Evolutionary Consequences of Mutation and Selection Within an Individual

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ABSTRACT

Whether in sexual or asexual organisms, selection among cell lineages during development is an effective way of eliminating deleterious mutations. Using a mathematical analysis, we find that relatively small differences in cell replication rates during development can translate into large differences in the proportion of mutant cells within the adult, especially when development involves a large number of cell divisions. Consequently, intraorganismal selection can substantially reduce the deleterious mutation rate observed among offspring as well as the mutation load within a population, because cells rather than individuals provide the selective "deaths" necessary to stem the tide of deleterious mutations. The reduction in mutation rate among offspring is more pronounced in organisms with plastic development than in those with structured development. It is also more pronounced in asexual organisms that produce multicellular rather than unicellular offspring. By effecting the mutation rate, intraorganismal selection may have broad evolutionary implications; as an example, we consider its influence on the evolution of ploidy levels, finding that cell-lineage selection is more effective in haploids and tends to favor their evolution.

EVOLUTION by natural selection is generally pictured as the constant struggle for existence among individuals; it is the individual that lives or dies, reproduces or remains barren. Yet, in many organisms, selection happens at a lower level, among the cell lineages within an individual. Somatic mutations in organisms without sequestered germ lines can create genetic mosaics, in which differential rates of cell mortality and replication can alter the fate of the mutation within an individual (WHITHAM and SLOBODCHIKOFF 1981; BUSS 1983; KLEKOWSKI 1988; SUTHERLAND and WATKINSON 1986; HUGHES 1989). Despite its potential importance, little theoretical attention has been paid to selection among cell lineages (HUGHES 1989). In a model of development specific to plants, KLEKOWSKI and KAZARINOVA-FUKSHANSKY (1984) showed that intraorganismal selection could have a large influence on the abundance of mutations. ANTOLIN and STROBECK (1985) came to the same conclusion based on a model of selection among the buds of a plant. Modeling intracellular selection among mitochondrial genomes within a cell, BIRKY (1991) found that selection could substantially reduce the rate at which deleterious mutations fix within a cell lineage. In this article, we develop models of selection acting within an individual, focusing on the role that such selection may play in reducing the frequency of deleterious mutations and the mutation load borne by a population (CROW 1970). We begin by reviewing the evidence for intraorganismal selection and the data on mutation rates.

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Evidence for intraorganismal selection: Selection among cell lineages would be impossible were it not for the fact that the expression of characters at the cellular level is often independent across cells (STEWART 1978). For example, anthocyanin synthesis, pH, cell size, and fragrance were found to be independently expressed in the different cells of a chimeric Camellia (STEWART *et al.* 1972). The majority of mutations are deleterious at the individual level (CROW and SIMMONS 1983), and it is reasonable to expect that most (*e.g.*, loss of function mutations) will also be detrimental to cell function. DEMEREC (1936) provided evidence for this supposition: 23 out of 39 mutations that were recessive lethal at the individual level were also cell lethal when homozygous in hypodermal tissue; the remaining 16 may have been detrimental but not lethal to the cells or may have been cell-lethal in other tissues (see also DEAROLF *et al.* 1988). There are, however, important exceptions where cell variants are detrimental to organismal fitness, most notably cancer cells (BUSS 1982; MOYRET *et al.* 1994; WOLF *et al.* 1994). We will focus on mutations that negatively affect both cell and individual replication, although the mathematical equations will be kept general and may be applied to either case.

The best evidence for selection among cells within an organism comes from chimeric plants (reviewed by STEWART 1978; WHITHAM and SLOBODCHIKOFF 1981; BUSS 1983). For example, variegated maple with white-tipped leaves grow at one-third the rate of the parental maple with green leaves; on average, mutations that revert a bud to the parental type grow to dominate

95% of a tree's foliage within 10 years (WHITHAM and SLOBODCHIKOFF 1981). That intraorganismal selection can eliminate mutations has been noted by plant breeders, who often want to control the appearance of new mutations (see GAUL 1958 and references therein). GAUL noted that, after X-ray irradiation of barley seeds, 30–50% more mutations appeared in early developing tillers than in late developing tillers. These results were explained by the fact that the first tillers to grow are formed from primordial cells already present within the embryo, allowing little opportunity for intercellular selection. Late tillers, on the other hand, develop from initial cells determined late in life, after extensive exposure to intraorganismal selection.

Other experimental studies have shown that selection can act upon genetic variation among organelles within an individual. Selection upon mitochondrial and chloroplast mutations can act at two levels: among the many copies of the organelle genome within a cell and among the cells of a multicellular organism. In *Saccharomyces cerevisiae* BACKER and BIRKY (1985) showed that intracellular selection was responsible for an increased fixation rate of mitochondrial mutants conferring resistance to erythromycin in the presence of this antibiotic. TILNEY-BASSETT and BIRKY (1981) found that selection, either at the intracellular or intercellular level, was needed to explain the distribution of plastids among embryos of Pelargonium. Similar evidence has been obtained for selection among mitochondria from heteroplasmic Drosophila lines (MATSUURA 1993) and from crickets (RAND and HARRISON 1986).

Mutation rates and mutation load: While mutations provide the ultimate fuel for evolution (heritable variation), their predominant effect is deleterious (CROW and SIMMONS 1983). Although the rate of deleterious mutations per diploid zygote per generation, U, has not been measured in many cases, it is estimated to be a minimum of 0.6 in Drosophila (MUKAI *et al.* 1972; CROW and SIMMONS 1983; HOULE *et al.* 1994), at least 0.5 in several plant species (CHARLESWORTH *et al.* 1990a, 1994), and very possibly greater than one in humans (KONDRASHOV and CROW 1993). In this paper, the "observed" mutation rate measures the rate of deleterious mutations in an individual generation (from a parent cell to an equivalent offspring cell) when selection acts within the generation. This measure will be compared to the expected rate of mutations in the absence of intraorganismal selection.

The effect of deleterious mutations on the average fitness of a population has been investigated in a series of models that largely ignore intraorganismal selection. These models focus on the mutation load, L, defined as the relative amount by which deleterious mutations reduce the mean fitness of a population, $\bar{w}$, from the value it could obtain in the absence of mutations, $\bar{w}_0$,

$$L = \frac{\bar{w}_0 - \bar{w}}{\bar{w}_0} \qquad (1)$$

(for a review of the load concept see CROW 1970). Although the interpretation of genetic loads is the subject of some controversy, we will assume that the evolutionary cost of a mutation load is that it increases the probability of extinction of a population, either in the absence of other species or in direct competition with them (*e.g.*, LYNCH and GABRIEL 1990).

The most straightforward calculation of the mutation load was made by KIMURA and MARUYAMA (1966) for a large diploid asexual population. Without recombination and segregation to regenerate lost combinations, the genome with the least number of mutations will be maintained at equilibrium only if selection exactly counterbalances mutation. Assuming that the number of new mutations follows a Poisson distribution with a mean rate per diploid genome per individual generation of U, the probability, P, that an individual produces an offspring with no more mutations than itself is e^{-U}. If the frequency of the least mutant class is p_0 and its fitness is w_0, the frequency of the least mutant class in the next generation is

$$p_0' = \frac{w_0 e^{-U} p_0}{\bar{w}}, \qquad (2)$$

ignoring backwards mutations. Combining (1) with (2) at equilibrium, the mutation load becomes $L = 1 - e^{-U}$, or 0.63 for $U = 1$. For haploid asexual populations, the mutation rate per individual and the mutation load are reduced, with the latter equal to $L = 1 - e^{-U/2}$.

In a sexual population that is large and haploid, the load remains $L = 1 - e^{-U/2}$ when the fitness consequences at each locus are independent (CROW 1970). For a diploid sexual population, the load lies between $L = 1 - e^{-U/2}$ and $L = 1 - e^{-U}$, depending on the dominance of mutations at each locus (CROW 1970). Fitnesses at different loci are not, however, independent. What little data exist suggest that each additional deleterious mutation decreases the fitness of an individual more than expected (synergistic epistasis) (MUKAI 1969). Using the quadratic equation estimated by MUKAI (1969) to describe the decrease in fitness as a function of the number of mutations, the load calculated for asexual populations remains the same, but the load for sexual populations is reduced by approximately one-half (KIMURA and MARUYAMA 1966; MUKAI 1969; CROW 1970).

KONDRASHOV (1994) extended the analysis of load in asexual populations to include the possibility that multicellular offspring are produced through budding or fission. In this case, newly produced offspring generally bear more mutations since they are composed of several genomes, each carrying their own mutations. KONDRASHOV showed that if there are n originating cells, the load is never less than it would be if single-cell reproduction were assumed (*i.e.*, for asexual diploids, $L \geq 1 - e^{-U}$).

