Genotype-Environment Interactions and the Estimation of the Genomic Mutation Rate in Drosophila melanogaster

Author(s): Alexey S. Kondrashov and David Houle

Source: *Proceedings: Biological Sciences*, Vol. 258, No. 1353 (Dec. 22, 1994), pp. 221-227
Published by: Royal Society
Stable URL: http://www.jstor.org/stable/50084
Accessed: 21-08-2017 16:38 UTC

Genotype–environment interactions and the estimation of the genomic mutation rate in *Drosophila melanogaster*

ALEXEY S. KONDRASHOV[1] AND DAVID HOULE[2]

[1] *Section of Ecology and Systematics, Cornell University, Ithaca, New York 14853, U.S.A.*
[2] *Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1*

SUMMARY

We have studied the relative fitnesses of three genotypes of *Drosophila melanogaster* in 50 environments. Two genotypes, the MA lines, had accumulated mutations in the absence of natural selection over 62 generations. The third was a related strain where selection had continued to act. The environments differed in three factors: parental density, dilution of the medium, and the temperature régime and medium composition. Our measure of fitness assessed fecundity and viability relative to a reference genotype. Both MA lines always had lower fitnesses than the selected line, but the difference increased dramatically with dilution of the medium and, especially, crowding. Under the most severe conditions, the performance of the MA lines approached 0. This increased difference in harsh conditions may be caused both by a uniform increase in the magnitude of deleterious effects of all mutations and by the exposure of mutations which are essentially neutral under benign conditions. If the second cause is important, previous experiments are likely to have underestimated the genomic deleterious mutation rate in *Drosophila melanogaster* more than previously thought.

1. INTRODUCTION

Genotype–environment interactions for relative fitness are widespread in nature. For example, studies of inbreeding depression and heterosis in crops (Barlow 1981) and natural populations (Mitton & Grant 1984; Zouros & Foltz 1987; Dudash 1990) show that average fitness differences are magnified in harsh conditions. The interactions over a set of genotypes and environments may be classified into one of three categories, depending on whether inferior genotypes in the sample are always deleterious (unconditionally deleterious), deleterious under some environments and neutral under the rest (conditionally neutral), or deleterious in some environments and beneficial in others (conditionally beneficial).

Conditionally beneficial variation has received a great deal of attention, as switching of fitness ranks of genotypes may maintain polymorphism (see Gillespie & Turelli 1989; Gillespie 1991). In *Drosophila pseudoobscura*, different inversions are favoured at different temperatures (Wright & Dobzhansky 1946; Birch 1955). Numerous other examples have also been documented (see, for example, Place & Powers 1984; Takano *et al.* 1987), although there are few cases where it is clear that polymorphisms are indeed protected.

In the unconditionally deleterious case, the environment affects only the magnitude of differences among genotypes. Many morphological mutants seem always to be deleterious (see, for example, Lindsley & Zimm 1992), although their effects may be increased in a poor environment (see, for example, Lewontin & Matsuo 1963).

Conditionally neutral variation is more difficult to document. Still, stocks bearing morphological mutations often have fitnesses indistinguishable from those of wild-type stocks in benign conditions, but become quite inferior in stressful environments (Lewis 1954; Birch 1955; Bakker 1961; Lewontin & Matsuo 1963). Other cases may include some allozyme polymorphisms in *Escherichia coli* (Dykhuizen & Hartl 1983) and temperature- and nutrition-sensitive mutations (see, for example, Suzuki *et al.* 1975). Kimura (1983) has emphasized the potential importance of such variation for evolution.

New non-neutral mutations are usually assumed to be mostly unconditionally deleterious (see Crow & Simmons 1983). Here we report data showing that the relative fitnesses of genotypes where spontaneous mutations were allowed to accumulate are lower in harsh environments, suggesting the possibility that conditionally neutral mutations may be common. If so, fitness assays under benign conditions only may underestimate the total genomic deleterious mutation rate.

2. METHODS

We have studied three lines which were derived from a large laboratory population, IV (Charlesworth & Charlesworth 1985). During their initiation, the eye mutant sparkling-poliert was introduced. Two were obtained from an experiment where mutations accumulated freely in the second chromosome (Houle *et al.* 1994). The third line, Selected, is related to the MA lines, but was drawn from a population maintained under conditions where natural

Proc. R. Soc. Lond. B (1994) **258**, 221–227
Printed in Great Britain

selection minimized the accumulation of new mutations (Houle *et al.* 1994). For this experiment, 16 second chromosomes were extracted from this population and then pooled. To choose the MA lines, a sample of 45 lines at generation 62 of accumulation were assayed for fitness by the Sved technique (Sved 1971; Houle *et al.* 1992). Wild-type chromosome frequencies at the end of the experiment ranged between 0.68 (line 51) and 0.58 (line 58). These two lines thus have the highest and the lowest fitness, and were used here.

