The Ecology and Genetics of Fitness in Chlamydomonas. VII. The Effect of Sex on the Variance in Fitness and Mean Fitness

Jack da Silva; Graham Bell


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of all cytonuclear disequilibrium measures. Recently a few papers have reported codominance in nuclear RAPD markers (Williams et al. 1990; Hunt and Page 1992; Tinker et al. 1993; Cruzan and Arnold 1994). These papers suggest that codominance is observed in relatively low frequency as compared to dominance. A comparative disequilibrium analysis of dominant and codominant markers can be pursued, provided Mendelian inheritance of RAPD markers is verified. For instance, it may be of interest to know whether or not the cytonuclear disequilibrium measure $D_1$ is the same for both the codominant marker(s) and the dominant markers.

The analyses in this paper suggest that alone or together with data from isozymes and RFLPs, RAPDs can be a valuable tool for the evolutionary biologist in the study of hybrid zones.

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LITERATURE CITED


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THE ECOLOGY AND GENETICS OF FITNESS IN CHLAMYDOMONAS. VII. THE EFFECT OF SEX ON THE VARIANCE IN FITNESS AND MEAN FITNESS

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The idea that deleterious mutations can be more efficiently eliminated by selection in an infinite, sexual population than in a similar asexual population has recently been resurrected (Kondrashov 1982, 1984a; Charlesworth 1990) and is reviewed as the deterministic mutation hypothesis by Kondrashov (1988). Two necessary conditions for the hypothesis are that the populations be at or near mutation-selection equilibrium, where the rate at which deleterious mutations arise

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in each generation is equal to the rate at which they are removed by selection, and that there is positive epistasis selection against mutations, where each additional deleterious mutation produces a greater decrease in relative fitness. The extreme form of positive epistasis is truncation selection, in which individuals with more than a threshold number of mutations (assuming mutations are of equal effect) do not reproduce. The argument is as follows. The proportion of a population that does not reproduce because of selection against mutations is the mutation load \( L \). For an infinite asexual population at mutation-selection equilibrium the mutation load \( L_{\text{ase}} \) is \( 1 - e^{-u} \) under any mode of selection (Kimura and Maruyama 1966), where \( u \) is the deleterious mutation rate per genome per generation. The mutation load of a sexual population \( L_{\text{sex}} \) may be lower, and therefore its relative mean fitness \( (1 - L) \) higher, under positive epistasis. This is most easily understood by assuming truncation selection. Under truncation selection, an infinite, asexual population at mutation-selection equilibrium will consist of individuals carrying exactly the threshold number of mutations. Recombination and outcrossing in this population will increase the variance in the number of mutations per individual and, depending on \( u \), this effect of sex may or may not lower \( L \). If \( u \) is high enough to cause more than half of asexual individuals to cross the selection threshold, that is, \( L_{\text{ase}} > 0.5 \), then the increase in variance in the number of mutations per individual due to sex will mean that a smaller proportion of the population will cross the threshold, thus lowering \( L \). If \( u \) is low enough that less than half of the asexual population crosses the threshold, that is, \( L_{\text{ase}} < 0.5 \), then sex, by increasing the variance in the number of mutations, increases the proportion of individuals that cross, and thus increases \( L \). The critical value of \( u \) is 0.69 (\( L_{\text{ase}} = 0.5 \)) (see Kondrashov [1984a] for a similar argument). Kondrashov (1984a), through numerical studies of selection on recombination modifiers, has identified the critical condition under truncation selection for selection to increase the rate of recombination. This is the genome degradation rate \( v = u/\sigma > 0.35 \), where \( \sigma \) is the standard deviation in the number of mutations per genome. However, this value may be much smaller for less extreme forms of positive epistasis (Kondrashov 1984a; Charlesworth 1990).

Since it is not possible with current methods to count the number of mutations per genome, we instead consider the effect of sex on the genetic variance in fitness \( V_w \), which can be measured. With truncation selection, \( V_w = (1 - L) L \), according to the binomial theorem, and is maximal at \( L = 0.5 \). Since the predictions outlined above are \( 0.5 < L_{\text{ase}} < L_{\text{ase}} \) and \( 0.5 > L_{\text{ase}} > L_{\text{ase}} \), depending on whether \( u \) is high or low, respectively, sex is predicted to increase \( V_w \) regardless of whether it also increases mean fitness \( W \).

