Copyright © 1990 by the Genetics Society of America

Increased Selection Response in Larger Populations. II. Selection for Ethanol Vapor Resistance in *Drosophila melanogaster* at Two Population Sizes

K. E. Weber[1] and L. T. Diggins

Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

Manuscript received July 11, 1989
Accepted for publication April 2, 1990

ABSTRACT

The effect of large population size on selection response was investigated using *Drosophila melanogaster*, with four "small" lines of 160 selected parents/generation compared to two "large" lines of 1600 selected parents/generation. All lines were selected under similar conditions at a selection intensity of approximately 0.55 standard deviations, for 65 generations, for increased ethanol vapor resistance (measured in minutes required to become anesthetized). Two unselected control lines of 320 parents/generation were also maintained. A significant effect of population size was found. The final treatment means and standard errors were: 27.91 ± 1.28 min (two "large" lines); 19.40 ± 1.54 min (four "small" lines); and 4.98 ± 0.35 min (two control lines). To estimate the mutation rate for the trait, two isogenic lines of about 400 selected parents were selected for 29 generations. The mean increase in additive genetic variance per generation was 0.0009 times the initial environmental variance of the outbred lines. This is comparable to other reported mutation rates. Mutation can explain part of the difference in evolved resistance between treatments, but it appears that even at rather large population sizes, a large difference in long-term response can be obtained in larger outbred lines, from more complete utilization of the initial genetic variation.

LARGER populations have two related evolutionary advantages, as explained by FISHER (1930). One advantage of larger size is the greater accumulation of new genetic variation, arising from all types of mutation and from the synthetic aspect of recombination. Another factor is the greater efficiency of selection in sorting existing genetic diversity: as population size increases, the threshold of effective neutrality (FISHER 1930, p. 102) is lowered, so that more genes of smaller effect can be enlisted.

Sorting efficiency alone, as an effect of population size, was first modeled in quantitative genetic terms by ROBERTSON (1960), with some debt to DEMPSTER (1955) and KIMURA (1957). Mutation was omitted in the belief (CLAYTON and ROBERTSON 1955) that it was negligible on the time scales of artificial selection. More recently, FRANKHAM (1980a) and YOO (1980b) have demonstrated that mutation can contribute to response during selection experiments, especially in large populations. Since then mutation has been incorporated into the Robertsonian model by HILL (1982a,b) and FRANKLIN (1982).

In ROBERTSON's (1960) model assuming infinitesimal gene effects, selection of intensity i on a population with size N_e, phenotypic standard deviation S_p, and additive genetic variance S_a^2, produces a maximum eventual response of $2N_eiS_a^2/S_p$. By extension (HILL 1982a,b), if new mutational variance accumulates steadily at S_m^2 per generation, then, after all the original genetic variability is depleted, $2N_eiS_m^2/S_p$ is the maximum eventual response *rate* per generation, for continuous evolution fueled by fresh mutations.

It is not clear whether significant response rates can be sustained by mutation. DARWIN (1859), FISHER (1930), MULLER (1964), KIMURA (1979), and others, have all pointed out in different arguments that larger populations can accumulate higher levels of heritable variability, and should therefore evolve faster than smaller populations when equal selection is applied. However, complete divergence of opinion has existed over the ability of mutation to supply continual new additive genetic variation to populations under selection, at rates sufficient for substantial continuous change. If new additive mutations are rare (WRIGHT 1977a: "exceedingly rare"), then after the initial additive variation is used up, continuing gains in large populations might only come with selection between interaction systems among subpopulations, diversified by drift. According to WRIGHT, DARWIN and others since him have overlooked the indispensable role of between-population selection (and of interactive

[1] Present address: Department of Ecology, Evolution and Behavior, University of Minnesota, Minneapolis, Minnesota 55455.

genes) in evolution and in the improvement of domestic breeds (WRIGHT 1982). However, if additive variation arises continuously, and if it provides the main material of major change (MULLER 1932), then sustainable rates of evolution may actually rise indefinitely with population size (FISHER 1930; MULLER 1964). An intermediate view is also possible (MAYNARD SMITH 1976; HILL 1982a), in which mutation rates suffice to raise eventual response in proportion to population size, but only up to some size range where all potential sites are represented by their full range of potential mutations.

How large a part of the genome can contribute, immediately or potentially, to the additive genetic variation affecting any trait? If much additive genetic variation resides in many genes of individually small effect (FISHER 1930; GREGORY 1965), then the increased efficiency of selection in larger populations could extract significantly larger gains. At the other extreme, if quantitative traits are typically determined by few loci with few alleles, very large size in a panmictic population becomes superfluous, under any model of gene action.

The experimental results presented here include assays of long-term response rates in large, replicated inbred and outbred lines, for the trait of ethanol vapor resistance. The outbred populations were of two sizes—the "small" populations were nearly as large as the largest previously reported long-term selection lines for this type of study (JONES, FRANKHAM and BARKER 1968), and the "large" populations were ten times larger than the "small." The population size of the inbred lines (during selection) was nearly intermediate to these on a log scale. Response in the inbred lines can be used to estimate the rate at which new additive genetic variance is generated for this trait. In the outbred lines, response results partly from new mutation and partly from preexisting variability. As the outbred lines of different size were all founded from the same genetic base and selected by identical procedures, the combined results give some idea of the relative importance of old and new variation, and of population size itself, in a size range where no previous long-term studies exist.

MATERIALS AND METHODS

Experimental apparatus: Selection for ethanol vapor resistance was performed using an apparatus which sorts large samples of flies automatically for resistance to gas-phase agents. The original "inebriometer" and methods of its operation are described in detail in WEBER (1986, 1988). Replicas of this system have been used by COHAN and GRAF (1985), WHITAKER and NASH (1987), and FRANKHAM, YOO and SHELDON (1988). The measurement of ethanol vapor resistance requires only a stream of air diffusing through liquid ethanol, then passing through an anesthetization chamber and out an exhaust. With this arrangement, if the air flow rate and the temperature of the evaporating ethanol are strictly controlled, a constant and uniform vapor concentration can be maintained throughout the anesthetization chamber. Flies placed in the chamber are gradually anesthetized and lose the ability to cling. Eventually they fall through an exit funnel in the bottom of the chamber and into successive test tubes of a fraction collector, where they recover. This gives a bell-shaped distribution with time as a parameter of resistance. The key to the system is the design of the anesthetization chamber, which is a 122 cm × 7 cm vertical glass cylinder, filled with a series of sloping mesh baffles on alternate sides. These interpose a large surface area of excellent footing below the flies, so that they are unlikely either to wander or fall through the exit until fully incapacitated. The precision of fractionation increases with this area.

Selection on outbred lines: The base stock for the present experiments came from wild flies caught at a cider press near Lincoln, Massachusetts, in September 1981. Out of 350 isofemale lines, 10 were chosen for good growth on cornmeal medium. Equal numbers of flies from these 10 lines were combined to set up the lines for the present study, which began in December 1981. Four "small" lines were set up to be continued each generation with 160 selected parents, and two "large" lines to be continued with 1600 selected parents. Two control lines were also maintained with 320 parents/generation. All cultures were grown in half-pint milk bottles with cornmeal medium, set up with 40 flies per bottle. Insertion of half a paper towel into each bottle made cultures highly productive.

Progeny were always allowed to mature and mate at random in the culture bottles before selection. Selection was always of the top 20% of each sample, and sexes were not distinguished during selection nor in setting up the next generation. Since females are a little stronger than males, unequal selection differentials are applied to the sexes in this system (about 30% of females were selected, and 10% of males). Because females mate randomly before selection, selection on males is actually approximately at random, and therefore the true selection differential is one half the selection differential applied to females. The average true selection intensity (i) was thus only about 0.55 standard deviations. Since about 75% of selected flies were premated females, each representing a pair of parents, the number of parents per line is actually 2×0.75 times as large as the number of flies used to set up the cultures, so that in "small" and "large" lines about 240 and 2400 parents per line could be represented genetically each generation. The control lines were set up with unselected flies and therefore with equal numbers of males and females, so that the true founding density was about 20 pairs per bottle for the 8 bottles in each control line.

