules and other sterile hybrids that also cannot reproduce, the grafted watermelon-pumpkin entity does not fuse genetic material, so its sterility is not necessarily due to a failure of genetics but instead a failure of configuration. It seems plausible that a different configuration would allow successful reproduction of the whole entity. In this chapter we consider possible configurations of multi-species chimeras that can reproduce and potentially evolve. Identifying such configurations may reveal the structure of existing multi-species chimeras or help explain their relative rarity.

17.2 Multi-species “organisms”

Before we identify possible configurations of multi-species chimeras, it is useful to have some benchmark for what kind of entity we are after. We use the term “entity” because it is not clear if or when a group of multiple species might be an “organism” or “individual”. Certainly there are many groups of species that are simply populations living in a shared environment, e.g. an arbitrary group of animals in the Serengeti. Yet, there are other examples of groups formed by different species that function like organisms or individuals (Bourrat and Griffiths, 2018; Godfrey-Smith, 2009; Queller and Strassmann, 2009). Lichens, for instance, are formed by a partnership between fungi and photosynthetic species (algae and/or cyanobacteria) and differ from an arbitrary group of species in fundamental ways: they exhibit complex traits that are not expressed by either constituent species on its own, they interact with their environment and other species in novel ways, and they can reproduce (at least vegetatively) and gain adaptations (Nash, 2008). If lichens are organisms and arbitrary groups of animals in the Serengeti are not, then it raises the question: where is the boundary between a community and a multi-species organism?

One difficulty with this question lies in how to disentangle a species or organism from others in its environment (Bourrat and Griffiths, 2018). For example, a human is often considered to be an

example of a single-species organism, but it is intimately associated with a large community of microbes that play a significant functional role. Disruptions of these communities can impair fitness or even development of their hosts (Gilbert et al., 2012). The associations have led some to describe such host-microbiome associations as new types of biological entities, called holobionts, similar in some ways to a lichen (Bordenstein and Theis, 2015; Gilbert et al., 2012; Rosenberg and Zilber-Rosenberg, 2016). Others view holobiont associations more as ecological communities of organisms, e.g. a human and many environmentally-associated microbial species (Douglas and Werren, 2016; Moran and Sloan, 2015; Queller and Strassmann, 2016; Skillings, 2016).

A simple approach to delineating between populations and multi-species organisms is to adopt a restrictive organism definition such that only entities formed by a single species (or genome) are organisms and everything else is a community. A problem with this approach is the classification of eukaryotes. Eukaryotes have intracellular organelles such as the mitochondria and chloroplasts that evolved from endosymbioses between different species. These organelles retain their own distinct DNA, which means eukaryotes violate the restrictive, single-species definition of an organism and would be considered communities rather than organisms.

Regardless of whether eukaryotes are communities or organisms, there is a broader, underlying issue that groups of species seem different in terms of their functional integration and evolvability (Godfrey-Smith, 2009). For example, contrast a unicellular eukaryote such as a yeast or algal cell with an arbitrary community of soil bacteria. Besides the unicellular eukaryote's higher level of functional integration, it can give rise to offspring that resemble the parent. Furthermore, cell walls make offspring distinct from their parents, which helps selection fix mutations that increase the fitness of the unicellular eukaryote. In contrast, the ability of a community of soil bacteria to reproduce depends on what it means for the community as a whole to reproduce—is it at least one cell of each species or do the ratios somehow matter? Moreover, without physical boundaries between parent and offspring soil communities, it can be difficult for selection to act and fix beneficial mutations.

The lack of a clear distinction between a community and a multi-species organism and the recognition that multi-species populations vary in terms of functionality and evolvability suggest a different approach. Strassmann and Queller in (Queller and Strassmann, 2009) propose viewing the term “organism” as a spectrum, such that some entities are more organismal than others (Godfrey Smith also adopts a spectrum approach in (Godfrey-Smith, 2009) to classify “Darwinian populations”). The minimum requirement for any organismal entity is that it has the capacity to reproduce and gain adaptations. So the watermelon-pumpkin grafting is not organismal because it cannot reproduce, but a lichen is organismal. After satisfying the minimal criteria, the extent to which an entity is organismal, its “organismality”, depends on other factors. Strassmann and Queller consider cooperation and conflict between the entity's constituent parts such that more organismal entities have high cooperation and low conflict. The key idea for our purposes is that there is a spectrum of organismality, and multi-species entities may evolve within this spectrum to become more/less organismal.

