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Cooperation and Conflict Mediation during the Origin of Multicellularity

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ABSTRACT

The basic problem in an evolutionary transition 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, its 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

Evolutionary individuals are units of selection and must satisfy Darwin's conditions of heritability and variation in fitness. Darwin's principles apply to different levels in the hierarchy of life, including genes, chromosomes, cells, cells within cells (eukaryotic cell), multicellular organisms, and social groups of organisms (Lewontin 1970). Because of the hierarchical nature of selection I take a multilevel selection approach to the origin of multicellularity and to evolutionary transitions. The multilevel selection approach to evolutionary transitions seeks to understand how a group of preexisting individuals becomes a new evolutionary individual, possessing heritable fitness variation at the group level and protected from within-group change by conflict mediators.

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 16.1). Because cooperation exports fitness from lower to higher levels, cooperation is central to the emergence of new evolutionary individuals and the evolution of increased complexity. I 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 and Szathmáry 1995; Michod and Nedelcu 2003a).

The flip side of cooperation is defection and selfishness leading to conflict among lower-level individuals between 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).

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

Table 16.1 Cooperation and conflict among cells within organisms. The effects on size assume growth is indeterminate and that the sizes of adults vary depending upon composition of cells. The notation +/- means positive or negative effects on fitness at the cell or organism level.

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

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 nonreplicative soma that, in turn, restricted the potential for further evolution (Nedelcu and Michod 2003; Michod et al. 2002).

A MODEL OF THE ORIGIN OF MULTICELLULARITY

Model Life Cycle

The model life cycle we have used to study cooperation and conflict mediation in the evolution of multicellularity is represented in Figure 16.1. 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.

In the basic model, adult size is not fixed but rather depends on rates of cell division and time available for development (however, for consideration of fixed size, see section below on Determinant Growth). The fitness of the cell group or organism is the expected number of propagules it produces; this depends both on the size of the organism and on the frequency of mutant cells in the adult.

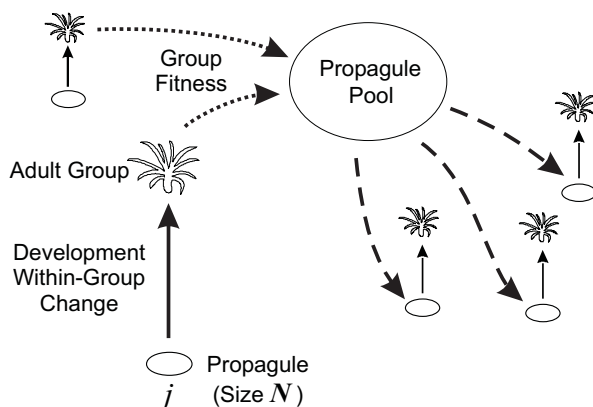


Figure 16.1 Model life cycle. The subscript j refers to a property of the propagule offspring group, typically its genotype or the number of mutant cells in the propagule; $j = 0, 1, 2, \dots, N$, where N is the total number of cells in the propagule, termed propagule size ($N = 1$ for single-cell reproduction). 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. The additional variables used in the model, but not specified here, include (a) the number and frequency of mutant and nonmutant cells in the adult cell group (that is after the propagule develops into an adult); (b) the change in mutant frequency within the cell group during development; and (c) fitness parameters at the cell and group level which stem from the interactions of the cell. For an application of the model to the case of the evolution of programmed cell death, see Table 16.3 below.

The complexity of the interactions 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-defecting cells D . Mutant-defecting cells (those carrying the D allele) no longer cooperate and this lowers the fitness of the cell group, as in Table 16.1. 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 nonmutant cells.

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 16.2). 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 16.1. 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, v generations (Michod and 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.

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 find 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

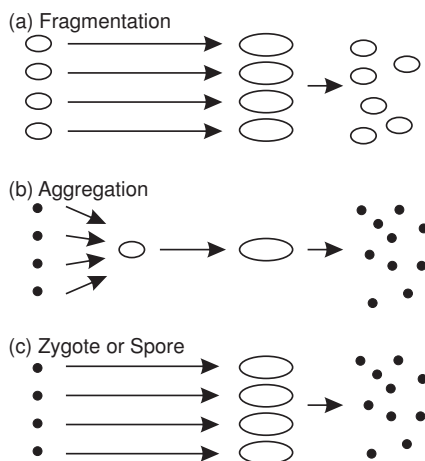


Figure 16.2 Modes of propagule formation (see also Figure 16.1). Small solid circles indicate single cells. Hollow ellipses indicate groups of cells. Small and large groups are shown. The small ellipses (of size N in the model) correspond to offspring propagule cell groups before cell division and development. The large ellipses correspond to adult groups. Under fragmentation (a), small offspring groups grow into larger adult cell groups, which produce offspring groups of the next generation. Under aggregation (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 (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.

