

Cooperation and Conflict in the Evolution of Complexity

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ABSTRACT

The basic problem in an evolutionary transition in complexity is to understand how a group of individuals becomes a new kind of individual, having heritable variation in fitness at the new level of organization. We see the formation of cooperative interactions among lower-level individuals as a necessary step in evolutionary transitions; only cooperation transfers fitness from lower levels (costs to group members) to higher levels (benefits to the group). As cooperation creates a new level of fitness, it creates the opportunity for conflict between the new level and the lower level. Fundamental to the emergence of a new higher-level individual is the mediation of conflict among lower-level individuals in favor of the higher-level unit. We define a conflict mediator as a feature of the cell-group (the emerging multicellular organism) that restricts the opportunity for fitness variation at the lower level (cells) and/or enhances the variation in fitness at the higher level (the cell-group). There is abundant evidence that organisms are endowed with just such traits and numerous examples are reviewed here from the point of view of a population genetic model of conflict mediation. Our model considers the evolution of genetic modifiers that mediate conflict between the cell and the cell-group. These modifiers alter the parameters of development, or rules of formation, of cell-groups. By sculpting the fitness variation and opportunity for selection at the two levels, conflict modifiers create new functions at the organism level. An organism is more than a group of cooperating cells related by common descent and requires adaptations that regulate conflict within itself. Otherwise their individuality and continued evolvability is frustrated by the creation of within-organism variation and conflict between levels of selection. Conflict leads to greater individuality and harmony for the organism, through the evolution of adaptations that reduce it.

INTRODUCTION

Life is organized hierarchically and the major transitions in biological complexity have involved transitions between the levels in the biological hierarchy: genes, gene networks, chromosomes, bacteria-like cells, eukaryotic cells with organelles (cells within cells), multicellular organisms, and social organisms (Buss, 1987; Maynard Smith, 1988; Maynard Smith & Vida, 1990; Maynard Smith, 1991; Maynard Smith & Szathmáry, 1995; Michod, 1999). These transitions in the basic units of life, what we term evolutionary individuals, share two common themes: (i) the emergence of cooperation among the lower level units in the functioning of the new higher level unit and (ii) regulation of conflict among the lower level units. Evolutionary individuals are units of selection, and must satisfy Darwin's principles of heritability and variation in fitness, which may apply at different levels in the hierarchy of life (Lewontin, 1970). Because of the hierarchical nature of selection we take a multi-level selection approach to the origin of multicellularity and to evolutionary transitions generally. The multilevel selection approach to evolutionary transitions seeks to understand how a group of pre-existing individuals becomes a new evolutionary individual, possessing heritable fitness variation at the group level and protected from within group change by conflict mediators. We focus here on the unicellular multicellular transition, but the principles we describe are applicable to all the major evolutionary transitions in the hierarchy of life. The origin of multicellular organisms from unicellulars is the premier example of the integration of lower level units into a new higher level. This transition has occurred multiple times in all the major groups of life.

The transition to a new higher-level individual is driven by *cooperation* among lower-level individuals. Only cooperation trades fitness from the lower level (its costs) to the higher level (the benefits of cooperation for the group) (Table 1). Because cooperation exports fitness from lower to higher levels, cooperation is central to the emergence of new evolutionary individuals and the

Cell Behavior	Level of Selection	
	Cell	Group (organism)
Defection (<i>D</i>)	(+) replicate faster or survive better	(+) larger (-) less functional
Cooperation (<i>C</i>)	(-) replicate slower or survive worse	(-) smaller (+) more functional

Table 1. Cooperation and conflict among cells within organisms. The positive and negative effects of cell behavior on group size stem from the assumption that growth (cell division within groups) is indeterminate and that the sizes of adult groups vary depending upon composition of cells. The notation +/- means positive or negative effects on fitness at the cell or organism level.

evolution of increased complexity. We believe this to be the case, even if the groups initially form via antagonistic interactions, as may have been the case during the origin of the eukaryotic cell (e.g., Maynard Smith & Szathmary 1995; Michod & Nedelcu 2002).