The approach of Timofeev-Resovsky (1934) was used to assay the relative fitness of lines. Mated females from the marked test line (51, 58 or Selected) and a wild-type reference line are allowed to oviposit in the same bottle. The females were then removed and the offspring counted. The ratio of counts of test and reference phenotypes, which reflects both female fecundity and egg-to-adult viability, is a measure of relative fitness, and will be called productivity. The original IV population was used as a reference line.

Starting two generations before the experiment, a constant number of young flies were used to initiate the culture bottles. Then, 15 d before the initiation of this experiment, 12 male and female flies (5–7 d old) of the IV or test genotypes were allowed to oviposit for 48 h in bottles with a diameter of 60 mm. This resulted in 200–300 eggs per bottle, well below the carrying capacity of the bottles, which was about 500 flies.

Emergence began 9 d later and, 5 d before the experiment, all flies which had eclosed from each line were mixed together, and transferred into fresh bottles, seeded with abundant live yeast, at a density of about 100 flies per bottle. The measurements of productivity were taken in bottles with a bottom diameter of 52 mm containing 30 ml of medium. To initiate the experimental bottles, the males were removed and test and reference females combined in a ratio of three test to one reference female. This ratio was chosen to allow adequate estimation of productivity of all genotypes. Groups of females were placed on the appropriate medium (see below), seeded with 10 mg of live yeast, and placed at 25 °C. After 24 h the females were discarded. Eclosing flies were counted daily from 9 d to 21 d after oviposition.

Except during the experimental generation, flies were reared at 25 °C, on a medium consisting of 2 g brewers yeast, 8 g corn flour, 8 g sucrose, 1.0 g agar, and 0.2 ml of proprionic acid for each 100 ml of deionized water. Live dry bakers yeast was added to enhance the fecundity of females. Flies were maintained on a 12:12 h L:D cycle. CO_2 anaesthesia was used when necessary. The only difference of these conditions from those in previous experiments with these lines (Houle *et al.* 1992, 1994) is the use of corn flour instead of corn meal and the addition of live yeast.

Environmental variation was introduced on three axes. First, two combinations of temperature and medium ('environments') were used. In the 'constant' environment flies were reared on standard medium at 25 °C. In the 'variable' environment, flies were reared with a diurnal alternation of temperature between 29 °C during the 18 h day, and 8 °C during the 6 h night. The medium included of 2 g brewers yeast, 5 g of corn flour, 5 g of sucrose, 5 ml ethanol, 3.5 g agar, and 0.2 ml of propionic acid for each 100 ml deionized water.

Secondly, the dilution of the medium was varied. The concentrations of all ingredients except agar and propionic acid were 100%, 75%, 55%, 40% and 30% of that described above; these are referred to as dilutions 1–5, respectively. The concentration of propionic acid was 0.2 ml per 100 g in dilutions 1 to 3, and 0.1 ml per 100 g in dilutions 4 and 5. Third, total parental female densities of 8, 16, 32, 64 or 128 per bottle were used, each with a 3:1 ratio of test to reference females. Each of the three test genotypes were assayed in each of the 50 environments in two replicates, so the total sample size of the experiment was 300 bottles. Statistical analyses were performed by using SAS (1988*a*, *b*). The raw data from these experiments are available from the authors.

3. RESULTS

In the constant environment, feeding by the larvae liquefied the medium within a few days. Thus only medium to a depth of approximately one larval body length was available; the microflora of the cultures would have been primarily anaerobic (Gordon & Sang 1941). In the variable environment the medium remained solid because of the higher concentration of agar. Larvae were unable to burrow into the medium until the second instar. Larvae reached the bottom of the bottles starting on the fourth day after oviposition and the resulting tunnels remained open. Thus the whole volume of the medium was utilized by the older larvae, and, probably, by aerobic microorganisms. The emerging flies were much larger at low densities and on undiluted media under both environments.

