Cooperation and Conflict in the Evolution of Individuality. III. Transitions in the Unit of Fitness

Richard E. Michod and Denis Roze

ABSTRACT. The evolution of multicellular organisms is the premier example of the integration of lower levels into a single, higher-level individual or unit of fitness. Explaining the transition from single cells to a multicellular organism is a major challenge for evolutionary theory. We provide an explicit genetic framework for understanding this transition in terms of the increase of cooperation among cells within-groups and the regulation of conflict within the cell group—the emerging organism. Cooperation is the fundamental force leading to new levels of organization and selection. While taking fitness away from lower level units (its costs), cooperation increases the fitness of the new higher level unit (its benefits). In this way, cooperation may create new levels of selection and higher levels of fitness. However, the evolution of cooperation sets the stage for conflict, represented here by the increase of deleterious mutants during development. The evolution of a means to regulate this conflict is the first new function at the organism level. The developmental program evolves so as to reduce the opportunity for conflict among cells. An organism is more than a group of cells related by common descent; to exist organisms require adaptations that regulate conflict within. Otherwise, continued improvement of the organism is frustrated by within-organism variation and change during development. The evolution of modifiers of within-organism change are a necessary prerequisite to the emergence of individuality and the continued well being of the organism. Heritability of fitness and individuality at the new level emerge as a result of the evolution of organismal functions that restrict the opportunity for conflict within and ensure cooperation among cells. Conflict leads—through the evolution of developmental adaptations that reduce it—to greater individuality and harmony for the organism.

1. Introduction

1.1. Fitness. Lewontin once remarked that evolution by natural selection should explain "fitness" [29]. In a recent book [39], one of us has taken the approach that to explain fitness we need to understand three things: (i) how fitness originated in the transition from the non-living to the living, (ii) the role of fitness in the mathematical theory of natural selection, and (iii) how new levels of fitness are created during evolutionary transitions to greater levels of complexity. The present paper is concerned with the last question, and in particular how fitness emerges at the level of the

organism out of a group of independently replicating cells. More generally, we are interested in understanding evolutionary transitions to higher levels of organization and complexity. Although, we primarily address the transition from single cells to multicellular organisms, we believe our results are applicable to other evolutionary transitions, such as the transition from replicating genes to cooperating gene networks, from gene networks to the origin of the first cell, from bacteria cells to eukaryotic cells, and the transition from multicellular organisms to societies.

1.2. A scenario for the origin of multicellular life. To help fix ideas, let us consider a scenario for the initial stages of the transition from unicellular to multicellular life (Figure 1).
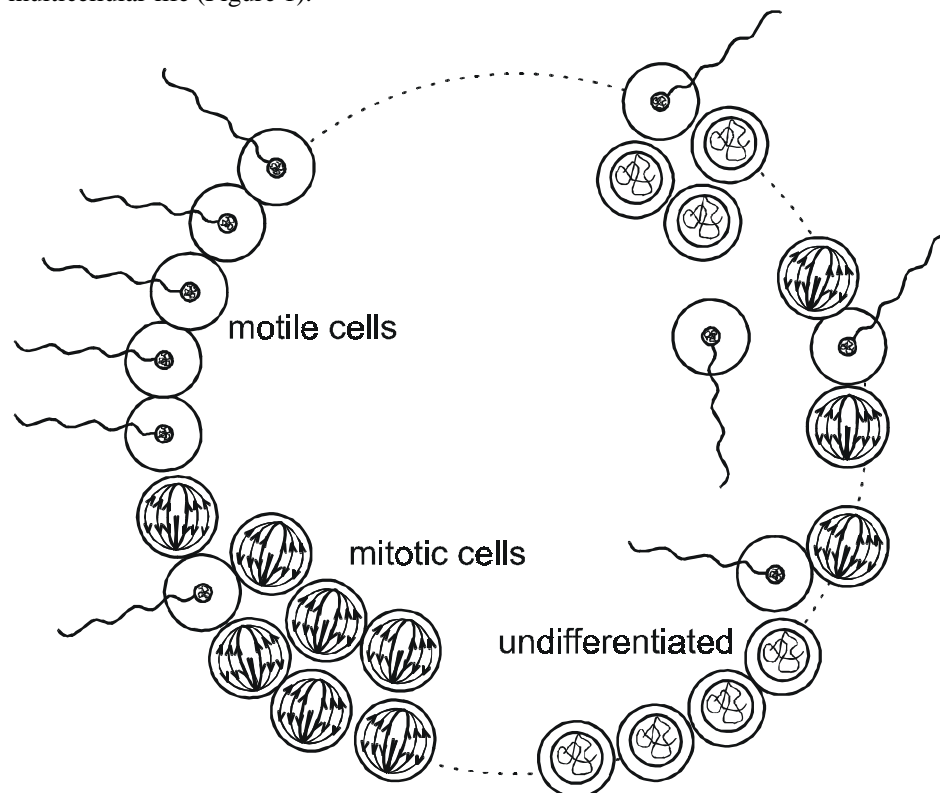

Figure 1. Scenario for the first organisms (groups of cells). Shown in the figure are motile cells with a flagella, non-motile mitotically dividing cells and cells which have yet to differentiate into either motile or mitotic states. Because of the constraint of a single microtubule organizing center per cell, cells cannot be motile and divide at the same time. As explained in the accompanying text, motile cells are an example of cooperating cells and mitotically reproducing cells are an example of defecting, or selfish, cells.

In simple multicellular organisms like Pleodorina in the Volvocales [5] or the sponge Leucosolenia [9], the organism is a hollow sphere with at the top a fixed proportion of cells remaining ciliated and dying at the end of the life cycle; the other cells keeping the capacity to divide. We assume that reproduction and motility are two basic characteristics of the early single celled ancestors to multicellular life, and these single cells were able to differentiate into reproductive and motile states [9,30-32]. Cell

development was likely constrained by a single microtubule organizing center per cell, and, consequently, there would have been a trade off between reproduction and motility, with reproductive cells being unable to develop flagella for motility and motile cells being unable to develop mitotic spindles for cell division [9,30,31]. Single cells would switch between these two states according to environmental conditions. Finally, the many advantages of large size (escape from predators being just one possible advantage [6,19,46,47]) might favor single cells coming together to form cell groups. We imagine the appearance of a new mutation perhaps coding for a cell adhesion molecule or the collar structures that hold cells together in a *Proterospongia* colony [27]. It is at this point that our investigations begin.

If and when single cells began forming groups, the capacity to respond to the appropriate environmental inducer and differentiate into a motile state would be costly to the cell, but beneficial for the group (assuming it was advantageous for groups to be able to move). Because having motile cells is beneficial for the group, but motile cells cannot themselves divide, or divide at a lower rate within the group, the capacity for a cell to become motile is a costly form of cooperation, or altruism. Loss of this capacity is then a form of defection, as staying reproductive all the time would be advantageous at the cell level (favored by within-group selection), but disadvantageous at the group level (disfavored by between cell-group selection). We are lead, according to this scenario (and many others), to consider the fate of cooperation and defection in a multi-level selection setting during the initial phases of the transition from unicellular life to multicellular organisms.

1.3. Cooperation and conflict. New evolutionary units begin as cooperative groups of existing units. Cooperation is the primary creative force in the emergence of a new unit of selection, because it trades fitness at a lower level (its costs) for increased fitness at the group level (its benefits). In this way, cooperation can create new levels of fitness (Table 1). Two issues are central to the creation of a new unit of selection—promoting cooperation among the lower level units in the functioning of the group, while at the same time mitigating the inherent tendency of the lower level units to compete with one another through frequency dependent fitness effects.

Cooperation represents the benefit of group living; groups of existing units can behave in new and useful ways. Frequency dependent interactions among evolutionary units are both a source of novelty for the group

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

Table 1. Effect of cooperation on fitness at cell and organism level. The notation +/− means positive or negative effects on fitness at the cell or organism level.

as well as a threat to its collective well-being. Cooperation is usually costly to the fitness of the individuals involved. Defection (that is, non-cooperative behaviors) may reap the benefits of the cooperative acts of others and spread in the population, thereby destroying the very conditions upon which its spread depended in the first place. As a result of the spread of defection, cooperation is lost and so is any hope for the creation of a new higher level. Certain conditions are required to overcome the inherent limits posed by frequency dependent selection to the emergence of new levels of selection:

kinship, population structure, and conflict mediation. Conflict mediation is the process by which lower level change is modulated in favor of the new emerging unit. The definition or usage of certain fundamental terms and concepts used in this paper are given in Table 2.

Term or Concept	Definition and Usage
Self-replication	The capacity to make copies, so that even mistakes can be copied
Individual	A unit of selection satisfying Darwin's three conditions of variation, heritability and self-replication with mechanisms to modulate lower level change
Fisherian Fitness	Per capita rate of increase of a variant
Cooperation	An interaction that possibly decreases the fitness of the individual while increasing the fitness of the group
Frequency Dependent Selection	When fitness depends upon interactions within a group or population
Conflict	Competition among lower level units of selection leading to defection and a disruption of the functioning of the group
Selfish Mutation	Mutations that are deleterious at the organism level and advantageous at the cell level (as opposed to mutations that are deleterious at both levels)
Conflict Mediation	The process by which the fitnesses of lower level units are aligned with the fitness of the group
Fitness Covariance	The covariance between individual fitness and heritable genetic properties (used to study the emergence of individuality)

Table 2. Definitions and usage of terms and concepts used in the paper.

1.4.   Multi-level selection. A recent commentary on multi-level selection theory observes whether there is anything in biology that can't be explained by individual selection acting on organisms, that requires selection acting on groups [41]. Although rhetorical, this remark reflects a view often taken in biology that most interesting questions can be addressed by viewing organisms as the sole unit of selection. But where do organisms come from? From single cells, of course. And what are multicellular organisms but cooperative groups of cells related by common descent. In this paper, we extend a multi-level selection framework recently developed to study the evolutionary transition from single cells to multicellular organisms [36]. We argue that multi-level selection theory is needed to explain the origin of the organism—that very creation which is supposed to deny the usefulness of multi-level selection in evolutionary biology.

Organisms can be thought of as groups of cooperating cells. Selection among cells could destroy this harmony and threaten the individual integrity of the organism. For the organism to emerge as an individual, or unit of selection, ways must be found of regulating the selfish tendencies of cells while at the same time promoting their cooperative interactions.

During the proliferation of cells throughout the course of development, deleterious mutation can lead to the loss of cooperative cell functions (such as the ability to become motile in the scenario above). We represent cell function in terms of a single cooperative strategy. Because deleterious mutation leads to the loss of cell function, mutation may produce defecting cells from cooperative cells. Mutant cells no longer take time and resources to cooperate with other cells and as a result may

replicate faster or survive better than cooperating cells. Such mutations are disadvantageous at the organism level, but advantageous at the cell level. Deleterious mutation can also produce completely defective cells with no capacity to replicate or survive. Such mutations are disadvantageous at both the cell level and the level of the group. In previous papers, we considered only defecting mutations, however, in the present paper we consider both kinds of mutations. We also extend the multi-level selection framework developed previously [36] by considering other forms of reproduction involving fragmentation in addition to zygote based reproduction, because we wish to understand the origin of the single cell stage which is almost universal in the life cycles of complex organisms. In addition, we have discovered new equilibria for the model at which linkage disequilibrium is positive and at which the population is polymorphic for conflict mediation; for example, cell groups with and without a germ line may coexist.

2.   Multi-level Selection Model

2.1. Overview. The sequence of life cycle events involve the creation (through gamete production or fragmentation or aggregation) of a founding propagule or "offspring" group of cells of size N. These offspring grow and develop into an adult and the adult then produces the offspring of the next generation. An overview of the model life cycle is given in Figure 2. In the case of single cell reproduction considered previously [36,37,39,40], $N = 1$, and sex (fusion and splitting with recombination) may occur among offspring propagules.
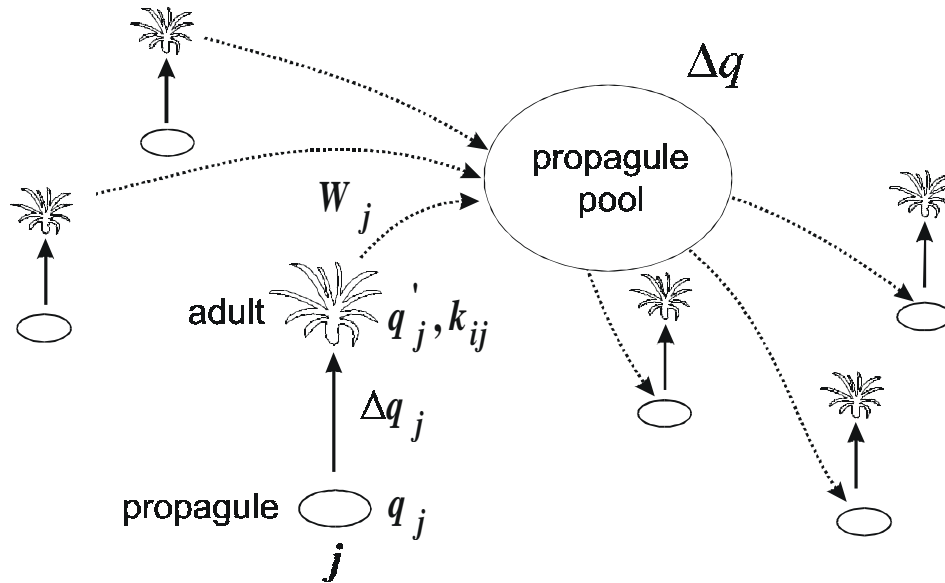

Figure 2. Model life cycle for the origin of organisms. The subscript j refers to the number of *cooperating* cells in a propagule; $j = 0$, 1, 2, ...N, where N is the total number of cells in the offspring propagule group, assumed constant for simplicity. The variable k_{ij} refers to the number of cells of type i (either type C or D) in the adult cell group (that is after development) produced by a propagule of type j. The variables q_j ($q_j = j/N$) and q'_j $\left(q'_j = k_{Cj}/k_j\right)$ refer to the frequency

of cooperation before and after development, respectively, while Δq_j is the change in gene frequency at the C/D locus during development. W_j is the fitness of group j, defined as the expected number of propagules produced by the group, assumed to depend both on size of the adult group after development and its functionality (or level of cooperation among its component cells) represented by parameter β in the models below.

After formation of the offspring, cells proliferate during development to produce the adult form. This proliferation and development is indicated by the vertical arrows in Figure 2. Because of mutation and different rates of replication of different cell types, there will be a change in gene and genotype frequency of cells within-organisms represented by Δq_j in Figure 2. There will also be a change in gene frequency in the population due to differences in fitness between the adult forms. These two components of frequency change, within-organisms and between-organisms give rise to the total change in gene frequency Δq. The number of cooperating and mutant cells of type i in the adult stage of a j offspring is represented by the variables k_{ij} Figure 2. A model to calculate these numbers is given below in Figure 3 and Table 3. The fitness of the adult form, W_j, is the absolute number of offspring produced and this is assumed to depend both upon the number of cells in the adult and how the cells interact. Cooperation among cells increases the fitness of the adult (parameter β in the models below) but non-cooperating cells may replicate faster, survive better, and produce a larger but less functional adult. Organism size is assumed to be indeterminate and to depend on the time available for development as well as rate at which cells divide. Determinate size can also be modeled. The evolution of conflict and cooperation in organisms with a fixed size can be studied by standardizing the frequencies before offspring propagule production much as is done in models of hard and soft selection [52].

2.2. Within-organism change. There are two components of gene frequency change: (i) between-organisms within populations, and (ii) between-cells within-organisms. In this subsection we consider a model of within-organism change stemming from mutation during cell division and selection among cells caused by differences in cell replication or cell survival. The basic variables are defined in Table 3 and explained in Figure 3.

As cells proliferate within the developing organism (development time t), mutations (rate μ) occur leading to loss of tissue function and cooperativity among cells. We consider only mutations that lead to a loss of cooperation (C to D) not its gain (no back mutation from D to C) as this represents a worse case for the evolution of cooperation. This is reasonable for biological reasons, because it is far easier to lose a

μ	mutation rate from C to D per cell division
t	generation time (for development)
c	rate of cell division for cooperating cells
b	effect of mutation on cell replication rate
cb	rate of cell division for mutant cells
$s_C\ s_D$	probability of cell survival for C and D cells, respectively

$$k_{Cj} = j\left[2s_C(1-\mu)\right]^{ct}$$

$$k_{Dj} = j\sum_{x=1}^{ct}2\left[2s_C(1-\mu)\right]^{x-1}\mu s_D\left[2s_D\right]^{b(ct-x)}$$
$$+(N-j)\left[2s_C\right]^{bct}$$

$$k_j = k_{Cj} + k_{Dj}$$

Table 3. Mutation and cellular selection model for haploidy. See also Figure 3 for an explanation of how the numbers of different cell types in the adult stage are calculated. Based on model of [42].

complex trait like cooperativity among cells than it is to gain it.

