Group maintenance in clonal multicellularity: Controlling intra-organismal evolution

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Abstract

For groups to become units of evolution, within-group variation has to be lower than among-group variation such that selection at the group level overrides selection at the cell level. During the transition to multicellularity, the capacity of multicellular groups to become stable evolutionary units was dependent on their ability to control intra-organismal evolution. That is, mechanisms to control both intra-organismal genetic variation and the selective advantage of within-group variants had to evolve. A number of mechanisms have been proposed to control intra-organismal evolution in clonal multicellular organisms. Although in most cases their contribution to the evolutionary stability of a multicellular lineage is obvious, it is not always clear whether they evolved specifically to control cell-level variation and selection. A full understanding of the mechanisms underlying the success of clonal multicellularity in terms of evolutionary stability and increased complexity requires a comparative approach that must take into account both the evolutionary history of the lineage – including the genetic and structural background on which multicellularity evolved, as well as the selective forces and various life history traits that shaped multicellularity in each lineage.

11.1 Introduction

Multicellularity has evolved independently in many lineages from both the prokaryotic and eukaryotic domains of life (e.g., Grosberg and Strathmann 2007). Generally, multicellular phenotypes can evolve via two very distinct pathways: cell aggregation (such as in myxobacteria and social amoebae) or failure to separate following cell division (e.g., in filamentous bacteria and most eukaryotic multicellular groups). The latter evolutionary strategy is known as clonal multicellularity, as the constituent cells are, by definition, clonal. Extant multicellular lineages exhibit very different levels of complexity – from simple multicellular forms without specialized cells to very complex organisms with hundreds of cell types and diverse developmental patterns and life histories. Remarkably, complex multicellular organisms with large bodies and many specialized cell types are only known among clonal multicellular lineages (e.g., land plants and animals).

Several theoretical frameworks (including kin selection/inclusive fitness, multi-level selection, cooperation/cheating, conflict/conflict mediation, self-limitation/limitation of exploitation from inside; e.g., Michod and Roze 2001; Libby and Rainey 2013; Bourke 2019; Aktipis 2020) and mechanistic views (mostly in the context of cancer suppression; e.g., Aktipis et al. 2015; Nedelcu and Caulin 2016; Nedelcu 2020) have been used to address how multicellular groups can be maintained and evolve into complex multicellular organisms. Most commonly, the increased evolutionary success of clonal multicellularity – in terms of prevalence and complexity, is thought to have been facilitated by the high relatedness of cells in clonal multicellular organisms (e.g., Fisher et al. 2013). Increased relatedness allows kin selection to operate and promotes cooperative and altruistic behaviours among cells (e.g., Queller 2000; Bourke 2019). However, as in all cooperative behaviours, cheaters – that is, cells that enjoy the benefits of cooperation without paying the costs, are still expected to occur even in clonal multicellular organisms (Buss 1987; Michod 1996; Queller 2000; Aktipis et al. 2015). Thus, to facilitate the evolutionary stability of multicellular lineages, the occurrence and success of selfish/cheater cells have to be limited (Michod 1996).

Here, we are taking a *first-principles approach* to explore different aspects involved in the evolutionary stability of clonal multicellular lineages. Specifically, we are using the framework of evolutionary transitions in individuality (Michod 1998) and consider multicellular groups as units of evolution – used here to imply both that (i) they are levels of selection and (ii) adaptations occur at the group level. For that to be the case, multicellular groups need to possess heritable variation in fitness at *their level of organization* (Michod 2007). However, because multicellular individuals evolved from groups of previously independent units of evolution (i.e., single-celled entities) that still possess the necessary conditions to evolve (heritable variation in fitness), variation can still occur and selection can still act at the cell level. Thus, for selection to act at the group level and for multicellular groups to be maintained and become stable evolutionary units, within-group variation and selection has to be lower than among-group variation and selection (Michod 1997). In other words, intra-organismal evolution needs to be *controlled*.

At the mechanistic level, controlling intra-organismal evolution requires both reducing the incidence of mutations (limiting genetic variation within the group) and lowering the negative effects of such mutations by decreasing their selective advantage (limiting cell-level selection). Many different processes have likely contributed to decreasing intra-organismal evolution and increasing group stability in clonal multicellular systems. Nevertheless, a series of pre-conditions, constraints, and life history traits specific to each lineage can also affect the type of mechanisms involved and the outcome in terms of the evolutionary stability and evolvability of the extant multicellular lineages. Here, we explore the relative contribution of these factors, both during the early evolution of multicellularity as well as in lineages that achieved high levels of morphological and developmental complexity, *with a focus on animal, green algal and plant lineages***,** which have been studied more extensively.

11.2 Within-group *variation*: Factors and mechanisms

By definition, cells in clonal multicellular organisms are genetically related, and thus within-group variation is expected to be low. However, different *factors* can affect variation within groups, and a series of *mechanisms* are thought to have evolved specifically to lower within-group variation.

11.2.1 Mode of reproduction

Although the evolution of clonal multicellularity is predicated on the inability of daughter cells to separate following the division of a single cell, a distinction has to be made between the origin of clonal multicellular organisms and their subsequently evolved ways of reproduction and development (i.e., life cycles). This is because although all clonal multicellular lineages evolved from single-celled ancestors, during their life cycles, the offspring can develop from either a single cell (spore or zygote) or multiple cells (propagule) (Fig. 11.1A and Fig. 11.1C). These two distinct strategies result in marked differences in the potential for within-group genetic variation in the offspring (Fig. 11.1B and Fig. 11.1D) and the fate of mutations during the early evolution of multicellularity (Ratcliff et al. 2017).

