

## THE ECOLOGY AND GENETICS OF FITNESS IN *CHLAMYDOMONAS*. XIII. FITNESS OF LONG-TERM SEXUAL AND ASEQUAL POPULATIONS IN BENIGN ENVIRONMENTS

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**Abstract.**—We measured the mean fitness of populations of *Chlamydomonas reinhardtii* maintained in the laboratory as obligately sexual or asexual populations for about 100 sexual cycles and about 1000 asexual generations. Sexuality (random gamete fusion followed by meiosis) is expected to reduce mutational load and increase mean fitness by combining deleterious mutations from different lines of descent. We found no evidence for this process of mutation clearance: the mean fitness of sexual populations did not exceed that of asexual populations, whether measured through competition or in pure culture. We found instead that sexual progeny suffer an immediate loss in fitness, and that sexual lines maintain genetic variance for fitness. We suggest that sexual populations at equilibrium with selection in a benign environment may be mixtures of several or many epistatic genotypes with nearly equal fitness. Recombination between these genotypes reduces mean fitness and creates genetic variance for fitness. This may provide fuel for continued selection should the environment change.

**Key words.**—*Chlamydomonas reinhardtii*, epistasis, evolution of sex, experimental evolution, mutation, mutation clearance, recombination.

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The sexual cycle usually acts to interrupt reproduction and thereby to reduce the rate of vegetative proliferation. The characteristic sexual processes of gametogenesis, gamete fusion, zygote maturation, and meiosis are costly and time consuming. In well-known eukaryotic microbes such as yeasts, chlorophytes, and ciliates, a sexual cycle will extend over several or many vegetative cycles, while producing no net population growth. In many multicellular organisms in which sex is obligately associated with reproduction, this effect is exacerbated by the involvement of individuals (usually males) who contribute few resources to the zygote (Maynard Smith 1971). Sex is not necessary for regular development, error repair, or any other physiological process in eukaryotic microbes, because large asexual populations can be cultured indefinitely with no signs of impairment. It seems likely, therefore, that a cumbersome sexual cycle is maintained despite reducing the number of progeny because it improves the quality of progeny.

An outcrossed sexual cycle, comprising the fusion of two unrelated gametes followed by genetic recombination, brings together mutations that arose independently in different lines of descent. This will increase genotypic diversity, because progeny will inherit different numbers of mutations from their sexual parents through random segregation. Sex may then increase the rate of response to selection, to the extent that it increases the genetic variance of fitness. This argument goes back to Weismann (1889) and was summarized recently by Burt (2000). It explains the maintenance of sex, provided that the conditions of growth deteriorate fast enough that enhanced adaptation more than compensates for retarded reproduction. This might happen for two reasons. The first is that current adaptedness is eroded because the environment becomes more stressful, the situation first envisaged by Weismann (1889) and subsequently explained in terms of Men-

delian genetics by Fisher (1930) and Muller (1932). Adaptation to the new conditions then requires mutation assembly, bringing together mutations that were previously deleterious but are now beneficial into the same genome, which then spreads through selection. The second reason is that current adaptedness is eroded because the genome becomes more corrupt. Adaptedness can then be maintained in a benign environment through mutation clearance, with sex acting to bring together currently deleterious mutations to create severely crippled genomes that are then eliminated from the population (Kondrashov 1984). Sexual populations are expected to have greater mean fitness than asexual populations through their more efficient process of mutation clearance, provided that specific conditions of mutation rate and epistatic interactions are met.

Mutation clearance is an attractive explanation for the maintenance of sex because deleterious mutation degrades adaptedness in all populations at all times, whereas a continual increase in environmental stress requires a special auxiliary explanation such as host-parasite coevolution. For sex to accelerate mutation clearance, however, two necessary conditions must be met. The first is that the rate of deleterious mutation should exceed one per genome per generation in order to provide a substantial advantage for sex (Kondrashov 1984). The rate estimated from mutation accumulation experiments in microbes is substantially lower, with values of 0.001–0.01 being reported in most studies (Drake et al. 1998; Zeyl and de Visser 2001). The magnification of mutational load through the germ line may increase this rate in multicellular organisms, but recent careful estimates fell short of a value of one in *Drosophila melanogaster* (Fernández and López-Fanjul 1996; Fry et al. 1999; Keightley and Eyre-Walker 2000) and in *Caenorhabditis elegans* (Keightley and Caballero 1997; Vassilieva and Lynch 1999; reviewed by

