Multi-level selection of the individual organism

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Abstract

Multi-level selection (MLS) occurs when populations are structured into groups within which frequency-dependent fitness interactions, such as cooperation and conflict, are more likely to occur. The foundational models and concepts in MLS theory are reviewed. The concept of counterfactual fitness is discussed and how it may be used to partition selection between levels in a causal sense during the evolution of multicellularity. MLS hypotheses about the evolution of development during the transition from unicellular to multicellular life show how developmental modifiers may coevolve with group structure and create the first true group-level functions, such as a sequestered germ line or cell policing. These modifiers take the population from groups of cooperating cells to integrated groups of cooperating cells with organism level functions that mediate conflict within the group and enhance the heritability, reproduction and individuality of the cell group. After these developmental modifiers evolve, fitness at the group level is no longer the average of cell fitness. The power of individual selection and the primacy of organisms is often used to deny the need for group selection in evolutionary biology, however the multicellular organism is a derived state and MLS theory is needed to explain its origin and evolution.

3.1 Introduction

“Is there anything in evolution that can’t be answered by individual selection, that needs to be explained by selection acting on groups?’’ asks Jerry Coyne, an evolutionary geneticist at the University of Chicago. “I can’t think of any.” (Morrell, 1996)

Although rhetorical, this remark reflects a common view in evolutionary biology that most questions can be addressed by viewing organisms as the sole unit of selection. For billions of years there were only unicellular organisms on earth; where did multicellular organisms come from? The answer, of course, is that multicellular organisms evolved from unicellular organisms when unicellular organisms started forming cell groups. In this chapter, I review work concerning the role of multi-level selection (MLS) in evolutionary transitions in individuality (ETIs), with focus on theoretical work on the transition from single cells to multicellular organisms. As we will see, MLS is needed to explain the origin of the multicellular organism, that very entity that is supposed to deny the need for MLS in evolutionary biology. The central question I wish to address with these models is, how do groups of individuals become a new kind of individual, or, with respect to the evolution of multicellularity, how do groups of cells become an individual multicellular organism?

3.2 Background

3.2.1 Evolutionary transitions in individuality (ETIs)

In the present chapter, I use multilevel selection theory (MLS) to study evolutionary transitions in individuality, or ETIs, with focus on the transition from unicellular to multicellular organisms. ETIs are changes in the unit of selection and adaptation, changes in the evolutionary individual. Examples of ETIs include the evolution of the cellular genome from replicating molecules, the evolution of complex eukaryotic cells from groups of bacterial and archaeal cells, the evolution of multicellular organisms from unicellular organisms and the evolution of eusocial societies from solitary organisms. ETIs are rare events having happened dozens of times during the history of life. While rare, they have contributed to one of life’s most fundamental characteristics, its hierarchical structure. While not all levels in the hierarchy of life are evolutionary individuals (for example, tissues or organs), all evolutionary individuals are levels in the hierarchy of life (for example, bacterial cells, eukaryotic cells, multicellular organisms, eusocial insect societies). ETIs involve the conversion of a group of existing individuals into a new kind of individual, such as the conversion of a group of cells into a multicellular organism. ETIs involve multilevel selection, but, in addition, involve the evolution of traits, such as conflict mediators discussed below, that modify the development of the groups to enhance the individuality of the group.

3.2.2 Wright’s shifting balance theory

Multi-level selection has been part of population genetics since the foundations of population genetics in the early part of the last century in the work of Sewell Wright and his shifting-balance theory of evolution (1932, 1977, Chapter 13). In Wright’s view, a large global population partially subdivided into local groups is the most favorable for continued evolution. The groups in this theory are local subpopulations partially isolated from other such subpopulations. In the terminology of multilevel selection (MLS) theory introduced later in this chapter, Wright’s shifting balance theory is in the realm of MLS1. Wright’s shifting balance theory does not focus on the fitness interactions that can occur within groups, and the groups in

Wright's theory are not evolving into new kinds of evolutionary individuals. Nevertheless, Wright viewed group structure as advantageous to continued evolution. Stochastic variation in local subpopulations allows groups to explore different fitness peaks. This within-group selection leads to an ensemble of groups, each attracted to local and likely different fitness optima. If one of the local optima is also a global optimum, then between-group selection mediated by, for example, differential migration among subpopulations (Wright termed this group selection phase "asymmetric diffusion") could result in transformation of the larger population or entire species. This group selection phase of Wright's theory builds upon individual selection; in the language of MLS theory introduced below, individual effects are filtering up to the group level; there are no true group effects. Still, in a partially subdivided population, local adaptation is possible, and so is mass transformation of the species. Large homogeneous populations without subdivision become trapped on local optima unable to explore the fitness surface, and small isolated populations suffer inbreeding and deleterious effects of genetic drift. Partially subdivided populations are more favorable for continued evolutionary change or movement towards fitness maxima according to Wright.

The group selection Phase 3 of Wright's shifting balance process depends on differences among groups in their average fitness. The average fitness of the group describes its growth and output into the global population through a set of equations that Wright developed (Wright, 1931, 1932, 1969, 1977). The discussion here is primarily based on the second volume of his 4-volume treatise (Wright, 1969). When fitness is constant, the average fitness of a group also controls within group change resulting from selection among individuals within the group. Consequently, when selection is constant, there is a harmony between selection at two levels in a selection hierarchy, in the sense that traits that increase the fitness of individuals also increase the average fitness of groups. When selection is constant, there is no conflict between the two levels in the selection hierarchy, and Wright's shifting balance process can operate with the third phase of group selection building upon the gene frequency change occurring during the second phase of within group selection.

Frequency-dependent selection based on fitness interactions within the group changes all this, because the phase of within-group selection and the phase of between-group selection are determined by different functions leading to the possibility of conflict between levels of selection in the direction of gene frequency change. Wright's (1969, p. 121) "fitness function" is maximized by the dynamics of within-group change in both cases of frequency-dependent and constant selection. However, under frequency-dependent selection, Wright's fitness function no longer equals average individual fitness, as it does with constant selection. Population growth is still determined by average individual fitness with frequency-dependent selection, as is the case with constant selection. In Wright's theory we are in the pre-ETI realm of MLS1; after an ETI the fitness of the group is decoupled from the fitnesses of its members, but that is not what Wright is concerned with in the shifting balance process.

Frequency-dependent selection and group selection can work in different directions. In frequency-dependent models, within-population change among organisms can lead to demise of the group and local extinction (for some simple examples, such as the evolution of spite, see Wright (1969, p. 127)). ETIs by their nature depend upon the frequency-dependent evolution of cooperative interactions within groups. The tension between the well-being of the group and selection dynamics among its members leading to conflict between levels is the basic problem

that must be solved during an evolutionary transition to a new unit of selection and adaptation, a transition to a new kind of evolutionary individual (Michod, 1999).

Although the maximization of individual fitness no longer occurs generally under frequency-dependent selection, maximization principles may be developed in specific cases. For example, during multilevel selection in populations that are structured into family groups, Wright's fitness function (1969, p. 121) equals the average inclusive fitness effect which is maximized by the population dynamics (Michod and Abugov, 1980). Future work is needed to determine what property might be maximized during an ETI. In the modifier models discussed below, the ratio between selection at the group level and selection within groups appears to increase during the ETI (Figure 3.2 C), but more work is needed to show if this is indeed a maximization process. Having a maximization principle for the evolution of individuality, even in simple limiting cases, would be extremely useful for understanding the concept of biological individuality. There is a large literature devoted to understanding biological individuality, with several collections of papers providing an overview of this exciting field (Bouchard and Huneman, 2013; Calcott and Sterelny, 2011; Gissis, Lamm, and Ayelet, 2017; van Baalen and Huneman, 2014).

3.2.3 Multilevel selection (MLS)

MLS occurs in a population that is structured into subpopulations, or groups. I use the term group and subpopulation interchangeably in this chapter. Group structure in a population has several consequences. Most relevant to our concerns in this chapter is that interactions affecting fitness are more likely to occur within the group, especially cooperative interactions that are costly to individuals but beneficial to the group. Migration is reduced between groups, so interactions occur preferentially within the group. The environment of each group may differ leading to different forces of selection within each group that in turn leads to different traits and variation between groups in genetic composition. The population size is smaller in groups than in the global population with the possibility of increased genetic drift producing different gene frequencies in each group and increased variation between groups. As a result of variation between groups there is a possibility for selection at the group level, such as when some groups persist longer, survive better, or produce more offspring and migrants than other groups. In a group-structured population there may be selection among individuals within a group and selection between groups.