Selection on inbred lines: The inbred selection lines were derived from the Bowling Green Stock Center strain of the mutant *raised*, which was inbred for 26 generations by crossing brothers to virgin sisters, through a single line of descent. This line was then expanded to give three identical lines. Lines A and B were selected at 25% of all flies, equivalent to approximately 35% of all females. These females mated randomly before selection, so that $i = 0.529$ (FALCONER, 1981). Approximately 400 selected flies were used as parents each generation in lines A and B (respective harmonic means were 425 and 379). Line C was maintained without selection or further inbreeding, with 50 to 400 parents per generation. There were 29 generations of selection. Four additional interspersed unselected generations occurred, but these were near the end of the experiment so their effect on the average mutation rate is ignored.

The scale of ethanol resistance. Resistance time is a

function of vapor concentration, which is controlled by the temperature of the evaporating ethanol. Thus the mean resistance time of a sample depends on the ethanol temperature setting, and with higher or lower means the whole distribution expands or contracts proportionately. The coefficient of variation is constant for replicated samples measured at different ethanol temperatures (WEBER 1986).

The temperature of the evaporating ethanol is controlled by means of an enclosing circulating water bath. In the early generations of selection lower ethanol temperatures were used to elevate and expand the distributions, for increased precision in determining the 20% truncation point. But as the selected lines evolved longer resistance times, it became a practical necessity to raise the ethanol temperature for all lines periodically, so that selection runs could still be kept within the convenient time of 60 min maximum, while control runs were compressed to shorter times. Eventually resistance time in some selected lines was over 500% as long as resistance time in the controls. Meanwhile the ethanol temperatures used to measure resistance time had been increased from 19° to 24°. Tests showed that the ratio between real-time resistance means of any two lines is conserved within the range of temperatures that were used (WEBER 1986). Therefore over the long term, all resistance parameters of selected lines can best be given as ratios to the control lines' mean for the same generation, measured at the same ethanol temperature. The units of this scale are minutes/control minute, so that any parameter of a selection line is convertible to real minutes for some ethanol temperature if multiplied by the mean of controls at that temperature (or by the square of the mean in the case of variances).

Ethanol vapor resistance appears to be a typical quantitative trait under control of multiple loci (WEBER 1986; COHAN, HOFFMANN and GAYLEY 1989). Neither the frequencies (COHAN and GRAF 1985; WEBER 1986) nor the activities (G. CHAMBERS and WEBER, unpublished) of the fast and slow allozymes of alcohol dehydrogenase predict ethanol vapor resistance in this system.

RESULTS

Selection on outbred lines: The evolution of ethanol resistance in all 6 lines is shown in Figure 1, averaged over periods of 10, 15, 15, 15, and 10 generations. Resistance is graphed as the ratio of selected mean to control mean, *i.e.*, in units of minutes/control minute (see MATERIALS AND METHODS). Mean resistance rose from 1 (equal to controls) to over 5 (>500% of controls) in the large lines by the last generation. At the beginning, response rates were indistinguishable, with realized heritabilities of approximately 0.22, as determined by regression of response on cumulative selection differential over the first 9 generations. The response rate declined a little in the larger lines, but declined much more in the smaller lines. As early as generation 20, total response in all lines was already in order of population size. During a period halfway through the experiment, response appeared to lag and then rise again in some lines, but this was due to a temporary environmental change. During this period, malfunctioning of the walk-in culture chamber kept the relative humidity almost at zero, depressing the measured resistance of some lines more than others. After this was corrected all the lines were again clearly differentiated in order of population size, and the magnitude of the differences continued to increase.

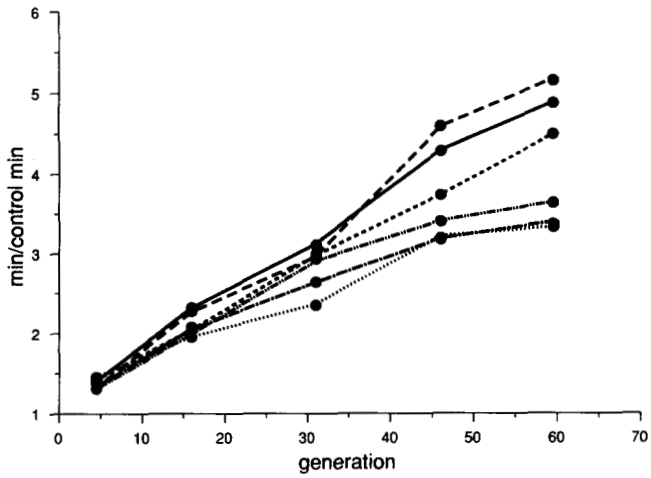

FIGURE 1.—Response to selection for ethanol resistance, averaged over intervals of 10, 15, 15, 15 and 10 generations. Resistance is in minutes/control-minute, *i.e.*, the ratio to control resistance in the same generation. Large populations: L1 (——); L2 (– – – –). Small populations: S1 (–·–·–·–·); S2 (·····); S3 (-----); S4 (–·–·–·–).

TABLE 1

Ethanol vapor resistance

Source	Response to selection d.f.	SS	MS	*F*
Population size	1	96.498	96.498	12.15*
Replicate lines	4	31.778	7.944	

* $P = 0.025$.

The total response of each line was estimated from the mean of the last three generations, measured with ethanol evaporating at 24°. The final treatment means of resistance time, with standard errors, were 27.91 ± 1.28 min for the two large lines, 19.40 ± 1.54 min for the four small lines, and 4.98 ± 0.35 min for the two control lines. The final mean response (selected − control) of the larger lines is therefore 59% greater than the mean response of the smaller lines. A one-way analysis of variance on the total response means of the six selected lines, with treatment as main effect, shows a significant effect of population size (Table 1, $P = 0.025$).

By the end of the experiment, the six selection lines appear to fall into two distinct categories, with a lower group comprising S1, S2 and S4, and an upper group comprising L1, L2 and S3. However, the resistance of flies in line S3 is not actually as high as it appears. In all other selection lines flies tumble out when they become unconscious, and then recover. The flies in line S3 developed a unique unconscious clinging response which prevented many of them from falling

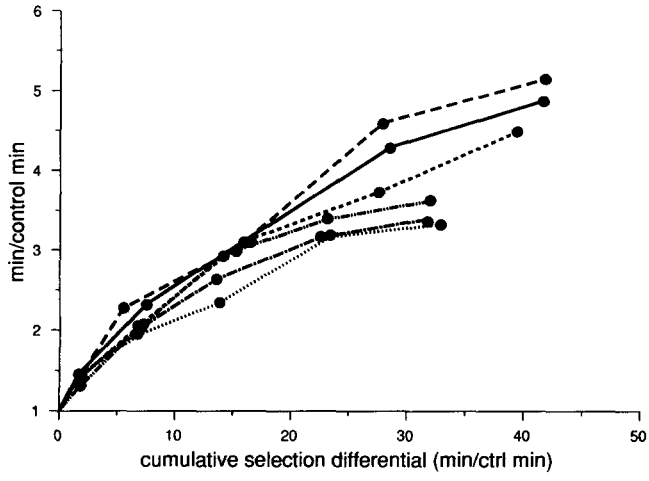

FIGURE 2.—Response to selection as a function of cumulative selection differential. Same symbols as in Figure 1.

until actually dead. The upper end of each sample exhibits a long tail of extremely high scoring individuals which inflate the mean by their suicide, requiring larger samples in order to obtain enough survivors. [A comparable outcome of ethanol vapor selection was observed by COHAN and HOFFMANN (1986 and personal communication) in a pair of lines from one locality.] Thus S3 is not actually in the same resistance category as L1 and L2, or any other line. Since we do not know how to correct the apparent resistance of S3, the scores of this line are taken at face value here, although this reduces the difference between treatment means and the significance level of the analysis of variance (Table 1). Except for the anomaly of line S3, the means of the lines are well grouped by treatment.

The standard deviation in the early generations of all lines was nearly constant (0.578 in units of minutes/control mintue), but began to increase in selection lines as they continued to evolve. Consequently selection differentials also increased proportionately as lines became more resistant. Figure 2 shows the same response data as Figure 1, plotted as a function of cumulative selection differential (again in minutes/control minute). This removes the "scale effect" inflation of response rates, caused by rising selection differentials, and makes the slope of the plot equal to the realized heritability (FALCONER 1981). Realized heritabilities were falling in all the lines, but the effect of this decline was offset more in the more resistant lines, by the increasing selection differentials. The total response of the large lines is 59% higher than the response of the small lines, but the mean cumulative selection differential is only 24% higher. This means that the higher accumulation of realized selection differentials is only a partial explanation for the higher response of larger lines. If the total responses are normalized for scale increase by division by the average standard deviations, the mean responses for large and small lines are 3.71 and 3.01.