Bataillon 2000). The second necessary condition is that there should be strong interactions among loci, such that the fitness of the double mutant is much less than expected (Kondrashov 1988; reviewed by Charlesworth 1990). This is plausible on theoretical grounds (Charlesworth 1990; Szathmary 1993), although the experimental detection of epistasis has been difficult, and only a few studies exist on the interaction of deleterious mutations. There is some evidence for synergistic epistasis in *Chlamydomonas moewusii* (de Visser et al. 1996), *Escherichia coli* (Elena and Lenski 1997), *Drosophila* (Whitlock and Bourget 2000), and the parasitic wasp *Nasonia vitripennis* (Rivero et al. 2003), although synergistic and antagonistic epistasis may be more or less equally frequent and thus cancel one another out (Elena and Lenski 1997; Rice 2002). Synthetic lethals occur at surprisingly high levels in yeast, where they may account for several percent of zygote mortality (Tong et al. 2004).

The properties of sexual and asexual populations have attracted numerous theoretical and comparative studies (Maynard Smith 1971; Williams 1975; Bell 1982; Michod and Levin 1987; Stearns 1987), but few experiments have addressed the issue directly (recent reviews by Rice 2002; Peters and Otto 2003). They are difficult to perform in multicellular organisms, although the manipulation of chromosomes in *Drosophila* has provided some evidence that suppressing recombination reduces the rate of response to selection (McPhee and Robertson 1970; Rice 1994; Rice and Chippendale 2001). Even in eukaryotic microbes, where sexual and vegetative cycles are distinct, few experiments have been reported. Selection experiments in *Chlamydomonas* (Colegrave 2002; Colegrave et al. 2002; Kaltz and Bell 2002) and yeast (Greig et al. 1998) have shown that sexually reproducing strains are able to adapt more rapidly than their asexual counterparts. Zeyl and Bell (1997) failed to find evidence for mutation assembly in yeast cultures adapting to growth on an exotic medium (galactose), perhaps because adaptation involved very few beneficial mutations of large effect. They suggested instead that performance on a benign medium (glucose) demonstrated mutation clearance. Goddard et al. (2005), also working with yeast, failed to show any effect of sex in benign environments, while finding evidence for adaptation to new harsh environments. There does not seem to be any clear demonstration that sexual and asexual populations diverge in mean fitness after many generations of culture on a benign medium to which they are well adapted.

We have conducted an experiment designed to provide a convincing test of the mutation clearance theory. This involved maintaining sexual and asexual cultures of a eukaryotic microbe for about 1000 vegetative generations, during which time the sexual lines passed through about 100 sexual cycles and the asexual lines never experienced sex. We measured fitness in competitive and noncompetitive conditions to find out whether the sexual populations expressed greater mean fitness or greater genetic variance of fitness. The mutation clearance hypothesis predicts that the variance of fitness should be greater because of an excess of superior lightly loaded genotypes, leading through selection to an elevation of mean fitness. We then isolated spores to make crosses within lines to provide a direct demonstration of the effect of genetic interaction on fitness. Crosses between spores of

similar fitness (i.e., similar mutation load), should produce offspring of fitness lower than the midparent value, provided that there is strong synergistic interaction among loci (de Visser et al. 1996).

## MATERIALS AND METHODS

### *Model Organism*

The unicellular chlorophyte *Chlamydomonas reinhardtii* can be grown in the light on liquid or solid Bold's minimal medium, a mixture of inorganic salts lacking a carbon source (Harris 1989), as a haploid photoautotroph. It can also grow heterotrophically in the dark on medium supplemented with acetate as sole source of carbon and energy. Gametogenesis is induced by nitrogen deprivation, which induces differentiation into mating type plus ( $mt^+$ ) or mating type minus ( $mt^-$ ) gametes, followed by fusion of gametes of opposite gender to form a diploid zygote that secretes a thick wall and acts as a resting stage (Harris 1989). Laboratory cultures are mated in the light, transferred to plates containing solid medium, and then placed in the dark for 4–5 days to allow zygotes to mature. The plates are then exposed to chloroform vapor for 45 sec to kill all unmated vegetative cells, while most of the zygotes survive. The plates can then be placed back in the light, where the zygotes will germinate within a few hours, and the haploid meiospores are used to initiate the next cycle of vegetative growth.

