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Cooperation and conflict in the evolution of individuality. II. Conflict mediation

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SUMMARY

Evolutionary transitions in the units of selection require the promotion of cooperation and the regulation of conflict among the lower level units. For multicellular organisms to emerge as a new unit of selection, the selfish tendencies of their component cells had to be controlled. Theoretical results indicate organisms may regulate this internal conflict and competition in several ways: by reducing the somatic mutation rate, by sequestering cells in a germ line and by directly reducing the benefits to cells of defecting.

1. INTRODUCTION

An organism is a group of cooperating cells. Selection among cells could weaken this harmony and threaten the organism's individual integrity. Unbridled competition at the cell level could favour cancer-like defecting cells that pursue their own interests at the expense of the organism. Defecting cells could either over replicate or migrate quickly to the gametes, thereby increasing their chances of being represented in the gametes and detracting from organism fitness. For the organism to maintain its integrity and individuality in the face of this internal conflict, ways must have been found to regulate the selfish tendencies of cells and to promote their cooperative interactions. Otherwise the organism could not exist as a unit of selection.

According to Buss (1987), the individual integrity of complex animal organisms is made possible by the germ line: the sequestered cell lineage set aside early in development for production of gametes. By sequestering a group of cells early in development, the opportunity for variation and selection is limited. Maynard Smith and Szathmary disagree with Buss's adaptive explanation of the germ line and argue that kinship among cells in an organism is sufficient to regulate the selfish tendencies of cells (Maynard Smith & Szathmary 1995; Szathmary & Maynard Smith 1995). By often reproducing through a single cell stage – the zygote – organisms insure close genetic relatedness among their component cells. This close relatedness helps to preserve the organism's individual integrity (Maynard Smith & Szathmary 1995; Maynard Smith 1988*b*). Another hypothesis argues that organisms evolve means of directly 'policing' the selfish tendencies of their component cells, thereby reducing the benefits of defection even if this costs the organism (Frank 1995).

Missing from these discussions is a quantitative framework for evaluating and comparing the different hypotheses. Indeed, Maynard Smith and Szathmary do not find their own arguments decisive and invite

quantitative work on the subject, 'the question is important, and we do not regard our arguments as decisive' (Maynard Smith & Szathmary 1995, p. 245). In a previous paper (Michod 1996), I develop a framework that may be used to investigate these hypotheses. I find that the levels of cooperation can be low in diploid organisms that do not have a germ line or do not directly regulate the selfish tendencies of cells, even with zygote reproduction and high levels of kinship among cells. My results suggest there is a significant problem in coping with within-organism variation and selection even in zygote derived organisms. Here I show this problem will select for a germ line once within-organism variation reaches a critical level. My results also indicate that adaptations to reduce the somatic mutation rate and to police the selfish tendencies of cells can be expected to evolve once organisms reach a critical size.

2. WITHIN-ORGANISM VARIATION AND SELECTION

Deleterious mutation leads to loss of cell and tissue function leading to the proliferation of uncooperative cells. The model presented in §3 represents this process in terms of four basic variables: β is the benefit to organisms of cooperation among their component cells, b is the benefit to cells of not cooperating in terms of their rate of replication, μ is the rate of mutation leading to loss of cell and tissue function and t is the time available for development. Organism size scales with the number of cells (Schmidt-Nielsen 1984) which in turn depends upon t , the time available for cell division. The complexity of interaction among different cell types and tissue functions is assumed to be represented by two kinds of interactions: cooperate and defect. Representing different synergistic and competitive forms of cell interactions by this dyad is really no different from representing the interactions of a wasp colony, with diverse castes and functions, by positing cooperative and non-cooperative strategies. This approach has aided the understanding of the

evolution of social behaviour of organisms within social groups and I hope a similar approach will prove useful in the study of the behaviour of cells within organisms.

Because of the hierarchical nature of selection within and between organisms there are two levels of selection at which to consider mutational effects: the cell and organism. This leads to a 2×2 classification scheme, $+/+$, $+/-$, $-/+$, $-/-$, with the effect of the mutation on the cell given on the left and the effect of the mutation on the organism given on the right. Mutations ($+/+$) which by luck benefit both the fitness of cells and the fitness of the whole organism will sweep through the population: there is little reason to model them explicitly. Likewise for mutations which detract from the fitness of both levels ($-/-$), except they will of course be lost from the population. There is some evidence for the $-/-$ kind of effect (Demerec 1936). In this case ($-/-$) the occurrence of selection among cells within the organism may have the benefit of lowering the overall mutation load in the population of organisms and this effect has been considered by several authors (Crow 1970; Whitham & Slobodchikoff 1981; Otto & Orive 1995). Mutations which benefit the cell's replication rate but detract from organism fitness ($+/-$) are the case of interest here, since they threaten the integrity of the organism. Considerable evidence exists for this kind of mutation in animals – most notably malignant cancer mutants. In plants malignant cancer is rarely a problem because plant cells have a cell wall and are not highly mobile. The other class of mutations which harm the cell but benefit the organism ($-/+$) can be addressed by a simple adjustment of the parameters in the models given below.