Selection on inbred lines: No response was detectable for at least 5 generations in the replicate inbred lines A and B, but after 29 generations of selection a clear difference was measurable. In the final generation, five separately cultured replicate samples of each line were measured with ethanol at 12°. The means and standard errors of each group of means were 11.20 ± 0.13 min for line A, 10.68 ± 0.09 min for line B, and 9.64 ± 0.09 min for line C (the control line). The mean resistance times of selected lines (A and B) were thus 1.162 and 1.108 minutes/control minute, relative to their own inbred, unselected control (inbred line C); for a mean response of 0.135 minutes/control minute.

Previous published calculations of mutation rates from selection experiments on inbred lines have employed the "infinitesimal" model, where it is assumed that mean gene frequencies undergo little change, and that the change in genetic variance from selection itself is small compared to the loss from drift (CLAYTON and ROBERTSON 1955; LYNCH 1988). This loss is taken to be approximately $1/2N_e$ of total additive genetic variance per generation. The same method is applied in the present case. It is assumed that the additive genetic variance of the selection lines was initially at equilibrium between drift loss and mutation for long-established sib-mated lines, *i.e.*, $S_a^2 = 4S_m^2$ (LYNCH and HILL 1986). Some additional genetic variance would be accumulated during the initial expansion of each population, in two generations, from a single pair to about 400 individuals, bringing the amount of genetic variance to an estimated $5.8S_m^2$ before selection began. Additional genetic variance then accumulated during the 29 generations of selection, at the rate of S_m^2 per generation. During this period, the decrease in genetic variance each generation by drift loss of $1/2N_e$, would be an insignificant amount in populations this size. Ignoring drift loss, the cumulative response after t generations of selection is given approximately by:

$$R_t = \left(5.8t + \sum_{n=1}^{t} n\right) i S_m^2 / S_p.$$

The summation term is just $0.5\,(t^2 + t)$. The estimated mean selection intensity (i) was 0.529; the mean standard deviation (S_p) was 0.527 ratio units (mean of selected and unselected lines). Solution for S_m^2 gives 0.00022 unit of variance. (A value of 0.00023 was obtained when drift loss was simulated.) For comparisons between traits the mutational increment is usually divided by the environmental variance of outbred lines (here estimated as 0.248): $S_m^2/S_e^2 = 0.0009$. When this ratio is calculated separately for the two lines, the two values have a standard deviation of 0.00018. This

mean mutation rate is within the lower range of similar determinations for other traits in *D. melanogaster* (LYNCH 1988).

Observations of an increase in the relative liveliness, viability, and productivity of the selected inbred lines, compared to their controls, suggested that mutations alleviating the inbreeding depression of fitness could contribute to the measured ethanol resistance and be selected for. Therefore the measured mutation rate might overestimate the rate at which variance specific for ethanol resistance would be generated in an outbred line.

DISCUSSION

This and the companion paper (WEBER 1990) both report substantial increases in long-term selection gains in populations unusually large for this type of study. Both studies will be discussed here, and compared with other long-term selection experiments in the context of existing theory.

The effect of large *vs.* very large population size: We can only approximately estimate effective population sizes in these experiments. A conventional estimate would be about 60% of the estimated numbers of parents ($N_e/N_p = 0.6$). This is comparable to or below past experimental determinations of N_e/N_p, as listed in CROW and MORTON (1955) and NOZAWA (1970). A mean proportion of $N_e/N_p = 0.60$ was also estimated by COHAN and HOFFMANN (1986), based on the divergence in ADH frequencies, in ten control lines in a culture system resembling ours. In the two sets of large, long-term experiments most comparable to these, N_e/N_p was estimated at 0.60 (YOO 1980a) and 0.70 (JONES, FRANKHAM and BARKER 1968; as estimated in FRANKHAM 1983). This proportion is also predicted for the selected lines by the experimemtal data of NOZAWA (1970) on the effect of founding density on N_e (Figure 3), although the comparison does not take into account productivity, which was probably higher in our cultures.

Another influence on N_e, evident in NOZAWA's data, is the depression of N_e by variance in male mating success when populations are founded with virgin females. The same effect was measured by CROW and MORTON (1955), who found that N_e/N_p was only 0.48 for males when it was 0.71 for females. This effect would depress N_e in typical selection experiments where selected virgins are mass-mated to selected males, but in the present experiments this factor is virtually eliminated, since females mated at random before selection. Because of the high yields of cultures, even more males were available for mating than the numbers that were measured during selection. Under these circumstances the probability that any two families are sired by the same male becomes very low.

Although the precedents discussed above would be consistent with a value of N_e/N_p in the neighborhood of 0.60, there is one piece of direct evidence for a lower value. During the course of the experiments, 43 determinations of the frequency of ADH allozymes were made in different generations of the two ethanol-resistance control lines. The small shifts in frequency between successive determinations allow estimates of inbreeding rate by the method of KRIMBAS and TSAKAS (1971), with a resulting value of $N_e/N_p = 0.22$. This estimate may be imprecise, because on a few occasions founders for the next generation were not drawn from all control bottles (this would reduce N_e), and also because there were some instances of simultaneous fluctuation in ADH frequency between the two control lines, which might imply influences of natural selection (if true this would reduce apparent N_e). Nevertheless, in our judgment this determination is not definitely compromised by these facts nor is it impossibly low, bearing in mind that our cultures were extremely productive, and that the founding density per bottle in terms of premated females was 50% higher in selected cultures than in these controls. In any case the arguments to be presented here do not depend critically on whether N_e/N_p was nearer 20% or 60%, although they are somewhat reinforced at the higher figure. Because there is no other direct empirical evidence in regard to these effective sizes, they will be given here provisionally as 20% of the estimated number of parents (as noted in MATERIALS AND METHODS), *i.e.*, 48 and 480 for ethanol resistance, and 11.2, 56, and 280 for wing height (WEBER 1990), although these may be regarded perhaps as low-end estimates. Even by these cautious estimates, the largest

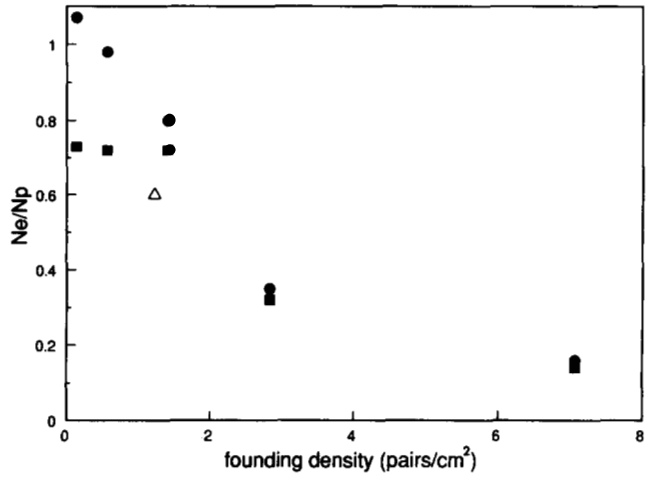

FIGURE 3.—Experimental determinations of N_e/N_p for males (■) and females (●) in cultures of *D. melanogaster* on cornmeal medium. Abscissae are founding densities in pairs/cm² of medium surface area. Calculated from data of NOZAWA (1970). Approximate founding densities of ethanol resistance and wing height lines (WEBER 1990), estimated from average number of (premated) females/half-pint bottle, would be consistent with conventional estimates of $N_e/N_p = 0.6$ (△).

lines are much larger than any previous long-term selection lines.

