Loss of electrophoretic variation in serially bottlenecked populations

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Experimental bottleneck lines were generated from a single outbred population of the housefly using one, four, or 16 pairs of flies. These lines were followed through one, three, or five successive founder-flush cycles. After each flush 30 individuals from each line were assayed for electrophoretic variation at four initially polymorphic loci. Electrophoretic variation largely followed neutral expectation, the major exception being that the differentiation among 16-pair lines was greater than expected. All bottleneck sizes showed significant losses in genetic variation compared to the control, particularly for losses in numbers of alleles. These findings contrast theoretical predictions in that few lines and loci would be required to detect historical bottlenecks.

INTRODUCTION

When an outbred population passes through a bottleneck genetic variation should decline in direct proportion to the severity of the bottleneck (Chakraborty and Nei, 1977; Maruyama and Fuerst, 1984, 1985; Nei *et al.*, 1975). These predictions suggest that one can use observed losses in heterozygosity and/or number of alleles to infer historical bottlenecks in natural populations. However, because of the large expected sampling variance among loci (Nei and Roychoudhury, 1974; Fuerst and Maruyama, 1986) many loci might have to be sampled to detect differences in level of average variation between populations. Moreover, random linkage associations within populations and differential level of inbreeding among populations will further inflate sampling variances for genetic variation (Avery and Hill, 1977; Cockerham and Weir, 1983 *et al.*, 1980), so any given set of loci and/or lines could easily deviate from expected losses by chance alone (Fuerst and Maruyama, 1986; Lynch, 1988; Nei and Roychoudhury, 1974).

Nevertheless studies have used reduced genetic variation at moderately few electrophoretic loci to infer historical bottlenecks in natural populations (e.g., Bryant, 1984; Bryant *et al.*, 1981; Johnson,

1987; O'Brien *et al.*, 1987; Schwaegerle and Schaal, 1979; Taylor and Gorman, 1975). The practice of using reduced genetic variation to infer putative bottlenecks will likely continue, particularly using electrophoretic loci, so it is important to evaluate the extent the reduction in genetic variation due to bottlenecks can be detected in natural populations. It is unlikely that a full theoretical treatment will be forthcoming, given the complexities of selection, hitchhiking, and linkage, so that an empirical check on the reliability of existing models to predict changes in genetic variation is warranted. However, this requires genetic data on populations of precisely known ancestry, and few such data exist.

In a study examining changes in variation of quantitative traits in experimental populations of the housefly, *Musca domestica* L., that were serially bottlenecked (Bryant *et al.*, 1986*a, b*) we also examined electrophoretic variation at four loci (that were initially polymorphic in the ancestral line) in these same lines. Evidence from both quantitative trait variation and measures of fitness indicates that selection occurred within these lines during the course of the serial founder-flush cycles (Bryant *et al.*, 1986*a, b*; Bryant *et al.*, 1989). In addition because the housefly has only five autosomal pairs of chromosomes considerable linkage disequilibria should be generated as a result of a bottleneck (Avery and Hill, 1977; Franklin and

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Lewontin, 1970). Hence, we might predict little correspondence between observed and theoretical changes in variation in these lines. These data thus allow a test of how well limited electrophoretic data may reflect historical bottlenecks in a case where the effects of linkage and selection should be pronounced. We specifically address two questions. First, can electrophoretic variation be used to detect known bottlenecks in experimental populations? Secondly, how well do losses in variation follow neutral expectation?

MATERIALS AND METHODS

An initial outbred population of the housefly was established in the laboratory by taking a single sample of flies (>100 females) from a refuse site in Alvin, TX. In the third generation in the laboratory, four replicate bottleneck lines were established from this outbred line, for each of three bottleneck sizes, by using offspring from one, four, or 16 pairs of flies. After the populations increased to normal size (about 1000 pairs) samples of thirty adult flies were taken from each line and frozen at -70°C until electrophoresis. This procedure was repeated for a total of three successive founder-flush cycles for each line. Two of these replicate lines per bottleneck size were further subjected to two additional founder-flush episodes, totalling five founder-flush cycles for these lines.