A consequence of the spectrum approach to organismality is that it suggests an evolutionary process by which a multi-species community may become a multi-species “organism”. If an initial configuration of multiple species can somehow form entities capable of reproduction and evolution, then it may become more organismal by gaining mutations that reduce conflict or enhance cooperation—though it is not a certainty that it will evolve into something more

organismal. Based on studies of the evolution of multicellularity within single species, it is likely that the evolutionary trajectory of the multi-species entity will depend heavily on its initial configuration. In the next sections, we consider the various starting configurations of organismal multi-species entities with an aim of understanding their evolutionary potential for organismality.

7.3 Multi-species group configurations

We now consider how an organismal multi-species entity first arises within a community. Since a multi-species entity must keep its different genomes distinct, it is likely to resemble some form of multicellularity—assuming that cells from different species do not fuse. Thus, we draw upon studies of the evolutionary origins of multicellularity to understand how a population of cells (or species) may evolve into something organismal. In particular, we focus on two basic requirements of any multicellular entity's life cycle (Black et al., 2020; Libby and Rainey, 2013; Ratcliff et al., 2017; Van Gestel and Tarnita, 2017): 1. a group structure and 2. a mode of group reproduction. We consider these aspects separately in order to produce a combinatorial framework that exhaustively describes initial configurations of an organismal multi-species entity.

Although the concept of a group is useful in studies of multicellularity as a way to distinguish nascent multicellularity from its ancestral unicellular population, the nature of what constitutes a group varies greatly between studies. For example, in studies that use experimental evolution techniques to evolve multicellularity, groups often form because mutations cause cells to stay physically attached following reproduction (Herron et al., 2019; Ratcliff et al., 2012, 2013). In contrast, social evolution studies often consider groups that form through cells aggregating and temporarily binding (Kessin, 2001; Muñoz-Dorado et al., 2016; Strassmann et al., 2000; Velicer et al., 2000). Groups can also form without any direct binding or attachment, rather by enclosing cells within a membrane or boundary (Black et al., 2020; Doulcier et al., 2020). If groups can form through temporary interactions or by enclosure within a boundary, it can be difficult to specify precise requirements that distinguish groups from populations. For our purposes we consider a broad interpretation of groups and only require some direct interaction between constituent cells. We also note that since an organismal group needs some mechanism for reproduction and evolution, the group structure should make this possible if not straightforward.

In terms of how groups form, there may be many possible factors that determine whether different species can form a group together. For example, lifestyle or chemical repertoire might play a role such that organisms that produce extracellular glues to attach to surfaces might be more likely to form multi-species groups, by adhering to other species (Niklas and Newman, 2013; Rokas, 2008). In this chapter we choose to focus on whether each species is unicellular or multicellular, because it is likely to be influential in group formation. If one species is already multicellular, then it has the capacity to form a group of cells that can give rise to new groups. Adding another species to this system might be easier due to the pre-existing multicellularity, i.e. multicellularity could provide a scaffold for other species to join and modify.

If we consider all possible combinations of two species that can be either unicellular or multicellular, we arrive at three possible routes to forming a multi-species multicellular group: 1) both species are unicellular, 2) one is unicellular and the other is multicellular, and 3) both species are multicellular (See Figure 1). Each of these initial configurations constitutes a class

with representative features. We discuss these features below and provide examples of organismal multi-species entities that may have evolved from similar starting configurations.