The largest previous study of the effect of population size on long-term selection response was that of JONES, FRANKHAM and BARKER (1968), whose largest lines (with 80 parents) have been estimated to have an N_e of 56 (FRANKHAM 1983). This is approximately the size we estimate here (at $N_e/N_p = 0.20$) for our "small" ethanol resistance lines and for the "medium" wing height lines in the companion paper (WEBER 1990). These results therefore cover a new size range, and show that larger population size can have a significant benefit, even above the largest sizes previously tested. If we consider only population sizes of about 50 or higher, it is permissible to combine the ten largest lines from both present studies in a significance test since they are independent tests and represent, without exclusion, all known comparisons of long-term response among lines in this new size range. The random probability of the rank order found among the 4 larger wing height lines is one in 6, and the probability of the rank order found among the 6 ethanol resistance lines is one in 15. The probability of the combined results is then one in 90, or 0.011 (one-tailed test). Therefore with significant frequency, populations that were approximately the size of the largest lines in JONES, FRANKHAM and BARKER (1968), were surpassed by even larger populations.

The sources of increased response in very large populations: In larger populations more mutations eventually accumulate, allowing greater response to selection. According to the infinitesimal model of gene effects as applied to finite populations, an equilibrium variance of $2N_eS_m^2$ (*e.g.*, CLAYTON and ROBERTSON 1955; HILL 1982a; HILL and KEIGHTLEY 1988) would be approached, so that eventually the difference in response rates could become very large. But large populations would require so long to approach mutational variance equilibrium that there is no practical information in mathematical expressions for their asymptotic response rates, particularly in the time range of the present experiments. The only immediate prospect for the present populations would be an almost linear (and almost identical) accumulation of new mutational variance arising at the size-independent rate of S_m^2, with a very small loss by drift of $1/2N_e$ per generation. Since we have an estimate of S_m^2 for this trait, the predicted cumulative response from new mutation can be calculated by the following recurrence equations, assuming an initial value of zero for genetic variance:

$$S_{a,t+1}^2 = S_{a,t}^2(1 - 1/2N_e) + S_m^2$$

$$R_{t+1} = R_t + iS_{a,t}^2/S_p.$$

This predicts cumulative responses from mutation alone of 0.47 and 0.39 standard deviations for the large and small ethanol resistance lines. Since the actual normalized total responses in the two treatments were about 3.7 and 3, it appears that although mutation can account for part of the response in both treatments, it can only explain about 11% of the observed difference in response.

Even less of the observed difference in response could be explained by mutation if population sizes were estimated at 60% instead of 20% of parent number. This is because as the estimated population sizes become larger the amount of response attributable to mutation in 65 generations not only increases but also becomes more nearly identical at both population sizes, approaching a limit. It should also be pointed out that the estimate of S_m^2 applied here is not based on small population results but on inbred populations in the same size range as the outbred populations under consideration. Since the role of size in the prediction of mutational contributions is still somewhat untested, this can increase our confidence in these predictions.

The tentative conclusion is that the accumulation of mutational variance for ethanol resistance must have contributed to the elevation of response in the larger lines, but it is insufficient to explain much of the observed difference in as short a time as 65 generations. Therefore, although the smaller populations of $N_e = 48$ would already be considered rather large for long-term experimental selection lines, it seems that the tenfold increase in population size in the larger lines ($N_e = 480$) nevertheless facilitated a significant gain in response rates simply from the recruitment of more and smaller existing allelic differences. Expectations about the amount of useful genetic variance that can be extracted from an outbred gene pool are still based upon a tradition of mostly short-term or small experiments. A comparison of our results with other long-term selection experiments and with theoretical predictions will make this point clearer.

The classical model and the available evidence: Some implications of the infinitesimal model for finite populations were derived by ROBERTSON (1960). If R_1 is the response to selection in the first generation, the Robertsonian limit for response to selection on existing variation is $2N_eR_1$, proportional to population size. This implies a limiting response *rate*, given constant mutation, of $2N_eR^*$ (where R^* is the response from mutation in the first generation), as has been shown by HILL (1982a,b) and FRANKLIN (1982).

ROBERTSON's model is convenient to use here as a limiting case to compare with the experimental data. It applies particularly to small populations, or to large populations under weak selection, but there are no explicit limits on the population size to which the Robertsonian formula can be applied at typical selec-

tion pressures. It is only required that the value of $N_e s$ at individual loci be small, where s is the coefficient of selection. If the number of contributory loci is sufficiently large, this condition may be approached even for moderately large populations (BULMER 1980, FALCONER 1981; HILL 1985), and thus if effects were infinitesimal the limits $2N_eR_1$ and $2N_eR^*_1$ would apply to populations of any size.

These limits are frequently involved in interpretation or prediction of selection results (*e.g.* JAMES 1965; JONES, FRANKHAM and BARKER 1968; YOO 1980a; FALCONER 1981; FRANKHAM 1983; HILL 1982a, 1985; ENFIELD 1986; OLLIVIER 1988). However, identification of limits is rather subjective. It would be more useful to have, instead, a form of the theory that makes specific predictions of response as a function of the number of generations. This can be derived in a few steps.

The critical assumption of ROBERTSON's (1960) model is that during selection, the additive genetic variance decreases each generation by a constant proportion. This proportion represents drift loss and is based on population size. It is hard to formulate ROBERTSON's model of geometric decay mathematically without briefly addressing the unruly fact that in long term selection experiments the additive genetic variance, as measured by traditional techniques, does not in fact regularly decrease during selection. It may indeed decrease to zero (BROWN and BELL 1961; ROBERTS 1966b), but it frequently is not reduced by selection. It may even increase, despite long periods of gradually declining response (*e.g.*, ENFIELD 1980; YOO 1980c), or even while response declines completely to zero (*e.g.*, LERNER and DEMPSTER 1951; TANTAWY, MALLAH and TEWFIK 1964; WILSON *et al.* 1971). Conversely, response may continue after additive genetic variance appears to have been exhausted (SHELDON 1963). Thus, in the context of the standard prediction equation for short-term response ($r = iS_ph^2$), the factor h^2 (the "narrow-sense heritability" defined as S_a^2/S_p^2) becomes nonpredictive in long-term selection, as was already noted some time ago (CLAYTON and ROBERTSON 1957; SHELDON 1963). It is also well known that gains in the total phenotypic variance frequently occur during protracted artificial selection (*e.g.*, CLAYTON and ROBERTSON 1957; WILSON *et al.* 1971; ENFIELD 1980; YOO 1980c), which increasingly inflate the factor iS_p (the apparent selection differential). In the typical long-term selection experiment, then, it is often only the factor i (the selection intensity) which remains constant, reflecting the constant percent selected.

These observations make it desirable to reformulate ROBERTSON's original model of the steady geometric decay of selectability in a way that avoids assuming either that additive genetic variance declines, or that phenotypic variance does not increase. To do this one could simply say that "selectability" (η) declines, if this parameter is only defined as the ratio of observed response (r_t in generation t) to the selection intensity i, based on the percent selected from an assumed normal distribution. Then if z is the factor by which η declines, response in generation t is given by:

$$r_t = i\eta_0 z^{t-1}$$

and cumulative response by

$$R_t = i\eta_0 \sum_{j=0}^{t-1} z^j$$

$$= i\eta_0(1 - z^t)/(1 - z).$$

If at some point mutation produces an immediate increment of η^* in selectability, then it follows that the cumulative response from that increment over the following t generations is:

$$R_t^* = i\eta^*(1 - z^t)/(1 - z)$$

so that if a similar increment is added every generation by continuous mutation, the cumulative response from all mutation is:

$$R_t^* = i\eta^* \sum_{j=1}^{t} (1 - z^j)/(1 - z)$$

which solves as:

$$R_t^* = i\eta^*[t/(1 - z) - (z - z^{t+1})/(1 - z)^2].$$

The combined cumulative response from preexisting and new mutational variation is given by:

$$R_t = i\eta_0(1 - z^t)/(1 - z) + i\eta^* \sum_{j=1}^{t} (1 - z^j)/(1 - z)$$

$$= R_1(1 - z^t)/(1 - z) + R_1^* \sum_{j=1}^{t} (1 - z^j)/(1 - z).$$

As t becomes large, the first term approaches the limit $R_1/(1 - z)$. The second term approaches the simpler form $tR^*_1/(1 - z)$, and increases perpetually. The exact difference between consecutive values of the second term is:

$$R^*_{t+1} - R^*_t = R^*_1(1 - z^{t+1})/(1 - z)$$

so that its continuous rate of increase approaches the limit $R^*_1/(1 - z)$ as t becomes large. If z is equal to the rate of drift loss of genetic variance per generation ($z = 1 - 1/2N_e$), then the limit to the first term converts to $2N_eR_1$ and the limit rate of the second term converts to $2N_eR^*_1$. These are, respectively, the same limits found by ROBERTSON (1960) for the gain from preexisting variation, and by HILL (1982a) for the asymptotic rate of gain. This demonstrates the identity of the present formulation with the original of ROBERTSON (1960).