The flip side of cooperation is defection and selfishness leading to *conflict* among lower-level individuals in terms of their effects at the cell and cell-group levels; such conflicts must be mediated for *heritable variation in fitness* to increase at the cell-group level (Michod, 1999). We define a *conflict mediator* as a feature of the higher level (the group) that restricts the opportunity for fitness variation at the lower level (cells) and/or enhances the variation in fitness at the higher level (the cell-group or organism). Several examples are given below.

The way in which the conflicts are mediated can influence the potential for further evolution (i.e., *evolvability*) of the newly emerged individual. We expect greater individuality to generally enhance evolvability by increasing the potential for cooperation and restricting within group change. However, evolution can be short sighted, and in *Volvox* it appears that conflict mediation led to a non-replicative soma, that, in turn, restricted the potential for further evolution (Nedelcu & Michod, 2002; Michod, Nedelcu & Roze, 2002).

A MODEL OF THE ORIGIN OF MULTICELLULARITY

Model Life-Cycle

The model we have used to study cooperation and conflict mediation in the evolution of multicellularity is represented in Figure 1. Development of the multicellular

group starts from an offspring or propagule group of N cells. This propagule may be formed in several ways as discussed in the next section (see Figure 2 below).

In the basic model, adult size is not fixed, but depends on rates of cell division and time available for development. The fitness of the cell-group or organism is the expected number of propagules it produces; this depends both on the size of the adult group and on the frequency of mutant cells in the adult.

The many forms of positive interaction among different cell types is represented by a single variable—cooperativity. We assume a single genetic locus controls the way in which cells interact. There are assumed to be two alleles, cooperate *C* and mutant defection *D*. Mutant defecting cells (those carrying the *D* allele) no longer cooperate and this lowers the fitness of the cell-group as in Table 1. The fitness of the cell in terms of its replication and or survival rates during development may be higher (selfish mutants) or lower (uniformly deleterious mutants) than non-mutant cells.

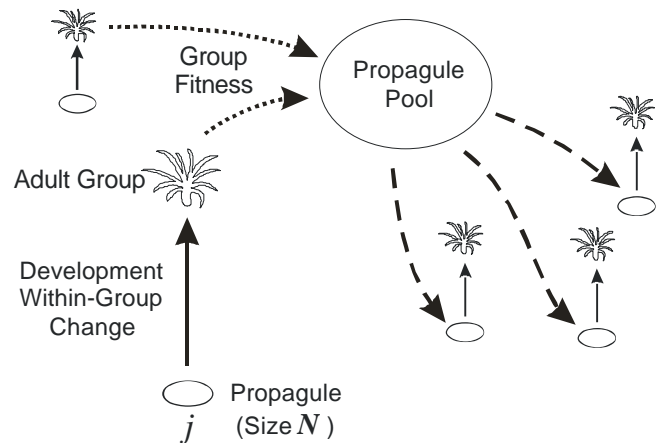
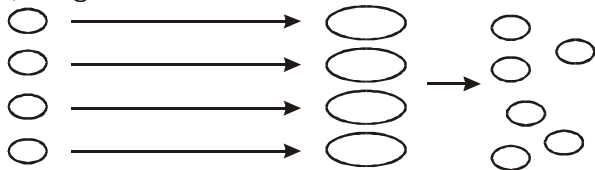


Figure 1. Model life cycle. The subscript j refers to a property of the propagule offspring group, typically its genotype or the number of mutant cells in the propagule; $j = 0, 1, 2, \dots, N$, where N is the total number of cells in the propagule, termed propagule size ($N = 1$ for single-cell reproduction). The fitness of group j is defined as the expected number of propagules produced by the group (dotted lines). Two components of group fitness are considered: the size of the adult group and its functionality. After production, the propagules form offspring groups of the next generation (dashed arrows). Sex may occur during this phase. The additional variables used in the model but not specified here include (i) the number and frequency of mutant and non-mutant cells in the adult cell-group (that is after the propagule develops into an adult); (ii) the change in mutant frequency within the cell-group during development; and (iii) fitness parameters at the cell and group level which stem from the interactions of the cell.

