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Short-term evolution of competition between genetically homogeneous and heterogeneous populations of *Drosophila melanogaster*

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ABSTRACT

Sexual reproduction may confer an advantage in intraspecific competition. We tested this by studying the evolution of larval competition between two populations of the fruit fly *Drosophila melanogaster*, one of them having genetic variability and the other being highly homogeneous, like an asexual strain would be. We found a clear response in competitive ability in the heterogeneous populations competing with three of the four genotypes assayed as homogeneous populations. In only six generations was this response enough to cancel out the double advantage in reproductive rate that would be expected in an asexually reproducing female. However, the outcome of the competition was genotype-dependent, as the fourth homogeneous genotype developed a competitive advantage through the generations. Thus, selection for larval competitive ability could play an important role in the maintenance of sexual reproduction in *Drosophila*, but would not be enough to ensure it, as some genotypes could overcome its effect if switching to asexual reproduction.

Keywords: co-existence, evolution of sex, genetic homogeneity, intraspecific competition.

INTRODUCTION

The relative rarity of asexual reproduction in nature is problematic, since a rare asexual female would have a two-fold advantage in per-capita rate of population increase over sexual females of the same species, and should therefore displace them (Williams, 1975; Maynard Smith, 1978; Bell, 1982, 1985; Kondrashov, 1993). One of the mechanisms most frequently used (Seger and Hamilton, 1988; Stearns, 1990; Lively, 1993; Vrijenhoek, 1994; Howard and Lively, 1994) to explain this rarity is the microevolutionary version (Lively, 1996) of the Red Queen hypothesis. It suggests that, for species locked in co-evolutionary struggles with biological enemies, the production of variable progeny neutralizes the genetic or ecological disadvantages of sex (Bell, 1982), because new, rare genotypes can better avoid the action of competitors, parasites and predators.

Most studies of the operation of Red Queen mechanisms have focused on the action of parasites. These studies have compared the parasite load of sexual and asexual strains

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within species, or sexual and asexual twin species sharing the same habitat, in fishes (Lively *et al.*, 1990), grasses (Yahara and Oyama, 1993; Kelley, 1993), lizards (Moritz *et al.*, 1991; Moritz, 1993) and snails (Lively, 1987, 1992; Jokela and Lively, 1995). Other studies have compared the incidence of parasitism in progenies obtained by outcrossing with those obtained through selfing in snails (Schrag *et al.*, 1994) and asexually in trees (Burt and Bell, 1991). Overall, these studies found that outcrossed progenies suffered less parasitism, but resistance to parasites might not be the only mechanism providing an advantage for sexual reproduction, because the reverse result has also been found (Strauss and Karban, 1994).

The ability to respond to shifting selection pressures by producing variable progenies could also be important for resisting conspecific competitors. If a female began to reproduce asexually, her clonal progeny would spread in the population, given that she has a two-fold reproductive advantage. As the clone becomes common, its conspecifics may be under stronger selection to compete with it, and the asexual clone could become rare again (Marrow *et al.*, 1992). This frequency-dependent selection is a requisite of co-evolutionary theories of the evolution of sex (Parker, 1994), and has been described for intraspecific competition in the laboratory among *Drosophila* fruit flies and *Tribolium* flour beetles (reviewed in Antonovics and Kareiva, 1988). The competition by conspecifics would not exclude a simultaneous parasite pressure, and in fact both mechanisms could operate together and reinforce each other (Hamilton *et al.*, 1990; Yan, 1996).

The aim of this study was to determine the effect of producing variable progeny on the short-term evolution of intraspecific competition. We forced two laboratory populations of *Drosophila melanogaster* to compete. The first had genetic variability and could respond to selection for competitive ability, whereas the second was genetically homogeneous and was prevented from responding to this selection. The study of the evolution of competition between these two lines could help in understanding the role of genetic variability for intraspecific competition in sexually reproducing populations.

MATERIALS AND METHODS

We took samples from a wild-type *D. melanogaster* population and placed them in competition with genetically marked and highly homogeneous F1s.

Base population

The Santiago population was founded with around 200 *D. melanogaster* females collected in the autumn of 1992 in Santiago de Compostela, Galicia, Spain, and kept since then in the laboratory in the dark at 24°C. Every generation of this laboratory population was started with at least 400 fertilized females that were uniformly distributed to at least 20 culture bottles.