Within-organism variation after development is represented by the expected number of cells of different types in the adult form—the k_{Cj} and k_{Dj} variables given in Figure 2 and defined in Figure 3 and Table 3. Many aspects of the analysis, the various equilibria and their stability for the different reproductive systems, can be obtained analytically without explicitly specifying values for these variables. However, more quantitative analyses require a specific within-organism mutation selection model. The model of within-organism variation and selection presented in this section is a first attempt at such a model. It has the virtue of being comparatively simple, at least in principle, although it becomes complex computationally. There may be other more realistic mutation selection models that could be used to obtain values for the k_{ij} variables, and the model proposed in the last section was set up with this in mind.

$$\sum_{x=1}^{ct} 2\left[2s_C(1-\mu)\right]^{x-1}\mu s_D\left[2s_D\right]^{b(ct-x)}$$
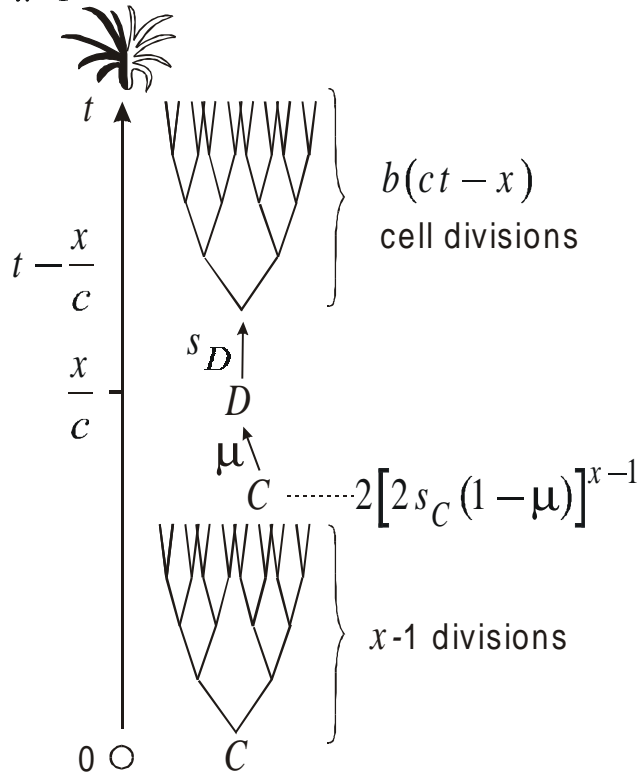

Figure 3. Calculation of the Adult. The figure explains the calculation of the number of defecting cells (D) in the adult stage descended from a single cooperate (C) cell in the offspring propagule (formula given in Table 3). For any single cell generation we assume replication, mutation and then survival. A C cell divides for x-1 divisions and then mutates to D during cell division x. Assume there are N_x cells in generation x. After cell division there are $2N_x$ cells and these survive with probability s_C or s_D, for C and D cells, respectively. The total number of D cells in the adult organism is represented by the color black in the adult form. Consider C cells that have divided, survived and not yet mutated for x-1 divisions and are now in the process of cell division x. The total number of these cells is $2[2s_C(1-\mu)]^{x-1}$. Some of these cells (μ) will mutate for the first time and the resulting mutants will survive to the next cell division with probability s_D and then undergo $b(ct-x)$ more cell divisions. We would like to choose the fitness effect of the mutation at the cell level (b and s_D) from a probability distribution of mutational effects, but for the deterministic treatment given here these mutational effects are fixed parameters of the model and the same for all mutations. The time taken to get x cell divisions is x/c. The time left is $t-x/c$. The number of cell divisions the mutant will undergo is then $cb(t-x/c) = b(ct-x)$. The sum is over all possible cell divisions x. The formulae in the figure simplify to

those published previously, if there is no selection on cell survival, $s_C = s_D = 1$ [38]. This approach gives k_{Cj}, k_{Dj} in Table 3 and Table 4 below.

The mutation model given in Table 3 and Figure 3 generalizes to the diploid case following the arguments given elsewhere for a similar model but one without differences in cell survival [37]; however the details are more complex and the steps involved are not presented here for reasons of space. For example, to study the branching process of mutations within cell lineages which start out as a *CC* cell requires considering four classes of events: (i) *CC* cells that mutate to *CD* during cell division *x* and then mutate to *DD* during cell division *y*, (ii) *CC* cells that mutate to *CD* during cell division *x* and remain *CD* for the rest of development, (iii) *CC* cells that mutate directly to *DD* during cell division *x* and, of course, (iv) *CC* cells that remain *CC* for all of development.

The number of cells in an adult depends on the time available for development, the rate of cell division and cell death. Rates of cell division vary widely among tissue types. In some tissues cells stop dividing (brain, muscle, liver) while in other tissues cells continue to divide throughout life (blood, intestine lining). To help fix ideas we can estimate the number of cells that exist after a given development time, *t*, for cooperating cells as follows. Ignoring mutation, cell death, and the different rates of replication of the different cell types, $t = 40$ would allow 40 cell divisions (assuming *c* = 1) implying around 10^{12} cells in the adult—a number similar in magnitude to the number of cells in an adult human. Including cell death would require a greater number of divisions to get the same number of cells in the adult. Cell death is likely present in most cell lineages. For example, it has been estimated that the number of cell divisions between the zygote and an average human male sperm is around 400 cell divisions [51]. Such a large number of cell divisions is needed to account for cell death. A typical human female egg is separated from the zygote by about 20 cell divisions [51].

2.3. Mutation. Because there are two levels of selection, there are two levels at which to consider the effects of mutation—the cell and the cell-group, or organism. The mutations we consider always lead to the loss of the cooperative function, that is the loss of the benefit β at the group level. This loss may be a pleiotropic consequence of the disruption of other cell functions not directly related to cooperation. In this case, the loss of cooperation may also be deleterious at the cell level, if these functions are important for replication and survival of the cell. We refer to such mutations as uniformly deleterious. According to the relative magnitude of the deleterious effects at the two levels (β and *b* in the simplest model), uniformly deleterious mutations are best eliminated by within-organism (between cell) or between-organism selection (see Figure 5 below and accompanying discussion).

However, the loss of cooperation may not be a pleiotropic consequence of the loss of cell functions related to cell fitness. Indeed, in Figure 1, mutant cells that are defective in the capacity to differentiate into the motile state may replicate faster or survive better than cooperating cells (because of the constraint of a single microtubule organizing center per cell). By freeing energy or time spent on cooperation, the loss of cooperation may lead to an increase of fitness at the cell level. Such selfish mutations are deleterious at the organism level but advantageous at the cell level. Selfish mutations are only eliminated by between-organism selection, because they are favored by within-organism selection at the cell level.

The model is constructed to study both selfish and uniformly deleterious mutations by considering the effects of mutation on the replication of cells within-organisms in terms of the parameters c and b in Figure 3 and Table 3 (or s_C and s_D if effects on cell survival are to be considered). For purposes of discussion, we consider only effects of deleterious mutation on the replication rate of the cell (parameters c and b), but effects of mutation on survival rates can also be studied (parameters s_C and s_D, in Figure 3 and Table 3). The case of altruism given in Figure 1 is modeled by assuming that mutation produces selfish cells that replicate faster than cooperating cells ($c = 1$, $b > 1$). The case of complete altruism occurs when cooperating cells are not able to survive or replicate at all, but here we focus on intermediate cases of altruism. In addition to the case of selfish mutations, we study uniformly deleterious mutations by considering ranges of the b parameter from zero (completely defective mutants) to unity (mutant cells replicate at the same rate as cooperating cells) ($c = 1$, $0 \leq b \leq 1$).

The deleterious mutation rate in the model pertains to the loss of cooperation and perhaps other functions that affect cell replication and/or survival. Although the model considers a single locus affecting cell behavior, there are likely to be many loci that affect tissue function and cooperativity among cells. For this reason, we like to think of this single locus as representing the cumulative effect of deleterious mutation at all loci leading to loss of cooperation. This is not realistic modeling but provides a better understanding of what we would like the model to represent. The mutation rate parameter in the model does not pertain to cells in modern multicellular organisms but rather to cells on the brink of coloniality during the period in which multicellular life first arose. Indeed, an earlier version of the model predicts that one of the ways of coping with within-organism change is to select for modifiers that lower the mutation rate [36,40]. Modifiers are genes whose effect is to change the value of one of the parameters in the model, such as the mutation rate, or in Section 3 the number of cells in a propagule group, or in Section 4 the parameters of within-organism change. Consequently, the mutation rate in modern organisms likely results from the very processes being modeled here. For these reasons, we consider mutation rates that are high when viewed as pertaining to a single locus in modern organisms.

Modern microbes as diverse as viruses, yeast, bacteria, and filamentous fungi, have a genome-wide deleterious mutation rate of 0.003 per cell division even though they have widely different genome sizes and mutation rates per base pair [13]. For independently acting mutations, a mutation rate of 0.003 yields a low value for the mutation load; the average fitness is approximately $e^{-0.003} \approx 0.997$ [22,23]. The genome wide mutation rate in modern multicellular organisms is much higher than in microbes, for example; *Drosophila* has a genome wide rate (expressed on a haploid basis) of approximately 0.5. Of course, the "organism" we have in mind here is nowhere near as sophisticated as *Drosophila*, being on the threshold of the transition from unicellular to multicellular life. The mutation rate in modern microbes of 0.003 has been attained through the evolution of modifiers over billions of years; these modifiers presumably balanced the benefits of reducing the mutation rate with the physiological costs of doing so. For this reason, we think a much higher overall deleterious mutation rate likely held for the unicellular progenitors of multicellular life.

2.4. Fitness. There are interesting issues in how to model fitness in simple proto-organisms. As already mentioned the fitness of the organism, or cell-group, W_j, is simple enough to define; following standard models in population genetics, we take it

to be the absolute number of offspring groups produced. Likewise, the fitness of the cell is straight-forward to define in terms of its rate of replication and survival. The interesting issues concern the relationship between fitness at the two levels and the dependence of organism (cell-group) fitness on the fitness properties of component cells. Cooperation is fundamental to the emergence of cell groups and, so, we take organism fitness to depend upon the level of cooperation among its component cells—using terms of the form $1 + \beta q_j^{'}$ in the models below, where β is the benefit of cooperation and $q_j^{'}$ is the frequency of cooperative cells in the adult stage.

Cooperation among cells increases the fitness of the adult (parameter β) but non-cooperating cells may replicate faster, survive better, and as a consequence produce a larger but less functional adult. The question then concerns whether and how to include the effects of organism (cell group) size in organism fitness. If we ignore the contribution of group size to fitness, we may take $W_j = 1 + \beta q_j^{'}$. The effect of organism size in a j-organism (organisms that start out as propogules with j cooperating cells) may be represented by the term k_j / N, where the total number of cells after development is k_j and N is the number of cells in an offspring propagule (Table 3). If we include the contribution of group size, fitness of the organism may then be taken to be the product of organism size and the effects of cooperation or $W_j = \left(1 + \beta q_j^{'}\right) k_j / N$.

We note that if there were no mutation, including the term k_j / N in the expression of fitness would not cause any variation of fitness with N. If there were no mutation, there would be no within-group variation in replication or survival rates and the adult group size, k_j, would be a linear function of its starting size, N, $\left(k_0 = N2^{bct} \text{ and } k_N = N2^{ct}\right)$ and so N would cancel in the expression for W_j. For example, considering just the size component of fitness, k_j / N, for $ct = 3$, with $N = 50$ the adult size is $50 \times 2^3 = 400$ and the organism fitness is $400 / 50 = 8$, and with $N = 1$ the adult size is 8 and the fitness $8 / 1 = 8$. Consequently, it is not true that just because organism fitness is divided by N, selection will always act to reduce N, so as to increase W_j.

We think it most appropriate to include group size in organism fitness, for simple organisms on the threshold of multicellular life. However, constructing fitness in this way, $W_j = \left(1 + \beta q_j^{'}\right) k_j / N$, underscores the lack of true individuality in these early cell groups, since there is a direct contribution of cell fitness to organism fitness. Even in the case of no interactions between cells, that is no cooperation or defection, if C and D cells had different intrinsic rates of replication or survival, there would be different sizes among cell groups and different fitnesses of these groups according to k_j / N. Yet, these differences in group fitness would have nothing to do with the interactions among the component cells. For individuality to emerge at the cell-group level, fitness at the new level must be decoupled from the fitness of the component cells.

As individuality at the cell group level becomes better defined, we expect organism size to be regulated by factors other than intrinsic cell replication rates. Modifiers affecting propagule size are studied in 3 and those affecting within-organism

change and conflict are studied in Section 4. Conflict modifiers regulating organism size are expected to evolve so as to remove one of the advantages of cell defection, that advantage being larger groups (J. Li and R. E. Michod, unpublished results). By forcing all organisms, to attain a constant size, regardless of their proportion of cooperating and defecting cells, determinant size can be seen as an adaptation to regulate conflict. With determinate size, organism fitness would no longer depend on the term k_j / N.

The simple models considered here (Table 3 above or Table 5 below) assume a linear dependence of adult fitness on numbers of cooperating cells in the group, although more complex models could be easily incorporated into the framework considered there. At some point in the evolution of organisms, tissue function and interaction became so integral that the organism could not survive without it. Representing this (zero fitness below a threshold level of cooperation) requires nonlinear fitness models. Multiplicative models and other nonlinear formulations can also be considered in the same framework and can be expected to affect the outcome of the model, much as is the case in the theory of kin selection studied during previous project periods (see, for example, [33,34]).

3.   Origin of the Single-Cell Stage of the Life Cycle

3.1. Overview. We wish to understand why the life cycle of most multicellular organisms passes through a single cell stage. This single cell stage is present in a wide variety of plants, animals, and fungi, from simple multicellular organisms in the Volvocales to complex animals such as humans. We follow the terminology proposed by others and use the word "zygote" to refer to the single cell produced during sexual life cycles and the word "stem cell" to refer to the single cell produced during asexual life cycles [15]. Our initial modeling framework involves asexual life cycles and the origin of the stem cell. However, our longer term goal is to study sexual life cycles. To understand why most life cycles involve a single cell stage we must consider alternative forms of reproduction such as fragmentation (as occurs in *Hydra* [8], in colonial choanoflagellates such as *Proterospongia* [27], or in colonial green algae [6]), vegetative reproduction (as occurs in many plants) or aggregation (as occurs in the social Myxobacteria and in the cellular slime molds). As already mentioned, we use the word "propagule" to refer to the offspring of a reproductive process in which a sample (assumed below to be of size N) of cells is taken from the adult to produce the next generation. The way in which the sample is taken affects the level of variation between and within-groups (binomial sampling is assumed below for simplicity).

As discussed in the previous section, organisms are viewed as groups of cooperating cells. As cells divide the group increases in size and propagule groups may fragment off giving rise to the offspring of the next generation. Although, this may happen continuously, for mathematical simplicity a discrete generation approach is taken initially. Organisms (cell groups) are assumed to be composed of cooperating cells (for example motile cells as in Figure 1) and mutant cells. Mutant cells may be completely defective in all functions or defective in the capacity to differentiate into the motile state, as considered in the motivating scenario given in Figure 1. Offspring propagules are denoted by the number of cooperating cells in the propagule group, $j = 0, 1, 2, ...N$, where N is the number of cells in the offspring propagule group, assumed

constant for simplicity. The problem of the evolution of single-cell reproduction is basically the problem of the reduction of N to $N = 1$.

During development deleterious mutations (C to D) occur at rate μ. These mutations may be caused by errors in DNA replication (genetic mutations) or errors in the chromosome marking systems. Because the framework for individuality considered here involves two levels of selection, the cell and the group or whole organism (see Figure 1 and Figure 2), mutation may have deleterious effects at either level (or both). Deleterious mutation may lead to the loss of the benefits of cooperation at the cell group or organism level (for example, loss of motility or the capacity to respond to the motility inducer as in Figure 1), or to the loss of other cell functions related to cell replication and survival (as modeled by effects on the cell replication and survival parameters in Figure 3 and Table 3. The number of cooperating and mutant cells in the adult stage of a j propagule is k_{Cj} and k_{Dj}. A model to calculate these numbers is given in Figure 3 and Table 3 above.

3.2. Propagule model There are assumed to be two alleles, cooperate C and mutant D. The notation D is for <u>D</u>efect to describe the interesting case of selfish mutations that lead to the loss of cooperation with enhanced rates of cell replication or survival. However, deleterious mutations that disrupt cell function at both the level of the group and the cell are also studied as described in the last section.

We refer to "organisms" in terms of the composition of the offspring with regard to the number of cells that are cooperative at the cooperation locus. Because of within-organism mutation and selection during development, the adult stage may have cells that differ genetically from those in the offspring. The k-variables in Table 4 refer to numbers of cells of different genotypes in the adult stage. There are two forces that may change gene frequency between the zygote and adult stages and determine the values of the k-variables: mutation and cellular selection. Mutation leads to the loss of cooperation, and other aspects of cell function. Mutation increases the variance among cells within-organisms and enhances the scope for selection and conflict among cells. Cellular selection is assumed to depend on differences in cell survival or the

i, j	number of cooperating cells in offspring propagule group i, $j = 0, 1, 2, ...N$
N	number of cells in the offspring propagule group, assumed constant for simplicity
x_i	frequency of propagules with i C cells at time t
f_{ij}	frequency of propagule i produced from the adult form of propagule j
k_{Cj}	number of C cells in the adult stage of a j-propagule
k_{Dj}	number of mutant cells in the adult stage of a j-propagule
k_j	total number of cells in adult stage of j-propagule after development, $k_j = k_{Cj} + k_{Dj}$
W_j	adult fitness (expected number propagules) of a j-propagule taken in Section 3 to be either $$W_j = 1 + \beta q_j' \text{ or } W_j = \left(1 + \beta q_j'\right)\frac{k_j}{N},$$ depending on whether we assume that adult fitness depends on adult size (see subsection 2.4 for discussion)
β	benefit to adult organism of cooperation among cells
q_j', q_j	freq. in adult and offspring for C gene for propagules of type j
$q', \Delta q$	freq. and change in freq. of C gene in total population

Table 4. Haploid Propagule Model.

rate of cell replication (Table 3, Figure 3). The definition of terms and variables in the haploid model is given in Table 4. Diploidy has also been considered previously in a simpler model [37], but here we focus on the haploid case. With these definitions it is straight forward to write down the new frequency in the next generation of propagules of type i (given in Equation 1),

$$x_i' = \sum_{j=0}^{N} f_{ij} \frac{W_j}{\overline{W}} x_j ,$$

Equation 1

with $\overline{W} = \sum_{i=0}^{N} W_i x_i$.