All of the above derivations have assumed, however, that there is no selection within an individual. Yet cell-

lineage selection may play an important role in eliminating deleterious mutations and coping with DNA damage (MICHOD 1995). In this paper, we evaluate the effect of intraorganismal selection on mutation rates under a variety of models. Specifically, we calculate P, the probability that a mutant-free zygote cell gives rise to a mutant-free reproductive cell in the adult in the presence of intraindividual selection. For asexual populations, P replaces e^{-U} in (2) and the mutation load becomes $L = 1 - P$. We begin by developing models that allow cells to replicate at different rates according to whether or not they bear new mutations. Both completely plastic development and development from meristems are considered. Reproduction via multicellular offspring is then analyzed. Next, we review results exploring selection among organelle genomes within a cell. Finally, we discuss the broader evolutionary influence exerted by intraorganismal selection, focusing on the specific example of the evolution of ploidy levels.

MODELS WITH INTRAORGANISMAL SELECTION

Differential rates of cell replication with unstructured development: We begin with a model of selection affecting the replication rates of different cell lineages within a developing individual. We imagine an organism with plastic development and without a separate germ line, such as a coral or a hydra; it may be either haploid or diploid. The cells of this organism grow and divide for an amount of time τ before producing a single-celled offspring. Let c be the rate of cell divisions among nonmutant cells, so that in the absence of intraorganismal selection there would be $k = c\tau$ cell divisions per organismal generation and 2^k cells in the organism when it reproduces. Let the probability that at least one mutation occurs somewhere within the genome in a single cell generation be U_C (which is related to the genomic rate of mutations per organismal generation by $U_C = 1 - e^{-U/(2k)}$ for haploids and $U_C = 1 - e^{-U/k}$ for diploids). We will determine the proportion of cells in the fully developed adult that are nonmutant. These calculations are analogous to ones describing the spread of mutant cells in exponentially growing populations of bacteria (LURIA and DELBRÜCK 1943; LEA and COULSON 1949; KOCH 1982; LENSKI *et al.* 1989). These papers, however, assumed that mutant cells were always rare and that the number of mutations was constant even as the class of mutant-free cells dwindled. Since we are especially concerned with mutation events throughout the genome, we relax the assumption that mutant cells are rare and calculate the exact frequency of mutations arising in nonmutant cells.

If mutations did not effect cell survival or replication, the probability (P) that a reproductive cell in an adult is nonmutant given that the founding cell of the individual was nonmutant cell is

$$P = (1 - U_C)^k, \tag{3}$$

i.e., the probability that a mutation does not happen in any of the k cell divisions. As expected, upon substitution for U_C, the mutation load for a diploid asexual population equals $L = 1 - P = 1 - e^{-U}$.

To consider intraorganismal selection, assume that the replication rate of any mutant cell is reduced by an amount β, from c to βc, regardless of how many mutations accumulate within the cell (an assumption that is relaxed in APPENDIX A). To begin, consider mutations that arise during the xth cell division within nonmutant parent cells, *i.e.*, cells that have not mutated in any of the previous $x - 1$ cell divisions. The total number of daughter cells that will be produced by these nonmutant cells is $2^x(1 - U_C)^{x-1}$. Of these, a fraction, U_C, will mutate for the first time. These mutants will now undergo only $\beta(k - x)$ more cell divisions before reproduction because of their lowered cell division rate. In the fully developed adult, the expected number of mutant cells that originated in the xth cell division is therefore $2^x(1 - U_C)^{x-1}U_C 2^{\beta(k-x)}$. The expected number of mutant cells originating sometime within an organismal generation (M) would then be

$$M = \sum_{x=1}^{k} 2^x(1 - U_C)^{x-1}U_C 2^{\beta(k-x)} = U_C \frac{2^k(1 - U_C)^k - 2^{\beta k}}{1 - U_C - 2^{\beta-1}}$$

and the expected number of nonmutant cells would be $2^k(1 - U_C)^k$. Therefore, the probability (P_S) that a cell is nonmutant at the end of development, given unequal cell division rates, is approximately

$$P_S = 1 - \frac{M}{2^k(1 - U_C)^k + M}. \tag{4}$$

In this formula, a ratio of expectations is used to approximate an expected ratio. From a simulation testing all combinations of $\beta = \{0.05, 0.1, 0.015, \ldots, 1\}$, $U = \{0.001, 0.01, 1, 10\}$, and $k = \{20, 50, 100\}$, we have demonstrated that the error introduced by this assumption is <3%, except when $U = 10$ and $\beta \leq 0.5$ when the error can be as large as 10%. If, in addition, nonmutant cells compensate for the reduced replication of mutant cells by increasing their replication rate, the denominator in (4) becomes $2k$, making the probability that offspring cells are nonmutant even higher. Although we have assumed that mutant cells have the same fitness regardless of the number of mutations they accumulate, we generalize (4) in APPENDIX A to account for the fitness effects of multiple mutations within a cell lineage.

When $\beta = 1$, there is no intraorganismal selection and (4) reduces to (3). With $\beta < 1$, differential rates of cell replication within an organism increase the probability that a reproductive cell in an individual bears no more mutations than a founding cell (P_S increases). In Figure 1, the probability that an initially nonmutant parent gives rise to a mutant reproductive cell, $1 - P_S$, is plotted against the degree of cell-lineage selection; this also describes the mutation load in an asexual pop-

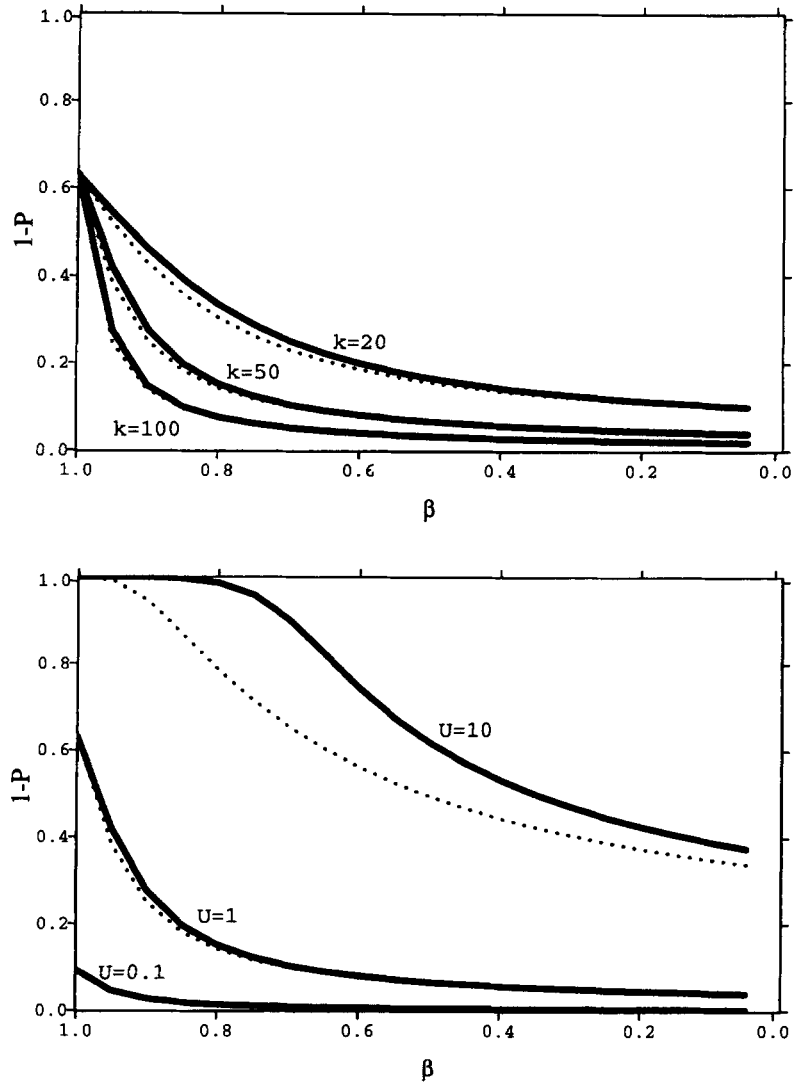

FIGURE 1.—Observed mutation rate with intraorganismal selection. Unstructured development. The probability of a nonmutant producing a mutant offspring cell (which equals the mutation load in an asexual population) is plotted against β, the growth rate of mutant cells relative to nonmutant cells. β is decreasing along the axis, from no cell-lineage selection at $\beta = 1$ to no mutant cell growth at $\beta = 0$. —, for $1 - P_S$ from (4); ---, for $p_k(0)$ from (A2) that allows for multiple mutations under multiplicative selection. (A) A genome-wide mutation rate of $U = 1$ for various values of k, the number of cell divisions per organismal generation for nonmutant cells. (B) $k = 50$ for various values of U.

ulation. $1 - P_S$ drops rapidly as β decreases so that even small differences in cell replication rates can substantially alter the probability of producing mutant offspring. This effect is especially pronounced when there are many cell generations per individual generation. Conversely, when mutant cells have higher rates of replication, $\beta > 1$, intraorganismal selection increases the proportion of mutant cells within the adult.