There is considerable evidence that within-organism mutation and selection among cells threatens the individual integrity of organisms. Both somatic mutation (Nowell 1976; Dennis *et al.* 1981; Farber 1984; Temin 1988; Blumenthal 1992; Ramel 1992; Coppes *et al.* 1993; Hague *et al.* 1993; Ionov *et al.* 1993; Kupryjanczyk *et al.* 1993; Chigira & Watanabe 1994; Miyaki *et al.* 1994; Nielsen *et al.* 1994; Shibata *et al.* 1994; Akopyants *et al.* 1995; Hoff-Olsen *et al.* 1995; Talbot *et al.* 1995; Tsiotou *et al.* 1995) and within-organism selection (Nowell 1976; Dennis *et al.* 1981; Michelson *et al.* 1987; Temin 1988; Gatenby 1991) are critical in the development of many human cancers. Human somatic mutation rates in tissue culture are similar to rates of naturally occurring mutations in the germ line when expressed on a per cell division basis (Kuick *et al.* 1992). Consequently, I expect naturally occurring rates of somatic mutation to be higher than germ line rates on a per-cell-division basis. Evidence for selection among mutant somatic cells exists in plants (Gaul 1958; Stewart *et al.* 1972; Stewart 1978; Whitham & Slobodchikoff 1981), where somatic mutation and selection creates genetic mosaics (Whitham & Slobodchikoff 1981; Klekowski Jr & Kazarinova-Fukshansky 1984). Selection among cells within the organism depends upon cells being able to express their own characteristics based on their own genotype, as has been observed in plants (Stewart *et al.* 1972; Stewart 1978). Somatic selection is likely to be

strong in modular organisms which undergo continuous mitotic proliferation and/or develop by budding as in corals, aspens, creosote or *Hydra* (Hughes 1989). Cellular selection may be an important defence against aging in constantly replicating cell lineages (Bernstein & Bernstein 1991). For example, blood-forming cells and the epithelial cells that line the intestines replicate continuously and do not appear to age, while liver, brain or muscle cells do not divide once they are fully differentiated and do age. As a result of within-organism selection, asexual plants may be able to cope with DNA damage and live for a long time (Michod 1995).

3. TWO LOCUS MODIFIER MODEL

For simplicity and analytical tractability, I consider two gene loci in a model haploid organism. The first locus determines whether cells within the adult organism cooperate or defect. The second locus is a modifier locus and modifies the parameters of selection and variation at the first locus. The definition of all terms and variables is given in table 1. The fitness of the zygote, W_j , is the absolute number of gametes produced by an adult organism that developed from a zygote of genotype j . Fitness is assumed to depend both upon the number of cells in the adult form and how the cells interact. Cells either cooperate or defect with other cells in the organism according to the cell's genotype at the C/D locus. Cooperation among cells increases the fitness of the adult (parameter β in table 1) but non-cooperating cells replicate faster (parameter b in table 1) and produce a larger but less functional adult. The simple model considered in table 1 assumes a linear dependence of organism fitness on frequency of cooperating cells in the adult stage, although more complex models could be easily incorporated into the framework considered here. Organism size is assumed to be indeterminate and to depend on the time available for development as well as the rate at which cells divide. Organisms are referred to by the genotype of the zygote they started from. Even though using a probabilistic model of development (table 2), the

Table 1. *Two locus haploid model of cell interaction and development*

variable	definition
i, j	index for genotype 1, 2, 3, 4 = CM, Cm, DM, Dm
k_{ij}	number of i cells in the adult stage of a j -zygote
k_j	total number of cells in adult stage of j -zygote
\bar{K}_{ij}	number of i cells in the germ line of a j -zygote
\bar{K}_j	total number of cells in the germ line of j -zygote
W_j	adult fitness of j -zygote: $W_j = k_j + \beta(k_{1j} + k_{2j})$
β	benefit to adult organism of cooperation among cells
r	recombination rate between C/D and M/m loci
x_j	frequency of j genotype in total population
μ	mutation rate from C to D per cell division
t	time for development
c	rate of cell division for cooperating cells
b	advantage to cell of defection (replication rate)
cb	rate of cell division for defecting cells ($b > 1$)

Table 2. Numbers of different cell types in adult and gamete stages

(The entries in the table equal k_{ij} in the case of a cost free germ line modifier. Zygote genotype is given across the top (column) and the genotype of the cells after development is given down the rows. In the case of a germ line allele, M , the numbers of different genotypes in the gametes, K_{ij} , are given by the same table except that t is replaced by t_M and μ is replaced by μ_M in the CM column. 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 given in Otto & Orive (1995) and Michod (1996). I do not consider mutations at the germ line locus, as I am interested in 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 mutual policing allele.)

