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COOPERATION AND CONFLICT IN THE EVOLUTION OF INDIVIDUALITY. I. MULTILEVEL SELECTION OF THE ORGANISM

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Abstract.—This article studies the transition in evolution from cells to multicellular organisms. The issues considered are applicable to all major transitions in the units of evolution that share two themes: the emergence of cooperation and the regulation of conflict among the lower-level units, in this case, cells. Explicit genetic models of mutation and selection both within and between organisms are studied in sexual and asexual haploid and diploid organisms without a germ line. The results may be understood in terms of the differing opportunities for within- and between-organism selection under the different reproductive modes and parameter values. Cooperation among cells increases when the fitness covariance at the level of the organism overcomes within-organism change toward defecting cells. Selection and mutation during development generate significant levels of within-organism variation and lead to significant variation in organism fitness at equilibrium. The levels of cooperativity attained can be low, even with reproduction passing through a single-cell zygote stage and the high kinship that entails. Sex serves to maintain higher levels of cooperation and lower levels of within-organism change. Fixed size may help organisms reduce conflict among cells.

The major transitions in evolution are from individual genes to networks of genes, 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: 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 (Eigen and Schuster 1979; Buss 1987; Maynard Smith 1988, 1990, 1991; Maynard Smith and Szathmary 1995). Eigen and Schuster's (1979) hypercycle was proposed as a way to keep individual genes from competing with one another so that cooperating gene networks could emerge (Eigen and Schuster 1979; Eigen 1992). Localizing genes in the cell 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 and Szathmary 1995). Meiosis serves to police the selfish tendencies of genes and usually ensures 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 increase their representation in the next generation by cooperating with other genes to

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help make a better organism. Uniparental inheritance of cytoplasm may serve as a means of reducing conflict among organelles (Hastings 1992), although it is worth noting that biparental inheritance occurs in some angiosperms. Finally, concerning the final transition—that from organisms to societies of cooperating organisms—the well-developed theories of kin selection, reciprocation, and group selection provide three mechanisms for the regulation of conflict among organisms: genetic relatedness, repeated encounters, and group structure (see, among many others, Michod and Hamilton 1980; Brown et al. 1982; Michod 1982, 1993; Ferriere and Michod 1995). 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.

Organisms can be thought of as groups of cooperating cells. Selection among cells—below the level of the organism—could destroy this harmony and threaten the individual integrity of the organism. This competition could favor defecting cells that pursue their own interests at the organism's expense. For the organism to emerge as an individual, or unit of selection, ways must have been found of regulating the selfish tendencies of cells while at the same time promoting their cooperative interactions.

The purpose of this article is to study the levels of within-organism variation that may arise by mutation and selection during development and to explore the consequences of this variation for the levels of cooperation and fitness attained in the adult form. My more general goal is to develop a theoretical framework to study the emergence of the organism from groups of interacting cells.

MODEL

Recurrence Equations

An overview of the model life cycle is given in figure 1. After zygote formation, cells proliferate during development to produce the adult form. This proliferation and development is indicated by the vertical arrows in figure 1. Because of mutation and different rates of replication of different cell types, gene and genotype frequencies of cells change within the organism, as represented by Δq_j in figure 1. Gene and genotype frequencies also change in the population of organisms because of 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 in the population.

The fitness of the adult form, W_j, is the absolute number of gametes produced, which is assumed to depend on both 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 noncooperating cells replicate faster (parameter b in the haploid model of app. A) and produce a larger but less functional adult. The simple model considered in appendix A (Haploid model and Additional terms for diploid model) assumes a linear dependence of adult fitness on frequency of cooperating cells, although more complex models could easily be incor-

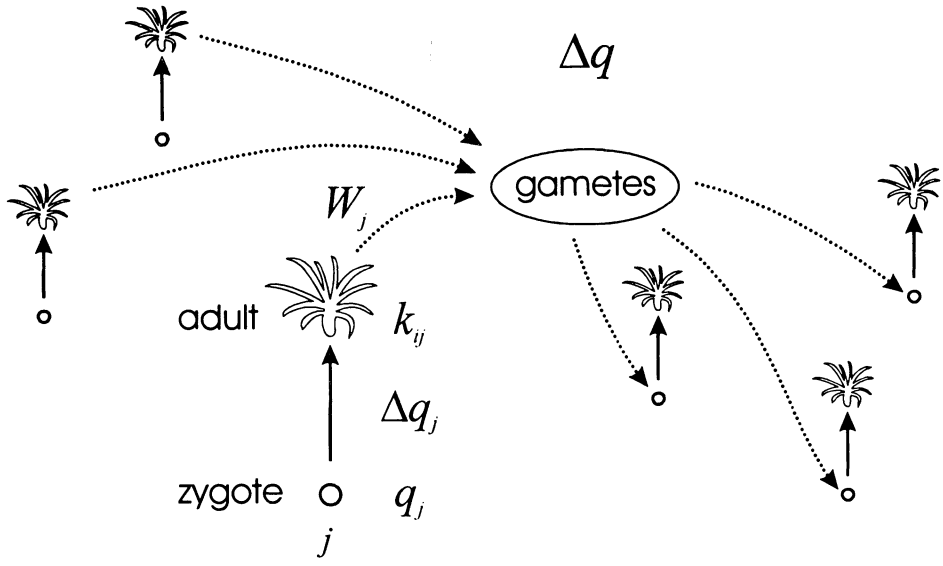

FIG. 1.—Life cycle of model organism; see text for explanation

porated into the framework considered here. Organism size is assumed to be indeterminate and to depend on the time available for development and the rate at which cells divide. Indeterminate growth is most applicable to organisms such as plants or clonal invertebrates. We find here that indeterminate growth makes matters more difficult for organisms. It allows selfish cells to reap additional fitness benefits by virtue of larger adult size, because selfish cells replicate faster than do cooperative cells. Viewed in this way, fixed adult size could be an adaptation to reduce within-organism conflict (J. Li and R. E. Michod, unpublished data).

A single locus with two alleles, cooperate *C* and defect *D*, is assumed to control the way cells interact. I refer to organisms in terms of the genotype of the zygote at the cooperate defect locus, either *C* or *D* in the case of haploid organisms or *CC, CD,* and *DD* in the case of diploidy. Because of within-organism mutation and selection during development, the adult stage may have cells with different genotypes than those of the zygote. The *k* variables in appendix A (Haploid model and Additional terms for diploid model) refer to numbers of cells of different genotypes in the adult stage. Two forces may change gene frequency between the zygote and adult stages and determine the values of the *k* variables: mutation and cellular selection. Mutation is assumed to lead to a loss of cooperation and tissue function. Mutation also increases the variance among cells and enhances the scope for selection and conflict among cells within organisms. Cellular selection is represented later (app. A, Mutation and cellular selection model for haploidy and Mutation and cellular selection model for diploidy) as differences in cell replication rate. Differences in viability between cells are ignored for mathe-

matical simplicity, but cell death is known to be common in cell lineages during development.

The definition of terms and variables in the haploid model is given in appendix A. With these definitions, it is straightforward to write down the new gene frequency in the next generation in equation (1):

$$q' = \frac{qW_C\left(\frac{k_{CC}}{k_C}\right) + (1 - q)\,W_D\left(\frac{k_{CD}}{k_D}\right)}{\overline{W}} \qquad (1)$$

$$\overline{W} = qW_C + (1 - q)\,W_D.$$

The terms in parentheses in equation (1) are the frequencies of C alleles in the cells of a C or D adult and appropriately weight the total gametic output to consider only those gametes containing C alleles. In the model of unidirectional mutation considered later (app. A, Mutation and cellular selection model for diploidy), there is no mutation from D to C, and so the second term in the numerator is 0. Sex in a haploid organism has no effect unless there is recombination between two or more loci or unless other evolutionary forces operate during the short diploid stage to change diploid genotype frequencies.

For diploids, the subscript i indicates the number of C alleles in the cell type: $i = 0, 1, 2$ for DD, CD, and CC genotypes. There are also two dominance parameters describing the effects of the CD genotype on the two levels (on cooperation in the adult stage, d, and on the replication rate during development, h). As the dominance parameter at the cell (or organism) level ranges from 0 to 1, the behavior of a CD heterozygote at the cell (or organism) level ranges from that of a DD (or D) cell to that of a CC (or C) cell. Overdominance has not been considered. Using the terms defined in appendix A (Additional terms for diploid model), it is straightforward to write down in equation (2) the new gene frequency in the next generation assuming random mating:

$$q' = \frac{\sum_{i=0}^{i=2} f_i W_i\left(\frac{k_{2i} + \frac{1}{2}k_{1i}}{k_i}\right)}{\overline{W}} \qquad (2)$$

$$\overline{W} = \sum_{i=0}^{i=2} f_i W_i.$$

The term in parentheses in equation (2) is the frequency of C alleles in the cells of an i adult and weights the total gametic output to consider only those gametes containing C alleles.

Asexual reproduction of diploid cells requires three separate equations for the three cell types, of which two are independent since they must sum to 1. The definitions in appendix A (Additional terms for diploid model) apply without modification to the case of asexual diploidy. It is straightforward to calculate the new

zygote frequencies directly, and they are given in equation (3):

$$f_i' = \frac{\sum_{j=0}^{j=2} f_j W_j \frac{k_{ij}}{k_j}}{\overline{W}}, \quad i = 0, 1, 2. \tag{3}$$

Within-Organism Mutation Selection Model

As cells proliferate within the developing organism, mutations (rate μ per cell division) occur, leading to loss of tissue function and cooperativity among cells. We consider only mutations from *C* to *D* (no back mutation) as this represents a worst case for the evolution of intercellular cooperation. This is reasonable, because it is far easier to lose a complex trait like cooperativity among cells than it is to gain it. Although the model considers a single locus, there are likely to be many loci that affect tissue function and cooperativity among cells. A simple model of mutation and cellular selection is given in appendix A for haploidy (Otto and Orive 1995). Consider *C* cells that are in the xth cell division and have not yet mutated in any of previous $x - 1$ divisions. The total number of these cells is $2^x(1 - \mu)^x - 1$. Some of these cells will mutate for the first time, and the resulting mutants will undergo $b(ct - x)$ more cell divisions. (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 $cb[t - x/c] = b[ct - x]$). This approach gives k_{CC}, k_{DC}, and W_D in appendix A (Mutation and cellular selection model for haploidy). The summation in k_{DC} can be simplified as shown in that part of appendix A. The generation time, t, is measured on the scale of time taken for a cooperating cell to divide (since I set $c = 1$), and so it gives the number of cell divisions per individual generation for cooperating cells.

The mutation model given in appendix A (Mutation and cellular selection model for haploidy) generalizes to the diploid case, but 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 organisms that start out as a *CC* zygote requires considering four classes of events: *CC* cells that mutate to *CD* during cell division x and then mutate to *DD* during cell division y; *CC* cells that mutate to *CD* during cell division x and remain *CD* for the rest of development; *CC* cells that mutate directly to *DD* during cell division x; and, of course, *CC* cells that remain *CC* for all of development. Nevertheless, we can obtain the expressions given in appendix A, Mutation and cellular selection model for diploidy (which are similar in kind to those given in the preceding part of app. A for haploidy), for the different numbers of cell types in adults derived from the three kinds of zygotes. The variable b_h is a cell division parameter for heterozygotes (app. A, Additional terms for diploid model), and $\Theta = (t - [x/c])cb_h$ in the expression for k_{02} is the number of remaining cell divisions possible for a *CD* cell assuming it mutated from a *CC* cell during cell division x.

Covariance Methods

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 the covariance approach developed by Price (1970, 1972, 1995) and extended by several workers (Arnold and Fristrup 1982; Wade 1985; Heisler and Damuth 1987; Frank and Slatkin 1990; Frank 1995). Although the covariance approach gives the same change in gene and genotype frequencies as the direct methods used here, it will help us to better understand the results. Price's approach posits a hierarchical structure in which there are two levels—in our case, between cells within organisms, viewed as a group of cells, and between organisms within populations. Both levels of selection can be described by a single equation (Price 1972):

$$\Delta q = \frac{\text{cov}[W, q_I]}{\overline{W}} + \text{E}[\Delta q_I]. \tag{4}$$

Variables q and q_I are the frequencies of a gene of interest in the total population and within zygotes; cov$[x, y]$ and E$[x]$ indicate the weighted covariance and expected value functions, respectively.

The covariance framework expressed in equation (4) is well suited to studying conflict and cooperation among cells within organisms. Conflict among cells may result in within-organism change, as represented by the within-group component of equation (4), E$[\Delta q_I]$. The fitness of the emerging higher-level unit is represented by the first component of equation (4), cov$[W, q_I]$. One hypothesized function of the germ line (Buss 1987) is to uncouple the organism's fitness from the fitness of the cells comprising it so as to reduce E$[\Delta q_I]$ to 0. Evolution then depends on the fitness of organisms and the covariance of adult fitness with zygote genotype, not the fitnesses of their component cells. The heritability of traits encoded in the zygote is thereby protected. The trait of interest here concerns the level of specialization and differentiation among cells within organisms—that is, the level of cooperativity among the cells.