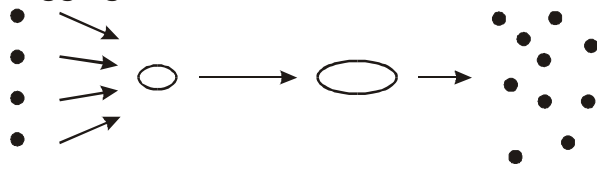
Mode of Propagule Formation

Concerning the formation of the propagule, we have considered three basic modes of reproduction: fragmentation, aggregation and spore or zygote reproduction (with or without sex; Figure 2). In all three cases, the sequence of life cycle events involve the creation of a founding propagule or offspring group of N cells shown in Figure 1. This propagule could be a single cell if $N = 1$, as in the case of spore or zygote reproduction. Indeed the case of spore reproduction can be seen as the limiting case of both fragmentation and aggregation modes (by setting $N = 1$). We have also considered the case of alternating fragmentation and spore reproduction every, say, ν generations (Michod & Roze, 1999). A fundamental difference between aggregation and the other reproductive modes is the opportunity for horizontal transfer of mutants to cell-groups that contain no mutant cells. This is important because aggregation continually reestablishes mixed groups and concomitantly the opportunity for within group selection and conflict between the two levels of selection.

(a) Fragmentation



(b) Aggregation



(c) Zygote or Spore

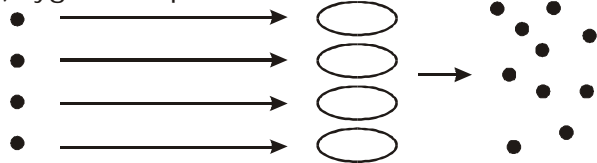


Figure 2. Modes of Propagule Formation. See Figure 1. Small solid circles indicate single cells. Hollow ellipses indicate groups of cells. Small and large groups are shown. The small ellipses (of size N in the model) correspond to offspring propagule cell-groups before cell division and development. The large ellipses correspond to adult groups. Under fragmentation (panel (a)), small offspring groups grow into larger adult cell-groups, which produce offspring groups of the next generation. Under aggregation (panel (b)), single cells aggregate to form an offspring group which grows into an adult group which produce single cells of the next generation. Under zygote or spore

reproduction (panel ((c)), single cells divide and grow into adult cell-groups which produce single cells of the next generation. If there is sex, fusion among single cells may occur in (c) prior to development into the multicellular form. Size of propagule (number of cells in propagule) is N .

Propagule size, N , influences fitness in several ways. First, propagule size affects the within and between group variance and opportunity for selection at the two levels, that is it affects the opportunity for conflict. Smaller N may be seen as a conflict mediator, because smaller N increases the between group variance and decreases the within group variance. Second, propagule size has direct effects on fitness, because smaller N increases the number of possible fragments, but decreases adult size. As discussed below, we have found that the direct effects of propagule size dominate the indirect effects in the evolution of reproductive mode, except when some mutations are selfish. When some mutations are selfish, the opportunity for selection at the two levels becomes the critical factor affecting the evolution of N .

Within Organism Change

As cells proliferate during the course of development, mutations occur leading to loss of cell function and cooperativity among cells. The mutants have a deleterious effect on the fitness of the group, while at the cell level, mutant cells may replicate slower (uniformly deleterious mutants) or faster (selfish cancer-like mutants) than non-mutant cells. A simple branching model of mutation from C to D and cellular selection has been considered (Michod, 1997). The mutation model allows the calculation of the expected number and frequency of mutant cells at the adult stage, and these variables are included in the recurrence equations for gene frequency change. Because of recurrent mutation from C to D , a mutation selection balance is achieved at the C/D locus. This balance takes into account selection at the cell and cell-group levels. A mathematical description of the mutation selection model is given elsewhere (Michod, 1997; Michod, 1999; Roze & Michod, 2001).