Other theories of sex may also predict that sex will increase \( V_w \), but these require small populations (Muller’s Ratchet, Fisher-Muller) or nonequilibrium conditions (Fisher-Muller, Red Queen; Felsenstein 1988; Maynard Smith 1988). We have measured the effect of sex on \( V_w \) and \( W \) in very large populations of the unicellular chlophyte alga \textit{Chlamydomonas reinhardtii} that had been maintained asexually in a standard environment for \( > 500 \) generations. Since the form of positive epistasis was unlikely to be truncation selection, we measured \( W \) for a number of generations after sex was induced to detect any delayed effect. Populations contained substantial \( V_w \) just before we induced sex. Just after inducing sex we observed an increase in this variance in one of three populations and a decrease in \( W \) in some assay cultures of all three populations. No effect of sex on \( W \) was observed after subsequent growth under standard conditions. We discuss this result in relation to estimates of genomic mutation rates for DNA-based, haploid microbes such as \textit{C. reinhardtii} and in relation to our experimental design.

**Materials and Methods**

**Chlamydomonas reinhardtii**

The life cycle of \textit{C. reinhardtii} lends itself well to the experimental manipulation of gametogenesis and gamete fusion and to the measurement of clonal reproduction. Haploid, unicellular spores reproduce by mitotic cell division. Photosynthetic spore growth and mitotic division require various inorganic nutrients, carbon dioxide, and light; and under optimal conditions cells divide about every 6 h. When starved of nitrogen, cells undergo gametogenesis either through two or more successive mitotic divisions or by direct differentiation. The organism is heterothallic, any clonal culture expressing only one of two mating types, plus (mt\(^+\)) and minus (mt\(^-\)). The gametes are equal in size, and only those of opposite mating type fuse, so that any culture that includes only one mating type is necessarily asexual. A pair of gametes will fuse spontaneously to produce a diploid zygospore, which is a resistant, resting stage. Zygospores can be stored for long periods in the dark but will germinate if exposed to light. Germination involves one or two meiotic divisions, producing four or eight meiospores that subsequently reproduce by mitotic division, producing haploid spores. The biology of \textit{Chlamydomonas} and the basic procedures used in our experiment are described by Harris (1989).

**Strains**

The wild-type strains CC-1010, CC-1952, CC-2342, and CC-2343, each from a different isolate maintained in the laboratory for many years, were obtained from the \textit{Chlamydomonas} Genetics Center, Duke University (Harris 1989).

**Lines**

Figure 1 outlines the experiment. Lines were established as part of a long-term study (Bell et al., unpubl.). Three pairs of sibling lines were established by crossing three pairs of strains, isolating a single random zygospore from each cross and then randomly isolating a single mt\(^+\) and a single mt\(^-\) spore from each germinated zygospore. The following initial crosses were used: cross b, CC-1010 × CC-2343; cross c, CC-2343 × CC-1952; cross d, CC-2343 × CC-2342. The spores isolated from each of these crosses were used to establish lines in clonal culture in a standard environment (300 mL of Bold liquid medium, a mixture of inorganic salts lacking a carbon source, in 500-ml Erlenmeyer flasks, approximately 25°C, constant illumination, light aeration) for 50
serial transfers to fresh medium, with transfers alternating between every 3 and 4 d. This amounted to 500–600 generations without sex. Serial transfers were made using approximately $5 \times 10^5$ cells and cultures reached densities of approximately $5 \times 10^8$ cells/mL (or approximately $1.5 \times 10^9$ cells in a 300-mL culture). Sibling lines were established because asexual cultures containing both mating types may lose one mating type if one clone (one mating type) has a higher fitness than all of the others, which would preclude the induction of sex within cultures.
Populations