Price's regression form of the gene frequency equation for this model is given in equation (5). This is the same as equation (4) but uses regression instead of covariance. Either equation (5) or equation (4) give exactly the same change in gene frequency as the direct method given in equation (1),

$$\Delta q = \frac{\text{reg}_{\boldsymbol{q}}[W, q_I]\,\text{var}_{\boldsymbol{q}}[q_I]}{\overline{W}} + \text{E}_{\boldsymbol{Wq}}[\Delta q_I], \tag{5}$$

with the following vectors used as weights, $\boldsymbol{q} = (1 - q, q)$, $\boldsymbol{Wq} = (W_D[1 - q], W_C q)$.

Under diploidy and sexual reproduction, gene frequency change is still given by equations (4) and (5), except that the classes are the diploid cell types, and the covariance in equation (4) is between the adult fitness of the three diploid zygote cell types and the frequency of the C allele at the zygote stage—$q_I = 0$, 0.5, 1 for *DD*, *CD*, and *CC* zygotes—using the vector of genotype frequencies, f_i, as weights. The gene frequency change within organisms with zygote genotype i is $([k_2 i + 0.5k_1 i]/k_i) - q_i$, for *DD*, *CD*, and *CC* zygotes. The regression form of the Price equation (eq. [5]) becomes equation (6) for diploid sex,

$$\Delta q = \frac{\mathrm{reg}_f[W, q_1]\,\mathrm{var}_f[q_1]}{\overline{W}} + \mathrm{E}_{Wf}[\Delta q_1], \tag{6}$$

with the following vectors used as weights: $\boldsymbol{f} = (f_0, f_1, f_2)$ and $\boldsymbol{Wf} = (W_0 f_0, W_1 f_1, W_2 f_2)$. With random mating in a sexual population, the zygote frequencies may be expressed by their Hardy-Weinberg proportions. Either equation (6) or equation (4) can be shown to be identical to equation (2).

Calculating the covariance form of the equations for asexual diploidy is different from the sexual case. In the case of sex, random mating allows the specification of genotype frequencies from gene frequencies, and so a single gene frequency equation can be used to describe evolution. Without sex, each of the three cell types requires a separate covariance equation:

$$\Delta f_i = \frac{\mathrm{cov}_f[W, f^i]}{\overline{W}} + \mathrm{E}_{Wf}[\Delta f_i], \quad i = 0, 1, 2, \tag{7}$$

with

$$f^0 = (1, 0, 0), f^1 = (0, 1, 0), f^2 = (0, 0, 1)$$

and

$$\Delta f_0 = \left(\frac{k_{00}}{k_0} - 1, \frac{k_{01}}{k_1} - 0, \frac{k_{02}}{k_2} - 0\right),$$

$$\Delta f_1 = \left(\frac{k_{10}}{k_0} - 0, \frac{k_{11}}{k_1} - 1, \frac{k_{12}}{k_2} - 0\right),$$

$$\Delta f_2 = \left(\frac{k_{20}}{k_0} - 0, \frac{k_{21}}{k_1} - 0, \frac{k_{22}}{k_2} - 1\right).$$

In each of these three covariance equations, the variable of interest is genotype frequency instead of gene frequency, as it is with sex and haploidy. Although it is possible to calculate the covariance of fitness using individual gene frequency as is done for sex and haploidy, this covariance does not play a direct role in the evolution of the system. Each of the three equations in equation (7) can be shown to equal its corresponding equation in equation (3).

RESULTS

Within-Organism Change during Development

One component of the total change in the population occurs within organisms during development (recall the second term on the right-hand side of the Price equation, eq. [4]). The change in gene frequency within organisms during development may be studied in both haploids and diploids. In diploid *CC* zygotes, mutation produces *CD* and *DD* cells, while in *CD* zygotes mutation produces *DD* cells. In haploids, mutation in *C* zygotes produces *D* cells. Since two zygote

types may accumulate variation during development in diploids, the average within-organism change in diploids is reported in figures 2 and 3 for a global gene frequency of $q = 0.1$. The case of $q = 0.1$ is reported because a lower gene frequency is most relevant to understanding the initial increase of cooperation studied in the next section. The global gene frequency only affects the weighting of the change that occurs within *CC* and *CD* cells. Typically, there is more change in *CC* cells than *CD* cells, but never as much as in haploid *C* cells. For example, for a high global gene frequency of $q = 0.9$, more weighting is given to the change occurring in *CC* cells, which is similar to that occurring in haploid *C* cells depending on dominance. Consequently, for higher global gene frequency the diploid curve approaches that of the haploid, again depending on the dominance parameters. A range of global gene frequencies were studied, and there is not a qualitative effect on the conclusions.

In figure 2 variation during development of a *CC* zygote is plotted as a function of time for development, t, and the mutation rate, μ, for additive mutations ($h = 0.5$), which have a 10% replication advantage over *CC* cells. The frequency of the *C* allele decreases in the manner shown as the mutation rate, μ, and time for development, t, increase. More time for development means greater variation in the adult form. Mutations begin accumulating when the rate of mutation reaches 10^{-5} and when the time for development reaches $t = 30$ or about 10^9 cells in the adult form. (In nature, the exact number of cells is a stochastic event, depending on mutation to the different cell types and the rate of replication of these cell types.) Even in small organisms ($t = 15$ or about 30,000 cells), mutations accumulate, but the mutation rate must be 10^{-3} or higher (see fig. 2*C*, *D*). The accumulation of variation is very sensitive to the replication advantage of defecting cells, b, which in figure 2 is 10%. This effect is studied further in figure 3. In figure 3 the change in gene frequency after development is complete is plotted as a function of the replication advantage of selfish cells, b, and the mutation rate for the case of additive mutations ($h = 0.5$) in a *CC* zygote. The time available for development, $t = 40$, means that the adult form contains around 10^{12} cells (assuming no cell death), similar to the number of cells in a human. The replication rate of *CC* cells is set equal to $c = 1$ with the replication advantage of defection ranging between 1 and 1.3. A value of $b = 1.0$ means that there is no difference in the replication rates of *CC*, *CD*, and *DD* cells. A value of $b = 1.1$ means that *DD* (or *D*) cells replicate 10% faster than *CC* (or *C*) cells. In diploid zygotes, the number of *CC* cells decreases, and *CD* and *DD* cells increase as the mutation rate, μ, and replication advantage, b, increase, giving rise to the changes in gene frequency. Defecting *D* cells increase in a similar fashion in *C* haploid zygotes. Mutations accumulate more rapidly in haploid zygotes than in diploid zygotes, because of the lower within-organism variance in diploids that results from the assumption of intermediate dominance (of the heterozygous *CD* cells).

The change in gene frequency within organisms is sensitive to the selection parameter, b, the mutation rate, μ, and the time available for development, t. In addition, the parameters interact, especially selection and mutation (fig. 3). While being an essential ingredient, the mutation rate alone is not the critical force. Rather, it is both mutation and selection (as determined by the development time

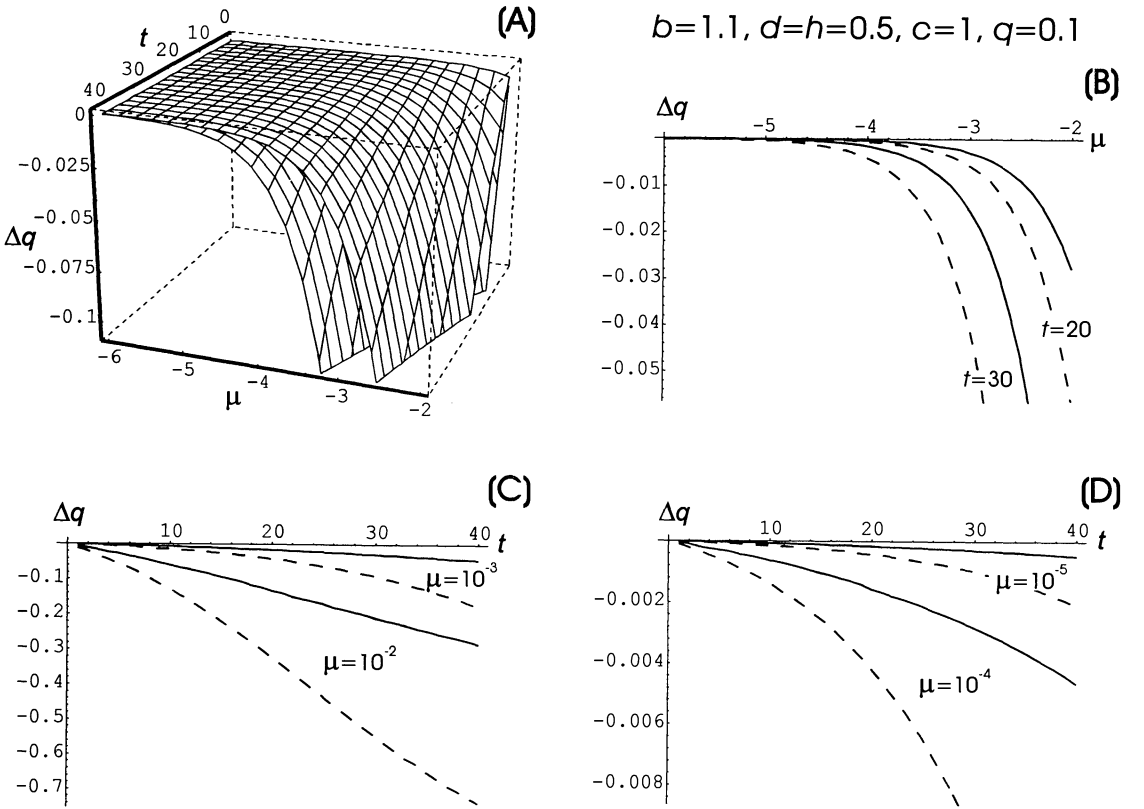

FIG. 2.—Within-organism change during development. Shown is the change in frequency of the *C* allele within haploid *C* and diploid *CC* and *CD* zygotes during development, as a function of the time for development, t, and the mutation rate, μ. Gene frequency, $q = 0.1$. The selection/mutation model is given in appendix A for parameters $b = 1.1$, $c = 1$, and $d = h = 0.5$. In panel *A*, the bottom surface is for haploidy, and the top surface is for diploidy. Panels *B–D* are slices through the three-dimensional surface given in panel *A;* dashed lines represent haploidy, and solid lines, diploidy. In panel *B*, there are two slices for $t = 20$ and $t = 30$; in panel *C*, there are two slices for $\mu = 10^{-2}$ and $\mu = 10^{-3}$; and in panel *D*, there are two slices for $\mu = 10^{-4}$ and $\mu = 10^{-5}$. A parameter value is indicated by the haploid (*dashed*) curve. The corresponding slice for the diploid surface is the first solid curve above the haploid curve. See the text for more discussion.

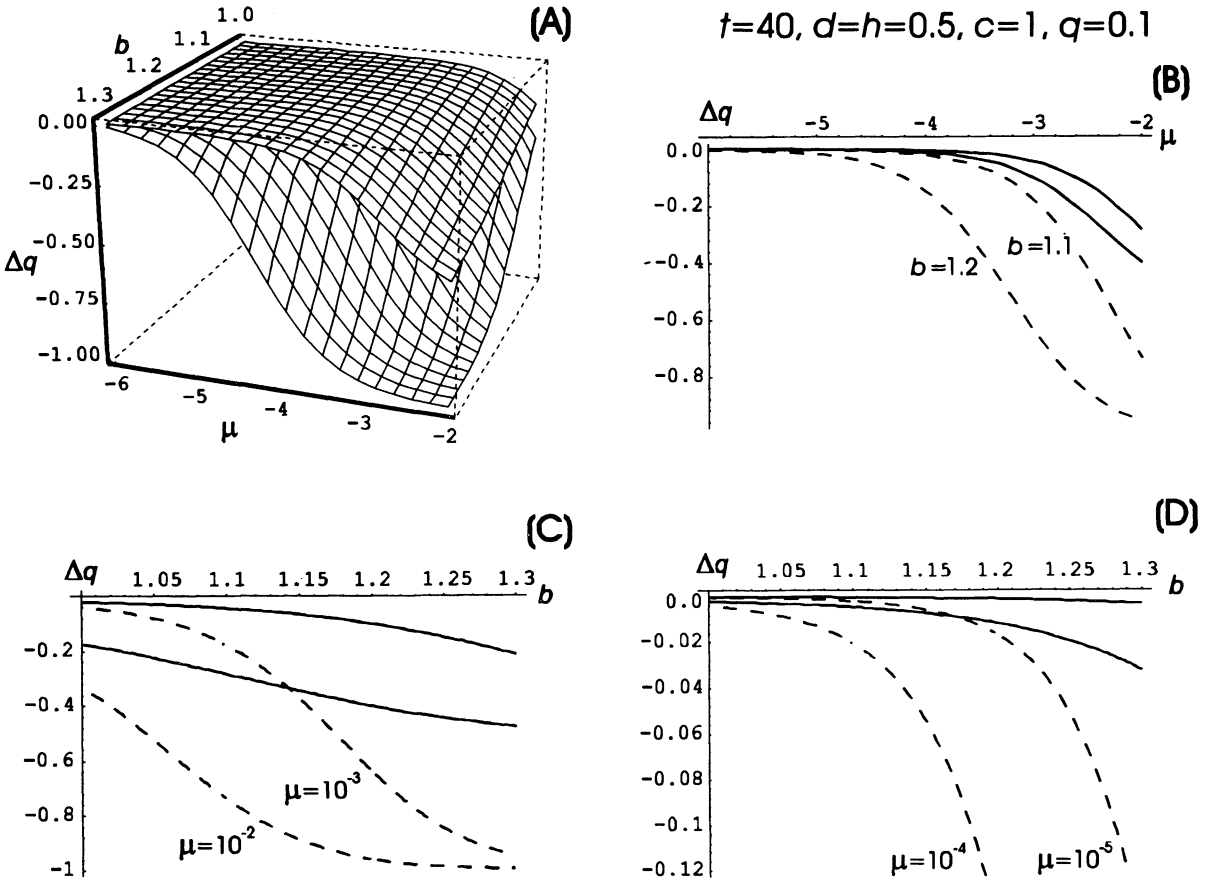

FIG. 3.—Within-organism change after development. The same legend applies as that in figure 2 except t is fixed, $t = 40$, and b varies. In panel *B*, there are two slices for $b = 1.1$ and $b = 1.2$. Labels are by haploid curves.

and replication rate at the cell level) that determine the amount of within-organism change.