	CM	Cm	DM	Dm
CM	$2^{ct}(1-\mu)^{ct}$	0	0	0
Cm	0	$2^{ct}(1-\mu)^{ct}$	0	0
DM	$\frac{\mu 2^{bct} - 2^{ct}(1-\mu)^{ct}\mu}{-1 + 2^{b-1} + \mu}$	0	2^{bct}	0
Dm	0	$\frac{\mu 2^{bct} - 2^{ct}(1-\mu)^{ct}\mu}{-1 + 2^{b-1} + \mu}$	0	2^{bct}

resulting dynamical equations are deterministic because they make use of the expected frequencies of different cell types in the different life history stages.

Three life history stages are considered: the zygote, the adult and the gamete. Zygote reproduction represents a 'worst case' for the creation of within-organism variation. Many organisms, especially plants and invertebrate animals, reproduce asexually by budding or fragmentation of many cells at a time. In these cases the within-organism variation present in the parent may be passed on to the offspring and these forms of reproduction may also be incorporated into the framework proposed here. Because of within-organism mutation and selection during development both the adult stage and the gamete stage may have cells of different genotype than the zygote. The k and K variables in tables 1 and 2 refer to numbers of cells of different genotypes in the adult and gamete stages, respectively. There are two forces that may change gene frequency between the zygote and adult stages and determine the values of the k and K variables: mutation and cellular selection. Mutation leads to loss of cooperation and tissue function. Mutation also increases the variance among cells within organisms and enhances the scope for selection and conflict. Cellular selection results from differences in cell replication rate.

The second locus is a modifier locus with two alleles M and m . The modifier locus may either be 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). In either case it is straight forward to write down the new genotype frequency in the case of asexuals. Define the column vector of genotype frequencies $\mathbf{x}_t = [x_{1t}, x_{2t}, x_{3t}, x_{4t}]^T$ and the \mathbf{W} matrix in which the ij element is the number of gametes of genotype i produced by a zygote of genotype j : $\mathbf{W}_{ij} = W_j(K_{ij}/K_j)$. The new vector of genotype frequencies is given in equation (1).

$$\mathbf{x}_{t+1} = \frac{\mathbf{W}\mathbf{x}_t}{\bar{W}_t} \quad (1)$$

In the case of sexual reproduction, I assume a haploid life cycle with gamete production, fusion of gametes into diploid zygotes followed immediately by meiosis with recombination and generation of the haploid state. For each haploid genotype i , I define a recombination matrix, \mathbf{R}_i , in which the k, l element of the recombination matrix for gamete i is the frequency of genotypes produced by a mating between genotypes k and l . Using these recombination matrices (given in Eq. 2 of Michod & Hasson (1990)), the four recurrence equations for sexual haploids may be written in a compact matrix form as in equation (2).

$$x_{i,t+1} = \frac{(\mathbf{W}\mathbf{x}_t)^T \mathbf{R}_i (\mathbf{W}\mathbf{x}_t)}{\bar{W}_t^2} \quad i = 1, 2, 3, 4. \quad (2)$$

When will the modifier allele increase if it were to arise in a population? Evolution of the complete two locus system is described by equation (1) for the case of asexual reproduction and by equation (2) for the case of sexual reproduction. Consider the initial situation in which the population does not have the M modifier allele so that the m allele is fixed. Because of mutation from C to D , fixation of the C allele is not possible. An internal equilibrium given in equation (3) exists when $W_2 > W_4$ ($W_4 = 2^{bct}$, $W_2 = k_{22} + k_{42} + \beta k_{22}$).

$$\hat{x}_1 = 0, \hat{x}_2 = \frac{W_2 \frac{k_{22}}{k_2} - W_4}{(W_2 - W_4)}, \hat{x}_3 = 0, \hat{x}_4 = 1 - \hat{x}_2. \quad (3)$$

The equilibrium given in equation (3) applies to both the sexual and asexual system, since when there is fixation of the m allele there is no effect of recombination (the stability of the equilibrium differs for the two reproductive systems, however).

For quantitative analysis, I need to specify the numbers of different cell types in the adult, k_{ij} , and in the germ line, K_{ij} . As cells proliferate during development of the adult form (time t), mutations (rate μ) occur leading to loss of tissue function and cooperativity among cells. I consider only mutations from C to D (no back mutation) as this represents a worse case for the evolution of intercellular coop-

eration. It is also reasonable for biological reasons because it is far easier to lose a complex trait like cell and tissue function than to gain it. A simple probabilistic model of mutation and cellular selection based on cell replication (not viability) has been studied previously (Otto & Orive 1995; Michod 1996) and is extended to the problem of a separate germ line in table 2. I now consider the stability of the equilibrium in equation (3) to introduction of a new modifier allele which either produces a germ line or polices the benefits of defection.