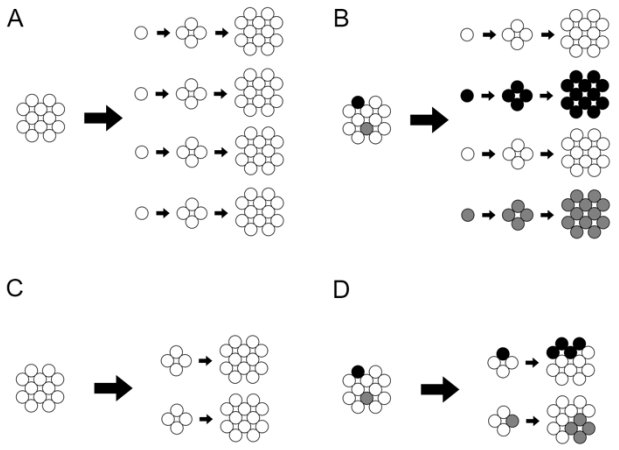

Figure 11.1. Simplified representation of two modes of asexual reproduction involving single-celled stages (A and B) and multicell propagules (C and D), highlighting the advantages of unicellular bottlenecks both in terms of fecundity (A versus C) as well as lowering intra-organismal variation and purging of deleterious (gray circles) and selfish (black circles) mutations (B versus D).

Single-cell reproduction has been proposed to be an adaptation to ensure that the clonality of cells in a multicellular organism is restored at the start of each generation (Szathmáry and Maynard Smith 1995; Grosberg and Strathmann 1998; Kuzdzal-Fick et al. 2011). The passage through a *"single-cell bottleneck"* is considered critical for the evolutionary stability of multicellular organisms by ensuring high cell relatedness, which is thought to be very important both in the early evolution of multicellular groups and as a means to prevent genetic conflicts in each generation, especially in multicellular lineages that evolved large body sizes and/or long lifespans (discussed later). But going through a single-celled stage every generation can also contribute to the elimination of deleterious mutations from the population by segregating and exposing the cell variants to inter-organismal selection in the next generation (Grosberg and Strathmann 1998). Such variants can include mutations that negatively affect both the cell and the multicellular group (uniformly deleterious; Roze and Michod 2001) as well as mutations that increase the fitness of a cell lineage at a cost to the group (selfish; Queller 2000) (Fig. 11.1B and Fig. 11.1D).

Nevertheless, single-celled stages have also been proposed to be necessary for complex development (Wolpert and Szathmáry 2002) or as means to maximize fecundity and population growth (Pichugin et al. 2017). The latter is supported by evidence from the experimental evolution of a multicellular life cycle with a single-cell bottleneck in the unicellular alga *Chlamydomonas reinhardtii*; the evolution of a unicellular bottleneck prior to any genetic conflicts in the multicellular group can imply that single-cell propagation is a preadaptation later co-opted for conflict suppression (Ratcliff et al. 2013). Furthermore, a series of life cycle models developed by Ratcliff et al. (2017) suggested that genetic bottlenecks and clonal development can also link the selection on heritable multicellular traits to that of the genes that affect them, and maximize the variance of group-level traits.

Although single-cell bottlenecks can be involved in many evolutionary processes (see Grosberg and Strathmann 1998 for a discussion), it is important to note that for lineages that reproduce sexually, the single cell stage is a *de facto* phase unrelated to the evolution of multicellularity. That is, by definition, sexual reproduction requires passing through single-cell phases – the gametes and the zygote. Thus, the question of the role of the unicellular stage in the life cycle of obligately sexual multicellular lineages cannot be fully dissociated from the question of the role of sexual reproduction. Notably, many multicellular lineages are facultatively sexual, reproducing asexually for much of their life cycle (e.g., green algae, sponges, cnidarians). Therefore, if single-cell bottlenecks are required to maintain the evolutionary stability of multicellular phenotypes in terms of reducing intra-organismal evolution, asexual reproduction should also employ a single-celled stage in facultatively sexual multicellular lineages.

However, in many clonal multicellular lineages, asexual reproduction can involve groups of cells (e.g., fragmentation in green, red or brown algae; stolons in land plants; budding in cnidarians) whose relatedness coefficient can vary depending on development and cell division patterns (discussed below). Accordingly, all else being equal, the potential for within-group variation in the developing offspring should be higher in these lineages. The potential for purging deleterious mutations is also expected to decrease, especially when propagules are large and/or the initiating cells in the propagule are distantly related (Kondrashov 1994; see Roze and Michod 2001 for a discussion). Nevertheless, if within-organism selection is stronger than selection among individuals, the mutation load can decrease as propagule size increases (Otto and Orive 1995). The fitness effect of mutations (uniformly deleterious vs selfish) can also influence the propagule size (which can affect the fitness of the offspring), with smaller propagules being favored when mutations are selfish, despite the cost in terms of smaller offspring (Roze and Michod 2001).