To illustrate the estimation of the parameter β, we use data examining the strength of selection against cells that are homozygous for the recessive lethal mutation in genetic mosaics of *Drosophila melanogaster* (DEAROLF *et al.* 1988). Somatic recombination, induced by gamma-irradiation of third-instar larvae, created some homozygous cells in a heterozygous background. The frequency of clones carrying somatic recombinants on the head of adult flies was then scored using bristle markers. In a control line, the frequency of awd^{+} homozygous clones was 0.559, but in the experimental lines the frequency of awd^{b3} homozygous clones was only 0.225. The control line estimates the frequency of somatic recombination events (γ) induced by irradiation, while the difference between the experimental and control lines provides an estimate for the strength of selection against awd^{b3} homozygous cells. Given that awd^{b3} homozygous cells replicate at a reduced rate, β, the expected frequency of awd^{b3} clones is

$$\frac{2^{\beta k}\gamma}{2^{\beta k}\gamma + 2^{k}(1 - \gamma)},$$

where k is now the number of cell generations between irradiation and the initiation of an epidermal clone on

the head. Using a rough estimate of $k = 10$ and letting $\gamma = 0.559$, we obtain an estimate for the selection coefficient against homozygous *awd*^{b3} cells of $\beta = 0.787$. More extreme selection against *awd*^{b3} cell lineages was found in other tissues and in larvae irradiated at the second-instar stage. Further experiments, such as the one performed by DEAROLF *et al.* (1988), that use visible or molecular markers to track the fate of mutations in genetic mosaics would be extremely useful, especially if extended to nonlethal mutations.

Selection within a developmentally structured individual: We now consider intraorganismal selection within organisms that develop primarily from meristematic tissue. In many pteridophytes, the apical meristem is dominated by an initial cell that repeatedly divides (KLEKOWSKI 1988). In seed plants, by contrast, the apical meristem is stochastic, consisting of a group of cells whose composition changes over time (KLEKOWSKI and KAZARINOVA-FUKSHANSKY 1984; ANTOLIN and STROBECK 1985). We will model development in stochastic apical meristems, where cell-lineage selection can affect which cells compose the meristem. Intraorganismal selection was addressed in such organisms by KLEKOWSKI and KAZARINOVA-FUKSHANSKY (1984). They considered the case where α apical cells are chosen at particular intervals to form the group of cells from which development occurs over the next interval. In their model the number of cell divisions is specified, but cells are allowed to die as a result of selection. KLEKOWSKI and KAZARINOVA-FUKSHANSKY considered the question of how selection within an individual alters the probability that mutant alleles, present in the first apical meristem, will be transmitted. We will adapt their model to consider mutations that arise continually.

Consider an organism that begins with α apical cells, each of which is nonmutant. The life cycle of the organism is again τ time units long but is divided into η rounds in which a new apical meristem is chosen and develops for an amount of time τ/η. We can easily adapt (4) for our purposes. We wish to determine the probability that a cell chosen to be one of the apical meristem cells is nonmutant given that there were i mutant cells and $(\alpha - i)$ nonmutant cells in the previous apical meristem. Each of the i mutant cells will grow to $2^{\beta k/\eta}$ cells when the next meristem is chosen. The $(\alpha - i)$ nonmutant cells will give rise to $2^{k/\eta}$ cells unless a mutation occurs, in which case the number of cells is reduced depending on when the mutation occurs. Overall, the probability that a cell chosen to be part of a new apical meristem is nonmutant given that the previous apical meristem had i mutants equals

$$P_M(i) = 1 - \frac{i2^{\beta k/\eta} + (\alpha - i)\sum_{x=1}^{k/\eta} 2^x 2^{\beta(k/\eta - x)} U_C(1 - U_C)^{x-1}}{(\alpha - i)2^{k/\eta}(1 - U_C)^{k/\eta} + i2^{\beta k/\eta} + (\alpha - i)\sum_{x=1}^{k/\eta} 2^x 2^{\beta(k/\eta - x)} U_C(1 - U_C)^{x-1}} \quad (5)$$

Since there will be α new apical cells, the probability that $(\alpha - j)$ of these will be nonmutant and j will be mutant is given by the binomial probability

$$P_{ij} = \binom{\alpha}{j}(P_M(i))^{\alpha - j}(1 - P_M(i))^j. \quad (6)$$

After $\eta - 1$ rounds of development from meristems, the probability distribution for the number of mutant cells, j, in the final apical meristem is given by $P_{0j}^{\eta-1}$, assuming that development began from a nonmutant meristem. From this last meristem, development occurs and reproductive cells are produced. The probability that a given reproductive cell is nonmutant is

$$P_M^* = \sum_{j=0}^{\alpha} P_{0j}^{\eta-1}(P_M(j)). \quad (7)$$

In Figure 2, we plot $1 - P_M^*$ (which equals the mutation load in an asexual population). Intraorganismal selection again acts to reduce the observed mutation rate and the mutation load; however, this reduction is not as great as when development was unstructured (curve with $\eta = 1$). The mutation load with structured development is reduced most by selection among cell lineages when there are few points at which apical meristems are established (low η) and when there are many cells within the meristem (high α). These conditions make development from apical meristems more similar to unstructured development.

Selection among the initial cells of a multicelled offspring: KONDRASHOV (1994) examined the mutation load in an asexual population that reproduces offspring with n original cells, as when reproduction occurs by budding or fragmentation. An asexual parent can produce multicellular offspring by a number of mechanisms. KONDRASHOV (1994) described four ways by which n cells can be chosen from a parent: false, sectorial, random, and structured. We shall discuss the effects of intraorganismal selection on mutation load under each of these mechanisms in turn.

In the first false mode, the n cells are as closely related as possible, that is, they all originate from a single cell in the parent that existed only g cell generations ago, where g is the minimum number of doublings sufficient to produce n cells ($2^g \leq n < 2^{g+1}$). The mutation load under false reproduction may be calculated by simply shifting the beginning of the life cycle back by g cell generations and imagining that one-celled progeny are produced at that point, spending the first g cell generations on the parent. In the absence of intraorganismal selection, the mutation load is thus the same as calculated by KIMURA and MARUYAMA (1966) for single-celled asexual reproduction, $L_f = 1 - e^{-U}$. The load is reduced, however, in the presence of selection among cell lineages to $L_f = 1 - P_S$, where P_S is given by (4) (see Figure 1). In this case the load does not depend on the initial number of cells that make up an offspring (KONDRASHOV 1994).