Sexual populations were established after the 50th serial transfer of line cultures by mixing 5 mL of sibling line cultures in nitrogen-free medium. Mating was halted in several 100-μL samples of each mating culture by thinly spreading the samples separately on solid medium (Bold’s liquid medium mixed with agar (15 g/L) and set in petri plates). Plated cultures were kept in light for 1 d and dark for 5 d to mature zygospores. Zygospores were selected by exposing cultures to chloroform vapor for 45 sec, which selectively kills non-resting stages, and then germinated by placing cultures in light for 7 d. Unmated controls (mt+ and mt-) were treated in the same way except that they were not mixed in nitrogen-free medium and plated culture were not exposed to chloroform. This gave, for each initial cross (crosses b, c, and d), two sibling, asexual populations (mt+ asexual and mt- asexual) and a population of their sexually derived progeny (sexual). This is the only time in the experiment, after the establishment of lines, when sex was induced.

Fitness

We define fitness as clonal growth in pure culture and have not investigated competitive interactions between sexual and asexual lineages. Clonal growth was measured for populations immediately after sibling lines were crossed, that is, after sex but before any subsequent, substantial selection on growth, then once every 3–4 d for a total of seven times during which populations were grown under the standard conditions used to maintain lines, and finally in a separate assay after the seventh interval of growth of the previous assay. Details of these assays are given below.

Immediately after sex, clonal growth was measured for pairs of clones of 15 randomly isolated spores from each population. Spores were grown in standard medium in 80-mL capped tubes to facilitate the measurement of large numbers of spores. Ideally, fitness would have been assayed using the conditions under which lines had been established. However, such an assay was not feasible for the large number of assay cultures (3 initial crosses × 3 populations × 15 spores × 2 clones = 270 tubes) required to obtain reasonable estimates of Vw, even if spores from each initial cross were assayed at different times. Moreover, growth in tubes can be measured more accurately than in flasks and is positively correlated with growth in flasks (see Results). The measurement of growth of a pair of clones for each spore allowed genetic variance Vg in growth to be estimated as the among-spore component of total spore variance (including clones). Growth was measured by measuring the density of tube cultures every 1–3 d for 17 d as optical transmittance. The logistic growth parameters r and K were estimated for each culture by fitting the logistic equation to the growth data (NLIN procedure [METHOD = GAUSS]; SAS Institute 1988; Bell 1991). The logistic equation consistently explained more than 98% of variation in density through time in cultures. Because culture density could not be measured without error, r and K were linearly related to the estimate of initial culture density i. To allow more precise comparisons of the variance in r and K between treatment and control populations, log r and log K were standardized to log i = 0 (i = 1% turbidity) by using residuals from their regressions on log i separately for populations within initial crosses on each of two growth chamber shelves (Fig. 2). Logarithmic transformations tended to normalize frequency distributions of residuals and ensured that variances and means were independent for populations with equal coefficients of variation (Lewontin 1966). The validity of using standardized values rests on the assumption that there are no interaction effects on r and K involving i and treatments across whose levels r and K are compared. This assumption was supported: analyses of covariance (GLM procedure; SAS Institute 1988) showed that there were no significant interaction effects on log r or log K involving log i and shelf, population, and initial cross, either singly or in combination (log r; P > 0.10; log K; P > 0.07; in all cases).

Growth was also measured for batch cultures in flasks under the standard conditions used to establish and grow lines. In this assay, approximately 103 cells from the populations established immediately after sex were transferred to liquid medium in 500-mL Erlenmeyer flasks. Equal numbers of cells from sibling lines were mixed to produce asexual populations containing both mating types. Sexual populations were established by simply transferring cells from the previously established sexual populations. For each initial cross, one asexual and one sexual flask culture was replicated four times, giving a total of 3 initial crosses × 2 populations × 4 replicates = 24 flasks. Cultures were transferred to fresh medium after periods of growth alternating between 3 and 4 d, for seven such growth cycles, which amounted to about 90 generations of cell division. Growth rates were measured as the number of cell doublings per day for each 3- or 4-d growth cycle, which were calculated from cell densities at the beginning and the end of the growth cycle. Cell densities of inocula and of cultures at the end of each growth cycle were measured with a particle counter (Coulter Multisizer II, Coulter Electronics Ltd.). Cultures contained approximately 5 × 106 cells/mL at the end of a cycle and 103–104 cells were transferred.