3.3. Modes of fragmentation. The parameter f_{ij} is the frequency of type i propagules among the offspring of a type j organism. This frequency depends on the fragmentation mode considered. Kondrashov has studied the mutation load (defined as the difference in average fitness with and without mutation) in a population where the individuals produce offspring composed of N cells (as in our model) [26]. The model that he used is similar to ours, but he doesn't include within-organism selection. He considers four possible modes of fragmentation (see his figure 1 [26]).

False mode: the N cells of each offspring are as genetically closed as possible. In fact this case is equivalent to a single-cell-stage, and N has no influence on the mutation load.

Sectorial mode: the N cells come from the same sector; this means that they all come from the same initial cell. One can calculate f_{ij} in the following way. The number of cells in the adult that arise from each of the j initial C cells is $k_C = 2^{ct}(1-\mu)^{ct} + \dfrac{\mu 2^{bct} - 2^{ct}(1-\mu)^{ct}\mu}{-1 + 2^{b-1} + \mu}$, so the probability of choosing a sector that arise from a C cell is $\dfrac{jk_C}{jk_C + (N-j)2^{bct}}$. The probability of having an offspring with i C cells and N-i D cells is for $i > 0$: $f_{ij} = \dfrac{jk_C}{jk_C + (N-j)2^{bct}} \dbinom{N}{i}(q_C)^i(1-q_C)^{N-i}$, with $q_C = \dfrac{2^{ct}(1-\mu)^{ct}}{k_C}$ being the frequency of C cells among the cells arising from a C initial.

Random mode: the N cells are taken randomly from the adult. In this case we have Equation 2.

$$f_{ij} = \binom{N}{i}\left(q_j'\right)^i\left(1-q_j'\right)^{N-i}.$$

Equation 2

Recall, q_j' is the frequency of cooperating cells in the adult form. The frequency of C cells in the offspring and adult stage of a j offspring is $q_j = j/N$ and $q_j' = k_{Cj}/\left(k_{Cj} + k_{Dj}\right)$, respectively. The k_{Cj} and k_{Dj} variables are calculated Table 3 and

Figure 3. To illustrate the model framework, we assume for simplicity Equation 2 that involves random sampling across all cells in the adult form (with replacement). The hypergeometric distribution would be more appropriate if the number of cells in the offspring is big, but it can be approximated by a binomial distribution if the number of cells in the offspring is small compared with the number of cells in the adult k_j (approximately $N < k_j/10$).

Structured mode: each of the N cells come from a different initial cell (case 4). Kondrashov shows that in this case the genetic load is maximal. We will consider the sectorial and random cases in the analysis of the model.

To sum up, the different parameters of the model are represented in Table 3 and Table 4. We note that this model could easily be modified to study the case of groups formed by aggregation of cells (like slime molds). The model is deterministic and it assumes an infinite population with discrete generations. Organisms are haploid and asexual. Each organism begins its development with N cells. Cells can be of two types: C (cooperative) or D (selfish with $b > 1$, or uniformly deleterious with $b < 1$). During development, mutations $C \rightarrow D$ occur at a rate μ per cell division. The parameters representing the relative importance of intra- and inter-group selection are b and β. Two modes of fragmentation are considered next (sectorial and random) in addition to spore reproduction, and fitness may depend upon the size of the organism as well as on the frequency of mutants in the organism.

3.4. Mutation load. Kondrashov calculates the mutation load (difference in average fitness of populations with and without mutation) under vegetative reproduction, using a similar model to ours but without including intraorganismal selection [26]. Otto and Orive extended Kondrashov's model to include intraorganismal selection for uniformly deleterious mutations (they did not study selfish mutations), and show that with moderate intraorganismal selection, the opposite result obtains, that is mutation load decreases when N increases [42]. This indicates that it can be advantageous to produce multicellular propagules if intraorganismal selection can act upon the initial cell variants to eliminate mutations which are deleterious at both levels. In Otto and Orive as in Kondrashov's model, the fitness of the organism doesn't depend on its size (whereas it can in our model as discussed in subsection 2.4). As we will see including organism size in organism fitness modifies the results.

The model with $N+1$ recurrence equations $(x_0, x_1, \dots x_N)$ is too complex to be treated analytically. Nevertheless Kondrashov shows that the mutation load at equilibrium $\left(L = 1 - \overline{W}/W_{max}\right)$ can be calculated and is equal to 1 - λ, λ being the first eigenvalue of the matrix $\left[f_{ij}W_j\right]$. The equilibrium distribution of the different types of organisms is given by the first eigenvector of the matrix. He shows that the load increases with N, and is more important with mode 2 of fragmentation than with mode 1, with the mode 3 than with mode 2 and with mode 4 than with mode 3 (1: false mode, 2: sectorial mode, 3: random mode, 4: structured mode, see figure 1 of Kondroshov [26]). This seems logical, indeed the variance between-organisms decreases as N increases, and also from the mode 1 to the mode 4 of fragmentation, and so selection is less effective at eliminating mutants.

There is no density-dependence in Kondrashov's model: his recurrence equations are written $x_i' = \sum_{j=0}^{N} f_{ij} W_j x_j$ (with constant W_j), and not $x_i' = \sum_{j=0}^{N} f_{ij} \frac{W_j}{\overline{W}} x_j$ as in our model. So the matrix $\left[f_{ij} W_j \right]$ is the transition matrix of his system, whereas in our model the transition matrix can be written $f_{ij} W_j / \overline{W}$, $\overline{W}$ changing at each generation. Kondrashov's model is linear, but not ours, and *a priori* we cannot use his technique to calculate the mutation load and the distribution at the equilibrium. Nevertheless, Cushing showed that in this particular case of non-linearity (when the system can be written as the product of a scalar depending on the variables and of a matrix independent of the variables), the equilibrium distribution is still given by the first eigenvector of the matrix [11]. So we obtain the same frequencies at the equilibrium with both models, and thus the same mutation load (this has been checked by simulation).

Otto and Orive add to this model a kind of intraorganismal selection, with $0 < b < 1$ (they don't study the case of selfish mutants with $b > 1$). They show that the mutation load increases with *N* only if the intraorganismal selection is weak (*b* close to 1). When the within-organism selection is more important the results are opposite and the load decreases when *N* increases. One can interpret this by saying that when the inter-organism selection is important compared to the intra-organism selection, the mutants are better eliminated when the variance between-organisms increases, whereas if the intra-organism selection is more important the mutants are better eliminated when the variance decreases (with more mixed groups).

Our preliminary results presented in subsection 3.5 confirm Otto and Orive's results, so long as there is no effect of organism size on fitness. However, when organism size is included in organism fitness mutation load always decreases with decreasing *N*. It is not clear in the work of Kondrashov [26] and Otto and Orive [42] if the effects on mutation load are indicative of the direction of selection on genes modifying the reproductive system by changing *N*. However, our results indicate that modifiers for a single-cell stage of the reproductive cycle do the honorable thing and increase when mutation load is decreased.

3.5. Preliminary results. We are currently investigating the equilibrium distribution of propagule states. In Figure 4, the results of simulations of Equation 1 are given assuming binomial sampling as specified in Equation 2 and the mutation selection model specified in Figure 3 and Table 3. The equilibrium frequency distribution appears to qualitatively shift to a U-shaped distribution for small *N* from a bell-shaped distribution for larger *N*. The U-shaped distribution is characteristic of loss of intermediate states in which there are mixed groups of the two types of cells, while the bell-shaped distribution is characteristic of maintaining intermediate mixed groups.

This result anticipates a fundamental advantage (at least for selfish mutations) of passing the life cycle through a bottleneck in cell number─increasing the variance at the cell-group level and reducing the within-group variation and conflict. This favors cooperation and helps to restrict the opportunity for defecting mutants to spread. Notice that in Figure 4, the frequency of cooperation is much higher in panel A ($N = 5$) than in panel B ($N = 15$). The limiting case of $N = 1$ corresponds to the transition from fragmentation to spore production. A simple regulatory model has been proposed in which the transition from fragmentation to spore production is caused only by a few changes [48]. The origin of the single cell spore stage is a matter of great importance for individuality and development.

As a first approach to the problem of selection on a modifier reducing propagule size, we consider a population composed of two distinct reproductive types, a "propagule reproducer" and "spore reproducer." Propagule reproducers reproduce according to the model given above (Equation 1 and Equation 2). There are $N+1$ recurrence equations for the propagule reproducer. The spore reproducer reproduces according to the model studied in our previous work and requires just two equations, since there are just two spore types for the haploid case, either C or D. The full vector of state variables is then $\left[x(0),\dots, x(N), x(C), x(D) \right]$, with $x(i)$ = frequency of individuals without modifier with i non-mutant initial cells, $x(C)$ = frequency of C-individuals (with the modifier, starting their development with a non-mutant cell), $x(D)$ = frequency of D-individuals (with the modifier, starting their development with a mutant cell). An individual reproducing via spores starts its development with only one cell and so the number of cells in the adult stage is lower than the number of cells in the adult stage of an individual without the modifier (because t is the same for both kinds of organisms). This can have an effect on fitness, nevertheless we don't include this effect here. We have shown that the frequencies go to an equilibrium given by the first right eigenvector of the $\left[W_j f_{ij} \right]$ matrix (constructed in a similar manner to Kondrashov [26] but including the equations for the spore reproducers).
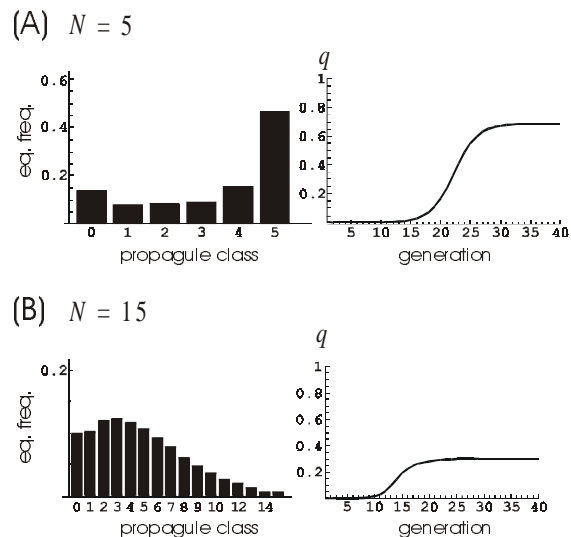

(A) $N = 5$

(B) $N = 15$

Figure 4. Simulation of propagule model for selfish mutations assuming $W_j = \left(1 + \beta q'_j\right) k_j / N$. Using Equation 1 and Equation 2. Parameter values in panel (A): $c = 1$, $t = 10$, $\beta = 3$, $\mu = 0.003$, $b = 1.1$; and in panel (B): $c = 1$, $t = 10$, $\beta = 10$, $\mu = 0.01$, $b = 1.05$. As explained elsewhere [40] these parameter values are appropriate for the transition involving cell groups similar in size to some species of Volvocales. The ordinate is either the equilibrium frequency of a propagule class ("eq. freq.") or the frequency of cooperation, q.

When mutations are selfish the spore reproducer always wins, because it is always advantageous to increase the variance between-organisms (and there is no cost of doing so because we have assumed no effect of N on adult size). This is always the case, for both fitness functions in Table 4.

Results for uniformly deleterious mutations are given in Figure 5 for the two fitness functions: when organism fitness is independent of group size (panel (A)) and when organism fitness depends upon group size (panel (B)). When fitness depends only on the frequency of cells (Figure 5 panel (A)), we have found that, when the intraorganismal selection is weak (b close to 1), the spore reproducer is selected, because between-organism selection is more effective at eliminating the deleterious mutations. When intraorganismal selection becomes stronger, the spore reproducer is no longer selected. We have studied the critical value of b for single cell (spore) reproduction to be selected, for several values of the other parameters. In Figure 5, this critical value of b occurs when the $N = 5$ curve intersects the $N = 1$ curve (at a value of b slightly greater than $b = 0.95$ in the figure). The critical value of b doesn't change much when the value of N for the propagule reproducer changes. In other words, the condition for selection of spore reproduction is about the same regardless of the size of the propagules of its competitor. In addition, the mode of fragmentation has a small, but barely noticeable effect, on this critical value. As just mentioned, the curves for other values of $N > 1$ intersect approximately at the same
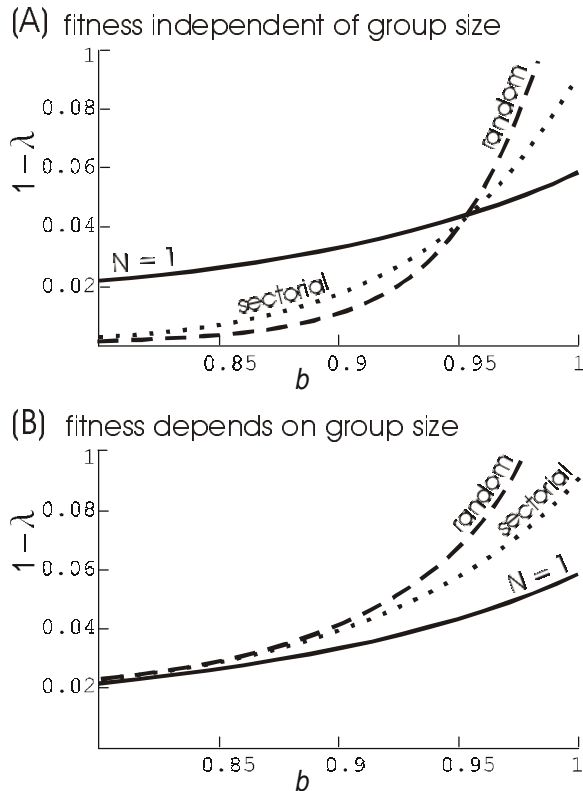

Figure 5. Mutation load (1- λ) and selection on spore (stem cell) reproduction for mutations deleterious at both levels of selection. Parameter values are $\mu = 0.003$, $t = 20$, $\beta = 3$. The parameter b on the abscissa is the replication rate of mutant cells. Non-mutant cells replicate at rate 1. Spore production is always selected for selfish mutations ($b > 1$). In panel (A), spore reproduction ($N = 1$) wins when the mutation load decreases with N (to the left of the point where the solid line crosses the others). Key in both panels: dashed lines for $N = 5$ and random mode of fragmentation (Equation 2); dotted lines for $N = 5$, and sectorial mode of fragmentation; solid lines for $N = 1$. The differences between the panels involve the way in which fitness is modeled at the organism (cell-group) level. In panel (A) fitness is independent of group size $W_j = 1 + \beta q_j'$, while in panel (B) fitness depends on group size $W_j = \left(1 + \beta q_j'\right) k_j / N$. See text for explanation.

point and behave qualitatively as does the $N = 5$ curve in that mutation load is greater (or smaller) than the $N = 1$ curve when b is to the right (left) of the intersection. Consequently, the main result concerning the evolution of spore reproduction when fitness is independent of group size is that, when the mutation load increases with N (to the right of the point at which the curves intersect in panel (A) of Figure 5), the modifier is selected (the spore reproducer wins); and when the mutation load decreases with N (to the left of the point of intersection), the modifier is not selected (the propagule reproducer wins).

However, when organism fitness depends upon group size, as it should for simple groups on the threshold of multicellularity, mutation load always decreases with N, and the spore reproducer is always selected (Figure 5 panel (B)). For the values of b lower than 0.8 the value of the load almost doesn't depend on N. Indeed the between-organism selection is reinforced by the effect of size (the more the proportion of deleterious mutants is important, the smaller is the organism). For the values of b lower than 0.8 the proportion of mutants in the population is low, whatever the value of N.

Under the sectorial mode of fragmentation, all cells of a propagule come from the same initial cell in the parent. The kinship among cells in the propagule is higher, and the variance between-organisms is higher, under sectorial sampling than under random sampling. As we see in Figure 5 (panel (A)), when the between-organism selection is more effective at eliminating deleterious mutations (b less than but close to unity), the mutation load is greater with random sampling than with sectorial sampling. The opposite result obtains when within-organism selection is more effective (b significantly less than unity) and the mutation load is lower under random than under sectorial sampling. When fitness depends upon group size (Figure 5 panel (B)), the load is always greater under random sampling than under sectorial sampling.

There are other aspects of the single cell state that require serious consideration, but these have been ignored here. First, there is the opportunity it provides to reset development. The second aspect of the single cell state is its role in the sexual life cycle. It is difficult to imagine sex between-organisms that does not involve a single cell state.[1] We intend to address these viewpoints in due time.