All three environmental factors affected either the number of flies emerging or their average development time. Figure 1 shows the average numbers of flies eclosing per bottle in each of the 50 environments. Analyses of variance show that all three factors, as well as all interactions, are significant at $p < 0.0001$. The average number of flies eclosing was 204 in the constant and 459 in the variable environment. We attribute this to drowning of larvae in the liquefied medium in the constant environment, and to the greater utilization of the medium and perhaps the presence of ethanol, which can stimulate oviposition or enhance survivorship, in the variable environment.

Dilution of the medium reduced the number of eclosing flies. The regression slope (b) with dilution was -56 ($p < 0.0001$) in the constant and -91 ($p < 0.0001$) in the variable environment. In the constant environment, the number of offspring decreased slightly with $\log_e$ parental density ($b = -14$, $p < 0.0005$). This suggests that the carrying capacity of the bottles was already reached with the lowest density, and increasing the number of parents markedly increased competition. Overall, in the variable environment, the number of offspring grew with density ($b = 90$, $p < 0.0001$). Analyses within each dilution show that the relation changes from significantly positive in dilutions 1–3 to significantly negative in dilution 5. Even with undiluted food, while density increases 16-fold, offspring number increases less than threefold, showing that competition is stronger at high density.

Figure 2 presents the average development time of all flies in each environment. Analysis of variance (ANOVA) shows that all factors and interactions are significant, except dilution × environment and dilution × density. Mean development time was 12.83 d in the constant, and 13.99 d in the variable environment. Within both environments development

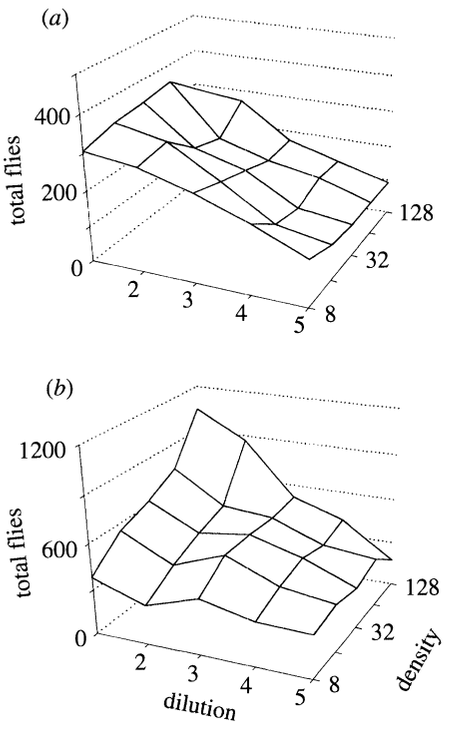

Figure 1. Mean number of flies of all genotypes eclosing in each environment: (*a*) constant, (*b*) variable.

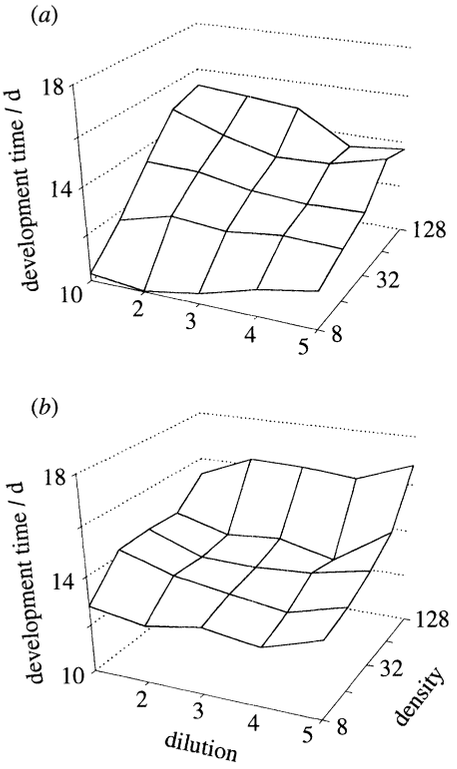

Figure 2. Mean development time in days over all genotypes in each environment: (*a*) constant, (*b*) variable.

time increases significantly with $\log_e$ parental density (constant, $b = 1.4$; variable, $b = 1.0$; $p < 0.0001$ for both), consistent with our interpretation of the relative insensitivity of the number of emerging flies to density. Surprisingly, there is no significant relation between development time and dilution in either environment.