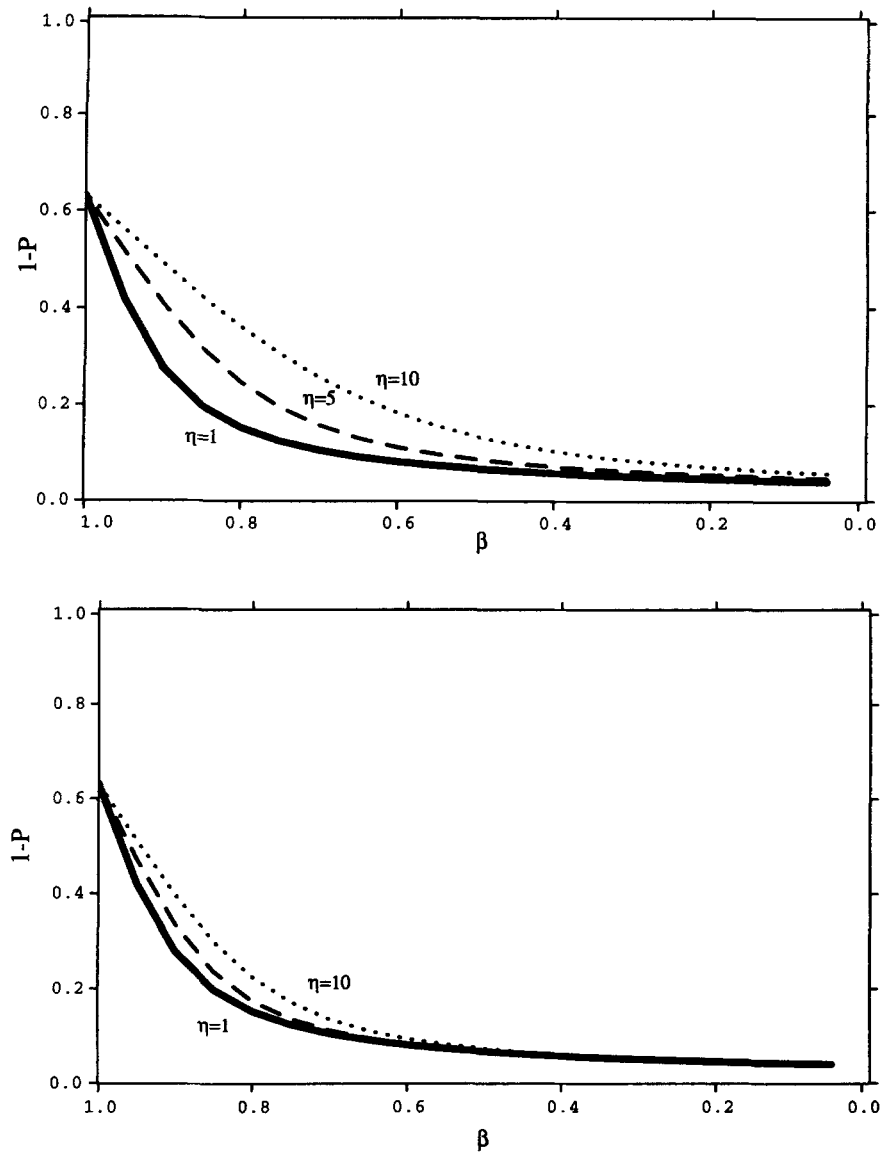

FIGURE 2.—Observed mutation rate with intraorganismal selection. Development from meristematic tissue. The asexual mutation load, $1 - P_M^*$ (see equation 7), is plotted against β, the cell replication rate among mutants. The number of rounds of development from a meristem, η, is set to one (—) (development is unstructured in this case), five (- - -) and ten (· · · · ·). The genomic mutation rate, $U(U_C = 1 - e^{-U/K})$, is set to one and the number of cell divisions per organismal generation for nonmutant cells, k, is set to 50. (A) Two initial cells in an apical meristem ($\alpha = 2$). (B) Four initial cells in an apical meristem ($\alpha = 4$).

Under the structured mode of reproduction, each of the n cells of the offspring must be a descendant of a different one of the n cells that originally composed the parent. Consider an individual that develops from all nonmutant cells. It will produce an equally fit offspring only if a mutation does not occur within any of the n cell lineages leading to an offspring cell. Without intraorganismal selection, this probability is e^{-nU}, leading to a load of $L_s = 1 - e^{-nU}$ (KONDRASHOV 1994). With intraorganismal selection, the mutation load in an asexual population is $L_s = 1 - P_S^n$. This is because each of the n offspring cells must be nonmutant and any one of them is nonmutant with probability P_S [the probability that an offspring cell is nonmutant when it originated from a nonmutant cell, (4)] The load under structured reproduction is always lower in the presence of intracellular selection but remains an increasing function of n. Producing multicellular offspring by this mechanism will only increase the mutation load within the population.

Under both the sectorial and the random mode of reproduction, all n cells are chosen randomly, either from the same sector (defined as the descendant cells of one initial cell, sectorial mode) or from all parental cells (random mode). In either case, an organism that initially has i mutant cells and $n - i$ nonmutant cells ($n - i > 0$) can produce an offspring with any number of nonmutant cells, including zero and n. Let P_{ij} equal the probability that a parent developing from i mutant cells produces an offspring with j mutant cells. KON-

DRASHOV (1994) found that the load of an asexual population depends only on the transition probabilities among the class of best individuals, *i.e.*, those that have at least one nonmutant cell ($i < n$ and $j < n$). In an equilibrium population at stable size, KONDRASHOV showed that the mutation load under sectorial and random modes of reproduction is $L_r = 1 - \lambda$, where λ is the largest nonunit eigenvalue of the matrix given by $s(j)P_{ij}$, where $s(j)$ is the average fitness of an offspring developing from j mutant cells. P_{ij} may be derived from previous calculations. With sectorial reproduction, one randomly chooses a sector and, if this sector arose from a nonmutant, each of the n offspring cells will be nonmutant with probability P_S (Equation 4). Assume that a sector is chosen with a probability that depends on its relative size in the adult organism. Each of the original i mutant cells produce $2^{\beta k}$ cells within the adult organism. Similarly, the number of cells within the adult that originate from each of the $n - i$ nonmutant cells is

$$f = 2^k(1 - U_C)^k + \sum_{x=1}^{k} 2^x 2^{\beta(k-x)} U_C(1 - U_C)^{x-1}.$$

Consequently, the probability of choosing a sector that arises from a nonmutant cell is

$$\frac{(n - i)f}{(n - i)f + i2^{\beta k}}.$$

Only from these nonmutant sectors can a nonmutant cell be chosen (with probability P_S). The overall probability of choosing an offspring with j mutant cells and $n - j$ nonmutant cells ($n - j > 0$) is

$$P_{ij} = \frac{(n - i)f}{(n - i)f + i2^{\beta k}} \binom{n}{j} (P_S)^{n-j}(1 - P_S)^j. \quad (8)$$

With random reproduction, the probability that one of the offspring cells is nonmutant is $P_M(i)$ with $\eta = 1$ and $\alpha = n$ from (5), since development occurs from a single starting group of cells like an apical meristem and continues until the end of the organismal generation. Therefore, the probability that j mutant cells are chosen from a parent that started with i mutant cells is

$$P_{ij} = \binom{n}{j} (P_M(i))^{n-j}(1 - P_M(i))^j. \quad (9)$$

To illustrate the effects of intraorganismal selection, we determine the mutation load numerically in these two cases. We make the simplifying assumption that the fitness of an individual developing from j mutant cells decreases linearly with j, that is

$$s(j) = 1 - \frac{j}{n}(1 - s_1). \quad (10)$$

Figure 3 shows the mutation load calculated for an asexual population that undergoes either random or sectorial reproduction. When intraorganismal selection is absent or very weak, the mutation load and hence the observed frequency of mutations increases with the number of cells within the offspring, as found by KONDRASHOV (1994). With moderate intraorganismal selection ($\beta < 0.98$), however, the mutation load is decreased when offspring are produced with more than one initial cell. These results indicate that a potential advantage to producing multicellular offspring is that intraorganismal selection can act upon the initial cell variants to eliminate deleterious mutations.

Selection among organelles: Another biologically important situation in which selection acts within an individual is in the replication and inheritance of the many organelles within a cell. It is well established that the frequency of different mitochondrial or chloroplast genomes varies within a heteroplasmic cell during development as a result of both stochastic and selective forces (THRAILKILL *et al.* 1980; TILNEY-BASSETT and BIRKY 1981; BIRKY 1983; BACKER and BIRKY 1985). Consequently, a new organelle mutation may disappear from a cell lineage or may rise in frequency to become the only organelle type found within a cell (a cell fixation). BIRKY (1991) examined the probability of fixation of a mutant organelle within a cell by treating the set of organelles within a cell as an asexual population. He showed that the rate, U_o, at which deleterious organelle mutations arise per organismal generation that are not ultimately lost from the population of organelles within a cell equals the product $n_o U_g E[\phi]$, where n_o is the number of organelle genomes in a cell, U_g is the deleterious mutation rate per organelle genome per organismal generation, and $E[\phi]$ is the average probability of fixation of an organelle mutation within a cell. The probability of fixation of a single organelle mutation within the population of organelles can be calculated using the WRIGHT-FISHER model, giving

$$\phi = \frac{1 - e^{2(n_e/n_o)s}}{1 - e^{2n_e s}}, \quad (11)$$

where s is the selective disadvantage during organelle replication of a mutation and n_e is the variance effective number of organelle genomes (EWENS 1979; BIRKY 1991). Assuming weak selection ($s \ll 1/n_e$), the fixation probability is approximately

$$\phi \approx \frac{1}{n_o} - \frac{n_e}{n_o} s, \quad (12)$$

leading to a cell mutation rate of

$$U_o \approx U_g(1 - n_e\bar{s}), \quad (13)$$

where $\bar{s}$ is the average selection coefficient. Intracellular selection against mutant organelles reduces the rate that organelle mutations survive to fix within a cell. With moderately strong selection ($s > 1/n_e$), the fixa-