4.   Evolution of Conflict Mediation

4.1. Overview. The preliminary results reported in the last section lend credence to the view that, by passing the life cycle through a single cell stage (the stem cell, spore or zygote), the opportunity for between-group selection is enhanced while the opportunity for within-group conflict is reduced. This can favor the transition between single cells and cell groups. We now (and in the next section) consider further aspects of the transition from single cells to organisms by assuming throughout section 4 that

---

[1] Perhaps, bacterial transformation or recombination in viruses (such as occurs during multiplicity reactivation) comes closest to the case in which recombination may occur among DNA molecules from multiple partners. In the slime mold, spores from a single individual may involve mating and recombination of cells from different parents. In the life cycle of the slime mold *Dictyostelium discoideum*, the single cell products of the organism, the spores, form amoebae that ultimately aggregate in groups to form a new multicellular individual, first the migrating slug and then the fruiting body. During the single cell amoebae stage before aggregation, sex (fusion and meiosis) may occur (for review see [50]). As a consequence, a single individual (fruiting body) may involve cells that are recombinations among different parent cells (the degree to which this occurs depends upon the relatedness of the aggregating cells).

cell groups originate from single cells. In our view an organism is more than a group of cooperating cells, more even then a group of cooperating cells related through common descent from a single cell. An organism must have functions that maintain its integrity and individuality. We are interested in understanding the first true emergent level functions during the emergence of the first true individual, the multicellular organism.

All models make assumptions about the processes they study, in the present case, these assumptions involve parameters representing within-organism mutation and cell-cell interaction during development. The purpose of the modifier model introduced here is to study how evolution may modify these assumptions and parameter values, and further how these modifications affect the transition to a higher level of selection. Unlike the classical use of modifier models, say in the evolution of dominance and recombination, the modifiers studied in the present paper are not neutral. Instead, they have direct effects on fitness at the cell and organism level by changing the parameters of within-organism change. By molding the ways in which the levels interact so as to reduce conflict among cells, for example by segregating a germ line early or by policing the selfish tendencies of cells, the modifiers construct the first true emergent organism level functions.

4.2. Model parameters. Recall the parameters of the model studied above. These parameters describe fitness effects at the organism (β) and cell level (b, s_C, s_D), the mutation rate per cell division (μ) and the development time (t). The parameter β is the benefit to organism fitness of cooperation among its component cells. Fitness is assumed to depend linearly (through β) on the number of cooperating cells in the organism's adult stage (see Table 5). The parameter bc is the replication rate of defecting cells (c is the replication rate of cooperating cells, assumed equal to unity). The parameters s_C, s_D are the survivals of C and D cells per cell generation (assumed equal here for simplicity). The parameter μ is the rate of mutation per cell division during development (interpreted here as the genome wide deleterious mutation rate at all loci leading to a loss of cell function). The parameter t is the time available for development (development allows for within-organism change resulting from mutation, μ, and selection, b, s_C, s_D, at the cell level). A new parameter is needed in the case of sexual reproduction with the possibility of recombination between two loci; r is the rate of recombination between the cooperate/defect locus and the modifier locus. Again, the complexity of interaction among different cell types and tissue functions is assumed to be represented by two kinds of interactions—cooperate and not cooperate (or defect). Heritability of fitness at the organism level is measured by the regression of offspring fitness on adult fitness.

A per cell division mutation rate of $\mu = 0.003$ is assumed in many of the studies reported in this section. The reason for assuming this value has been discussed above, in Section 2.3. Nevertheless, smaller mutation rates also result in the evolution of modifiers of within-organism change (that is the transition from equilibrium 3 to 4 discussed below, see, for example, panels (D) and (E) of Figure 6). Higher mutation rates mean more within-organism change and this facilitates evolution of the modifier.

4.3. Two-locus modifier model. We now consider a second locus which is assumed to modify the parameters of within-organism change at the cooperate/defect (C, D) locus (see Table 5 for the additional terms for the modifier model).

The modifier locus is interpreted here as either as a germ line locus or a mutual policing locus, according to whether the modifier allele *M* affects the way in which cells are chosen for gametes (germ line modifier), or the parameters of selection, and variation at the organism and cell level (policing modifier). A *germ line modifier* is assumed to sequester a group of cells with shorter development time and possibly a lower mutation rate than the soma and less selection at the cell level. A *self-policing modifier* causes the organism to spend time and energy monitoring cell interactions and reducing the advantages of defection at a cost to the organism. In either case, the modifier locus is assumed to have two alleles *M* and *m*, with no mutation at the modifier locus. Because there is no mutation at the modifier locus and groups start out as single cells, there can be no within-group change at the modifier locus because there is no within-group variation at the modifier locus. A group of cells expressing allele *m* is assumed to have the same properties as the cell-groups studied in the previous section (assuming *N* = 1). A group of cells expressing allele *M* is assumed to have different properties that represent the ideas of a germ line or self policing.

Variable	Definition
i, j	indices for genotype 1, 2, 3, 4 = *CM*, *Cm*, *DM*, *Dm*
k_{ij}	number of *i* cells in the adult stage (soma) of a *j*-zygote
k_j	total number of cells in adult stage (soma) of *j*-zygote, $k_j = \sum_i k_{ij}$
K_{ij}	number of *i* cells in the germ line of a *j*-zygote
K_j	total number of cells in the germ line of *j*-zygote, $K_j = \sum_i K_{ij}$
W_j	individual fitness of *j*-zygote (assumed to depend on group size): $W_j = k_j + \beta(k_{1j} + k_{2j})$
r	recombination rate between *C/D* and *M/m* loci
x_j	frequency of two locus *j* genotype in total population

Table 5. Additional notation and terms for the two locus modifier model. The genotype frequencies, x_j, are measured at the gamete stage, before mating, meiosis, development and within and between-organism selection. The variables, x_j, are now completely different than those defined in the propagule model in Table 4. The table considers germ line modifiers that create a separate germ and somatic line, each of which may have different numbers of cell types (the K_{ij} and k_{ij} variables). In the case of self-policing modifiers, there is no distinction between the germ line and the soma (all cells are potential germ line cells).

The additional terms and definitions for the two-locus modifier model are given in Table 5. Some explanation of the haploid life cycle considered here may be helpful. In the diploid life cycle, a generation typically begins with the diploid zygote, followed by development, within and between-organism selection, the adult stage, meiosis and the production of haploid gametes which fuse to produce the zygotes of the next generation. In previous analyses of the two-locus haploid modifier model studied here, it was also assumed that the generation began with the zygote stage (Michod 1996, Michod and Roze 1997). In the haploid life cycle, the haploid zygote stage is followed by development, within and between-organism selection, the adult stage (still haploid), production of haploid gametes, fusion of gametes to produce a transient diploid stage which undergoes meiosis to produce the haploid zygotes of the next generation. Although it doesn't affect the results, the two-locus recurrence equations are simpler

for the haploid life cycle, and easier to understand, if we begin a generation with the gametes and let the x_j variables be the frequencies of the different genotypes among gametes (instead of among zygotes). Consequently, the following sequence of events in each generation is assumed: gametes, fusion of gametes to make the transient diploid stage, meiosis and recombination to form haploid zygotes, development, within and between-organism selection to create the adult stage, and then formation of the gametes to start the next generation.

The recurrence equations for the two-locus haploid life cycle may be constructed as follows. Recombination may change the genotype frequencies according to the level of linkage disequilibrium in the population. *Linkage disequilibrium* measures the statistical association between the frequencies at the two loci. If $G = 0$, the joint distribution of alleles in gametes is the product of the allele frequencies and recombination has no effect on the genotype frequencies. In Equation 3, we define linkage disequilibrium between the locus determining cell behavior and the modifier locus (using the variable G for gametic phase imbalance).

$$G = x_1 x_4 - x_2 x_3 \qquad \text{Equation 3}$$

After meiosis, the frequencies of the four genotypes may change from what they were before fusion, depending on the rate of recombination (r) and the level of linkage disequilibrium, G. The new frequencies after recombination are $x_1 - rG$, $x_2 + rG$, $x_3 + rG$, and $x_4 - rG$, for the *CM*, *Cm*, *DM* and *Dm* genotypes, respectively. It is upon these new genotype frequencies that selection and mutation operate, as shown in Equation 4. The full two-locus dynamical system is given in Equation 4,

$$x_1' \overline{W} = \left(x_1 - rG \right) W_1 \frac{K_{11}}{K_1}$$

$$x_2' \overline{W} = \left(x_2 + rG \right) W_2 \frac{K_{22}}{K_2}$$

$$x_3' \overline{W} = \left(x_3 + rG \right) W_3 + \left(x_1 - rG \right) W_1 \frac{K_{31}}{K_1} \qquad , \qquad \text{Equation 4}$$

$$x_4' \overline{W} = \left(x_4 - rG \right) W_4 + \left(x_2 + rG \right) W_2 \frac{K_{42}}{K_2}$$

with $\quad \overline{W} = (x_1 - rG)W_1 + (x_2 + rG)W_2 + (x_3 + rG)W_3 + (x_4 - rG)W_4 \quad$ and $\quad$ linkage disequilibrium given by Equation 3. Only three of the equations are independent, since the frequencies must sum to one. Equation 4 gives the same results as the equations studied previously (equations 1 and 2 of Michod 1996 and in Michod and Roze 1997). The primary difference is that genotype frequencies are measured among gametes and not zygotes, and this allows a simpler form of the recurrence equations.

4.4. Equilibria of the system. The transition to a new level of selection proceeds in two general stages. First, cooperation must increase. Deleterious mutation leads to the loss of cooperation. A mutation selection balance may be reached at which the increase of cooperation (by group selection) is offset by its loss through mutation. As discussed previously, 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 towards defection and a loss of cooperation [37]. The second stage in an evolutionary transition is the appearance 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 only then is the group is truly indivisible in that it possesses higher level functions that protect its integrity.

The main equilibria of the system of are given in Table 6. These equilibria are obtained by setting linkage disequilibrium to zero ($G=0$, using Equation 3) and by setting the change in genotype frequencies equal to zero (using Equation 4). The equilibria given in Table 6 are the ones we expect based on the biological assumptions of the model, however they assume that linkage disequilibrium (Equation 3) is zero. In the case of germ line modifiers discussed in the next section, we have found examples of other equilibria at which $G \neq 0$, but they are confined to narrow regions of the parameter space. These equilibria with $G \neq 0$ are, nevertheless, quite interesting as the populations are polymorphic at the modifier locus and maintain both germ line and non-germ line phenotypes in the same population.

Eq.	Description of Loci	Interpretation
1	no cooperation; no modifier	Non-functional cell groups, single cells[2]
2	no cooperation; modifier fixed	Not of biological interest, never stable
3	polymorphic for cooperation and defection; no modifier	*Groups of cooperating cells:* no higher level functions
4	polymorphic for cooperation and defection; modifier fixed	*Individual organism*: integrated group of cooperating cells with higher level function mediating within-organism conflict

Table 6. Equilibria for modifier model without linkage disequilibrium. See especially Table A 1 and Table A 2, for a mathematical description of the equilibria and eigenvalues.

For the moment we focus on the equilibria given in Table 6. The eigenvalues of the three independent genotypes $(x_4 = 1 - x_1 - x_2 - x_3)$ are given in Table A 2 for the different equilibria and for sexual and asexual reproduction. The evolution of cooperation corresponds to equilibrium 3 and the evolution of the modifier corresponds to equilibrium 4. Consequently, the question of the transition to individuality boils down to the conditions for a transition from equilibrium 3 to equilibrium 4. The condition for increase of the modifier is given as λ_{31} in Table A 2. In the case of asexual reproduction, $\lambda_{31} > 1$ means that a *CM* organism must produce more *C* gametes than a *Cm* organism.

The evolution of functions to 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 (at equilibrium 3) which sets the stage for a transition between equilibrium 3 and 4 and the evolution of individuality.

4.5. Evolution of the germ line. The essential feature of a germ line is that gamete producing cells are sequestered from somatic cells early in development. Consequently, gametes have a different developmental history from cells in the adult form (the soma) in the sense that they are derived from a cell lineage that has divided for a fewer number of cell divisions with, perhaps, a different mutation rate per cell replication and a different selective context. The main parameters influencing the

---

[2] The model assumes cell groups. Nevertheless, we think of Eq. 1 as representing single cells, because there is no cooperation and no interaction between cells to maintain the group structure assumed in the model.

evolution of the germ line is the reduction in development time in the germ line relative to the soma, δ (with the development time in the germ line being $t_M = t - \delta$), the deleterious mutation rate in the germ line, μ_M, and the nature of cell selection in the germ line.

	Adult Stage	Genotype of Zygote			
		CM	Cm	DM	Dm
Germ Cells, K_{ij}	CM	$2^{ct_M}(1-\mu_M)^{ct_M}$			
	Cm	0			
	DM	$\dfrac{\mu_M 2^{bct_M} - 2^{ct_M}(1-\mu_M)^{ct_M}\mu_M}{-1+2^{b-1}+\mu_M}$			
	Dm	0			
Somatic Cells, k_{ij}	CM	$2^{ct}(1-\mu)^{ct}$	0	0	0
	Cm	0	$2^{ct}(1-\mu)^{ct}$	0	0
	DM	$\dfrac{\mu 2^{bct} - 2^{ct}(1-\mu)^{ct}\mu}{-1+2^{b-1}+\mu}$	0	2^{bct}	0
	Dm	0	$\dfrac{\mu 2^{bct} - 2^{ct}(1-\mu)^{ct}\mu}{-1+2^{b-1}+\mu}$	0	2^{bct}

Table 7. Numbers of different cell types in the soma and gamete stages for germ line modifiers. Zygote genotype is given across the top (column) and the genotype of the cells after development is given down the rows. For genotypes containing the *m* allele, there is no germ line; so there is no difference between the germ line stage and the somatic adult stage. The germ line is ignored in *D* containing zygotes since by assumption there is no mutation from *D* to *C* and so no within-organism variation in *D* containing zygotes. The steps involved in obtaining the formulae in the table are described in Table 3 and Figure 3. We do not consider mutations at the germ line locus, since we are interested in the loss of tissue function and cooperation among somatic cells. A modification of the model given in the text relates the table to the study of a self-policing allele. Selection is allowed in the germ line stemming from the different rates of replication of cooperate and defecting cells. This makes matters more difficult for the origin of the germ line. We also consider in Table 8 the case that, since germ line cells don't cooperate and otherwise function in somatic functions, the replication advantage of defecting cells no longer obtains in the germ line.

We are interested in modeling the origin of germ cells in an ancestor that had none. Furthermore, we wish to consider the hypothesis that the germ line serves to increase the heritability of traits in the organism, as opposed to directly increasing organism fitness. For this reason, we assume that the germ line is costly to fitness (expected number of offspring) although it increases the heritability of fitness (regression of fitness of offspring on fitness of parents). Where do the germ line cells come from? We imagine that the total number of cells is conserved and allocated between the germ line and the soma. Cells sequestered in the germ line are no longer available for somatic function, and, for this reason, the germ line allele may detract from adult organism function. One way of representing this cost is by subtracting the

germ line cells from the somatic cells in the adult form (k_{ij} - K_{ij} in Table 7 and Table 8). The number of cells is directly related to the development time, t. So if δ is very small, then the number of cells in the germ line is quite large leaving few cells for somatic function. For this reason, we find that the transition from equilibrium 3 to 4 cannot occur by a continuous increase of δ from zero. There is a threshold value of δ resulting from the cost of the germ line which must be overcome for the modifier to increase. If there were no cost, the germ line allele always spreads in the population.

There is a limitation in our approach to modeling the origin of the germ line. Fitness is assumed to depend on the number of cells in the soma and not on the number of cells in the germ line. Fitness is maximal when no cells are allocated to the germ line, because there are more cells available for somatic function (although the heritability of fitness is less, if there is no germ line). Presumably, the number of cells allocated to the germ line will have some effect on the number of gametes produced, but we have ignored the problem of modeling this effect.

In Table 8 we give the numbers of different cell types in the gamete stages for germ line modifiers, assuming complete differentiation between germ and soma. In other

Adult Stage		Genotype of Zygote CM
Germ Cells, K_{ij}	CM	$2^{ct_M}\left(1-\mu_M\right)^{ct_M}$
	Cm	0
	DM	$2^{ct_M}\left(1-\left(1-\mu_M\right)^{ct_M}\right)$
	Dm	0

Table 8. Numbers of different cell types in the gamete stages for germ line modifiers assuming no selection in the germ line. See Table 7 for explanation of table. Only the CM zygote is shown here, the other zygotes have germ and soma properties as specified in Table 7.

words, we assume that there is no expression of cooperate/defect phenotype in germ line and so no selection in the germ line ($b = 1$ in the germ line). Deleterious mutation may still occur in the germ line, however it is neutral at the level of the cell in the germ line (mutation still disrupts cell function in the soma, however). We represent no selection in the germ line by assuming $b = 1$ in the table cell corresponding to the DM gametic output of a CM zygote. No selection in the germ line only makes sense for selfish mutations ($b > 1$), since cells that are defective at both levels of selection ($b < 1$) may be expected to replicate more slowly in both the germ and the soma because they are generally defective (not just defective in the cooperative function).