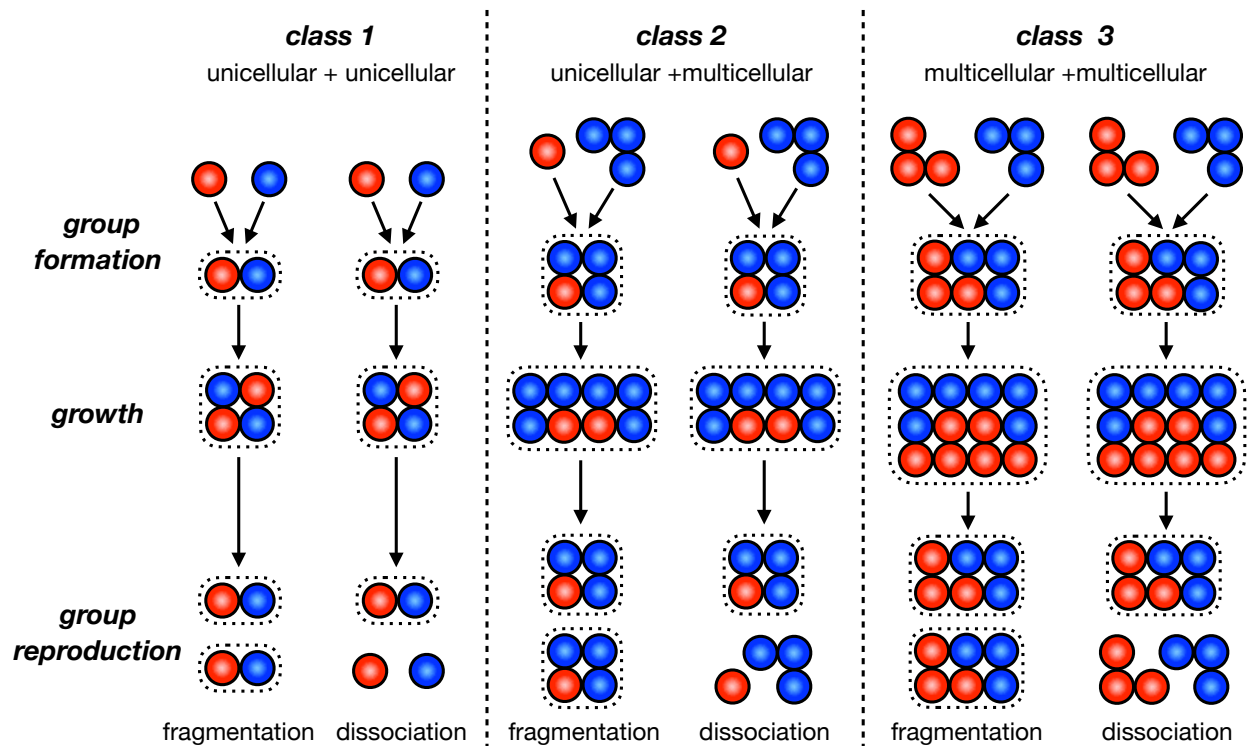


Figure 1: Multi-species multicellular life cycles. We outline a set of six life cycles that describe how an organismal multi-species group may form and generate group offspring. The life cycles are organized into three classes based on whether the partner species are unicellular or multicellular. The dotted lines distinguish groups from free-living species. Following group formation, there is some time needed for groups to grow via cell reproduction so that they can reproduce. Each multi-species group can reproduce through fragmentation and/or dissociation. In all cases of fragmentation, a multi-species group splits into smaller groups. We show an equal splitting but this is not required. In dissociation some set of the constituent species leave the multi-species group, with the potential to form new groups.

7.3.1 Class 1, Two unicellular species:

In the first class, both species are unicellular and come together to form a multicellular group. There are many examples of organismal groups that likely started this way. For example, microbial syntrophies often feature interdependent, metabolic coupling between different unicellular microbial species (Morris et al., 2013; Schink, 2005; Stams and Plugge, 2009). Unlike typical microbial communities, these syntrophies exhibit a high degree of interdependence, specificity, and coupling between species. Nonetheless, these syntrophies are quite far from paradigmatic organisms and have not evolved to be much more than mutualistic communities.