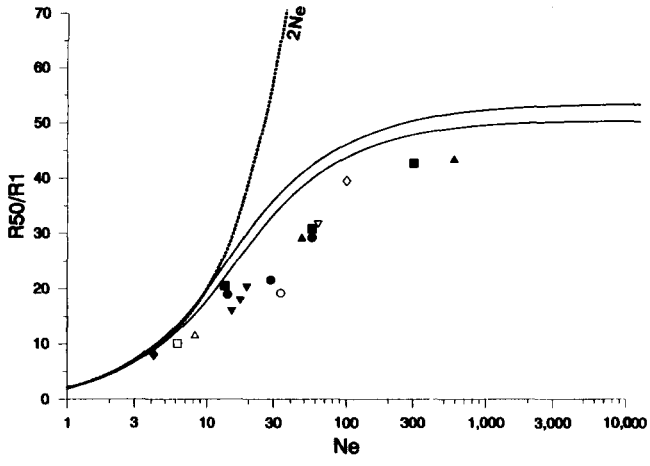

FIGURE 4.—Cumulative response at generation 50, divided by response in the first generation, as a function of effective population size on a log scale. The broken line is the Robertsonian limit for total response with no mutation, equal to $2N_eR_1$. The pair of sigmoid curves represent the Robertsonian predictions for generation 50, with no mutation (lower curve), and with mutation at a rate of $S_m^2/S_g^2 = 0.001$. The data points represent mean values of R_{50}/R_1 from DUDLEY (1977) (○); ENFIELD (1980) (◇); JONES, FRANKHAM and BARKER (1968) (●); MATHER and HARRISON (1949) (△); RASMUSON (1955) (◆); REEVE and ROBERTSON (1953) (□); ROBERTS (1966a,b) (▼); WEBER (1990) (■); YOO (1980a,b) ▽; and this paper (▲).

The point of this rederivation is to obtain a form of ROBERTSON's model which is suitable for calculation of the predicted cumulative response in any given generation. Previously the *limits* which arise from ROBERTSON's approach ($2N_eR_1$ for the approximate "plateau" and $2N_eR\dagger^*$ for the asymptotic response rate) have been compared to experimental results (*e.g.*, YOO 1980a; HILL 1985), but it is certainly more objective to compare real data with finite-time predictions.

Accordingly, some data have been assembled here (Figure 4) representing cumulative response in the 50th generation from published selection experiments over a wide spectrum of population size. (The data represented in Figure 4 are tabulated in the APPENDIX, with methods of analysis.) The set of experiments included here is not exhaustive at the low end of the range where most work has been done, but it represents nearly all work above $N_e = 40$, for experiments of 50 or more generations under constant conditions. Although some of these studies were continued for even longer than 50 generations, one of the most important studies for the effect of population size stopped at that point (JONES, FRANKHAM and BARKER 1968), and another was altered by a major change in regime soon after that point (DUDLEY 1977), so that at longer durations much less information exists. In ROBERTSON's model, heritabilities and selection intensities cancel out of the ratio R_{50}/R_1, permitting these diverse experiments to be compared on a single graph.

The response values suffer from the imprecision of ratios with small denominators, and are subject to the usual interline and intergenerational variability of selection results. The particular choice of scale for each trait also affects the result. But the condensation of many disparate lines into the fewest possible data points yields a coherent pattern, within and even between experiments. Initial rates of response tend to be sustained longer in larger populations, even up to the largest sizes.

In Figure 4 the order of publication is virtually the same as the order of population size. Early long-term selection experiments on small populations explored the phenomena of limits. Later long-term experiments, with larger populations, suggested upward estimates of the limits, the number of loci, and the potential role of mutation (ENFIELD 1986). In Figure 4 most of the data points for $N_e < 20$ represent response plateaus, which were attained even before generation 50. Larger populations were usually still responding at generation 50.

The pair of solid lines in Figure 4 shows the ratio R_{50}/R_1 as a function of population size, as calculated from the model with and without mutation. For this calculation the model can be expressed in the form:

$$R_t = R_1\left[(1 - z^t)/(1 - z) + \mu \sum_{j=1}^{t} (1 - z^j)/(1 - z)\right]$$

where $\mu = \eta^*/\eta_0$ is the spontaneous mutation rate. The mutation rate for quantitative characters has usually been expressed as a ratio to base population environmental variance since LANDE (1975), but was originally expressed as a ratio to base population additive genetic variance (CLAYTON and ROBERTSON 1955), as it is here. In this form the rate would be higher, but of the same order of magnitude. The rate μ was assumed to be 0.001 in these calculations.

This finite-time form of the model permits derivation of another limit, which is the response in a finite time, in an infinite population. If $z = 1 - 1/2N_e$, then as population size becomes very large the previous equation approaches the limit:

$$R_t = R_1 t + \mu R_1 t(t + 1)/2.$$

The two terms on the right are the cumulative responses from preexisting variation (rising as t), and from new mutation (rising as $t^2 + t$), at generation t in an infinite population. Both limits are closely approached on the right-hand margin of the graph in Figure 4. In terms of this model, populations of 10,000 would be effectively infinite in the time span of fifty generations, and populations of 1000 nearly so. In only 50 generations, little of the cumulative response is attributable to mutation under this model, at any population size, although eventually the contribution from mutation would be overwhelming.

The experimental data show response falling short

of the model's predictions, but at 50 generations the infinitesimal model is still not too unrealistic as an extreme upper bound for finite populations. This argues that many loci can contribute to selection response. However, some of the largest populations in Figure 4 owe part of their high responses to scale inflation (*e.g.*, YOO 1980a; ENFIELD 1980; and the present ethanol resistance lines), which may be due partly to multiplicative gene action (YOO 1980b; COMSTOCK and ENFIELD 1981).

The broken line in Figure 4 shows ROBERTSON's final selection limit of $2N_eR_1$, for the total response from preexisting variation. This limit was not expected to apply to very large populations unless selection were very weak, but the sizes at which it does apply, in typical experiments, can only be determined empirically. Figure 4 suggests that as N_e increases, the value of the expression $2N_eR_1$ swiftly exceeds any rational significance as a limit. Populations that have actually been selected to the point of near-exhaustion of response (e.g., REEVE and ROBERTSON 1953; ROBERTS 1966a,b; ENFIELD 1980; YOO 1980) indicate that ROBERTSON's upper limit of $2N_eR_1$ cannot be attained except in very small populations. For populations of 10 it is realistic; for populations of 50 it is not remotely possible.

The half-life of selection response for the infinitesimal model was given by ROBERTSON (1960) as $1.4N_e$ generations. Using the present terms, if g is the half-life then:

$$R_1(1 - z^g)/(1 - z) = (1/2)R_1/(1 - z).$$

This solves as $g = \ln(0.5)/\ln z$, and, using the approximation $1 - 1/2N_e = e^{-1/2Ne}$, it follows for the case where z is a function of drift that $g = 2N_e\ln(0.5)$ or $1.386N_e$. This is the same as the half-life for the decay of neutral variation (FISHER 1930). In the infinitesimal model gene effects are too small for frequences to be affected by selection, and only drift acts.

BULMER (1974, 1976) has demonstrated that selection reduces initial genetic variance by generating linkage disequilibrium. In his model of this effect in "very large" populations (BULMER 1980), the main effect is in the first generation. A fixed equilibrium genetic variance is attained within a few generations and maintained without drift loss. If the Bulmer effect were incorporated into the present model, differences in selection intensity and heritability would no longer cancel out of the response ratio R_{50}/R_1, and the proper estimation of R_1 would not be by linear regression as used here (see APPENDIX). Any influence of this effect would reduce the response ratios, bringing the theoretical predictions somewhat closer to experimental results.

Selection on a finite genome in a finite population: At 50 generations, the predictions of the Robertsonian infinitesimal model for finite populations seem to bear an encouraging resemblance to experimental results, as long as one disregards information not presented in Figure 4 such as the degree to which highly selected lines typically plummet when selection is relaxed. But clearly this model of simple additive genes, of such vanishingly small effect that they do not rise in mean frequency, has limited validity in the interpretation of selection experiments at typical population sizes and selection pressures. Selection response frequently is controlled as much by the opposition of natural selection as by the decline of additive genetic variance, and many experiments show individual alleles of major effect rising in frequency.