Although present in the foundational work of Wright (1932, 1977, Chapter 13), MLS did not emerge as a subfield within evolutionary biology until the 1970's, along with interest in the evolution of fitness interactions within the group leading to social and anti-social behavior. Interest in MLS has continued more recently by its role in ETIs, beginning especially with the work of Buss (1987). Contemporary MLS theory began with the foundational work of Price, who developed a covariance approach to selection (1970) in a multi-level selection context (1972) that is discussed in more detail below. Wilson (1975) developed an MLS model, termed the "trait-group" model, that has been extremely influential in the study of the evolution of cooperative and social behavior. Like Wright, Wilson distinguished two phases of selection, within- and between-group selection, but Wilson was interested in the effects of this multilevel selection on the evolution of fitness-affecting interactions within the group such as the evolution of cooperation. During the within-group phase, a cooperative trait will usually decline in frequency, because of the costs paid by cooperative individuals relative to non-cooperative or defecting individuals in the same group. During the between-group selection phase, groups with more cooperation survive at higher rates or output more offspring to the next generation than

groups with less cooperation. Wilson showed how between-group selection in favor of cooperation can overcome within group selection against cooperation. In other foundational works, Heisler and Damuth (1987) developed a contextual analysis approach to study selection in structured populations and Damuth and Heisler (1988) distinguished between two kinds of MLS. MLS-type 1 (termed MLS1 here) occurs when the focal entities are the individuals within the group and the group provides context for selection on the individuals. MLS-type 2 (termed MLS2 here) occurs when the groups themselves are the focus and the groups differentially survive and reproduce as groups. As discussed more below, ETIs have been characterized as a transition between MLS1 and MLS2 (see, for example, Okasha, 2005). There are several general models of MLS (Frank, 2012; Gardner, 2015; Gardner and Grafen, 2009). The best introduction to MLS in evolutionary biology is Okasha's book (2006).

We have used MLS theory to study the development of cell groups during the evolution of multicellularity (Michod, 1996, 1999; Michod, Nedelcu, and Roze, 2003; Michod and Roze, 1997, 1999, 2001; Roze and Michod, 2001). There are three analytical tools or modeling approaches we use in our work. The first tool is Price's covariance approach to selection (1970) which he developed in a multi-level selection context (1972). The second tool involves kin selection and the study of evolution in genetically structured populations (Hamilton, 1964a, 1964b; Michod, 1982; Michod and Abugov, 1980). The third tool involves game theory and its use in the study of the evolution of cooperation and conflict in structured populations. Kin selection is implicit in the MLS models discussed here because the models involve groups that develop clonally from a single cell propagule (Figure 3.1). I begin with Price's equation, since its analysis in a MLS context leads to the notion of counterfactual fitness that is useful in quantifying evolution through an ETI.

3.2.4 Price equation and counterfactual fitness

Following Darwin, a population evolves by natural selection when there is heritable variation in fitness. The Price equation or Price's theorem (Price, 1970, 1972) can be thought of as a mathematical version of this conditions approach to natural selection (Okasha, 2006, pp. 36–37), in which the Darwinian conditions are represented in equation form (Shelton and Michod, 2020). For an overview of the Price equation in evolutionary biology see a recent collection of papers on this topic (Lehtonen, Okasha, and Helanterä, 2020). The logic of natural selection is general (Lewontin, 1970), and Darwin's conditions may apply at several hierarchically nested levels at the same time. The occurrence of selection at multiple levels simultaneously is multi-level selection (MLS). Price (1972) and Hamilton (1975) showed how the Price equation can be applied recursively to represent selection at different levels giving Equation 3.1, in which Δq and Δq_s are the frequencies of an allele of interest in the global population and subpopulation *s*, respectively, and $\overline{W}$ and $\overline{W}_s$ are the average fitnesses of the global population and subpopulations (indexed by *s*), respectively. The derivation of Equation 3.1, or similar forms of the Price equation, can be found in many places; here I follow Michod (1999, Chapter 4).

$$\Delta q \overline{W} = Cov\left[\overline{W}_s, q_s\right] + E\left[\overline{W}_s \Delta q_s\right] \qquad \text{Equation 3.1}$$

Hamilton (1975) interpreted the MLS version of the Price Equation 3.1 as accomplishing a "formal separation of levels of selection," in which the first term on the right-hand-side represented the effects on gene frequency change of between-group selection (or just "group

selection") and the second term represented the effects of within-group or individual selection. Unfortunately, further analysis of the Price partition has shown the causal analysis is not so simple, and different partitions are needed to formally separate group and individual selection in explicit MLS models. See Okasha (Okasha, 2006) and Shelton and Michod (2020) for a fuller discussion of these issues.

The basic issue that must be addressed has been termed the "pseudo-group" problem (Shelton and Michod, 2014). Pseudo-groups meet Darwin's conditions of heritable variation in fitness, without there being "true" group effects on fitness. Following Williams (1966b), consider a herd of fleet deer, that is, a population of deer in which there is variation in the running speed of individual deer. If this population is subdivided into groups, by chance, groups will contain different compositions of deer and the groups will vary in average group running speed. This between-group variation in average running speed, likely an important component of group fitness, is not due to any interaction among the deer within the group and so it is not a "true" group effect. Rather, the between group variation in average running speed is determined solely by sampling individual properties of the deer, properties the deer have in isolation from one another. The situation in which the evolution of a group-level trait is influenced only by natural selection at the individual-level and not by natural selection at the group level has been termed a "fortuitous benefit" (Williams, 1966b) or "cross-level byproduct" (Okasha, 2006). In this chapter, I use the term "pseudo-group" for these kinds of groups; in pseudo-groups, the fitness variation at the individual level "filters up" to the group level (Shelton and Michod, 2014).

Beginning with Darwin, as represented in, for example, Lewontin (1970), it was assumed that the conditions for natural selection were sufficient for understanding natural selection and the partitioning of the contribution of each level in a selection hierarchy to overall genetic change. It is now clear that there is more to the problem of partitioning "group selection" in MLS scenarios than the conditions approach alone can resolve, and explicit mathematical models are needed to clarify the causation of selection, for example, whether selection is caused by true group effects in which interactions within groups play a causal role.

An approach based on counterfactual fitness, the fitness an individual would have were it to leave the group, allows for a clear separation between levels of selection and a possible resolution of the pseudo-group problem (Shelton and Michod 2020), without restricting the use of the term "group" to cases when there are true group effects, an approach taken by other workers (Clarke, 2016). Shelton and Michod assumed that in MLS models (models without genetic drift, pleiotropy, and epistasis) only "group selection" can lower counterfactual fitness, that is, only group selection can lower the fitness of a cell were it to leave the group. Based on this assumption, Michod and Shelton (2014, 2020) developed a partition of group and individual selection that attributes the degree of group and individual selection in problem cases like when there are pseudo groups. Unlike the Price approach, the counterfactual approach has the feature that the degree of group-specific selection increases continuously with the degree of group effect. Furthermore, as the ETI proceeds, group fitness becomes decoupled from counterfactual fitness, in the sense that fitness in a group may be quite high, even as counterfactual fitness decreases to zero. In this way, counterfactual fitness can be used for quantifying progression through an ETI. Although more work needs to be done on this problem, our analyses to date based on counterfactual fitness suggest that there are at least three kinds of selection that can be occurring at the same time in MLS models: group-specific selection along with two kinds of individual selection, within-group selection and global individual selection. "Global individual selection"

refers to the aspects of individual selection that are independent of interactions within a group (more on global individual selection below).

3.3 MLS models of the evolution of organismal development

3.3.1 Overview

We have used MLS to study the development of cell groups and the conditions under which modifiers of development evolve that increase the heritability of group fitness, reduce within group selection, and lead to a decoupling of fitness between levels (Michod, 1996, 1999; Michod and Roze, 1997, 1999, 2001; Roze and Michod, 2001). These models are hypotheses for the transition between MLS1 and MLS2 and the origin of multicellular individuality. These models are intended as heuristic devices for understanding the evolutionary transition to multicellularity and how a genotype-phenotype map could be reconstructed at the group level when initially it is present only at the cell level. The basic setup is given in Figure 3.1, for concreteness a volvocine green alga is shown, but the models are abstract and general population genetics models; they are not specific to the volvocine algae. Using these models we have studied the evolution of developmental mechanisms by which cooperative cell groups are constructed during the clonal cell divisions that create the adult group from a zygote or propagule.