As already mentioned, the essence of a germ line is that gametes come from a different cell lineage from somatic cells in the adult form. Consequently in M zygotes, $k_{ij} \neq K_{ij}$ and $k_i \neq K_i$. In a zygote with the m allele there is no germ line in the sense that gametes are taken from the cells that make up the adult organism. In this case the 'germ line' stage is identical to the adult stage. As a result $k_{ij} = K_{ij}$ and $k_i = K_i$ in tables 1 and 2 (m zygotes). Regardless of whether an organism has a germ line or not, its fitness – number of gametes produced – depends upon the cell frequencies and interactions in the adult organism (k_{ij} and k_i). However, the genotypic makeup of the gametes depends on the within-organism variation in the germ line (K_{ij} and K_i). The number of cell divisions may be smaller in the germ line than in the soma. This is modelled by assuming a different time available for cell replication in the germ line: $t_M = t - \delta$. The mutation rates may also differ between the germ line, μ_M , and the soma, μ .

Consider the stability of the equilibrium given in equation (3) to the increase of a germ line modifier, the M allele. The elements of the vector of genotype frequencies \mathbf{x}_i must sum to one so there are three independent equations describing evolution. The three eigenvalues of the system at the equilibrium given in equation (3) determine whether fixation of the m allele is stable or not. If the equilibrium is unstable the new germ line allele M will increase and the population will evolve a germ line. The eigenvalues are given in equation (4) for the case of asexual reproduction. Each eigenvalue involves a zygote fitness multiplied by a diluting factor equal to the frequency of cells in gametes that are of the same genotype as the zygote. The diluting factors represent the essence of the problem of development: the risk of generating within-organism variation. The first eigenvalue describes the one locus system without a germ line locus and describes increase of the Dm genotype (it is the inverse of the eigenvalue in Eq. 5 of (Michod 1996)). I assume the equilibrium is stable in the one locus setting, $\lambda_1 < 1$, or else there would be little point in considering its stability to the new modifier allele.

$$\lambda_1 = \frac{W_4}{\frac{K_{22}W_2}{K_2}}, \quad \lambda_2 = \frac{\frac{K_{11}W_1}{K_1}}{\frac{K_{22}W_2}{K_2}}, \quad \lambda_3 = \frac{W_3}{\frac{K_{22}W_2}{K_2}}. \quad (4)$$

The second and third eigenvalues are new and correspond to the modifier locus. If I assume the germ line modifier is cost free, it has no effect on the parameters describing variation and selection in the

soma ($W_1 = W_2$, $W_3 = W_4$). In this case, the third eigenvalue is equal to the first and less than one, $\lambda_3 = \lambda_1 < 1$. Increase of the germ line modifier depends on the second eigenvalue which equals the ratio of the diluting factors for the new modifier to the resident genotype. As the presumed effect of the modifier is to reduce the risk of development for the germ line either by reducing the number of cell divisions or by lowering the mutation rate, I assume $K_{11}/K_1 > K_{22}/K_2$. In this case, the modifier will always increase as $\lambda_2 > 1$. The germ line allele may impose a cost on the organism, if only because germ line cells are no longer available for cooperative interactions and tissue function. In this case I subtract from the soma the cells used in the germ line. The stability of the fixation equilibrium given in equation (3) is more complex for sexual reproduction. Again there are three independent equations and three eigenvalues. The first equals λ_1 in equation (4). The second and third eigenvalues are roots of a quadratic equation given in the Appendix and are studied numerically in the figures below. In the case of a policing allele, the definitions given above still apply except for the following changes: in table 2, $K_{ij} = k_{ij}$; in table 2 in the CM column, $b = b - \epsilon$; in table 1 for CM zygotes only ($j = 1$), $\beta = \beta - \delta$ and $b = b - \epsilon$.

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 human tissues cells stop dividing (brain, muscle, liver) while in other tissues cells continue to divide throughout life (blood, intestine lining). To help fix ideas, $t = 20$ would make an organism with a possible 2^{20} or approximately 10^6 cells. However, these numbers should be treated with caution, as they do not include cell death. Ignoring mutation and the different rates of replication of the different cell types, $t = 40$ would allow 40 cell divisions ($c = 1$) implying around 10^{12} cells in the adult: a number somewhat similar in magnitude to the number of cells in an adult human. But cell death will require a far greater number of divisions to get the same number of cells in the adult. 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 (Vogel & Rathenberg 1975). However, a typical human female egg is thought to be separated from its zygote by about 20 cell divisions (Vogel & Rathenberg 1975).

4. 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. Let δ be the decrease in development time caused by a germ line allele. As discussed in §3, if the germ line allele accrues no cost, it will always spread. However, cells sequestered in the germ line are no longer available for tissue differentiation and