### *Base Population*

The base population for the experiment was a line constructed by C. Zeyl (Wake Forest University, Winston-Salem, NC) as a complex cross involving three wild types that was propagated through 16 sexual cycles and about 200 vegetative doublings under standard laboratory conditions. It carries a marker that causes cells to form yellow (rather than green) colonies when grown on acetate in the dark; this marker is inherited uniparentally through the  $mt^+$  parent and is therefore presumed to be located on the chloroplast. We have never observed reversion, despite examining many thousands of colonies. Several such mutants, involving the deletion of a gene involved in photosynthesis, are known and produce the yellow phenotype by causing the accumulation of a chlorophyll precursor (see Ford and Wang 1980).

### *Selection Experiment*

Lines were extracted as samples of about  $10^5$  cells from the base population and subsequently propagated in flasks containing 300 ml of Bold's medium with continuous light (four soft white fluorescent elements, 20 W each, at 30 cm) and aeration (sterile-filtered room air) at room temperature (22–26°C) for about a week (the period varied somewhat over the experiment), during which they achieve about 10 doublings. We used four treatments to create a total of 12 lines with different sexual histories: (1) mass sexual (four replicates): an obligate sexual cycle was imposed at the end of each period of vegetative growth, and about 200–500 germinated zygotes were used to inoculate fresh medium; (2) single-zygote sexual (four replicates): an obligate sexual cycle was imposed at the end of each period of vegetative

growth, and a single germinated zygote was used to inoculate fresh medium; (3) unselected (two replicates): gametogenesis was induced but zygotes were not selected; hence, the population transferred (250  $\mu$ l of culture containing about  $1-4 \times 10^5$  cells) was an unspecified mixture of sexual and vegetative products; and (4) asexual (two replicates): lines were transferred directly without either gametogenesis or zygote selection by pipetting 250  $\mu$ l of culture containing about  $1-4 \times 10^5$  cells.

The sexual lines went through 113 sexual cycles between September 1997 and September 2002, and all lines went through about 1000 vegetative doublings. In the latter half of the experiment, due to the strong selection pressure, zygotes were produced without a nitrogen starvation treatment in the sexual lines. Thus, nitrogen starvation was not performed anymore to induce gametogenesis; plates were simply exposed to chloroform to kill all unmated vegetative cells. The number of zygotes produced in the mass sexual lines also greatly increased during the course of the selection experiment, such that 200–500 diploid zygotes should be considered as a conservative estimate of population transfer size (see Bell 2005).

#### *Competitive Assay*

We measured the fitness of the ancestor (base population) and the experimental lines by competing them as populations against one of two wild-type strains, CC-2931 and CC-3079. These independently isolated wild types form green colonies on acetate-supplemented medium in the dark. The lines cannot be used directly, because powerful and long-continued selection for mating had caused zygotes to be formed spontaneously in the sexual lines, and zygote formation would reduce the rate of vegetative growth. Consequently, we isolated heterothallic spores of known mating type from each line and used these to set up populations derived from the experimental lines but comprising only *mt*<sup>-</sup> spores. We used 16–18 spores to reconstruct the mass sexual lines; 2–7 for the single-zygotes sexual lines; 23–31 for the unselected lines; and 28–39 for the asexual lines. Each reconstructed population was mixed with a roughly equal quantity of the wild types and propagated in the same fashion as in the experiment for four transfers. At the beginning of the assay and after each transfer, a sample was thin-spread on acetate-supplemented plates and incubated in the dark for 2–3 weeks, after which colony color could be scored. The fitness of the experimental lines relative to the wild type was estimated as the slope of the regression of the ratio of the yellow (experimental lines) and green colonies (wild-type CC-2931 for replicate 1 and wild-type CC-3079 for replicate 2) on the number of generations of competition.

#### *Pure Culture Assay*

Fitness was also estimated as growth in pure culture. It is impracticable to conduct extensive pure culture assays in flasks, because only a few replicates can be used, and considerable variance among flasks is created by differences in bubbling rate. Consequently, we used culture tubes and 384-well microtiter plates to achieve repeatable estimates and high levels of replication. According to pilot experiments,

culture tubes provide the most repeatable assay (correlation coefficients between independent replicate blocks of cultures is  $r = 0.826$  for limiting density and  $0.852$  for rate of increase; data not shown), but 384-well microtiter plates permit much higher levels of replication and are satisfactory if border rows are used to control evaporation ( $r = 0.302$  for limiting density and  $0.306$  for rate of increase; data not shown). Pure culture growth in culture tubes and 384-well plates are also known to give concordant results (correlation coefficients between tube and well plates is  $r = 0.789$  for limiting density and  $0.714$  for rate of increase; data not shown). Two types of assays were conducted.