The norms of reaction for the productivity of each test line, calculated as the number of spa^{pol} progeny over three times the number of wild-type progeny, are shown in figure 3. Test genotypes generally have mildly reduced productivity in benign conditions but, as density or dilution increases, the productivity of all test genotypes drops. Analyses of covariance (ANCOVAS) within each environment were performed with line as a classification variable, and dilution and $\log_e$ density as covariates. In both environments, Selected and MA lines are significantly different by SNK tests ($p < 0.0001$), but lines 51 and 58 are not. In the variable environment, the slopes for the other factors are homogeneous over lines. For dilution the slope is -0.10 ($p < 0.0009$), and for $\log_e$ density the slope is -0.37 ($p < 0.0001$). In the constant environment, the slopes for $\log_e$ density for different genotypes are not homogeneous. This is due to a significant difference between Selected ($b = -1.07$, $p < 0.0001$) and MA slopes (for both lines, $b = -0.53$, $p < 0.0001$). The effect of dilution is homogeneous with a slope of -0.22. The larger slopes for density than for dilution are interesting, given the marked effect which dilution has on the total number of progeny. Lines 51 and 58 had the most dissimilar fitness among MA lines in a Sved cage test (see Methods), but were never significantly different in our assay, although line 51 did have higher mean productivity than line 58 (0.624 against 0.556 in the constant, 0.588 against 0.561 in the variable environment). This could reflect genotype–environment interactions, or the results of the Sved test might have involved aspects of fitness not included in productivity, such as mating ability.

The bumpy surface for the Selected line in the variable environment is at odds with the relatively smooth surfaces for the other treatments, suggesting that we may have made an experimental error, although we have no evidence as to its nature.

Figure 4 shows the productivity of the two MA lines relative to that of the Selected in the same treatments. The relative productivity of the MA lines decreases with increasing dilution and, especially, density. Within environments the effects of both dilution and $\log_e$ density are homogeneous over lines. In both environments, the effect of $\log_e$ density is significant at $p < 0.0001$ (constant $b = -0.133$; variable $b = -0.229$). The effect of dilution is significant in the constant ($b = -0.051$, $p < 0.0001$) but not in the variable environment ($b = -0.022$, $p < 0.4$).

The development times of the test genotypes relative to those of the reference are shown in figure 5. In the constant environment, slopes are homogeneous over lines. Line 58 has a significantly longer development time than the Selected or line 51. Relative development time is more sensitive to $\log_e$ density than to dilution (dilution, $b = -0.0508$, $p < 0.0001$; density, $b = -0.12$, $p < 0.0001$). The effects of density and dilution are not homogeneous between lines in the variable environment, but there are no significant differences in overall means of lines. The effects of dilution are significant at $p < 0.01$ for each line, with slopes for the Selected, line 51 and line 58 of -0.017, -0.044 and -0.030, respectively. The effect of $\log_e$