Horizontal electrophoresis was carried out using 12 per cent (w/v) starch gels (Electrostarch, Lot 392) with apparatus and procedures as described in Selander *et al.* (1971), Brewer (1970), and Shaw and Prasad (1970). Four polymorphic enzymes were assayed for each individual fly: two phosphoglucosmutases (PGM-1, PGM-2; EC 2.7.5.1), an esterase (EST; EC 3.1.1.1), and a superoxide dismutase (SOD; EC 1.15.1.1). Progeny tests were used to confirm Mendelian inheritance of electromorphs. The esterase locus had three alleles, one of which was null, and estimates of allele frequencies used Bernstein's correction (Cavalli-Sforza and Bodmer, 1971, p. 55). The first measure of genetic variation was the average heterozygosity computed for each locus as $H = 1 - \sum x(i)^2$, where $x(i)$ is the frequency of the i th allele, and then these individual H values were averaged over the four loci. Assuming rapid rate of return to equilibrium after a bottleneck the expected loss in heterozygosity would be $(1 - 1/2N)$ per bottleneck episode (where N is the

bottleneck number of individuals), so observed values can be compared directly to this expectation. For multiple bottlenecks an average rate of decay of heterozygosity was obtained as the slope of the linear regression of $\log_e [H_t/H_0]$ onto bottleneck episode number with the statistical constraint of the regression to pass through the origin (Bliss, 1967; H_0 and H_t are the respective heterozygosities observed initially and after the t th founder-flush cycle). The residuals about these linear regressions can be used in significance testing but the errors may not be independent (e.g., Felsenstein, 1985; Hill, 1972a, b). A more conservative and unbiased test is to convert these regression slopes back into expected losses in heterozygosity per line after the specified number of bottleneck episodes (i.e., three or five). These single values per line can be used in significance tests comparing observed to expected levels of variation (i.e., $n = 4$ or $n = 2$ lines per bottleneck episode). For the bottleneck lines subjected to five successive bottleneck episodes separate regression estimates were made for the loss rate up to the third bottleneck and then over the five bottlenecks.

A second measure of variation was n , the observed number of alleles per locus. The expected rate of loss in numbers of alleles is conditional on the initial number of alleles as well as their frequencies (Maruyama and Fuerst, 1985), so a general formula relating loss in alleles to bottleneck size is not possible. To determine expected loss in numbers of alleles per locus requires use of a multinomial distribution to enumerate all possible outcomes of sampling (Denniston, 1978; Engels, 1980), so one needs to know the frequencies of alleles after each successive bottleneck episode as a basis of determining expected losses for the next episode. In order to generate these expected losses in number of alleles we adopted a bootstrap approach (Efron, 1982; Efron and Gong, 1983) whereby a sample of $2N$ genes was drawn from an infinite population with the number and frequency of alleles available equal to that in the base population. Once the number and frequency of the alleles in the sampled population were determined, these were used as the basis for the next sampling cycle (bottleneck episode). The process was repeated for 1000 sequences of five successive bottleneck episodes starting with the same initial number of alleles and allele frequencies as in the base population. These were averaged to obtain a single expectation for each bottleneck size over the successive bottleneck episodes that was compared to the observed losses in the same manner as for heterozygosities.

RESULTS

There were 13 initial alleles over the four loci in the control population, and no allele was lost in the control line over the course of the experiment. As a check for possible changes in the frequencies of alleles in response to the laboratory environment *per se* independent of bottleneck effects, we analyzed the control population at the start of the experiment and after each bottleneck episode. Levels of heterozygosity per enzyme and bottleneck episode for the control are shown in fig. 1. No monotonic changes are evident over the course of the experiment for any of the loci, and only

Figure 1 Levels of heterozygosity for the control line at the start of experiment and after each of the five consecutive bottleneck episodes at each polymorphic locus. Horizontal line indicates the average of the six values. Only the variation in heterozygosity for *Sod* was significantly greater than neutral expectation, based on the expected sampling variance of average heterozygosity of Nei and Roychoudhury (1974).

