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The variance in inbreeding depression and the recovery of fitness in bottlenecked populations

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Theoretical analyses of inbreeding suggest that following an increased degree of inbreeding there may be a temporary recovery of fitness, because of selection either within or among inbred lineages. This is possible because selection can act more efficiently to remove deleterious alleles given the greater homozygosity of such populations. If common, recovery of fitness following inbreeding may be important for understanding some evolutionary processes and for management strategies of remnant populations, yet empirical evidence for such recovery in animals is scant. Here we describe the effects of single-pair population bottlenecks on a measure of fitness in *Drosophila melanogaster*. We compared a large number of families from each of 52 inbred lines with many families from the outbred population from which the inbred lineages were derived. Measures were made at the third and the 20th generations after the bottleneck. In both generations there was, on average, substantial inbreeding depression together with a highly significant variance among the inbred lines in the amount of fitness reduction. The average fitness of inbred lines was correlated across generations. Our data provide evidence for the possibility of recovery of fitness at two levels, because (i) the average fitness reduction in the F_{20} generation was significantly less than in the F_3 generation, which implies that selection within lines has occurred, and (ii) the large variance in inbreeding depression among inbred lines implies that selection among them is possible. The high variance in inbreeding depression among replicate lines implies that modes of evolution which require a low level of inbreeding depression can function at least in a fraction of inbred populations within a species and that results from studies with low levels of replication should be treated with caution.

Keywords: bottlenecks; *Drosophila melanogaster*; fitness variance; inbreeding depression

1. INTRODUCTION

One of the oldest observations important for evolution and breeding is that inbred individuals are very likely to have lower fitness than outbred ones (Darwin 1876), a pattern known as inbreeding depression. This reduced fitness is usually thought to be caused by the increase in genetic homozygosity associated with inbreeding, either by the increase in expression of deleterious recessive alleles or by the loss of overdominant allele combinations (Charlesworth & Charlesworth 1987). Whatever its cause, inbreeding depression can be large and is of potential significance in many aspects of evolutionary biology through effects on both the fitness of individuals and the evolutionary trajectories of populations. Inbreeding depression can be important in relation to the maintenance of endangered populations (Soulé 1986), the evolution of mating systems (Charlesworth & Charlesworth 1987) and dispersal (Thornhill 1993; Greenwood 1983), and the interpretation of selection experiments (Falconer & Mackay 1996).

Theoretical analyses of the consequences of inbreeding suggest that an increased degree of inbreeding leads to a drop in mean fitness (due to the fixation of deleterious alleles) and then eventually a decreased frequency of recessive or partially recessive deleterious alleles (Lande & Schemske 1985; Barrett & Charlesworth 1991; Hedrick 1994; Lande 1994; Frankham 1995; Lynch *et al.* 1995). Deleterious alleles can be reduced in frequency either by increased effectiveness of selection within populations (due to more efficient selection with increased homozygosity) or by the biased loss of populations with high inbreeding depression. These 'purging' effects can therefore diminish the fitness reduction expected due to homozygosity for deleterious mutations and have been detected experimentally in a small number of cases (Barrett & Charlesworth 1991; Saccheri *et al.* 1996). If widespread, purging will be important for conservation biology, which often assumes that endangered populations of small size are under threat from inbreeding depression (Frankel & Soulé 1981). It is important to establish the extent to which natural selection can restore inbred populations to high levels of fitness, so that appropriate management strategies of such populations can be devised (Lande 1988, 1995).

The empirical evidence for purging is limited. The best evidence is a study by Barrett & Charlesworth (1991), who assayed the fitness of outcrossed and selfed progeny of artificially inbred populations of *Eichhornia paniculata* and concluded that deleterious recessive alleles

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were the major cause of inbreeding depression. They observed increased fitness relative to the source population in crosses between inbred lines, which could be explained by purging against these deleterious recessive alleles. Such an increase in the F_1 hybrids is not expected if inbreeding depression is caused by overdominant loci. The evidence on purging in animal species is conflicting. Rapid recovery in fitness was recorded in inbred lines of the butterfly, *Bicyclus anynana*, that had initially displayed severe inbreeding depression (Saccheri *et al.* 1996). In contrast, a large-scale study of the consequences of prolonged periods of small population size in *Drosophila melanogaster* failed to find any such increase (Gilligan *et al.* 1997), and a review of captive-bred populations failed to find strong evidence of purging (Ballou 1997).