The first assay measured the growth of the experimental lines as populations (using the reconstructed populations described above) in culture tubes. The populations were first grown in bubbled flasks for 4 days and then used to inoculate 20 ml of Bold's medium in a screw-top culture tube. Five replicates of each line were arranged in tube racks as a single completely randomized block with a single border row. Tubes were scored every day for 3 weeks by vortexing briefly and recording transmittance at 665 nm. Calibration against serially diluted suspensions showed a tight relation ( $r^2 > 0.99$ ) between transmittance ( $T$ ) and log values of cell density (cell density =  $-0.2399 \ln[T] + 9.3289$ ). The cultures are assumed to grow logistically according to the equation  $N_t = K/[1 + (K/N_0 - 1)\exp(-r_{\max}t)]$ , where  $t$  is time,  $N_0$  is the initial population,  $K$  is the limiting density, and  $r_{\max}$  is the maximal rate of increase approached as  $N$  becomes very small (for further discussion on the choice of the logistic equation as an appropriate population growth model, see Bell 1990). We used the estimates of cell density to estimate  $r_{\max}$  and  $K$  for each line. The whole assay was performed twice consecutively. This assay provides the basic comparison of mean fitness between sexual and asexual lines.

The second assay measured the growth of spores isolated from the experimental lines to provide estimates of both the mean and variance of fitness. The first experiment was conducted in culture tubes with 20 spores/line and 2 replicates/spore for all the lines, using the same procedure and method of estimation of  $r_{\max}$  and  $K$  as previously described. The second experiment was conducted in 384-well plates with 128 spores/line and 4 replicates/spore for the asexual and mass sexual lines only. The spores isolated from each line were transferred to the central 128 wells of the plate, with four border rows of control cultures to eliminate edge effects. After a few days of growth on pre-inoculation plates, cultures were transferred to the experimental plates via pin replicator, using 4 replicates line arranged randomly in a growth chamber with constant light (four soft white fluorescent elements, 20 W each, at 30 cm), temperature (26°C), and humidity (60%). We scored absorbance (wavelength: 665 nm) every day for 2 weeks on an automated plate reader (Synergy, Fisher Scientific, Hampton, NH). Calibration against serially diluted suspensions showed a tight relation ( $r^2 > 0.9$ ) between absorbance ( $A$ ) and cell density (cell density =  $9 \times 10^6 \ln[A] + 2 \times 10^7$ ). Maximal absorbance values were then used to estimate  $K$  values and provide an estimate of fitness in 384-well plates.

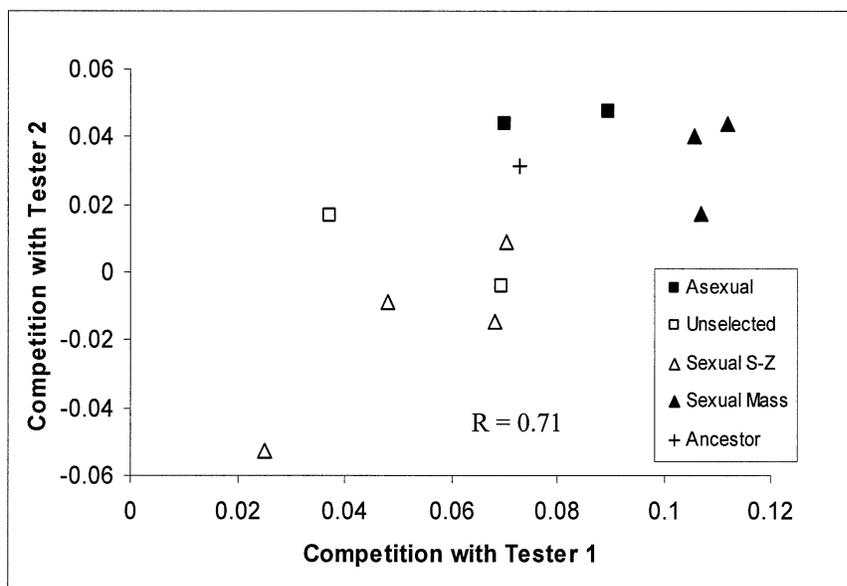


FIG. 1. Competitive assays with arbitrary testers. Plotted values are selection coefficients estimated from the regression of the log ratio of spores from the experimental line to tester spores (tester 1: CC-2931, tester 2: CC-3079) over time for a total of four successive transfers. Two replicate flasks from one of the sexual mass-transfer experimental line were lost through contamination by bacteria.