Inbred strains and F1s

The X-linked eye-colour mutant gene *white* (provided by the Department of Biological Sciences, Bowling Green State University, Ohio) was introduced into the wild-type Santiago *D. melanogaster* population using a four-generation backcross scheme. Two hundred individuals from the Santiago population were used in every backcross generation, with mass mating in the first three generations and individual mating in 200 vials in the last generation.

The expected proportion of neutral, non-*white*-linked Santiago population genes was 0.9375. The individuals obtained from the last backcross were mated with their full sibs, and 100 homozygous *white* progenies, each from different parents, were submitted to 11 generations of full-sib mating, so that the resulting individuals had an inbreeding coefficient of 0.908, with a variance of 0.00426. The 10 most productive (in terms of number of pupae) of the 36 surviving inbred strains were preselected and allocated at random to a row or to a column of a 5×5 mating square. The 25 resulting F1 crosses were used in a competition test, in which a sample of 200 eggs from each F1 was introduced into a competition vial with another sample of 200 eggs from the base, wild-type population, following the protocol of the competition experiment described below. Two repetitions of this test were made for each F1. The four best competitors of the 25 crosses (i.e. those producing most *white* pupae when competing with the wild-types) were used for the competition experiment. All inbred strains used to obtain these four *white* F1 crosses (which will be called F1 number 1, 2, 3 and 4) were different, except the maternal strain of F1 number 1 and the paternal strain of F1 number 2, which were the same. Taking the most productive inbred strains and the best competitiors among the resulting F1s, we tried to ensure that they made a strong selection pressure on the genetically varied wild-types. It was also for this reason that we used F1s instead of the inbred strains, as these could suffer an inbreeding depression in competitive ability.

Egg collection

Flat, rough-surfaced pieces of black plastic (2.5×5.0 cm) were covered with a 3 ml (about 2 mm thick) layer of culture medium made up of 100 g of sucrose, 100 g of live baker's yeast, 16 g of agar, 5 ml of propionic acid and 22.8 ml of acetic acid per litre of water. Every medium-covered piece of plastic was introduced, together with adult flies, into a 250 ml empty culture bottle. The flies laid eggs on the medium surface; after 12 h, the pieces of plastic were removed from the bottles to facilitate easy collection of the eggs.

Competition experiment

We made four replicate competition lines for each of the four F1s (16 lines in total). In each line, 200 eggs of the corresponding *white* genotype were removed with a needle from the egg-laying pieces of plastic and introduced, together with 200 eggs from the wild-type Santiago population, into a 150 ml cylindrical plastic vial covered with a 5.5 cm diameter plastic cap, in which ventilation holes had been made with a needle. These vials contained a 30 ml layer of 'water medium' to conserve humidity within the vial, made up of 16 g of agar and 5 ml of propionic acid per litre of water. This was covered by a 4.5 ml layer of 'food medium', made up of 11 g of agar, 50 g of live baker's yeast and 5 ml of propionic acid per litre of water. This medium contained no sugar. Since yeast does not grow without sugar, the amount of food available for larvae can be determined accurately (Mueller, 1985).

The pupae produced in each vial were withdrawn, and their genotype, pupation day and individual weight recorded. We were able to separate pupae by genotype because eye colour can be seen through the puparium of an almost emergent pupa.

All wild-type individuals emerging in a vial were mass-mated in a 250 ml bottle and a random sample of 200 of their eggs was used to obtain the next wild-type generation in that line. They competed again with 200 eggs of the same *white* F1, which were obtained in every

generation from the cross of the corresponding parental strains. These parental strains were maintained under standard conditions in culture bottles throughout the experiment. We did not use the *white* F1's progeny because this progeny would be a genetically varied F2 that could evolve together with the wild-types. Thus, each line was started in every generation with a constant *white* F1 and the progeny of the wild-type individuals that survived competition with the same F1 in that line the previous generation, so that we had two populations of *D. melanogaster* in every line, the wild-type one having genetic variability between individuals and the *white* one being highly homogeneous. Both suffered selection for survival in a competitive environment. The first could respond to this selection, but the other could not.