Although inbreeding depression is known to vary among populations within species (e.g. Thornhill 1993), there is very little data on the variance of inbreeding depression among different inbred lines or the stability of the pattern of inbreeding depression over time. An experiment on *Tribolium* flour beetles (Pray & Goodnight 1995) examined components of fitness among multiple lines founded from a source population. Inbred lines and families within inbred lines were found to vary significantly in the amount of inbreeding depression.

Despite the importance of inbreeding depression in evolutionary biology, there are considerable gaps in our knowledge. It is important to know both the generality of purging within populations and whether there is a large variance in inbreeding depression among inbred lineages. Any such test needs to be conducted on a sufficiently large scale to ensure that estimates of the variance of inbreeding depression per line are accurate; this requires high replication in the number of independent lines and families within those lines (Lynch 1988).

Here we describe a study of the effects of inbreeding on fitness in the fruit fly, *D. melanogaster*, a species for which there is a large literature about the effects of inbreeding (see Lynch & Walsh 1998). We used a biologically realistic degree of inbreeding ($F = 0.25$) in combination with a high level of replication between and within lineages. We describe the fitness consequences of single-pair population bottlenecks used to found 52 inbred lineages. Fitness measures were obtained at the third generation after the bottleneck and at the 20th generation. Each inbred line had been subjected to a single bottleneck event followed by random mating within lines in subsequent generations. This allowed rapid expansion to large population sizes similar to those of the outbred control lines. This design has sufficient statistical power to test whether the fitness effects of inbreeding are variable across populations. The use of a pair of assay generations allowed the estimation of the temporal stability of the pattern of inbreeding depression.

The term 'inbreeding depression' has been used in many ways in the evolutionary literature; here we use the term in its most general way, to mean the proportional reduction in fitness of inbred individuals relative to outbred individuals. Individuals in a bottlenecked population remain inbred relative to randomly chosen individuals in the source population, and we will refer to the difference in fitness between individuals from a bottlenecked line and those from an outbred source population as inbreeding depression.

2. MATERIAL AND METHODS

(a) *Stocks*

The flies used in this experiment came from a stock established in 1972 from a large collection in Dahomey (now Benin), West Africa. This stock has been maintained in large numbers in population cages and exhibits large amounts of genetic variation (see Wilkinson *et al.* 1990; Whitlock & Fowler 1996, 1999). All flies were kept at 25 ± 1 °C, with a fixed illumination cycle of 12 L : 12 D. All handling was performed at room temperature using carbon dioxide or ice anaesthesia. Throughout the experiment, unless stated otherwise, flies were fed in standard food vials (75 mm × 25 mm diameter) with 7 ml of medium.

(b) *Pedigrees*

Full details of the derivation of the experimental populations are given in Fowler & Whitlock (1999). In brief, the experiments reported here were done in six blocks, in pairs two to three months apart. For each block, a large sample of eggs was taken from the Dahomey stock and allowed to mature to adulthood under conditions of low density. The progeny were collected as virgins. Single pairs were chosen at random and mated in standard vials for 24 h, transferred to other vials for three successive 24 h samples, and then discarded. For each pair of blocks, 200 such pairs were established.

Inbred lines were established from the offspring of single fertile pairs. In each block lines were established from a random sample of the successful pairs, for a total of 52 inbred lines. Each line was maintained in four-bottle cultures founded by random samples of 18 males and 18 females taken from the progeny of a single pair. At the same time, random samples of progeny from all fertile pairs were combined to create outbred lines. Within each block, an outbred line was established as 14 bottles of 18 flies per sex per bottle. Inbred and outbred lines were then maintained at large population sizes. Breeding individuals were obtained by mixing emergees between culture bottles within lines, for each subsequent generation until the F_{20}.