Population Genetic Analysis

To study the evolution of conflict mediators, we employ a standard two locus population genetic framework using genetic modifiers. As already discussed, the first locus controls cell behavior, that is, whether cells cooperate or not in their interactions with other cells. The recurrence equation for change in gene frequency at this first locus has been analyzed elsewhere in terms of the levels of cooperation and fitness variation and heritability maintained in the system (Michod, 1997; Michod, 1999).

The level of cooperation among cells and fitness heritability at the cell-group level depends on a variety of assumptions about development, mutation, and selection within the cell-group. So as to study the consequences of development for the emergence of fitness heritability at the higher level, a second modifier locus is considered that changes these assumptions. For example, the modifier locus may create a germ line, allow for cell policing, change the propagule size, change the way in which cells are sampled to put in the propagule, or the modifier may limit the size of the group. Virtually any aspect of the development of the groups may be studied in this way to see if it serves to mediate conflict in favor of the multicellular group. The resulting two locus population genetic model is analyzed using standard techniques.

The transition to multicellular individuals involves two general steps. First, cooperation must increase among cells in the group. Without cooperation the cells are independently evolving units. However, the increase of cooperation within the group is accompanied by an increase in the level of within group change and conflict as mutation and selection among cells leads to defection and a loss of cooperation. Organisms are more than cooperating groups of related cells. The second general step is the evolution of modifier genes that regulate this within group conflict. Only after the evolution of modifiers of within group conflict, do we refer to the group of cooperating cells as an “individual.” Because then the group possesses higher level functions, conflict mediators, that protect its integrity.

One way of using the model to study the evolution of multicellular individuals is to investigate the model’s equilibrium structure. The equilibria of the system with no linkage disequilibrium are verbally described in Table 2. The evolution of cooperation in multicellular groups corresponds to the transition from equilibrium 1 to 3. The evolution of individuality supported by the spread of

conflict mediators corresponds to a transition from equilibrium 3 to equilibrium 4. The question of the transition to individuality, then, boils down to the conditions for a transition from equilibrium 3 to equilibrium 4.

As discussed in more detail elsewhere (Michod & Roze, 1999), conflict mediators increase by virtue of being associated with the more fit genotype and by increasing the heritability of fitness of that type. For example, at equilibrium 3 in Table 2, cooperating zygotes are more fit than defecting zygotes; the cooperating groups must be more fit, because for equilibrium 3 to be stable, the fitness of groups with cooperators must compensate for directional mutation towards defection (from *C* to *D*). The modifiers increase the heritability of fitness of the cooperating type and hitchhike along with these more fit chromosomes. They increase the heritability of fitness of the more fit type by decreasing the within-group change created by deleterious mutation.

The evolution of conflict mediators—functions that protect the integrity of the organism—are not possible, if there is no conflict among the cells in the first place. It is conflict itself (the mutation selection balance at equilibrium 3 and the conflict between levels of selection) which sets the stage for a transition between equilibrium 3 and 4 and the evolution of individuality.

CONFLICT MEDIATION

Kinds of Conflict Mediation

We now consider briefly the kinds of conflict mediators we have studied to date using the models discussed in the last section. As already mentioned, we define a conflict mediator as a feature of the cell-group that restricts the opportunity for fitness variation at the lower level (cells) and/or enhances the variation in fitness at the higher level (the cell-group or organism). Accordingly, one can think

Eq.	Description of Loci	Interpretation of Equilibrium (Eq.)
1	No cooperation; no modifier	<u>Single cells, no organism</u>
2	No cooperation; modifier fixed	Not of biological interest, never stable
3	Polymorphic for cooperation & defection; no modifier	<u>Group of cooperating cells</u> : no higher level functions
4	Polymorphic for cooperation & defection; modifier fixed	<u>Individual organism</u> : integrated group of cooperating cells with higher level conflict mediation function