We performed six competition generations for all lines except the four lines competing with F1 number 4, in which only five generations were possible. All inbred strains and competition lines in the experiment were maintained at 24°C in the dark in the same culture chamber.

Statistical analysis

An analysis of covariance was applied to survival, pupation day and weight measured in the competition experiment. The model used *white* F1 as a random factor, generation as a covariate, and their interaction to test for the between-F1 homogeneity of slopes for the generation covariate. When this interaction was significant, a model was fitted to the data for each genotype separately, the model then including only generation as a covariate.

We had previously analysed models including the line within the *white* F1 and its interaction with generation, but these were not significant for any variable studied, so that the line and its interactions were deleted from the models in the analyses presented in the Results section. The values reported were averaged for the four lines corresponding to each *white* F1.

RESULTS

Changes in the number of individuals by generation are shown in Fig. 1. An analysis of these data is presented in Table 1. Separate analyses were performed for each *white* F1 for the number of *white* pupae, because the interaction F1 × generation was significant ($F_{3,84} = 12.77$, $P < 0.001$) for this variable. Separate analyses are also presented for the total number of pupae (wild-type + *white*) per vial, because the *F*-value for the F1 × generation interaction was very nearly significant ($F_{3,84} = 2.67$, $P < 0.053$). Independently of the initial situation for each F1 genotype, the number of wild-type individuals increased with the generations. There were, however, significant differences between genotypes. The wild-types competing with F1s number 2, 3 and 4 increased their number of pupae at the expense of the *whites*, which experienced an equivalent reduction in survival. In F1 number 1 lines, which were kept in the same culture chamber and the same physical environment, the increase in wild-type numbers was accompanied by an increase in the number of *white* individuals. Therefore, in these F1 number 1 lines, wild-type individuals were apparently able to evolve to identify new resources in the same environmental conditions, thus leaving more resources for the *whites*, whose survival also increased with the generations (Fig. 1). The wild-types seemed to be evolving towards co-existence with the F1 number 1 *whites*. This interpretation is supported by the analysis of the total (*white* + wild-type) number of

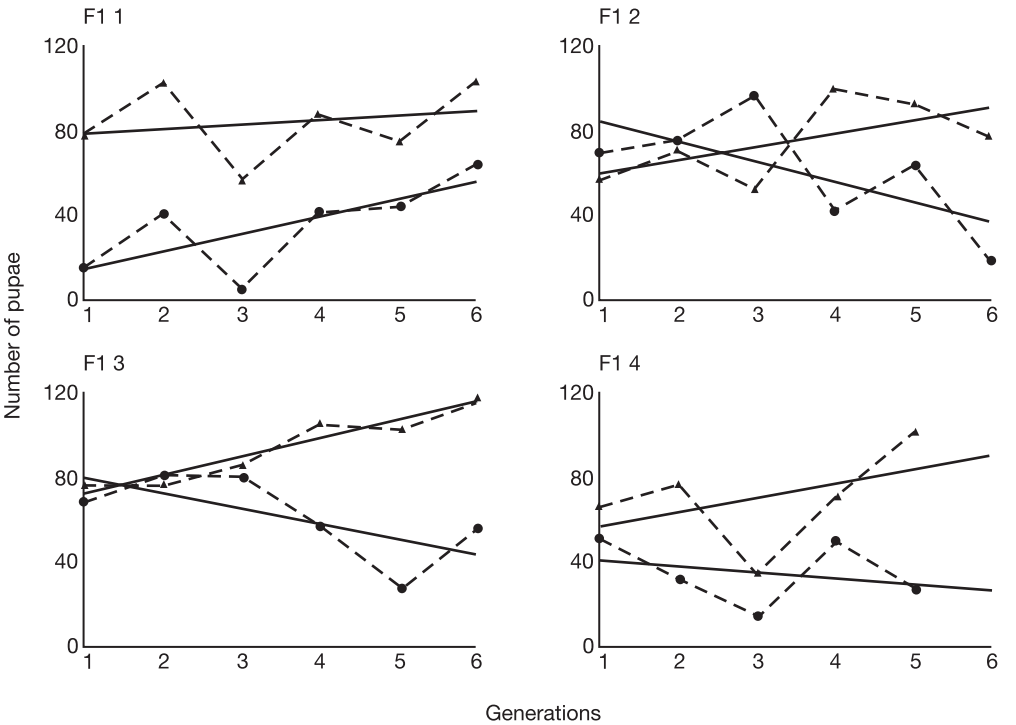

Fig. 1. Average number of pupae per vial (broken lines) of the wild-type (▲) and *white* F1s (●). Continuous lines are the linear regression of this trait on generation number.