Often a large part of the genetic variance of a quantitative trait can be explained by a few major genes (THOMPSON 1975). For example, in a classic study of polygenic inheritance, SPICKETT and THODAY (1966) identified five loci or factors contributing 87.5% of the variance in sternopleural bristle number. However, genetic analysis of this trait was only undertaken in the first place because response to selection had occurred in such a way that a few easily locatable genes of major effect were to be anticipated (THODAY, GIBSON and SPICKETT 1964). Also, since this selection experiment had an N_e of only 8 it could hardly have concentrated the effects of many minor genes in any case. The point is that such data do establish the importance of major genes (see also DAVIES 1971), but cannot be used as evidence against a large potential component of additional response from genes of small effect.

The data presented here can be well explained if it is assumed that for any trait there are likely to be a few common segregating genes of major effect, which can be selected to rise in frequency even in the relatively inefficient context of a small population; but that a large pool of genes of progressively lower effects or frequencies also exists which can only be tapped efficiently by larger populations. For example, selection with a gamma distribution of gene effects and several frequency distributions, in a finite genome in finite populations, has been modeled by HILL and RASBASH (1986), under the assumptions of additivity and no linkage. However, fitness interactions are a large part of the story, and linkage may become important if in fact the potential number of contributory loci is high. Negative effects on fitness may arise due to nonrandom linkages between deleterious alleles and selected alleles in a finite population. Such disadvantageous combinations can be better overcome in a larger population because a more exhaustive set of recombinants is available each generation. Negative pleiotropic effects of selected alleles can also be circumvented in larger populations in several ways. In the first place the greater efficiency of selection in larger populations allows the more consistent utiliza-

tion of alternative genes with smaller pleiotropic effects. Secondly, larger population size allows the persistence of more variety, permitting balancing or compensatory combinations in which pleiotropic effects are ameliorated. Second-order effects of selection, in which the selected allele in turn selects for modifiers in the genetic background which alleviate its pleiotropic effects, will only be possible in large populations with their many recombinants. Such considerations may provide some real insight into the trend of data in Figure 4.

Implications for evolutionary and quantitative genetics: On the scale of evolutionary processes, populations even of a million might be considered small, and a hundred thousand generations short-term, at least by the criterion that phenomena within both of these limits are generally invisible in the fossil record. This is a thousand times the scale of time and size of the present experiments. It is interesting to consider how far the advantages of larger population size indicated here could be extrapolated toward such horizons, where mutation becomes the limiting factor.

If even a part of the large response improvement in larger lines is due to a higher accumulation of new mutation, as seems clearly to be true, this contradicts any view (*e.g.*, WRIGHT 1977b) of large panmictic populations as evolutionary dead ends, and supports instead the more open-ended views on population size and evolutionary potential of, for example, FISHER (1930) and MAYNARD SMITH (1983). It is conceivable that selection between small randomizing subpopulations (WRIGHT 1977b) might have yielded even greater increases in response. Nevertheless, the demonstrated rates of generation of new additive genetic variance, in this trait and in a variety of others (LYNCH 1988), are evidently high enough to challenge the idea that large size *per se* makes a panmictic population incapable of perpetual evolution except at "extremely slow" rates (WRIGHT 1977b, p. 441). Given any appreciable additive genetic mutation rate, larger size in a panmictic population does not limit its evolutionary potential, but instead increases it (FISHER 1930; HILL 1982a).

Very small populations are routinely invoked as the arena for evolutionary creativity. Some experimental evidence (BRYANT, MCCOMMAS and COMBS 1986) suggests that episodes of small population size can increase heritability for some traits, but there is no evidence that this improves the capacity for sustained evolution. Evidence to the contrary is available (FRANKHAM 1980b, and references therein). There is almost no experimental evidence on the long-term evolutionary potential of large populations, although exploration of this area is not quite technically infeasible.

In breeding for agricultural production, selection on massive numbers could often be simply engineered. For example, in Soviet silkworm breeding an apparatus capable of selecting on 40,000 pupae per day for cocoon silk content has been reported (STRUNNIKOV 1983). In selection on oil content in corn, nondestructive biochemical analyses of whole kernels can be accomplished "in seconds" (SILVELA *et al.* 1989) using nuclear magnetic resonance spectroscopy. For large animals such as dairy cattle, advances in data processing and reproductive control can eventually integrate populations over any area; this is already well underway (FRANKLIN 1982; HAMMOND and MCCLINTOCK 1982; SKJERVOLD 1982).

In every feature including scale, the present experiments can be regarded as pilot studies: only two traits are involved, replication is limited, and effective population sizes can only be estimated crudely. Nevertheless, these studies demonstrate large effects of population size on long term response in populations almost an order of magnitude larger than those previously investigated for these effects. No asymptote in the effects of population size on long term response was encountered.

We thank FRED COHAN, FRANK ENFIELD, DICK FRANKHAM, GREG MAYER, DAVID TOWNSEND, MICHAEL TURELLI and two anonymous reviewers for useful comments on both manuscripts. This research was supported by U.S. Public Health Service Fellowship 5-T32-GM07620-07-0131, and National Institutes of Health grants 5-RO1.GM 21,179; KO4.HD 00638; and RO1.GM 40907.

LITERATURE CITED

BROWN, W. P., and A. E. BELL, 1961 Genetic analysis of a "plateaued" population of *Drosophila melanogaster*. Genetics **46:** 407–425.

BRYANT, E. H., S. A. MCCOMMAS and L. M. COMBS, 1986 The effect of an experimental bottleneck upon quantitative genetic variation in the housefly. Genetics **114:** 1191–1211.

BULMER, M. G., 1974 Linkage disequilibrium and genetic variability. Genet. Res. **23:** 281–289.

BULMER, M. G., 1976 The effect of selection on genetic variability: a simulation study. Genet. Res. **28:** 101–117.

BULMER, M. G., 1980 *The Mathematical Theory of Quantitative Genetics.* Clarendon Press, Oxford.

CLAYTON, G. A., and A. ROBERTSON, 1955 Mutation and quantitative variation. Am. Nat. **84:** 151–158.

CLAYTON, G. A., and A. ROBERTSON, 1957 An experimental check on quantitative genetical theory. II. The long-term effects of selection. J. Genet. **55:** 152–170.

COHAN, F. M., and J. GRAF, 1985 Latitudinal cline in *Drosophila melanogaster* for knockdown resistance to ethanol fumes and for rates of response to selection for further resistance. Evolution **39:** 278–293.

COHAN, F. M., and A. A. HOFFMANN, 1986 Genetic divergence under uniform selection. II. Different responses to selection for knockdown resistance to ethanol among *Drosophila melanogaster* populations and their replicate lines. Genetics **114:** 145–163.

COHAN, F. M., A. A. HOFFMANN and T. W. GAYLEY, 1989 A test of the role of epistasis in divergence under uniform selection. Evolution **43:** 766–774.

COMSTOCK, R. E., and F. D. ENFIELD, 1981 Gene number esti-

mation when multiplicative genetic effects are assumed—growth in flour beetles and mice. Theor. Appl. Genet. **59:** 373–379.

CROW, J. F., and N. E. MORTON, 1955 Measurement of gene frequency drift in small populations. Evolution **9:** 202–214.

DARWIN, C., 1859 *The Origin of Species.* Washington Square Press, New York.

DAVIES, R. W., 1971 The genetic relationship of two quantitative characters in *Drosophila melanogaster.* II. Location of the effects. Genetics **69:** 363–375.

DEMPSTER, E. R., 1955 Genetic models in relation to animal breeding problems. Biometrics **11:** 535–536.

DUDLEY, J. W., 1977 76 Generations of selection for oil and protein percentage in maize, pp. 459–473 in *Proceedings of the International Conference on Quantitative Genetics.* edited by E. POLLAK, O., KEMPHORNE and T. B. BAILEY. Iowa State University Press, Ames, Ia.

ENFIELD, F. D., 1980 Long term effects of selection; the limits to response, pp. 69–86 in *Selection Experiments in Laboratory and Domestic Animals,* edited by A. ROBERTSON. Commonwealth Agricultural Bureaux, Slough, U.K.