Initial Increase of Cooperation

For organisms to emerge as a new evolutionary unit, greater levels of harmony and cooperation among cells must be attained. A basic issue concerns the increase of a rare mutation favoring more cooperation among cells within organisms. Under what conditions will such a mutation increase? This question is answered by studying the conditions under which the fixation equilibrium of complete defection is unstable. Analysis can be done on the eigenvalues for the basic set of equations in terms of the k_{ij}'s (app. A, Haploid model; eqq. [2], [3]) without assuming a particular model of within-organism mutation and selection such as that given in appendix A (the mutation and cellular selection models for haploidy and diploidy). We do, however, assume that there is no back mutation from *D* to *C* so that $k_{21} = k_{20} = k_{10} = 0$. As already mentioned, this assumption is generally reasonable, because it is much harder for mutation to produce a complex function like cooperation than it is to destroy it.

Eigenvalues

In the case of asexual haploidy, the eigenvalue is given in equation (8):

$$\lambda_H = \frac{W_C}{W_D}\frac{k_{CC}}{k_C}. \tag{8}$$

In the case of sexual diploidy, there is a single eigenvalue given in equation (9):

$$\lambda_1 = \frac{W_1}{W_0}\frac{k_{11}}{k_1}. \tag{9}$$

In the case of asexual diploidy, there are two eigenvalues since the rare perturbation from all *DD* can involve *CC* and/or *CD* cells. In the case of sex, *CC* cells immediately produce *CD* offspring when they mate with the common *DD* type. The first eigenvalue for asexual diploidy is identical to the sexual one in equation (9) and corresponds to increase of the *CD* genotype. The second eigenvalue for asexual diploidy is given in equation (10) and corresponds to increase of the *CC* genotype:

$$\lambda_2 = \frac{W_2}{W_0}\frac{k_{22}}{k_2}. \tag{10}$$

In all cases studied, $\lambda_2 > \lambda_1$. For this reason, only results for λ_1 are reported in the case of asexual diploidy presented later.

These eigenvalues are a product of two components. The first component is a fitness ratio that is identical to the standard condition for increase based on between-organism "individual" selection when there is no within-organism selection. The second component is a diluting effect equal to the fraction of the cells in the adult organism that contains the *C* allele. The second component tends to unity as the mutation rate and within-individual variation tend to 0. The haploid

eigenvalue (eq. [8]) and the second asexual diploid eigenvalue (eq. [10]) are qualitatively similar, although not identical because of the different opportunities for selection and variation in developing haploid and diploid zygotes. They approach one another as the mutation rate tends to 0 and there is no within-individual variation.

No Within-Organism Variation

Typically in group-structured models, the effects of cooperation are positive at the group level and negative at the organism level. The situation is more complicated when the groups are organisms composed of cells. Even in the absence of within-organism variation, the benefit to the organism of increased cooperation must overcome the cost to the organism of a smaller adult size. Recall that the model assumes that growth is indeterminate and that adult fitness is a function of both adult size and the level of cooperativity among its cells. A cell pays for cooperating by replicating more slowly, which in turn results in a smaller but more functional adult. Fixed adult size for all genotypes would remove one of the temptations of defection at the cell level, since there would be no effect on group (organism) size of a defecting cell's faster rate of replication. There would still be an advantage to defection in the form of greater representation in the gametes; however, adult size would be fixed. Viewed in this way, fixed organism size may be seen as an adaptation to help maintain the integrity of organisms by removing one of the costs of cooperation (J. Li and R. E. Michod, unpublished data).

When will cooperation increase when there is no within-organism variation and selection? From the limiting values of the eigenvalues ($\mu = 0$), critical levels of β may be determined for haploidy, β_H, and diploidy, β_D (eq. [11]):

$$\beta_D = \frac{-1 + 2^{(b-1)cht}}{d}, \quad \beta_H = -1 + 2^{(b-1)ct}. \tag{11}$$

Equation (11) (β_H from eq. [8] and β_D from eq. [9]) gives the minimum benefit required for cooperation to evolve given the costs to the organism of a lowered division rate for cooperating cells. The C allele must have some heterozygous effect $d > 0$, or else it would be initially neutral. Within-organism variation can only make matters worse for cooperation, requiring more extreme benefits than those given in equation (11).

Reproductive mode affects the increase of cooperation through the dominance parameters h and d. Recall that as h ranges from 0 to 1, the growth rate of a CD cell ranges from that of a DD cell to that of a CC cell. Likewise, as d ranges from 0 to 1, the behavior of a CD cell in the adult form ranges from the selfish behavior of a DD cell to the cooperative behavior of a CC cell. It seems wishful thinking to suppose that a heterozygous cell could cooperate as CC cells do ($d \cong 1$) while not paying the cost in replication rate assumed for a CC cell ($h \cong 0$). Consequently, the dominance effects at the two levels of selection must be coupled in some way, although I am not familiar with any data bearing on this issue. In figure 4, combinations of parameter values for which $\beta_H = \beta_D$ are graphed. These surfaces define critical values for which the increase of cooperation is the same

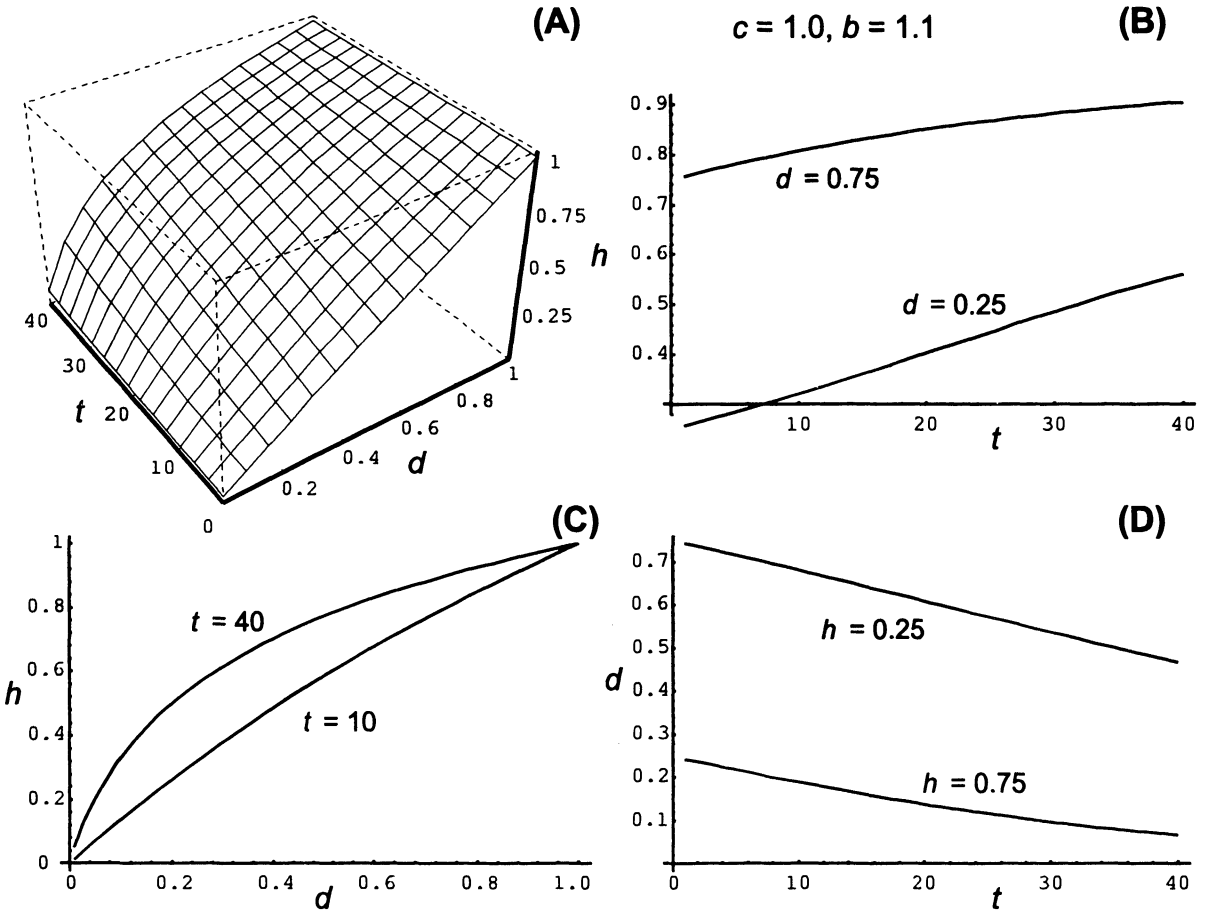

FIG. 4.—The case for $\beta_D = \beta_H$, $\mu = 0$. The surfaces give values of parameters for which the critical β's defined in equation (11) are equal. For these values, the conditions for cooperation to increase are equal for haploidy and diploidy, assuming no within-organism variation. Panels *B–D* are slices through the three-dimensional surface given in panel *A*.

under diploidy and haploidy. For combinations of values below the curves, cooperation fares better under diploidy; above the curves, haploidy is better. If the heterozygous effects at the two levels are assumed to be equal, diploidy is more conducive to the increase of cooperation than haploidy ($d = h$ surface is always less than the curves in panels *A* or *C* of fig. 4).

The reasons for the advantage of diploidy are the masking effects in heterozygotes, especially at the level of cell replication since these effects are compounded multiplicatively. For intermediate h, the adult size of *CD* zygotes is between those of the two homozygotes, something that is, of course, impossible for the new mutant genotype under haploidy. The expression of cooperation among cells is also less in *CD* zygotes than in *CC* or *C* zygotes; however, the effect of d is linear, whereas the effect of h is compounded multiplicatively and dominates the relationship between the two eigenvalues (see eq. [11]).

The effects of reproductive mode can be dramatic. In figure 5, equation (11) is graphed for additive genes ($h = d = 0.5$). Figure 5 shows that the level of β required for cooperation to increase depends on the replication benefits of defection and the time needed for development (alternatively size of the adult form). The larger b and t are, the greater is the size of the less integrated organisms (those with uncooperative cells), which necessitates larger benefits of cooperation. For organisms of about 10^9 cells ($t = 30$), replication benefits greater than about $b = 1.1$ require increasingly extreme levels of cooperation benefits. Smaller organisms with about 30,000 cells ($t = 15$) can tolerate replication benefits approaching 20% ($b = 1.2$) without extreme benefits at the organism level.