The extent to which syntrophic communities resemble populations as opposed to multicellular organisms depends in part on their physical structure. Some microbes establish physical connections between cells in order to share resources while others rely on diffusion in a shared environment (D'Souza et al., 2018). Since paradigmatic multicellular organisms, like plants and animals, use physically attached cells to construct tissues, it is tempting to consider multi-species communities that rely on physical connections as more organismal than those that rely on diffusion. Yet, if the species occupy an environment in which communication via diffusion is effective or even better than rigid physical connections, then the environment would limit the evolution of physical structures associated with more paradigmatic organisms.

This first class of multi-species entities is not limited to groups that resemble communities. The endosymbiosis that gave rise to eukaryotes would also fall in this category. Here, we are not equating the current, derived form of a eukaryotic cell with a multicellular organism. Instead, we are considering the earliest stage of the endosymbiosis when the two species were unicellular organisms and the endosymbiont could leave the host without threatening the survival of either species. At that initial stage, the two unicellular species would have formed a multicellular group. It may seem unusual to equate an endosymbiosis with a multicellular group, but the fact that one species is inside the other does not seem so different from a group of clonal cells in which there are interior cells completely surrounded by exterior cells, e.g. some of the volvocine algae (Kirk, 2005). Of course, in the case of the eukaryotic endosymbiosis the initial multicellular group did not stay multicellular but rather evolved into a more complex form of unicellularity that in some lineages later re-evolved multicellularity (often clonal).

On the one hand, it seems surprising that this class of multi-species multicellularity did not evolve into large, complex chimeric organisms. Since both species are unicellular and build the multicellular group from the bottom up, there is the potential to produce integrated structures that harness the functional capacities of the two species. If, by contrast, they had already evolved complex multicellular tissues prior to forming a group, then it might be difficult to combine them in an effective way. On the other hand, it could be that building a complex structure is difficult for two different unicellular species because of possible breakdowns in cooperation. The ecological and evolutionary time scales for unicellular organisms often overlap, which means cheating mutations can arise and disrupt cooperation (Ennis et al., 2000; Velicer et al., 2000). Cheating mutants also present a problem for clonal multicellularity, but kin selection can help to stabilize the multicellular group and maintain cooperation (Gilbert et al., 2007; Kuzdzal-Fick et al., 2011). Without kin selection, multi-species groups composed of unicellular species may be limited in the extent to which they can evolve large, complex structures—this constraint may be lessened if one of the species has already evolved multicellularity as there would be some stable structure on which to add. Ultimately, we lack observations of large complex, multicellular forms being built from two different unicellular species.

7.3.2 Class 2, A unicellular and a multicellular species:

In the second class of multi-species group formation, a unicellular species is incorporated as part of an existing multicellular species. There are many possible scenarios that can lead to such a multi-species group. For example, a unicellular species may share the same environment as a multicellular species and begin to grow on its surface, or it may be internalized (either accidentally or as a parasite or food). Whatever the case, the multicellular species provides a structural—and possibly ecological—niche for the unicellular species. And since the

multicellular species would have already evolved a way of reproducing its multicellular form, it would regularly occur and be available for interactions with unicellular species.

This class includes lichens, which are probably the best example of a multi-species chimeric organism. Lichens evolved through associations between multicellular fungal lineages and unicellular photobionts (algae and/or cyanobacteria). Even though the original nature of the relationship varied between parasitic to mutualistic (Gargas et al., 1995), the evolved relationship is fairly similar across lineages. The fungal partner provides an organized physical structure that surrounds and protects the photobionts from harsh environmental conditions, and the photobionts provide the fungi with energy (Honegger, 1998; Nash, 2008). When lichens reproduce sexually it is via the fungal partner, whose offspring must associate with a free-living photobiont to produce a new lichen (Bowler and Rundel, 1975). Thus, it is the multicellular fungal species that drives the repeated formation of groups.