Many multicellular lineages (including among animals and plants) are known to successfully employ reproductive modes involving fragmentation or budding during their asexual phase or as their only means of reproduction over many generations. While for some lineages their eventual passage through a single cell stage during the sexual phase would reset the clonality within the multicellular group and "purge" them of deleterious mutations (Grosberg and Strathmann 1998), it is unclear if the unicell sexual stage is in fact an adaptation to decrease intra-organismal evolution or a by-product of sexual reproduction. In this context, it has also been suggested that unicellular bottlenecks are in fact "exaptations conferring immunity to future cell-cell conflicts rather than being adaptations *per se*" (Niklas and Newman 2020). Furthermore, in some instances (e.g., filamentous cyanobacteria and some green algae) both single and multicell asexual propagules can be produced, arguing that the two modes of reproduction are adaptations to selective pressures unrelated to preventing within-group variation (Singh and Montgomery 2011). Thus, it is possible that the frequent use of single-celled stages in the life cycle of most multicellular lineages is the result of multiple distinct selective forces. Moreover, even in lineages that always go through a single-celled stage, the potential for intra-organismal variation still exists, and additional mechanisms are required to control variation acquired during ontogeny. Overall, although a single-cell bottleneck is definitely an efficient way to decrease intra-organismal evolution, other factors and mechanisms are also equally important for the early evolutionary stability of clonal multicellularity (Queller and Strassmann 2009; Libby et al. 2016).

11.2.2 Developmental modes

One of the most fundamental differences among multicellular phenotypes is related to the presence of specialized cells, and in particular reproductive/germ and non-reproductive/somatic cells. The *absence of a germ-soma separation* in simple multicellular lineages (e.g., cyanobacteria, some green algae) implies that all intra-organismal variation will also be transmitted to the offspring. Whether that variation will affect the offspring depends on the reproductive mode. In lineages that always go through a single-cell bottleneck during the asexual phase (i.e., each cell in the group will produce a multicellular offspring; such as in multicellular volvocine green algae) each generation will start from a single cell founder. However, in lineages that reproduce by fragmentation (e.g., filamentous cyanobacteria and many green, red and brown algae) the fate of intra-organismal variation will be dependent on the cell division pattern and growth mode (apical,

intercalary, lateral; see below). Nevertheless, since multicellular organisms without a germ-soma separation have simple developmental and growth patterns, the cells in the propagule are expected to be closely related (Ratcliff et al. 2017).

In multicellular lineages with a defined soma and germline, the fate of the within-group variation will be affected by both the reproductive and developmental modes (Figure 11.2). The main difference in developmental modes is with respect to the timing of *germline segregation* (Fig. 11.2). In the so-called "ancestral mode of development" (Buss 1987), the germline is segregated late in development. In groups with this mode of development (e.g., sponges, cnidarians, land plants), the somatic cell lineages are incapable of continuous division or re-differentiation and thus they have to be replenished from one or a few pluripotent lineages that remain mitotically active throughout ontogeny, and can later differentiate into germ cells (Fig. 11.2A). These lineages are also capable of vegetative reproduction via fragmentation or budding, which allows somatic variation to be transmitted to offspring. In contrast, in the "derived mode of development" (Buss 1987) – such as in most animals and some (but not all) multicellular volvocine algae (e.g., *Volvox carteri*), multipotent stem cells with various degrees of mitotic capacity (approaching immortality in some stem cell lineages) and/or potential for differentiation are produced from a totipotent lineage which then differentiates into germ cells early in the development (Fig. 11.2C). The evolution of an early segregated germline is thought to mediate potential conflicts among cell lineages – including cheaters, in terms of access to the germline and representation in the next generation (Michod 1996; Michod et al. 2003). Nevertheless, since multicellular lineages with an early-segregated germline do also go through a bottleneck, such mutants will ultimately be removed from the population through among-group selection (as a multicellular group composed exclusively of cheaters will be less fit) (Fig. 11.1B).

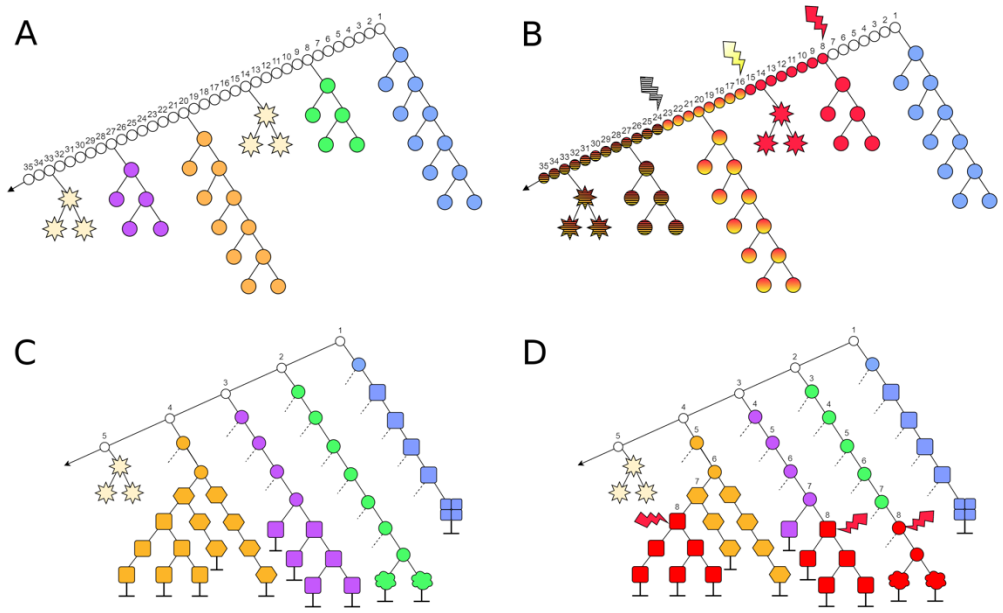

Figure 11.2. Simplified representation of the "ancestral" (A) and "derived" (C) modes of development (Buss 1987), highlighting the different impact that mutations have on intra-