A final tube assay was conducted at the end of the flask assay to confirm the results of the flask assay. Ten spores were randomly isolated from each flask at the end of the last growth cycle but were not clonally replicated as in the first tube assay (Vw was not estimated). Growth was measured using optical transmittance, and the logistic growth parameters were estimated by fitting the logistic equation to the growth data, as before. Standardized log r and log K (to log i = 0) were used in analyses.

Results

Variance in Fitness and Mean Fitness before Selection

Genetic variances in log r and log K for sexual and asexual populations before selection are given in Table 1. Significant Vg in one or the other parameter was detected in one or the other sibling line of each initial cross. In addition, analyses of variance showed significant variances in log r and log K among spores within populations and crosses (Table 2), which correspond to Vg in Table 1. When these analyses of variance were repeated with the sexual populations excluded, significant variation among spores within populations and crosses remained: among-spore variance components were, for log r, 0.001781 (F64,84 = 2.92, P < 0.001) and, for log K, 0.001134

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Fig. 2. The relationships of log $r$ and log $K$ to log $i$ measured for individual spore clones grown in assay tubes before selection. For the purposes of presentation, data points are means across replicate clones of a spore, one located on each of two growth chamber shelves. Initial crosses are represented by symbol shape (b: circle; c: triangle; d: diamond) and populations by symbol fill (sexual: closed; asexual [mt$^+$ and mt$^-$]: open). Least-squares, linear-regression lines are for sexual (solid) and asexual (dashed) populations of each initial cross. Logarithmic transformations tended to normalize frequency distributions of residuals. $T$ is optical transmittance. As indicated in the text, analysis of covariance showed that there were no interaction effects on log $r$ or log $K$ involving log $i$ and shelf, population, or cross. Equations for overall least-squares

$F_{84,84} = 4.94$, $P < 0.001$, indicating that generally there was significant $V_G$ in the logistic parameters in asexual populations.

For the asexual populations, $V_G$ in log $r$ ranged from 0 to 0.004814, and in log $K$, from 0 to 0.004597 (Table 1).

Sexual populations contained significant $V_G$ in the logistic parameters in crosses b and c (Table 1): $V_G$ in log $r$ ranged from 0.001976 to 0.005899, and in log $K$, from 0 to 0.000968.

We compared $V_G$ between the sexual population and the sibling population with the highest value within each initial cross. For both logistic parameters in crosses b and d, one of the asexual populations had the higher $V_G$, and in every case this was the mt$^+$ population. For both logistic parameters in cross c, the sexual population had the higher variance, but the differences were not statistically significant (one-tailed variance-ratio tests: $F_{14,14} < 1.98$, $P > 0.10$ for both cases), possibly because these tests had low statistical power ($\leq 0.3$; estimated following Kramer and Thiemann [1987]).

Table 1 also shows the means of the logistic parameters for each population within each initial cross. Sexual populations had means intermediate to, or lower than, the means of sibling asexual populations in five out of six visual comparisons; only for log $r$ in cross c was the sexual mean higher than that of both asexual populations. In statistical comparisons of means we controlled for the blocking effect of shelves and for spore replicates. Analyses of variance show no main effect of population, but do show significant population-by-cross and population-by-cross-by-shelf interaction effects on both logistic parameters (Table 2). To investigate these interactions, analyses were repeated for each cross-shelf combination, and Tukey's studentized range test (GLM procedure [MEANS statement, TUKEY option]; SAS Institute 1988) was used to test the a posteriori null hypothesis that pairs of population means are equal. For log $r$, on shelf 1 only two of the three crosses showed significant differences among populations (maximum experimentwise error rate $= 0.05$); the sexual population had a lower mean than the two asexual populations in cross b, but had a higher mean in cross c, which explains the higher sexual mean log $r$ in cross c shown in Table 1. On shelf 2, the sexual mean was intermediate between that of the two asexual populations in all three crosses. For log $K$, on shelf 1 only cross c showed a significant difference, with the sexual population having an intermediate mean, whereas on shelf 2 the sexual population had significantly the lowest mean in all three crosses. In summary, the sexual population had the highest log $r$ in only one cross and on only one shelf, whereas the sexual population had the lowest log $K$ in all three crosses, but only on the other shelf.