Table 2. Equilibria for two locus modifier model without linkage disequilibrium. The first locus controls cell behavior with two alleles cooperate, *C*, and defect, *D*. Recurrent mutation from *C* to *D* occurs during development. The second locus modifies aspects of development, group formation or policing of mutant cells. The first stage in an evolutionary transition involves the increase of cooperation, the transition from Eq. 1 to Eq. 3. The second stage of an evolutionary transition involves the evolution of conflict mediation, the transition from Eq. 3 to Eq. 4. The effect of linkage disequilibrium and a mathematical description of the equilibria and eigenvalues are given elsewhere (Michod & Roze, 1997; Michod, 1999)

of two general classes of conflict mediators, those that restrict within group change and those that increase the variation in fitness between groups, although both have the effect of increasing the heritability of fitness at the group level. It should be recognized that we focus on conflict mediation among cells, and that we do not cover conflict at lower levels, such as among genes, chromosomes and organelles. Conflict mediators that operate at these lower levels are also important to the origin of multicellularity and are discussed elsewhere (Lachmann, Blackstone, Haig, Kowald, Michod, Szathmáry, Werren & Wolpert, 2003).

Propagule size

Multicellularity presumably evolved because of advantages for cells of group living. However, most multicellular organisms begin their life-cycle as a single cell. If group living is so advantageous, why going back to a unicellular stage at the start of each generation? A common hypothesis is that the unicellular bottleneck acts as a conflict mediator, by increasing the kinship among cells in the organism, thereby aligning the interests of cells with the interest of the organism (Bell & Koufopanou, 1991; Maynard Smith & Szathmáry, 1995; Grosberg & Strathmann, 1998). Smaller propagule size increases between group variation; however, propagule size has direct effects on the adult group size, in addition to its effects on conflict mediation. All things being equal, smaller propagules produce smaller adults. For this reason, we have studied the evolution of propagule size in simple cell colonies in the context of both selective factors: the direct effects on adult organism size and the more indirect effects on conflict mediation through the opportunity for selection on mutations at the cell and cell-group levels (Michod & Roze, 2000; Roze & Michod, 2001). Our results show that the evolution of propagule size is determined primarily by its direct effects on group size, except when mutations are selfish. So long as some mutations are selfish, smaller propagule size may be selected, including single cell reproduction, even though smaller propagule size has a direct fitness cost by virtue of producing smaller organisms (all other factors being equal).

Germ and Soma

By developing cell types specialized at vegetative and reproductive functions, the evolutionary opportunities of the majority of somatic cells are limited, because genes in somatic cells may spread in the population only if they cooperate with other genes in other cells, thereby doing something useful for the cell-group or organism. There are four basic issues concerning the reproductively specialized germ cells: (i) how many cells are selected to form the

propagule for the next generation, (ii) the way in which these cells are sampled (two extremes would be cells are selected randomly from all cells in the adult, or they are selected from cells that are descendents of a single cell in the adult), (iii) the time in development at which these cells are selected, and (iv) the number of cell divisions between the propagules of two successive generations. Although these issues range on a continuum, often, the term “germ line” is used for the special case in which a single cell (the spore or egg) is chosen from a distinct cell lineage set aside early in development. It is also often assumed there are fewer cell divisions in the “germ line” than in the soma. Using a conflict mediator model, we have specifically modeled the selective forces acting on the time of sequestration, the number of cells sequestered, and the number of cell divisions in the germ line (Michod, Nedelcu & Roze, 2002).

When discussing the role of reproductive specialization as a conflict mediator, one must remember that other factors, such as division of labor, may have been important in the evolution of germ and soma. Specialization of cell types into reproductive and vegetative functions may do both. For example, in the *Volvocales*, the soma likely evolved to lower the survival costs (due to compromised motility) of reproducing increasingly large groups (Koufopanou, 1994; Solari, Nedelcu & Michod, 2003; Michod & Nedelcu, 2003b; Solari, Nedelcu & Michod, 2004). Still, the time of sequestration and number of cell divisions may be adjusted to reduce the opportunity for mutation (Michod, Nedelcu & Roze, 2002).

Mutation Rate

The vast majority of mutations are disadvantageous and so our models of germ line sequestration considered mainly deleterious mutation, of either the uniformly deleterious or selfish varieties. Modifiers that lower the mutation rate are always selected for in our models because they reduce the opportunity for selfish mutations which create conflict between the levels of selection.