The model assumes that the development of the group starts with a propagule. The propagule may be a single cell, as is the case in many multicellular organisms that develop from a fertilized egg, like the volvocine green alga shown for example in the figure. Alternatively, the model has been used to study the evolution of this single cell bottleneck that is so common in the development of multicellular groups by considering propagules comprised of multiple cells sampled in various ways from adult groups of the previous generation (Michod and Roze, 2000; Roze and Michod, 2001). In the model, the concept of a genotype-phenotype map at the group level involves the mapping between genetic traits present in the propagule and those present in the adult group stage. In particular, the models study how various ways of constructing adult groups, such as using a germ line or cell policing, affect this genotype-phenotype map.

The models embed a cooperate/defect game within a two-locus, multilevel selection framework to study how modifiers of development evolve at a second locus in response to mutation and selection at the primary cooperate/defect locus. Before the second modifier locus is considered, the primary cooperate/defect locus embodies a standard MLS1 group selection model (see, for example, Michod, 1997a, 1997b). For simplicity, haploidy is assumed, except for a transient diploid stage during sex. Development involves the conversion of a cell propagule into an adult cell group through cell division described by a variety of parameters given in Figure 3.1. As already mentioned, propagules contain one or more cells sampled from an adult group in the previous generation (or from several adult groups in the case of aggregation). Sex may occur in the case of single celled propagules that fuse with propagules from other groups to start a new group.

The genotype phenotype map is a mapping between the propagule's genotype and the phenotype of the adult cell group derived from the propagule. The main group phenotype of interest is the degree to which cells in the adult group cooperate with each other to benefit the group.

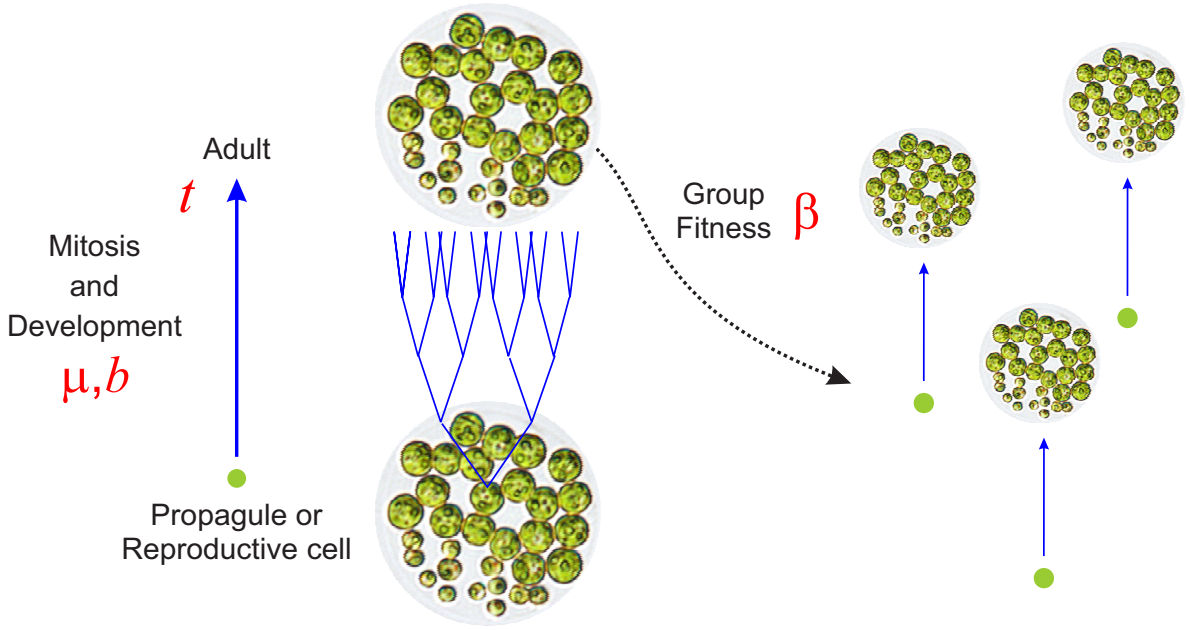

Figure 3.1. Development and multi-level selection in a model of a multicellular organism. Mitotic cell divisions produce an adult cell group from a propagule. There can be selection at both the group level and the within group or cell level during development of the adult group. Blue lines indicate development, dashed black lines indicated group productivity which is determined by the frequency of cooperative cells in the group. Parameters μ, b, t in red refer to the parameters of development: μ is the mutation rate (from cooperate to defect), b cell division rate, and t is the time available for cell division and development, respectively. The parameter □ refers to the beneficial group fitness effect of cooperation on the number of propagules produced by the group. Other parameters may be studied such as the survival of cells during development and cell size, but these details are not considered here. There are, however, additional parameters related to the nature of modifier locus and how it modifies the developmental parameters μ, b, t. Several different kinds of modifiers have been considered: cell policing, programmed cell death, determinant size and germ line modifier alleles (Michod, 2003). For example, the germ line modifier considered in Figure 3.2 below reduces the development time of the germ line by amount ™, in other words, the soma develops for time t and the germ line develops for time t - ™, so there is less time available for deleterious mutation in the germ line relative to the somatic line. In an extension of the germ line model, the timing of sequestration of the germ line is another parameter that has been considered (Michod et al., 2003).

The model is an abstract population genetics model; for concreteness a colonial volvocine green algal species like *Pleodorina starrii* is shown in the figure, a species in which the adult group develops from a single cell propagule. Shown in Figure 3.1 is the adult cell group created by a single reproductive cell in a parental *P. starrii* colony. The adult group has non-specialized reproductive cells (they participate in both reproductive and survival functions) and smaller

specialized somatic cells appearing at the bottom left of the colony image (the somatic cells specialize in somatic functions like flagellar action).

3.3.2 Cooperation and conflict

The cell behavior locus is assumed to have two alleles, *C* and *D*, which express cooperation and defection, respectively, among cells interacting in the group. Cooperation benefits other cells in the adult group at a cost to the cooperating cell paid during the mitotic divisions that produce the adult group. Cooperating cells pay a cost of cooperation by either replicating more slowly or surviving less often compared to defecting cells. Defecting cells do not cooperate and do not pay a cost, but they may receive benefits from cooperating cells present in their adult group, because groups with cooperating cells are more productive. During development of the adult group, there is recurrent mutation from *C* to *D* at each cell division; back mutation is ignored on the assumption that there are many more ways to lose a functional trait like cooperation than to gain it. These defector mutations disrupt the functioning of the adult cell group by reducing the level of cooperation. Mutation increases the variance and opportunity for selection at the within-group or cell level during the mitotic divisions that create the adult stage. After the adult group is formed, a propagule is made either asexually or sexually. The output of propagules depends on the degree of cooperation at the adult group according to a parameter β. Depending on the parameters of development (mutation rate, cell replication rate, time for cell division), the costs and benefits of cooperation, and the mode of propagule formation, a polymorphism may be maintained at the *C*/*D* locus by mutation selection balance. This polymorphism sets the stage for the evolution of modifiers of development assumed to be encoded by a second locus.

3.3.3 Mutation and multilevel selection

Mutation from cooperate to defect is assumed at rate *m*, while back mutation is ignored. Back mutation from defection to cooperation is ignored, relative to mutation leading to the loss of cooperation, on the assumption that it is more likely to lose a complex trait like cooperation than gain it through random mutation. Because of the hierarchical nature of selection within and between organisms, there are two levels of selection at which to consider mutational effects: the cell and the cell group or emerging organism. This leads to a classification scheme, +/+, +/- , -/+, -/- , with the effect of the mutation on the cell given on the left and the effect of the mutation on the organism given on the right. Uniformly advantageous mutations (+/+) which benefit both the fitness of cells and the fitness of the whole organism will sweep through the population: there is little reason to model them explicitly, given the deterministic assumptions of the model (the effects of finite population sampling are ignored). Likewise for uniformly deleterious mutations which detract from the fitness of both levels (-/-) , except they will be lost from the population. There is some evidence for the -/- kind or effect (Demerec, 1936). In this case (-/-), the occurrence of selection among cells within the organism may have the benefit of lowering the overall mutation load in the population of organisms and this effect has been considered by several authors (Crow, 1970; Otto and Orive, 1995; Whitham and Slobodchikoff, 1981). Mutations which benefit the cell's replication rate but detract from organism fitness (+/-) are the case of interest here, since they arise when *C* cells mutate to *D* cells during mitotic cell division. Considerable evidence exists for this kind of mutation in animals - most notably malignant cancer mutants. The other class of mutations which harm the cell but benefit the organism (- /+) can be addressed by an adjustment of the parameters in the models given below but have not been studied in detail.