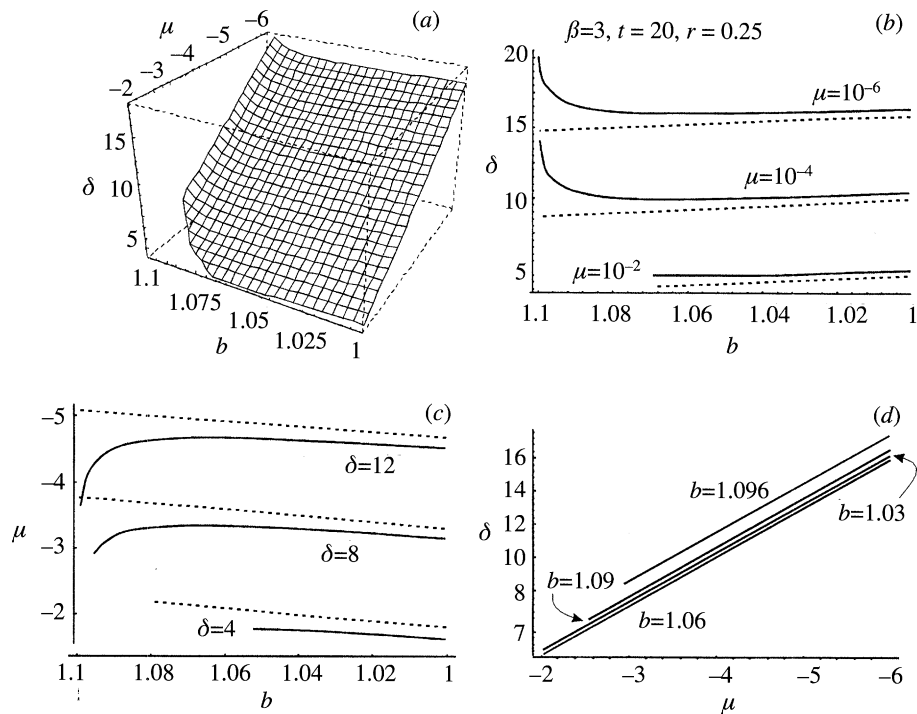


Figure 1. Increase of costly germ line allele. The critical level of δ , the reduction in development time in the germ line, which must be exceeded for the germ line allele to increase is given as a function of two parameters describing the opportunity for within-organism variation – the mutation rate, μ , and the replication advantage of defection at the cell level, b – for a fixed organism development time ($t = 20$), a fixed organism benefit of cooperation ($\beta = 3$) and equal rates of mutation in the germ line and soma ($\mu_M = \mu$). Both sexual and asexual reproduction are considered in organisms without mutual policing. The overall qualitative shape of the curves are not significantly affected by changes in t and β . The germ line allele will increase for parameter values above the three-dimensional surface (this means above the curves in (b) and (d) and below the curves in (c)). The solid curves in (b), (c) and (d) are two-dimensional slices through the three-dimensional surface in (a). Only combinations of parameter values for b and μ that beget a stable internal equilibrium of the one locus cooperate/defect system are shown. Panel (a) and solid curves in other panels are for sexual reproduction with a recombination rate of $r = 0.25$. Dashed curves in two-dimensional panels are for asexual reproduction. In (b) and (c) there are three slices each for sexual and asexual reproduction corresponding to $\mu = 10^{-2}$, 10^{-4} , 10^{-6} (b) and $\delta = 4, 8, 12$ (c). In (d), the asexual curves are not shown as they are extremely close to the sexual curves. The nature of the selection mutation balance equilibrium attained in this case is described in (Michod 1996). Note that the b and μ axes run from higher to lower values. This was done to show the nature of the three-dimensional surface in (a) more clearly. Methods underlying the figure are explained in §3.

cooperation. For this reason, the germ line allele may detract from adult organism function. I represent this cost by subtracting the germ line cells from the somatic cells in the adult form ($k_{ij} - K_{ij}$ using table 2). The conditions for increase of a costly germ line allele are given in figure 1. In figure 1a the critical level of δ which must be exceeded for the germ line allele to increase is given as a function of two parameters describing the opportunity for within-organism variation – the mutation rate, μ , and the replication advantage of defection at the cell level, b – assuming zygote reproduction and equal rates of mutation in the germ line and soma ($\mu_M = \mu$). Both asexual and sexual reproduction are considered in panels 1b–d.

For any specific value of the germ line parameter, δ , there is a critical level of within-organism variation, as represented by the mutation rate, that must be exceeded for the germ line strategy to be cost effective (figure 1c). Nevertheless, a germ line allele will always increase so long as it reduces the time available for cell replication in the germ line by a critical amount. Greater opportunities for within-organism variation

(increasing b and μ) are more conducive to the evolution of a germ line in the sense that smaller levels of δ are required for the modifier to increase. Continuous evolution via small changes in the time for development of the germ line ($\delta = 1$) is not possible. In previous studies (Michod 1996), selection at the cell level was a more critical parameter than the rate of mutation in determining the outcome of selection on cooperation among cells. However, this priority is switched for selection on a modifier of the germ line. Over the range of values begetting a stable internal equilibrium frequency of cooperation, selection on a germ line modifier is more sensitive to the mutation rate than the replication advantage of defection (the surface in figure 1a drops dramatically along the μ axis and only slightly along the b axis). This suggests that lowering the mutation rate may be an important function of a germ line modifier. Indeed any decrease in the mutation rate ($\mu_M < \mu$) in the germ line is immediately selected for in the model studied in §3.