Policing

Another means of reducing conflict among cells is for the organism to actively police and regulate the benefits of defection. How might organisms police the selfish tendencies of cells? The immune system and programmed cell death are two examples. To model self-policing, we let the modifier allele affect the parameters describing within and between organism selection and the interaction among cells. Within organism selection is still assumed to result from differences in replication rate, not cell survival. Cooperating cells in policing organisms spend time and energy monitoring cells and reducing the

advantages of defection at a cost to the organism. Explicit analyses are given elsewhere of immune system policing (Michod, 1996; Michod, 1999; Michod & Nedelcu, 2003b) and programmed cell death (Michod, 2003; Michod & Nedelcu, 2003a).

Determinant Growth

In our model, growth of the cell-group was assumed to be indeterminate, and many factors influenced the number of cells in the adult organism. The main factors influencing the size of the adult were the replication and death rates of the cooperative and defecting cells, along with the time available for development. Mutant defecting cells are assumed to replicate faster and so produce larger, though less functional, adults. Because organism fitness is assumed to depend upon the size of the adult, in addition to the level of cooperation, there is an advantage of defection at the organism level resulting from the organism's larger size, in addition to its advantage at the cell level (recall defecting cells replicate faster). One way of reducing the temptation of defection, that is conflict, is to control adult size, thereby removing the advantage of defection (cost of cooperation) at the organism level. Even if adult size is fixed, defecting cells still have a selection advantage within organisms; fixing adult size only removes the positive effect of defection at the organism level of defection.

Conflict Mediation and Fitness

Figure 3 considers the evolution of a germ line modifier and shows how the average heritable fitness of the group increases, while the average fitness of the cells decreases, after modifier evolves. Both these effects increase as the mutation rate increases. When cooperation is beneficial at both levels, mutations are deleterious at both levels, and one may think that there is no conflict between the two levels of selection. However, as the modifiers evolve during the transition to multicellularity, fitness at the group level increases more than at the cell level as shown in Figure 3b.

Why do groups fare better than cells after the evolution of conflict modifiers that reduce the effective mutation rate? Modifiers evolve in this case by virtue of increasing the heritability of fitness in the more-fit non-mutant (cooperative) subpopulation (Michod & Roze, 1999). When cooperation is beneficial at both levels, on average both cells and cell groups are fitter after the transition, as the modifier decreases the effective mutation rate and mutation is deleterious at both levels. However, cell groups benefit twice from the lower mutation rate, because of their much larger size and enhanced functionality. To put the matter another way, when cooperation is beneficial at both levels, cell fitness may

not be directly increased by mutation; however relative to cell groups, cell fitness is increased, because groups are harmed more by mutation than are cells.