**Mean Fitness through Selection**

Fitness was estimated for the sexual and asexual populations while they were grown under the physical conditions used to establish and maintain lines (liquid batch cultures in flasks). Cell doubling rates were calculated for each growth cycle of the seven-cycle assay. Doubling rates were higher

$y = 0.82 - 0.30x$ ($r^2 = 0.39$, $F_{1,133} = 83.92$, $P < 0.001$) and $y = 1.83 + 0.10x$ ($r^2 = 0.17$, $F_{1,133} = 28.11$, $P < 0.001$).
Table 1. The mean and variance in standardized log \( r \) and log \( K \) estimated from the tube assay before selection. Data were pooled across two growth shelves in estimating variance components. The genetic component \( (V_G) \) of phenotypic variance was estimated as the among-spore variance component in a variance component analysis (NESTED procedure; SAS Institute 1988). The environmental variance component \( (V_E) \) was estimated as the within-spore (between replicate clones) variance component. \( V_E = 0 \) was tested with the F-ratio (among-spore mean square)/(within-spore mean square) with 14 and 15 df. \( N = 15 \) spores \( \times 2 \) replicates. \( T \), optical transmittance at 665 nm. \(* P < 0.05; ** P < 0.001.\)

<table>
<thead>
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<th>Cross</th>
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<th>( N )</th>
<th>Mean (SE)</th>
<th>( V_E )</th>
<th>( V_G )</th>
<th>Mean (SE)</th>
<th>( V_E )</th>
<th>( V_G )</th>
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<td>30</td>
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for 3-d cycles (approximately 4.3 doublings per day) than for 4-d cycles (approximately 3.4 doublings per day) because of the logistic growth of cultures. We have expressed the relative fitness of a sexual population as the ratio of its doubling rate to that of the population consisting of its mixed, parental sibling lines. For all initial crosses, and both growth chamber shelves, the ratio is initially unity or less, increases for three or four cycles, and finally decreases to, or stabilizes at, a value close to unity (Fig. 3). The effects of shelf, cross, population, and their interactions on doubling rates were tested for each cycle using ANOVA. In these analyses, shelf was a block effect, cross a random experimental effect, and population a fixed experimental effect. Doubling rates of sexual and asexual populations did not differ significantly in any growth cycle (overall \( F_{1,12} < 2.22 \) and \( P > 0.09 \) for cycles 1, 2, and 4–7; for cycle 3, \( F_{1,12} = 4.75 \) and \( P = 0.01 \); but for the test of a population effect, \( F_{1,12} = 0.213 \) and \( P = 0.72 \). The significant effects that was a shelf-by-cross interaction in cycle 3 (\( F_{2,2} = 19.22, P = 0.005 \)). There was a large between-replicate-flask variance component (comprising half the total variance in cycle 1, for example), which contributed to the error variance in these analyses, and may have obscured variance among crosses and populations. In fact, the statistical power of these tests was consistently \(< 0.5\) because of the small sample size per cell (two flasks) of the experimental design matrix (power estimated following Lindman [1974]).

The results for cycle 1 (Fig. 3) are comparable to those of the tube assay conducted immediately after sibling lines were crossed because both assays were inoculated with cells from cultures plated immediately after mating (see Fig. 1). The correlation between doubling rate in flasks and growth in tubes was estimated from analyses of covariance in which the population (sexual and asexual) mean of each logistic parameter was regressed on the population mean doubling rate, with initial cross as the covariate. Correlations were positive, although weak, perhaps because of the few degrees of freedom available for comparing populations within crosses (Pearson’s product-moment correlations: \( r = 0.19, F_{1,2} = 0.02, P = 0.91 \) for log \( r \); \( r = 0.68, F_{1,2} = 10.40, P = 0.08 \) for log \( K \)).