The mutation selection balance model embedded in the two-locus modifier approach to the evolution of individuality is different from the standard mutation selection model of population genetics. Two levels of selection are assumed in the developmental modifier model and so there are two levels of mutational effects that must be considered as discussed above. Mutation may be uniformly deleterious, or uniformly beneficial, in that it decreases, or increases, the fitness at both the cell and the group levels simultaneously. On the other hand the effects of mutation at the two levels could go in different directions, such as is the case with an altruistic mutation that increases the fitness at the group level, while decreasing the fitness at the cell level.

3.3.4 Modifiers of development

A second modifier locus is considered that modifies the parameters and mode of development of the adult group and how cells are sampled to create propagules for the next generations (gametes in the case of sexual reproduction). For example, in the case of a germ line modifier, we assume the propagules for the next generation are sampled from a group of cells that is separate from the somatic cell line, and that has a lower mutation rate and/or less time available for cell division. Consequently, the mutation rate parameter m and/or the development time parameter t in the germ line are reduced relative to what these parameters are in the somatic line. Another means of reducing conflict among cells is by cells actively policing and regulating the benefits of defection. We assume that cooperating cells expressing policing spend time and energy monitoring cells and reducing the advantages of defecting at a cost to the cell group. As a result of policing, the benefits of cooperation to the adult group are reduced, while the advantages to cells of defecting are reduced. In similar ways, we have considered modifiers creating a unicellular propagule (Michod and Roze, 2000; Roze and Michod, 2001), programmed cell death (the modifier reduces the survival and replication rate of defecting cells) and determinate adult group size, which fixes the size of the adult group and has the effect of reducing the opportunity for within group change (different kinds of modifiers are reviewed in Michod, 2003).

By modifying the parameters of development and mode by which cells are sampled to create the next generation, the modifier locus molds the genotype phenotype map and the degree to which the propagule produced by an adult resembles the propagule that founded the group. This resemblance is a measure of heritability at the group level and is used to interpret the results of the models. By molding development, the modifier M allele creates group heritability and the capacity of the groups to reproduce themselves (Griesemer, 2001). Modifier alleles create "higher level" functions, in the sense that their traits are selected by virtue of their tilting the balance in favor of selection at the group level and away from selection at the individual level.

The case of germ soma specialization is presented here (Hanschen, Shelton, and Michod, 2015; Michod, 1999; Michod et al., 2003; Michod and Roze, 1997). A future project is to apply these MLS models to study development of specialized somatic cells in a species like *Pleodorina starrii* as illustrated in Figure 3.1. Doing so would be of interest to understanding the evolution of division of labor in the volvocine green algae lineage, because soma specialization evolved before germ specialization in this lineage. The reproductive cells in a species like *Pleodorina starrii* (shown in Figure 3.1) are not specialized at reproduction and additionally participate in somatic activities like flagellar beating.

3.3.5 Evolutionary transitions in individuality

The results of these models show that cooperation among cells in a proto-organism may be vulnerable, because traits that benefit the cell and harm the cell group can increase within each

cell group during the mitotic proliferation that forms the adult group. This is similar to issues of cooperation and conflict that have been much discussed in a synchronic context in the sociobiology literature. The diachronic context in these models comes from explicit consideration of the evolution of modifier traits that affect development. Modifier traits affect development of the multicellular group and can tip the balance in favor of cooperation by changing aspects of development that affect the interplay of levels of selection. By subverting within-group natural selection, modifiers can set the stage for enhanced cooperation and elaborate integration of cell-groups into adaptive wholes and multicellular individuals.

Table 3.1 Equilibria and their interpretation in the two-locus modifier model introduced in Figure 3.1. The cooperate/defect locus has two alleles, *C* for cooperate and *D* for defect. The modifier locus has two alleles *m* and *M* that affect development. The non-modifier *m* allele has the basic parameters of development μ, *b*, *t* shown in Figure 3.1, while the modifier allele *M* involves changes in these and other parameters depending on the kind of modifier considered. For example, the germ line modifier considered in Figure 3.2 reduces the development time of the germ line by amount ™; the soma develops for time *t* and the germ line develops for time *t* - ™. As a result, there is less opportunity for mutation in the germ line.

Equil.	Genotype	Description of Loci	Interpretation
1	*D*, *m*	no cooperation; no modifier	Single cells, no organism
2	*D*, *M*	no cooperation; modifier fixed	Not of biological interest, never stable
3	*C*/*D*, *m*	polymorphic for cooperation and defection; no modifier	*Group of cooperating cells or proto-organism*: no higher-level functions
4	*C*/*D*, *M*	polymorphic for cooperation and defection; modifier fixed	*Individual organism*: integrated group of cooperating cells with higher-level functions mediating within organism change

Why do these modifier *M* alleles evolve and how do they lead to the capacity of a group to reproduce itself? In the Michod and Roze (1997) model, four possible equilibria were studied as described in Table 3.1. Equilibrium 1 has only *Dm* cells; there is no cooperation and no group fitness. Equilibrium 2 has only *DM* cells and is not stable or biologically interesting. Equilibrium 3 is polymorphic at the primary locus and has *Cm* and *Dm* cells, and may be viewed as a proto-organism, a cell group with cooperation and fitness variance, but no higher-level functions. Finally, equilibrium 4 is polymorphic at the primary locus, fixed for the modifier *M* allele, and so has *CM* and *DM* cells (Michod, 1999, p. 114). At equilibrium 4 the population has transitioned to existing as groups of cooperating cells with higher-level group functions that mediate conflict at the lower level. Consequently, I refer to the groups at equilibrium 4 as individual organisms. Equilibrium 1 occurs when the advantage of defection is high. In this model, within-group selection favors defector (*D*) cells and between-group selection favors cooperator (*C*) cells. Thus, for there to be a population that is polymorphic for *C* and *D* (equilibriums 3 and 4), selection at

the two levels must be in balance. This balance means that the first and second term of the right-hand side of the Price equation (Equation 3.1) are equal in magnitude. At equilibrium 3, the population is fixed for no modifier (*m*). The *C/D* polymorphism consists of cooperating cells being maintained at relatively low frequencies (Michod, 1999, p. 123, see Figure 6-3 in that reference). The exact level of cooperation depends on several parameter values, but the general observation of lower cooperation in a population fixed for the *m* allele holds. For a population in equilibrium 4, again cell- and group-level selection are in balance. However, the frequency of the cooperator (*C*) allele in this equilibrium can be much higher as the cell groups have higher-level group-specific functions that suppress lower-level selection for defection.

The mutation-selection balance equilibrium at the *C*/*D* locus implies that *C* alleles are fitter than D alleles, to compensate for mutation from *C* to *D*. Under certain conditions, alleles at the modifier locus evolve due to hitchhiking with the fitter *C* allele. This has the effect of increasing the between-group variance and decreasing the within-group variance, thereby increasing the level of cooperation and the fitness of the group. Examples of conflict modifiers studied by this approach include germ-soma specialization, reduced mutation rate, policing, programmed cell death, passing the life cycle through a single-cell zygote stage, and fixed group size (reviewed in Michod (2003)). By increasing the variance at the group level and decreasing the variance at the cell level, the modifiers have the effect of decoupling the fitness at the group level from the counterfactual fitness of cells as well as enhancing the capacity of the group to reproduce itself.