Other organismal entities that evolved from this second class of group formation share a similar organization to each other. In general, the multicellular species constitutes most of the group's cells and maintains its body while the unicellular species is an endosymbiont or is at least kept localized to a specific section or organ. Examples include termites that maintain unicellular endosymbionts in their hindguts (Lombardo, 2008) and bobtail squid that grow populations of *Vibrio fischeri* in specialized light organs (McFall-Ngai, 2014; Ruby and Lee, 1998). The similar organization of this second class of multi-species entities could help maintain and regulate the inter-species relationship (Estrela et al., 2016). For instance, if the unicellular species lived on the outside surface of the multicellular species, then the partnership would be susceptible to disruption or invasion from other species in the environment. Alternatively, if the unicellular species were free to move around inside the multicellular species, this could interfere with other internal structures or functions of the multicellular species.

Unlike single-species multicellularity or the first class of multi-species multicellularity, the second class features different time scales between constituent parts. The time it takes a multicellular species to complete its life cycle is likely to be significantly longer than its partner unicellular species (Marbà et al., 2007). The difference in time scales may lead to the multicellular species evolving mechanisms to manage the evolution of its partner unicellular species. Without such mechanisms the inter-species cooperation could be lost. For example, in the case of the bobtail squid, when a population of its *Vibrio fischeri* evolves to produce less light, the squid expels them and acquires a new population from the environment (McFall-Ngai, 2014; McFall-Ngai et al., 2012). Examples from this class that involve an endosymbiont often have intricate regulation that allows the multicellular species to control reproduction of its unicellular endosymbionts, preventing their loss or rampant growth (Lowe et al., 2016; Ratzka et al., 2012). Indeed, the need for multicellular species to manage their unicellular partners might point to why many examples from this class have specialized physical structures that contain the unicellular species.

7.3.3 Class 3, Two multicellular species:

Finally, in the third class of multi-species groups, two multicellular species unite to form a single group. Since both species are multicellular, they have traits that can be co-opted to assist in evolving multi-species multicellularity. For instance, by virtue of being multicellular, both species would already have the ability to create groups, perhaps by secreting extracellular glues or maintaining cell-cell attachment following reproduction (Rainey and Kerr, 2010; Ratcliff et

al., 2012). If the watermelon-pumpkin grafting had a mechanism for reproduction and gaining adaptations it would form an organismal group belonging to this class, as would the mythological chimera (Knox and Fagles, 1990).

Both the grafting and the mythological chimera demonstrate a key challenge within this class. Since the two multicellular species have evolved to build complex structures, it can be difficult to combine them in an effective way. If we return to our grafting example, there are many ways to combine parts of watermelons and pumpkins that would not create a surviving, reproducing entity. Many representative examples of this class of group formation also do not feature intimate physical integration; instead, the multicellular species are mostly distinct. For example, an acacia and the protective ants it houses could be viewed as a member of this class (Janzen, 1966), as would a fig and the pollinating wasps it houses (Janzen, 1979; Weiblen, 2002). In both examples, cells of each multicellular species are kept segregated, and the amount of cell-cell contact between species is limited (i.e. most cells are only in contact with clonemates).

The acacia-ant and fig-wasp groups are examples of mutualisms that because of partner specificity and co-evolution may be considered more organismal than other multi-species communities. Yet, they may be limited in the extent of organismality they can achieve. As a point of comparison, we consider honeybee colonies, which can contain tens of thousands of multicellular organisms cooperating together to grow and sustain a hive (Seeley, 2009). Their scale, functional integration, and complexity have led them to being placed in a class of highly organismal groups called superorganisms (Seeley, 1989). Unlike the acacia-ant and fig-wasp mutualisms, superorganisms contain only a single multicellular species, and despite their comparative genetic homogeneity they have evolved intricate mechanisms to control who reproduces so as to maintain cooperation (Ratnieks et al., 2006). Multicellular mutualisms may not be able to attain similar levels of reproductive control because it would need to emerge from the interaction between two genomes rather than being encoded in a single genome. Lack of reproductive control provides evolutionary opportunities for both genomes to exploit their partner species or abandon the partnership all together, which would constrain the amount of interdependence and functional integration that evolves between the species in this third class of multispecies groups.