mutants) than nonmutant cells. A simple branching model of mutation from C to D and cellular selection has been considered, which extends previous work (Otto and Orive 1995) to include survival selection among cells in addition to differences in cell replication rate (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, 1999; Roze and 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. Thus, 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 described in Table 16.2. 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 in Table 16.2.

As discussed in more detail elsewhere (Michod and 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 16.2, 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 toward 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. Conflict itself (the mutation selection balance at equilibrium 3 and the

Table 16.2 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 and Roze 1997; Michod 1999).

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

conflict between levels of selection) sets the stage for a transition between equilibrium 3 and 4 and the evolution of individuality.

CONFLICT MEDIATION

Kinds of Conflict Mediation

Let us consider briefly the kinds of conflict mediators 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 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 not on conflict at lower levels (e.g., among genes, chromosomes, and organelles). Conflict mediators that operate at these lower levels are also important to the origin of multicellularity and are discussed by Lachmann et al. (this volume).

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: (a) how many cells are selected to form the propagule for the next generation, (b) the way in which these cells are sampled (two extremes would be cells selected randomly from all cells in the adult or selected from cells that are descendents of a single cell in the adult), (c) the time in development at which these cells are selected, and (d) the number of cell divisions between the propagules of two successive generations. Although these issues range on a continuum, the term “germ line” is often 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.

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. Nevertheless, specialization of cell types into reproductive and vegetative functions may still act to reduce conflict. 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; Michod and Nedelcu 2003b). Even in this case, the time of sequestration and number of cell divisions may be adjusted to reduce the opportunity for mutation (Michod et al. 2002).

Propagule Size

Multicellularity presumably evolved because of advantages for cells of group living (see Lachmann et al., this volume). However, most multicellular organisms begin their life cycle as a single cell. If group living is so advantageous, why return 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 kinship among cells in the organism, thereby aligning the interests of cells with the interest of the organism (Bell and Koufopanou 1991; Maynard Smith and Szathmáry 1995; Grosberg and Strathmann 1998). Smaller propagule size does increase 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 and Roze 2000; Roze and Michod 2001). Our results show that 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.

Time of Sequestration and Number of Cell Divisions in Germ Line

In our initial studies of the evolution of a germ line, we assumed for simplicity that the germ line was sequestered as a *single* cell set aside during the *first* cell division (Michod 1996; Michod and Roze 1997, 1999). However, most organisms depart from this ideal, and sequester cells later in development. For example, in the green alga, *V. carteri*, the precursors of the germ line are formed after five cell divisions, but the germ line is sequestered only after the ninth cell division. For this reason 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 et al. 2002). Our results depend upon how the cost of germline sequestration is interpreted. We may interpret the cost of the germ line as stemming either from the *new* germ cells or the *missing* somatic-like cells (by that we mean, cells no longer available for somatic function). In the case where the germline cost is assumed to be proportional to the new germ cells, it is easier for a germ line to evolve (“easier” in the sense that the conditions on the parameters in the model are more relaxed) the earlier the germ line is sequestered, the lower the number of times it divides, and the fewer number of cells that are sampled. This is because there are only advantages to early segregation and low replication (in terms of a lower effective deleterious mutation rate resulting from the fewer number of cell divisions), and the cost of the germ line is smaller the fewer cells that are sampled. Organisms following this model should form a germ line by sequestering a single, nondividing cell during the first cell division.

What about when the cost of the germ line depends upon the number of missing cells unavailable for vegetative (somatic) function? The missing cells are those that would have been formed by the cells sequestered to form the germ cells. In this case, there is a cost to early sequestration of the germ line in terms of more missing somatic cells, and thus there is an intermediate optimum time for sequestration. Early sequestration is better in terms of coping with the threat of deleterious mutation; however, there is a greater penalty to pay in terms of missing cells unavailable for somatic function.

Mutation Rate

The vast majority of mutations are disadvantageous and therefore our models of germline sequestration considered mainly deleterious mutations, 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.