**Mean Fitness after Selection**

The main result of the flask assay, that there is no evidence of selection increasing \( W \) of sexual populations relative to asexual populations, was confirmed by the tube assay of the growth of individual spores at the end of the last growth cycle of the flask assay. Means and standard errors of the logistic parameters for sexual and asexual populations from each initial cross are presented in Table 3. Means are higher for the sexual population in five of six visual comparisons. However, ANOVA shows no main or interaction effects of population on either log \( r \) or log \( K \) (Table 4). The only significant main effect was that of replicate flask from the flask assay, for both logistic parameters, and the only significant interaction effect was that of shelf-by-cross for log \( K \) (Table 4). The effect of replicate flask was large (\( F_{12,214} = 95.05, P < 0.001 \) for log \( r \); \( F_{12,214} = 90.89, P < 0.001 \) for log \( K \)) and is consistent with the large between-replicate-flask variance component in the flask assay. These results are consistent with the flask assay; sexual populations did not have higher \( W \) after selection for growth in flasks. However, the statistical power of testing for an effect of population with the observed variation in log \( r \) between populations was low (< 0.5) (the power of testing for a population effect on log \( K \), given the observed variation, could not be estimated since \( F < 1 \)).

**Discussion**

The asexual sibling populations from each initial cross exhibited substantial \( V_G \) in log \( r \) and log \( K \) after 500–600 generations in a standard environment. This genetic variation represents the accumulation of new mutations, because each population was established from a single vegetative colony. The average rate of accumulation of \( V_G \) can be calculated from Table 1 as about \( 8.0 \times 10^{-3} V_E \) per generation for log
TABLE 2. Randomized, complete-blocks, mixed model ANOVAs (VARCOMP procedure [METHOD = TYPE1]; SAS Institute 1988) of the effect of sex on standardized log r and log K estimated from the tube assay before selection. Each of two replicate-clone assay cultures of a spore was placed on a different shelf of a growth chamber, each shelf being laid out as a complete, unreplicated, randomized block with 15 spores × 3 populations (mt−, mt+, and sexual) × 3 crosses (b, c, and d), giving 135 assay cultures per shelf and 270 cultures in all. Spores are nested within crosses and populations. Population is a fixed effect, all other effects are random. F-test denominator MS (mean square) and df were calculated on the basis of Expected MS (Zar 1984). Residual MS includes shelf-by-spor interactions MS. * 0.01 < P < 0.05; ** P < 0.001.

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<td>126</td>
<td>0.0019</td>
<td>3.51**</td>
<td>0.0027</td>
<td>126</td>
<td>0.0006</td>
<td>4.32**</td>
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<tr>
<td>Shelf × cross</td>
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<td>4</td>
<td>0.0147</td>
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<td>4</td>
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<td>0.0321</td>
<td>4</td>
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<td>7.01*</td>
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<tr>
<td>Cross × population</td>
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<td>0.1476</td>
<td>7</td>
<td>0.0194</td>
<td>7.60*</td>
<td>0.0386</td>
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<td>5.81*</td>
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<tr>
<td>Shelf × cross × population</td>
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<td>0.0147</td>
<td>126</td>
<td>0.0019</td>
<td>7.71**</td>
<td>0.0046</td>
<td>126</td>
<td>0.0006</td>
<td>7.37**</td>
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r and about 1.6 × 10⁻³ V_E per generation for log K. These values are consistent with previous estimates of about 10⁻³ V_E per generation (Lynch 1988). One interpretation of this variation is that it represents a balance between the rate at which deleterious mutations arise in every generation and the rate at which they are removed by selection.

We did not observe any general effects of sex on fitness. This could be explained if there were no fitness interactions among mutations, since sex would then have had no effect on V_E or W. This is because without epistasis the number of mutations per genome would be randomly distributed in an asexual population at mutation-selection equilibrium and, therefore, recombination with random mating would have no effect on this distribution. Although there is little empirical evidence to support one mode of selection against mutations over another (Charlesworth 1990), mutation-accumulation experiments with Drosophila melanogaster suggest positive epistasis (Mukai 1969). Another possibility is that both u and the truncation selection threshold, or, to generalize to other degrees of positive epistasis, the mean number of accumulated mutations per genome, were so low that sex would have had only a very small effect on fitness, which went undetected.