organismal variation in the two developmental modes (B versus D). (A) In the "ancestral" mode (such as in cnidarians and plants, for instance), a pluripotent lineage (white circles; numbers indicate cell divisions) gives rise to both differentiated cells (various gray shapes) and gametes (white stars) throughout life time (and into offspring, during vegetative reproduction; arrow). (B) Mutations (lightning signs) that occur in this pluripotent lineage (for simplicity and to allow comparison of effects, mutation events are indicated every 8th cell division) will be inherited in all subsequent differentiated cell lineages (including gametes and asexual offspring) and can have cumulative effects (indicated by increased hatching pattern). (C) In the "derived" mode (such as in animals and *V. carteri*, for example) a totipotent lineage (white circles; numbers indicate cell divisions) gives rise to several multipotent stem lineages that self-renew (various gray shapes with dashed arrows) and produce differentiated cells with limited replication potential (blunt arrows), and then terminally differentiates into gametes (white stars) early in development. (D) Mutations (lightning signs) occurring with the same frequency as in panel B (every 8th cell division) will largely only affect the terminally differentiated cells and will be removed from the group as cells senesce and die.

Important to controlling intra-organismal variation is also the *cell division potential* associated with the various developmental modes. For instance, in the ancestral mode of development, all cells in the multicellular organism (including the germ cells) are descendants from one or a few pluripotent cell lineages that divide continuously throughout ontogeny. Furthermore, in multicellular groups that reproduce through fragmentation, budding or stolons, the offspring inherits these long-lived proliferative lineages; thus, the potential for variation in the vegetatively produced offspring could be high (Fig. 11.2B). On the other hand, in organisms with an early-segregated germline (i.e., the "derived mode of development"), somatic cells are descendants of a limited number of stem cell lineages that "delegate" proliferative tasks to a battery of progenitor/amplifying cells that replenish the terminally differentiated cells as needed (Fig. 11.2C). This pattern of distribution of the cell proliferation potential is thought to represent an adaptation to limit the number of cell divisions (and thus potential for mutation) in the stem cells as a means to reduce the occurrence of oncogenic somatic mutations (DeGregori 2011).

Multicellular lineages also differ vastly in their embryonic development and ontogeny in terms of *cell division and multicellular growth patterns*. In multicellular organisms that can reproduce vegetatively and/or do not have an early-segregated germline, these aspects have the potential to influence the genetic composition of the multicellular offspring. For instance, plants employ complex cell division and growth patterns (even in the same individual), including apical and lateral cell division as well as intermediate/indeterminate growth (i.e., throughout life; e.g., roots, shoots) and determinate growth (i.e., stops when a final size is reached; e.g., leaves, flowers). Indeterminate growth (also characterizing some animal lineages; e.g., corals, many fishes, amphibians, snakes), by definition, will result in an increasing number of cell divisions and thus potential for high intra-organismal genetic variation. Nevertheless, the distribution of this intra-organismal variation is also under selection, depending on the tissue's contribution to the next generation and longevity. For instance, in perennial (but not annual) plants, the rate of mutation accumulation (per unit time) in shoot apical meristems is lower than that in root apical tissues (Wang et al 2019). But even when growth is limited to a final size (such as in insects, mammals), cell divisions do occur during ontogeny to replace damaged as well as senescent cells, which can

result in intra-organismal variation. A notable exception is among some invertebrates (e.g., *Caenorhabditis elegans;* Pearson and Sánchez Alvarado 2008) and volvocine algae (Kirk 1998), in which there are no post-embryonic cell divisions; in these lineages, the potential for intra-organismal variation is restricted to the embryonic stage.

Another difference in developmental modes that can influence the potential for intra-organismal genetic variation is the ability of cells to move within the group. The lack of cell mobility in plant lineages decreases the potential for variants to invade nearby tissues and/or migrate to distant locations, which might explain both the absence of malignant tumours and the high incidence of vegetative reproduction (including fragmentation, stolons/runners, bulbs) in many plant lineages. On the other hand, the ability of animal cells to move both during embryonic development as well as in adults reflects in the high incidence of malignancy and might contribute to the low incidence of vegetative reproduction involving fragmentation and budding in animal lineages.

11.2.3 Body size

The potential for intra-organismal genetic variation is also expected to increase with group/body size. That is because – all else being equal, more cells require more cell divisions, and more cells equal more targets for mutation. This correlation is generally expressed in an expected increase in cancer incidence in multicellular lineages with large *body sizes*. However, this expected correlation has not been confirmed, a situation known as Peto's paradox (Peto et al. 1975). For instance, large organisms such as elephants and whales do not show the high cancer incidence expected based on their body size (Caulin and Maley 2011). The lack of correlation between body size and cancer rates is commonly attributed to the evolution of better/additional tumour suppression mechanisms in larger animals (Tollis et al. 2017). However, cancer-unrelated life history traits and pressures associated with the evolution of a large body size (e.g., low metabolic rates that could reflect in less oxidative damage; late maturation and low fecundity that would result in, or require, increased investment in somatic maintenance) could also have affected mutation rates or shaped the development in these lineages in a way that has resulted in lower than expected – based on size alone, cancer rates (Brown et al. 2015; Møller et al. 2017; Nedelcu and Caulin 2016; Nedelcu 2020). Interestingly, large body sizes are achieved both in lineages with single-cell reproductive modes and early segregated germline (animals) as well as in lineages that do not segregate their germline early in development and do not necessarily go through a single-cell bottleneck in every generation (plants). Large long-lived plants also do not show the expected (based on size alone) increase in the per-generation mutation rate, and are assumed to have evolved mechanisms to reduce mutation rates per unit growth (Orr et al. 2020).