Maynard Smith and Szathmary (1995) suggest that germline cells may enjoy a lower mutation rate but do not offer a reason why. Bell (1985) interpreted the evolution of germ cells in the Volvocales as an outcome of specialization in metabolism and gamete production to maintain high intrinsic rates of increase while algae colonies got larger in size (see also Maynard Smith and Szathmary 1995, pp. 211–213). I think there may be a connection between these two views.

As metabolic rates increase, so do levels of DNA damage. Metabolism produces oxidative products that damage DNA and lead to mutation. It is well known that the highly reactive oxidative by-products of metabolism (e.g., the superoxide radical O_2^- , and the hydroxyl radical $\bullet OH$ produced from hydrogen peroxide H_2O_2) damage DNA by chemically modifying the nucleotide bases or by inserting physical cross-links between the two strands of a double helix, or by breaking both strands of the DNA duplex altogether. Deleterious effects of DNA damage make it advantageous to protect a group of cells from the effects of metabolism, thereby lowering the mutation rate within the protected cell lineage.

This protected cell lineage — the germ line — may then specialize in passing on the organism’s genes to the next generation in a relatively error-free state. Other features of life can be understood as adaptations to protect DNA from deleterious effects of metabolism and genetic error (Michod 1995): keeping DNA in the nucleus protects the DNA from energy-intensive interactions in the cytoplasm, nurse cells provision the egg so as to protect DNA in the egg, sex serves to repair genetic damage effectively while masking the deleterious effects of mutation. The germ line may serve a similar function of avoiding damage and mutation; by sequestering the next generation’s genes in a specialized cell lineage, these genes are protected from the damaging effects of metabolism in the soma.

According to Bell (1985), the differentiation between the germ and the soma in the Volvocales results from increasing colony size, with true germ soma differentiation occurring only when colonies reach about 10^3 cells as in the *Volvox* section *Meriliosphaera*. Assuming no cell death, this colony size would require a development time of approximately $t = 10$ in our model (in reality, because of cell death, larger t with more risks of within-colony variation would be needed to achieve the same colony size). Although Bell interpreted the dependence of the evolution of the germ line on colony size as an outcome of reproductive specialization driven by resource and energy considerations, this relation is also explained by the need for regulation of within-colony change (see panel F of Figure 6.1 in Michod 1999).

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 thus 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 selection.

Jie Li and I have considered an extension of the discrete generation model introduced above, in which a constant adult size is attained for all groups by assuming that the different kinds of zygotes develop for different periods of time (Li 1998, unpublished). For example, we allow *C* zygotes to develop for a longer period of time than *D* zygotes, so that both have the same number of cells in the adult stage (we maintain the assumption used here that *C* cells divide more slowly than *D* cells). We further assume there is a fixed time for reproduction, so that *D* zygotes reproduce for a longer period of time, since they reach adult size quicker. Because of the exponential nature of cell growth, only small differences in development time are needed to attain a fixed adult size. Consequently, there is little difference between organisms in the time available for reproduction. We have not yet considered a model with overlapping generations, although this is clearly in need of study.

Our results indicate that determinate growth acts as a conflict mediator. Constant adult size makes it much easier for cooperation to increase, and this effect is more pronounced for smaller mutation rates. In addition, much greater levels of harmony and cooperation are maintained within the organism if adult size is regulated. Cell death may have important effects with regard to organism size. Cell death increases the number of cell divisions required to reach a given adult size, and this has the additional consequence of increasing the opportunity for within-organism change and variation.

Policing

Another means of reducing conflict among cells is for the organism to actively police and regulate the benefits of defection (Boyd and Richerson 1992; Frank 1995). 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.

An explicit analysis is given elsewhere of immune system policing (Michod 1996, 1999; Michod and Nedelcu 2003b). In general, self-policing increases, if the cost of policing is not high.

As an explicit illustration of our study of conflict mediation and self-policing in the evolution of multicellularity, we consider the evolution of programmed cell death (PCD). PCD, sometimes termed apoptosis, is an evolutionarily conserved form of cell suicide that enables metazoans to regulate cell numbers and control the spread of cancerous cells that threaten the organism. It is best studied in *Caenorhabditis elegans* and mammals; however, similar traits have also been described in unicellular organisms. Presumably, in unicellular organisms, PCD is a form of kin-selected altruism, although there is little direct evidence of this.