ENFIELD, F. D., 1986 Quantitative genetic variation from new mutations in *Tribolium,* pp. 144–151 in *Proceedings of the 3rd World Congress of Genetics Applied to Livestock Production.* Lincoln, NE.

FALCONER, D. S., 1981 *Introduction to Quantitative Genetics.* Ed. 2. Longman, New York.

FISHER, R. A., 1930 *The Genetical Theory of Natural Selection.* Clarendon Press, Oxford. 2nd ed. (1958) Dover, New York.

FRANKHAM, R., 1980a Origin of genetic variation in selection lines, pp. 56–68 in *Selection Experiments in Laboratory and Domestic Animals,* edited by A. ROBERTSON. Commonwealth Agricultural Bureaux, Slough, U.K.

FRANKHAM, R., 1980b The founder effect and response to artificial selection in *Drosophila,* in *Selection Experiments in Laboratory and Domestic Animals,* edited by A. ROBERTSON. Commonwealth Agricultural Bureaux, Slough, U.K.

FRANKHAM, R., 1982 Contributions of *Drosophila* research to quantitative genetics and animal bredding, pp. 43–56 in *Proceedings of the 2nd World Congress on Genetics Applied to Livestock Production,* Vol 5.

FRANKHAM, R., 1983 Origin of genetic variation in selection lines, pp. 1–18 in *Proceedings of the 32nd Annual National Breeders' Roundtable.*

FRANKHAM, R., B. H. YOO and G. L. SHELDON, 1988. Reproductive fitness and artificial selection in animal breeding: culling on fitness prevents a decline in reproductive fitness in lines of *Drosophila melanogaster* selected for increased inebriation time. Theor. Appl. Genet. **76:** 909–914.

FRANKLIN, I. R., 1982 Population size and the genetic improvement of animals, pp. 181–196 in *Future Developments in the Genetic Improvement of Animals,* edited by J. S. F. BARKER, K. HAMMOND and A. E. MCCLINTOCK. Academic Press, New York.

GREGORY, W. C., 1965 Mutation frequency, magnitude of change and the probability of improvement in adaptation, in *The Use of Induced Muations in Plant Breeding.* Supplement to Radiat. Bot. **5:** 429–441.

HAMMOND, K., and A. E. MCCLINTOCK, 1982 Implications of developments in engineering and computing for the genetic improvement of animals, pp. 215–221 in *Future Developments in the Genetic Improvement of Animals,* edited by J. S. F. BARKER, K. HAMMOND and A. E. MCCLINTOCK. Academic Press, New York.

HILL, W. G., 1982a Rates of change in quantitative traits from fixation of new mutations. Proc. Natl. Acad. Sci. USA **79:** 142–145.

HILL, W. G., 1982b Predictions of response to artificial selection from new mutations. Genet. Res. **40:** 255–278.

HILL, W. G., 1985 Effects of population size on response to short and long term selection. Z. Tierz. Zuechtungsbiol. **102:** 161–173.

HILL, W. G., and P. D. KEIGHTLEY, 1988 Interrelations of mutation, population size, artificial and natural selection, pp. 57–70 in *Proceedings of the Second International Conference on Quantitative Genetics,* edited by B. S. WEIR, J. EISEN, M. M. GOODMAN and G. NAMKOONG. Sinauer Associates, Sunderland, Mass.

HILL, W. G., and J. RASBASH, 1986 Models of long term artificial selection in finite populations. Genet. Res. **48:** 41–50.

JAMES, J. W., 1965 Response curves in selection experiments. Heredity **20:** 57–63.

JONES, L. S., R. FRANKHAM and J. S. F. BARKER, 1968 The effects of population size and selection intensity in selection for a quantitative character in *Drosophila.* II. Long-term response to selection. Genet. Res. **12:** 249–266.

KIMURA, M., 1957 Some problems of stochastic processes in genetics. Ann. Math. Stat. **28:** 882–901.

KIMURA, M., 1979 Model of effectively neutral mutations in which selective constraint is incorporated. Proc. Natl. Acad. Sci. USA **76:** 3440–3444.

KRIMBAS, C. B., and S. TSAKAS, 1971 The genetics of *Dacus oleae.* V. Changes of esterase polymorphism in a natural population following insecticide control—selection or drift? Evolution **25:** 454–460.

LANDE, R., 1975 The maintenance of genetic variability by mutation in a polygenic character with linked loci. Genet. Res. **26:** 221–235.

LERNER, I. M., and E. R. DEMPSTER, 1951 Attenuation of genetic progress under continued selection in poultry. Heredity **5:** 75–94.

LYNCH, M., 1988 The rate of polygenic mutation. Genet. Res. **51:** 137–148.

LYNCH, M., and W. G. HILL, 1986 Phenotypic evolution by neutral mutation. Evolution **40:** 915–935.

MATHER, K., and B. J. HARRISON, 1949 The manifold effect of selection. Heredity **3:** 1–52 and 131–162.

MAYNARD SMITH, J., 1976 What determines the rate of evolution? Am. Nat. **110:** 331–338.

MAYNARD SMITH, J., 1983 Current controversies in evolutionary biology, pp. 273–286 in *Dimensions of Darwinism,* edited by M. GRENE. Cambridge University Press, Cambridge.

MULLER, H. J., 1932 Some genetic aspects of sex. Am. Nat. **66:** 118–138.

MULLER, H. J., 1964 The relation of recombination to mutational advance. Mutat. Res. **1:** 2–9.

NOZAWA, K., 1970 Estimation of the effective size in *Drosophila* experimental populations. Drosophila Inform. Serv. **45:** 117–118.

OLLIVIER, L., 1988 Current principles and future prospects in selection of farm animals, pp. 438–450 in *Proceedings of the Second International Conference on Quantitative Genetics,* edited by B. S. WEIR, J. EISEN, M. M. GOODMAN and G. NAMKOONG. Sinauer Associates, Sunderland, Mass.

RASMUSON, M., 1955 Selection for bristle numbers in some unrelated strains of *Drosophila melanogaster.* Acta Zool. **36:** 1–49.

REEVE, E. C. R., and F. W. ROBERTSON, 1953 Studies in quantitative inheritance. II. Analysis of a strain of *Drosophila melanogaster* selected for long wings. J. Genet. **51:** 276–316.

ROBERTS, R. C., 1966a The limits to artificial selection for body weight in the mouse. I. The limits attained in earlier experiments. Genet. Res. **8:** 347–360.

ROBERTS, R. C., 1966b The limits to artificial selection for body weight in the mouse. II. The genetic nature of the limits. Genet. Res., **8:** 361–375.

ROBERTSON, A., 1960 A theory of limits in artificial selection. Proc. R. Soc. Lond. B **153:** 234–249.

ROBERTSON, F. W., and E. REEVE, 1952 Studies in quantitative inheritance. I. The effects of selection of wing and thorax length in *Drosophila melanogaster*. J. Genet. **50:** 414–448.

SHELDON, B. L., 1963 Studies in artificial selection of quantitative characters. I. Selection for abdominal bristles in *Drosophila melanogaster*. Aust. J. Biol. Sci. **16:** 490–515.

SILVELA, L., R. RODGERS, A. BARRERA and D. E. ALEXANDER, 1989 Effect of selection intensity and population size on percent oil in maize, *Zea mays* L. Theor. Appl. Gen. **78:** 298–304.

SKJERVOLD, H., 1982 The results of 20 years selection for production in cattle, sheep and pigs—which way now? pp. 3–14 in *Future Developments in the Genetic Improvement of Animals*, edited by J. S. F. BARKER, K. HAMMOND and A. E. MCCLINTOCK. Academic Press, New York.

SPICKETT, S. G., and J. M. THODAY, 1966 Regular responses to selection. 3. Interaction between located polygenes. Genet. Res. **7:** 96–121.

STRUNNIKOV, V. A., 1983 Control of silkworm reproduction, development and sex, in *Advances in Science and Technology in the USSR: Biology Series*. MIR Publishers, Moscow.