Within-Organism Variation

We now consider the effects of within-organism variation by allowing the mutation rate to be greater than 0, $\mu > 0$. Increase of the *C* allele when rare requires instability of the *D* fixation equilibrium, which occurs when the eigenvalues given in equations (8)–(10) are greater than 1. The conditions $\lambda > 1$ define the critical values of the benefit of cooperation β given in equation (12). These critical values must be exceeded for the benefit of cooperation to the organism (β) to offset the benefit of defecting at the level of the cell (b) for a given mutation rate (μ) and time of development (t).

$$\lambda_H > 1 \Rightarrow \beta > -\frac{(k_{CC} - k_{DD})k_C}{k_{CC}^2} \equiv \beta_H,$$

$$\lambda_1 > 1 \Rightarrow \beta > -\frac{(k_{11} - k_{00})k_1}{dk_{11}^2} \equiv \beta_1, \tag{12}$$

and

$$\lambda_2 > 1 \Rightarrow \beta > -\frac{(k_{22} - k_{00})k_2}{k_{22}^2 + dk_{12}k_{22}} \equiv \beta_2.$$

The critical β's defined in equation (12) are all positive, since the term in parentheses in the numerator is negative for reasonable models of mutation and selection

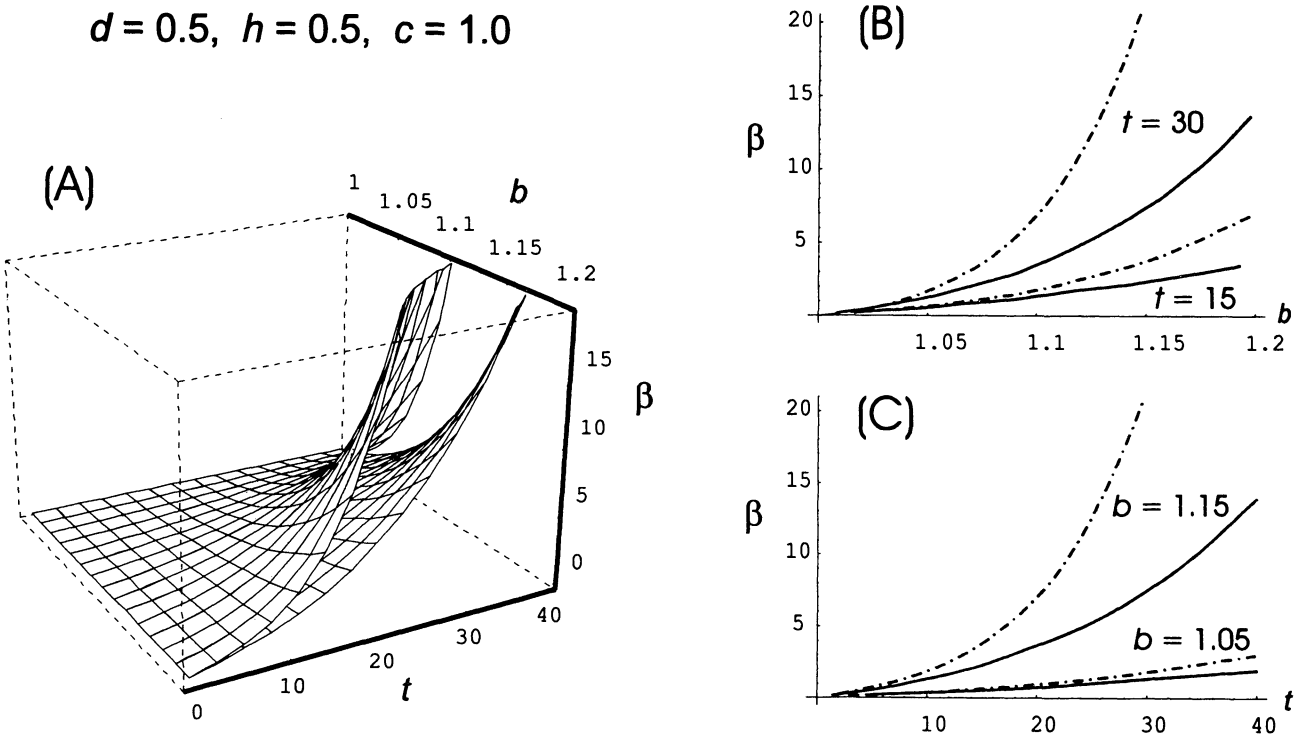

FIG. 5.—Minimum values of β for cooperation to evolve, with equation (11) as a function of the replication advantage of defection, b, and the time for development, t. In panel A, the upper surface (greater values of b) is for haploidy and the lower surface is for diploidy. Panels B and C are slices through the three-dimensional surface given in panel A, with dashed-dotted lines for haploidy and solid lines for diploidy. In panel B, there are two slices for $t = 15$ and $t = 30$. In panel C, there are two slices for $b = 1.05$ and $b = 1.15$. For each slice, there is a pair of lines, one dashed-dotted and one solid, corresponding to haploidy and diploidy, respectively. The parameter values for the slice is indicated near each pair of lines.

like those defined in appendix A (the mutation and cellular selection models for haploidy and diploidy). The critical value β_1 clearly requires $d > 0$ because if $d = 0$, then the corresponding eigenvalue $\lambda_1 = 1$. The critical values of β defined in equation (12) approach the values defined in equation (11) as the mutation rate goes to 0.

Numerical calculations of the critical values of β given in equation (12) show that of the three modes of reproduction—haploidy, diploid sex, and diploid asexuality—it is usually easier for cooperation to increase from rarity in diploids assuming intermediate dominance. An example is given in figures 6 and 7 for additive genes. The only difference between these figures is that figure 6 is for fixed $b = 1.05$ with t and μ varying, and figure 7 is for fixed $t = 30$ with b and μ varying. With $t = 30$ time units for development, there will be approximately 10^9 cells in the adult form (again, assuming no cell death). As can be seen by the similarity of the graphs in the two figures, the replication advantage, b, and the time for development, t, have similar effects on the critical level of benefit, β, for instability of the all-D equilibria. Again, we see in figure 7 the sensitivity of the outcome to b, the selection parameter at the cell level, while the mutation rate does not have much effect until it reaches very high levels of 10^{-3} to 10^{-2}. An advantage in cell replication, increased b, is compounded during development. The challenge in coping with such renegade cells is a special case of the general problem with group selection: groups typically turn over more slowly than the entities of which they are composed.

For most parameter values, it is more difficult for cooperation to increase in haploids. The top surface in the three-dimensional panels of figures 6 and 7 is for the critical level of benefit of cooperation for haploid organisms, which can be seen to depart significantly from diploids over most of the parameter space. However, this is not always the case. As shown in the $b = 1.01$ panel in figure 7 (1% advantage to the defecting cell's replication rate), for mutation rates greater than about 10^{-4}, cooperation among cells fares better in haploids. There are two eigenvalues and hence two critical β's for asexual diploidy. One critical value, β_2, is similar to that for asexual haploids. The other critical value is identical to that for sexual diploids (eq. [12]). In all cases studied, $\beta_2 > \beta_1$, so only β_1 is reported in the figures. In the $b = 1.001, 1.01, 1.1$ panels of figure 7, the curves tend to the levels of benefit defined by equation (11) as the mutation rates get small.

These results may be understood in terms of the differing opportunities for within- and between-organism selection under the different reproductive modes and parameter values. Indeed, the critical values of β graphed in figures 6 and 7 are identical to those derived by an alternate approach of requiring the between-organism component of equation (4) to be greater than the within-organism component: $\text{cov}[W, q_I]/\overline{W} > \text{E}[\Delta q_I]$ (for small q; results are not reported here for reasons of space). Three factors determine the outcome: within- and between-organism variation and differences in adult size. For intermediate dominance, diploids have lower levels of within-organism variation, giving less opportunity for defecting cells to increase. The flip side is that haploids typically have greater between-organism variances in the level of cooperativity (again assuming intermediate dominance in diploids), which gives haploidy an advantage—one that becomes dominant as the frequency of the cooperate allele reaches significant levels

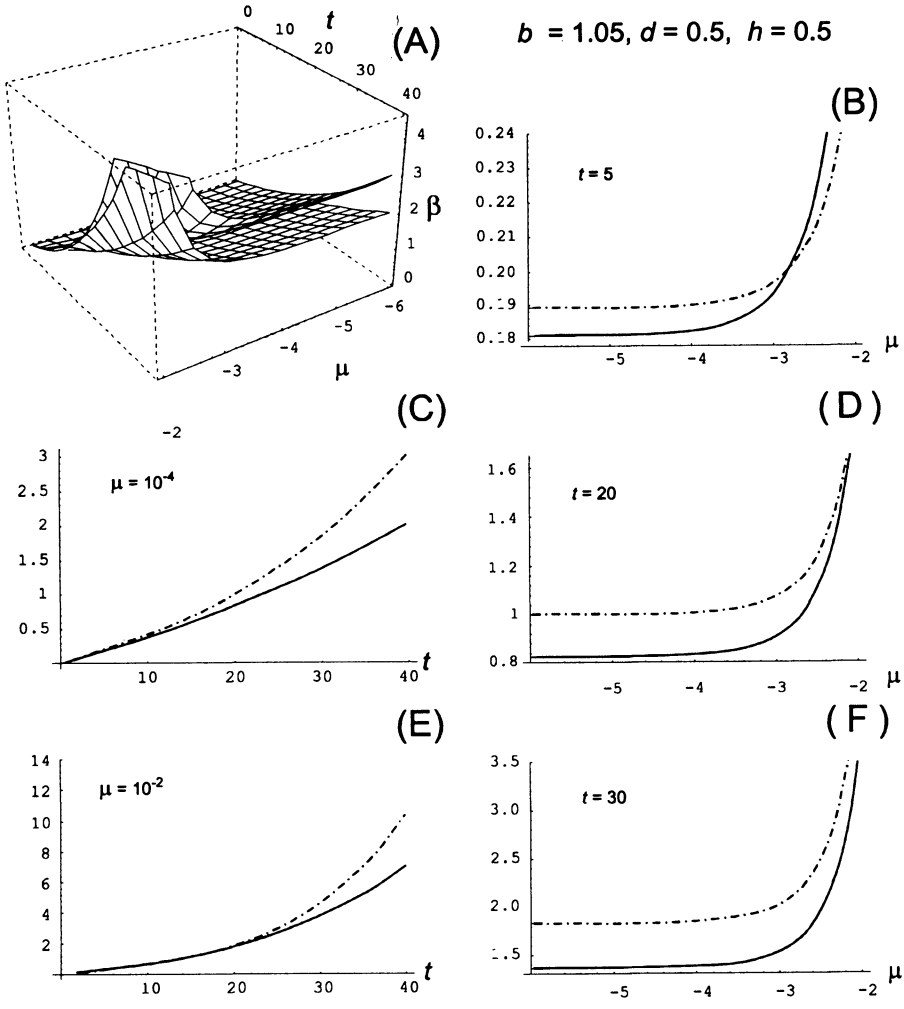

FIG. 6.—Critical β for cooperation to increase from rarity, with equation (12) as a function of the time for development, t, and the mutation rate, μ, assuming the replication advantage for defection, $b = 1.05$, $c = 1$, and additive genes. In the three-dimensional panel, the upper surface (greater values of β) is for haploidy, and the lower surface is for diploidy. In the two-dimensional slices in the seven remaining panels, dashed-dotted lines are for haploidy, and solid lines are for diploidy. There are three slices in the m-β plane for values $t = 5$, 20, 30, and two slices in the t-β plane for values $\mu = 10^{-2}$, 10^{-4}. See the text for more discussion.

in the population. In addition, the reduced difference in adult size in diploids discussed in the previous section gives an advantage to diploidy. For initial increase conditions, the between-organism variance effect is concealed by the effects of adult size and within-organism variation, giving an advantage to diploidy. However, for high mutation rates, the heterozygous effect within organisms becomes less important because *CD* cells quickly mutate to *DD* cells, and the

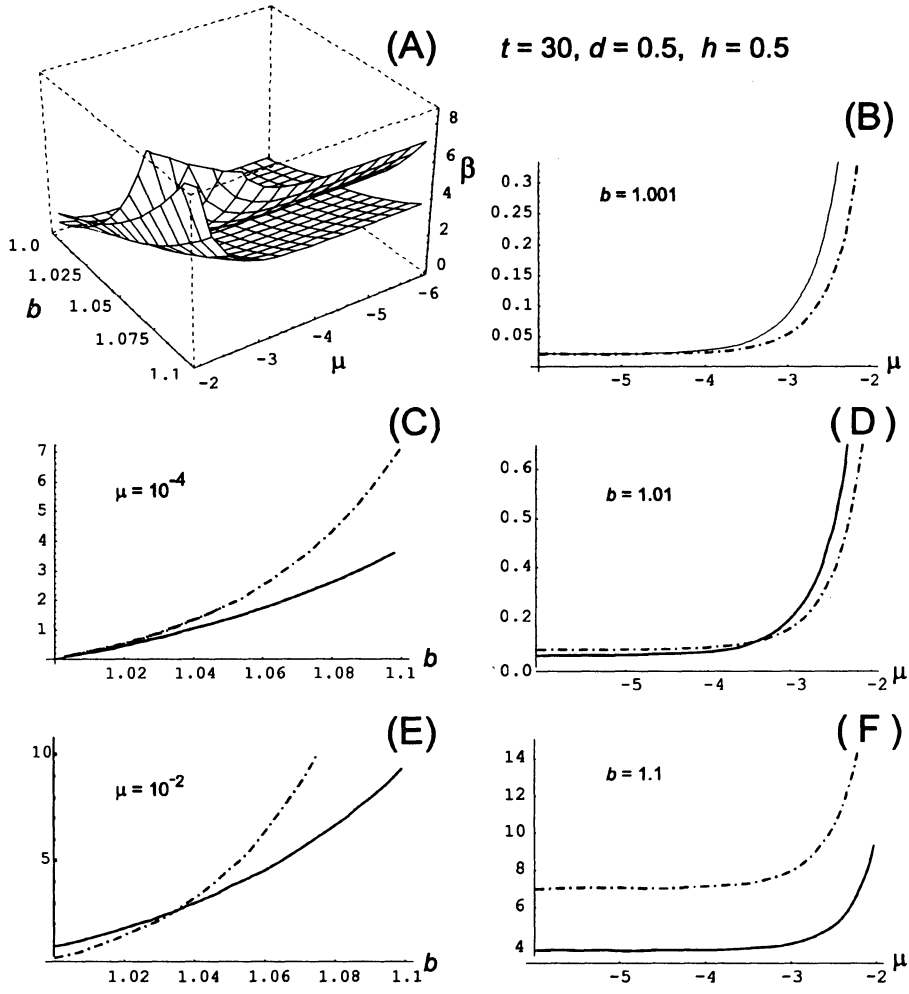

FIG. 7.—Critical β for cooperation to increase from rarity. This figure is similar to figure 6 except equation (12) is a function of the replication advantage of defection, b, and the mutation rate μ assuming the time for development $t = 30$ and additive genes. There are three slices in the μ-β plane for values $b = 1.001$, 1.01, 1.1, and two slices in the b-β plane for values $\mu = 10^{-2}$ and 10^{-4}. See the text for more discussion.

advantage of haploidy becomes manifest: so long as the opportunity for differential replication of defecting cells is small, b and t are small (see $t = 5$ panel of fig. 6 and $b = 1.001$, $b = 1.01$, or $\mu = 10^{-2}$ panels of fig. 7).