Fig. 3. Ratios of sexual-(sex) to asexual-population (asex) cell doublings per day (d.dp) in relation to cycle of growth in flasks. Initial crosses b (solid), c (long dash), and d (short dash) are each represented by two shelves of a growth chamber (numbered). Thin, solid line at y = 1 is a reference. For each cross, two sexual- and two asexual-population assay flasks were positioned randomly on each shelf. Ratios were calculated at the end of each cycle from doubling rates averaged across the two replicates of each population on a shelf. As stated in the text, ANOVAs for each cycle showed no main or interaction effect of population on d.dp.

Table 3. Means of standardized log r and log K estimated from the tube assay after selection. Data were pooled across spores isolated from four replicate flasks from the flask assay of mean fitness through selection (see text). Ten spores were isolated per flask, so that sample sizes (N) are typically 40 spores. In cases with only 39 spores, one spore culture failed to grow. T, optical transmittance at 665 nm.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Population</th>
<th>N</th>
<th>Log₁₀ (r/(1/d))</th>
<th>Log₁₀ (K(100 - %T))</th>
</tr>
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<tbody>
<tr>
<td>b</td>
<td>Asexual</td>
<td>39</td>
<td>1.171 (0.019)</td>
<td>1.811 (0.006)</td>
</tr>
<tr>
<td></td>
<td>Sexual</td>
<td>40</td>
<td>1.097 (0.015)</td>
<td>1.825 (0.013)</td>
</tr>
<tr>
<td>c</td>
<td>Asexual</td>
<td>40</td>
<td>1.041 (0.033)</td>
<td>1.663 (0.016)</td>
</tr>
<tr>
<td></td>
<td>Sexual</td>
<td>40</td>
<td>1.231 (0.028)</td>
<td>1.711 (0.022)</td>
</tr>
<tr>
<td>d</td>
<td>Asexual</td>
<td>40</td>
<td>0.611 (0.063)</td>
<td>1.677 (0.030)</td>
</tr>
<tr>
<td></td>
<td>Sexual</td>
<td>39</td>
<td>0.863 (0.028)</td>
<td>1.680 (0.019)</td>
</tr>
</tbody>
</table>
in our experiment due to the low statistical power of comparisons. It seems likely that $u$ was very low. Estimates of genomic mutation rates for DNA-based, haploid microbes (including bacteriophages, a bacterium, a yeast, and a filamentous fungus, based on reports in the literature on mutant frequencies for specific genes) place $u$ fairly consistently at 0.003 (Drake 1991). This overestimates $u$ since presumably a small fraction of these mutations are beneficial. We have no knowledge of the mean number of accumulated mutations per genome except to say that, as previously mentioned, the accumulation of $V_G$ in both logistic parameters is consistent with previous estimates.

With $u = 0.003$, $L_{asex} = 0.003$. Since $L_{asex} < 0.5$, the hypothesis predicts that, with truncation selection, sex will increase $V_W$ and decrease $W$. Indeed, we observed a nonsignificant increase in $V_W$ in one initial cross and a significant decrease in $W$ (log $K$) in all three crosses on one incubator shelf. Also consistent with these predictions, da Silva and Bell (1992) found, in an experiment with *C. reinhardtii* designed to measure the correlated response of viability (clonal growth) to selection for fast mating (syngamy), that sexual populations had a lower intrinsic rate of increase than asexual populations after these had been maintained in a standard environment for > 200 generations.

In contrast to the low $u$ estimated for some microbes, a study of mutation-accumulation in *D. melanogaster* estimated $u$ to be near one (Mukai et al. 1972). This gives $L_{asex} = 0.63$ and leads to the prediction that under truncation selection and at mutation-selection equilibrium sex will increase $V_W$ and $W$. Experiments with *Drosophila* have consistently shown that crossing over in the second chromosome increases $V_G$ in fitness components, but also that it reduces their means (Spies 1958; Mukai and Yamaguchi 1974; Charlesworth and Charlesworth 1975). These results may be irrelevant to the hypothesis being tested here since, although chromosomes were extracted from large wild populations, fitness components were measured in the novel environment of the laboratory (Kondrashov 1988). Alternatively, it may not be crossing over, but segregation that contributes mostly to an advantage of sex, as Charlesworth (1990) has shown in his numerical studies of the deterministic mutation hypothesis.