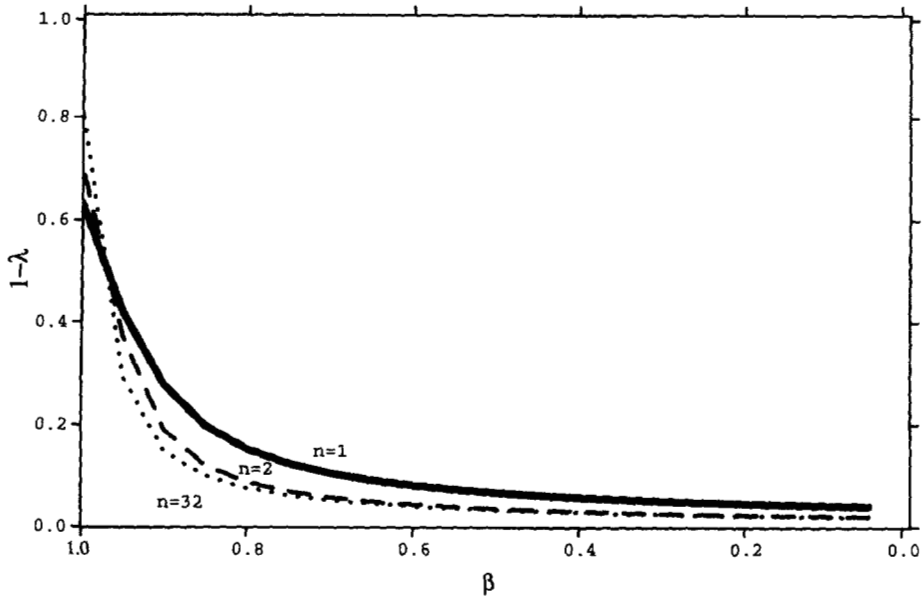

FIGURE 3.—Mutation load with intraorganismal selection. Development from multicellular tissue. The asexual mutation load, $1 - \lambda$ (using equation 9), is plotted against β, the reduction in cell replication rate among mutants, for the case when cells are randomly chosen from the parent to form the offspring (random mode). The figure is nearly identical to that obtained when cells are chosen according the sectorial mode of reproduction. The number of initial cells, n, is set to one (—; reproduction is unicellular), two (- - -) and 32 (· · · · ·). U (the genomic mutation rate, $U_G = 1 - e^{-U/k}$) is set to one, k (the number of nonmutant cell divisions per organismal generation) is set to 50, and s_1 (the fitness of an individual developing from n mutant cells relative to 0 mutant cells) is set to 0.5.

tion probability of a new organelle mutation within the cell is even more dramatically reduced,

$$\phi \approx \frac{2(n_e/n_o)s}{e^{2n_e s} - 1}, \tag{14}$$

which becomes vanishingly small as $n_e s$ increases. Consequently, the organelle mutation rate per organismal generation, U_o, is effectively zero under moderately strong selection acting intracellularly against deleterious mutations.

The mutation load due to uniparentally inherited organelle mutations follows the derivation for asexual populations, that is, $L = 1 - e^{-U_o}$. In the presence of weak intracellular selection (equation 13), the load becomes

$$L = 1 - e^{-U_g(1-n_e\bar{s})}.$$

Under moderately strong intracellular selection, however, the load is completely eliminated. If, in addition, intercellular selection acts upon cells with differenct frequencies of mutant organelles (*e.g.*, by equation 4), the load will be even further reduced. As an example, consider KONDRASHOV's (1993) estimate of $U_g \approx 10^{-3}$, and BIRKY's (1991) estimate of $n_o = 1000$ and $n_e = 500$, for the mitochondrial genome. Even in the absence of any intraorganismal selection, the mutation load in this case is low (10^{-3}), since most mutations are lost due to drift within a cell. This estimate drops even further to 4.6×10^{-7} with selection of $\bar{s} = 0.01$ against mutant organelles. These calculations show that, in contrast to previous conclusions (KONDRASHOV 1994), the mutation load arising from organelle mutations may be safely ignored. Organelle mutations rarely fix within a cell, being frequently lost due to random drift, and are even less likely to fix when selection acts against deleterious organelle mutations.

EVOLUTIONARY IMPLICATIONS OF INTRAORGANISMAL SELECTION

We have demonstrated that in a wide variety of models even fairly weak selection acting upon the cells of a developing individual can have a large impact on the probability that an individual will produce mutant offspring. This suggests that evolutionary processes that depend on the frequency of mutations may themselves be dependent on the extent of intraorganismal selection. For example, many models examining the evolution of sexual reproduction or the evolution of recombination are sensitive to the mutation rate (*e.g.*, KONDRASHOV 1982, 1988; CHARLESWORTH 1990). Similarly, calculations of inbreeding depression and its effect on the evolution of selfing depend on the mutation rate (*e.g.*, CHARLESWORTH *et al.*, 1990b). In this section, we develop a model examining the impact of intraorganismal selection on the evolution of ploidy levels.

Diploid cells, like diploid individuals, can mask the deleterious effects of mutations that arise in the heterozygous state during development [for example, the lethal mutations studied by DEMEREC (1936) were recessive at both the cellular and individual levels]. This suggests that the rate of replication of mutant cells in diploids (β_D) may be much nearer one than in haploids (β_H). Consider

a particular viability locus subject to recurrent deleterious mutations at a rate μ per organismal generation. Dropping terms that are $O(\mu^2)$ from (4), it can be shown that cell-lineage selection reduces the observed rate of mutations to μ_D in diploids and μ_H in haploids, where

$$\mu_D = \mu \frac{2(2^k - 2^{\beta_D k})}{k2^k(2 - 2^{\beta_D})} \text{ and } \mu_H = \mu \frac{2(2^k - 2^{\beta_H k})}{k2^k(2 - 2^{\beta_H})}. \quad (15)$$

Whenever diploid cells mask mutations from intracellular selection ($\beta_D > \beta_H$), the mutation rate is higher in diploids than in haploids. We now generalize a two-locus model examining the evolution of ploidy levels to include different mutation rates in haploids and diploids (PERROT *et al.* 1991; OTTO and GOLDSTEIN 1992; ORR 1995).

The evolution of haploidy and diploidy with unequal mutation rates: We examine the dynamics of a two-locus, two-allele model in which one locus is subject to deleterious mutations ($A \xrightarrow{\mu} a$) and the second locus (C) alters the life cycle. Specifically, the genotype of a diploid zygote at the life cycle, or ploidy, locus (C_iC_j) determines the probability that meiosis will occur late in life (d_{ij}), with the consequence that the organism experiences selection as a diploid. With probability $1 - d_{ij}$, the organism undergoes meiosis early and experiences selection as a haploid. Regardless of genotype, all organisms are sexual and produce haploid gametes at the same time that unite at random to produce the next generation of diploid zygotes. This model has been studied extensively, focusing on the evolution of the parameter d_{ij} to determine the conditions under which evolution favors increased diploidy or increased haploidy (PERROT *et al.* 1991; OTTO and GOLDSTEIN 1992; ORR 1995). Previous derivations, however, have assumed that mutation rates are equal in haploids and diploids. We allow different mutation rates in haploids (μ_H) and diploids (μ_D in homozygotes and μ_{HET} in heterozygotes). Since diploids generally mask mutations that arise during development (ORR 1995), we expect cell-lineage selection to be less effective at eliminating mutations in diploids, leading to a higher observed mutation rate.

The fitnesses of each genotype are given in Table 1. Following ORR (1995), we allow mutations that occur during development to affect fitness by an amount proportional to the parameter m_e (m_e will be related to the proportion of mutant cells in an adult and to the strength of cell-lineage selection in APPENDIX B). Let the haplotype frequencies among gametes equal

x_1 = frequency of C_1A,

x_2 = frequency of C_1a,

x_3 = frequency of C_2A,

x_4 = frequency of C_2a.