SOD showed significantly greater variance over time ($P < 0.025$) compared with the expected variance in heterozygosity given by Nei and Roychoudhury (1974). Since there was no clear trend in heterozygosity change over time even for this locus, we utilized averages over the six tests (one initial and five bottleneck episodes) as initial heterozygosities at each locus in evaluating bottleneck effects.

The proportional losses in heterozygosity for the bottleneck lines in relation to the control and to neutral expectation are given in table 1 for the three bottleneck sizes after one, three, or five successive bottleneck episodes. Significant losses in heterozygosity were detected for the single-pair lines after the first, third, and fifth bottleneck cycles (t_a in table 1). For the larger bottleneck sizes, the only significant losses were observed for the four-pair lines after the first bottleneck. No significant deviations from neutral expectation were detected for any bottleneck size or founder-flush cycle (t_b in table 1).

Interestingly, after the fifth bottleneck the level of heterozygosity for the four-pair lines was as high as that for the control, despite being significantly lower in heterozygosity after the first bottleneck (table 1). This was not a consequence of loss of replicate lines: In examining the two replicate lines that were assayed after the fifth bottleneck, there was still significantly lower heterozygosity after the first bottleneck in these lines. Since there was a significant loss in numbers of alleles in these lines (see below), there was an apparent reshuffling of frequencies of remaining alleles in these lines that recovered heterozygosity to the level of the control.

Proportional losses in numbers of alleles in relation to the control and to neutral expectation are shown in table 2, for the three bottleneck sizes. Significant losses in numbers of alleles were obtained for all bottleneck sizes, after one, three, or five bottleneck episodes (t_a in table 2). For the single-pair or four-pair lines there was no significant departure of observed losses from neutral expectation, whereas for the 16-pair lines there was a significantly greater loss in number of alleles over expectation after both the first and third bottleneck episodes (t_b in table 2).

As genetic variation erodes within bottleneck populations it should be transformed into genetic variation among populations in proportion to bottleneck size (Wright, 1951; Falconer, 1981). Specifically, the variance among bottleneck lines of size $2N$ would be $2FS^2 = S^2/N$ where S^2 is the initial [additive] genetic variation at a particular

locus within the base population, provided the lines increase in size rapidly after each bottleneck (e.g., Lynch, 1988). When sample sizes are large $[n(n-1)S_B^2]/S^2$ would be distributed approximately as chi square with $n-1$ degrees of freedom, where S_B^2 is the genetic variance among lines of equal bottleneck size (e.g., Lande, 1977). The chi square values for the four loci and three bottleneck sizes are given in table 3. Only the chi squares for

the 16-pair lines were significantly different from expectation for any locus. Hence, genetic differentiation among lines was consistent with neutral expectation for the single-pair and four-pair lines but not for the 16-pair lines, in which significantly greater differentiation among lines occurred than expected by random drift.

To determine if any loci were linked, progeny from 38 single-pair matings were obtained, and

Table 1 Proportion of heterozygosity for bottleneck lines in relation to control after one, three, or five bottleneck episodes for the three bottleneck sizes of one, four, or 16 pairs of flies