(c) *Fitness measurements*

The F_3 generation flies were reared under standard conditions of low larval density. For each inbred line, 12 vials were set up with initial densities of 50 first-instar larvae (see Fowler & Partridge (1986) and Whitlock & Fowler (1996) for methods). From each of the density vials, we collected four virgin females and four virgin males. We made 48 mating pairs per line in a reciprocal mating scheme that combined flies from different vials, to avoid additional inbreeding. For each outbred line we collected virgins from each of 42 standard density vials and established 168 mating pairs. On day 1 after mating, the pairs were placed in standard culture vials for 24 h (± 5 min) and then transferred to another vial for 48 h (± 5 min). These manipulations were labour intensive, so for both the inbred and the outbred populations two-thirds of the pairs measured in this way were four days old (as adults) when mated while the other one-third were six days old when mated.

The culture vials from the 24 h and 48 h lays were then kept until all F_4 progeny had emerged, and the number of these offspring was counted. Thus for each of the pairs there are two point samples that span a three-day period early in adult life and record the product of fecundity, fertility and survivorship, a

Table 1. *The mean fitness and inbreeding depression for the two generations*

generation	mean outbred fitness	mean inbred fitness	mean inbreeding depression (s.e.)	proportion of steriles	
				outbred	inbred
F_3	196.196	140.614	0.284 (0.013)	0.023	0.070
F_{20}	221.795	175.616	0.209 (0.016)	0.019	0.050

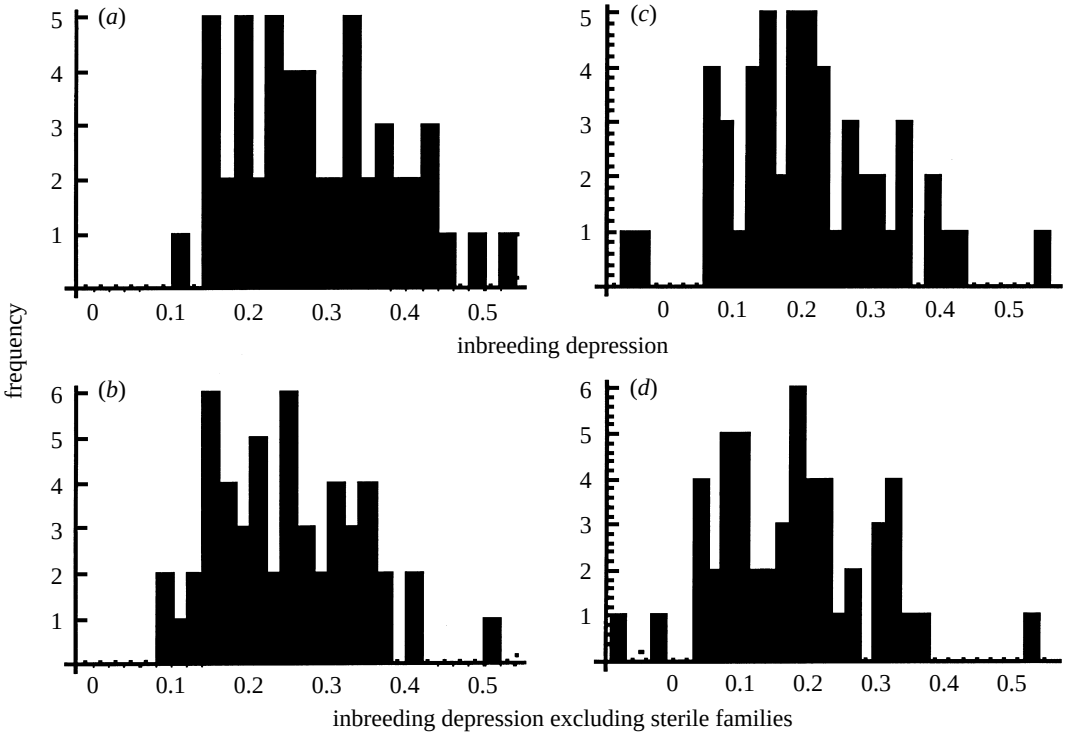

Figure 1. The distributions of inbreeding depression among inbred lines. These data are plotted both including and excluding the sterile families. (*a*,*b*) Distribution among lines in the F_3. (*c*,*d*) Distribution for the F_{20} generation. There is strong inbreeding depression on average, and the fitness declines are extremely variable across lines.