Levels of Cooperation among Cells within Organisms

Fixation of cooperation cannot occur no matter how large the benefit, β, small the organism, t, or weak the selection among cells, b, because of recurrent mutation, $\mu > 0$, leading to loss of cell and tissue function. The best we can hope for

is that cooperation among cells increases when rare and reaches high levels in the population and within the organism. The internal equilibria of the system are of interest for this reason. There are two kinds of internal equilibria possible in the models: mutation selection balance and heterozygote superiority in the case of diploidy.

The internal equilibria are defined in equations (B1)–(B4) of appendix B along with the methods used to study their existence and stability. It can be shown that if cooperation increases from rarity, it reaches a stable internal equilibrium. Consequently, the regions for increase of cooperation given in figures 6 and 7 are also regions for the existence and stability of biologically meaningful equilibria. There is a further complication for asexual diploidy described in appendix B. For haploidy and sexual diploidy, there is just one internal equilibrium. However, for asexual diploidy, there are two internal equilibria possible in the diploidy regions of figures 6 and 7 (see app. B).

From figure 6 and 7, we see that the region for cooperation to exist and be stable is usually larger under diploidy than haploidy. However, as already discussed, there are regions of parameter space, especially for high mutation rates in organisms of small size or small advantage of defection, in which haploidy fares better. The regions in figures 6 and 7 indicate existence and stability of equilibria but tell us nothing about the levels of cooperation attained at equilibrium. I consider this question next.

The level of cooperation attained among cells in organisms using different reproductive systems can be studied by comparing the equilibrium values of the frequency of cooperation (eqq. [B1]–[B4]). This is done in figure 8 for mutations with intermediate dominance as a function of the development time (panels *A*, *B*), the mutation rate (panels *C*, *D*), the benefit to organisms of cooperating (panel *E*), and the advantage to cells of defecting (panel *F*). Many combinations of parameter values have been studied; however, figure 8 is typical. Only two-dimensional graphs are shown because three-dimensional graphs are confusing in this case as one surface tends to block the others. In the figures for asexual diploidy (esp. figs. 8 and 11), there appears to be a discontinuity in the curves at the point when the two equilibria exchange stability. The humped curve for asexual diploidy is really a product of two separate curves that cross and exchange stability at the point of apparent discontinuity. The unstable portions of the curves are not shown for reasons of clarity.

Several conclusions may be drawn from figure 8. Haploid organisms maintain higher levels of cooperation among their component cells than do diploids but cannot tolerate as much within-organism selection—haploids must be smaller in size ($t < 30$ in panels *A* and *B*), and the advantage of defection must not be too large ($b < 1.08$ in panel *F*). There is little effect of the mutation rate over a wide range (10^{-6} to 10^{-3}); however, for haploid organisms to become bigger, a way must be found of limiting the mutation rate to less than about 10^{-3} ($\mu < 0.001$ in panel *D*). There is an abrupt limit in size for haploid organisms at about 10^9 cells ($t = 30$). Cooperation remains high up to this threshold and then drops off precipitously in an almost steplike manner. The weaker the mutation rate is, the more precipitous is the drop (compare the haploid curves in panels *A* and *B*). The

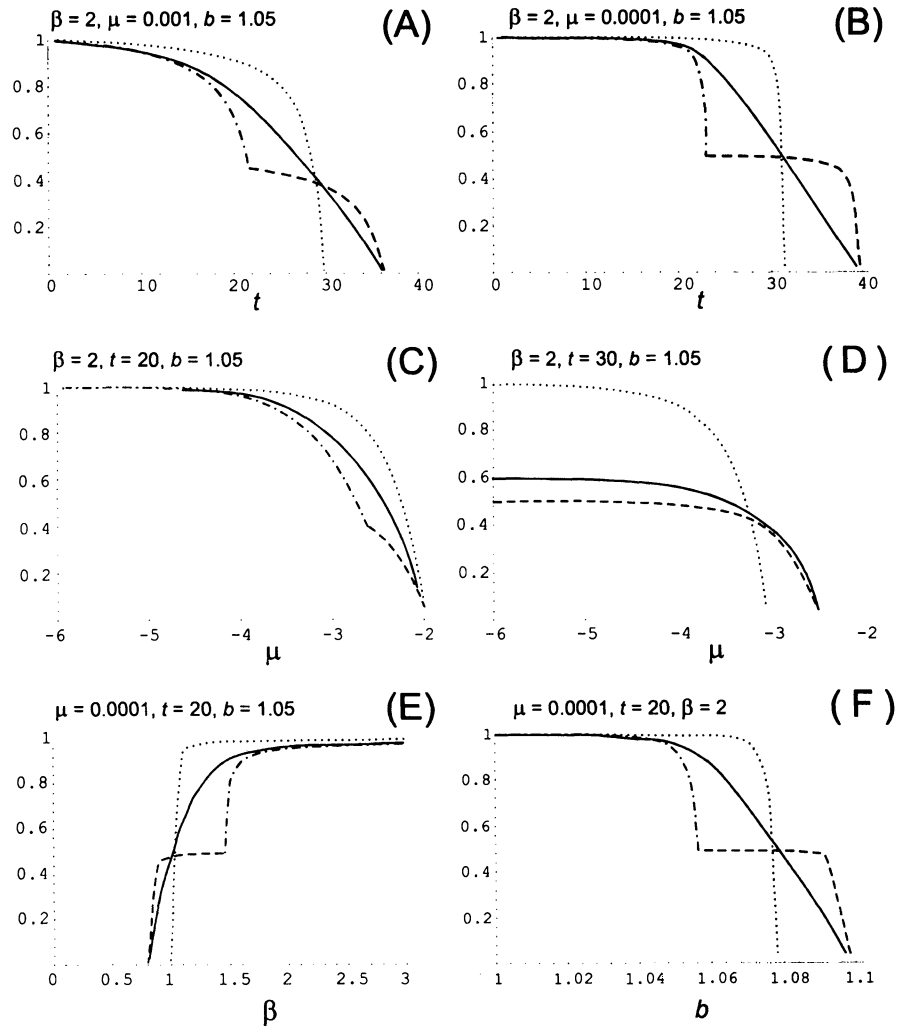

FIG. 8.—Level of cooperation for mutations with intermediate dominance ($h = d = 0.5$). The vertical axis in all panels is the equilibrium frequency of cooperation allele. Parameter values are given above each panel. The horizontal axis varies between panels. Haploidy is indicated by dotted lines; diploid sex, by solid lines; diploid asexuality, by dashed lines (first equilibrium eq. [B3]) and dashed-dotted lines (second equilibrium eq. [B4]). Only locally stable equilibria are shown.

exact position of these thresholds depends on the other parameters, especially β. However, the conclusions still hold in a qualitative sense but for different threshold values.

Diploid organisms may be bigger than haploids, but the level of cooperation among cells drops off quickly. Indeed, it is questionable whether the less than 50% level of cooperation permitted for diploids in this size range ($t > 30$) is

sufficient for organisms—groups of cooperating cells—to exist at all. In diploids, the level of cooperation begins dropping off at the same size of about 10^6 cells regardless of whether the organisms are sexual (the level of cooperation begins dropping off when t is in the low twenties in the $\mu = 0.001$ and $\mu = 0.0001$; panels *A* and *B*). This size of 10^6 cells is significantly less than the similar threshold in size for haploidy ($\approx 10^9$ cells, assuming no cell death). Haploidy is the system of choice for smaller organisms ($t < 30$), in terms of the level of cooperation attainable among component cells.

More intense selection among cells (higher levels of b) does not qualitatively affect the lessons drawn from figure 8*A–E* (results are not presented here for reasons of space). The general shape of the curves are the same but are offset to the left in all panels except in panel *E*, where the curves move to the right. In other words, for larger b, the thresholds mentioned with regard to figure 8 occur for smaller values of t (smaller organisms) and μ and greater values of β.

The *D* allele is a deleterious mutation, and most deleterious mutations are recessive or nearly so. In figure 9, the frequency of the cooperation allele is graphed for the case of *D* recessive and *C* completely dominant ($h = d = 1.0$) for the same set of parameters and conditions as those used in figure 8. The haploid curves in figure 9 are identical to those in figure 8, but they are graphed again for purposes of comparing the diploid curves. The advantage of diploidy and sex in coping with higher levels of within-organism variation and selection for additive alleles (higher t, b, and μ) goes away when mutations are recessive (in all panels in fig. 9, the diploid and haploid curves reach the *X*-axis for identical parameter values). Complete dominance removes the previously discussed advantage of intermediate dominance in reducing differences in adult size. The frequency of the cooperation allele is improved for both sexual and asexual diploidy (for sex, cf. especially the *D* panels of the two figures). For asexual diploidy, it appears that cooperation is doing worse (cf. any of the panels except *D*). However, this is misleading because the *C* allele is dominant, and most of the *C* alleles are present *CD* heterozygotes. Consequently, a frequency for *C* of about 0.5 (which is the case in most panels in fig. 9) means that almost all of the cells are *CD* heterozygotes and all are expressing the cooperation phenotype.

Fitness of Organisms

As a means to further clarify the forces and factors shaping the evolution of cooperation among cells within organisms, I consider organism fitness, and its covariance with genotype, as a function of the parameters describing within-organism change.

In figure 10, the average organism fitness is graphed for the equilibrium populations studied in figure 8. Population fitness declines with the parameters describing within-organism variation and selection: development time, t (panels *A* and *B*), mutation rate, μ (panels *C* and *D*), and the advantage of defection to cells, b (panel *F*). Average fitness increases with the benefit of cellular cooperation, β (panel *E*). Apart from these predictable relations, the average fitness of organisms is remarkably similar for the different reproductive modes considered (note the expanded scale in panel *D*). Average fitness departs slightly in the regions of

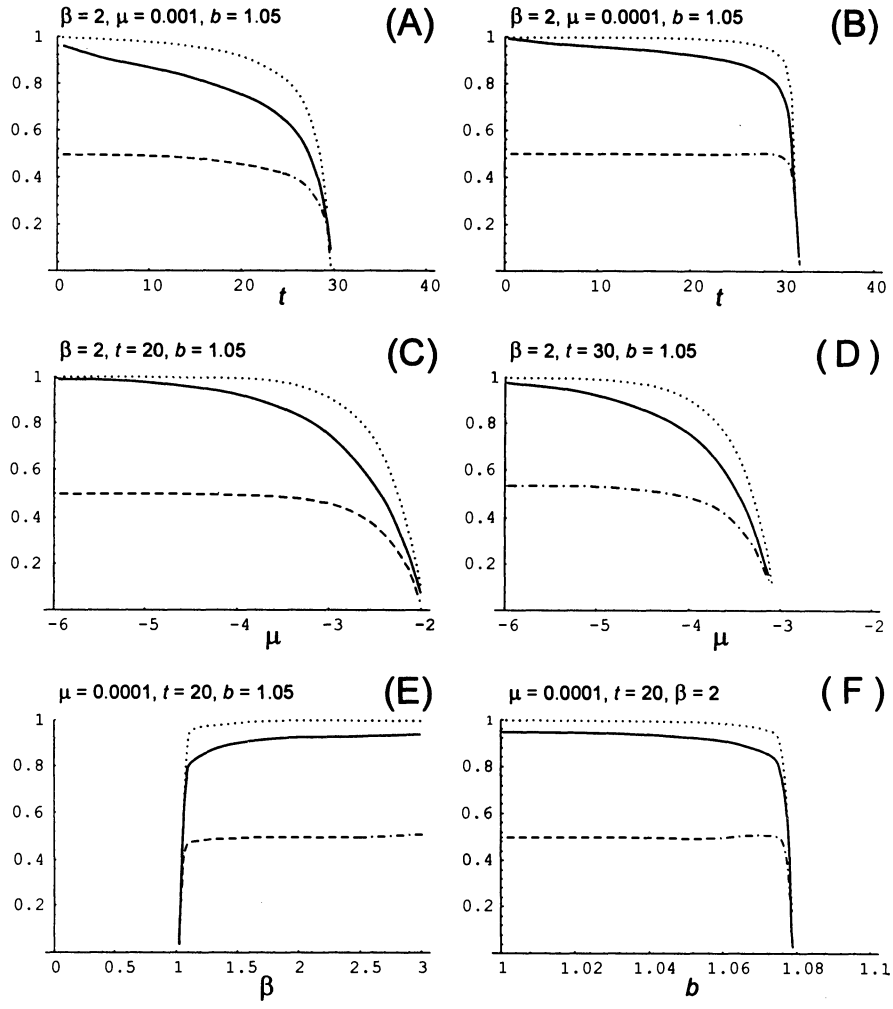

FIG. 9.—Frequency of cooperation allele for recessive mutations ($h = d = 1.0$). The legend is the same as that for figure 8.

differences observed in figure 8 but does not explain the patterns and degree of differences between reproductive modes observed in figure 8. Similar results are observed for recessive mutations (results are not shown here).