individuals pupating in the vials (Table 1). For F1 number 1, this number increased with the generations, showing that the wild-type populations changed to avoid competition with the *whites*, and that the community exploited the medium more intensively. No change in this variable was detected for the rest of the vials, in which the two populations (*white* and wild-type) seemed to compete for a fixed set of resources, any advance by the wild-types being at the expense of the *whites*.

Changes in relative competitive ability are shown in Fig. 2. The results for each F1 are presented separately because the interaction F1 × generation was significant ($F_{3,84} = 13.04$, $P < 0.001$) when all data were analysed together. The covariate effect was significant for F1s number 1, 2 and 3 ($F_{1,22} = 8.86$, $P < 0.010$; $F_{1,22} = 30.31$, $P < 0.001$; $F_{1,22} = 12.90$, $P < 0.010$, respectively), but not for F1 number 4 ($F_{1,22} = 2.71$, $P > 0.050$). In a very short time, the wild-type populations competing with F1s number 2, 3 and 4 had a larval competition advantage that was enough to compensate for the double advantage in rate of increase of a genetically homogeneous, asexually reproducing population. The result was the reverse with F1 number 1 vials, which showed a reduction in wild-type relative competitive ability.

The F1 genotype had a clear effect on the survival of *white* individuals (Table 1), indicating that there was genetic variability for larval competitive ability in the population from which these *white* F1s were taken. Similar results had already been reported for *Drosophila* (Lewontin, 1955; Moya *et al.*, 1988). Moreover, we found significant F1 effects

Table 1. Analysis of variance for number of pupae per vial

	F1	Generation	F1 × generation	d.f. error	bgen
Wild-type	7.24 ***	21.77 ***	1.46	84	6.30
White					
F1 1	—	18.59 ***	—	22	8.07
F1 2	—	15.07 ***	—	22	–9.87
F1 3	—	11.13 **	—	22	–7.16
F1 4	—	1.09	—	18	–3.07
Total					
F1 1	—	7.00 *	—	22	9.96
F1 2	—	1.75	—	22	–3.84
F1 3	—	0.31	—	22	1.32
F1 4	—	0.27	—	18	3.22

Note: Shown are *F*-values for the effects considered in the analyses. bgen is the estimated value for the generation covariable. Separate analyses were performed for each *white* F1 for the number of *white* pupae, because the interaction F1 × generation was found to be significant ($F_{3,84} = 12.77$, $P < 0.001$) for this variable. Separate analyses were also performed for the total number of pupae (wild-type + *white*) per vial, because the *F*-value for the F1 × generation interaction was very nearly significant ($F_{3,84} = 2.67$, $P < 0.053$). In the wild-types analysis, there were 3, 1, 3 and 84 degrees of freedom for the competing *white* F1, generation, their interaction and the error sum of squares, respectively, *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

on the survival of the wild-types, indicating that the *white* F1s also differed in the response they generated in the wild-type populations competing with them. We found similar F1 genotype effects for pupa weight and development time (results not shown).

In the F1 number 1 vials, there were different effects of generation on developmental time. For this variable, the interaction F1 × generation was significant ($F_{3,84} = 4.91$, $P < 0.010$) when all data were analysed together. These significant differences led us to perform separate analyses for each F1. Only for F1 number 2 was the generation effect on pupation day significant ($F_{3,84} = 12.45$, $P < 0.010$) in the separate analysis. Figure 3 shows that, in the F1 number 1 vials, in which both kinds of flies were experiencing increasing numbers with the generations, wild-type individuals tended to pupate later and at a smaller size than the *whites*, while the reverse happened in the rest of the vials. Figure 3 also shows the results for average pupa weight. No significant effects of generation were found in the analysis of this variable.

The evolution of phenotypic variability within the vials is shown in Table 2. We used coefficients of variation to assess this variability, as we found that the variance of the traits tended to change in line with the mean. There was an effect of *white* F1 on variation in wild-type developmental time; some of these *white* F1s induced more variation in the response of the individuals that competed with them than others. The variability in weight increased with the generations in *whites* and wild-types.