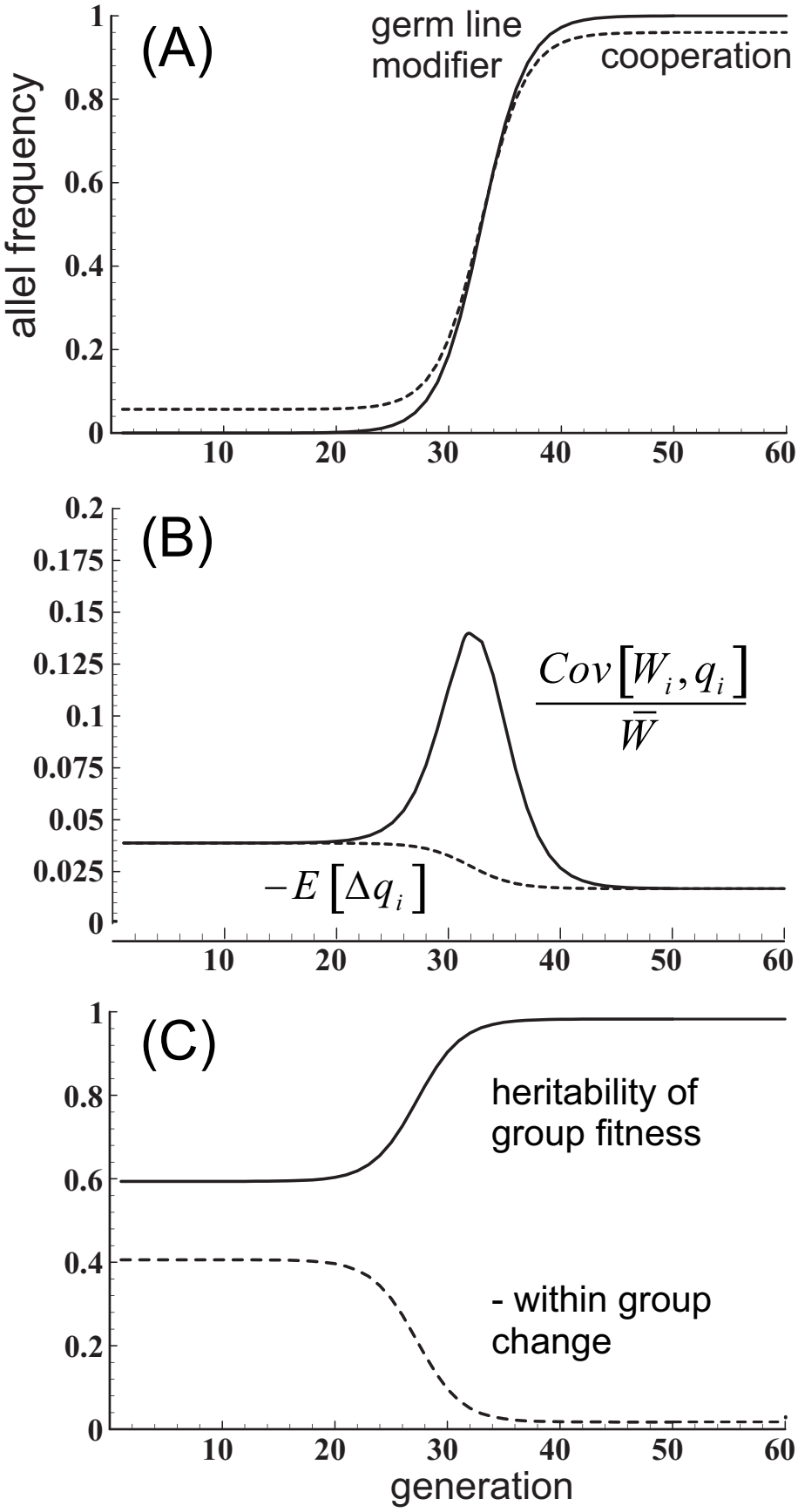
(A)
germ line
modifier
cooperation
allel frequency
1
0.8
0.6
0.4
0.2
0
10
20
30
40
50
60
(B)
$\frac{Cov[W_i, q_i]}{\bar{W}}$
$-E[\Delta q_i]$
0.2
0.175
0.15
0.125
0.1
0.075
0.05
0.025
0
10
20
30
40
50
60
(C)
heritability of
group fitness
- within group
change
1
0.8
0.6
0.4
0.2
0
10
20
30
40
50
60
generation

Figure 3.2. Components of an evolutionary transition to multicellular individuality as modeled by a two-locus MLS modifier model given in Figure 3.1 and Table 3.1. Figure modified from Michod and Roze (1997). The figure considers the case of a transition between equilibrium 3 and 4 (see Table 3.1 and associated text) for a costly germ line modifier of within organism change (mathematical model presented in Michod (Michod, 1996)). The modifier is assumed to decrease the development time for the germ line (when compared to the soma) by amount ™. The parameter values studied are ⁄ = 0.003, ® = 30, t = 40, ™ = 35 and b = 1.1 (b is the replication rate of defecting cells relative to unity for cooperating cells). The parameter ™ is the reduction of time for cell division in the germ line compared to the somatic line. In other words, the somatic line divides for time t, while the germ line divides for time t - ™, so there is less time available for deleterious mutation in the germ line. The x-axis for all panels is time in organism generations. The y-axis in panel (A) is gene frequency (frequency of either the C allele or the M allele); the y-axis in panels (B) and (C) correspond to the different curves as labeled. Modifier M alleles may increase and sweep through the population as shown by the solid curve of panel (A) leading to an increase in cooperativity among cells (dashed curve in panel (A)). As shown in panel (B), the underlying cause of the modifier's success during the transition is the fact that the heritable covariance in fitness for the organism, $\frac{Cov(W_i, q_i)}{\overline{W}}$ (solid curve), is greater than the average within organism change, $E(\Delta q_i)$ (dashed curve). The derived gene frequency dynamics under multilevel selection can be represented in terms of Price's Equation 3.1 which becomes in this case $\Delta q = \frac{Cov(W_i, q_i)}{\overline{W}} + E(\Delta q_i)$, with $q_i, \Delta q_i$ being the frequency, and change in frequency, respectively, of the C (cooperate) allele within zygotes of type i, and $q, \Delta q$ the frequency, and change in frequency, respectively, of the C gene in the total population (i = 1, 2, 3, 4 for CM, Cm, DM and Dm zygotes respectively). As occurs in panel (B), the two components of the Price equation must be equal at both equilibria, before (at equilibrium 3) and after the transition when the population returns to equilibrium (now equilibrium 4). As a consequence of the modifier's success and fixation, the level of cooperation in the population increases dramatically from nearly zero initially to greater than 0.90 after the transition (panel (A) dashed curve) and the heritability of fitness at the emerging organism level increases from approximately 0.6 to close to unity (panel (C), solid curve), while the within organism change in groups with cooperating cells drops from about 0.4 to near zero (panel (C), dashed curve). In this figure, heritability of fitness at the group level is measured by the regression of offspring fitness on adult fitness. See text for further explanation.

Although the Price equation does not always partition selection correctly between levels because of the pseudo-group problem discussed above, it may be used to help us understand the transition to equilibrium 4 in the modifier model. In Figure 3.2(B), the two components of the Price covariance Equation 3.1 are plotted for the case of the modifier model. These components partition the total change in gene frequency into heritable fitness effects at the organism level (solid line) and within-organism change (dashed line). In the model studied here, within-organism change is always negative, because defecting cells replicate faster than cooperating cells and there is no back mutation from defection to cooperation. At equilibrium, before and

after the transition, the two components of the Price equation must equal one another in magnitude, or else the population could not be in equilibrium (this is shown in Figure 3.2(B)). However, during the transition we see that the covariance of fitness with genotype at the emerging organism level (solid curve, Figure 3.2(B)) is greater than the average change at the cell level (dashed curve, Figure 3.2(B)). This greater heritable covariance in fitness at the higher level forces the modifier into the population. Note that after the transition, the within-organism change is smaller than before. Recall that the $E(\Delta q_i)$ term includes the effect of lower-level selection, which is seen as "property change" at the group level. Since the levels of property change are lower in equilibrium 4 compared to equilibrium 3, the population has evolved to have higher heritability of traits (and therefore higher heritable fitness) at the group level.

3.3.6 Group reproduction and group fitness heritability

Several approaches to quantifying "group reproduction" and group fitness heritability have been used in this work. The capacity of a group to reproduce itself may be measured by the degree to which a group created by a propagule resembles the group the propagule came from. Alternatively, since the group is made from a propagule, and the two-locus recurrence equations are in terms of the gene and genotype frequencies at the propagule stage, we may measure the capacity for reproduction and heritability of group traits as the degree to which the propagules produced by a group are similar to the propagule(s) that created the group. Heritability of fitness at the organism level may be measured in the standard way by the regression of offspring fitness on adult fitness (Michod, 1999, Chapter 6 and Appendix). Using this definition of heritability of fitness and the Price Equation 3.1, Figure 3.2 shows how the heritability of fitness increases during a model ETI (transition from Equilibrium 3 to 4 given in Table 3.1) involving the evolution of a developmental modifier that creates germ-soma specialization (Michod and Roze, 1997). Note that for the notation used in deriving Figure 3.2, the Price Equation 3.1 becomes $\Delta q = \frac{Cov(W_i, q_i)}{\overline{W}} + E(\Delta q_i)$, where *i* indexes cell groups, the proto-organisms.

Like any trait, heritability of fitness may be defined as the regression of offspring fitness on the fitness of parents. During the transition in the model in Figure 3.2, heritability of fitness at the group level increases. It can be shown that the evolutionary transition always leads to an increase of heritability of fitness (Michod and Roze, 1997, 1999). The heritability of fitness is further studied in Michod (1999, especially Appendix pp. 203-218).