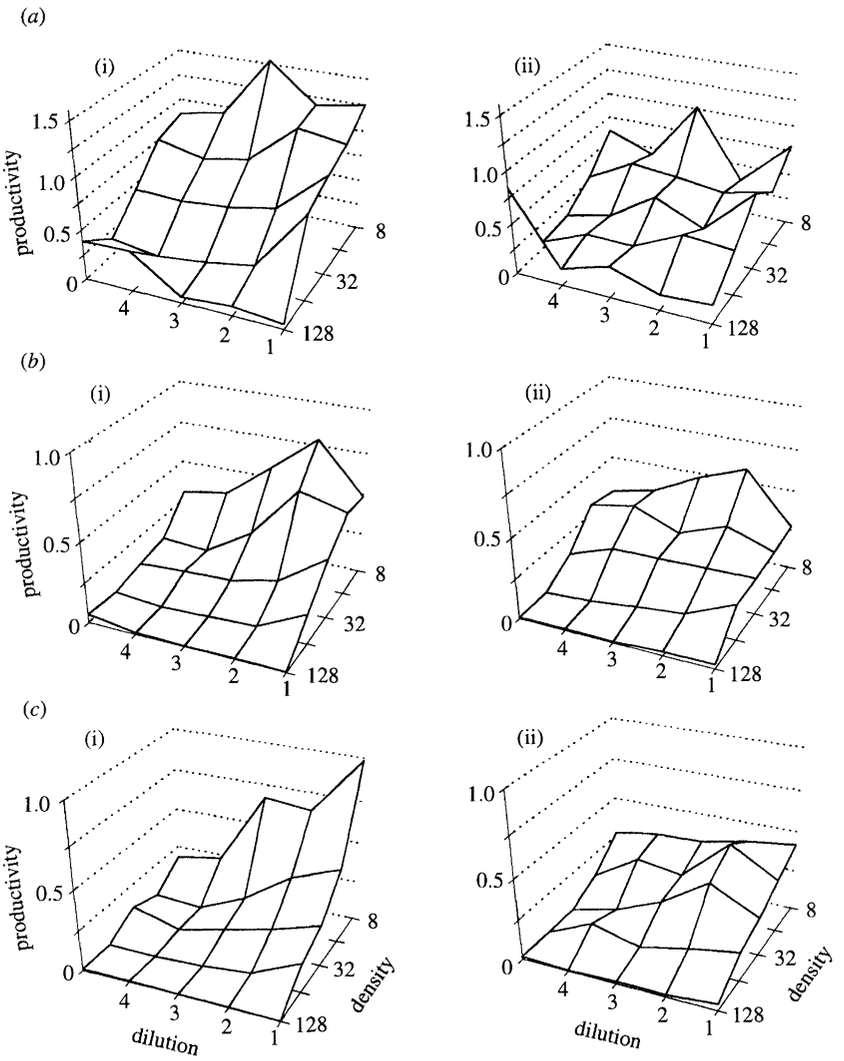

Figure 3. Mean productivity of each test genotype, relative to the IV reference: (*a*) Selected, (*b*) line 51, (*c*) line 58; in each case, (i) constant, (ii) variable.

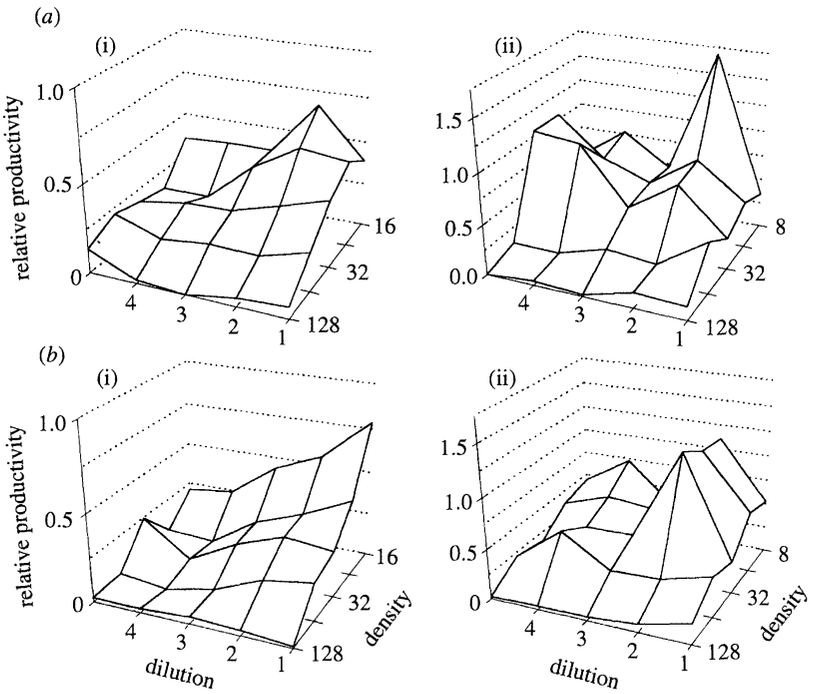

Figure 4. Mean productivity of the MA genotypes, relative to the productivity of the Selected genotype: (*a*) line 51, (*b*) line 58; in each case, (i) constant, (ii) variable.