We now return to considering the evolutionary equilibria of the system. The equilibria of Equation 4 correspond to different evolutionary outcomes. Regions of stability of the different equilibria for different parameter values are given in Figure 6 in terms of the reduction of development time caused by the germ line modifier, δ, and the replication rate of mutant cells, b. The replication rate of cooperating cells is unity ($c = 1$). Recall that mutations are beneficial (selfish mutants), neutral or deleterious at the cell level when $b > 1$, $b = 1$, and $b < 1$, respectively.

The transition involving the increase of cooperation (Eq. 1 to Eq. 3 in Figure 6) has been considered previously (Michod 1997a). This transition occurs for parameter values in the regions marked Eq. 3 in the panels in Figure 6, while cooperation will not increase for parameter values in the Eq. 1 region. The transition which interests us here involves the increase of modifiers of within-group change and this transition occurs after cooperation increases (Eq. 3 to Eq. 4 in Figure 6). The conditions under which the population evolves from equilibrium 3 to 4 were studied previously [36]. This transition occurs for parameter values in the Eq. 4 regions in Figure 6. In a later

section (Transitions in Individuality), we study the components of this transition in terms of the emergence of fitness and the heritability of fitness at the new organism level.
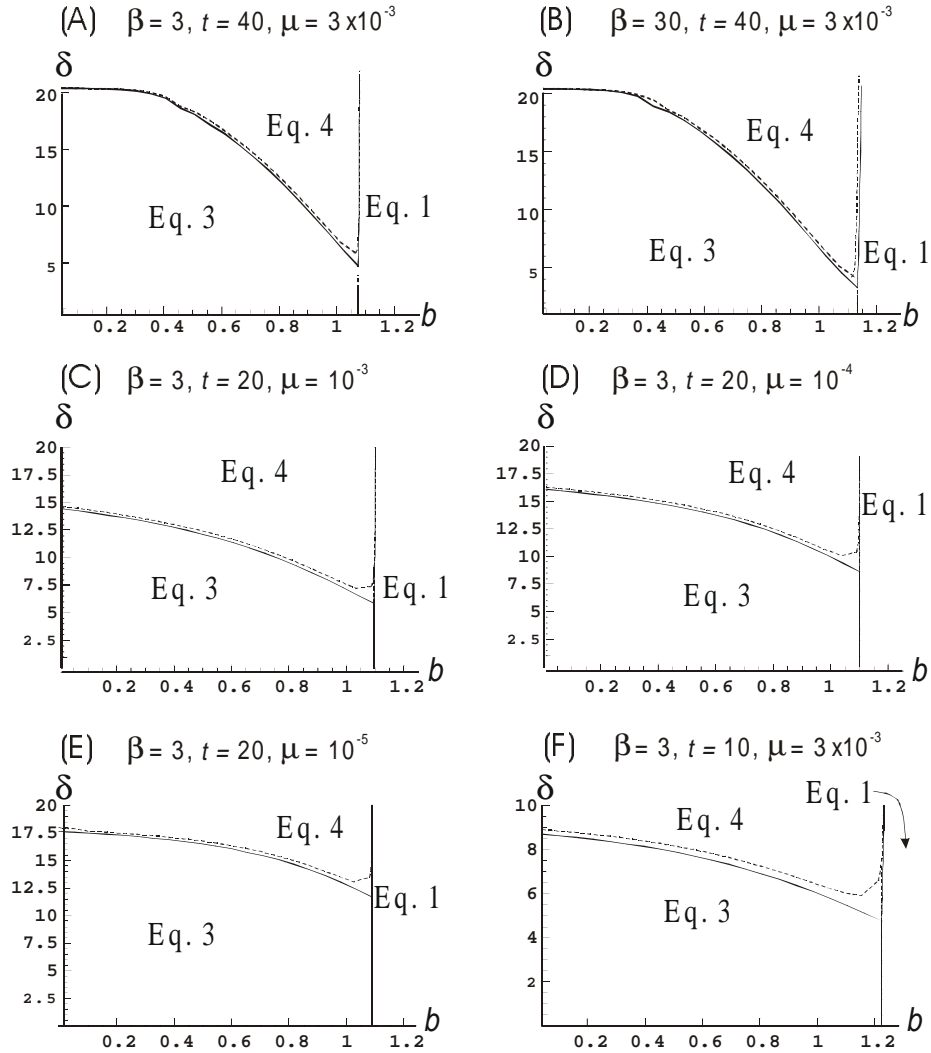

Figure 6. Stability of evolutionary equilibria for germ line modifiers. Results here assume selection in the germ line (Table 7). The modifier is assumed to decrease the development time for the germ line (when compared to the soma) by amount δ. Selfish ($b > 1$) and uniformly deleterious ($b < 1$) mutations are considered. Regions of stability for the different equilibria described in Table 6 are given as a function of the replication rate of mutant cells (relative to cooperating cells), b, and δ, for different values of the mutation rate, μ, development time, t, and advantage of cooperation, β. Solid curves are for asexual reproduction and dashed curves for sexual reproduction assuming a recombination rate of $r = 0.25$ between the modifier and cooperate/defect locus. Cells sequestered in the germ line are not available for somatic function. The mutation rate is assumed to be the same in the soma and the germ line. See also Figure 1 of Michod [37] for more detailed treatment of the boundary between equilibrium 3 and 4 in three dimensions (b, μ, δ).

The general result apparent in Figure 6 is that the modifier increases for all kinds of mutations at the cell level (beneficial, neutral or deleterious) so long as they are deleterious at the organism level (as is assumed throughout) and the reduction in time for replication, δ, is large enough. As the mutants change from being beneficial at the cell level to deleterious, it becomes more difficult for the germ line modifier to increase, in the sense of requiring a larger decrease in the time available for replication in the germ line (all panels in Figure 6). Selfish mutants are most efficacious in selecting for a germ line, nevertheless all mutations deleterious at the organism level promote selection for the germ line modifier.

The parameters have understandable effects on the regions of stability of the different equilibria described in Table 6. For example, as the benefit of cooperation to the group increases from $\beta = 3$ to $\beta = 30$, larger replication benefits of defection at the cell level are tolerated as shown in panels (A) and (B) of Figure 6. Likewise, as the size of the organism decreases from $t = 40$ to $t = 20$ to $t = 10$ (shorter development times), larger benefits of defection at the cell level are tolerated although the reduction in development time for the germ line to evolve is about the same (see panels (A), (C) and (F) of Figure 6). As the mutation rate decreases from 10^{-3} to 10^{-4} to 10^{-5}, it becomes more difficult for germ line modifiers to increase, in the sense that larger reductions in development time are required for there to be a transition from equilibrium 3 to 4 (see panels (C), (D) and (E) of Figure 6; see also Figure 1 of Michod [37]). Higher mutation rates mean more within-organism change and this makes it easier for conflict modifiers to increase.

Figure 6 shows the limits of these regions of stability of the four equilibria in Table 6 in (b,δ) space, for different values of the time for development, t, the benefit of cooperation, β, and μ the mutation rate. Recall, the parameter δ is the difference between the development time of the soma (t) and of the germ line. In the case of asexual reproduction these different regions don't overlap. With sexual reproduction, there are regions in which more than one equilibrium can be stable at the same time (bi-stability) and regions in which none of the four equilibria given in Table 6 are stable. Such a situation is shown in Figure 7, for selfish mutations ($b > 1$).

In Figure 7, there is assumed to be no selection in the germ line (Table 8), although the conclusions discussed now also apply when there is selection in the germ line (Table 7). There are three curves given in Figure 7 which overlap in different regions. First there is a nearly vertical dotted line at about $b = 1.1$. To the right of this line, equilibrium 1 is stable and there is no cooperation among
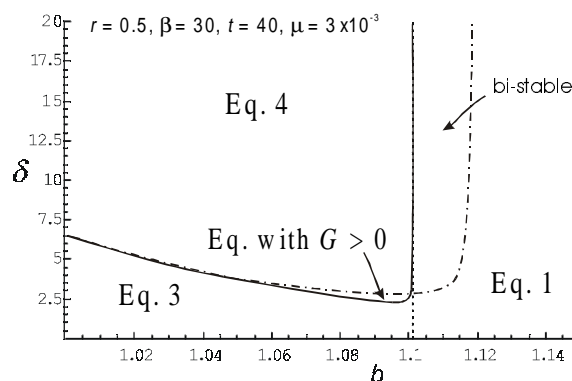

Figure 7. Equilibria and regions of stability for germ line modifier and selfish mutations. No selection in germ line (Table 8), although the qualitative relationships of the three curves (bi-stable and Eq. with $G > 0$ regions) are similar if there is selection in the germ line (Table 7), or if the recombination rate is smaller so long as $r > 0$. See text for explanation.

cells. Second there is a solid curve which defines the region of stability for equilibrium 3. Below this curve equilibrium 3 is stable and there is cooperation but the germ line modifier won't increase. Third, there is a dashed-dotted curve which defines the region of stability for equilibrium 4. Above this curve equilibrium 4 is stable; both cooperation and the germ line modifier are present.

The region in which both equilibrium 1 and 4 are stable is labeled bi-stable in Figure 7. Depending on initial conditions the population can switch between no cooperation to a fully functional organism. This requires the introduction of the two alleles C and M at the same time and on the same chromosome. We consider this to be a rare and unlikely event. Nevertheless this suggests that with sex, mixed populations of individual cells (no organisms, Eq. 1) and organisms, interpreted as well integrated groups of cells (Eq. 4), may coexist. However, the transition from equilibrium 1 to equilibrium 4 is less interesting in terms of an evolutionary scenario towards individuality, because it supposes the simultaneous appearance of C and M alleles in the population (cooperation and germ line). It is more reasonable to consider the evolutionary transition via equilibrium 3. Consequently, the boundary which interests us is the one between the region of stability of equilibrium 3 and of equilibrium 4.

In Figure 7, the curves defining the regions of stability of equilibrium 3 and 4 are disjunct for a narrow region of parameter space (approximately δ around 2.6 and b in between 1.06 and 1.1). This region is labeled "Eq. with $G > 0$." In this region none of the four equilibria with zero linkage disequilibrium are stable. There is, however, in this region a stable equilibrium with positive linkage disequilibrium, so that the CM and Dm chromosomes are more common than predicted by the product of the allele frequencies. For parameters in this region, selection and recombination are balanced at the $G \neq 0$ equilibrium. Selection is favoring the CM chromosome and disfavoring the Dm chromosome. However, recombination is breaking these chromosomes apart generating Cm and DM chromosomes, configurations for which the modifier is either not present (Cm) or has no effect (DM).

We have only recently discovered the existence of these equilibria with positive linkage disequilibrium. Although the range of parameters permitting this equilibrium are so far small, the $G > 0$ equilibrium interests us greatly since the reproductive system is polymorphic in the population; both organisms with and without germ lines coexist in such equilibrium populations. An example of this is given in the computer simulation in Figure 8. As just mentioned there is a stable polymorphic equilibrium at the germ line locus so that both germ line and no germ line phenotypes are maintained in the population.
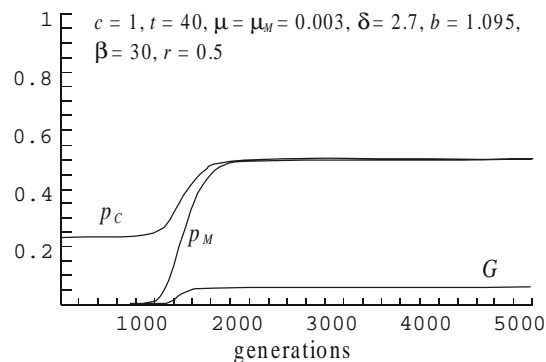

Figure 8. Population converges to an equilibrium at which $G > 0$ and is polymorphic for the germ line modifier. The variables p_C, p_M and G are the frequencies of cooperation, the modifier allele, and linkage disequilibrium, respectively. See Figure 7 and text for explanation.

In summary, the transition to individuality via equilibrium 3 involves two steps: initial increase of cooperation within

the group, and concomitantly of the level of within-group change since mutation leads to loss of cooperation, and then appearance of the germ line to regulate this within-group conflict. Only after the evolution of modifiers of within-group conflict, can we refer to the integrated group of cooperating cells as an "individual," since the group is now truly indivisible and possesses higher level functions that protect its integrity.

4.6. Evolution of the mutation rate. Modifiers lowering the mutation rate ($\mu_M < \mu$) are also selected for in this model. Maynard Smith and Szathmáry suggest that germ line cells may enjoy a lower mutation rate but do not offer a reason why [32]. Bell interpreted the evolution of germ cells in the Volvacales as an outcome of specialization in metabolism and gamete production to maintain high intrinsic rates of increase while algae colonies got larger in size ([4], see also Maynard Smith and Szathmáry [32] pp. 211-213). We 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 (for example, the superoxide radical O_2^-, and the hydroxyl radical $\cdot OH$ 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. The 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 the deleterious effects of metabolism and genetic error [35]: keeping DNA in the nucleus protects the DNA from the energy intensive interactions in the cytoplasm, nurse cells provision the egg so as to protect the DNA in the egg, sex serves to effectively repair genetic damage 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.

As just mentioned, according to Bell [4], 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 *Merillosphaera*. Assuming no cell death, a colony size of 10^3 cells would require a development time of approximately $t = 10$ in our model (see panel (F) of Figure 6 for the case $t = 10$; 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. Colony size increases as t increases, but so does the opportunity for conflict and the need to regulate within-group change.

4.7. Evolution of self-policing. We now consider another means of reducing conflict among cells, that of self-policing. In the model analyzed now there is assumed to be no germ line, although presumably a germ line and self-policing may operate together, that case is not explicitly studied here. Organisms may reduce conflict by actively policing and regulating the benefits of defection [7,17]. How might organisms police the selfish tendencies of cells? The immune system and programmed cell death are two possible examples. There are several introductions to the large and rapidly developing area of programmed cell death, or apoptosis [1,2,10]. 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, by assuming $s_C = s_D = 1$. Cooperating cells in policing organisms spend time and energy monitoring cells and reducing the advantages of defection to b - ε at a cost to the organism, δ. The parameter δ is now completely different from the germ line modifier δ; δ is now the fitness cost of self-policing at the organism level. To sum up, m genotypes are described by the parameters b and β, while in M genotypes, the benefit of cooperation becomes β - δ and the benefit of defection becomes b - ε.

(A)

$\beta = 3, t = 40, \mu = 3 \times 10^{-3}, \varepsilon = 0.05$

(B)

$\beta = 10, t = 40, \mu = 3 \times 10^{-3}, \varepsilon = 0.05$

(C)

$\beta = 3, t = 30, \mu = 3 \times 10^{-3}, \varepsilon = 0.05$

(D)

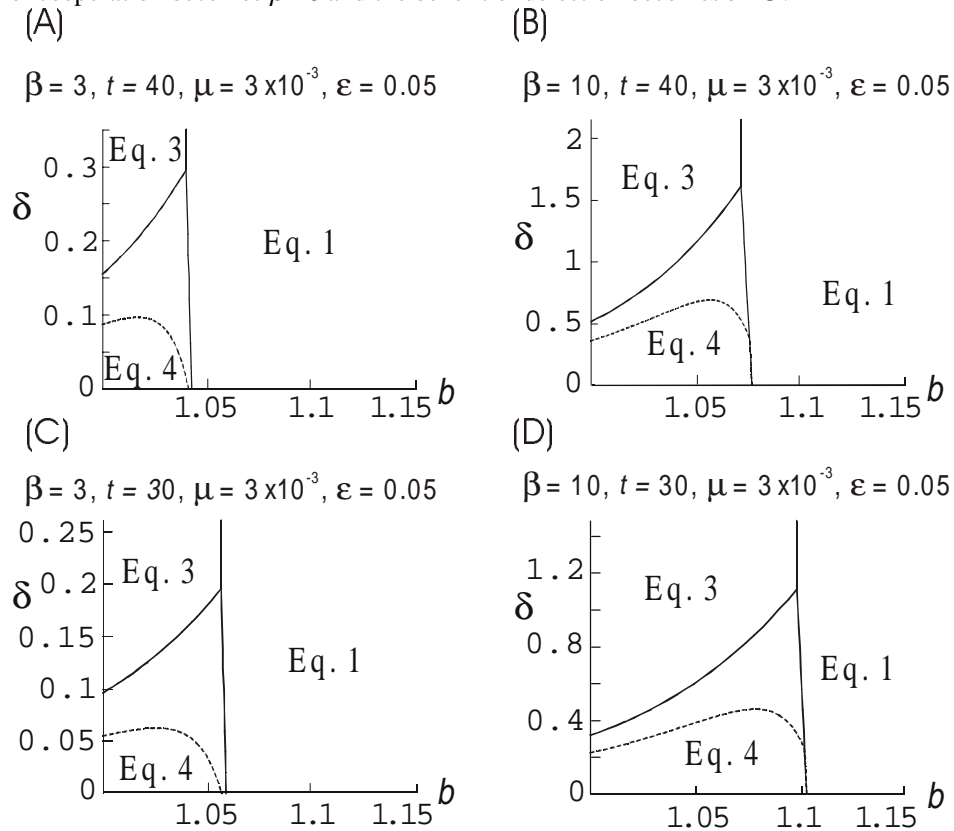$\beta = 10, t = 30, \mu = 3 \times 10^{-3}, \varepsilon = 0.05$

Figure 9. Stability of evolutionary equilibria for self-policing modifiers. Regions of stability of the different equilibria as a function of the advantage of defection, b, and the cost of policing, δ, for different values of the development time, t, and benefit of cooperation to the organism, β. The equilibria are described in Table 6. The modifier is assumed to decrease the advantage of defection by amount ε to b -ε ($\varepsilon = 0.05$ in all panels). Solid curves are for asexual reproduction

and dashed curves for sexual reproduction assuming a recombination rate of $r = 0.25$. See Figure 3 of Michod [36] for more detailed treatment of the boundary between equilibrium 3 and 4 in three dimensions (b, t, δ).