A low $u$ does not mean that the hypothesis cannot explain the evolutionary maintenance of sex in DNA-based, haploid microbes. Sex may be advantageous to these organisms if and when the truncation selection threshold decreases such that fewer mutations are required to cross the threshold, to the extent that $L_{asex} > 0.5$. Kondrashov (1984b) uses this argument to explain cyclical parthenogenesis. He assumes that the selection threshold is higher during lenient times of the year than during harsher times. This may explain why *C. reinhardtii* like many other microbes and animals (Bell 1982) undergo some form of sex in response to deteriorating environmental conditions. This implies that, if the hypothesis is correct, we would have had to apply more stringent selection than the asexual lines had experienced before sex was induced to have observed an advantage of sex. Future experiments will investigate this hypothesis as well as the mode of selection on mutations and the genomic mutation rate.

We are uneasy about two other aspects of the design of our experiment. The first is the separate culture of mt+ and mt− lines, which might not produce the same result as culturing a single population in which genotypes were always competing directly with one another. The second is that we have used growth in pure culture rather than competitive ability in mixtures as a measure of fitness. These difficulties could be overcome by using homothalic or heterothalic strains in mixed-mating type cultures, marking them so that the frequencies of different types in mixed populations can be followed through

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**Table 4.** Randomized, compete-blocks, mixed model ANOVAs (VARCOMP procedure [METHOD = TYPE1]; SAS Institute 1988) of the effect of sex on standardized log $r$ and log $K$ estimated from the tube assay after selection. Two growth chamber shelves were each laid out as a complete, unreplicated, randomized block with 10 spores $\times$ 2 replicate flasks $\times$ 2 populations (sexual and asexual) $\times$ 3 initial crosses (b, c, and d), giving 120 assay cultures per shelf and 240 cultures in all. Two cultures failed to grow (one asexual and one sexual; see Table 3), so the total number of cultures analyzed is 238 and the ANOVA is slightly unbalanced. Replicate flasks are nested within shelves, crosses, and populations. Population is a fixed effect, all other effects are random. F-test denominator MS (mean square) and df were calculated on the basis of Expected MS (Zar 1984). Residual MS includes spore MS. * $0.01 < P < 0.05$; ** $P < 0.001$.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
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<td></td>
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<tr>
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<td>1</td>
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</tr>
<tr>
<td>Cross</td>
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<td>95.05**</td>
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</tr>
<tr>
<td>Shelf $\times$ cross</td>
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<td>2</td>
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<td>0.03</td>
<td>0.0043</td>
<td>2</td>
<td>0.0241</td>
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<td>1.96</td>
<td>0.0111</td>
<td>2</td>
<td>0.0241</td>
</tr>
<tr>
<td>Shelf $\times$ cross $\times$ population</td>
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<td>12</td>
<td>0.7346</td>
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time. Although this procedure would have its own drawbacks, we plan to conduct such experiments in the future to test the generality of the results that we have reported here.

ACKNOWLEDGMENTS

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LITERATURE CITED


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TESTING COLD FUSION OF PHYL: MATERNITY IN A TUNICATE X SEA URCHIN HYBRID DETERMINED FROM DNA COMPARISONS

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Key words.—Ascidia mentula, Ascidiacea, cytochrome oxidase I, Echinoidea, Echinus esculentus, horizontal gene transfer, hybridization, mtDNA, 28S ribosomal RNA.

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Many marine invertebrate groups with different adult forms have similar larval (such as the trochophore larva of mollusks and annelids, or the pluteus larva of sea urchins and brittle stars), and these similar larval forms are usually considered to be examples of either convergent evolution by natural selection or functional constraints on the evolution of early stages in development (Jagersten 1972; Strathmann 1988; Raff and Kaufman 1991; Wray 1992, 1995). However, Williamson (1992) recently proposed that many of these similarities are due to horizontal genetic transfer of larval forms between members of different taxonomic orders, classes, or phyla by rare cross-fertilizations. This improbable hypothesis implies that the evolutionary history of major taxa is an anastomosing web of hybridizations and divergences, resulting in the insertion of larval forms (or new complex life cycles) into lineages that previously lacked them. The hypothesis

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