In figure 11, I consider the level of covariance of fitness with individual frequency for the equilibrium populations studied in figure 8. The different components of the Price equation—the variances, covariances, and regressions involving organism fitness and individual frequency—all have something different to tell about the underlying causes of gene frequency change. In figure 11, I graph the first part of the right-hand side of the Price equation, $\text{cov}[W, q_I]/\overline{W}$ (eq. [4]) in the case of haploidy and sexual diploidy and the population average of

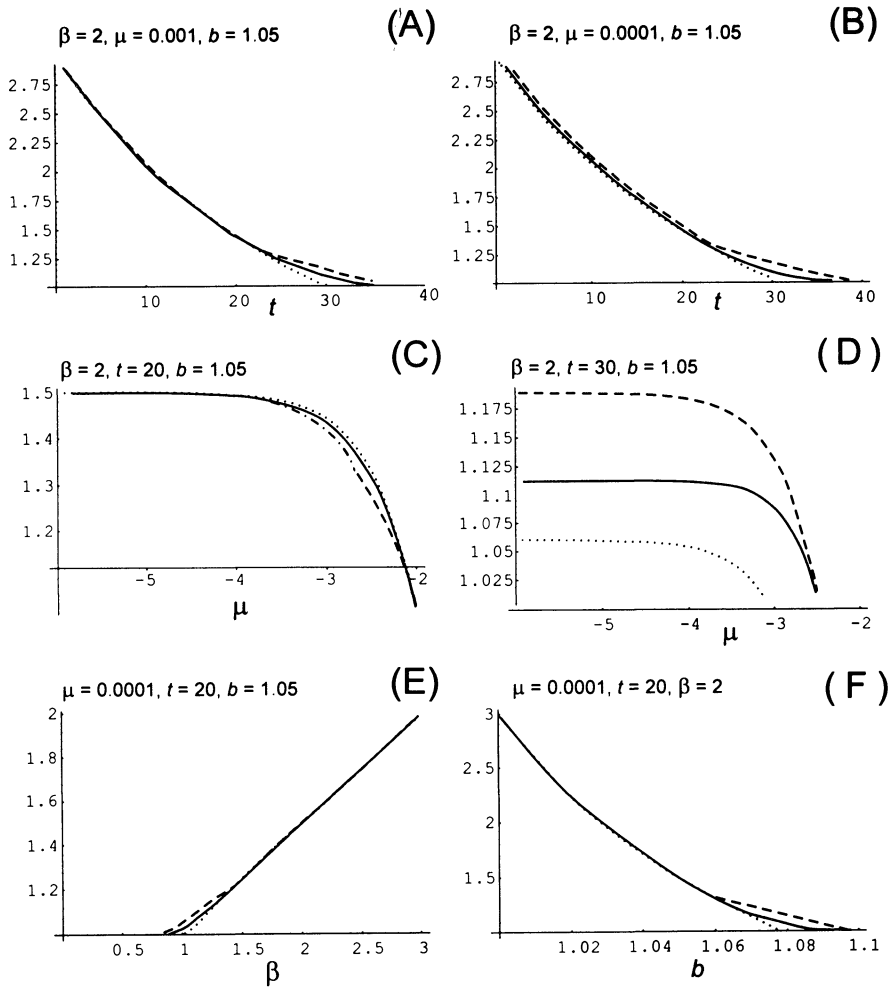

FIG. 10.—Average fitness at equilibrium for mutations with intermediate dominance. Panels and parameter values correspond to those given in figure 8. Statistics are based on relative organism fitness obtained by dividing absolute fitness, W_j, by the absolute fitness of the defecting genotypes, D or DD, so that $W_D = W_{DD} = 1$ at all points in all panels. Note that organism fitness does not depend on the gene and genotype frequencies in the population of organisms (it does depend on the frequencies of cell types within the organism, however). The legend is the same as that for figure 8.

$\mathrm{cov}[W, f^i]/\overline{W}$ (eq. [7]) in the case of asexual diploidy. The panels and parameter values correspond to those in figure 8. Populations at equilibrium must exactly balance the two levels of selection—within and between organisms—because within-organism change is never 0 because of mutation. Higher values of fitness covariance imply correspondingly high levels of within-organism change, or else the population could not be in equilibrium. For this reason, the weighted covari-

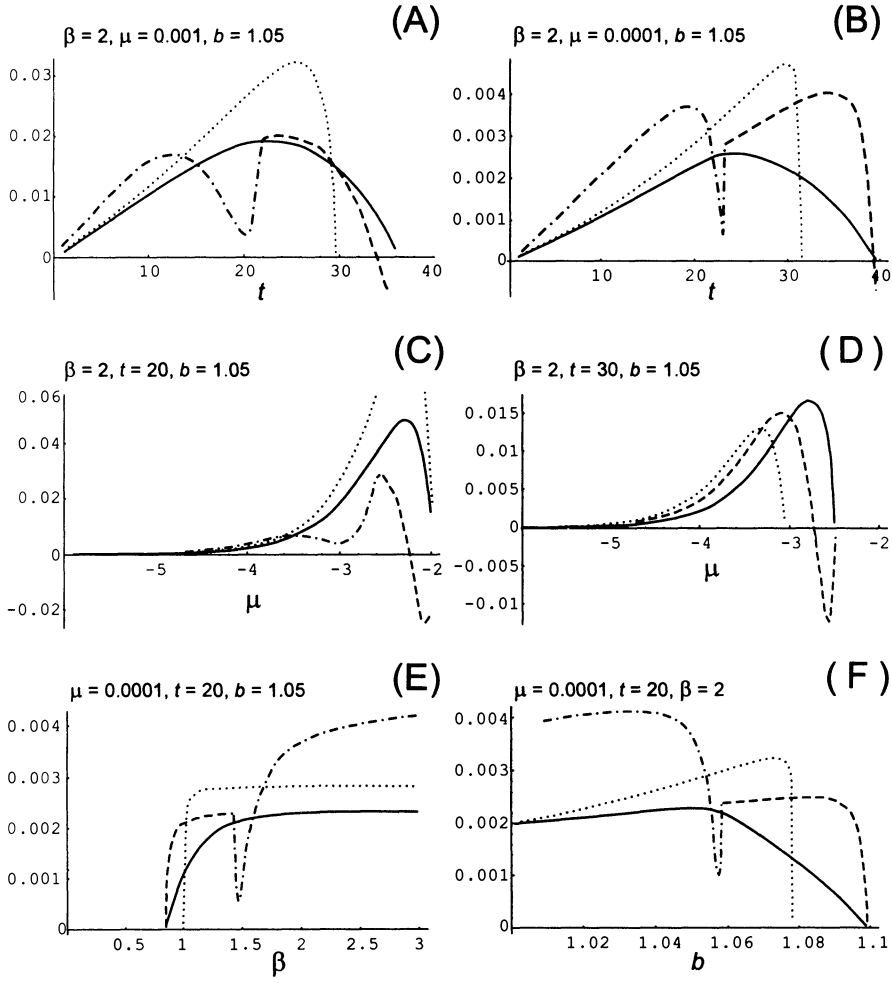

FIG. 11.—Fitness covariance at equilibrium for mutations with intermediate dominance. Panels and parameter values correspond to those given in figure 8. The vertical axis in all panels is the first part of the right-hand side of the Price equation, $\text{cov}[W, q_I]/\overline{W}$ (eq. [4]) in the case of haploidy and sexual diploidy and the population average of $\text{cov}[W, f^i]/\overline{W}$ (eq. [7]) in the case of asexual diploidy (using f_0, f_1, f_2 to take the average). Since the populations are at equilibrium, these weighted covariances must also equal the negative of the average within-organism change—that is, the second part of the right-hand side of equation (4) (and the average of the right-hand side of eq. [7] for asexual diploidy). Statistics are based on relative organism fitness as discussed in the legend to figure 10. The rest of the legend is the same as that for figure 8.

ances graphed in figure 11 must equal the negative of the average within-organism change (i.e., the second part of the right-hand side of eq. [4] for haploidy and sexual diploidy or the average of the right-hand side of eq. [7] for asexual diploidy). Consequently, the curves in figure 11 may be interpreted in two ways: either as the weighted covariance of organism fitness with individual frequency or as the amount of within-organism change.

Figure 11 implies that there is significant fitness variation at the organism level in equilibrium populations. The figure underrepresents the amount of fitness variation underlying the covariation graphed there, because the covariance is divided by the average population fitness, which takes on values between 1 and 3 for the equilibria considered. So the covariances are larger than the values plotted in figure 11 by a factor of 2 or 3.

Although the level of cooperation is often higher in haploids than in diploids, the level of within-organism change can show the opposite relationship—being lower in diploids than in haploids; this seems to be especially true for sexual diploids. In other words, although sexual organisms may not maintain as high a level of cooperation for some regions of parameter values, they more ably modulate selection and variation within organisms. This makes the high level of cooperation attained in equilibrium haploid populations even more striking because it is attained in the face of higher levels of within-organism change by equally high levels of fitness covariance. For asexual diploids, the average of the covariances of fitness and individual frequency become negative (alternatively, the average within-organism change becomes positive) as development time and mutation rate get large (see panels *A–D*). This is because the fitnesses of *DD* and *CD* genotypes have less to do with their own properties and more to do with the properties of the genotypes that mutate to them. This also occurs in sexual populations, but it does not affect the covariance in the same way.

Sex allows the integration of the genotypic covariances in a way not possible in asexual populations. As the mutation rate increases in sexual organisms, the regression of fitness on zygote gene frequency actually increases (results are not reported here). In other words, as the mutation rate increases, and along with it the amount of within-organism change, more of the variance in fitness is explained by the cooperative alleles carried in the zygote. How can this be? The greater mutation rate must result in greater levels of within-organism change. At equilibrium this within-organism change must be balanced by a larger covariance of fitness with zygote frequency. This is what equation (4) says. However, in haploid populations this is accomplished by a greater variance in zygote gene frequency, while in sexual populations this is accomplished by a greater regression of fitness on zygote frequency (results are not reported here). Although it is unclear whether equilibrium covariances predict anything about evolutionary transitions, this greater precision in the mapping of cooperative propensity onto organism fitness may, if it were to extend into the nonequilibrium realm, allow sexuals to make the transition from cells to organisms more easily under more challenging circumstances.

As already mentioned (and discussed in app. B), under asexual diploidy there

are two equilibria: the first (eq. [B3]) has no *CC* genotypes present, and the second (eq. [B4]) maintains all three genotypes in nonzero frequencies. For lower levels of within-organism change (smaller b, t, and μ) and higher benefits of cooperation (β), the *CC* genotype may exist at appreciable frequencies (the second equilibrium given in eq. [B4] is biologically meaningful and stable). However, as the levels of within-organism variation increase and/or the benefit of cooperation decreases, *CC* genotypes decrease in frequency as the second equilibrium merges into the first (figs. 8 and 9). As this point is approached, the variances and covariances first decrease as the *CC* genotype gets lower in frequency. However, as the *CC* genotype goes extinct, the covariances increase dramatically to reach the curve for the second equilibrium, giving rise to the spikes in figure 11.

As seen in figure 12, these spikes do not occur for recessive mutations, because the two asexual diploid equilibria merge into each other in a more continuous fashion (notice the reduced nature of the humps for the asexual diploid equilibria in fig. 9). The fitness covariances for diploids assuming recessive mutations (fig. 12) are generally similar to those for additive mutations (fig. 11), in the sense that diploids have less within-organism change at equilibrium than do haploids, and sexual diploids usually have less within-organism change than do asexual diploids (except in regions of high levels of within-organism change where asexual diploids undergo the sign reversal discussed earlier).

DISCUSSION

Main Conclusions

The evolution of organisms is a multilevel selection process. Cooperation alleles increase in frequency when the fitness covariance at the level of the organism overcomes within-organism change toward defection (figs. 6, 7). Selection and mutation during development generate significant levels of within-organism variation (figs. 2, 3, 11, 12). As a result of this within-organism variation, significant variation in organism fitness is maintained in equilibrium populations (figs. 11, 12). It is easier for cooperation to increase in diploid organisms (figs. 6, 7), although the levels of cooperation attained are usually higher in haploids (figs. 8, 9). The levels of cooperativity attained in diploid organisms can be low, even with reproduction passing through a single-cell zygote stage and the high kinship that entails (fig. 8). Haploid organisms should be smaller in size than diploids (fig. 8*A*, *B*), while diploids have lower levels of within-organism change than do haploids (figs. 2, 3, 11, 12). Among diploids, sex serves to maintain higher levels of cooperation and lower levels of within-organism change (figs. 8, 11, 12). Finally, fixed organism size may help organisms reduce conflict among cells.