Figure 9 shows the regions of stability of the different equilibria given in Table 6 as a function of b and δ, for several values of development time, t, and benefit of cooperation at the whole organism level, β. The modifier increases (Equilibrium 4 is stable), if the cost of policing, δ, doesn't exceed the boundary between regions Eq. 3 and Eq. 4 in Figure 9.

In Figure 9, there is a threshold level of the benefit of defection, b, above which the organism cannot be maintained, with or without the modifier; equilibrium 1 is stable in this region. This region is defined as the nearly vertical line defining Eq. 1 in Figure 9. This threshold increases, permitting greater levels of defection, as the development time decreases (compare panel (C) with panel (A) and panel (D) with panel (B)). Once the modifier evolves (region Eq. 4), greater levels of defection are tolerated as the threshold slants to the right. The effect is more pronounced for higher levels of β ($\beta > 10$; results not shown, but one can see the general effect by comparing panels (B) and (A) of Figure 9). This effect also occurs in the case of the germ line modifier (see panel (B) of Figure 6).As the benefit of defection begins increasing, larger costs of policing are tolerated and the modifier still increases (boundary between Eq. 3 and Eq.4 tends upward as b increases from 1). Recombination (dashed curve) reduces the prospects for the policing modifier, as it did in the case of germ line modifiers (Figure 6), although the effect is larger in magnitude in the case of policing modifier. This effect of recombination becomes most pronounced as the Eq. 1 threshold is reached (Figure 6 and Figure 9), leading to the humped curve defining the boundary between Eq. 3 and Eq. 4.

There are important differences in how self-policing and germ line modifiers are modeled. In the case of the germ line, both the cost and the benefit of the M allele vary with δ, the reduction in development time in the germ line. The cost of the germ line increases as δ decreases. In the case of self-policing, the cost and the benefit are independent. In the graphics of Figure 9, the benefit of defecting at the cell level is fixed at $\varepsilon = 0.05$ (the replication rate of the D cells is lowered by 5%), while the cost to the organism of the policing defecting cells, δ, is given on the y-axis.

4.8. Summary of modifier evolution. Modifiers increase by virtue of being associated with more fit genotypes and by increasing the heritability of fitness of these types. For example, at Eq. 3, 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). This is what the eigenvalue conditions tell us, as can be seen by considering the eigenvalues in Table A 2. As discussed further elsewhere [39], the eigenvalues in Table A 2 are products of cell-group (organism) fitness and heritability at the cell group level (heritability decreasing with the amount of within-group change). It can be seen (Table A 2) that we need $W_2 > W_4$, for Eq. 3 to be stable ($\lambda_{33} < 1$) and Eq. 1 unstable ($\lambda_{12} = 1/\lambda_{33} > 1$) in the single locus case assuming asexual reproduction. Modifiers increase by virtue of increasing the heritability of fitness of the already more fit type and by hitchhiking along with these more fit chromosomes. They

increase the heritability of fitness of the already more fit type by decreasing the within-group change.

For the modifiers to increase, it is not necessary for cooperation per se to exist, only that there be a fitness difference between genotypes and further that the modifiers increase the heritability of fitness of the favored type. For example, if we considered a parallel situation of mutation from defection to cooperation (from D to C), the defection equilibrium (Eq. 1) would involve a mixture of types (just like Eq. 3). For equilibrium 1 to be stable in the first place (before the modifier is introduced) D groups must be more fit than groups composed of cooperators (more fit because of the assumed size advantage of D groups). We suspect that germ line modifiers would then increase, again by increasing the heritability of fitness, but this time heritability of fitness of D zygotes. However, in this situation, even though the modifiers increase, we would not want to speak of there being a transition to a new higher level individual or unit of selection. There cannot be a new individual, or unit of selection, at the level of the group without there being interactions among its members by virtue of which the group gains new functionality.

5.   Transitions in Individuality

5.1. Effect of transition on the level of cooperation. We now consider the consequences of an evolutionary transition from equilibrium 3 to 4 on the level of cooperation and synergism attained (this subsection) and on the heritability of fitness at the new organism level (in the next two subsections). For reasons of space, we only consider the evolution of the germ line, but qualitatively similar results have been obtained for the other forms of conflict mediation such as self-policing and determinate size. We also assume the case of cell selection in the germ line (Table 7), although similar results exist if there is no selection in the germ line (Table 8).

In Figure 10 the frequencies of the

Figure 10. Frequencies of cooperation and modifier during evolutionary transition. Germ line modifier refers to the M allele and cooperation to the C allele; asexual reproduction is assumed. The values of the parameters are $c=1$, $t=40$, $\delta=35$, $b=1.1$, $\beta=30$, $r=0$, $\mu=\mu_M=0.003$. These parameter values were chosen to illustrate the components of an evolutionary transition, as they produce large changes in the frequencies before and after the transition. However, as shown in Figure 6 (and Figure 9 for self-policing modifiers) a transition occurs for all parameter combinations in the "Eq. 4" regions of the different panels.

cooperation and modifier allele are plotted along with the linkage disequilibrium. We see that the transition dramatically increases the level of cooperation in the population,
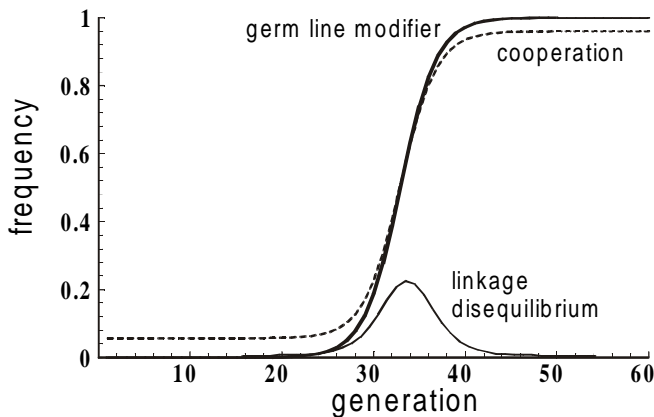
and that during the transition the coupling chromosomes (*CM* and *Dm*) predominate. The level of cooperation always increases during a transition, whatever the values of the parameters (if equilibrium 4 is stable). To understand the effect of this evolutionary transition on the regulation of the within-organism change and the heritability of fitness at the new level, we need to adapt the covariance methods of Price to the present system of equations.

5.2. Increase of fitness covariance at organism level. The recurrence equations above are derived by directly monitoring the numbers and frequencies of cells at the different life stages. An alternative method for representing selection in hierarchically structured populations is Price's covariance approach [43-45]. The covariance approach to the present situation is discussed in more detail elsewhere [37,39,40]. Price's approach posits a hierarchical structure in which there are two selection levels—in our case, (i) between cells within-organisms—viewed as a group of cells—and (ii) between-organisms within populations. Gene frequency change at the cooperate/defect locus is given in Equation 5,

$$\Delta q = \frac{\text{Cov}_\mathbf{q}[W_i, q_i]}{\overline{W}} + \text{E}_\mathbf{Wq}[\Delta q_i], \qquad \text{Equation 5}$$

with the following vectors used as weights $\mathbf{q} = (1-q, q)$, $\mathbf{Wq} = (W_D(1-q), W_C q)$. Variables q and q_i are the frequencies of a gene of interest in the total population and within zygotes; $\text{Cov}_\mathbf{q}[x, y]$ and $\text{E}_\mathbf{Wq}[x]$ indicate the weighted covariance and expected value functions respectively. The Price covariance Equation 5 partitions change to the two levels of selection. The first term of the Price equation is the covariance between fitness and genotype and represents the heritable aspects of fitness; the second term is the average of the within-organism change. The first term can be considered as representing the selection between-organisms within the population, and the second term the selection between cells within the organism. When the population is at an equilibrium, $\text{D}q = 0$ and so it must be the case that the two terms on the right hand side of Equation 5 equal one another in magnitude $\frac{\text{Cov}_\mathbf{q}[W_i, q_i]}{\overline{W}} = -\text{E}_\mathbf{Wq}[\Delta q_i]$.

In Figure 11, the two components of the Price covariance Equation 5 are plotted during the transition from equilibrium 3 to equilibrium 4 given in Figure 10 during the increase in frequency of the germ line allele, from 0 to 1 [43]. 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, since 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. This can be seen in Figure 11 by the fact that the two curves begin and end at the same point. However, during the transition we see that the covariance of individual fitness at the emerging organism level with zygote genotype (solid curve of Figure 11) is greater than the average change at the cell level (dashed curve of Figure 11).

This greater covariance in fitness at the higher level forces the modifier into the population. In Figure 11, we see that modifiers of within-organism change evolve by making the covariance between fitness at the organism level and zygote genotype more important than the average within-organism change. This implies that modifiers increase the heritability of fitness at the new level.
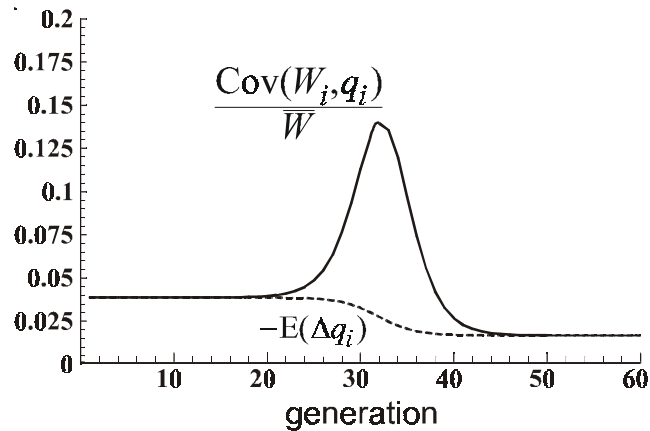

Figure 11. Study of evolutionary transition by Price equation. Same parameter values as Figure 10. Figure adapted from Michod and Roze [40].

5.3. Heritability of fitness and the evolution of individuality. Darwin argued that natural selection requires heritable variations in fitness [12]. Levels in the biological hierarchy—genes, chromosomes, cells, organisms, kin groups, groups—posses heritability of fitness to varying degrees according to which they may function as evolutionary individuals, or units of selection [24,28]. Beginning with Wilson [54] and the study of the transition from solitary animals to societies, then Buss [9] with the study of the transition from unicellular to multicellular organisms, and more recently Maynard Smith and Szathmáry [32,49], attention has focused on understanding transitions between these different levels of selection or different kinds of evolutionary individuals.

Before the evolution of cooperation, in the present model, the population is composed of Dm cell types (equilibrium 1 in Table 6). In such a population the heritability of fitness equals unity, because there is either no sex, or no effect of recombination if there were sex (in Dm x Dm matings), and there is no within-organism variation or change (we assume no mutation from D to C). When the C allele appears in the population, evolution (directed primarily by kin selection) may increase its frequency leading to greater levels of cooperation (from equilibrium 1 to equilibrium 3). With the evolution of greater cooperation, within-organism change increases, because of mutation from C to D and selection at the cell level. As a consequence of the evolution of cooperation, and increasing within-organism change, the heritability of fitness must decrease.

The organism cannot evolve new adaptations, such as enhanced cooperation, if these adaptations are costly to cells, without increasing the opportunity for conflict within and thereby decreasing the heritability of fitness. Deleterious mutation is always a threat to new adaptations by producing cells that go their own way. By regulating within-organism change, there is less penalty for cells to help the organism. Without a means to regulate within-organism change, the "organism" is merely a group of cooperating cells related by common descent. Such groups are not individuals, because they have no functions that exist at the new organism or group level.

The existence of a zygote stage in the life cycle serves to decrease the within-organism change by increasing the relatedness among cells. However, as showed elsewhere [37], within-organism change can be significant even in this case. The main criteria of significance is whether within-organism variation leads to selection of modifiers to reduce it. We have found that such modifiers increase in frequency leading to an evolutionary transition that we have interpreted as individuality because these modifiers represent the first higher level functions. However, does heritability of fitness—the defining characteristic of an evolutionary individual—actually increase during the transition between equilibrium 3 and 4?

Heritability of fitness at the new cell group or organism level may be defined as the regression of the fitness of offspring cell groups on fitness of the parent cell groups (see, for example, reference [16]). It can be shown that when the population is at equilibrium 3 or at equilibrium 4, this definition gives a simple expression for heritability equal to $h_W^2 = \dfrac{k_{22}}{k_2}$ at equilibrium 3 and equal to $\dfrac{K_{11}}{K_1}$ at equilibrium 4.

We always have $\dfrac{K_{11}}{K_1} > \dfrac{k_{22}}{k_2}$, so the evolutionary transition always leads to an increase in the heritability of fitness. If we go back to the eigenvalues of the different equilibria given in Table A 2 we can see that these eigenvalues are ratios between products of fitnesses and heritabilities. This illustrates clearly that what determines if a new characteristic can increase in frequency in the population is the heritability of fitness of individuals with the new feature.

During the transition between equilibrium 3 and equilibrium 4, all four genotypes are present in the population. It's not possible to simplify the heritability as above, however the following expression for heritability may still be used $h_W^2 = \dfrac{\mathrm{Cov}(W_P, W_O)}{\mathrm{Var}(W_P)}$, where W_P is the fitness of each parent cell group and W_O is average fitness of the offspring cell groups produced by the parents.

Initially, before the evolution of cooperation between cells, the heritability of fitness is unity. After cooperation evolves, because of high kinship, heritability is significant at the group (organism) level ($h_W^2 \approx 0.6$, Figure 12), but this value is still low for asexual haploidy (heritability at the organism
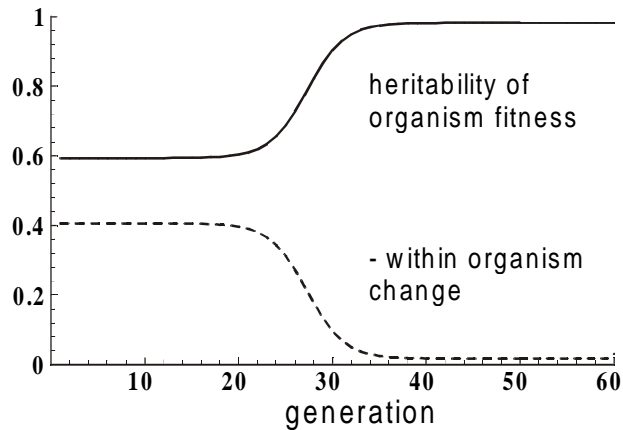

Figure 12. Heritability of organism fitness and within-organism change during evolutionary transition. Same parameter values as Figure 10. Figure adapted from Michod and Roze [40].

level should equal unity in the case of asexual organisms when there is no environmental variance). Low heritability of fitness at the new level resulting from

significant within-organism change posses a threat to continued evolution of the organism. In the case considered in Figure 12, development time, and hence organism size, could not increase without the evolution of conflict modifiers. Indeed, as already noted, the continued existence of cell-groups at all is highly unlikely, since the cooperation allele is at such a low frequency and stochastic events would likely lead to its extinction. Before the evolution of modifiers restricting within-organism change, the "organism" is just a group of cooperating cells related by common descent from the zygote. As the modifier begins increasing, the level of within-organism change drops (dashed curve Figure 12) and the level of cooperation among cells increases dramatically (dashed curve of Figure 10) as does the heritability of organism fitness (solid curve of Figure 12).

The essential conclusion is that even in the presence of high kinship among cells, there remains significant within-organism change, by "significant" we mean this change leads to the evolution of a means to regulate it, such as the segregation of a germ line during the development or the evolution of self-policing. Once within-organism change is controlled, high heritability of fitness at the new organism level is protected. Individuality at the organism level depends on the emergence of functions allowing for the regulation of conflict among cells. Once this regulation is acquired, the organism can continue to evolve new adaptations without increasing the conflict among cells, as happened when cooperation initially evolved (transition from equilibrium 1 to equilibrium 3).

6.   Conclusions

The models studied here support the view that single cell or spore reproduction, the germ line, self policing and determinant size evolved to increase the heritability of fitness and to help mediate conflict between cooperating and defecting cells. As a consequence, these adaptations served to facilitate a transition between cells and multicellular organisms. Development evolves, at least during its initial phases, so as to reduce the opportunity for conflict among cells. Having a germ line functions to reduce the opportunity for conflict among cells and promote their mutual cooperation both by limiting the opportunity for cell replication [9] and by lowering the mutation rate [32]. Mutual policing [7,17] is also expected to evolve as a means of maintaining the integrity of the organisms once they reach a critical size. Any factors that directly reduce the within-organism mutation rate are also favored. These modifiers of within-organism change during development increase by virtue of being associated with the cooperating genotype, which is more fit than the defecting genotype at the time when the modifiers are introduced (at equilibrium 3). The modifiers increase the heritability of fitness of the more fit type, in our case the cooperating type.