In their review of the evolution of multicellularity, Rainey and de Monte (2014) observe that collective level or group heritability is a derived state, something that must be explained. They state, "Although MLS theory appropriately describes the state of a population of cells before and after the transition to multicellularity, it provides no explanation for how selection shifts from lower-level entities to collectives." I agree that collective level heritability is a derived state, however, I do not think their criticism of MLS applies to the explicitly diachronic setting of the MLS modifier models discussed here. Indeed, these models provide a hypothesis for how MLS will act on modifiers of development, and, by so doing, increase the heritability at the group level. These diachronic MLS models explain the shift in selection from lower-level entities to higher-level collectives (Figure 3.1 and Figure 3.2).

This section reviewed MLS models for the evolution of modifiers of the development of cell groups and propagules that are sampled from the adult cell groups to produce the next

generation. These developmental modifiers create the first true group-level functions, such as specialized germ and somatic cell lines, that mold development to increase group heritability and the mapping of offspring genotype to adult phenotype. In this way, these models provide a hypothesis for how a group of individuals may become a new kind of individual, the central question posed by ETI theory.

These models take a decidedly diachronic view on levels-of-selection questions. As already mentioned, ETIs raise the questions of how the group level emerges and takes on properties of an evolutionary individual. The two-locus MLS modifier models present a hypothesis in which developmental features of the group-level reproductive system can themselves evolve by MLS, and the Price Equation 3.1analysis helps to highlight the within-group versus between-group selective dynamics as shown in Figure 3.2 (B).

The sampling of populations of Darwinian individuals easily creates groups with Darwinian properties giving rise to the problem of pseudo-groups and the need to distinguish true group effects, like those created by the genetic modifiers in the MLS models of development. There are at the least three kinds of selection that occur in MLS models, within-group individual selection, between-group selection and global individual selection (Shelton and Michod, 2020). Michod and Roze (1999, p. 10) discuss the issues of pseudo-groups and the global individual selection that comes up in the MLS modifier models. Colonies with more defecting *D* cells (cells that replicate faster, all else equal) would be fitter than those with fewer *D* cells, even if there were no interactions between the cells within the groups. The high-replication-rate cells do better in competition within each cell group, and these are the same cells that (for the same reason) would do better without any group context at all. This issue has also been discussed by Okasha (2006, Chapter 8.4).

These models also contribute to our understanding of fitness during ETIs and how it is reorganized during the evolution of ETIs generally and the transition to multicellularity specifically. I now turn to the issue of fitness and how it is reorganized during the evolution of multicellularity to create individuality at the group level.

3.4 Fitness reorganization and germ-soma specialization

Recall, as the modifier allele increases in frequency in the population, heritable variation in fitness increases at the group level and decreases at the cell level (Figure 3.1(C)). In the case of the germ line modifier, as it spreads in the population, fitness becomes reorganized, in the sense that cells become specialized in the fitness components of the group. Fitness always has two basic components, survival and reproduction, that must be rebuilt at the group level when initially they are present only at the cell level (Table 3.2). The evolution of germ-soma specialization accomplishes this, because germ cells specialize in the reproduction component of fitness of the group and somatic cells specialize in the viability component. As already discussed, the germ-soma modifier allele builds a new genotype-phenotype map for fitness at the group level when initially this mapping is present at the cell or individual level.

Table 3.2 Reorganization of Fitness during ETIs.

Fitness components	Viability (vegetative/somatic functions).
	Fecundity (reproductive functions).
Definition of fitness Reorganization	Transfer of fitness from lower to higher level. Lower levels specialize in fitness components. Heritability of fitness emerges at higher level.
Means of fitness reorganization	Stress responses. Fitness trade-offs.
	Somatic specialization. Group inseparability. Gene co-option.
Consequences of fitness reorganization	Individuality at higher level. Specialization at lower level. Complexity. Evolvability.

By "reorganization of fitness" I mean the increase of fitness heritability at the group level and decrease at the cell level, with the specialization of lower-level units (cells) in the fitness components of the cell group (the new individual) (Michod, 2005, 2006, 2007). As a consequence of this reorganization of fitness, fitness at the group level becomes decoupled from fitness at the cell level. "Decoupled" in the sense that the fitness of the group may be high, while the individual fitness of the specialized cells would be low were they to leave the group. A germ cell cannot survive well on its own and a somatic cell cannot reproduce, yet, together in a group, the group can survive and reproduce.

The MLS modifier model is intended as a heuristic device for understanding the general issues involved and overlooks a variety of practical issues that are involved in the reorganization of fitness (Table 3.2), such as how the genes for fitness reorganization arise. Genes previously used for life-history stress responses in a unicellular ancestor may be co-opted for the evolution of soma and division of labor in the group (Nedelcu and Michod, 2006, 2020; Olson and Nedelcu, 2016). Cell division, previously the reproduction component of cell fitness, is co-opted for growth of the group and organism body size (Nedelcu and Michod, 2003). A group life cycle must evolve from a cell cycle (Hanschen et al., 2016; Maliet, Shelton, and Michod, 2015; Shelton, Leslie, and Michod, 2017; Shelton and Michod, 2014), possibly through the coevolution of a cell life history trait (such as cell growth) and a group trait (such as time spent in the group) (Maliet et al., 2015; Shelton and Michod, 2014). Within the group, germ cells specialize in reproduction and somatic cells in viability of the group, as in the germ-soma modifier model.

Specialization of somatic cells is a key stage in the remapping of fitness to the group level, for as individuals specialize in the fitness components of the group, they lose their individual (counterfactual) fitness outside of the group, while the fitness of the group increases. Germ-soma specialization also increases the individuality of the group by making it indivisible. Germ and soma specialized cells have low fitness when removed from the context of the group, even as the fitness of the group may be quite high. In effect, the germ and soma specialized cells constitute a good team that together bring high fitness to the group. Group fitness may also be decoupled from the individual fitness through the evolution of life history traits; as individuals spend more time in the group, their individual properties will change from values optimal for living alone to values optimal for living in the group (Maliet et al., 2015; Shelton and Michod, 2014).

Trade-offs between fitness components have a special role to play in the reorganization of fitness. A simple trade-off at the individual level, say, between survival and reproduction, can lead to altruism when cells are in a group (Michod, 1999; Michod and Roze, 1999). Cells that put more effort into survival functions, if these same functions benefit the group, are behaving altruistically relative to cells that put less effort into survival. For example, in the volvocine green algae, flagellar motility is a significant survival component of both cells and cell groups. However, flagellar motility at the cell level interferes with the capacity of the cell to reproduce. As a consequence of this trade-off, when groups are first formed, cells that keep their flagella longer are behaving altruistically relative to cells that lose their flagella earlier.

The concept of fitness decoupling that arises from individuality modifiers like germ-soma specialization is similar to the ideas of MLS1 and MLS 2 developed by Damuth and Heisler (1988). In MLS1, group fitness is an aggregate property of individual fitness, while, in MLS2, fitness is a non-aggregate, or emergent, property of the group. In the case of cell groups, MLS1 would be the kind of group selection that occurs when group fitness is an average of cell fitness. With the evolution of germ-soma division of labor, the cell group enters the realm of MLS2, because group fitness is decoupled from the (counterfactual) fitness of cells (Okasha, 2004, 2006).

3.5 Criticisms and commentaries on the MLS approach to ETIs

3.5.1 Darwinian properties

A repeated concern with the MLS approach to ETIs has been the concern that MLS assumes the existence of groups with Darwinian properties, something that should be explained, not assumed (Clarke, 2014; Huneman, 2012; Rainey and Kerr, 2010; Rainey and Monte, 2014). For example, de Monte and Rainey (2014) say, "…it is possible to fall into the trap, as we and others have emphasized, of invoking Darwinian properties as the cause of their own evolution."

The concern is important; a hypothesis should not be circular, that is, an explanation should not assume what is to be explained. Groups formed by sampling Darwinian populations will often have Darwinian properties at the group level. The groups formed by sampling a population of individuals will likely be comprised of different frequencies of types of individuals; there will often be differences between groups in group fitness, taken as the average of the fitnesses of members of the group. The Darwinization of groups is the flip side of the pseudo-group problem discussed above, which recognizes that groups of Darwinian individuals are themselves easily Darwinized. The Darwinian properties filter up so-to-speak from the individual level to the group level during the sampling process that creates the group. The challenge is not explaining why groups may have Darwinian properties; this is rather easy to understand. The challenge is with explaining how groups may gain properties of an evolutionary individual. The evolution of developmental modifiers discussed above is a hypothesis for how individual properties may evolve beginning with a group-structured population of cooperating individuals. These groups will often themselves have Darwinian properties as a result of sampling a population of cooperating individuals.