DISCUSSION

The selection response for larval competitive ability in the genetically heterogeneous wild-types was overall very efficient in displacing the homogeneous *whites*. In three of the four

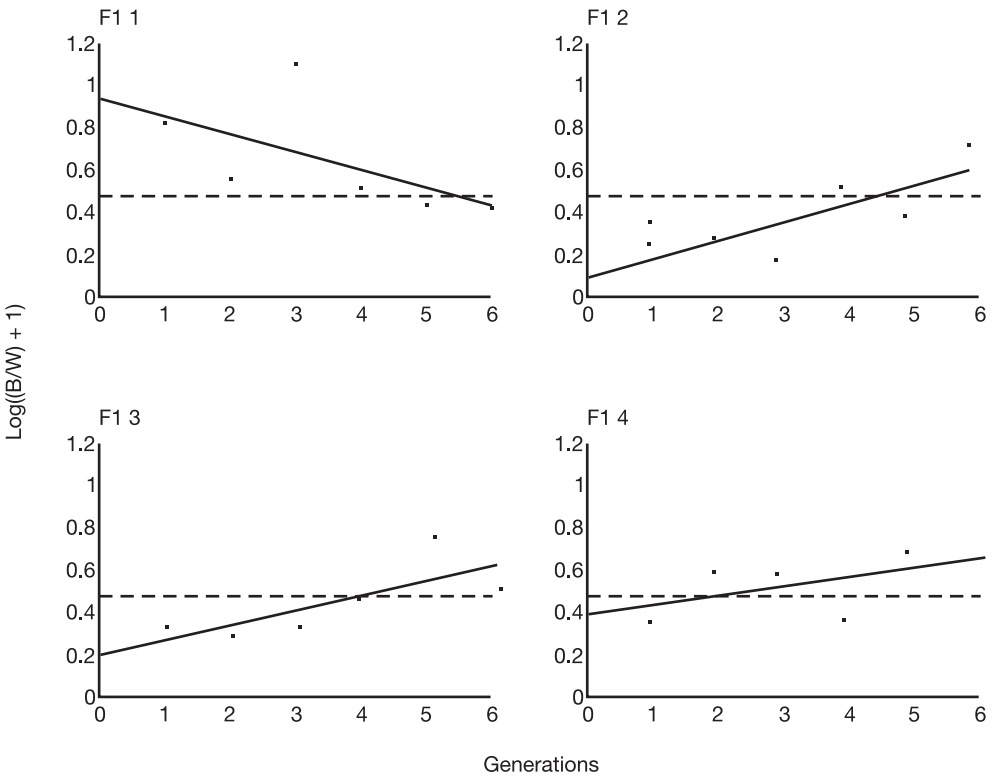

Fig. 2. Evolution of relative larval competitive ability, measured as the regression of the logarithm of the sum of 1 plus the quotient: (number of wild-type pupae (B)/number of *white* pupae (W)), on the generation number (continuous lines). The broken lines represent the value corresponding to a double advantage in competitive ability for the wild-types (log [(2/1) +1] = 0.477).

Table 2. Analysis of variance for the coefficient of variation (CV) of pupation day and pupa weight

	F1	Generation	F1 × generation	bgen
Wild-type				
CV day	2.78***	0.00	2.69	0.013
CV weight	0.52	18.63***	2.48	0.022
White				
CV day	2.07	0.02	2.11	0.001
CV weight	1.38	20.89***	0.37	0.036

Note: Shown are *F*-values for the effects considered in the analyses. The wild-types and the *white* F1s were analysed separately. bgen is the estimated value for the generation covariable. The degrees of freedom were 3, 1, 3 and 84 for the *white* F1, generation, their interaction and the error sum of squares, respectively, in all the analyses. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