The recursions for these gamete frequencies are simplified by defining terms, T, that describe the transmission of each haplotype across a generation, including the effects of both selection and mutation. Let T^{nm} refer to A haplotypes that do not mutate, T^{mut} to A haplotypes that do mutate, and T to a haplotypes. As an example, $T^{nm}_{Aa,A\cdot d_{ij}}$ describes the probability of survival without mutation of an A haplotype that is initially present in an Aa zygote that experiences selection as a diploid (Aa) with probability d_{ij} and as a haploid (A) with probability $(1 - d_{ij})$. Using the selection coefficients defined in Table 1, we get that

TABLE 1

Individual fitness in haploids and diploids in the presence of deleterious mutations

Ploidy level	Initial genotype	Fitness
Haploid	A	$(1 - m_{eH}S)$
Haploid	a	$(1 - s)$
Diploid	AA	$(1 - 2m_{eD}hs)$
Diploid	Aa	$(1 - hs - m_{eHET}s(1 - h))$
Diploid	aa	$(1 - s)$

$$T^{nm}_{AA,A\cdot d_{ij}} = (1 - d_{ij})(1 - m_{eH}s)(1 - \mu_H) + d_{ij}(1 - 2m_{eD}hs)(1 - \mu_D)$$

$$T^{nm}_{Aa,A\cdot d_{ij}} = (1 - d_{ij})(1 - m_{eH}s)(1 - \mu_H) + d_{ij}(1 - hs - m_{eHET}s(1 - h))(1 - \mu_{HET})$$

$$T^{mut}_{AA,A\cdot d_{ij}} = (1 - d_{ij})(1 - m_{eH}s)\mu_H + d_{ij}(1 - 2m_{eD}hs)\mu_D$$

$$T^{mut}_{Aa,A\cdot d_{ij}} = (1 - d_{ij})(1 - m_{eH}s)\mu_H + d_{ij}(1 - hs - m_{eHET}s(1 - h))\mu_{HET}$$

$$T_{Aa,a\cdot d_{ij}} = (1 - d_{ij})(1 - s) + d_{ij}(1 - hs - m_{eHET}s(1 - h))$$

$$T_{aa,a\cdot d_{ij}} = (1 - s)$$

Following random mating, early meiosis (for haploids), selection, late meiosis (for diploids), and gamete production, the gamete frequencies in the next generation are as follows:

$$x_1'\bar{T} = x_1^2 T^{nm}_{AA,A\cdot d_{11}} + x_1x_2T^{nm}_{Aa,A\cdot d_{11}} + x_1x_3T^{nm}_{AA,A\cdot d_{12}} + (1 - r)x_1x_4T^{nm}_{Aa,A\cdot d_{12}} + rx_2x_3T^{nm}_{Aa,A\cdot d_{12}}$$

$$x_2'\bar{T} = x_1^2 T^{mut}_{AA,A\cdot d_{11}} + x_1x_2T^{mut}_{Aa,A\cdot d_{11}} + x_1x_3T^{mut}_{AA,A\cdot d_{12}} + (1 - r)x_1x_4T^{mut}_{Au,A\cdot d_{12}} + rx_2x_3T^{mut}_{Aa,A\cdot d_{12}} + x_1x_2T_{Aa,a\cdot d_{11}} + x_2^2T_{aa,a\cdot d_{11}} + (1 - r)x_2x_3T_{Aa,a\cdot d_{12}} + rx_1x_4T_{Aa,a\cdot d_{12}} + x_2x_4T_{aa,a\cdot d_{12}}$$

$$x_3'\bar{T} = x_1x_3T^{nm}_{AA,A\cdot d_{12}} + (1 - r)x_2x_3T^{nm}_{Aa,A\cdot d_{12}} + x_3^2T^{nm}_{AA,A\cdot d_{22}} + x_3x_4T^{nm}_{Aa,A\cdot d_{22}} + rx_1x_4T^{nm}_{Aa,A\cdot d_{12}}$$

$$\begin{aligned} x_4'\bar{T} = {} & x_1x_3T^{mut}_{AA,A\cdot d_{12}} + (1-r)x_2x_3T^{mut}_{Aa,A\cdot d_{12}} \\ & + x_3^2T^{mut}_{AA,A\cdot d_{22}} + x_3x_4T^{mut}_{Aa,A\cdot d_{22}} + rx_1x_4T^{mut}_{Aa,A\cdot d_{12}} \\ & + (1-r)x_1x_4T_{Aa,a\cdot d_{12}} + x_2x_4T_{aa,a\cdot d_{12}} \\ & + x_3x_4T_{Aa,a\cdot d_{22}} + x_4^2T_{aa,a\cdot d_{22}} + rx_2x_3T_{Aa,a\cdot d_{12}}, \end{aligned} \quad (16)$$

where $\bar{T}$ is the mean fitness and r is the recombination rate between the two loci. In APPENDIX B, we use these recursions to determine the mutation-selection balance reached by a population in the presence of a single life cycle allele, C_1, and then determine the local stability of this equilibrium to invasion by a new life cycle allele.

We find that by reducing the observed mutation rate more strongly in haploids, intraorganismal selection greatly reduces the parameter space in which diploidy can invade, as shown in Figure 4. Under strong intraorganismal selection (selection against mutant cells is the same strength as selection against mutant individuals), the effect is substantial even when recombination rates are loose, so that haploid life cycles would be favored even in sexual species with free recombination between most genes. These results lead to the prediction that in organisms with fairly unstructured development (such as algae), cell-lineage selection may be strong and haploidy more common. Conversely, in organisms with highly structured development and with tissue-specific gene expression (such as metazoans), cell-lineage selection may be ineffective and diploidy more common. These results provide a plausible explanation for the observed correlation between diploidy and developmental complexity (BELL 1994).

As shown in APPENDIX B, however, the importance of cell-lineage selection to the evolution of ploidy levels is sensitive to assumptions made about the relationship between cell and individual selection coefficients. We need more information about how cell-lineage selection effects individual fitness. We also need data on the relationship between cell and individual selection coefficients and dominance coefficients, especially for nonlethal mutations. Of particular interest would be studies comparing mutation rates and cell-lineage selection in isomorphic haploid and diploid individuals (such as in the green alga Ulva). Such studies promise to tell us not only how selection acts at different levels but also what evolutionary importance the level of selection might have.

DISCUSSION

Even weak selection among cell lineages within a developing individual can substantially alter the frequency of deleterious mutations observed among offspring. As a result, the mutation load of a population can be greatly reduced by intraorganismal selection. We have developed models that investigate such selection in organisms with both plastic development and development from apical meristems. As we might expect, selection within an individual is more effective at reducing the frequency of deleterious mutations when development is completely plastic (Figure 1). Organisms with meristematic development undergo a series of "cellular bottlenecks" that slightly reduce the effectiveness of intraorganismal selection (Figure 2). If development is not plastic at all, if every cell has a role that cannot be played by other cells, then cell-lineage selection cannot act and the mutation load is maximal (Figure 1, $\beta = 1$).

In organisms that sequester a germ line (BUSS 1983), cell-lineage selection among the spermatogonia or oogonia of an individual can also reduce the mutation load, according to the simple model of cell-lineage selection (Figure 1). There are, however, fewer cell generations involved in oogenesis, which would limit the potential of cell-lineage selection among females. Furthermore, it is difficult to determine whether the same selective forces act within a germ line; selection upon the spermatogonia or oogonia may be weakened or strengthened relative to somatic cell lineages.

Intraorganismal selection can be even more effective when multicellular offspring are produced through fission or budding. Despite the fact that newly produced offspring carry more genomes capable of bearing mutations, the production of multicellular offspring can reduce the mutation load when there is intraorganismal selection, in contrast to results in the absence of intraorganismal selection (KONDRASHOV 1994). This is because genetic variation initially present within the individual increases the chance that nonmutant cells will exist and be selected for within the individual. The advantage of multicellular reproduction will be weakest when the offspring's cells are recently derived from a common progenitor cell within the parent (little variation exists), or when cells must be chosen from specific progenitor cells (selection cannot freely choose among the initial variants). The advantage of multicellular reproduction will be strongest when the cells that constitute an offspring are chosen from different parts of the parent in a manner that reflects selection within the parent. In any case, the mutation load is reduced by the action of intraorganismal selection no matter how many cells initially constitute an individual.