11.2.4 Life span

Increased *life span* is expected to increase intra-organismal variation as well, since cell lineages go through a higher number of cell divisions during the lifetime of a long-lived individual. For instance, long-lived plants (such as conifers) have been shown to have among the highest per-generation mutation rates for any eukaryote, in spite of their remarkably low annual somatic base substitution rate (Hanlon et al. 2019; Hofmeister et al. 2020). Similarly, the number of somatic mutations in normal human liver was shown to increase with age, with up to 3.3 times more

mutations per cell in aged humans than in young individuals (Brazhnik et al. 2020). This increase in the number of cell divisions and mutations with age should result in higher incidence of cancer in longer-lived multicellular lineages. The lack of correlation between lifespan and cancer incidence is another side of Peto's paradox, which is also commonly explained in terms of better cancer suppression mechanisms in long-lived organisms (Tollis et al. 2020). Nevertheless, as for body size, the lower than expected (based on lifespan alone) incidence of cancer can be an indirect by-product of life history traits and adaptations unrelated to suppressing cancer (Nedelcu and Caulin 2016). Interestingly, an increased intra-organismal mutation load in long-lived trees is considered adaptive as it generates important genetic variation that enable selection both among offspring (as such mutation can be inherited since plants do not have a segregated germline) and among cell lineages within individual trees (e.g., a branch can acquire resistance to herbivory) (Padova et al. 2013; Hanlon et al. 2019).

11.2.5 Somatic mutation suppression mechanisms

The potential for intra-organismal variation is dependent on the incidence of somatic mutations. Because of the impact such mutations have on the development of cancer, most studies on this topic are centered around animal systems. Although not all somatic mutations are associated with cancer (see below), it is generally believed that a series of mechanisms had to evolve specifically to prevent the initiation and progression of cancers in all multicellular lineages. These mechanisms are generally referred to as tumour suppression mechanisms. They include both *"caretakers"* (e.g., DNA damage sensing and DNA repair) and *"gatekeepers"* (premature senescence and apoptosis) (Kinzler and Vogelstein 1997; Hooper 2006). However, caretakers also function in indispensable cellular processes, and many, including p53 – the most frequently mutated tumour suppressor gene in human cancers, have evolved in single-celled lineages (Domazet-Lošo and Tautz 2010). On the other hand, the evolution of gatekeeper genes is thought to largely overlap with the emergence of metazoans and has been interpreted to reflect the need for both increased cooperation and cheating prevention (Domazet-Lošo and Tautz 2010). Yet, both premature senescence and apoptosis-like phenomena have been found in unicellular lineages, suggesting that they also predate the origin of multicellularity (Nedelcu et al. 2011; Nedelcu and Caulin 2016). Also, many tumour suppressor genes (including p53) do not seem to be involved in tumour suppression in invertebrates (Pearson and Sánchez Alvarado 2008). However, direct correlations between tumour suppressing mechanisms and the ability to decrease cancer potential have been reported. For instance, the lower than-expected (based on size and lifespan) cancer incidence in elephants was correlated with the finding of extra copies of the tumour suppressor gene p53, which is thought to result in increased sensitivity to DNA damage-induced apoptosis in elephants (Sulak et al. 2016). Nevertheless, additional p53 gene copies have not been found in the humpback whale, suggesting that if additional tumour suppressing mechanisms are required to control cancer (i.e., intra-organismal evolution) in large/long-lived multicellular bodies, they are lineage specific (Tollis et al. 2019). Lower per-year somatic mutations in long-lived angiosperms (such as poplar) – compared to annual plants, are also thought to be the result of mechanisms that can decrease the potential for somatic mutations (and thus intra-organismal variation). These include limiting the number of meristematic cell divisions and evolving ways to protect meristematic cells from DNA-damaging factors such as UV radiation (Hofmeister et al. 2020).