In the evolutionary transition to multicellularity, it is not difficult to understand why cell groups have Darwinian properties. The problem is with explaining how increased Darwinian properties of groups may arise from true group effects, such as the effects created by the developmental modifier traits discussed above. These modifiers tweak the developmental processes that create

cell groups, to enhance the evolutionary individuality of those groups. This is what panel C of Figure 3.2 and other analyses show.

Rainey and colleagues have argued for what they call a "take-nothing-for-granted account" (Black, Bourrat, and Rainey, 2019; De Monte and Rainey, 2014), in which they suggest that "Darwinian properties might emerge from non-Darwinian entities and, therefore, by non-Darwinian means" (Black et al., 2019). When one looks at the mathematical model for this "take-nothing-for-granted account" account, one finds the standard assumptions of MLS theory, most basically, a sampling process of Darwinian individuals that generates cell groups with Darwinian properties (termed "patches" in the model (Black et al., 2019)). These patches or groups possess Darwinian properties by virtue of sampling cells with Darwinian properties as occurs in all MLS models. The author's use the term "ecological scaffolding" for this process by which a sample of Darwinian individuals itself has Darwinian properties. For this reason, I do not see how the model shows that "Darwinian properties might emerge from non-Darwinian entities and, therefore, by non-Darwinian means" (Black et al., 2019).

The authors (Black et al., 2019) go on to argue that their model involves a "shift from levels to timescales [that] does much to clarify the kinds of conditions necessary to effect transitions in individuality." While a nice feature of their model is that it has two different time scales, it also has two different levels, the cell and the patch or group. The general assumption in their model, common to all MLS models for ETIs, including the models discussed in this chapter, is that a group of Darwinian individuals may itself have Darwinian properties and these groups can, through further evolution, be molded into a new multicellular individual.

3.5.2 Group reproduction

The issue of explaining group reproduction has received special concern. Rainey (2007) states "The catch-22 is that selection is powerless to act at the group level because newly emerged groups are incapable of differential reproduction." As we have seen, groups likely have Darwinian properties, including differential reproduction, by virtue of sampling individuals with these Darwinian properties. The model of Black et al. (2019) discussed above is based on this assumption as are all MLS1 type models.

In describing the MLS modifier models presented here, Rainey and Kerr (2010) state "While such a scenario describes plausible changes, the model assumes that the capacity to leave group offspring is already in place. But how such a new level of reproduction emerges requires explanation." I do not believe the models described here begin by assuming a new level of reproduction. In fact, as I have tried to explain, before variation is introduced at the modifier locus, there is a mutation-selection balance equilibrium at the first cooperate-defect locus at which some groups are more productive than others depending on the frequency of cooperation in the group. However, this is not a new level of reproduction. The new level of reproduction comes about because of variation introduced at the second modifier locus, the new modifier allele changes how groups develop so as to create a new level of reproduction. As I have already discussed, a feature of the MLS modifier models described here is that they explain the capacity of the group to leave offspring through the evolution of modifiers of development such as germ line modifiers and the effects of these changes in development on group heritability and the genotype phenotype map (panel C of Figure 3.2).

The central issue in explaining group reproduction is understanding the evolution of the process by which adult cell groups are formed and produce propagules for the next generation. As we

have seen, modifiers of development may mold both the development of adult groups and the sampling process by which cells are taken from the adult group to produce propagules for the next generation. These modifiers in turn increase heritability of fitness at the group level (panel C of Figure 3.2). The MLS modifier models discussed here assume that a generation starts with propagules sampled from adult groups in the previous generation, these propagules then give rise to the adult groups through "development." Development in the model refers to mitotic cell divisions, cell specialization along with the process by which cells are sampled from the adult to produce the next generation. The propagules may be sampled randomly from the entire adult group may, or the sample may come from a smaller group of cells set aside and sequestered at a certain stage in development as occurs with a germ line modifier (Michod et al., 2003; Roze and Michod, 2001). In the case of a germ line modifier, the propagule sample comes from a separate lineage of cells that may have fewer divisions or a lower mutation rate, because germ cells are separated from the metabolic activities present in the somatic line. A main feature of these modifier models is their capacity to explain the evolution of group reproduction through the evolution of development.

Reproduction of the group is quantified and explained in the modifier model, by studying the mapping of group properties, especially fitness, from the propagule to adult. As the modifiers spread, they increase the heritability of group fitness as shown in Figure 3.2 (C). The evolution of modifiers of development gives rise to increased heritability of fitness at the group level and by so doing give rise to group reproduction. The evolution of these modifiers of development accomplish what is needed for the acquisition of group reproduction, following Griesemer (2001) "Development from an evolutionary point of view can be thought of, in general, as the acquisition of the capacity to reproduce."

3.5.3 Fitness transfer and fitness decoupling

A concern with the MLS models described above is that the concepts of "fitness decoupling" and "fitness transfer" are metaphorical and descriptive (Black et al., 2019). These terms have been used as descriptions of the results of the MLS modifier models reviewed here, such as the results given in Figure 3.2 (C). There we see that, as the modifier increases, the fitness at the two levels diverge, with group fitness increasing and cell fitness decreasing. I have referred to this as fitness decoupling and/or fitness transfer. However, speaking of fitness "transfer" may incorrectly suggest fitness is a conserved quantity in the models. There is a mechanistic sense in which the evolution of altruistic forms of cooperation such as occurs in the model (Figure 3.1 (A)) can be seen as transferring fitness between levels (Shelton and Michod, 2020). An altruistic behavior is defined as having both a cost at the individual level and benefit at the group level. Consequently, as an altruistic allele spreads in a population, its costs decrease fitness at the cell level, while its benefits increase fitness at the group level. In this sense, the evolution of altruism transfers fitness between levels.

As discussed above, the idea that there is a decoupling of fitness between levels during an ETI relates to the idea that an ETI is a transition between MLS1 and MLS2. Libby and Rainey (2013) state that "The difficulty is that MLS theory fails to explain how the transition from MLS1 to MLS2 comes about." I hope it is clear from the presentation here that MLS theory can, when coupled with the evolution of developmental modifiers, explain the transition from MLS1 to MLS2. For example, when the germ line modifier evolves that average fitness of the cell group is no longer an aggregate property of the cell level (counterfactual) fitnesses and the heritability of

the group fitness increases as a result. For this reason, the transition from Equil. 3 to Equil. 4 in Table 3.1 and Figure 3.2 is a transition from MLS1 to MLS2.

3.5.4 Origin of individuating properties

Clarke (2014) warns of a possible "evolutionary chicken and egg" problem when discussing evolutionary transitions. "We must not presuppose the existence of higher-level organisms when offering evolutionary explanations [of higher-level organisms]," and she goes on to make clear that this warning applies not to just higher-level organisms themselves, but to the kinds of traits that define higher-level organisms and give rise to their individuality. Traits that define higher-level organisms Clarke calls "individuating mechanisms," traits like a germ line, single cell bottlenecks, policing; in short, the kind of modifiers of development considered in the two-locus modifier models discussed above and reviewed in (Michod, 2003). I agree that we must explain the existence of these traits and cannot assume them to be both causes and consequences of higher-level selection. I see the modifier models discussed here as an explanatory hypothesis for the evolution of such individuating traits, like germ soma specialization, that define higher-level individual organisms.

In these models, before the evolution of the modifier allele, there is variation in fitness at the group level because different groups will contain different allele frequencies at the cooperate/defect locus, as discussed above. Initially, there is no modifier allele *M*. The modifier of development allele is introduced at equilibrium 3 in Table 3.1. Equilibrium 3 is a mutation-balance selection equilibrium at the cooperate-defect locus; consequently cooperation is more fit than defection, so as to compensate for mutation from *C* to *D*. Mechanistically, in a population genetic sense, the modifier allele, *M*, may hitchhike with the more fit *C* allele, along with the new kinds of groups it creates, and the population may transition from equilibrium 3 (*C*/*D*, *m*) to equilibrium 4 (*C*/*D*, *M*) (Table 3.1). This transition from equilibrium 3 to equilibrium 4 takes cooperation and heritable group fitness to higher levels as it creates groups with more individuality (Figure 3.1).