We have only recently discovered cases in which the population remains polymorphic for two reproductive strategies, one involving conflict mediation and the other not (for example, the polymorphic germ line equilibria in Figure 7 and Figure 8). These cases are of interest to us, because of their possible relevance to the mixed reproductive mode so common in plants, in which vegetative (propagule) reproduction and seed (or spore) reproduction are maintained together in populations. As studied in section 3, seed (or spore) reproduction can be seen as a mechanism to reduce conflict and can evolve in populations reproducing by fragmentation. Furthermore, the mode of reproduction has profound implications for mutation load and the distribution of

mutations. The growth habits of plants are more indeterminate than animals and plant fitness probably often depends upon organism size and the replication rates of component cells. Furthermore, levels and mechanisms of within-organism change are well documented in plants [3,14,18,25,53], and the models studied here seem especially relevant. It is often noted that plants don't have a germ line. The consequences of within-organism change are not as severe in plants as in animals, because plant cells cannot move. Because of the presence of a rigid cell wall in plants, the opportunity for systematic infections of cancerous cells is severely reduced [9].

Godelle and Reboud consider a class of two level selection models that, although different from ours in orientation and purpose (they consider primarily segregation distortion and do not explicitly interpret their results in terms of evolutionary transitions), have some similar properties and comparable results [20,21]. A single diploid locus is considered affecting between-organism and within-organism selection. Within-organism change is represented by segregation distortion in the heterozygote. A gene frequency equation is derived giving the total gene frequency change through a generation accounting for selection at both levels and, in addition, for inbreeding. Through analysis of the gene frequency equation and computer tournaments between invading mutants and resident alleles the evolutionary dynamics are characterized in terms of replacement of alleles by new mutations. In general [21] no constraints on the effects of the mutants on the two levels of selection are considered, although in [20] there is considered to be a trade-off between the performance of a mutation at the two levels. In reference [21] a "synthetic fitness function" is proposed that is maximized during the course of evolution and is a product of the fitnesses at the two levels. Inbreeding serves to favor between-organism selection and reduce within-organism change.

In our model modifiers of within- and between-group change are introduced at an equilibrium that is polymorphic for two kinds of group, those stemming from cooperating or defecting zygotes. By definition, cooperative genotypes bias the selection towards the group (organism) level (by taking fitness away from cells while increasing the fitness of the group), while defecting genotypes do the opposite and bias selection toward the cell level. To maintain cooperation (at equilibrium 3) cooperative groups must be more fit (produce more gametes) than defecting groups, because the fitness benefits of cooperation at the group level must compensate for mutation towards defection.

As discussed in subsection 4.8, modifiers of within and between-organism change increase by virtue of being associated with the more fit type (cooperation) when they are introduced and by increasing the heritability of fitness of this type. As a result, the modifiers increase the heritable component of fitness at the organism level—that is the product of heritability and fitness. This product function is similar to the synthetic fitness function of Godelle and Reboud in which the product of selection at the two levels is taken [21]. In reference [21], selection within the organism is due to segregation distortion, which can be viewed as reducing the heritability of the genotypic properties of the heterozygote (the heterozygote begins with an equal ratio of the two alleles but produces a different ratio among its gametes).

Heritability is also a simple function of the level of within-organism change in our model. The question of a transition to individuality reduces in our model to the conditions for a transition from equilibrium 3 to equilibrium 4 in Table 6 (cooperation with and without the modifier). The condition for increase of the modifier in the case of asexual reproduction is given as λ_{31} in Table A 2. The condition $\lambda_{31} > 1$ simply means that a cooperating organism must produce more C gametes with the modifier (*CM*) than without (*Cm*). As discussed elsewhere [39] the eigenvalue λ_{31} is a comparison of product of fitness and heritability for the *CM* and *Cm* genotypes $\left(W_1 K_{11}/K_1\right)\big/\left(W_2 k_{22}/k_2\right)$. In our model, heritability is inversely related to the level of within-organism change and equals K_{11}/K_1 and k_{22}/k_2 after and before the transition, respectively (see Figure 6-5 of [39]). As already mentioned in the last subsection, we always have $K_{11}/K_1 > k_{22}/k_2$, so the evolutionary transition leads to an increase in the heritability of fitness.

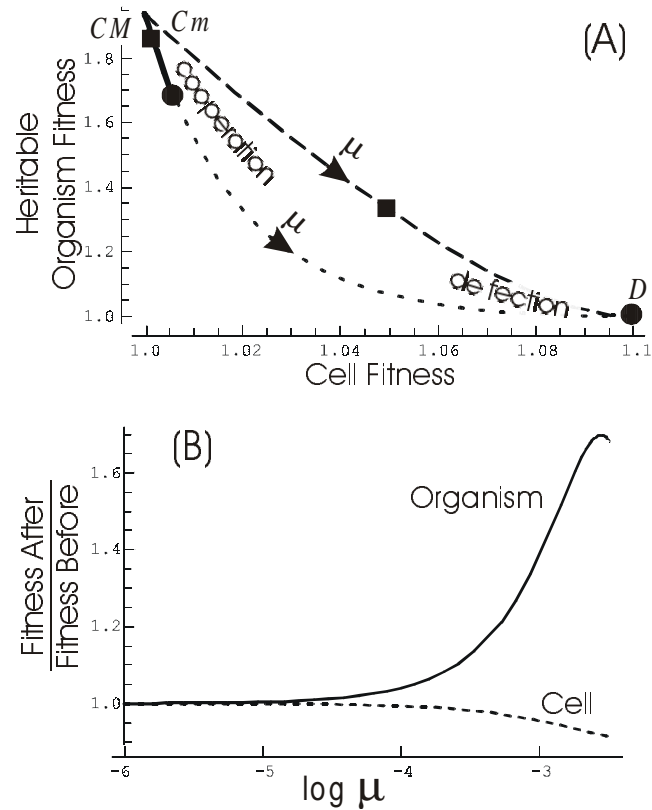An evolutionary transition is diagramed in Figure 13 for selfish

Figure 13. Transfer of fitness between levels for a evolutionary transition involving selfish mutations ($b = 1.1$). Same parameter values as in Figure 10, Figure 11, and Figure 12 except that in both panels the mutation rate μ is changing. Panel (A) is a parametric plot while panel (B) is a standard plot. In panel (A) the dashed line is for equilibrium 3 before the transition (*Cm*) and the solid-dotted line is for equilibrium 4 after the transition (*CM*). The modifier has no effect in defecting cells. Organism fitness depends on group size and functionality (Table 5). Average heritable organism fitness (expressed relative to defecting groups) is shown on the ordinate in panel (A) and is calculated as $\left(W_1 f_{11} x_1 + W_3(1 - x_1)\right)\big/W_3$ after the transition and $\left(W_2 f_{22} x_2 + W_4(1 - x_2)\right)\big/W_4$ before. Because the heritable fitness of cell groups is expressed relative to defecting groups, the heritable fitness of defecting groups is unity at all points in the figure. Average cell fitness is shown on the abscissa in panel (A) and is calculated as $cf_{11} x_1 + b(1 - x_1) + b(1 - f_{11}) x_1$ after the transition and $cf_{22} x_2 + b(1 - x_2) + b(1 - f_{22}) x_2$ before. Ratios of these fitnesses at the two levels are plotted on the ordinate in panel (B). See text for explanation. In panel (A), the points of equal mutation rates are indicated by solid squares for $\mu = 10^{-3}$, and solid circles for $\mu = 3.16 \times 10^{-3}$.

mutations with regard to the spectrum of fitness variation at the organism and cell levels before and after the transition as a function of the mutation rate, μ. The same parameter values are assumed in Figure 13 as in Figure 10, Figure 11, and Figure 12, except that in Figure 13 in both panels the mutation rate is not fixed but μ ranges from 10^{-6} to 3.16×10^{-3} (at which point equilibrium 3 no longer exists). In panel (A) the average heritable component of fitness (heritability times expected number of gametes) of the cell group, the organism, is plotted on the ordinate and the average fitness of the cell is plotted on the abscissa as a function of the mutation rate , μ. The calculation of these average heritable components of fitness is explained in the legend to the figure. Two curves are plotted parametrically in panel (A) as a function of the mutation rate. The top curve corresponds to the situation before the modifier increases (dashed line) and the bottom curve to the situation after the transition occurs (solid - dotted line). In both cases, as the mutation rate increases from near zero ($\mu = 10^{-6}$) to a high level, the population shifts from being primarily composed of cooperating groups (*CM* zygotes for the bottom curve and *Cm* zygotes for the top curve) to defecting groups (*D* zygotes). In other words, as the mutation rate increases, the fitness at the organism level must decrease and the fitness at the cell level must increase. This, after all, is the definition of selfish mutations (considered in the figure). However, the rate and manner in which this transfer of fitness occurs is quite different before and after the transition.

Before the transition (top dashed curve) the population shifts from nearly complete cooperation at $\mu = 10^{-6}$ to complete defection $\mu = 3.16 \times 10^{-3}$ (at which point equilibrium 3 no longer exists). For this same increase in the rate of mutation, the population after the transition (bottom curve in panel (A)) changes only a small amount in terms of its fitness at the two levels, as is shown by the solid portion of the curve (the solid circles correspond to the fitness at the two levels for a mutation rate of $\mu = 3.16 \times 10^{-3}$). Only the solid portion of the bottom curve is comparable to the entire dashed curve before the transition (panel (A) of Figure 13). In other words with conflict mediation in place the deleterious effects on fitness of selfish mutations are buffered for the organism. The rest of the bottom curve (the dotted portion) is generated by letting the mutation rate increase past 3.16×10^{-3} into ranges not permitted before the transition. The two curves must begin and end at approximately the same point because the modifier has little effect either when there is no mutation or a lot of mutation.

A study similar to that in Figure 13 where $b = 1.1$, can be made for uniformly deleterious mutations and this is done in Figure 14 for $b = 0.9$. As shown in Figure 6, the modifier increases in such cases (panel (B) of Figure 6 is closest to the situation studied in Figure 13 and Figure 14). The curves in panel (A) have a positive slope in Figure 14, because there is no longer any conflict between the fitness effects of mutation at the two levels. Mutation is deleterious at both levels. There is a dramatic effect of mutation on the fitness of the cell group, because mutation not only reduces the functionality of the group but decreases its size as well.

The ordinate axis in panels (A) of Figure 13 and Figure 14 are vastly different. Why does the heritability of fitness at the organism level change more drastically with the mutation rate, μ, when mutations are uniformly deleterious (as in Figure 14) than when they are selfish (as in Figure 13)? A given decrease in average cell fitness (the abscissa of panel A in both figures) has conflicting effects at the organism level in Figure 13 but not in Figure 14. Reducing the average deleterious nature of the mutations (moving from left to right along the abscissa of panel (A) of Figure 14),

dramatically increases the fitness of the organism by increasing its size as well as its functionality. On the other hand, reducing the advantage of defection (moving from right to left along the abscissa of panel (A) of Figure 13) has conflicting effects on organism fitness because it increases the functionality of the organism but also decreases its size. To be explicit, consider, the case with near zero mutation with little or no within-organism change. For selfish mutations the ratio of fitness of cooperating and mutant groups is about 1.9 (the upper left-hand corner of panel (A) Figure 13), while for uniformly deleterious mutations ($b = 0.9$) the ratio of fitness of cooperating and mutant groups is about 500 (the upper right-hand corner of panel (A) in Figure 14). Again, this is because with uniformly deleterious mutations the mutant groups are much smaller in size as well as being less functional than cooperating groups, while with selfish mutations the mutant groups are larger in size than cooperating groups (but less functional).
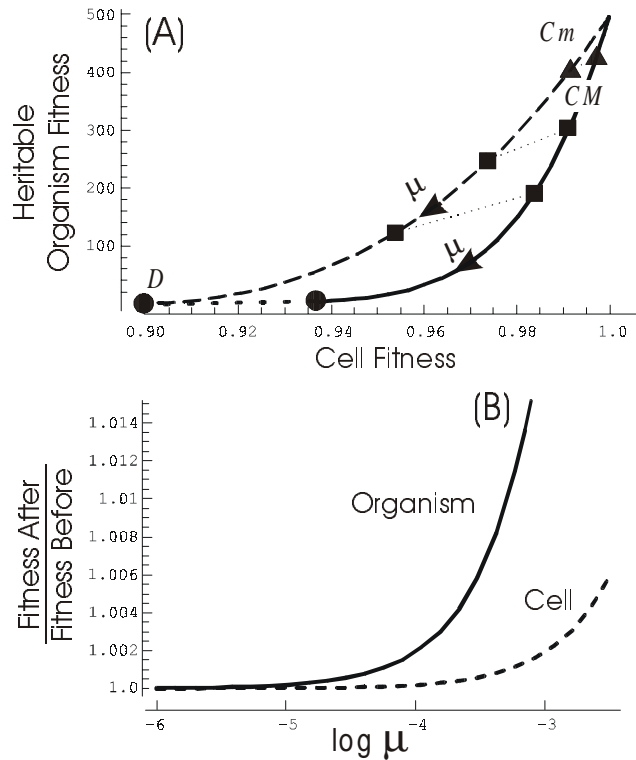

Figure 14. Fitness effects of increase of modifiers for uniformly deleterious mutations ($b = 0.9$). Same legend as Figure 13, except $b = 0.9$. In panel (A), the points of equal mutation rates are indicated by solid circles for $\mu = 10^{-1}$, solid squares for both $\mu = 10^{-2}$ and $\mu = 2 \times 10^{-2}$, and solid triangles for $\mu = 3.16 \times 10^{-3}$ in the top right portion of the panel (corresponding to the range of mutation rate considered in panel (B) and in Figure 13, do not confuse triangles with arrowheads by μ). In panel (B) the same range of mutation is considered as in panel (B) of Figure 13. Average heritable organism fitness (expressed relative to defecting groups) is shown on the ordinate in panel (A) and is calculated as $\left(W_1 f_{11} x_1 + W_3(1 - x_1)\right)/W_3$ after the transition and $\left(W_2 f_{22} x_2 + W_4(1 - x_2)\right)/W_4$ before. Because the heritable fitness of cell groups is expressed relative to defecting groups, the heritable fitness of defecting groups is unity at all points in the figure.

Why do the modifiers evolve in this case of uniformly deleterious mutations; there is no longer any conflict between levels to mediate? Genotypes with the modifier have a lower level of deleterious mutational error. As discussed in section 4.8, the modifiers increase by virtue of increasing the heritability of fitness of the more fit non-mutant type. Both cells and groups are more fit, as a consequence of the fact that the modifier decreases the effective mutation rate. Organisms benefit twice from their lower

mutation rate, because of their much larger size and enhanced functionality (panel (B) of Figure 14). The fitness of cells increases slightly as a result of the modifier although not as dramatically as at the level of the cell group.

The spectrum of fitness variation is similar for uniformly deleterious mutations (Figure 14) before and after the modifier increases, in dramatic contrast to selfish mutations (Figure 13). For example, a given decrease in the mutation rate (from 10^{-6} to 10^{-1}) affects the average heritable component of the fitness of the group by about the same amount (ordinate of Figure 14) whether the modifier is present or not, while the average fitness of cells has decreased to about 0.94 with the modifier compared to 0.90 without the modifier.

At all points along the curve in panels (A) of both Figure 13 and Figure 14, the heritable component of organism fitness is greater after the modifier increases than before. This result is shown clearly in panels (B) of both figures. The effect of the modifier on organism fitness is larger for selfish mutations than for uniformly deleterious mutations. There is one complication which is that when there is absolutely no mutation, the fitness at the organism level must be smaller after the modifier increases, because there is then no benefit of the modifier to offset the cost of allocating cells to the germ line. However, in the particular case considered in Figure 13 and Figure 14, the cost of the germ line is small because there are only five cell divisions in the germ line and so there is a relatively small number of cells in the germ line. This raises a limitation of the model, which is that the fitness of the organism (expected number of gametes produced) is assumed to scale with adult size and so to depend upon the number of cells in the soma, not the number of cells in the germ line. This may be reasonable, in many situations, but as the number of cells in the germ line decreases at some point the number of germ line cells must limit fitness. We have not included such an effect in our model to date, but plan to in the near future.

The basic consequence of an evolutionary transition is to move from a situation characterized by low between-group and high within-group change to the opposite situation characterized by high between-group and low within-group change. The fitness of defecting zygotes is not affected by the modifier, because there is no variation within D zygotes ($W_3 = W_4$). However, the heritability of fitness increases for cooperating zygotes. Concomitantly, the level of within-group change decreases as shown in Figure 12. As a result, after the transition, the fitness variation in Figure 13 is characterized by a steeper negative slope, indicating smaller variation within the cell group and greater variation between cell groups (organisms). After the transition the opportunity for within-group change is reduced while that for between-group change is enhanced.

The evolution of modifiers of within-organism change leads to increased levels of cooperation within the organism and increased heritability of fitness at the organism level. The evolution of these conflict mediators are the first new functions at the organism level. An organism is more than a group of cells related by common descent; organisms require adaptations that regulate conflict within. Otherwise their continued evolution is frustrated by the creation of within-organism variation and conflict. The evolution of modifiers of within-organism change are a necessary prerequisite to the emergence of individuality and the continued well being of the organism.