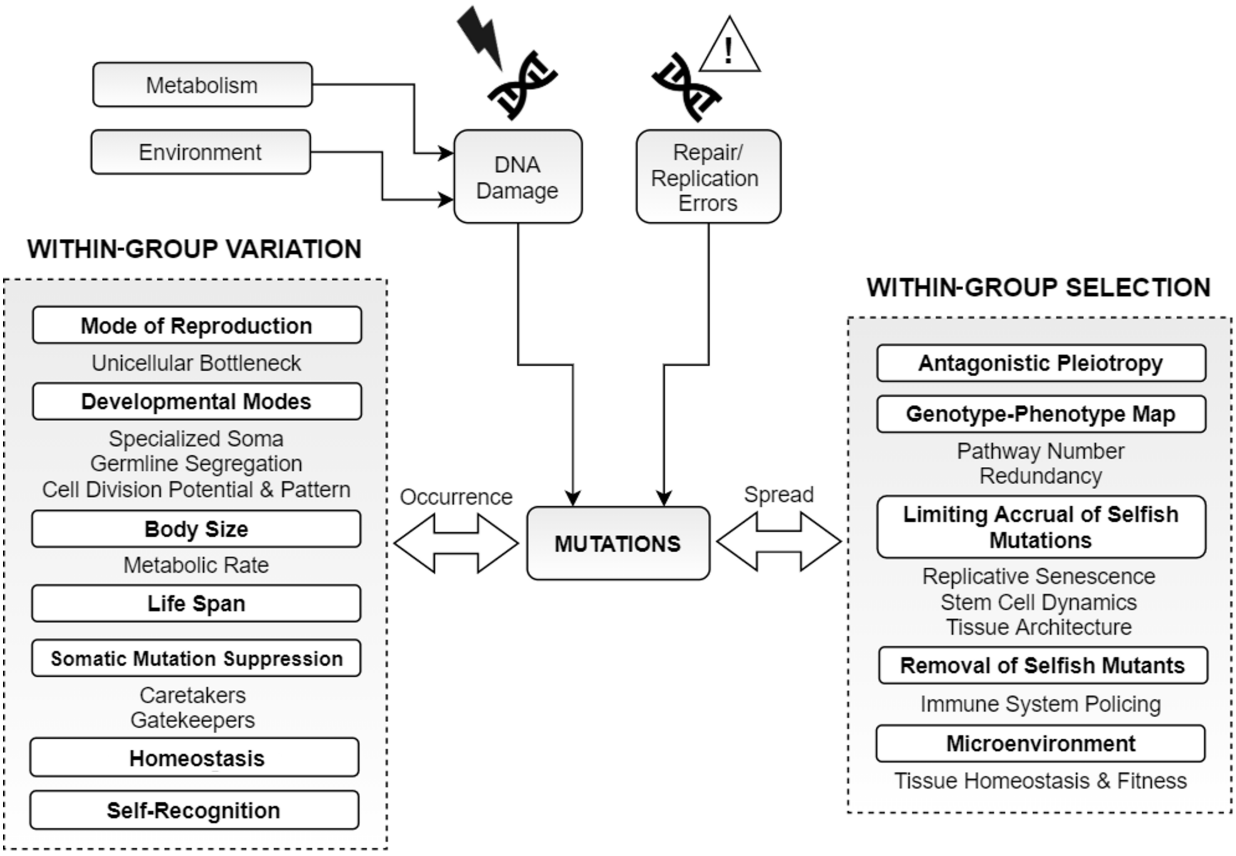

Figure 11.3. Simplified summary of the various factors and mechanisms that affect within-group variation and selection (see text for details and discussion).

11.2.6 Homeostasis

In addition to DNA replication/repair and metabolic-induced mutations, environmental factors can also result in DNA damage and mutations (Fig. 11.3). One of the proposed advantages of group living is homeostasis, which can provide protection from environmental stressors (Smukalla et al. 2008). Developmental modes that ensure stem cell lineages are protected from environmental challenges can also limit the potential of DNA damage-induced mutations and intra-organismal variation. These include, for instance, the location of stem cells in crypts and of meristems in buds.

11.2.7 Self-recognition

For multicellular organisms that developed clonally but faced a continual threat of chimerism, the evolution of a self-recognition system would have had obvious benefits against germline-invading or fitness-reducing cells from other individuals (Fernández-Busquets et al. 2009). However, as in the case of the unicellular bottleneck, the timing of the evolution of self-recognition systems may determine whether they evolved as a specific anti-cheating mechanism or were later co-opted into that role. Sponges are used as a model-system for studying the adhesive/recognition mechanisms during the evolution of animal multicellularity (Fernández-Busquets et al. 2009; Vilanova et al. 2016).

The ability to discriminate against non-self can also be beneficial to recognize self-cell variants and thus act to suppress intra-organismal variation. This aspect is extremely relevant in the context of the recognition of malignant cells by the immune system (see below). In animals, such systems are thought to be efficient mechanisms to both eliminate intra-organismal genetic variants as well as provide a strong barrier to the inter-organismal transmission of cancer. The few special cases of transmissible cancer are thought to be facilitated by low non-self-recognition systems (Belov 2012).

11.3 Within-group *selection*: Limiting the advantage of *selfish mutants*

Mechanisms that reduce intra-organismal variation will affect the incidence and distribution of *all* types of mutations. But the ultimate fate of mutations will be determined by the effect (positive +; negative -; or neutral ~) they have on the fitness of both the cell (C) and the multicellular group (M). In multicellular organisms with a differentiated soma, somatic mutations can be (i) deleterious only at the cell level (C-/M~), (ii) deleterious or advantageous at both the cell and organism level (C-/M-, or C+/M+), (iii) altruistic (C-/M+), (iv)selfish (C+/M-), or (v) uniformly neutral (C~/M~). C-/M~ mutants, by definition, will likely be eliminated through negative selection at the cell level (Otto and Orive 1995; Otto and Hastings 1998). However, uniformly deleterious mutations can accumulate in some cell types and have been associated with human diseases, neurodegeneration and aging (see Gonzalez-Perez et al. 2019 and Brazhnik et al. 2020 for examples and references). Thus, mechanisms that reduce the occurrence and spread of C-/M- could be favoured. Nevertheless, in long-lived woody plants such deleterious somatic mutations can accumulate, and this has been hypothesized to favour outcrossing by reducing the survival of inbred progeny (Bobiwash et al 2013).

The fate of cell-level advantageous mutants will be determined by their effect on organismal fitness. In plants, due to their developmental and reproduction modes, cell-level beneficial mutations occurring in the apical meristems can be selected for and transmitted to offspring, which might then affect inter-individual variation. Thus, in plants, intra-organismal variation can have implications for their ability to adapt to changing ecological conditions and, ultimately, for plant speciation (Hanlon et al. 2019; Schoen and Schultz 2019; Orr et al. 2020). However, in animals, mutants that gain selective advantages at the cell level are more likely to be costly at the organism level (Frank and Nowak 2004). Cancer is a reflection of such mutants, resulting in selection at the cell level overriding selection at organism level. If these selfish variants negatively affect the fitness of the multicellular group during the reproductive phase, they can affect the evolutionary stability of the lineage and have the potential to drive the groups to extinction directly (through decreasing their fitness) or indirectly through gaining access to the germline. Thus, several factors and mechanisms are known, or have been proposed, to limit or reduce the selective advantage of selfish mutants (Figure 11.3), especially in the context of cancer.