Bottleneck episode		Bottleneck size (No. of pairs)		
		1	4	16
1 ($n=4$)	Observed proportion	0.681	0.910	0.986
	Expected proportion	0.750	0.938	0.984
	t_a	4.99*	3.46*	1.53 ns
	t_b	0.64 ns	0.59 ns	0.12 ns
3 ($n=4$)	Observed proportion	0.386	0.891	0.934
	Expected proportion	0.422	0.824	0.954
	t_a	7.89**	3.11 ns	3.09 ns
	t_b	0.32 ns	0.90 ns	0.51 ns
5 ($n=2$)	Observed proportion	0.284	1.006	0.921
	Expected proportion	0.237	0.724	0.924
	t_a	15.21*	0.00 ns	4.22 ns
	t_b	0.81 ns	8.33 ns	0.02 ns

t_a = student t value of observed vs. control; t_b = student t value of observed vs. expected proportion based on neutral expectation. All tests based on arcsin transformed proportions. * = $P < 0.05$. ** = $p < 0.01$. ns = nonsignificant at $P < 0.05$.

Table 2 Proportion of alleles for bottleneck lines in relation to control after one, three, or five bottleneck episodes for the three bottleneck sizes of one, four, or 16 pairs of flies

Bottleneck episode		Bottleneck size (No. of pairs)		
		1	4	16
1 ($n=4$)	Observed proportion	0.595	0.846	0.885
	Expected proportion	0.592	0.854	0.984
	t_a	11.74**	8.81**	9.71**
	t_b	0.30 ns	0.11 ns	6.18**
3 ($n=4$)	Observed proportion	0.438	0.731	0.804
	Expected proportion	0.390	0.704	0.951
	t_a	37.81**	10.45**	7.62**
	t_b	2.19 ns	0.58 ns	3.98*
5 ($n=2$)	Observed proportion	0.391	0.756	0.801
	Expected proportion	0.308	0.616	0.896
	t_a	43.71*	25.24*	22.41*
	t_b	4.27 ns	7.40 ns	6.41 ns

t_a = student t value of observed vs. control; t_b = student t value of observed vs. expected proportion based on neutral expectation. All tests based on arcsin transformed proportions. * = $P < 0.05$. ** = $P < 0.01$. *** = $P < 0.001$. ns = nonsignificant at $P > 0.05$.

Table 3 Chi square values for significance of divergence among replicated lines within a bottleneck size after the first bottleneck, in relation to genetic variance within the base populations

Bottleneck size	Electrophoretic locus			
	<i>Pgm-1</i>	<i>Pgm-2</i>	<i>Est</i>	<i>Sod</i>
1	0.87	6.66	4.80	2.04
4	1.86	6.06	3.57	2.07
16	1.89	13.89***	29.55***	19.77***

Chi-square tests with 3 df.

*** = $P < 0.005$; all other values non-significant at $P > 0.05$.

the electrophoretic genotypes were compared between parents and offspring. Departures from expectation assuming independence were tested by chi square, and the only linkage indicated was between *Pgm-1* and *Sod* ($P < 0.004$). Based upon these small probabilities of independence the two loci appeared to be tightly linked and changes in levels of variation for these two loci would not be independent. In the bottleneck lines there was an apparent linkage between a common allele for *Pgm-1* and a rare allele for *Sod* and they both increased in frequency during the course of the experiment. As a result the losses in variation at the *Pgm-1* locus were mirrored by increases in variation at the *Sod* locus.

DISCUSSION

Because the alleles most at risk during a bottleneck episode are those which are initially in low frequency and contribute little to average heterozygosity, the major consequence of a bottleneck is loss in alleles and not in heterozygosity (e.g., Malécot, 1964; Lewontin, 1965). The prediction then is that the losses in numbers of alleles would be the most sensitive indicator of a bottleneck, and our data support this. In the comparisons of losses of variation against the control in tables 1 and 2, there were more cases of significant deviations as well as greater levels of significance attained for losses in numbers of alleles than in losses in heterozygosity.

One possible bias in comparisons of observed losses with those expected under the neutral theory was that the expected losses were assumed to be a direct result of the bottleneck with no additional losses in the period of recovery immediately following the bottleneck. This assumption would result in underestimates of the expected losses; however, the only significant deviations from

expectation occurred in the 16-pair lines (table 2). Since the rebound for the 16-pair lines after each bottleneck was extremely rapid and considerably faster than for the single-pair or four-pair lines, these additional losses are unlikely the result of slow population growth after each bottleneck.