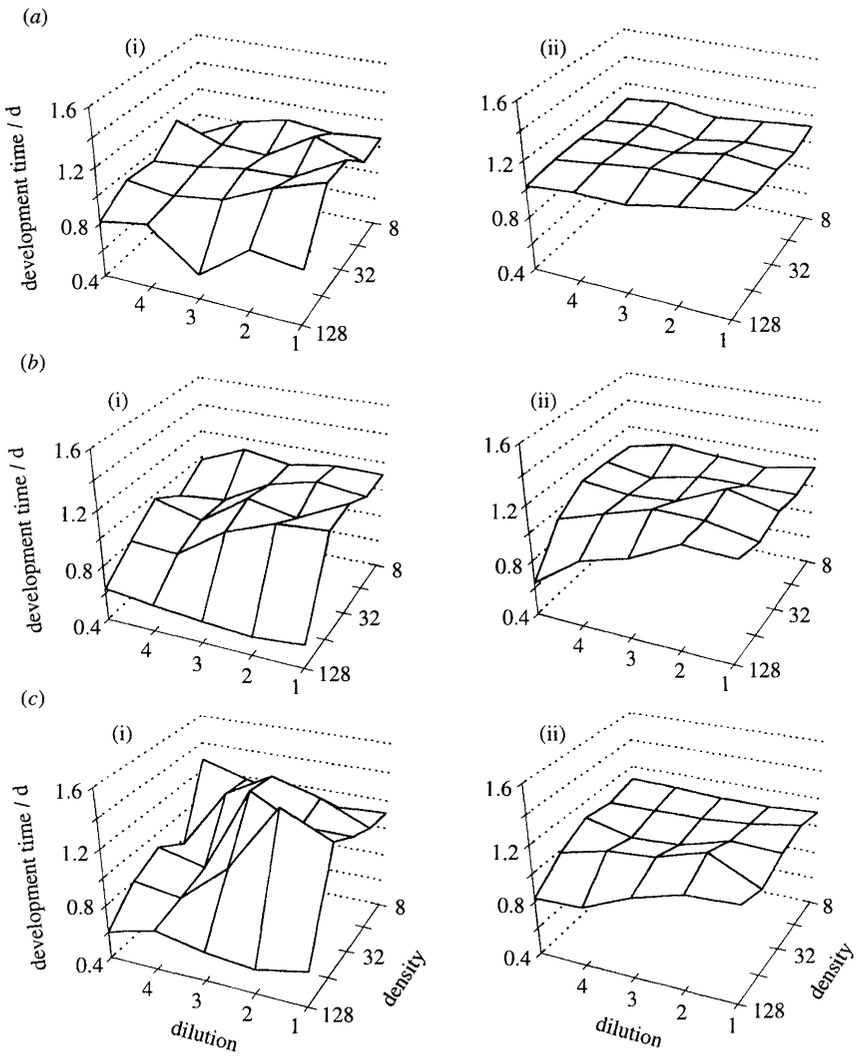

Figure 5. Mean development time of test genotypes relative to IV: (*a*) Selected, (*b*) line 51, (*c*) line 58; in each case, (i) constant, (ii) variable.

density is also negative in each case, with slopes for Selected, line 51 and line 58 of -0.001 (n.s.), -0.043 ($p < 0.01$) and -0.037 ($p < 0.01$), respectively.

Close examination of the pattern of eclosion suggests that the fitness of MA lines in the most competitive conditions is even lower than is suggested by the results above. The only MA flies eclosing at the highest density had relatively rapid development. They probably escaped the severest competition by initiating development early, while live yeast was still abundant. After this early peak of emergence, the fitness of line 51 is essentially 0 (data not reported).

4. DISCUSSION

We have estimated norms of reactions of a fitness component for three genotypes while varying three environmental factors. For each treatment, changing any one of the three factors had an effect on either the total number of flies eclosing or their development time. Both density and dilution had clear, simple and predictable (Sang 1950; Robertson 1960; Ashburner & Novitski 1978) effects. Density clearly affects competition in that offspring number does not increase proportionally with the number of parents. Development time increases with density. Dilution also seems to have been a source of stress for the larvae. It reduced the offspring number, although this may also have been due to reduced oviposition. In addition, offspring size was noticeably reduced at high densities and dilutions. The effect of 'constant' against 'variable' environment is less clear. The variable environment was designed to increase the complexity of the cultures, and it is difficult to compare their relative quality.

The productivities of all three test lines declined dramatically with the quality of the environment, relative to an outbred reference population. This effect was particularly marked as density increased. The productivity of the Selected line differed by a factor of at least 10 within each environment, while the productivity of both MA lines declined almost to 0 in the most crowded conditions. Similar observations led Wallace (1968) to argue that selection tends to be 'soft', i.e. acts in both a frequency- and density-dependent manner. We have also demonstrated a weaker dependence between fitness and the harshness of the physical environment.

Interestingly, the decline of productivity at high

density is most marked in less diluted medium. We suspect that in the high-density, high-dilution environments, genotype-independent differences in reaching the limited supply of live yeast may have been a major determinant of survivorship. In these treatments most of the larvae failed to complete development.