11.3.1 Antagonistic *pleiotropy*

One mechanism that can decrease the selective advantage of selfish mutants (and thus enforce clonal cooperation) – especially during the early evolution of multicellularity, is antagonistic

pleiotropy. Specifically, if cooperative genes are linked to individual-level traits, mutations in such genes will also negatively affect the fitness of the selfish mutants (Foster et al. 2004). A related evolutionary mechanism – coined as "type 1 ratcheting", has been proposed by Libby et al. (2016). This scenario envisions that reversion to unicellularity is hindered by the fact that the accumulation of mutations that increase cell-level fitness in a multicellular context are also costly in a single-celled context.

Pleiotropy has been shown to stabilize cooperation in aggregative multicellularity (Foster et al. 2004), but less is known about its role in the evolution of clonal multicellularity. However, at least in the volvocine alga *Volvox carteri*, selfish mutants that evade the developmental control of cell proliferation are also more sensitive to stress (Konig and Nedelcu 2021). Notably, the increased proliferation of cancer cells is also known to be linked to their lower ability (compared to normal somatic cells) to withstand nutrient stress due to their failure to trade-off cell proliferation for maintenance in stressful environments (Raffaghello et al. 2008; Lee et al. 2012). Similar trade-offs and pleiotropic effects have likely contributed to the evolutionary stability of early multicellular groups and have played roles in the evolution of other clonal multicellular lineages.

11.3.2 Genotype-phenotype *map* re-organization

During the transition to multicellularity, a new genotype-phenotype map (i.e., the relationship between genotype and phenotype; Alberch 1991) had to evolve to reflect both the loss of unicellular traits ("type 2 ratcheting"; Libby et al. 2016) and the emergence of new traits at the group level. How a new map is established can influence both the stability of the group and the evolvability of the lineage (Nedelcu and Michod 2004). For instance, the differentiation of somatic cells in *V. carteri* is achieved through the induction of a single gene whose expression suppresses cell proliferation. Mutations in this gene alone result in somatic cells gaining proliferative abilities, with drastic negative effects for the group. Thus, having such a simple genetic architecture (that can be lost via single mutations) endangers the stability of the group as well as limits the evolutionary potential of the lineage.

The genotype-phenotype map is also very important for the stability of complex multicellular organisms. In the context of cancer, this aspect is reflected in the *number of pathways* that need to be inactivated to induce malignancy, known as transformation stages. Interestingly, the number of stages differs between species. For instance, the transformation of fibroblasts requires that six signal pathways be affected in humans, compared to only two in mice (Rangarajan et al. 2004). Also, the development of retinoblastoma (an eye cancer that begins in the retina and mostly affects children) requires the inactivation of only one locus (Rb) in humans but two (Rb and p107) in mice (see discussion in Leroi et al. 2003). Furthermore, human cells require more mutations than mouse cells to create immortalized cultures; both the Rb and p53 pathways must be knocked out to immortalize human fibroblasts while mouse cells require only the p53 pathway to be inactivated (Hahn and Weinberg 2002).

Added *redundancy* in the form of extra tumor suppressor genes can also limit the selective advantage of selfish mutants. In this scenario, mutations in all copies would be required to result

in malignancy. In support of this possibility, transgenic mice that contain an extra copy of p53 (including its regulatory elements) gain an increased resistance to cancer (García-Cao et al. 2002). Redundancy in tumor suppressor genes is also thought to be responsible for the lower than-expected cancer incidence in large animals (Nunney 1999; Leroi et al. 2003). For instance, there are at least 19 copies of p53 in the African elephant genome, and though 18 of these appear to be a result of retrotransposition events, they are expressed and are thought to contribute to cancer suppression in elephants (Abegglen et al. 2015).

11.3.3 Limiting the potential for the progressive accrual of selfish mutations

In many multicellular lineages with an early segregated germline and determinate growth, most somatic cell lineages have limited proliferation potentials. In animals, this phenomenon is known as *replicative senescence* and is induced by telomere shortening (via repression of telomerase activity). In this way, the accumulation of mutations that could provide somatic lineages with selective advantages is limited (Campisi 2001). On the other hand, animals with indeterminate growth express telomerases in the tissues of adults (e.g., American lobster and rainbow trout; Klapper et al. 1998a, b). However, exceptions do exist. For instance, mouse somatic cells express telomerases and have very long telomeres; as these cells do not exhibit replicative senescence, senescence of mouse cells is thought to be a stress response (e.g., Seluanov et al. 2008). Furthermore, rodent species differ as far as telomerase activities in their somatic cells, and replicative senescence appears to correlate with body mass (Seluanov et al. 2007, 2008). Interestingly, the long-lived naked mole rat does express telomerase activities in its somatic cells, and despite its increased longevity relative to other rodents, spontaneous neoplasms have never been reported in this species (Buffenstein 2005). Notably, although it involves a different mechanism, replicative senescence is also known in yeast (Steinkraus et al. 2008) and the simple multicellular green alga, *Volvox carteri* (Shimizu et al. 2002). Similarly, although plants do express a form of replicative senescence known as mitotic senescence, this process does not involve shortening of telomeres and is involved in curtailing cell proliferation in germline-like apical meristems and during early stages of fruit development (Gan 2003).