#### Experimental Crosses

We made crosses within experimental lines to investigate the fitness of sexual progeny. We screened the 20 spores with highest growth and the 20 with lowest growth from each mass sexual line for mating type, to make high  $\times$  high and low  $\times$  low crosses. However, the changes in mating type distribution caused by sexual selection in these lines (Bell 2005) restricted the matings that could be made to 16 within-line crosses (2  $mt^+$  parents  $\times$  4  $mt^-$  parents for high and low sets, respectively, from a single line). The gametes for each cross mated in 1.5 ml of Bold's medium and were transferred to agar plates after 24 h. The plates were incubated in the dark for 5 days to allow zygote maturation, then exposed to chloroform vapor for 45 sec to kill unfused vegetative cells. The plates were then incubated under continuous light to stimulate zygote germination. Each zygote gives rise to four meiospores, which reproduce to form a small colony. One colony was chosen at random from each cross and restreaked to fresh agar to allow 24 random spores to be isolated. These were each cultured on 384-well plates, replicated four times, and their growth recorded, as described above.

## RESULTS

### Competitive Assay

Replicate experiments using the two tester strains were rather highly repeatable ( $r = 0.88$  for CC-2931,  $r = 0.87$  for CC-3079). The two wild types had markedly different fitness relative to the experimental lines (mean  $\pm$  SE selection coefficient against CC-2931,  $0.0748 \pm 0.0053$ ; against CC-3079,  $0.0157 \pm 0.0059$ ), but the response of the lines to the two testers was reasonably consistent ( $r = 0.71$ ). The mean fitnesses of the lines as expressed by competition are shown in Figure 1. In both cases, the asexual and mass sexual lines had greater overall mean fitness than the ancestor: asexual

lines exceeded the ancestor by 28.3% ( $\pm$ SE 9.4%) and mass sexual by 29.3% ( $\pm$ SE 9.5%). The estimates for unselected and single-zygote sexual lines fell short of the ancestor in both cases. Analysis of variance, with the two replicate assays as blocks and treatments as fixed effects, showed that mean fitness differed significantly among treatments ( $F_{3,39} = 14.0$ ,  $P < 0.001$ ). Post hoc comparisons showed that this overall effect was largely attributable to the difference between sexual mass-transfer and single-zygote sexual treatments ( $F_{1,25} = 75.5$ ,  $P < 0.001$ ) rather than to the difference between sexual mass-transfer and asexual ( $F_{1,17} = 3.1$ ,  $P = 0.1$ ).

### Mean Fitness in Pure Culture

Population growth in tubes was positively although weakly correlated ( $r = 0.38$  for  $K$ ) with competitive fitness (Fig. 2). The asexual and sexual mass-transfer lines had similar fitness ( $F_{1,50} = 3.2$ ,  $P = 0.08$ ). Neither was significantly different from the ancestor. The single-zygote and sexual mass-transfer lines did not differ ( $F_{1,50} < 1$ ), whereas the unselected lines had anomalously low growth. Results were similar using  $r_{\max}$  values (positive correlation between  $r_{\max}$  and competitive fitness,  $r = 0.43$ ), with asexual and sexual mass-transfer lines attaining similar fitness. Although the two replicate experiments yielded positively correlated fitness values for  $K$  ( $r = 0.68$ ), there was no positive correlation between the two independent replicate experiments for  $r_{\max}$  ( $r = -0.11$ ). For this reason,  $r_{\max}$  values were not used to analyze the results of the selection experiment.

### Variance of Fitness

Measurements of proliferation of random spores isolated from the experimental lines gave estimates of both the mean and the variance of growth in pure culture. The mass sexual lines again showed no consistent superiority, but spores from