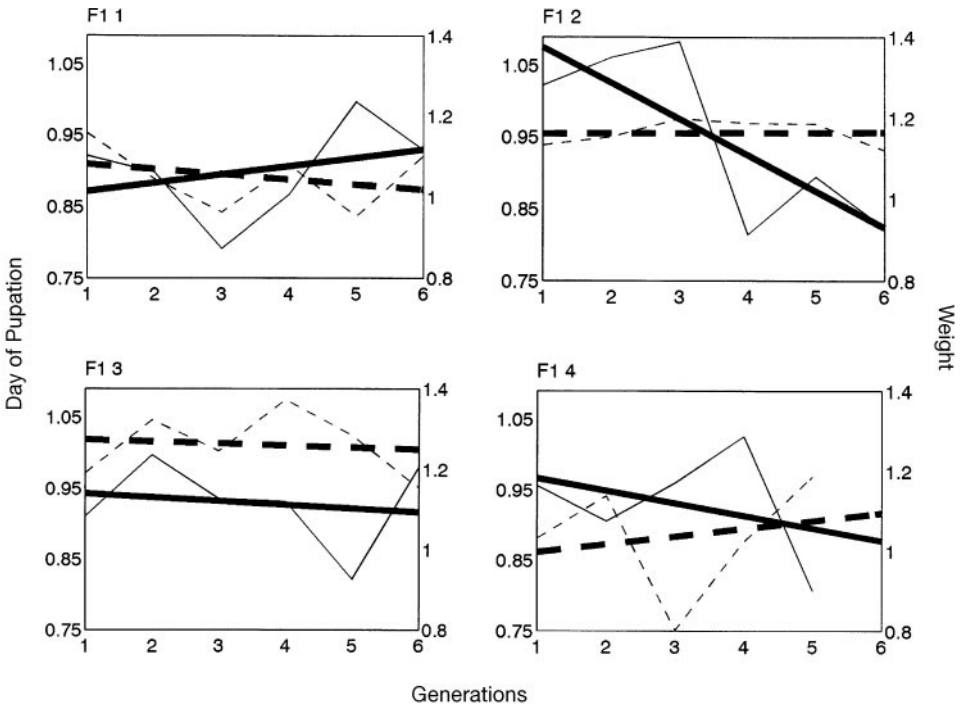

Fig. 3. Evolution of average pupation day (continuous lines) and average pupa weight (broken lines) in the wild-type populations, measured as proportions of the corresponding values for the *white* individuals (wild-type value/*white* value). The bold lines represent the regression of these values on generation number.

F1s studied, and in a very short time, this selection provided a double advantage in larval competition, enough to compensate for the double advantage in rate of increase that an asexual genotype could have. Rapid changes in competitive ability have already been noted in *Drosophila* (Seaton and Antonovics, 1967; Ayala, 1969; Mueller, 1988a). These changes in larval competition might constitute a serious threat to the extension of progeny of an asexual female in a *D. melanogaster* population, at least under laboratory conditions. Some examples of asexually reproducing females have been observed in *D. melanogaster*, but their reproductive efficiency was less than that of sexual females (Templeton, 1983). The above three *white* genotypes, even if reproductively efficient and therefore enjoying a double advantage in population rate of increase, could go extinct if they became asexual in our laboratory population, due to their decreasing relative competitive ability. Their extinction would be faster if the population was approaching demographic equilibrium, since, under these conditions, success depends more on competitive ability than on maximum population growth potential (Charlesworth, 1971; Warner, 1978).

An experiment that did find support for the possible role of intraspecific competition in the maintenance of sexual reproduction introduced competition between two populations of *Tribolium castaneum* (Dunbrack *et al.*, 1995). The first population was allowed to respond to selection for competitive ability, whereas the second was prevented from responding. The non-evolving population was eliminated in a few generations. This result is

similar but not directly comparable to ours, because Dunbrack *et al*. had genetic variability in both competing populations, and also introduced a deliberate environmental change at the beginning of the competition process.