Assumptions of Models

The evolution of conflict and cooperation among cells within organisms has been represented in terms of several basic parameters and variables at a single gene locus. This simplification permitted analysis of the consequences of development, but the limitations of the approach must be kept in mind. The limitations

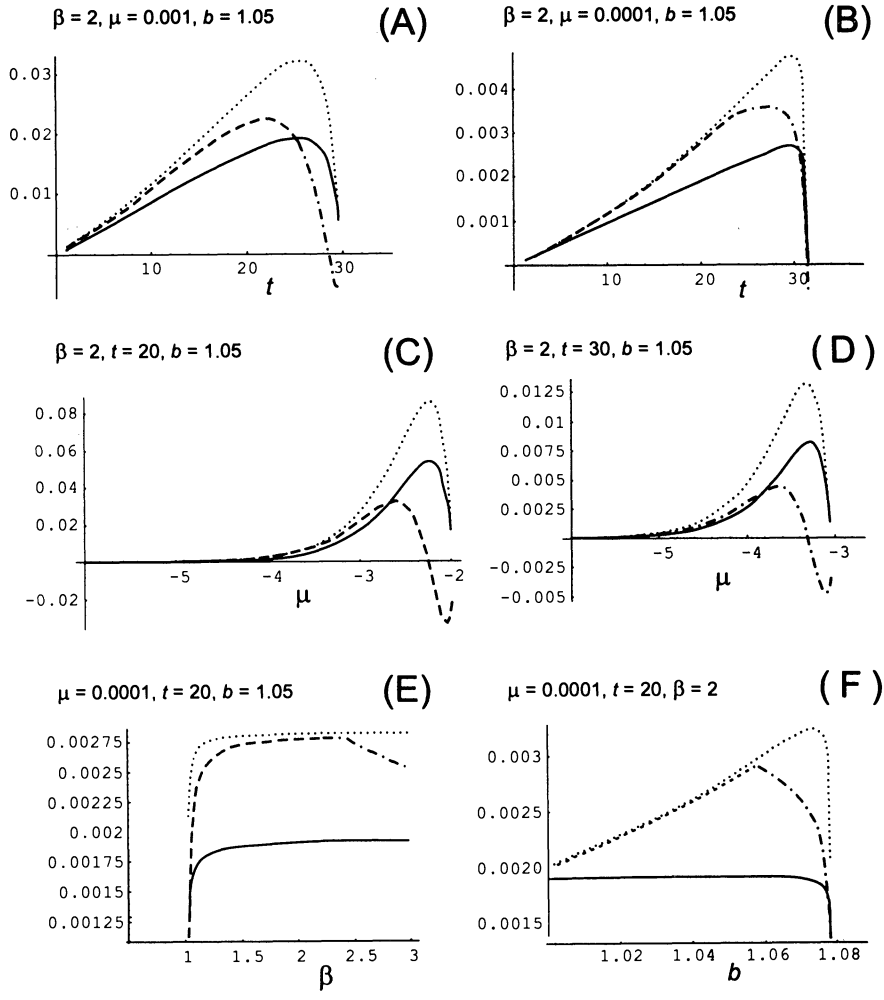

FIG. 12.—Fitness covariance at equilibrium for recessive mutations. Panels correspond to those for figure 9; otherwise, the legend is the same as that for figure 11.

and consequences of considering a single locus are discussed in the next section. Selection within the organism depends on the rate of cell division; cell death, known in practice to be common, was ignored. Within-organism variation after development is represented by the expected number of cells of different types in the adult form—the k_i and k_{ij} variables defined in appendix A (Haploid model and Additional terms for diploid model). Many aspects of the analysis, especially the various equilibria and their stability for the different reproductive systems, could be obtained without explicitly specifying values for these variables. For the numerical studies reported in the graphs, a specific within-organism mutation selection model is assumed (the models of mutation and cellular selection for

haploidy and diploidy). There may be other more realistic mutation selection models that could be assumed to obtain values for the k_{ij} variables, and the model was set up as a framework with this possibility in mind. Several additional key assumptions are necessary, permitting analytical analysis of the problem.

The complexity of interaction among different cell types and tissue function is assumed to be represented by one variable: cooperativity. The assumption of representing the complexity of interaction of different cell types into a single variable "cooperativity" is really no different than representing the cooperation of a wasp colony, with diverse castes and functions, by positing a single cooperative strategy. This approach has led to a deep understanding of the evolution of social behavior of organisms within social groups, and I believe a similar approach will prove useful in the study of the social behavior of cells within organisms.

Another issue of critical importance concerns the effects of mutation on fitness. 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. Mutations that by luck benefit both the fitness (replication rate) of cells and the fitness of the whole organism will sweep through the population—there is little reason to model them explicitly. This same point holds true for mutations that detract from the fitness of both levels, except that they will, of course, be lost from the population. There is some evidence for this 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 and Slobodchikoff 1981; Otto and Orive 1995). Mutations that 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. Malignant cancer is rarely a problem in plants because plant cells have a cell wall and are not highly mobile. The other class of mutations that harm the cell but benefit the organism can be addressed by a simple adjustment of the parameters in the models.

I assume that the organisms reproduce by passing through a single-cell zygote stage. This represents a "worst case" for the creation of within-organism variation. Many organisms, especially plants and invertebrate animals, may reproduce asexually by budding or fragmentation of many cells at a time. In this case, the within-organism variation present in the parent may be passed on to the offspring. The model was designed to incorporate these other forms of reproduction.

The models studied here are deterministic. A probabilistic model of within-organism variation and selection during development is given in the models for mutation and cellular selection for haploidy and diploidy in appendix A. However, the recurrence equations make use of the expected frequencies of the different cell types after development. To determine the range of validity and applicability of these results, Monte Carlo–type simulations of the branching process proposed in those appendix A models are planned.

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 (brain, muscle, liver), cells stop dividing; in other tissues (blood, intestine lining), cells continue to divide throughout life. To help fix ideas, I give estimates during the article of the number of cells that could be expected from a given development time, t. However, these numbers should be treated with great caution, since they do not include cell death. For example, if we ignore mutation and the different rates of replication of the different cell types, $t = 40$ would allow 40 cell divisions ($c = 1$), implying about 10^{12} cells in the adult—a number 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 approximately 400 (Vogel and Rathenberg 1975). In contrast, a typical human female egg is thought to be separated from its zygote by about 20 cell divisions (Vogel and Rathenberg 1975).

A theory of the emergence of organisms would be incomplete without addressing the emergence of tissue and cell types—a level between the cell and the organism. Including a third level in the hierarchical model presented earlier should not present any problem in theory. We already have in our simple formulation different cell types (cooperate, defect) in organisms of a specified type—that is what the k_{ij} variables represent. However, tissues are groups of cells, often spatially localized, in which the cell's phenotype is defined conditionally by interactions with other cells. There are immediate parallels with conditional behaviors among genetically identical organisms interacting in populations that suggest directions for future work (Brown et al. 1982; Michod and Sanderson 1985; Ferriere and Michod 1995; Ferriere and Michod 1996).

Effects of Parameters

The most critical parameters are the advantage to the adult organism of cooperation among its cells, β, and the parameters of within-organism mutation and selection: the difference in replication rates between cooperative and defecting cells, b, the time available for development, t, and the mutation rate per cell replication, μ.

A central result of the models studied here is that it is not the absolute rate of mutation that matters but rather the interaction between the mutation rate and selection and development time. Mutation (μ) provides variation that is acted on by selection (b) during development (t). The interaction between selection and mutation during development is critical in determining the overall levels of change within organisms (figs. 2, 3, 11), the initial increase of cooperation alleles (figs. 4–7), the levels of cooperation among cells (figs. 8, 9), and the final fitness characteristics of organisms once equilibrium is reached (figs. 10–12).

The levels of within-organism change observed here (figs. 2, 3, 11, 12) most certainly underestimate those occurring in nature because only a single locus was studied and cell death was ignored. Including cell death would increase the effect of selection and, as already discussed, would require a larger number of cell

divisions to attain a given size. More cell division means more opportunity for within-organism change (as seen in fig. 2, the levels of within-organism change increase with increasing t).

A single locus mutation rate of 10^{-5} gives rise to significant within-organism change at a single locus within one generation for reasonable differences in rates of replication (fig. 3). However, this rate of mutation is several orders of magnitude higher than what is the case in modern organisms. Higher mutation rates are realistic for more primitive organisms during the time in which individuality first evolved. Although it is helpful to use rates from modern organisms to provide parameter values for the models, it is not entirely valid to do so. Mutation rates in modern organisms are the outcome of evolution involving the very processes studied here. As I show in a follow-up article, one strategy to cope with within-organism change is to select for genetic modifiers that lower the mutation rate (Michod 1996).

A single locus was studied primarily for reasons of mathematical tractability and heuristic insight (Michod 1981). Because of this assumption and the assumption of no cell death, rather high mutation rates, when viewed from the perspective of rates per cell generation in modern organisms, are required to generate significant within-organism change. What really matters is the genome-wide mutation rate at different loci leading to the loss of a cell's ability to cooperate synergistically with its neighbors. An alternate modeling approach based on the distribution of numbers of mutants within organisms similar to that studied previously (Hopf et al. 1988) could be applied to this problem. However, such models do not lend themselves to the insights possible in an explicit genetic framework, such as those gained by partitioning change into within- and between-organism components. Admittedly, a small amount of mutation per cell division, say, 10^{-7}, would give rise to a small amount of within-organism change. Yet if many loci may potentially mutate and destroy a cell's capacity to cooperate, the total number of defecting cells in the adult could be quite substantial if each locus mutates with only a small probability. I expect that low mutation rates at many loci would have similar effects to the higher rates at a single locus studied here. This would likely depend on the assumptions that selection acts multiplicatively across loci and that the distribution of the number of mutations per genome under cell-lineage selection is Poisson. In any event, this is clearly a matter in need of further study.

Even low mutation rates and/or replicative differences would give rise to significant variation and change in organisms that live for longer periods of time, such as clonal invertebrates and some plants (corals, aspens, creosote). This can be seen by increasing the t parameter in the model. Naturally occurring rates of somatic mutation could be higher than germ line rates on a per–cell division basis. I say this because human somatic mutation rates measured 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).

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 and 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; Tsiòtou et al. 1995) and within-organism selection (Nowell 1976; Dennis et al. 1981; Michelson et al. 1987; Temin 1988; Gatenby 1991) occur in the development of many human cancers. Evidence for selection among mutant somatic cells also exists in plants (Gaul 1958; Stewart et al. 1972; Stewart 1978; Whitham and Slobodchikoff 1981) and clonal organisms (Buss 1985). In plants, mutation and selection create genetic mosaics that can be a problem in domesticated varieties (Whitham and Slobodchikoff 1981; Klekowski and Kazarinova-Fukshansky 1984). Selection among cells requires individual cells to express their own characteristics, as has been observed in plants (Stewart et al. 1972; Stewart 1978). Somatic selection is likely to be strong in modular organisms that undergo continuous mitotic proliferation and/or develop by budding, such as corals, aspens, creosote, or *Hydra* (Buss 1985; Hughes 1989). Cellular selection may be an important defense against aging in constantly replicating cell lineages (Bernstein and 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). Maynard Smith and Szathmary (1995) argue that the high degree of genetic relatedness among cells make organisms possible. However, we have found that the replication advantage of defecting cells, b, is a critical threat to organism integrity, even with reproduction passing through a single-cell zygote stage and the high intercellular kinship that entails. As shown in figures 7 and 5, there is a relatively small window of values of b, the replication advantage of defecting cells, that can be successfully offset by cooperation, β, among cells at the adult stage. This is because the advantage accrued by defecting cells is compounded like interest in the bank during cell division, while the benefit of cooperation after development, β, is expressed just during the adult stage. I believe that this is a general problem with the evolution of organisms and that it correctly represents biological reality, at least for many organisms. The functioning adult must offset the risks of development. It is a special case of the generally appreciated problem with group selection: groups replicate more slowly than their component units. The critical effect of b even for low mutation rates can also be seen in figure 3.

There is also a critical range of mutation rates between 10^{-3} and 10^{-2} that cannot be exceeded if cooperation is to succeed (see figs. 2, 3, 6, 7), but these values are so high that they are probably not of practical significance. However, the critical value of b is about 10% even for low mutation rates of 10^{-5} (see fig. 7), and this difference in cell replication rate is well within observed values; 30% differences have been observed in some plant systems (Whitham and Slobodchikoff 1981).

Longer times for development are necessary to make bigger organisms. Organism size can have a number of effects; even though these effects were not explicitly studied here, they are the underlying reasons for our interest in development time. Complex organisms require division of labor among cells, and more cell types require a larger number of cells. Having a large size, however, does not necessarily imply a large number of cell types, as one can see from clonal inverte-

brates, multicellular fungi, and plants. Larger size may also reduce predation, and there may be allometric costs of larger size (Bell 1985). Whatever its advantage, large size means more cells, and more cells means more opportunity for mutation during development. It is this last effect that we are primarily concerned with here.