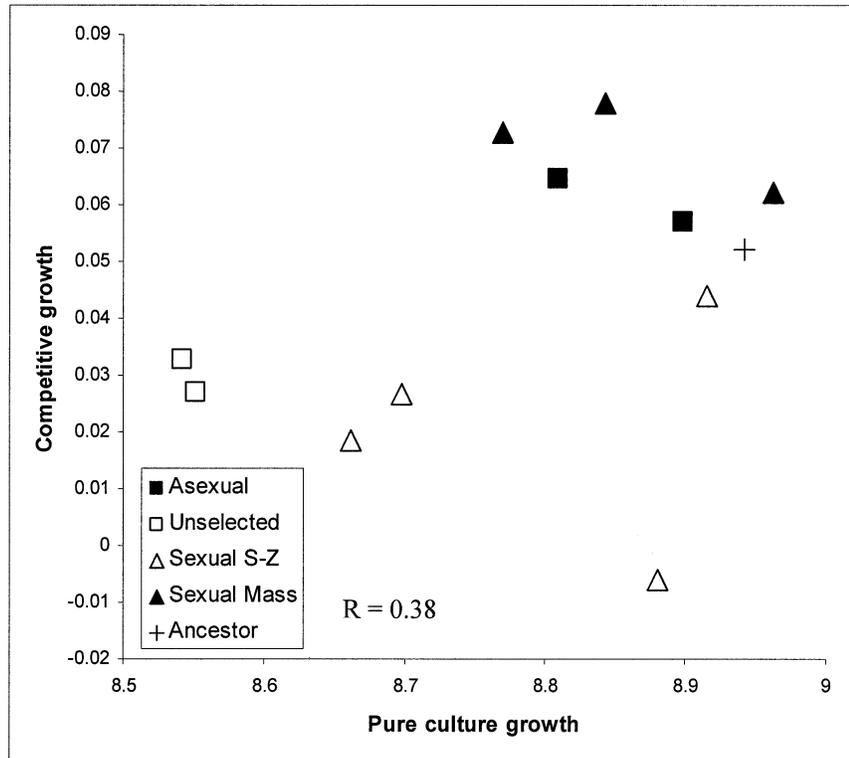


FIG. 2. Relationship between competitive and pure-culture performance ( $K$ ). Competitive values plotted are the mean of the selection coefficients (calculated from the regression of the log ratio of spores from the experimental line to tester spores over time) from the two replicate experiments for each arbitrary tester. Pure culture values are maximal cell densities (log number of cells/ml) estimated from the transmittance data. (Note: One of the mass sexual lines was not plotted because there were no data for one of the replicate competitive fitness assays because of bacterial contamination. However, it behaved similarly to the other sexual mass lines; selection coefficient = 0.0389 for competition with tester 2 and  $K = 8.77$  for pure culture assay.)

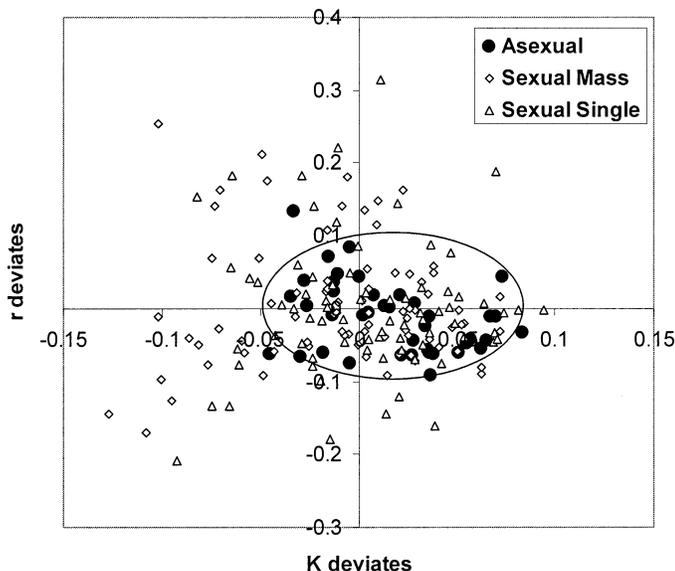


FIG. 3. Growth parameters  $r_{\max}$  and  $K$  of random spores isolated from experimental lines. Values plotted are deviations from overall means (each point is the average of two independent estimates). The ellipse was calculated as the mean  $\pm$  2 standard deviations in both dimensions for spores isolated from the asexual lines.

these lines were more variable (Fig. 3). There was no detectable within-line genetic variance among asexual spores for either  $K$  ( $F_{38,40} = 1.09$ , ns) or  $r_{\max}$  ( $F_{38,40} = 1.46$ , ns), whereas among spores from the mass sexual lines there was significant genetic variance for both  $K$  ( $F_{76,80} = 1.92$ ,  $P < 0.01$ ) and  $r_{\max}$  ( $F_{76,80} = 1.65$ ,  $P < 0.025$ ). The sexual single-zygote lines may have genetic variance for  $K$  ( $F_{76,80} = 1.53$ ,  $P = 0.05$ ) but not for  $r_{\max}$  ( $F_{76,80} = 0.94$ , ns). The magnitude of the within-line genetic variance relative to the phenotypic variance in the mass sexual lines was  $\sigma_G^2/\sigma_P^2 = 0.00871/0.003343 = 0.26$  for  $K$  and  $0.00317/0.01674 = 0.19$  for  $r_{\max}$ . The greater variability of sexually derived spores is clearly apparent in Figure 3, where the sexual populations have a conspicuous excess of inferior spores with low scores for both  $r_{\max}$  and  $K$ . The 384-well assay of random spores gave concordant results. Greater replication made it possible to detect a small amount of genetic variance of limiting density ( $K$ ) within asexual lines ( $\sigma_G^2/\sigma_P^2 = 0.06$ ) and a much larger quantity ( $\sigma_G^2/\sigma_P^2 = 0.81$ ) in the mass sexual lines.