In animals with an early segregated germline, many features of *stem cell dynamics* are thought to be adaptations to reduce the selective advantage of potentially selfish mutants. These include: asymmetric divisions, preserving an immortal DNA strand in the self-renewed stem cell, limiting the number of stem cells, interposing a series of transiently amplifying cells between the stem cells and the terminally differentiated cells, and the imposition of differentiation on proliferating stem cell progeny (Potten et al. 2002; Frank and Nowak 2004; Caussinus and Gonzalez 2005; DeGregori 2011). Overall, stem cells are indeed known to experience reduced spontaneous mutation loads compared with differentiated cells, although the mechanisms are not fully understood (Brazhnik et al. 2020).

Additional ways that can reduce the impact of selfish mutants on the fitness of the multicellular organism include traits associated with *tissue organization and architecture* – such as the organization of epithelial tissues in crypts, microenvironmental signals and niche or stromal matrix contacts, and serial differentiation (Cairns 1975; Frank and Nowak 2004; Gatenby et al. 2010;

DeGregori 2011). Differences in tissue architecture could influence the frequency of mutant cell lineages (and cancers) depending on the number of stem cells or the dynamics of the tissue itself (Leroi et al. 2003). For example, it has been suggested that under a model of serial differentiation it is possible to increase the number of cells and the amount of cell turnover per organism without increasing the number or proliferative activity of somatic cells, simply by increasing the number of non-stem stages (Pepper et al. 2007).

Tissue architecture and development also affect intra-organismal selection in plants. For instance, the large number of apical initials in conifers allows efficient selection among cells within the meristem, but the highly structured nature of their apical meristems might limit the potential for cells with higher fitness to remain within the meristem. Furthermore, conifers and angiosperms differ in both development and physical architecture, with most conifers having one dominant stem, little bifurcating branching, and a single layer of apical initials in a relatively simple meristem, all of which are thought to limit somatic selection (Hanlon et al. 2019). Generally, patterns of stem cell divisions – such as limiting the number of cell divisions between the meristem and the new branch, are thought to contribute to the low per-year somatic mutation rates and longevity in perennial plans (Burian et al. 2016).

11.3.4. Removal of potentially selfish mutants

Policing is a well-documented strategy to decrease the selective advantage of selfish mutants in all social groups. In many animal lineages, this role is performed by the *immune system* which, in addition to recognizing pathogens, can also identify and remove abnormal self-cells, including mutant cells that have the potential to become selfish (Dunn et al. 2004). The low incidence of cancer in Decapoda is credited in part to their immune system (Vogt 2008). Similarly, the immune system of vertebrates can recognize and eliminate primary developing tumors (Shankaran et al. 2001). However, following clonal escape and tumor formation, chronic activation or innate immune cells can also promote tumor growth (De Visser et al. 2006).

11.3.5. Tissue microenvironment and fitness

As in any system, the selective advantage of a cell variant is dependent on (or relative to) a specific environment. Recently, the role of *tissue microenvironment and fitness* in suppressing cancer (and thus, controlling intra-organismal evolution) has been acknowledged and is receiving a lot of attention. Healthy tissues are known to be able to provide a strong barrier to selection of mutant clones. For instance, *NOTCH1* mutant clones (often associated with cancer) have been found to increase with age in the human esophageal tissue and can coexist with normal clones (Martincorena et al. 2018). However, changes in the tissue microenvironment during aging as well as in response to environmental stimuli (e.g., smoking) and chronic inflammation can select for cancer clones better fit to those conditions and promote cancer development (Casás-Selves and DeGregori 2011; DeGregori 2018).

11.4 Conclusion

The capacity of multicellular groups to become stable evolutionary units is dependent on their ability to control intra-organismal evolution. For such groups to become units of evolution, within-group variation has to be lower than among-group variation such that selection at the group level overrides selection at the cell level. That is, mechanisms to control both intra-organismal genetic variation and the selective advantage of within-group variants have to evolve (Fig. 11.3). However, the specific mechanisms and their relative contributions are dependent on the genetic and structural background on which multicellularity evolved.

For instance, in multicellular plant lineages the presence of a cell wall inherited from their single-celled ancestors (reflected in the strong connections between plant cells) decreases the selective advantage of selfish mutants by limiting their ability to spread. Nevertheless, the potential for oxidative DNA damage due to their photosynthetic activities as well as the increased potential for UV-induced DNA damage associated with the sessile lifestyle of land plants are expected to increase levels of intra-organismal variation. These are in contrast to the high mobility of animal cells and the increased range of physiological and behavioral adaptations that animals evolved to cope with environmental stress. Also, in addition to differences inherited from their unicellular ancestors, plant and animal lineages also differ in their developmental and reproduction modes as well as life history traits and strategies, which together are expected to have distinct effects on intra-organismal evolution.

A number of mechanisms have been proposed to have evolved to control intra-organismal evolution during the evolution of clonal multicellularity. Nevertheless, although in most cases their contribution to the evolutionary stability of a multicellular lineage is obvious, it is not always clear whether they evolved specifically to control cell-level variation and selection. In other words, it is unclear whether other selective pressures or life history traits shaped these mechanisms that in turn also allowed a better control of intra-organismal evolution.

The astonishing diversity that independently-evolved clonal multicellular lineages achieved in a relatively short evolutionary time reflects independently successful strategies to control intra-organismal evolution. A full understanding of the mechanisms underlying the success of clonal multicellularity in terms of evolutionary stability and increased complexity requires a comparative approach that must take into account both the evolutionary history of the lineages and the specific selective pressures and life history traits that shaped the evolution of each multicellular lineage.

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