We now illustrate how PCD may be viewed as a conflict mediator using our theory. The definitions of additional terms are given in Table 16.3. A PCD modifier lowers the rate of division (or survival) of the mutated cell (parameter pcd). We assume this occurs at some cost, δ , to the cell group, or organism. If there were no costs for the modifier, the modifier would always increase so long as it was introduced in a population in which cooperation was present (the role of cooperation is discussed further below).

In Figure 16.3 we report results for the evolution of PCD modifier alleles, assuming sexual reproduction ($N = 1$ with sex) and a single class of mutant cells D with fixed effect b (the replication rate of mutant cells without the PCD modifier allele; the replication rate of nonmutant cells is unity). Cells with the modifier allele express the PCD phenotype; mutant cells replicate at rate $pcd \times b$, instead of rate b in nonmodified cells. A perfect PCD phenotype would mean that no mutant cells replicate; in this case we would set $pcd = 0$. Of course, it is unlikely that the first PCD response was perfect, so we consider the entire range of possible values for the PCD phenotype ($0 \leq pcd < 1$). The cost of the PCD phenotype at the organism level is assumed to be δ —the benefit of cooperation is reduced in PCD cells to $\beta - \delta$, instead of β in non-PCD cells ($\beta = 3$ in Figure 16.3).

Table 16.3 Additional terms and variables for programmed cell death (PCD) modifier model.

b	Replication rate of mutant cells (relative to unity for nonmutant cells)
β	Effect of cooperation on group fitness
δ	Cost of PCD modifier to group fitness
pcd	Effect of PCD modifier on replication rate of mutant cells
r	Recombination rate between C/D locus and modifier locus
t	Development time
μ	Mutation rate per cell division
M, m	Alleles at modifier locus; M allele creates PCD phenotype

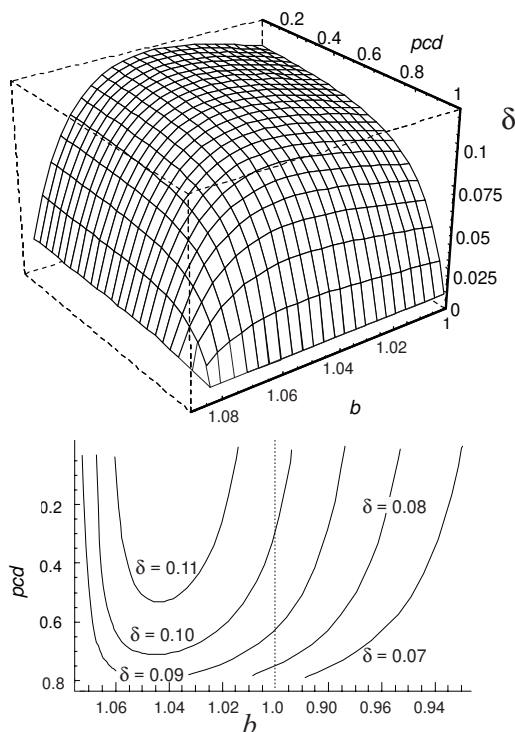


Figure 16.3 Evolution of apoptosis. Bottom panel gives 2D slices through the 3D surface in the top panel. PCD modifiers evolve for parameter values below the 3D surface and above the 2D curves. The parameter b is the replication rate of nonregulated mutants (relative to nonmutants) and pcd is the factor decrease in replication rate of PCD modified cells; modified cells replicate at rate $pcd \times b$. In the bottom panel five curves are plotted for different values of the cost to organisms of the PCD phenotype. Parameter values in the model (for specification of the model details, see Chapters 5 and 6 of Michod [1999]): offspring group size $N = 1$, time for development $t = 20$, benefit of cooperation $\beta = 3$, recombination rate between mutated locus and modifier locus $r = 0.2$, survival is incorporated in replication so $s_C = s_D = 1$, and mutation rate $\mu = 0.003$ (which is a value typical of the genome-wide rate in modern unicells [Drake 1974; Drake 1991]). In the bottom panel selfish mutations lie to the left of the vertical dotted line and uniformly deleterious mutations lie to the right.

An interesting feature of the results shown in Figure 16.3 is that uniformly deleterious mutations (ones that disrupt the functioning of the group and proliferate slower than normal cells, $b < 1$), may also select for PCD modifiers; however, to invade, the modifier requires lower costs of the PCD phenotype to the organisms than is the case for selfish mutations. It is common in the literature on PCD to assume that the risk of selfish mutations has led to the evolution of the PCD phenotype. We see in Figure 16.3 that both uniformly deleterious and selfish mutations can select for PCD. We have also observed that both kinds of

mutations select for the other kinds of modifiers that we have studied, such as germ line and self-policing modifiers.