#### Fitness of Sexual Progeny

All the within-line crosses done with spores isolated from the mass sexual lines yielded a positive estimate of genetic variance among progeny, and this was individually significant at  $P < 0.001$  in 13 of 16 crosses. The genetic variance comprised on average 62% of phenotypic variance in the high

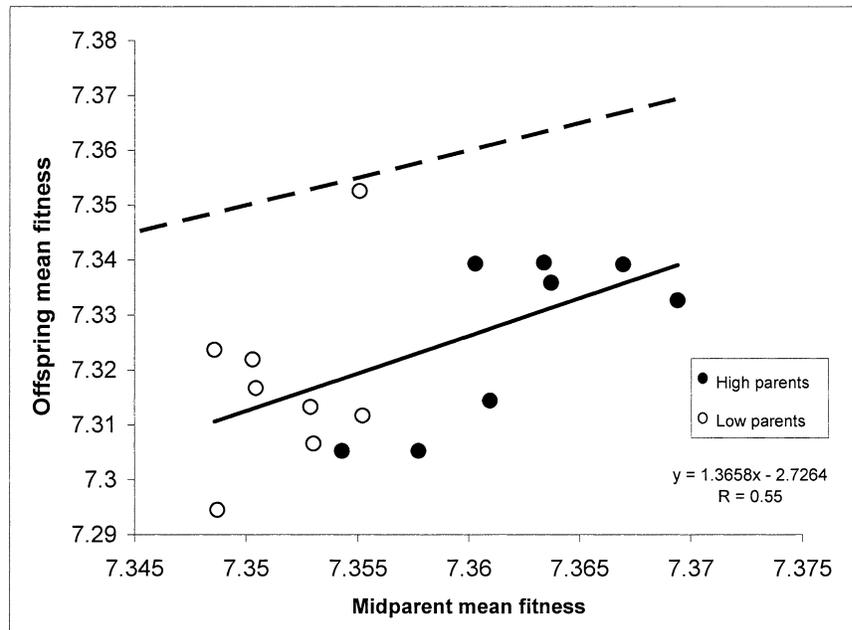


FIG. 4. Growth of sexual progeny (log values of  $K$ ) for crosses within lines. Values are estimates of maximal mean cell densities (number of cells/ml) obtained from the absorbance data (mean value of the two parents and corresponding mean value of the offspring).

crosses and 49% of phenotypic variance in the low crosses. The mean genetic variance component for the single-zygote families represented 28% of the overall genetic variance estimated for the line and was almost exactly the average genetic variance estimated for the sexual single-zygote lines.

The regression of the offspring mean on the midparent for within-line crosses (Fig. 4) had a slope of about  $1.366 \pm 0.556$  SE ( $t = 2.46$ ,  $df = 14$ ,  $P \approx 0.02$ ). That the slope was about unity suggests that most of the variance among family means was genetic. The intercept was negative, with all 16 offspring means falling below the corresponding midparent. There was thus a strong tendency for the limiting density ( $K$ ) of sexual progeny to fall below that of their parents, by a factor of about 0.5% in log values, equivalent to a factor of 7.7% in units of cell density.

## DISCUSSION

### *Mean Fitness*

We found no evidence that the sexual populations were consistently superior to the asexual populations, regardless of how fitness was measured. The low fitness of the single-zygote lines, at least under competitive conditions, suggests that deleterious mutations were arising at appreciable frequency, but this did not cause a detectable increase in mean fitness in the mass sexual experimental lines. If the rate of deleterious mutation is really only about 0.01 per genome per generation (Drake et al. 1998; Zeyl and de Visser 2001) or less, then the mean fitness of a sexual population cannot exceed that of a comparable asexual population by more than about 1%, which we would not have been able to detect. Consequently, these results are consistent with mutation-accumulation experiments. In short, our study failed to provide support for mutation clearance as a general consequence of sexuality.

### *Nature of Fitness*

Our results show a weak positive correlation between growth in competition and pure culture. This weak correlation is most likely due to substantial genotype-environment interactions that are expressed even between slightly different growth conditions. This is consistent with previous studies of genotype-environment interactions for growth in this experimental system (Bell 1990, 1991).