For these same lines after the first bottleneck episode we previously found an increase in additive genetic variation within and among lines for eight morphometric traits (Bryant *et al.*, 1986a, b). Since many of these traits were closely associated with fitness by virtue of their correlation to general body size and fecundity they might be expected to show nonadditive components of genetic variation, particularly directional dominance (Bryant *et al.*, 1986a). The increases in additive genetic variance we observed as a result of the bottleneck treatment were consistent with possible nonadditive components of genetic variance affecting our traits (Goodnight, 1987, 1988). Moreover, over the course of the five bottleneck episodes the inbreeding depression observed after the initial bottleneck (e.g., fig. 3, Bryant *et al.*, 1986) was largely ameliorated in that only one single-pair line among all the lines remained low in fitness by the fifth bottleneck episode (Bryant *et al.*, 1989). In contrast, loss of electrophoretic variation over the course of the experiment seemed to follow neutral expectation for both loss of genetic variance within lines and increase in genetic variance among lines, the major exception being the 16-pair lines.

One is compelled to ask why such differences in responses to bottlenecks could occur among suites of traits within lines. Our populations were allowed to flush after each bottleneck and before sampling for electrophoresis, so that random linkage effects generated by the bottlenecks would be greatly dissipated by the time we sampled our lines (e.g., Lynch, 1988). The consistency of our results to neutral expectation seems to confirm that sufficient independence of these loci from loci affected by other processes, particularly selection, has been achieved. Despite probable selection acting on some parts of the genome (i.e., quantitative traits) electrophoresis was still effective in detecting losses in genetic variation during bottlenecks as predicted by neutral theory.

In an experiment similar to ours, Sing *et al.* (1973) followed genetic variation at seven electrophoretic loci for 21 generations in lines of *Drosophila melanogaster* kept at small size by starting each generation with five randomly chosen inseminated females. Four of their seven electrophoretic loci deviated from neutral expectation, and as well, we observed that half of our loci

deviated from neutrality (*Pgm-1*, *Sod*). Despite these deviations, however, the decline in average heterozygosity over all loci in both cases closely followed neutral expectation. Hence, measures of average genetic variation may still be useful in detecting the effects of small population size, even though individual loci may deviate from expectation.

Our significance tests for loss in average genetic variability of bottleneck lines from the ancestral control were based on the observed variance in these losses among our replicate bottleneck lines. The most common question one wants to ask is whether or not a *single* population could have experienced a bottleneck during its establishment from a putative ancestral population. The variance of average heterozygosity is expected to be inversely proportional to the number of loci (for a given number of individuals; Nei and Roychoudhury, 1974). Our observed variance among replicate lines of [arcsin-transformed] proportion heterozygosity based on 30 individuals, S_H^2 , can then be used to estimate the number of polymorphic loci necessary to detect a bottleneck of a given size in a single derived population (e.g., Sokal and Rohlf, 1977). using $4S_H^2$ as the presumed variance among lines based on a single locus, and an expected decline of heterozygosity of $(1 - 1/2N)$ for a bottleneck size of N individuals, the number of polymorphic loci necessary to be 80 per cent sure of detecting the bottleneck at the 5 per cent level of significance would be seven, 15, and 46 for bottleneck sizes of one, four, and 16-pair, respectively (assuming electrophoretic samples of 30 individuals). Although no canonical estimates can be similarly generated for number of alleles, since they would depend upon particular allele frequencies, the number of loci needed should be less than that for average heterozygosity. These results suggest that few loci polymorphic in the putative ancestor may be needed to detect bottlenecks when they are severe (i.e., less than ten pairs), even when selection and linkage effects may be pronounced. Electrophoresis thus seems capable of detecting small bottlenecks, which are the ones that would most drastically alter genomic structure and therefore be important to detect.

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