What happens during an evolutionary transition to a new higher level unit of individuality, in this case the multicellular organism? While taking fitness away from lower level units, cooperation increases the fitness of the new higher level unit. In this

way, cooperation may create new higher levels of selection. However, the evolution of cooperation sets the stage for conflict, represented here by the increase of mutants within the emerging organism. The evolution of modifiers restricting within-organism change are the first higher level functions at the organism level. Before the evolution of a means to reduce conflict among cells, the evolution of new adaptations (such as the underlying traits leading to increased cooperation among cells) is frustrated by deleterious and defecting mutants. Individuality requires more than just cooperation among a group of genetically related cells, it also depends upon the emergence of higher level functions that restrict the opportunity for conflict within and ensure the continued cooperation of the lower level units. Conflict leads—through the evolution of adaptations that reduce it—to greater individuality and harmony for the organism.

References

1. Ameisen, J. C. 1996. The origin of programmed cell death. *Science (Washington, D.C.)* 272: 1278-1279.
2. Anderson, P. 1997. Kinase cascades regulating entry into apoptosis. *Microbiological Reviews* 61: 33-46.
3. Antolin, M. F. and Strobeck, C. 1985. The population genetics of somatic mutation in plants. *American Naturalist* 126: 52-62.
4. 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, A. Monroy. 221-256. Alan R. Liss, Inc., New York.
5. Bell, G. and Koufopanou, V. 1991. The architecture of the life cycle in small organisms. *Philosophical Transactions of the Royal Society of London B, Biological Sciences* 332: 81-89.
6. Boraas, M. E., Seale, D. B., and Boxhorn, J. E. 1998. Phagotrophphy by a flagellate selects for colonial prey: A possible origin of multicellularity. *Evolutionary Ecology* 12: 153-164.
7. Boyd, R. and Richerson, P. J. 1992. Punishment allows the evolution of cooperation (or anything else) in sizable groups. *Ethology and Sociobiology* 13: 171-195.
8. Buss, L. W. 1985. The uniqueness of the individual revisited. In *Population Biology and Evolution of Clonal Organisms*, ed. J. B. C. Jackson, L. W. Buss, R. E. Cook. Yale University Press, New Haven.
9. Buss, L. W. 1987. *The Evolution of Individuality*, Princeton, NJ: Princeton University.
10. Carson, D. A. and Ribeiro, J. M. 1993. Apoptosis and disease. *Lancet* 341: 1251-1254.
11. Cushing, J. M. 1989. A strong ergodic theorem for some nonlinear matrix models for the dynamics of structured populations. *National Resource Modeling* 3: 331-357.
12. Darwin, C. 1859. *The Origin of Species by Means of Natural Selection, or Preservation of Favoured Races in the Struggle for Life*, London: John Murray.
13. Drake, J. W. 1991. A constant rate of spontaneous mutation in DNA-based microbes. *Proceedings of the National Academy of Sciences, USA* 88: 7160-7164.
14. Fagerström, T. 1992. The meristem-meristem cycle as a basis for defining fitness in clonal plants. *Oikos* 63: 449-453.

15. Fagerström, T., Briscoe, D. A., and Sunnucks, P. 1998. Evolution of mitotic cell-linages in multicellular organisms. *Trends in Ecology & Evolution* 13: 117-120.

16. Falconer, D. S. 1989. *Introduction to Quantitative Genetics*, Burnt Mill, England: Longman.

17. Frank, S. A. 1995. Mutual policing and repression of competition in the evolution of cooperative groups. *Nature (London)* 377: 520-522.

18. Gill, D. E., Chao, L., Perkins, S. L., and Wolf, J. B. 1995. Genetic mosaicism in plants and clonal animals. *Annual Review of Ecology and Systematics* 26: 423-444.

19. Gillott, M., Holen, D., Ekman, J., Harry, M., Boraas, M. E. 1993. Predation-induced *E. coli* filaments: Are they multicellular? In *Proceedings of the 51st Annual Meeting of the Microscopy Society of America*, ed. G. Baily, C. Reider. San Francisco Press, San Francisco, CA.

20. Godelle, B. and Reboud, X. 1995. Why are organelles uniparentally inherited. *Proceedings of the Royal Society of London B, Biological Sciences* 259: 27-33.

21. Godelle, B. and Reboud, X. 1997. The evolutionary dynamics of selfish replicators: a two-level selection model. *Journal of Theoretical Biology* 185: 401-413.

22. Haldane, J. B. S. 1937. The effect of variation on fitness. *American Naturalist* 71: 337-349.

23. Hopf, F., Michod, R. E., and Sanderson, M. J. 1988. The effect of reproductive system on mutation load. *Theoretical Population Biology* 33: 243-265.

24. Hull, D. L. 1981. Individuality and selection. *Annual Review of Ecology and Systematics* 11: 311-332.

25. Klekowski, E. J., Jr. 1988. *Mutation, Developmental Selection, and Plant Evolution*, New York: Columbia University Press.

26. Kondrashov, A. S. 1994. Mutation load under vegetative reproduction and cytoplasmic inheritance. *Genetics* 137: 311-318.

27. Leadbeater, B. S. C. 1983. Life-history and ultrastructure of a new marine species of *Proterospongia* (Choanoflagellida). *Journal of the Marine Biological Association of the United Kingdom* 63: 135-160.

28. Lewontin, R. C. 1970. The Units of Selection. *Annual Review of Ecology and Systematics* 1: 1-18.

29. Lewontin, R. C. 1978. Adaptation. *Scientific American* 239: 212-230.

30. Margulis, L. 1981. *Symbiosis in Cell Evolution*, San Francisco: W. H. Freeman.

31. Margulis, L. 1993. *Symbiosis in Cell Evolution, Microbial Communities in the Archean and Proterozoic Eons*, New York: W. H. Freeman.

32. Maynard Smith, J., Szathmáry, E. 1995. *The Major Transitions in Evolution*, San Francisco: W.H. Freeman.

33. Michod, R. E. 1980. Evolution of interactions in family structured populations: mixed mating models. *Genetics* 96: 275-296.

34. Michod, R. E. 1982. The theory of kin selection. *Annual Review of Ecology and Systematics* 13: 23-55.

35. Michod, R. E. 1995. *Eros and Evolution: A Natural Philosophy of Sex*, Reading, Mass.: Addison-Wesley.

36. Michod, R. E. 1996. Cooperation and conflict in the evolution of individuality. II. Conflict mediation. *Proceedings of the Royal Society of London B, Biological Sciences* 263: 813-822.

37. Michod, R. E. 1997. Cooperation and conflict in the evolution of individuality. I. Multi-level selection of the organism. *American Naturalist* 149: 607-645.
38. Michod, R. E. 1997. Evolution of the Individual. *American Naturalist* 150: S5-S21.
39. Michod, R. E. 1999. *Darwinian Dynamics, Evolutionary Transitions in Fitness and Individuality*, Princeton, N.J.: Princeton University Press.
40. Michod, R. E. and Roze, D. 1997. Transitions in individuality. *Proceedings of the Royal Society of London B, Biological Sciences* 264: 853-857.
41. Morrell, V. 1996. Genes versus teams. *Science (Washington, D.C.)* 273: 739-740.
42. Otto, S. P. and Orive, M. E. 1995. Evolutionary consequences of mutation and selection within an individual. *Genetics* 141: 1173-1187.
43. Price, G. R. 1970. Selection and covariance. *Nature (London)* 227: 529-531.
44. Price, G. R. 1972. Extension of covariance selection mathematics. *Annals of Human Genetics* 35: 485-490.
45. Price, G. R. 1995. The nature of selection. *Journal of Theoretical Biology* 175: 389-396.
46. Shikano, S., Luckinbill, L. S., and Kurihara, Y. 1990. Changes of traits in a bacterial population associated with protozoal predation. *Microbial Ecology* 20: 75-84.
47. Stanley, S. M. 1973. An ecological theory for the sudden origin of multicellular life in the Late Precambrian. *Proceedings of the National Academy of Sciences, USA* 70: 1486-1489.
48. Szathmáry, E. 1994. Toy models for simple forms of multicellularity, soma and germ. *Journal of Theoretical Biology* 169: 125-132.
49. Szathmáry, E. and Maynard Smith, J. 1995. The major evolutionary transitions. *Nature (London)* 374: 227-232.
50. Urushihara, H. 1992. Sexual development of cellular slime molds. *Development Growth & Differentiation* 34: 1-7.
51. Vogel, F. and Rathenberg, R. 1975. Spontaneous mutations in man. *Advances in Human Genetics* 5: 223-318.
52. Wade, M. J. 1985. Soft selection, hard selection, kin selection, and group selection. *American Naturalist* 125: 61-73.
53. Whitham, T. G. and Slobodchikoff, C. N. 1981. Evolution by individuals, plant-herbivore interactions, and mosaics of genetic variability: the adaptive significance of somatic mutations in plants. *Oecologia (Berlin)* 49: 287-292.
54. Wilson, E. O. 1975. *Sociobiology: The New Synthesis*, Cambridge, MA: Belknap Press of Harvard Univ. Press.

Appendix: equilibria for modifier model (with $G = 0$)

There are four dynamical equations (Equation 4) and the equilibria described in Table 6, and given mathematically in Table A 1, are obtained by setting the change in genotype frequencies equal to zero, along with $G = 0$ (Equation 3). The modifier allele may either be absent (Eq. 3) or fixed (Eq. 4) in the population, because there is no mutation affecting this allele.

There are four state variables corresponding to the frequencies of the four gamete types, but only three are independent, since they must sum to one. Consequently, there are three eigenvalues determining the stability of the system stemming from the Jacobian matrix of the linearized system evaluated at the different equilibria in Table A

2. Some of these eigenvalues depend on whether reproduction is sexual or asexual and the value of the recombination rate r. As seen in Table A 2, the eigenvalues are ratios of fitnesses multiplied by cell type frequencies (C or D). These eigenvalues can be expressed as ratios of fitnesses multiplied by heritability of fitness at the organism level. Equilibrium 2 (no cooperation, modifier allele fixed) is never stable, as we assume that the modifiers of within-organism change accrue some cost. Because of the cost of the modifier, $W_4 > W_3$, whatever the values of the parameters, and so $\lambda_{23} > 1$. This means that the evolution of functions to 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 which sets the stage for the evolution of individuality.

Eq.	Genotype Frequencies	Allele Frequencies	Description of Loci	Interpretation
1	$x_1 = 0$, $x_2 = 0$, $x_3 = 0$, $x_4 = 1$	$q = 0$, $s = 0$	no cooperation; no modifier	*single cells, no organism*
2	$x_1 = 0$, $x_2 = 0$, $x_3 = 1$, $x_4 = 0$	$q = 0$, $s = 1$	no cooperation; modifier fixed	not of biological interest
3	$x_1 = 0$ $x_2 = \dfrac{W_2 \dfrac{k_{22}}{k_2} - W_4}{W_2 - W_4}$ $x_3 = 0$ $x_4 = \dfrac{k_{42} W_2}{k_2 (W_2 - W_4)}$	$q = \dfrac{W_2 \dfrac{k_{22}}{k_2} - W_4}{W_2 - W_4}$ $s = 0$	polymorphic for cooperation and defection no modifier	*group of cooperating cells*: no higher level functions
4	$x_1 = \dfrac{W_1 \dfrac{K_{11}}{K_1} - W_3}{W_1 - W_3}$ $x_2 = 0$ $x_3 = \dfrac{K_{31} W_1}{K_1 (W_1 - W_3)}$ $x_4 = 0$	$q = \dfrac{W_1 \dfrac{K_{11}}{K_1} - W_3}{W_1 - W_3}$ $s = 1$	polymorphic for cooperation and defection modifier fixed	*organism*: integrated group of cooperating cells with higher level function mediating within-organism conflict

Table A 1. Equilibria of Two Locus Modifier Model (Equation 4) with $G = 0$ (Equation 3). The variables q and s are the frequencies of the C and M alleles in the population.

The eigenvalues of the other three equilibria, in the case of asexual reproduction, indicate that two or three equilibria cannot be stable at the same time. In the case of sexual reproduction, this is no longer true. Although we have not been able to determine this analytically, because we have not obtained simple expressions for the eigenvalues ($\lambda_{31}, \lambda_{32}, \lambda_{41}$ and λ_{43} in Table A 2), as discussed in the text, using numerical experiments, we have discovered some regions in which equilibrium 1 and 4 are both stable and regions in which none of the four equilibria are stable. As discussed in the text, in the latter case there exist equilibria with $G > 0$.

	Equilibrium 1			Equilibrium 2		
	λ_{11}	λ_{12}	λ_{13}	λ_{21}	λ_{22}	λ_{23}
asexual	$\dfrac{W_1\dfrac{K_{11}}{K_1}}{W_4}$	$\dfrac{W_2\dfrac{k_{22}}{k_2}}{W_4}$	$\dfrac{W_3}{W_4}$	$\dfrac{W_1\dfrac{K_{11}}{K_1}}{W_3}$	$\dfrac{W_2\dfrac{k_{22}}{k_2}}{W_3}$	$\dfrac{W_4}{W_3}$
sexual	$\dfrac{(1-r)W_1\dfrac{K_{11}}{K_1}}{W_4}$	$\dfrac{W_2\dfrac{k_{22}}{k_2}}{W_4}$	$\dfrac{W_3}{W_4}$	$\dfrac{W_1\dfrac{K_{11}}{K_1}}{W_3}$	$\dfrac{(1-r)W_2\dfrac{k_{22}}{k_2}}{W_3}$	$\dfrac{W_4}{W_3}$

	Equilibrium 3			Equilibrium 4		
	λ_{31}	λ_{32}	λ_{33}	λ_{41}	λ_{42}	λ_{43}
asexual	$\dfrac{W_1\dfrac{K_{11}}{K_1}}{W_2\dfrac{k_{22}}{k_2}}$	$\dfrac{W_3}{W_2\dfrac{k_{22}}{k_2}}$	$\dfrac{W_4}{W_2\dfrac{k_{22}}{k_2}}$	$\dfrac{W_2\dfrac{k_{22}}{k_2}}{W_1\dfrac{K_{11}}{K_1}}$	$\dfrac{W_3}{W_1\dfrac{K_{11}}{K_1}}$	$\dfrac{W_4}{W_1\dfrac{K_{11}}{K_1}}$
sexual	$\dfrac{a_1+\sqrt{b_1}}{c_1}$	$\dfrac{a_1+\sqrt{b_1}}{c_1}$	$\dfrac{W_4}{W_2\dfrac{k_{22}}{k_2}}$	$\dfrac{a_2-\sqrt{b_2}}{c_2}$	$\dfrac{W_3}{W_1\dfrac{K_{11}}{K_1}}$	$\dfrac{a_2+\sqrt{b_2}}{c_2}$

Table A 2. Eigenvalues for asexual and sexual reproduction for increase of modifier. For each of the four equilibria described in Table A 1, the eigenvalues are given for both asexual (top) and sexual (reproduction). Although the equilibrium frequencies do not depend upon reproductive system, the stability does. The eigenvalues are ratios of products of group fitnesses and heritabilities [39]. For example, at equilibrium 3 the only genotypes in the population are genotypes 2 (*Cm*) and 4 (*Dm*). In the case of sexual reproduction a_1, b_1, c_1 and a_2, b_2, c_2 are complicated terms given in Table A 3.

$$a_1 = (1-r)K_{11}k_2W_1(W_2-W_4)^2$$
$$+K_1\begin{pmatrix} r(W_1-W_3)(W_2-W_4)(k_{22}W_2-k_2W_4) \\ +k_2W_3(W_2-W_4)^2 \end{pmatrix}$$

$$a_2 = K_{11}W_1\begin{pmatrix} -rK_{11}k_2W_1W_2 - (1-r)k_{22}K_1W_1W_2 \\ +(1-r)k_{22}K_1W_2W_3 + rk_2K_1W_2W_3 \\ +rK_{11}k_2W_1W_4 \\ -k_2K_1W_1W_4 + (1-r)k_2K_1W_3W_4 \end{pmatrix}$$

$$b_1 = -4K_{11}k_2^2K_1W_1W_3(1-r)(W_2-W_4)^3 +$$
$$\left(\begin{matrix} -(1-r)K_{11}k_2W_1(W_2-W_4)^2 \\ +K_1\begin{pmatrix} -r(W_1-W_3)(W_2-W_4)(k_{22}W_2-k_2W_4) \\ -k_2W_3(W_2-W_4)^2 \end{pmatrix} \end{matrix}\right)^2$$

$$b_2 = 4(r-1)k_{22}K_{11}^2k_2K_1^2W_1^2W_2(W_3-W_1)^2W_4$$
$$+K_{11}^2W_1^2\begin{pmatrix} rK_{11}k_2W_1W_2 + (1-r)k_{22}K_1W_1W_2 \\ -(1-r)k_{22}K_1W_2W_3 - rk_2K_1W_2W_3 \\ -rK_{11}k_2W_1W_4 \\ +k_2K_1W_1W_4 - (1-r)k_2K_1W_3W_4 \end{pmatrix}^2$$

$$c_1 = 2k_{22}K_1W_2(W_2-W_4)^2$$

$$c_2 = 2K_{11}^2k_2W_1^2(W_3-W_1)$$

Table A 3. Terms for eigenvalues under sexual reproduction given in Table A 2.

Richard E. Michod is in the Department of Ecology and Evolutionary Biology University of Arizona, Tucson, AZ 85721, email address: michod@u.arizona.edu.

Denis Roze is in the Department of Ecology and Evolutionary Biology University of Arizona, Tucson, AZ 85721, email address: roze@u.arizona.edu.