Why do the curves in Figure 16.3 fall off rapidly as b increases up toward a value of approximately 1.07? As the proliferation advantage of mutants, b , increases, the equilibrium frequency of nonmutant cooperating cells decreases, eventually reaching zero at about 1.07 (when within-group change overpowers between-group selection for cooperation). Without variation at the cell interaction C/D locus, the PCD modifier, M , is disadvantageous, because when the modifier is introduced, the only genotypes are MD and mD (assuming haploidy for explanation purposes; where D is the mutant and M and m are the PCD and non-PCD alleles, respectively, at the modifier locus). Cell groups initiated by PCD cells (MD) end up being smaller than groups initiated by non-PCD cells (mD), because of the lower replication rate (or higher death rate) of PCD cells. However, when cooperating cells are maintained in the population before the PCD modifier is introduced, the significant competition is between groups initiated by CM and Cm cells. The cooperating groups carrying PCD modifiers (initiated by CM) end up being more functional and having fewer mutant cells in the adult stage; the associated fitness advantage can make up for the cost of PCD, δ (in the regions under the curves shown in the figure). The dependence of the evolution of PCD on the maintenance of cooperation reflects the need for a higher-level unit of selection (the cell group, or organism). The PCD modifier increases by virtue of tilting the balance in favor of the cell group, by enhancing its individuality and heritable fitness (Michod 1999).

SEX AND INDIVIDUALITY

Sex and individuality are in constant tension, because sex involves fusion and mixis of genetic elements and, so, naturally threatens the integrity of evolutionary units (see Lachmann et al., this volume). Yet, sex is fundamental to the continued well-being of evolutionary units too. Although sex seems to undermine individuality, sex has been rediscovered as each new level of individuality emerges in the evolutionary process. Sex holds the promise of a better future and a more whole and undamaged individual. Genetic redundancy and repair occur during the sexual cycle and are the key to greater wholeness and well-being for the individual (Michod 1995). Theories for the evolution of sex are discussed in three collections of papers (Stearns 1987; Michod and Levin 1988; Birky 1993).

Sex affects evolutionary transitions in our models in several ways. Sex affects the quantitative conditions for the evolution of conflict mediators: with recombination, it takes longer for the transition to occur (Michod 1999). The modifier increases by virtue of being more often associated with cooperating C alleles in gametes and recombination breaks apart this association. Although recombination can retard the transition between equilibrium 3 and 4 in Table 16.2, I do not see these quantitative differences as presenting any real barriers to the

evolution of conflict modification and evolutionary transitions in sexual progenitors. More important, I think, is the way in which sex organizes variability and heritability of the traits and capacities that affect the fitness of the new emerging unit.

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 emerging organism in profound ways (see also 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 et al. 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 et al. 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 than 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 states (see, e.g., Equation 5-2 of Michod 1999). In haploid and asexual diploid populations, this is accomplished by a greater variance in zygote gene frequency, whereas 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 nonequilibrium 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 sexuals to make the transition from cells to multicellular organisms more easily under additionally challenging circumstances. This result is consistent with the view that the protist ancestor of multicellular life was likely sexual (Maynard Smith and Szathmáry 1995).

CONCLUSIONS

Multilevel selection theory predicts that for organisms to emerge from cooperating cell groups, 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, sexual reproduction facilitates the maintenance of fitness heritability in the face of within-group change driven by high mutation rates.

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REFERENCES

- Bell, G. 1985. The origin and early evolution of germ cells as illustrated by the Volvocales. In: *The Origin and Evolution of Sex*, ed. H.O. Halvorson and A. Monroy, pp. 221–256. New York: A.R. Liss.
- Bell, G., and V. Koufopanou. 1991. The architecture of the life cycle in small organisms. *Phil. Trans. Roy. Soc. Lond. B* **332**:81–89.
- Bernstein, H., H.C. Byerly, F. Hopf, and R.E. Michod. 1985. DNA damage, mutation, and the evolution of sex. *Science* **229**:1277–1281.
- Birky, W. 1993. American Genetics Society Symposium for the Evolution of Sex. *Journal of Heredity* **84**.
- Boyd, R., and P.J. Richerson. 1992. Punishment allows the evolution of cooperation (or anything else) in sizable groups. *Ethol. Sociobiol.* **13**:171–195.
- Drake, J.W. 1974. The role of mutation in bacterial evolution. *Symp. Soc. Gen. Microbiol.* **24**:41–58.
- Drake, J.W. 1991. A constant rate of spontaneous mutation in DNA-based microbes. *Proc. Natl. Acad. Sci. USA* **88**:7160–7164.
- Frank, S.A. 1995. Mutual policing and repression of competition in the evolution of cooperative groups. *Nature* **377**:520–522.