Organism size is a critical factor in the models studied here. A central result is that it is easier for cooperation to evolve and attain high levels in small organisms than it is in large ones. This suggests that well-integrated large organisms are more likely to have evolved from small organisms with harmoniously interacting cells rather than from less integrated large organisms.

Effects of Mode of Reproduction

Whether reproduction is sexual or asexual and the organisms are haploid or diploid all have profound effects on the evolution of cooperativity among cells, the levels of within-organism change, and the fitness characteristics of organisms. To attain ever-greater levels of harmony among cells, rare alleles increasing the levels of cooperation must increase. The prospects for rare cooperative alleles is generally highest for diploids and lowest for haploids (figs. 6, 7), except when mutation rates are high in organisms of small size ($t = 5$ panel in fig. 6). The advantage of haploidy in small organisms with high mutation rates is especially pronounced if the mutations are only slightly advantageous in terms of their replication advantage (see $b = 1.001$, $b = 1.01$, $\mu = 10^{-2}$ panels of fig. 7). Nevertheless, large organisms must be diploid for reasonable levels of β, the benefit to adult organisms of cooperation among their cells. For example, if $\beta = 2$, haploid organisms have a maximum size of about 10^9 cells ($t = 30$), while diploids may reach sizes of 10^{12} ($t = 40$), again assuming no cell death, before within-organism change dominates (figs. 2, 3).

If cooperation alleles increase when rare, they come to an internal equilibrium at which within-organism mutation and multilevel selection are in balance. The regions of existence and stability of cooperation are also greater under diploidy than under haploidy (figs. 6, 7), again except for small organisms with especially high mutation rates, in which case haploidy fares better. When cooperation is possible under haploidy, it is more extreme, as cooperative alleles attain higher frequency under haploidy than under diploidy (fig. 8). At equilibrium, significant variation in organism fitness is observed. Sexual systems tend to maintain lower levels of within-organism change and less fitness variation than asexual systems (figs. 11, 12).

The initial increase conditions are the same for sexual and asexual diploids because the dominant eigenvalues of the two systems at the complete defection equilibrium are identical (eq. [9]). However, the levels of cooperation attained by sexual and asexual reproduction in diploids are different (fig. 8). For most regions of parameter space, sexual reproduction can maintain higher levels of cooperation than can asexual reproduction. However, what is remarkable is how low the levels are. Once organisms reach about 10^9 cells, no reproductive system can maintain the high levels of cooperation that one would think are a prerequisite for a harmoniously functioning organism, even for reasonable mutation rates

(e.g., $\mu = 0.0001$) and selection coefficients ($b = 1.05$, $\beta = 2$). This raises the question of whether evolutionary modification of the parameters of within-organism change could help mediate within-organism conflict. In a subsequent article, I show that the within-organism variation studied here may select for a germ line and other modifiers of the parameters of selection and variation within organisms (Michod 1996).

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APPENDIX A

TABLE A1

TERMS IN MODELS

Term	Definition
Haploid model:	
k_{ij}	Number of i cells in the adult stage of a j zygote, $i, j = C, D$
k_j	Total number of cells in adult stage of j zygote after development; $j = C, D$
W_D	Adult fitness of D zygote; $\propto k_D$
W_C	Adult fitness of C zygote; $\propto k_{CC} + k_{DC} + \beta k_{CC}$
β	Benefit to adult organism of cooperation among cells
$q_1, \Delta q_1$	Initial frequency, and change in frequency, of C gene within organisms
$q, \Delta q$	Initial frequency, and change in frequency, of C gene in total population
Additional terms for diploid model:	
i, j	Subscript for cell genotype; $i, j = CC, CD, DD$
f_j	Frequency of cell genotype j in the zygote population
k_{ij}	Number of cells of genotype i in the adult stage of a zygote of genotype j
k_j	Total number of cells in adult stage of j zygote after development: $k_j = \sum_i k_{ij}$
W_j	Adult fitness of j: $W_j = k_j + \beta k_{2j} + d\beta k_{1j}$
$\overline{W}$	Average fitness: $\overline{W} = \sum_j f_j W_j$
d	Dominance parameter at adult stage ($0 \le d \le 1$)
h	Dominance parameter at cell level ($0 \le h \le 1$)
cb_h	Growth rate of CD cells: $b_h = h + b(1 - h)$
Mutation and cellular selection model for haploidy:	
μ	Within-organism 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 (in terms of replication rate; $b > 1$)
cb	Rate of cell division for defecting cells
k_{CC}	$= 2^{ct}(1 - \mu)^{ct}$
k_{DC}	$= \sum_{x=1}^{ct} 2^x(1 - \mu)^{x-1}\mu 2^{b(ct-x)} = \dfrac{\mu 2^{bct} - 2^{ct}(1 - \mu)^{ct}\mu}{-1 + 2^{b-1} + \mu}$
W_D	$= 2^{bct}$
W_C	$= k_{CC} + k_{DC} + \beta k_{CC}$
Mutation and cellular selection model for diploidy:	
k_{00}	$= 2^{cbt}$
k_{10}	$= 0$
k_{20}	$= 0$
k_{01}	$= \sum_{x=1}^{tcb_h} 2^x(1 - \mu)^{x-1}\mu 2^{cb(t-x/cb_h)}$
k_{11}	$= 2^{ctb_h}(1 - \mu)^{ctb_h}$
k_{21}	$= 0$
k_{02}	$= \sum_{x=1}^{x=ct} 2^{x+b(ct-x)}(1 - \mu)^{2(x-1)}\mu^2 + \sum_{x=1}^{x=ct-1}\sum_{y=x+1}^{y=x+\theta} 2^x(1 - \mu)^{2(x-1)}2\mu(1 - \mu)2^{y-x}$ $(1 - \mu)^{y-x-1}\mu 2^{cb[t-(x/c)-(y-x/cb_h)]}$
k_{12}	$= \sum_{x=1}^{x=ct} 2^{1+x-bh(-ct+x)}(1 - \mu)^{2x-1-bh(-ct+x)}\mu$
k_{22}	$= 2^{ct}(1 - \mu)^{2ct}$

APPENDIX B

Internal Equilibria

For haploidy there is a single possible internal equilibrium given in equation (B1) (it requires $W_C > W_D$ to be meaningful):

$$\hat{q}_H = \frac{W_C \frac{k_{CC}}{k_C} - W_D}{(W_C - W_D)}. \tag{B1}$$

The eigenvalue describing stability of this equilibrium $\hat{q}_H$ is the inverse of the eigenvalue at $q_H = 0$ or $1/\lambda_H$, where λ_H is given by equation (8). When cooperation increases from rarity, it reaches a stable internal equilibrium.

For diploid sex, the possible internal equilibria are defined by two roots of the quadratic equation (B2) that is obtained by setting $\Delta q = q' - q = 0$, where q' is given by equation (2):

$$\begin{aligned} 0 = {} & -2k_1k_2W_0 + 2k_{11}k_2W_1 + q^2(-2k_1k_2W_0 + 4k_1k_2W_1 - 2k_1k_2W_2) \\ & + q(4k_1k_2W_0 - 4k_1k_2W_1 - 2k_{11}k_2W_1 + k_1k_{12}W_2 + 2k_1k_{22}W_2). \end{aligned} \tag{B2}$$

Although symbolically tractable, these roots are complex functions of the k_{ij}'s and other parameters and will not be given here. In numerical calculations, I have found only one of the roots to be biologically realistic.

For diploid asexuality, I set $f_1 = 1 - f_0 - f_2$ in equation (3), giving two independent equations in f_0 and f_2. Setting $\Delta f_0 = \Delta f_2 = 0$ defines the possible equilibria $\hat{f}_0$, $\hat{f}_2$. Two kinds of internal equilibria are possible, although numerical calculations indicate that only one can be stable at a time (see fig. 8). The equilibria simplify considerably if we assume $k_{00} = k_0 = W_0$. This assumption means no mutation from D to C as is assumed in the selection mutation models given in appendix A. The first equilibrium assumes $f_2 = 0$ and is given in equation (B3):

$$\begin{aligned} \hat{f}_1 = 1 - \hat{f}_0 &= \frac{W_1 \frac{k_{11}}{k_1} - W_0}{W_1 - W_0}; \\ \hat{f}_2 &= 0. \end{aligned} \tag{B3}$$

It has the same form as the haploid equilibrium given in equation (B1), and its stability and other characteristics are similar. Indeed, in the case of recessive mutations ($d = h = 1.0$ in app. A, Mutation and cellular selection model for diploidy), the equilibrium frequency of CD cells given by equation (B3) equals the equilibrium frequency of C cells given by equation (B1). In this case of recessive mutations, the following relations can be seen to hold: $W_D = W_0$ (this is always true), and $W_D = W_0$, $W_C = W_1$, $k_C = k_1$, $k_{DC} = k_{01}$, $k_{CC} = k_{11}$.

The second asexual diploid equilibrium maintains all three cell types and is considerably more complex. It is given in equation (B4):

$$\hat{f}_0 = \frac{W_2(k_{02}k_1k_2W_1 - k_{01}k_2^2W_1 + k_{01}k_2k_{22}W_1 - k_{02}k_1k_{22}W_2)}{T}$$

and

$$\hat{f}_2 = \frac{(k_{00}k_2 - k_{22}W_2)(k_{01}k_2W_1 - k_1k_2W_1 + k_1k_{22}W_2)}{T}, \tag{B4}$$

with

$$T = k_2\begin{pmatrix} k_{00}k_{01}k_2W_1 - k_{00}k_1k_2W_1 - k_{00}k_{02}k_1W_2 + k_{00}k_1k_2W_2 \\ + k_{02}k_1W_1W_2 - k_{01}k_2W_1W_2 + k_1k_{22}W_1W_2 - k_1k_{22}W_2^2 \end{pmatrix}.$$

By studying the linear stability of the equilibria given in equations (B1)–(B4), I have found that certain eigenvalues serve to define these regions of existence and stability. As already mentioned in the case of haploidy, the eigenvalue at the internal equilibrium can be obtained analytically and is the inverse of the eigenvalue at the fixation equilibrium given in equation (8). Consequently, the internal equilibrium is stable when the fixation equilibrium is unstable and vice versa, so that $\lambda_H > 1$ defines a region of stability for the internal equilibrium. The surface $\lambda_H = 1$ is given in figures 6 and 7 where it appears as the usually upper surface in the three-dimensional plot and the dashed-dotted line in the two-dimensional plots. For parameter values above this surface, a stable, biologically meaningful internal equilibrium for haploidy exists, defined by equation (B1). The equilibrium $\hat{q}_H$ is close to 1 for parameter values far above the surface (small t and large β) and tends to 0 in a continuous fashion as the parameter values approach the surface (see fig. 8).

A similar situation holds for diploid sex: when $\lambda_1 > 1$ (see eq. [9]), a stable biologically meaningful internal equilibrium exists, defined by equation (9). The surface defined by $\lambda_1 > 1$ is given in figures 6 and 7 where it appears as the generally lower surface in the three-dimensional plot and the solid line in the two-dimensional plots. For parameter values above this surface, the internal equilibrium for sex defined by equation (B2) is stable and biologically meaningful.

The situation is slightly more complex for asexual diploidy, since there are two internal equilibria to consider given in equations (B3) and (B4). The region still defines the existence of a stable internal equilibrium for asexual diploidy, but which one is it? The two eigenvalues describing the stability of the first asexual diploid equilibrium given in equation (B3) are given in equation (B5), in which the "diluting effect" discussed in reference to equations (8)–(10) appears again:

$$\lambda_3 = \frac{W_0}{W_1\dfrac{k_{11}}{k_1}}, \quad \lambda_4 = \frac{W_2\dfrac{k_{22}}{k_2}}{W_1\dfrac{k_{11}}{k_1}}. \tag{B5}$$

The first eigenvalue in equation (B5), λ_3, is the inverse of the eigenvalue λ_1 given in equation (9) that describes the increase of diploidy from rarity. Thus, when diploidy can increase from rarity, λ_3 (eq. [B5]) is less than unity. The stability of the first diploid equilibrium is then determined by the second eigenvalue in equation (B5), λ_4. When $\lambda_4 < 1$, the first diploid equilibrium (eq. [B3]) is stable, and the second asexual diploid equilibrium given in equation (B4) is not biologically meaningful. This can be determined by setting $\hat{f}_2$ in equation (B5) and seeing that $\hat{f}_2 > 0 \Rightarrow \lambda_4 > 1$. Therefore, the condition $\lambda_4 = 1$ defines a surface that divides the diploid region into two regions. The $\lambda_4 = 1$ divides the diploid region in figures 6 and 7 into two regions (not shown here for reasons of space). For parameter values above the $\lambda_4 = 1$ surface, the second equilibrium defined by equation (B4) is meaningful and stable, while the first equilibrium defined in equation (B3) is either not biologically meaningful or not stable. The opposite is the case for the region of parameter values between the $\lambda_4 = 1$ surface and the $\lambda_1 = 1$ surface. In this region the first equilibrium defined by equation (B3) is meaningful and stable, while the second equilibrium (eq. [B4]) is not.

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