It is easier for the germ line allele to increase under asexual reproduction or with lower recombination

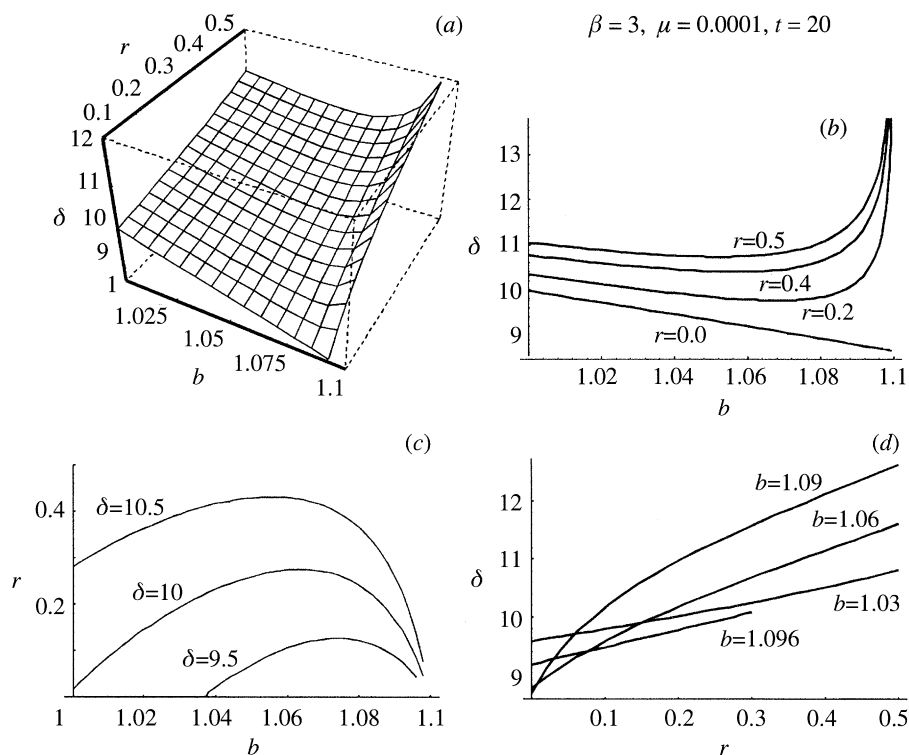


Figure 2. Effect of recombination on increase of germ line modifier. The critical level of δ , the reduction in development time in the germ line, which must be exceeded for the germ line allele to increase is given as a function of the recombination rate, r , and the replication advantage of defection at the cell level, b : for a fixed organism development time ($t = 20$), a fixed organism benefit of cooperation ($\beta = 3$) and equal rates of mutation in the germ line and soma ($\mu_M = \mu = 0.0001$). Both sexual ($r > 0$) and asexual reproduction ($r = 0$) are considered in organisms without mutual policing. The overall qualitative shape of the curves are not significantly affected by changes in t , β , and μ . The germ line allele will increase for parameter values above the three-dimensional surface (this means above the curves in (b) and (d) and below the curves in (c)). The solid curves in (b), (c) and (d) are two-dimensional slices through the three-dimensional surface in (a). Only combinations of parameter values for b and μ that beget a stable internal equilibrium of the one locus cooperate/defect system are shown. The slices are $r = 0.0, 0.2, 0.4$ and 0.5 in (b); $b = 1.03, 1.06, 1.09$ and 1.096 in (c); and $\delta = 9.5, 10, 10.5$ in (d). Methods underlying the figure are explained in §3.

rates, but the effect is not significant except for high levels of b , the benefit to cells of defecting. As b approaches a value of 1.1 (the limit at which the system can no longer maintain a polymorphism at the cooperate/defect locus), the critical values with sex and recombination diverge from the values for asexual reproduction. This means that the germ line allele must have a more marked effect on reducing the time for development in the germ line when compared to the soma (figure 1b). Put another way, the threshold level of within-organism variation for increase of a germ line allele is greater when there is more recombination. The effect of recombination is explored more thoroughly in figure 2.

In figure 2, I study the critical value of δ that must be exceeded for the germ line allele to increase as a function of the recombination rate, r , and the replication advantage to defection, b , for fixed values of somatic development time, $t = 20$, advantage to the organism of cooperation, $\beta = 3$, and mutation rate $\mu = 0.0001$. Only parameter values begetting a stable internal equilibrium in cooperation before the modifier is introduced are studied. For all values of b , increasing recombination requires more drastic effects (larger δ) of the modifier allele for it to increase (figure 2d).

However, for fixed values of the modifier allele (figure 2c), there is an intermediate benefit of defection that maximizes the chances of germ line evolution in the sense that the largest recombination rates may occur. Without sex ($r = 0$ curve in figure 2b) increasing b means increasing within-organism variation which means less drastic modifier alleles (smaller δ) may increase. Sex ($r > 0$ curves in figure 2b) has the effect of reversing this dependency for high values of b . In general, sex is a liability for the evolution of modifiers since it genetically decouples the modifier allele from its effect.