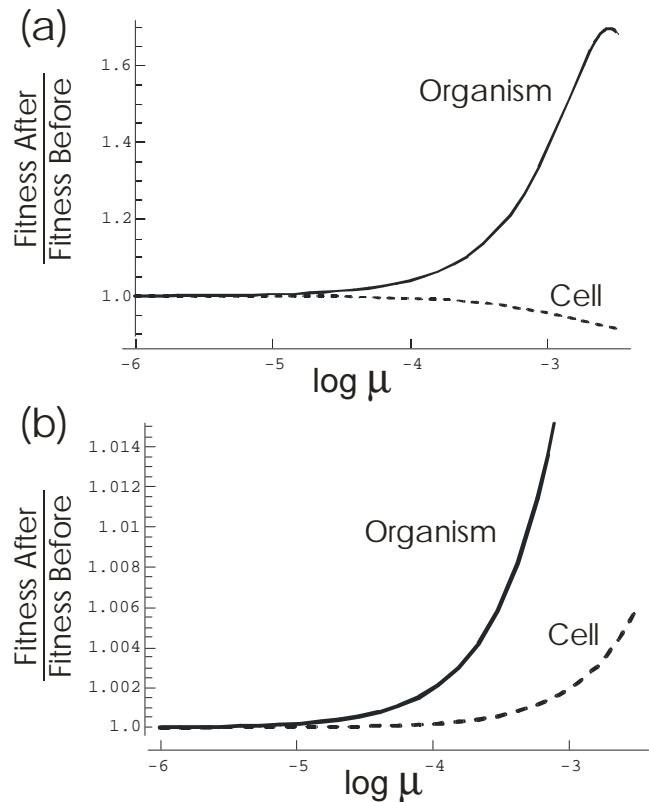


Figure 3. Effect of evolutionary transition on the fitnesses of the lower and higher levels. The effect of an evolutionary transition on the heritability of fitness at the group and the cell level is shown as a function of the deleterious mutation rate, μ , for the case of (a) cell-cell altruism (selfish mutations) and (b) cell synergism (uniformly deleterious mutations). In both cases, the basic effect of modifier evolution is to increase the relative heritability of fitness at the group level compared to the cell level. Calculation of average organism fitness and cell fitness and construction of the figure is explained in Section 6 of Michod and Roze (1999).

Sex and Complexity

The effects of sex on fitness variation and heritability at the group level are studied in detail in the Appendix of Michod (1999) where it is shown that sex affects the level of conflict and variation within the multicellular organism in profound ways (also see Michod (1997)). Sex helps diploids maintain higher heritability of fitness, under more challenging conditions especially when there is great opportunity for within organism variation and selection. With sex, as the mutation rate increases, and concomitantly, the amount of within organism change, more of the variance in fitness is heritable. Sex allows the

integration of the genotypic covariances in a way not possible in asexual populations.

The increase in complexity during the evolution of multicellularity required new gene functions and an increasing genome size, which led to an increase in the deleterious mutation rate. It is often noticed that diploidy helps multicellular organisms tolerate this increase in mutation rate by masking recessive or nearly recessive deleterious mutations. However, once a diploid species reaches its own mutation selection balance equilibrium, the mutation load actually increases beyond what it was under haploidy (Haldane, 1937; Hopf, Michod & Sanderson, 1988). There must be another factor that allows complex multicellular diploids to tolerate a high mutation rate and genetic error. This other factor may be sex. Sex helps cope with genetic error in a variety of ways: by masking deleterious recessive mutations (Bernstein, Byerly, Hopf & Michod, 1985), by avoiding Muller's ratchet (Muller, 1932), by removing deleterious mutations from the population (Kondrashov, 1988), and through recombinational repair of DNA damage (Bernstein et al., 1985). To these we may add how sex maintains a higher heritability of fitness in the face of within organism change resulting from somatic mutation.

As the mutation rate increases in sexual diploid organisms, the regression of fitness on zygote gene frequency actually increases (see Figure 9-2 of Michod (1999)). In other words, as the mutation rate increases, and along with it the amount of within organism change, more of the variance in fitness in sexual diploids is heritable, that is explained by the alleles carried in the zygote. How can this be? The greater mutation rate must result in greater levels of within organism change. At equilibrium this within organism change must be balanced by a larger covariance of fitness with zygote frequency. This is what the Price equation (see, for example, Equation 5-2 of Michod (1999)) says. In haploid and asexual diploid populations, this is accomplished by a greater variance in zygote gene frequency, while in sexual populations this can be accomplished by a greater regression of organism fitness on zygote frequency.

The fitness statistics we have studied (see Appendix of Michod (1999)) apply before and after the transition. It is unclear whether these equilibrium statistics can be extended into the non-equilibrium realm of evolutionary transitions and if the results will hold up under more realistic genetic models. If so, the greater precision in the mapping of cooperative propensity onto group fitness should allow sexual organisms to make the transition from cells to multicellular organisms more easily under more challenging circumstances.

Conclusions

For organisms to emerge from cooperating cell groups, multilevel selection theory predicts that they must acquire adaptations that reduce conflict so as to tilt the balance of selection away from the cell in favor of the multicellular group. There is abundant evidence that organisms are endowed with just such traits. Examples include a separate and sequestered germ line, passing the life cycle through a single cell stage, cell policing (including the immune system and programmed cell death), determinant growth, and a lowered mutation rate. In addition the transition to multicellularity and higher levels of complexity may be facilitated by sex. Sexual reproduction facilitates the maintenance of fitness heritability in the face of within group change driven by high mutation rates.

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