The extent of intraorganismal selection within an organism may itself be under evolutionary control. Modifier theory that pertains to the evolution of mutation rates has shown that mechanisms that reduce the deleterious mutation rate are generally favored (LIBERMAN and FELDMAN 1986; ALTENBERG and FELDMAN 1987). That is, a genetic variant (modifier) that reduces the mutation rate will increase in frequency. Therefore, any genetic change that enhances the ability of intraorganismal selection to eliminate deleterious mutations will have a selective advantage by reducing the observed deleterious mutation rate. Furthermore, unlike mechanisms that decrease mutation rate by reducing the error

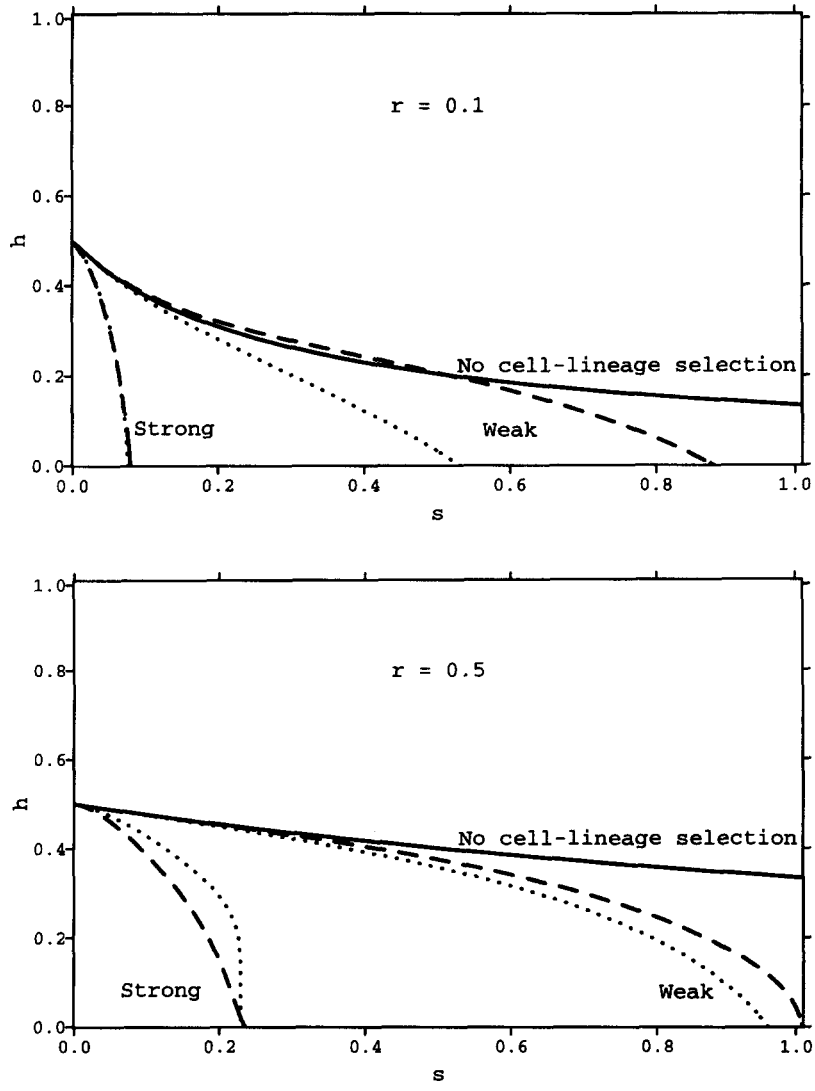

FIGURE 4.—The evolution of ploidy levels with intraorganismal selection. Alleles that increase the frequency of diploids within a haploid population can invade if h falls below the given curves (*i.e.*, if masking is sufficiently strong). In each case, a ploidy allele that causes equal numbers of haploids and diploids ($d_{12} = 0.5$) is introduced into a population that is initially all haploid ($d_{11} = 0$). The model with only individual selection [upper solid curves, from OTTO and GOLDSTEIN (1992)] is compared with the model of intraorganismal selection, under weak (middle curves, equation B2) and strong (lower curves, equation B3) cell-lineage selection. Fitness in genetic mosaics depends on the initial genotype (· · · · ·, equation B4) or on the proportion of mutant cells within the adult (- - -, equation B5). (A) $r = 0.1$. (B) $r = 0.5$.

in DNA replication, cell-lineage selection can enhance the rate of beneficial mutations (if cell replication is also enhanced, $\beta > 1$) as well as reduce the rate of deleterious mutations.

There are, however, potential costs to organisms that allow selection to act via differential cell replication. Intraorganismal selection that is too strong can lead to stunted growth, directly decreasing individual fitness, an effect that we have ignored within this paper. If development is structured or highly dependent on particular allometric relationships, differential cell replication can disrupt development and decrease fitness. This suggests that one cost of structured development is that it eliminates the ability of intraorganismal selection to act without completely disrupting development. Selection among cell lineages would also be costly when cell selection and individual selection act in opposition (BUSS 1982). In these cases, cells that grow fastest are those that are deleterious at the individual phenotypic level (such as cancerous cells, which may be modeled by setting $\beta > 1$ in our equations). It seems likely, however, that mutations acting in opposition at the cellular and individual levels would be much rarer than those that act in the same direction (*e.g.*, DEMEREC 1936; DEAROLF *et al.* 1988), especially in organisms in which cell and individual performance are highly correlated. If the bulk of mutations are deleterious or beneficial at both the cellular and the individual levels of selection, intraorganismal selection will act primarily to reduce the mutation rate to deleteri-

ous mutations while increasing the rate of beneficial mutations.

Cell-lineage selection, by reducing the rate of deleterious mutations, can have an impact on a broad range of evolutionary questions. In particular, it can affect the evolution of sexual reproduction, selfing, and recombination, whose advantages depend in part on the mutation rate (*e.g.*, KONDRASHOV 1982, 1988; CHARLESWORTH 1990; CHARLESWORTH *et al.* 1990b). In this paper, we have explored in greater detail how intraorganismal selection may effect the evolutionary transition between haploid and diploid life cycles. Since diploid cells are likely to mask deleterious mutations from cell-lineage selection as well as individual selection, diploids will tend to have a higher observed rate of deleterious mutations across an organismal generation than haploids. This increased mutation rate reduces the advantage to diploids of masking mutations and hinders their evolution. When cell-lineage selection and individual selection are equivalent in strength (strong selection), the evolution of haploidy is generally favored over the evolution of diploidy (Figure 4).

We conclude by noting that the observed mutation rate and the mutation load of a population are strongly dependent on the means by which organisms develop. Estimates obtained from organisms, such as Drosophila, with highly structured development are unlikely to apply to organisms, such as coral, with more plastic and modular development. Although the extent of intraorganismal selection is difficult to estimate, data from plants suggest that selection among cell lineages may be common (GAUL 1958). Further experiments, especially in clonal organisms, would be extremely valuable to assess the prevalence of intraorganismal selection and its importance to evolution.

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APPENDIX A

When mutation rates are high per individual generation, it is likely that more than one mutation will accumulate within a cell lineage. Equation 4 can readily be extended to allow for any number of mutations by subdividing the class of mutant cells into those that do or do not accumulate additional mutations. For example, if a first mutation occurs in generation x_1, no further mutations will occur with probability $(1 - U_C)^{k-x_1}$, while a second mutation will occur in another X_2 cell generations with probability $(1 - U_C)^{x_2-1}U_C$. Distinguishing between those cells that do or do not accumulate a second mutation, the number of mutant cells within the adult, M, in (4) becomes

$$M = \sum_{x_1=1}^{k} 2^{x_1}(1 - U_C)^{x_1-1}U_C\left[(1 - U_C)^{k-x_1}2^{\beta_1(k-x_1)} + \sum_{x_2=1}^{k-x_1} 2^{\beta_1 x_2}(1 - U_C)^{x_2-1}U_C 2^{\beta_2(k-x_1-x_2)}\right], \quad \text{(A1)}$$

where β_i is the replication rate of a cell containing i mutations. This procedure may be repeated indefinitely to account for any number of mutations, although the formula soon becomes unwieldy.