Density-dependent changes in relative fitness of laboratory cultures of *Drosophila* are common (see, for example, Dobzhansky & Spassky 1944; Lewontin 1955; Birch 1955; Lewontin & Matsuo 1963; Clark & Feldman 1981). Fitness differences are usually greatest at high (Lewis 1954; Bakker 1961; Moree & King 1961) or extreme densities (Lewontin & Matsuo 1963). Our data are exceptional only in the magnitude of the decline in fitness of the MA lines with increasing density. There are counter-examples where fitness differences seem to be greatest in benign conditions (Clark & Feldman 1981, Mueller & Ayala 1981), although neither of these studies used competitive measures of fitness.

The ratio of the decline of the mean fitness to the increase in its variance due to accumulation of mutations in the absence of selection provides a minimum estimate of the genomic deleterious mutation rate, U, and a maximum estimate of the average coefficient of selection against a mutation, s (Mukai 1964; Mukai *et al.* 1972; Crow & Simmons 1983). If all new mutations are unconditionally deleterious, a change of experimental conditions may alter s, but will not affect the estimate of U. However, if some mutations are conditionally neutral they may be missed by assaying performance in a good environment, causing an underestimate of U.

Productivity of MA lines relative to the Selected line is markedly lower under harsh conditions. To determine the contribution of conditionally neutral mutations to this, one needs to compare the estimates of U and s in good and simple with that in harsh and complex enironments. It is desirable to include as many potential components of fitness as possible. For example, conventional laboratory experiments do not assay dispersal, sensory physiology, and predator avoidance behaviours. The genes involved in these may be both numerous and to some extent independent of those whose effects are seen under simple conditions. If this is so, the conditions under which fitness is assayed could have a major impact on estimates of U and s, and it will be very difficult to avoid underestimating them.

However, the fact that most changes in relative productivity seem to result from changes in density suggests that previous experiments already include the sort of effects we have seen. Mukai (1964; Mukai *et al.* 1972) assayed viability under fairly crowded conditions which may have been similar to our high-density, low-dilution, constant environment. The situation is potentially more complex in the Sved cage test used by Houle *et al.* (1992). Here, density is unregulated over a series of generations, and so conditions are quite competitive. However, density in the cages has a positive correlation with the frequency of the test chromosome. This introduces a genotype–environment correlation, which, given our results, would tend to reduce the variance among lines. Only further measurements of fitness components under harsh and complex environments can help resolve these questions concerning estimation of the genomic deleterious mutation rate.

We thank B. Charlesworth and M. Lynch for discussions and support. This work was supported by NIH grants GM36827 to M. Lynch and AG07748 to B. Charlesworth.

REFERENCES

Ashburner, M. & Thompson, J. N. 1978 The laboratory culture of *Drosophila*. In *The genetics and biology of* Drosophila, vol. 2a, (ed. M. Ashburner & T. R. F. Wright), pp. 1–109. London: Academic Press.

Bakker, K. 1961 An analysis of factors which determine the success in competition for food among larvae of *Drosophila melanogaster*. *Arch. Neerl. Zool.* **14**, 200–281.

Barlow, R. 1981 Experimental evidence for interaction between heterosis and environment in animals. *Anim. Breed Abstr.* **49**, 715–737.

Birch, L. C. 1955 Selection in *Drosophila pseudoobscura* in relation to crowding. *Evolution* **9**, 389–399.

Charlesworth, B. & Charlesworth, D. 1985 Genetic variation in recombination in *Drosophila*. I. Responses to selection and preliminary genetic analysis. *Heredity* **54**, 71–83.

Clark, A. G. & Feldman, M. W. 1981 Density-dependent fertility selection in experimental populations of *Drosophila melanogaster*. *Genetics* **98**, 849–869.

Crow, J. F. & Simmons, M. J. 1983 The mutation load in *Drosophila*. In *The genetics and biology of* Drosophila, vol. 3c (ed. M. Ashburner, H. L. Carson & J. N. Thompson, Jr), pp. 1–35. London: Academic Press.

Dobzhansky, T. & Spassky, B. 1944 The genetics of natural populations. XI. Manifestation of genetic variants in *Drosophila pseudoobscura* in different environments. *Genetics* **29**, 270–289.

Dudash, M. R. 1990 Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* **44**, 1129–1139.

Dykhuizen, D. & Hartl, D. L. 1983 Functional effects of PGI allozymes in *Escherichia coli*. *Genetics* **105**, 1–18.

Gillespie, J. H. 1991 *The causes of molecular evolution*. Oxford University Press.

Gillespie, J. H. & Turelli, M. 1989 Genotype–environment interactions and the maintenance of polygenic variation. *Genetics* **121**, 129–138.