### *Variation of Fitness*

The simplest interpretation of the asexual lines is that they have each become fixed for a single genotype through a selective sweep or series of sweeps. The features that are consistent with this are the fixation of a single mating type in all asexual and unselected lines (Bell 2005) and the very low level of genetic variance for fitness components. The sexual lines might likewise have become fixed, if superior alleles at different loci have independent multiplicative effects on fitness. This is inconsistent, however, with the substantial genetic variance for growth present in the sexual lines. This could be generated by the recombination of deleterious mutations with synergistic epistasis for fitness, although this should result in a greater mean fitness, which was not detected. An alternative interpretation is that selection in sexual populations favors several or many epistatic combinations of alleles with similar phenotypes. No single combination can be fixed because recombination periodically creates a range of inferior genotypes from mating between parents with different superior combinations. This is consistent with the observation that the mass sexual lines contain inferior spores (Fig. 3) and with the results of crossing genotypes isolated from the sexual lines. Crosses between superior parents will yield offspring of lower mean fitness, because recombination

will tend systematically to break up epistatic combinations of beneficial mutations. Our results conform to this expectation, with the mean fitness of sexual progeny being consistently inferior to that of their parents. An immediate reduction of fitness following crossing was observed previously in this system (Colegrave et al. 2002; Kaltz and Bell 2002).

The most extensive and exact studies of the effect of recombination on fitness have involved manipulating chromosomes in *Drosophila*. Chromosomes can be extracted from individuals captured from natural populations and used in experimental crosses. Progeny that are made homozygous for recombinant chromosomes tend to have lower average viability than parents that are homozygous for nonrecombinant chromosomes (Spassky et al. 1958; Spiess 1958; Dobzhansky et al. 1959). These experiments suggested that the reduction of viability caused by recombinational load can be quite substantial, averaging about 10%. Flies that have been made homozygous for chromosomes from male parents captured in the wild are somewhat more viable than comparable flies made homozygous for chromosomes derived from females (Mukai and Yamaguchi 1974; Charlesworth and Charlesworth 1975). This difference was attributed to recombinational load because crossing-over occurs during female meiosis but not during male meiosis in *Drosophila*. These experiments provided modest estimates of recombinational load, representing as little as 1% reduction in mean viability.

An alternative explanation of our results is that the sexual cycle is itself mutagenic, and thereby directly causes a reduction in mean fitness and an increase in variance. This might be the consequence of the breakage and reunion of chromatids during meiosis (Tucic et al. 1981). This is unlikely to be the primary cause of recombinational load in *Drosophila*, because the mutation rate is the same in males and females, but it cannot be ruled out in other organisms. Alternatively, the stressful conditions used to induce the sexual cycle (nitrogen starvation in our case) might increase the mutation rate. This effect has been reported in *Chlamydomonas* (Goho and Bell 2000) but is unlikely to contribute to the reduction of fitness in the mass sexual lines. In the latter half of the experiment, due to a strong selection pressure for sexual reproduction, mass sexual lines started mating spontaneously, such that nitrogen starvation was not needed anymore to induce gamete formation, which halted the possible confounding effects of an increased mutation rate. This effect, however, may explain the anomalous behavior of the unselected lines, in which sex was induced (nitrogen starvation) but not selected (no chloroform treatment). Thus, although we cannot decisively rule out a direct effect of sexuality on fitness, we do not think that it is likely to account for our results.

### Conclusions

The main effect of sex in benign environments may be to prevent the fixation of any particular epistatic combination of beneficial mutations. We speculate that many different multilocus genotypes may have similar fitness. In an asexual population, one of these will rapidly become fixed. In replicate asexual populations, each experiencing the same novel conditions of growth, a different genotype may become fixed

in each particular instance (see MacLean and Bell 2003). Each evolving population is thought to follow a unique trajectory because successive mutations in a successful lineage increase fitness only in the context of the mutations that have occurred previously, while determining the range of mutations that will be successful in the future. A similar process may operate within a single sexual population, where several or many different genotypes may continually tend to spread through selection, but are continually disrupted through outcrossing and recombination. This process does not necessarily increase mean fitness, and it will reduce the fitness of progeny relative to their parents. However, it will necessarily increase the variance of fitness and in that way facilitate a more rapid response to selection if the environment should change. The accelerated adaptation to novel environments associated with sexuality (Colegrave 2002; Colegrave et al. 2002; Kaltz and Bell 2002) may involve the recruitment of this source of variation.

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