function closely related to fitness (Roper *et al.* 1993). For the analysis below, the two samples are summed to produce one estimate of fitness per family. The inbreeding depression was calculated for each line as

$$\delta_i = (W_o - W_i)/W_o,$$

where W_i is the mean fitness of inbred line i, and W_o is the mean fitness of the contemporaneous outbred control line.

The whole procedure was repeated at the F_{20} generation on a larger scale and yielded approximately 72 mating pairs per inbred line and 216 per outbred line.

3. RESULTS

(a) *Is there inbreeding depression?*

The average values for the fitnesses of the inbred and outbred flies is given in table 1. There is substantial inbreeding depression (*ca.* 28%) in the F_3 generation, as well as in the F_{20} (*ca.* 21%). Figure 1 shows the distribution of inbreeding depression across the lines. In the F_3 generation (figure 1*a*,*b*), no inbred line had a higher mean fitness than its control, which by a sign test has probability $p = 2.2 \times 10^{-16}$ under the null hypothesis of no inbreeding depression. In the F_{20} generation (figure 1*c*,*d*), there are two lines which have apparently higher fitness than their controls, but in neither case is this increase even close to significance (Kruskal–Wallis tests). There is still substantial and significant inbreeding depression in the F_{20} generation (sign test, $p = 3 \times 10^{-13}$), i.e. the mean fitness of the inbred lines is still less than the mean fitness of the outbred control population.

Substantial numbers of pairs did not produce any offspring at all in the three days of the experiment, which we will refer to as sterile families (table 1). The number of sterile families is substantially higher in the inbred lines than in the outbred population, for both the F_3 and F_{20} generations (Kruskal–Wallis test comparing proportions by batch: F_3, $p = 0.0014$; F_{20}, $p = 0.0163$). Excluding the sterile families does not change the rank order of the inbred lines versus their controls; the inbreeding depression in both generations is strongly significant even excluding the effects of outright sterility.

Table 2. *The distribution of variance in inbreeding depression*

(In both generations the fitness estimates varied significantly from one inbred line to another. The six groups of inbred lines were analysed separately (Kruskal–Wallis test), and the results from each block were combined by summation of the χ^2-values and associated degrees of freedom to yield an overall χ^2-test value.)

generation	data set	variance in inbreeding depression	χ^2	d.f.	p
F_3	all families	0.0109	219.4	46	1.9×10^{-24}
F_3	steriles excluded	0.0087	222.74	46	5.0×10^{-25}
F_{20}	all families	0.0133	390.8	46	3.5×10^{-56}
F_{20}	steriles excluded	0.0116	415.9	46	4.7×10^{-61}

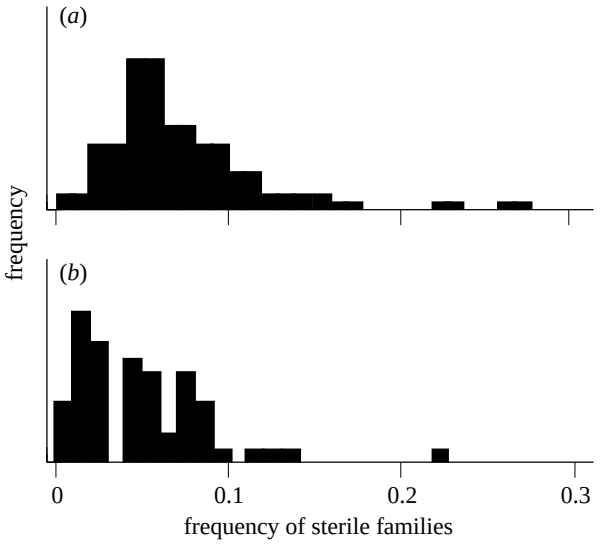

Figure 2. The distribution of the frequency of sterile families in the inbred lines, in (*a*) the F_3 and (*b*) the F_{20} generations.