Gordon, C. & Sang, J. H. 1941 The relation between nutrition and exhibition of the 'Antennaless' (*Drosophila melanogaster*). *Proc. R. Soc. Lond.* B **130**, 151–184.

Houle, D., Hoffmaster, D. K., Assimacopoulos, S. & Charlesworth, B. 1992 The genomic mutation rate for fitness in *Drosophila*. *Nature, Lond.* **359**, 58–60.

Houle, D., Hughes, K. A., Hoffmaster, D. K., Ihara, J., Assimacopoulos, S., Canada, D. & Charlesworth, B. 1994 The effects of spontaneous mutation on quantitative traits. I. Variance and covariance of life history traits. *Genetics* **138**, 773–785.

Kimura, M. 1983 *The neutral theory of molecular evolution*. Cambridge University Press.

Lewis, H. W. 1954 Studies on a melanoma-producing lethal in *Drosophila*. *J. exp. Zool.* **126**, 235–276.

Lewontin, R. C. 1955 The effects of population density and composition on viability in *Drosophila*. *Evolution* **9**, 27–41.

Lewontin, R. C. & Matsuo, Y. 1963 Interaction of genotypes determining viability in *Drosophila busckii*. *Proc. natn. Acad. Sci. U.S.A.* **49**, 270–278.

Lindsley, D. L. & Zimm, G. 1992 The genome of *Drosophila melanogaster*. San Diego: Academic Press.

Mitton, J. B. & Grant, M. C. 1984 Associations among protein heterozygosity, growth rate, and developmental homeostasis. *A. Rev. Ecol. Syst.* **15**, 479–499.

Moree, R. & King, J. R. 1961 Experimental studies on relative viability in *Drosophila melanogaster*. *Genetics* **46**, 1735–1752.

Mueller, L. D. & Ayala, F. J. 1981 Fitness and density-dependent population growth in *Drosophila melanogaster*. *Genetics* **97**, 667–677.

Mukai, T. 1964 The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. *Genetics* **50**, 1–19.

Mukai, T., Chigusa, S. I., Mettler, L. E. & Crow, J. F. 1972 Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. *Genetics* **72**, 335–355.

Place, A. R. & Powers, D. A. 1984 Kinetic characterization of the lactate dehydrogenase (LDH-B4) allozymes of *Fundulus heteroclitus*. *J. biol. Chem.* **259**, 1309–1315.

Robertson, F. W. 1960 The ecological genetics of growth in *Drosophila*. 1. Body size and development time on different diets. *Genet. Res.* **1**, 288–304.

Sang, J. H. 1950 Population growth in *Drosophila* cultures. *Biol. Rev.* **25**, 188–219.

SAS Institute 1988*a* *SAS procedures guide, release 6.03 edn.* Cary, North Carolina: SAS Institute, Inc.

SAS Institute 1988*b* *SAS/STAT user's guide, release 6.03 edn.* Cary, North Carolina: SAS Institute, Inc.

Suzuki, D. T., Kaufman, T., Falk, C. & University of British Columbia *Drosophila* Research Group 1976 Conditionally-expressed mutations in *Drosophila melanogaster*. pp. 207–263. In *The genetics and biology of* Drosophila, vol. 1a (ed. M. Ashburner & E. Novitski), pp. 207–263. London: Academic Press.

Sved, J. A. 1971 An estimate of heterosis in *Drosophila melanogaster*. *Genet. Res.* **18**, 97–105.

Takano, T., Kusakabe, S. & Mukai, T. 1987 The genetic structure of natural populations of *Drosophila melanogaster*. XX. Comparison of genotype–environment interaction in viability between a northern and a southern population. *Genetics* **117**, 245–254.

Timofeeff-Resovsky, N. V. 1934 Uber die Vitalitat einiger Genmutationen und ihrer Kombinationen bei *Drosophila funebris* und ihre Abhangigkeit vom genotypischen und vom ausseren Milieu. *Z. indukt. Abstamm.-u. Vererbehre* **66**, 319–344.

Wallace, B. 1968 *Topics in population genetics*. New York: W. W. Norton.

Wright, S. & Dobzhansky, T. 1946 Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. *Genetics* **31**, 125–155.

Zouros, E. & Foltz, D. W. 1987 The use of allelic isozyme variation for the study of heterosis. *Curr. Top. Biol. med. Res.* **15**, 1–59.

Received 2 August 1994; accepted 16 August 1994