A more elegant treatment of the expected fraction of mutant cells is possible if cell-lineage selection is multiplicative. When the number of new mutations every cell generation follows a Poisson distribution with mean $u = U/k$, the distribution of mutations will remain Poisson after multiplicative selection (KIMURA and MARUYAMA 1966). Let $(1 - s_c)^i$ be the fitness of a cell carrying i mutations [$(1 - s_c)$ is equivalent to $2^{\beta}/2$]. In the first cell generation, the population (really a single nonmutant cell) experiences no selection but undergoes mutation such that the probability that a cell carries i mutations is

$$p_1[i] = \frac{u^i e^{-u}}{i!}.$$

In the second generation, the offspring cells experience selection, changing the probability distribution for i to

$$p_2^s[i] = \frac{u^i e^{-u}}{i!}\frac{(1 - s_c)^i}{\sum_{j=0}^{\infty}\frac{u^j e^{-u}}{j!}(1 - s_c)^j} = \frac{(u(1 - s_c))^i e^{-(u(1-s_c))}}{i!},$$

which is Poisson with mean $u(1 - s_c)$. After another round of mutation, the distribution of mutations in these cells becomes Poisson with mean $u(1 - s_c) + u$. Repeating this procedure, it can be shown that after n rounds of selection followed by mutation, the distribution of the number of mutations is Poisson with mean

$$\frac{u(1 - (1 - s_c)^n)}{s_c}.$$

In particular, this implies that after k cell generations, the probability that a cell carries no mutations equals

$$p_n(0) = e^{[-u(1-(1-s_c)^k)]/s_c}. \quad \text{(A2)}$$

When genome-wide mutation rates are not much higher than one per organismal generation, accounting for multiple mutations makes little difference (compare dotted to solid curves in Figure 1). With high mutation rates ($U = 10$), however, multiple mutations within a cell lineage are reasonably frequent, and either (A1), which allows for epistasis, or (A2), which assumes multiplicative cell selection, is more accurate than (4), both leading to similar curves when selection is multiplica-

tive. Regardless of the mutation rate, the number of deleterious mutant cells in the adult is reduced when intraorganismal selection acts more strongly against multiple mutations. Therefore, for deleterious mutations, (4) provides a conservative estimate of the effect of intraorganismal selection on the proportion of nonmutant offspring cells.

APPENDIX B

In this Appendix, we examine the evolution of alleles that alter the life cycle, thereby changing the probability that individuals are haploid or diploid at the time of selection. We begin by determining the mutation-selection balance from (16) when the population is fixed upon a life cycle allele, C_1. Assuming that the amount by which mutation during development effects fitness (m_e) is on the order of the mutation rate at the viability locus (which is small), the frequency of the allele held at a mutation-selection balance is

$$\bar{x}_2 = \frac{(1 - d_{11})\mu_H + d_{11}\mu_D}{(1 - d_{11})s + d_{11}hs} + O(\mu^2)$$

unless both $h = 0$ and $d_{11} = 1$. Invasion of the C_2 allele will occur if the largest leading eigenvalue of the local stability matrix obtained from (16) is greater than one. As in the analysis of ORR (1995), this leading eigenvalue equals

$$\lambda_L = \frac{1}{T}\left(a + \frac{bc}{a - d}\right),$$

where now

$$a = (1 - \bar{x}_2)T^{nm}_{AA,A\cdot d_{12}} + (1 - r)\bar{x}_2 T^{nm}_{Aa,A\cdot d_{12}}$$

$$b = r(1 - \bar{x}_2)T^{nm}_{Aa,A\cdot d_{12}}$$

$$c = (1 - \bar{x}_2)T^{mut}_{AA,A\cdot d_{12}} + (1 - r)\bar{x}_2 T^{mut}_{Aa,A\cdot d_{12}} + r\bar{x}_2 T_{Aa,a\cdot d_{12}}$$

$$d = r(1 - \bar{x}_2)T^{mut}_{Aa,A\cdot d_{12}} + (1 - r)(1 - \bar{x}_2)T_{Aa,a\cdot d_{12}} + \bar{x}_2 T_{aa,a\cdot d_{12}}.$$

In the following analysis, we will focus on two alternative assumptions about the relationship between the strength of selection at the cellular level and at the individual level. With strong cell-lineage selection, we assume that selection at the cellular level is the same strength as selection at the individual level; that is,

$$\frac{2^{\beta_H}}{2} = 1 - s \quad \text{and} \quad \frac{2^{\beta_D}}{2} = 1 - hs. \tag{B1}$$

With weak cell-lineage selection, we assume that the growth of mutant cells across an entire organismal generation is reduced by an amount equal to the strength of individual selection:

$$\left(\frac{2^{\beta_H}}{2}\right)^k = 1 - s \quad \text{and} \quad \left(\frac{2^{\beta_D}}{2}\right)^k = 1 - hs, \tag{B2}$$

which makes selection at the cellular level much weaker per cell generation. These relationships can be used in (15) to determine the observed mutation rates in haploids and diploids. We now examine the stability of the population to invasion by a new ploidy allele under different assumptions concerning the effect of mutations during development on fitness (m_e). The results are depicted graphically in Figure 4, which shows the conditions under which diploidy can invade a haploid population.

Fitness depends only on initial genotype: In this case, mutations that arise during development do not effect fitness ($m_e = 0$), even though intraorganismal selection acts upon cells. To simplify the presentation, assume that the population is initially haploid ($d_{11} = 0$). In this case, increased diploidy is favored ($d_{12} > 0$) whenever the recombination rate between the two loci is sufficiently high

$$rK_1 > s(1 - F + Fh)(1 - d_{12} + hd_{12}),$$

$$K_1 = (1 - d_{12})s + F(1 - 2h - s + hs + d_{12}s - 2hd_{12}s + 2h^2 d_{12}s) \tag{B3}$$

where F is the ratio of haploid to diploid mutation rates μ_H/μ_D. When $r = 0$, the condition can never be met and only haploidy is favored. When $F = 1$, this formula reduces to the condition given by OTTO and GOLDSTEIN (1992). Intraindividual selection will, however, reduce F below one, making condition (B3) more difficult to satisfy (Figure 4).

Fitness depends on the proportion of mutant cells: In this case, intraorganismal selection again reduces the mutation rate in haploids, but those mutations that do survive development reduce fitness in haploids to a greater extent than in diploids where they are masked. Focusing on organisms in which there is no distinction between germ-line and soma, the proportion of mutant cells in the adult is simply equal to the mutation rate: $m_{eH} = \mu_H$ and $m_{eD} = \mu_D$. Again letting $d_{11} = 0$, we have that the evolution of diploidy is favored when

$$rK_2 > s(1 - F + Fh - Fs + 2hs)(1 - d_{12} + hd_{12}),$$

$$K_2 = (s + F)(1 - 2h - d_{12} + 2hs - 2hd_{12}s + 2h^2 d_{12}s) + F(hs + s^2 - d_{12} - 2d_{12}s - d_{12}s^2 + hd_{12}s^2). \tag{B4}$$

As found by ORR (1995), even when $r = 0$, alleles increasing the the amount of diploidy can be favored if the right side of (A6) is negative. However, decreasing F below one makes condition (B4) harder to satisfy for all recombination rates (Figure 4).

Fitness depends on the total number of cells in the adult: If mutant cells replicate at a slower rate, the size of an adult will be reduced unless nonmutant cells compensate with a faster rate of replication. In some organ-

isms, especially modular ones, size may be directly proportional to fecundity and hence to fitness. Letting μ equal the mutation rate in the absence of intraindividual selection, $2^k\mu$ cells are expected to be mutant in an A haploid, but this number is reduced to $2^k\mu_H$ by cell-lineage selection. Therefore, there will be $2^k(\mu - \mu_H)$ fewer cells with intraorganismal selection, and we now assume that the fitness of an A haploid is reduced from 1 to $1 - (\mu - \mu_H) = 1 - m_{eH}s$. Similarly, for AA diploids, there are $2^k(2\mu - 2\mu_D)$ fewer cells as a consequence of intraorganismal selection and their fitness becomes $1 - (2\mu - 2\mu_D) = 1 - 2m_{eD}hs$. Making these substitutions for m_e and letting $d_{11} = 0$, diploidy is favored whenever

$$rK_3 > s(1 - F_D + F_Hh)(1 - d_{12} + hd_{12}),$$

$$K_3 = 1 - d_{12}hs - (1 - F_D + F_Hh)(2 - s + d_{12}s - 2d_{12}hs), \quad \text{(B5)}$$

where $F_D = \mu_D/\mu$ and $F_H = \mu_H/\mu$. Assuming that all selection in this model is due to the reduced replication rate of mutant cells, *e.g.*, that mutant a haploids are $(1 - s)$ the size of completely nonmutant individuals, then (B2) may be used to relate the cell replication rates to the individual selective coefficients. In this case, the stability condition (B5) is very nearly the stability condition for the model with only individual selection [it reduces to condition (1) of OTTO and GOLDSTEIN (1992), which assumes that $F_D = F_H$ and $m_e = 0$, for s small but diverges slightly near $s = 1$ because of differences between cell and individual dominance coefficients]. In particular, diploidy is never favored when $r = 0$ but is more likely to be favored as r increases. Therefore if selection at the individual level is caused by selection at the cellular level, then the evolution of diploidy does not depend on the level of selection. In this case, cell-lineage selection does not particularly favor the evolution of haploidy because the reduction of mutation rates in haploids is exactly balanced by a reduction in individual fitness.