7.4 Multi-species group reproduction

In the previous section we discussed the different configurations of multi-species groups, but for these groups to be organismal they need some mode of reproduction. Across the different classes of multi-species multicellularity there are two basic modes of reproduction: 1) fragmentation and 2) cycles of dissociation and re-association (see Figure 17.1). In the case of fragmentation, the multi-species group simply splits into smaller groups, each of which contains at least both species and possibly maintains some type of physical or community structure. In the second mode of reproduction, one or both species abandons the group and at some later point re-associates with the same partner species—though not necessarily the same individuals or lineage—to recapitulate the multi-species group. Importantly, both modes of group reproduction allow groups to produce group offspring and gain adaptations.

A key distinction between the two modes of reproduction lies in whether the multi-species partnership is maintained or temporarily severed (or, alternatively, whether the offspring group is a result of staying together or coming together (Tarnita et al., 2013)). If the relationship is severed, as is the case in dissociation, there is the risk that the two species will not be able to re-

establish their partnership. If this risk is high enough then it will reduce the fitness of groups and interfere with the species evolving interdependence (Estrela et al., 2016). While there is no requirement that species are interdependent in organismal multi-species groups, it is a common trait of more organismal, clonal multicellular organisms, e.g. cells in different organs rely on one another for the organism as a whole to function. When faced with a recurring risk of not re-establishing a partnership, the different species are likely to maintain self-reliance or evolve more general reliance on species present in the environment (Estrela et al., 2016; Oliveira et al., 2014).

Although dissociation can sever the relationship between specific lineages, it may also facilitate some kinds of adaptation. Many clonal multicellular organisms reproduce through a single-cell bottleneck, which helps purge deleterious mutations, consolidate developmental changes, and maintain cooperation (Grosberg and Strathmann, 1998; Queller, 2000; Ratcliff et al., 2017). A cycle of dissociation and then re-association can function in a similar manner by reducing the size of group offspring and allowing species to swap members of the same species. Moreover, dissociation can be coupled with sexual reproduction to give partner species a chance to generate genetic variation. In lichens, when fungi reproduce sexually they dissociate from the lichens to disperse and form new lichen associations (Bowler and Rundel, 1975).

We note that the two modes of reproduction are not mutually exclusive, so the same multi-species group can use both to reproduce depending on the circumstances. For example, many lichens reproduce via fragmentation as part of an asexual reproductive life cycle and also dissociate their mycobionts (and possibly photobionts) as part of a sexual reproductive life cycle (Bowler and Rundel, 1975). Fragmentation allows for faster colonization of new niches, while dissociation increases genetic variation in lichens. Since the two modes of reproduction have different costs and benefits, lichens may incorporate both modes into their life cycles to better propagate the multi-species groups across generations.

At the onset of multi-species multicellularity, there is nothing that theoretically prevents multi-species groups from reproducing via either mode. But if we look at the different classes of multi-species groups, there appears to be a bias in favor of dissociation when at least one multicellular species is involved—especially if it has complex traits such as developmental programs or sexual reproduction. For example, in the acacia-ant system acacia trees reproduce through seeds, so in order to re-establish the relationship ants must disperse and colonize new acacia trees (dissociation). Similarly, in the squid-*Vibrio* system the squid reproduces via eggs, which means the symbiosis goes through cycles of dissociation and re-association (Nyholm and McFall-Ngai, 2004). The bias towards dissociation, however, is less apparent when both species are unicellular, e.g. microbial syntrophies can fragment or dissociate depending on environmental conditions and whether the species disperse (Cremer et al., 2012). Ultimately the prevalence of either mode of reproduction likely stems from the pre-existing life cycles of the partner species. Since complex multicellular species have a higher prevalence of dissociation-based life cycles, so too do their multi-species groups.