TANTAWY, A. O., G. S. MALLAH and H. R. TEWFIK, 1964 Studies on natural populations of *Drosophila*. II. Heritability and response to selection for wing length in *Drosophila melanogaster* and *D. simulans* at different temperatures. Genetics **49:** 935–948

THODAY, J. M., J. B. GIBSON and S. G. SPICKETT, 1964 Regular responses to selection. 2. Recombination and accelerated response. Genet. Res. **5:** 1–19.

THOMPSON, J. N., 1975 Quantitative variation and gene number. Nature **258:** 665–668.

WEBER, K. E., 1986 The effect of population size on response to selection. Ph.D. thesis, Harvard University, Cambridge.

WEBER, K. E., 1988 An apparatus for measurement of resistance to gas-phase agents. Drosophila Inform. Serv. **67:** 90–92.

WEBER, K. E., 1990 Increased selection response in larger populations. I. Selection for wing-tip height in *Drosophila melanogaster* at three population sizes. Genetics **125:** 579–584.

WHITAKER, J., and H. NASH, 1987 Anesthesia mutants of *Drosophila melanogaster* (abstract), p. 113 in *Molecular Neurobiology of Drosophila*, edited by B. GANETZKY and J. HALL. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

WILSON, S. P., H. D. GOODALE, W. H. KYLE and E. F. GODFREY, 1971 Long term selection for body weight in mice. J. Hered. **62:** 228–234.

WRIGHT, S., 1977a Modes of evolutionary change of characters, pp. 679–702 in *Proceedings of the International Conference on Quantitative Genetics*, edited by E. POLLACK, O. KEMPTHORNE and T. B. BAILEY. Iowa State University Press, Ames, IA.

WRIGHT, S., 1977b *Evolution and the Genetics of Populations*. Vol. III. University of Chicago Press, Chicago.

WRIGHT, S., 1982 Character change, speciation, and the higher taxa. Evolution **36:** 427–443.

YOO, B. H., 1980a Long-term selection for a quantitative character in large replicate populations of *Drosophila melanogaster*. I. Response to selection. Genet. Res. **35:** 1–17.

YOO, B. H., 1980b Long-term selection for a quantitative character in large replicate populations of *Drosophila melanogaster*. II. Lethals and visible mutants with large effects. Genet. Res. **35:** 19–31.

YOO, B. H., 1980c Long-term selection for a quantitative character in large replicate populations of *Drosophila melanogaster*. III. The nature of residual genetic variability. Theor. Appl. Genet. **57:** 25–32.

Communicating editor: M. TURELLI

APPENDIX

Each data point in Figure 4 is a mean value of R_{50}/R_1, representing all of the selection lines having the same N_e within one set of experiments, including lines selected in opposite directions or at different intensities. (Intensity cancels out of R_t/R_1 in the present model.) In Table 2 the same data are broken down to the level of individual treatment means. To derive these means, all raw data from experiments by others were first extracted from the graphs of selection response published in the cited papers, by measuring enlarged photocopies of the original graphs on a 50 × 50 cm digitizing pad. Response values for individual generations were estimated as the means of three repeated measurements.

Estimates of R_{50} were then improved by averaging response among adjacent generations before and after generation 50, including generations 45 through 55. If any value in this interval was missing, the value for the symmetric generation before or after generation 50 was also dropped. The study of JONES, FRANKHAM and BARKER (1968) stopped at generation 50 so in this case this method could not be applied. The values of individual generations are not all given in ENFIELD (1980), but values for generations 41, 47, 53 and 59 are given and are symmetric about generation 50 and so were averaged.

Estimates of R_1 represent the slopes of linear regressions of phenotypic mean on generation number, constrained to pass through the base population or R_o value. The number of generations included represents a compromise, since with too few generations, the estimate of R_1 may be distorted by random intergenerational variations; but if too many generations are included R_1 will be underestimated. The underestimation of the initial rate will tend to be more serious in smaller populations. For example, the Robertsonian response half-life for the smallest populations ($N_e = 4$) is only 5.6 generations. Because of these considerations the regression is based on generations 0 to 3 for all lines of $N_e < 10$ and on generations 0 to 6 for all lines of $N_e > 10$, except that for ROBERTS (1966b) the first 8 generations were used and for JONES, FRANKHAM and BARKER (1968) and ENFIELD (1980) only values for generations 0 and 5 were used as plotted in the cited figures. The value of R_1 for REEVE and ROBERTSON (1953) had to be obtained from ROBERTSON and REEVE (1952) as half the slope of the first 3 generations of the plotted divergence between the long-wing and the shorter-term short-wing selection lines, using the mean of both sexes. The value of R_1 for WEBER (1989) is based on the largest lines. One pair of divergent lines (RASMUSON, 1955; Figure 1b) was excluded because with an apparent response of zero over the first 5 generations, it could not be analyzed by these methods.

Wherever lines were replicated, the value of R_{50}/R_1 represents the ratio of the means of replicates within treatments (this includes RASMUSON 1955), but where different treatments (*e.g.*, divergent selection or dif-

TABLE 2

Long-term selection responses

Species and Trait	N_e	i	Replicates	R_{50}	R_1	R_{50}/R_1	Reference
D. melanogaster; sternopleural bristle number	4	+1.80	2	11.64	1.00	11.64	RASMUSON (1955; Figure 1, a and c)
D. melanogaster; sternopleural bristle number	4	−1.80	2	3.70	0.74	5.00	
D. melanogaster; wing length (0.01 mm)	6	+1.51	1	14.65	1.46	10.03	ROBERTSON and REEVE (1952; Figure 4), REEVE and ROBERTSON (1953; Figure 1)
D. melanogaster; abdominal bristle number	8	+1.72	1	17.33	1.50	11.55	MATHER and HARRISON (1949; Figure 1)
M. domesticus; body weight (g)	15	+1.00	1	7.27	0.45	16.16	ROBERTS (1966a; Figure 1)
M. domesticus; body weight (g)	17	+1.00	1	11.05	0.61	18.11	ROBERTS (1966b; Figure 1)
body weight (g)	19	+1.00	1	10.27	0.50	20.54	
D. melanogaster; abdominal bristle number	14	+1.59	4	16.29	0.78	20.88	JONES, FRANKHAM and BARKER (1968; Figure 1)
	14	+1.21	5	11.23	0.72	15.60	
	14	+0.76	4	8.05	0.39	20.64	
	28	+1.58	2	20.25	0.75	27.00	
	28	+1.25	3	14.71	0.82	17.94	
	28	+0.83	3	12.16	0.63	19.30	
	56	+1.68	1	31.69	0.85	37.28	
	56	+1.23	2	18.81	0.73	25.77	
	56	+0.85	2	16.39	0.67	24.46	
Zea mays; % protein content	34	+1.40	1	7.10	0.48	14.79	DUDLEY (1977; Figures 1 and 2)
% protein content	34	−1.40	1	7.12	0.33	21.58	
% oil content	34	+1.40	1	8.87	0.31	28.61	
% oil content	34	−1.40	1	3.68	0.27	13.63	
Tribolium castaneum; pupa weight (micrograms)	100	+1.00	2	2304	58	39.72	ENFIELD (1980; Figure 1)
D. melanogaster; abdominal bristle number	60	+1.40	6	18.34	0.57	32.18	YOO (1980a, Figure 1)
D. melanogaster; wing-tip height (mils)	11	+0.60	3	8.44	0.41	20.58	WEBER (1990)
	56	+0.60	2	12.49	0.41	30.46	
	280	+0.60	2	17.53	0.41	42.76	
D. melanogaster; ethanol resistance (min/control min)	48	+0.55	4	2.48	0.085	29.18	Present study
	480	+0.55	2	3.55	0.082	43.29	

ferent intensities of selection) were used at the same population size, it was necessary to use the mean of the ratio R_{50}/R_1 for lines of similar size. In general all these estimates of R_{50}/R_1 can be considered as overestimates since R_1's are underestimated by the regression method.

The estimates of N_e and i are those given in the cited references, except that the value of N_e of 70% of parent number for the study of JONES, FRANKHAM and BARKER (1968) is actually given in a review by FRANKHAM (1983). No estimate of N_e was given in DUDLEY (1977), and these lines are estimated here at 70% ($N_e = 34$) of parent number. In the three studies with extremely small populations (MATHER and HARRISON 1949; RASMUSON 1955; REEVE and ROBERTSON 1953) no estimate was given and these lines are estimated here at 100% of parent number, *i.e.*, at N_e's of 8, 4, and 6, respectively.