Is there a chicken and egg problem with this model as a hypothetical explanation for the ETI from unicellular to multicellular individuals? Does the model assume traits associated with higher level individuals, like the germ line modifier, are both a cause and an effect of higher-level individuality? I do not think so. Before the evolution of the modifier, the fitness variances at the individual and group levels are a result of both cell division and the sampling creating groups (as diagrammed in Figure 3.1). In addition, there are assumptions related to the cooperate/defect game, the mutation/selection balance, and the standard assumptions of haploid two-locus population genetics.

Once introduced, the modifier allele coevolves with its effects on enhanced cooperation and group heritability (Figure 3.1), but this is what we would hope to see in an explanation of an ETI. For example, Sober and Wilson (1998, p. 97) say: "The coevolution of traits that influence population structure with traits that are favored by the new population structure can result in a feedback process that concentrates natural selection at one level of the biological hierarchy' (Sober and Wilson 1998, p 97).

Concerning the MLS modifier models discussed above, Clarke (2014, p. 9) says that the modifiers, and the traits they cause, are present at the beginning of the model. I do not think this is the case. The modifier *M* allele is not present at equilibrium 3 (Table 3.1) where it is introduced as already explained. A locus where the modifier allele *M* might arise is assumed, but,

until the modifier is introduced, this locus has no effect on the model, the *m* allele assumed to reside there is neutral without any effect. It seems to me that the MLS modifier model is an excellent example of what Clarke advances in her paper as the remedy to the chicken and egg problem in explaining individuality. Clarke argues that individuality is built up over time with some aspects being present early in the process and other aspects arising later. In the MLS modifier model, cooperation, defection, and sampling into groups are present initially, with the evolution of modifier traits caused by the modifier *M* allele coming later. To assume a single gene locus encodes a complex trait, like a germ line, is, of course, an oversimplification of many steps. But, this limitation is found in all simple one and two locus population genetic models, and does not reduce the heuristic power of these models in explaining evolutionary processes (Michod, 1981).

3.6 Conclusions, open questions, and future work

3.6.1 General

I have given a brief overview and history of MLS in population genetics and evolutionary biology. MLS was key to Wright's shifting balance theory (Wright, 1977, fig. 13.1) in which groups of individuals selected to a local fitness peak may output more individuals into the global population leading to transformation of the species (if the local fitness peak is also a global peak). Using the terminology developed in this chapter, we see that the MLS in Wright's theory is MLS1 and group fitness is a direct reflection of individual fitness as in the fleet deer example discussed by Williams (Williams, 1966a). In Wright's process, the fitness of individuals filters up, so to speak, to create group fitness.

The determinants of group fitness during ETIs require something different. MLS always involves group fitness whether in Wright's theory or during ETIs. What is different is how group fitness is comprised. The fitness of a group of individuals that has become a new kind of individual is no longer a simple average of the fitness of its members, because the members will often specialize in different activities and components of fitness of the group. Alone group members would have little fitness but together in a group the fitness can be quite high. An ETI begins with the group as a collection of individuals and ends with the group being a new kind of individual. This requires the specialization and integration of activities of members in service of group fitness, the survivorship and reproduction of the group. The specialization and integration of the group involves the evolution of new developmental processes.

I have reviewed how MLS may be used to develop hypotheses about the evolution of development during the transition from unicellular to multicellular life. We have seen how developmental modifiers may coevolve with group structure and create the first true group level functions such as a sequestered germ line cell policing. In effect, these modifiers take the population from MLS1 to MLS2, from groups of cooperating cells to groups of cooperating cells with higher group-level functions, such as germ-soma separation, that mediate conflict within the group and enhance the heritability, reproduction and individuality of the cell group. After the transition, fitness at the group level is no longer the average of cell fitness, since the group is comprised of specialized somatic and germ cells that would be deficient, if they were to leave the group.

The germ-soma model discussed above assumes the modifier creates both germ and soma specialization. It would be useful to revisit these MLS modifier models to consider the evolution of somatic specialization without a specialized germ line. In the empirical systems we are most

familiar with, such as the volvocine green algae, somatically specialized cells evolved before germ specialized cells. The example, given for illustration in Figure 3.1, is of *P. starrii* a volvocine species with typically 64 total cells with smaller specialized somatic cells (seen at the bottom left of each colony in the figure) and non-specialized reproductive cells that first have flagella before losing their flagella during cell division and reproduction (most of the cells in the colony image). The modifier models could be used to develop hypotheses about the soma first (germ later) evolution observed in this clade.

3.6.2 Maximization principles and individuality

Maximization principles are useful in science because they may summarize complex dynamics in terms of a few variables and concepts. What might be maximized during an ETI? This measure could be used to quantify individuality, a concept that is difficult to understand as discussed above. In Figure 3.2 C, we see that as the ETI proceeds from equilibrium 3 to equilibrium 4 (Table 3.1), the fitness of the group increases and the degree of within group change declines. Within group change can be seen as a kind of transmission error that lowers the heritability of the adult group phenotype (Frank, 2012; Michod, 1999). Additional analyses of the effect of mutation rate on fitness after versus fitness before the ETI for the two levels of selection, group and cell level, are given in Michod and Roze (1999, figs. 13–14). The increase in heritability at the group level relative to the cell level holds for both uniformly deleterious mutations ((-, -) in the notation given above), as well as for selfish mutations (+/-) assumed to result from mutation from cooperation to defection. So, after the ETI, fitness at the group level has increased relative to the cell level. Cell level fitness here refers to the replication rate of cells within the group. In the MLS modifier models, the rate of cell division depends only on cell genotype and does not depend on group context; the benefit of cooperation is assumed to affect the functionality of the adult group, not the replication rate of cells that make up the adult group (Figure 3.1).

3.6.3 Fitness

Fitness is a unique and fundamental concept in biology. Lewontin remarked, “Natural selection of the character states themselves is the essence of Darwinism. All else is molecular biology (1972).” It is a challenge to understand and be clear about the meaning of “fitness” even when there is just one level of selection; we may expect challenges when considering multiple, simultaneous levels of selection with the goal of understanding the transition from one unit or level of fitness to another. When there are multiple levels of selection, we would like to know what level (or levels) of selection is (are) causing changes in the frequency of a trait.

Fitness is used in a variety of senses in this Chapter. In the MLS modifier models, there are two aspects of cell fitness, the cell replication rate (which depends only on cell genotype) and the cell’s cooperative behavior that contributes to group fitness of the adult cell group in a frequency-dependent manner. In these models, a cell’s replication rate depends only on the genotype of the cell and does not depend on the composition of the group. This is because in the model cell replication creates the group in which the cooperative behavior is expressed, as shown in Figure 3.1. The composition of the group affects group fitness, after the group has been made, but the group composition does not affect the replication rate of cells during the mitotic divisions that create the group. The overall fitness of a cell in the MLS models includes both its replication rate as well as its differential propagation through the differential output from groups, which is a frequency-dependent function of the frequency of cooperative cells in the adult group.

I have discussed "global individual selection" of cells, the contributions to individual fitness that do not depend on group membership, and give an example in the MLS modifier models of a defecting cell that has a higher replication rate regardless of context (see also, Okasha, 2006, Chapter 8.4). A defecting cell, in the MLS models, has two aspects to its fitness: a context-dependent component, by virtue of finding itself in groups with cooperators, and a context-independent component, such as a higher replication rate during cell division that gives rise to global individual selection. Although more work needs to be done, our analyses based on counterfactual fitness suggest that there are at least three kinds of selection that can be occurring at the same time in MLS models: group-specific between-group selection, along with two kinds of individual selection, within-group selection and global individual selection.

"Counterfactual fitness" refers to the fitness a cell that is inside a group would have were it to leave the group. The degree to which counterfactual fitness differs from the fitness of unicells, that have not evolved in the context of the group, may be used to quantify progression through the ETI, however much more work needs to be done.

There is also "fitness" in the sense of gene or allelic fitness, which considers the fitness effects of all the contexts the allele is in on the overall change in frequency of the gene. For example, I mean gene fitness when I say that "cooperation is fitter than defection at the mutation-selection balance equilibrium…". Likewise, one could refer to just "cell fitness," and ignore the different underlying fitness partitions that arise due to levels of selection. In this sense, cell fitness considers all sources of differential survival and reproduction of a cell. Likewise, group fitness may stem from different sources such as sampling of cell fitness or from "true group effects".

It is a challenge to get clear on "fitness" when levels of selection are changing, and modifier alleles are creating new kinds of groups and changing the degree to which selection is occurring at different levels. More work is needed to clarify fitness, how it is partitioned between levels and its causal basis during ETIs. The power of individual selection and the primacy of organisms were often used to deny the need for group selection in evolutionary biology, however the multicellular organism is a derived state and multilevel selection theory is needed to explain its origin and evolution.

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