Any modifier lowering the mutation rate ($\mu_M < \mu$) is selected for in the model studied in §3. Maynard Smith and Szathmary suggest that germ line cells may enjoy a lower mutation rate but do not offer a reason why (Maynard Smith & Szathmary 1995). Bell interpreted the evolution of germ cells in the Volvocale as an outcome of specialization in metabolism and gamete production to maintain high intrinsic rates of increase while algae colonies got larger in size (Bell 1985). I think there may be a connection between these two views: 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. It is therefore advantageous to protect DNA from the byproducts of metabolism. Other features of life can be understood as adaptations to protect DNA from the deleterious effects of metabolism: 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. The germ line may serve a similar function: by sequestering the next generation's genes in a specialized cell lineage they are protected from the damaging effects of metabolism in the soma.

5. EVOLUTION OF SELF POLICING

Another means of reducing conflict among cells is by the organism actively policing and regulating the benefits of defection (Frank 1995). To model this

hypothesis I let the modifier allele affect the parameters describing within-organism selection. Cooperating cells in policing organisms spend time and energy monitoring cells and reducing the advantages of defecting at a cost to the organism, δ . As a result of policing, the benefits of cooperation to the organism are reduced to $\beta - \delta$ while the advantages to cells of defecting are reduced to $b - \epsilon$.

When will a policing allele increase in frequency? A representative result is given in for a similar set of parameters as studied in figure 1 except that the within-organism mutation rate is fixed ($\mu = 0.001$) and the time for development, t , varies. Policing is assumed to result in a decrease of 0.01 in a defecting cell's rate of replication ($\epsilon = 0.01$). There is a critical cost to the organism of policing, δ , which must not be exceeded for policing to increase. This cost is rather small and is given as a function of the time for development, t , and the initial benefit to cells of defection before the modifier is introduced, b . For a fixed cost to the organism, δ , there is a threshold organism size (represented by t) needed for mutual policing to pay (figure 3*b*). This threshold size is smaller for asexuals than for sexuals and