We focused only on the effects of an inability to respond to selection for intraspecific larval competition, not all of the consequences of asexual reproduction. It is for this reason that there were several differences between the situation encountered in our experiment and what would be found in a real population after the appearance of an asexually reproducing female. First, we studied the role of genetic homogeneity in the evolution of larval competition. We found that such competition is enough to give sexual individuals an advantage, but it is of course unknown what the effect would be of any change in other traits. Second, our design tested the case where the homogeneous lineage had gained an important share of the population's reproduction (half the eggs in every generation would be 'asexual'), but did not simulate the effect of competition within a generation on the egg production of the next generation. We used an equal, fixed number of eggs from both lineages to start every generation, because we wished to isolate the effect of larval competition. We believe that the introduction of variation in the reproductive success of each genotype in the previous generation would have greatly reduced the precision of the experiment. Third, it would also have been better to have used females only in our *white* F1s, as a true asexual lineage would be composed of females only, and it is known that, in *Drosophila*, an individual's sex affects its competitive ability (Nunney, 1983). The use of the two sexes may have introduced some phenotypic variation in the *white* F1s, but would not have contributed to any selection response. Fourth, our *white* genotypes were F1 crosses of inbred strains of small population size, so that they could lose some competitive ability over the generations due to increases in inbreeding depression in maternal effects from their inbred mothers. This is unlikely, because no great changes in inbreeding could have occured during the competition experiment. The parental strains already had very high initial inbreeding values at the beginning of the experiment and were mass-mated to produce large numbers of F1 eggs. In addition, the viability of *Drosophila* eggs is not dependent on the mother's past history (Prout and MacChesney, 1985).

It could also be argued that the inbred strains used for our *white* F1s might have been genetically unstable during the experiment, because they may have been adapting to the conditions prevailing in their maintenance bottles. But this appears unlikely in the short term, given their high inbreeding coefficient, and the low mutational heritabilities found for fitness-related traits in this species (Houle *et al*., 1994, Fernández and López-Fanjul, 1996).

On the other hand, the wild-types could simply have been adapting to the novel environment of the competing vials. However, the culture medium and the physical environment in the competition vials were very similar to those used for the maintenance of the Santiago population, from which all individuals were sampled. In addition, most F1s at the start of the competition experiment had a high genetic level for competitive ability in these vials, later being displaced by the wild-types. One population was displacing the other over the generations in a highly competitive context, and was therefore adapting to these competition conditions.

The differences between the four F1 genotypes were remarkable. Unlike the other *white* F1s, F1 number 1 was not displaced by the selection response for competitive ability in the wild-types. Differences in the evolution of competitive ability between these F1s are compatible with the fact, explained above, that the same inbred line was used as the paternal strain of F1 number 1 and the maternal strain of F1 number 2, because there is a high

proportion of non-additive genetic variance for this trait in *Drosophila* (reviewed in Latter and Sved, 1994). It is also known that initial genotypic differences between lines can cause different evolutionary responses to competition in *Drosophila* (Joshi and Thompson, 1995); in the same way, the consequences for the population of the appearance of an asexual female could be heavily dependent on that female's genotype. It would seem that the spread of a new asexual line is likely in this species, as one of the four genotypes (F1 number 1) tested was not displaced in the short term by selection. An asexual genotype with characteristics like those of our *white* F1 number 1 could have resisted the larval competition of the sexual genotypes, even if lacking a double advantage in reproductive rate, as it had an advantage in terms of pupal weight and developmental time. These are two important components of competitive ability in this species (Nunney, 1983; Mueller, 1988b), because fast developing individuals can reproduce earlier and exploit scarce resources before their depletion (Bakker, 1961; Burnet *et al*., 1977), and big pupae result in bigger adults that lay more eggs (Chiang and Hodson, 1950; Robertson, 1957; Mueller, 1987).

Predicting the long-term outcome of competition in *white* F1 number 1 lines is not straightforward. The wild-types could go extinct, given their increasing disadvantage in competitive ability, but co-existence could also be promoted by resource partitioning. In fact, the wild-types competing with F1 number 1 increased their numbers over the generations and appeared able to avoid competition with this F1. When competition is intense, natural selection may favour genotypes that avoid competition, and produce a co-evolution-structured community (Rummel and Roughgarden, 1983). Examples of stable co-existence between sexual and asexual strains of animals have been reported (Harshman and Futuyma, 1985; Schenck and Vrijenhoek, 1986; Hebert *et al*., 1988; Honeycutt and Wilkinson, 1989; Weeks *et al*., 1992; Fox *et al*., 1996).

Our results were obtained in laboratory populations, but could be relevant to natural populations, as there is increasing evidence that intraspecific competition is an important ingredient of *Drosophila* larval life under field conditions (Prout and Barker, 1989; Santos *et al*., 1994). The selection response for competitive ability could be a powerful mechanism against the spread of asexuals, and thus may play an important role in the maintenance of sexual reproduction in *D. melanogaster*.

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