7.5 Conclusions

In the beginning of this chapter, we considered the peculiarity of plant grafting and the seeming rareness of multi-species chimeric organisms. To understand this rareness, we used a bottom-up approach informed by studies on the origins of multicellularity to explore how a group of multiple species might first form and then reproduce. A key idea in our approach is that these

simple, reproducing multi-species groups are starting points from which something more organismal might evolve (or not). We organized multi-species groups into three classes depending on whether the partner species are unicellular or multicellular. For each class, we gave examples of existing multi-species entities that may have evolved from similar initial configurations. We then considered the two ways in which such groups could reproduce—either via fragmentation or dissociation—and weighed the benefits and prevalence of their use. In this last section, we draw a few conclusions on the different types of multi-species multicellularity and their potential to evolve a chimeric organism.

Firstly, there are a dearth of examples of multi-species entities high on the organismality spectrum that were formed by two unicellular species. Apart from the examples of the eukaryotic cell and perhaps a dozen plastid endosymbioses (Hackett et al. 2007), the vast majority of multi-species entities from this class are likely to be similar to microbial syntrophies, i.e. unicellular communities with some degree of cooperation. The turbulent dynamics of microbial communities in which environments change and new species frequently invade likely inhibit these communities from persisting and reliably reproducing with the same partners (Oliveira et al., 2014). The exceptions from this class, including the original eukaryotic cell as well as primary, secondary, and tertiary endosymbiotic origins of plastids, established persistent and reliable relationships through endosymbiosis. The extreme rarity of unicellular host-endosymbiont pairs (Lane, 2017) suggests that there are important physical constraints that make this an unlikely route to multi-species multicellularity. So, while this first class of multi-species groups seldom becomes anything more organismal than a community, it has the potential to produce highly integrated, organismal entities.

Compared to the first class of multi-species group formation, the second and third classes describe many more existing multi-species entities. Both of these classes involve a multi-species group formed by at least one multicellular species. Their evolutionary trajectories often result in one of two characteristic configurations: 1) a multicellular species with a unicellular endosymbiont and 2) two multicellular species with a mutualistic relationship. These examples represent fundamental and ubiquitous ecological relationships. Although the organismality of any of these partnerships depends on partner specificity as well as the cooperation/conflict elements of their relationship, some symbioses in which there is a strong interdependence between partner species have been considered to be very organismal (Estrela et al., 2016). Thus, multi-species organisms may be quite prevalent, and if so they were likely formed by a merger involving at least one multicellular species.

Though multi-species organisms may be widespread, they have different conformations than the chimeras presented in mythology. Mythological chimeras were often odd assortments of parts of creatures, e.g. an amalgamation of a lion's head, a goat's body, and a serpent's tail (Knox and Fagles, 1990). In some sense this is similar to our grafting example of a watermelon fruit sewn on a pumpkin root. Yet, it is far removed from the best extant example of a multi-species chimera, lichens. As with other organismal representatives of the second and third classes, one species is contained within another. In lichens the photobiont is surrounded by the multicellular fungi, in the squid-*Vibrio* system the *Vibrio* are contained in specialized light organs in the squid, and in the fig-wasp system the wasp lives inside the fig fruit. The fact that one species is contained inside another establishes a more persistent relationship, which may facilitate the evolution of species-specific adaptations (Estrela et al., 2016).

Finally, we end by noting an interesting consequence to the prevalence of the dissociation mode of reproduction in multi-species multicellularity. By separating species in order to reproduce, there is the risk the species will not re-establish a group. This may disrupt the evolutionary trajectory of a multi-species group, but it could lead to the partner species forming new partnerships with other species. There are many biological examples of inter-species partnerships being lost and exchanged for others, e.g., the evolutionary history of lichens shows that the symbiosis has been lost and re-gained across diverse fungal lineages (Lutzoni et al., 2001; Nelsen et al., 2020). Such fluidity in forging new relationships may complement the evolution of new species to create new types of multi-species chimeric organisms, whose genomes contain an evolutionary legacy of previous inter-species cooperation as members of previous chimeras.

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