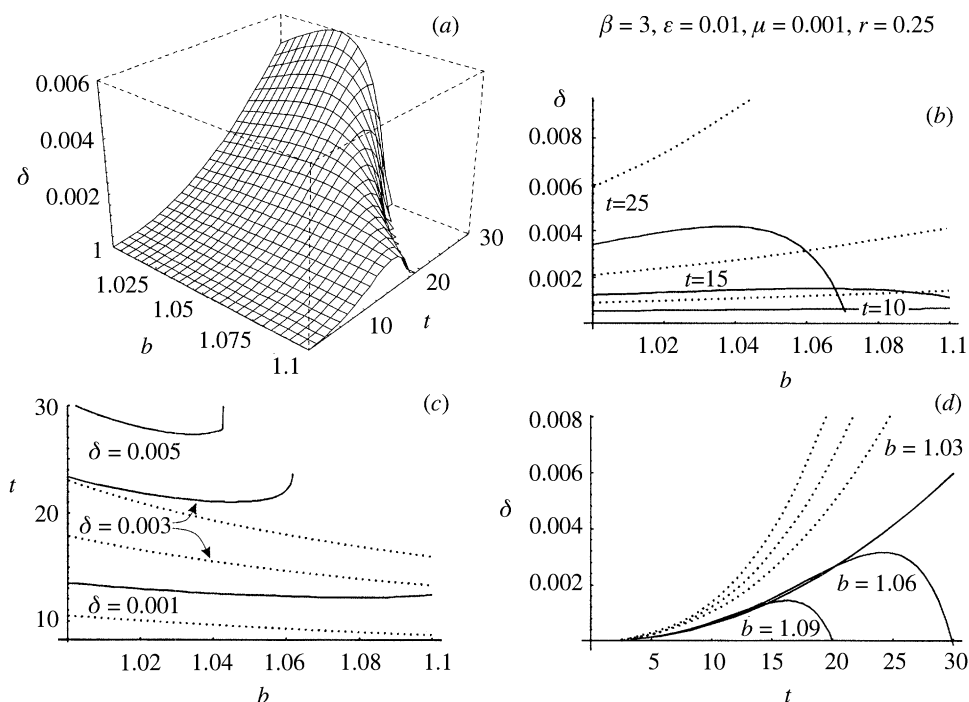


Figure 3. Increase of mutual policing modifier. The critical level of δ , the cost of policing to the organism, which must not be exceeded for the policing allele to increase is given as a function of two parameters describing the opportunity for within-organism selection – the development time, t , and the replication advantage of defection at the cell level, b – for a fixed mutation rate ($\mu = 0.001$), a fixed organism benefit of cooperation ($\beta = 3$) and equal rates of mutation in the germ line and soma ($\mu_M = \mu$). Both sexual and asexual reproduction are considered in organisms without a germ line. The overall qualitative shapes of the curves are not significantly affected by changes in t and β . Modifier will increase for parameter values below the three-dimensional surface (this means below the curves in (b) and (d) and above the curves in (c)). The solid curves in (b), (c) and (d) are two-dimensional slices through the three-dimensional surface in (a). In all panels $\mu = 0.001$ and $\beta = 3$. Only combinations of parameter values for b and t that beget a stable internal equilibrium of the one locus cooperate/defect system are shown. Panel (a) and solid curves in other panels are for sexual reproduction, $r = 0.25$. Dashed curves in two-dimensional panels are for asexual reproduction. In each panel there are three slices each for sexual and asexual reproduction corresponding to $t = 10, 15, 25$ (b); $\delta = 0.001, 0.003, 0.005$ (c); and $b = 1.03, 1.06, 1.09$ (d). Although a mutation rate of $\mu = 0.001$ may seem high it is well within reported values for some organisms. In any event, the basic qualitative features of the curves are not changed for lower mutation rates. The curves for $\mu = 0.0001$ are quite similar to those in figure 3. Methods underlying the figure are explained in §3.

increases with the recombination rate, r . Asexual reproduction and lower recombination rates are more able to accommodate larger costs of policing. Without sex, the larger the initial benefit of defection, b , the larger may be the costs of policing (figure 3*c*). Likewise, the larger the size of the organism, t , the greater may be the costs to the organism of policing (figure 3*d*). The effect of the initial benefit of defection, b , and time for development, t , is complex when there is significant recombination. For smaller organisms ($t = 10$) or smaller initial benefits of defection ($b = 1.03$) the dependence is similar to asexuals with larger initial benefits of defection or longer time for development allowing for larger costs of policing (figure 3*c, d*). However, for larger organisms ($t = 25$) or larger initial benefits of defection ($b = 1.06$) the curve is humped. With sexual reproduction, there is an intermediate level of within-organism variation, an intermediate size and intermediate benefit of defecting, for which the evolution of policing is most favourable.

6. TRANSITIONS IN THE UNITS OF SELECTION

The major transitions in the units of selection are from individual genes to gene networks, from gene networks to bacteria-like cells, from bacteria-like cells to eukaryotic cells with organelles, from cells to multicellular organisms, and from solitary organisms to societies. These transitions in the units of selection share two common themes: (i) the emergence of cooperation among the lower level units in the functioning of the new higher level unit and regulation of conflict among the lower level units (Buss 1987; Maynard Smith 1988, 1990, 1991; Maynard Smith & Szathmary 1995). Eigen and Schuster proposed the hypercycle as a way of keeping individual genes from competing with one another so that cooperating gene networks could emerge (Eigen & Schuster 1979; Eigen 1992). The cell itself keeps selfish parasitic genes from destroying the cooperative nature of the genome (Michod 1983; Eigen 1992). Chromosomes reduce the conflict among individual genes (Maynard Smith & Szathmary 1995). Meiosis serves to police the selfish tendencies of genes and usually insures that each of the alleles at every diploid locus has an equal chance of ending up in a gamete. As a result of the fairness of meiosis, genes can increase their representation in the next generation only by cooperating with other genes to help make a better organism. Uniparental inheritance of cytoplasm may serve as a means of reducing conflict among organelles (Hastings 1992). Concerning the final transition – that from organisms to societies of cooperating organisms – the well developed theories of kin selection, reciprocation and group selection can be understood as providing three mechanisms for the regulation of conflict among organisms: genetic relatedness, repeated encounters and group structure (Hamilton 1964*a, b*; Trivers 1971; Axelrod & Hamilton 1981; Brown *et al.* 1982; Michod 1982; Michod & Sanderson 1985; Ferriere & Michod 1995; Ferriere & Michod 1996). These are just a few of the ways in which the selfish tendencies of lower level

units are regulated during the emergence of a new higher level unit.

The models studied here support the view that the germ line and mutual policing facilitate the transition between cells and multicellular organisms. The germ line functions to reduce the opportunity for conflict among cells and promote their mutual cooperation both by limiting the opportunity for cell replication (Buss 1987) and by lowering the mutation rate (Maynard Smith & Szathmary 1995). Mutual policing (Frank 1995) is also expected to evolve as a means of maintaining the integrity of the organisms once they reach a critical size so long as the cost of policing the organization is small. Any factors that directly reduce the within-organism mutation rate are also favoured.

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APPENDIX

The characteristic equation for sexual reproduction is

$$-\lambda^2 K_{22}^2 W_2 (K_{11} + K_{31}) + \lambda \left(\begin{array}{c} K_{22}(K_{22} + K_{42})(K_{11}W_2 + K_{11}W_4 + K_{31}W_4) + \\ rK_{22} \left(\begin{array}{c} K_{22}K_{31}W_2 - K_{11}K_{42}W_2 - K_{11}K_{22}W_4 \\ -K_{22}K_{31}W_4 - K_{11}K_{42}W_4 - K_{31}K_{42}W_4K_{42}W_4 \end{array} \right) \end{array} \right) - (1-r)K_{11}W_4(K_{22} + K_{42})^2$$

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