(b) *The distribution of variance in inbreeding depression*

We tested whether the fitness estimates varied significantly from one inbred line to another. There were no significant effects of the natal vial of the parents. The six groups of inbred lines were analysed separately (Kruskal–Wallis test) and the results from each block were combined by summation of the χ^2-values and degrees of freedom to yield an overall χ^2-test value. The results are given in table 2. In the F_3 generation, the among-line heterogeneity in inbreeding depression was significant both when all families were used ($p = 1.9 \times 10^{-24}$) and when the steriles were excluded ($p = 5 \times 10^{-25}$). The F_{20} generation shows a similar pattern for the entire data ($p = 3.5 \times 10^{-56}$) and the fertile families alone ($p = 4.7 \times 10^{-61}$).

The frequency of sterile families also varies significantly among lines, in both generations (*G*-tests: F_3, $p < 0.001$; F_{20}, $p < 0.0001$). The distributions of the frequencies of sterile families in each generation is given in figure 2. The range of the frequencies of sterile families is quite high, from zero to as high as 28% in one line.

(c) *Does inbreeding depression decline between generations?*

The mean inbreeding depression in generation 20 is less than in generation 3, when measured by any standard. The average inbreeding depression excluding sterile families was 24.8% in the F_3, compared to 18.5% in the F_{20}. The difference was tested by computing the standard errors of the inbreeding depression estimates using the standard errors of the estimates of the fitness of the controls across residuals from the batch effects, the standard error of the mean of the inbred lines. These were combined to find the standard error of the ratio of the difference between inbreds and controls relative to the value of the control (see Lynch & Walsh 1998, Appendix 1). The difference between the two generations is extremely significant ($p = 0.001$). Thus inbreeding depression has dropped in the intervening generations, as measured on a multiplicative scale. Note that the fitness of the outbred controls is higher, on average, in the 20th generation relative to the third generation. This is likely to reflect subtle differences between generations in the environment in which fitness was measured rather than any genuine evolutionary change in the outbred control. Environmental variation through time in factors such as food quality are observed frequently (Falconer & Mackay 1996) and could give rise to a pattern of greater productivity in the 20th generation. If there were environmental differences between the generations, then an alternative explanation for the decrease in difference between inbred and outbred flies could possibly be an increased sensitivity of inbred individuals to stressful environments, a phenomenon which has been observed in some, but by no means all, other studies (Lynch & Walsh 1998).

It can also be seen that the frequencies of sterile families have dropped sharply between the generations. The fraction of sterile families does not vary significantly between the generations for the outbred flies ($p = 0.54$). Thus, we can simply compare the proportion of steriles in the inbreds, and the difference is extremely significant (one-tailed Fisher's exact test, $p = 0.0008$). The average frequency of sterile families within lines declined from 7% to 5% after 17 generations of relaxed inbreeding and selection.

(d) *Are relative rankings of lines similar across generations?*

The inbreeding depression of an inbred line averaged over the fertile families in the F_{20} generation is significantly correlated with its fitness in the earlier generation ($r = 0.34$, $p = 0.014$). The relationship is shown in figure 3. The proportion of sterile families is also positively correlated across generations (Spearman's rank-sum correlation, $r = 0.32$, $p = 0.02$).