- Grosberg, R.K., and R.R. Strathmann. 1998. One cell, two cell, red cell, blue cell, the persistence of a unicellular stage in multicellular life histories. *Trends Ecol. Evol.* **13**:112–116.
- Haldane, J.B.S. 1937. The effect of variation on fitness. *Am. Nat.* **71**:337–349.
- Hopf, F.A., R.E. Michod, and M.J. Sanderson. 1988. The effect of reproductive system on mutation load. *Theoret. Pop. Biol.* **33**:243–265.
- Kondrashov, A.S. 1988. Deleterious mutations and the evolution of sexual reproduction. *Nature* **336**:435–440.
- Koufopanou, V. 1994. The evolution of soma in the Volvocales. *Am. Nat.* **143**:907–931.
- Lewontin, R.C. 1970. The units of selection. *Ann. Rev. Ecol. Syst.* **1**:1–18.
- Maynard Smith, J., and E. Szathmáry. 1995. *The Major Transitions in Evolution*. San Francisco: W.H. Freeman.
- Michod, R.E. 1995. *Eros and Evolution: A Natural Philosophy of Sex*. Reading, MA: Addison-Wesley.
- Michod, R.E. 1996. Cooperation and conflict in the evolution of individuality. II. Conflict mediation. *Proc. Roy. Soc. Lond. B* **263**:813–822.
- Michod, R.E. 1997. Cooperation and conflict in the evolution of individuality. I. Multilevel selection of the organism. *Am. Nat.* **149**:607–645.
- Michod, R.E. 1999. *Darwinian Dynamics: Evolutionary Transitions in Fitness and Individuality*. Princeton, NJ: Princeton Univ. Press.
- Michod, R.E., and B.R. Levin. 1988. *Evolution of Sex: An Examination of Current Ideas*. Sunderland, MA: Sinauer.
- Michod, R.E., and A. Nedelcu. 2003a. Cooperation and conflict in the origins of multicellularity and the eukaryotic cell. In: *Evolution: From Molecules to Ecosystems*, ed. A. Moya and E. Font. Oxford: Oxford Univ. Press, in press.
- Michod, R.E., and A. Nedelcu. 2003b. Individuality during evolutionary transitions. *Integ. Comp. Biol.*, in press.
- Michod, R.E., A. Nedelcu, and D. Roze. 2002. Cooperation and conflict in the evolution of individuality. IV. Conflict mediation and evolvability in *Volvox carteri*. *BioSystems* **2190**:1–20.
- Michod, R.E., and D. Roze. 1997. Transitions in individuality. *Proc. Roy. Soc. Lond. B* **264**:853–857.
- Michod, R.E., and D. Roze. 1999. Cooperation and conflict in the evolution of individuality. III. Transitions in the unit of fitness. In: *Mathematical and Computational Biology: Computational Morphogenesis, Hierarchical Complexity, and Digital Evolution*, ed. C.L. Nehaniv, pp. 47–92. Providence, RI: American Mathematical Society.
- Michod, R.E., and D. Roze. 2000. Some aspects of reproductive mode and the origin of multicellularity. *Selection* **1**:97–109.
- Muller, H.J. 1932. Some genetic aspects of sex. *Am. Nat.* **66**:118–138.
- Nedelcu, A., and R.E. Michod. 2003. Evolvability, modularity, and individuality during the transition to multicellularity in volvoclean green algae. In: *Modularity in Development and Evolution*, ed. G. Schlosser and G. Wagner. Chicago: Univ. of Chicago Press, in press.
- Otto, S.P., and M.E. Orive. 1995. Evolutionary consequences of mutation and selection within an individual. *Genetics* **141**:1173–1187.
- Roze, D., and R.E. Michod. 2001. Mutation load, multilevel selection and the evolution of propagule size during the origin of multicellularity. *Am. Nat.* **158**:638–654.
- Stearns, S.C., ed. 1987. *The Evolution of Sex and Its Consequences*. Basel: Birkhauser.