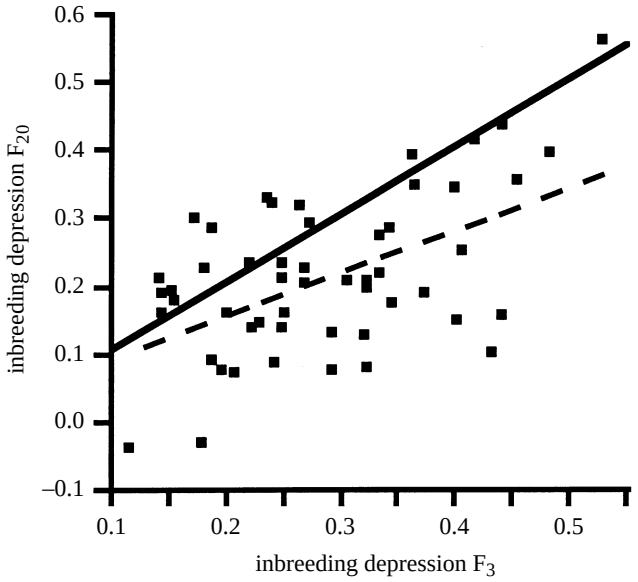

Figure 3. Inbreeding depression of individual lines between generations. These data are plotted including the sterile families. The dotted line plots the least-squares regression; the solid line shows the line of equivalence. There is a strong relationship between the relative decline in fitness in the two generations, but the fitness of the inbred lines has on average increased towards that of the outbred populations over time.

4. DISCUSSION

Our results provide new evidence for recovery of fitness following episodes of inbreeding. Recovery from inbreeding depression may reflect either the action of selection within inbred lines or differential survival among lines. Here we have followed a suite of inbred replicate lines for 20 generations after a bottleneck and found evidence for the possibility of both types of recovery of fitness. The evidence that selection against deleterious alleles has occurred within lines is that the average fitness of inbred lines increased relative to controls through time. The difference in the fitness of inbred and outbred lines was significantly lower in the F_{20} than in the F_3 generation after the bottleneck. Also, we observed a very large variance in inbreeding depression among the inbred lines. Thus selection between lines would be possible. Here this did not lead to the extinction of any lines, but this could occur if populations were exposed to less benign conditions than those of the present experiment.

Both the average fitnesses of the inbred lines and the proportion of sterile families within lines were positively correlated across generations. These correlations indicate that the fitness of a particular inbred line at one time is a good predictor of its fitness at another time, even several generations removed.

An important conclusion to be drawn from the large variance in inbreeding depression among inbred lines is that inferences made from studies with poorly replicated measures of inbreeding depression ought to be treated with caution. If different inbred lines can behave very differently then studies with low levels of replication may be misleading with results obscured by sampling error (Lynch 1988; Schultz & Willis 1995).

The large variance in the level of inbreeding depression suffered by these lines suggests that the conventional approach of describing inbreeding depression in terms of an average is insufficient (see Holsinger 1988). The change in fitness following a population bottleneck is not consistent; instead, replicate lines are extremely variable in the fitness depression that they suffer. In view of this, it is more appropriate to summarize the intensity of inbreeding depression in terms of variance as well as the mean. With variance among lines in inbreeding depression, if inbreeding depression affects the evolutionary future of populations, independent populations will evolve differently. For example, even if inbreeding depression at the level equivalent to the average were enough to prevent the establishment of a new population in a novel environment, some colonization events may be able to succeed, because in some inbred lineages the effect of inbreeding on fitness will be small. Similarly, if we wish to know the probability of establishment of a new mating system, the average level of inbreeding depression may be sufficient to prevent the evolution of inbreeding, but in particular populations or lineages this evolution would in fact be possible.

Finally, these results have implications for conservation biology in terms of the relative significance of inbreeding depression for the management of endangered populations. It appears that, based mainly on species kept in captivity, there is substantial variation among species in inbreeding depression (Frankham 1995). These studies are however unreplicated; in the light of the data presented here, such estimates can be seen to be drawn from a variable distribution. As such we can have little confidence in the accuracy of any particular estimate for a particular species. Decisions about conservation policy ought not to be based on restricted case histories of single species but need to use cross-species-based estimates of critical parameters. Furthermore, the inbreeding depression which seems to plague many species may, with enough time and replication, temporarily be largely purged from some populations.

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