

Mutational Variation and Long-term Selection Response*

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I. INTRODUCTION

Artificial selection on quantitative traits is the primary route for genetic improvement of domestic plants and animals. Genetic variation is the fuel for adaptation under artificial or natural selection, and ultimately

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this has its origin in mutation. Until the early 1980s an important contribution to artificial selection response from mutations arising during the course of an artificial selection experiment or breeding programme was largely discounted. This view existed because the new mutational variation appearing in one generation for the archetypal quantitative trait, bristle number in *Drosophila*, was known to be negligible in comparison to standing genetic variation (Clayton and Robertson 1955). Two studies led to a reassessment of this viewpoint. Frankham (1980) appraised the evidence from several selection experiments, and concluded that mutations with large effects, which could not plausibly have been present at the start of artificial selection, often contributed sizable responses late on in selection experiments for bristle number in *Drosophila*. He also reported a replicated selection experiment for high and low abdominal bristle number lasting 50 generations starting from an inbred base population that showed striking selection responses, and evidence for a large contribution from X-linked mutations at the *bobbed* locus. Hill (1982a,b) gave these observations a theoretical basis, and showed how new mutations could make an important contribution to the response sooner than the small amount of mutational variation arising per generation might suggest.

Here, I review the main empirical and theoretical evidence on new mutation as a source of genetic variation in sustaining responses under artificial selection in the laboratory, and by implication in breeding programs for domesticated plants and animals. The major features that differentiate mutational selection response from selection response due to standing genetic variation are discussed. These are a strong dependency on population size, the tendency for response from new mutations to be highly asymmetric, and the erratic and highly variable nature of the response. Also considered is what is known about the genetic basis of response from new mutations, in particular the joint effects of mutations on quantitative traits and fitness. The review is largely restricted to artificial selection experiments in the laboratory (as opposed to natural selection experiments) that have used inbred lines as a base population.

II. STUDYING SPONTANEOUS MUTATIONS AFFECTING QUANTITATIVE TRAITS

A. Experimental

The most straightforward methods for quantifying the contribution of spontaneous mutations to variation in a quantitative trait are to carry out an artificial selection experiment or a mutation accumulation (MA)

experiment using an inbred or isogenic line as the base population. Although an inbred line is not expected to be completely free of genetic variation [for example, the equilibrium genetic variance in a brother-sister mated line is $4V_m$, where V_m is the mean input of mutational variance per generation (Lynch and Hill 1986)], after a dozen or so generations of selection or mutation accumulation, the contribution from new mutations dominates. Specific inferences about new mutations are more difficult to draw from outbred lines due to the background genetic variation, but as discussed below the presence of large-effect mutations contributing to response was inferred in several artificial selection experiments in outbreds (Frankham 1980).

A contribution from new mutations is likely to show up more quickly under artificial selection than under mutation accumulation in inbred sublines, since selection can rapidly bring initially rare alleles with effects in heterozygotes to intermediate frequencies, a state in which they can make large contributions to the genetic variance and response. However, under artificial selection, objective inferences about the amount of variance arising per generation or the nature of the distribution of mutation effects are somewhat more difficult to make than in a MA experiment, because selection response depends on the properties of individual genes.

B. How Much Mutational Variance?

Clayton and Robertson (1955) suggested that V_m could be estimated from the selection response in an initially inbred line in a manner analogous to estimating the realised heritability. In order to facilitate comparisons across traits and species, estimates of V_m are usually scaled by the phenotypic variance for the trait; this is equivalent to scaling by the environmental variance (V_e) for an inbred line, and is expressed as V_m/V_e , a ratio that has become known as the "mutational heritability" (h_m^2). Two surveys (Lynch 1988; Houle et al. 1996) suggest that h_m^2 for multicellular organisms frequently falls in the range 5×10^{-4} to 5×10^{-3} . For the best-studied traits, abdominal and sternopleural bristle number in *Drosophila*, the commonly assumed value for h_m^2 of 10^{-3} is typical, a value suggested as an upper limit by Clayton and Robertson (1955). A positive relationship between the generation time of a species and both h_m^2 and the mutational coefficient of variation ($\sqrt{V_m}/M$, where M is the phenotypic mean of the trait), has been suggested (Lynch et al. 1999), although the relationship is derived from a comparison across many different kinds of traits. A comparison of similar traits in species with widely different generation times suggests that the relationship is

probably quite weak. For example, in laboratory mice estimates of h_m^2 for body weight from response to selection from an inbred base population are 0.0053 (confidence interval 0.0038 – 0.0072) (Keightley 1998), and from the response to selection in a cross between a pair of long-isolated inbred sublimes is 0.0008 (confidence interval 0.0004 – 0.0027) (Caballero et al. 1995), whereas estimates for body volume in the nematode *C. elegans* are 0.0044 (SE 0.0022) and 0.0035 (SE 0.00096), from two independent MA experiments in the N2 strain (Azevedo et al. 2002). Although laboratory mice and *C. elegans* differ in generation time by a factor of about 25, these estimates are surprisingly similar. There seems to be a positive relationship between the generation time of a species and the nucleotide mutation rate (Drake et al. 1998; Keightley and Eyre-Walker 2000), so the difficulty is in explaining why estimates of h_m^2 often tend to be close to 10^{-3} .

III. THEORY ON ARTIFICIAL SELECTION RESPONSE FROM NEW MUTATIONS: WHAT DO WE EXPECT?

Hill's (1982a,b) theoretical analysis showed that a mutational variance input as small as $10^{-3}V_e$ per generation could lead to a substantial contribution to the selection response, but that the dynamics depend heavily on the magnitude of mutational effects. He analysed models of unlinked genes in which new mutations affect a quantitative trait at biallelic loci. Under the infinitesimal model of many unlinked additive mutations, the cumulative response to generation t is given by

$$C_t \approx (2NiV_m/\sigma_p)(t - 2N(1 - e^{-t/2N})), \quad [1]$$

where N is the effective population size, i is the selection intensity, and σ_p is the phenotypic standard deviation of the trait (Hill 1982b). For V_m of $10^{-3}V_e$, unless the period of artificial selection is of very long duration, C_t is expected to be quite small under the infinitesimal model.

The asymptotic rate of response at a balance between the input of mutational variance and its loss through drift is given by

$$R = 2NiV_m/\sigma_p \quad [2]$$

(Hill 1982a,b). This formula also applies if the new mutational variation is due to additive mutations with large effects ($Ns = Nia/\sigma_p \gg 1$, where s and a are the selective advantage and effect, respectively, of the mutation in the homozygote), and if mutations have equal probabilities of

increasing or decreasing the trait. Essentially, for a given V_m , the higher lifetime contribution to the genetic variance from major beneficial mutations almost exactly offsets their smaller number. If mutation effects are asymmetrically distributed about zero, the response upwards is proportional to the fraction of the mutational variance explained by mutations that increase the trait. Predictions for response from selection acting simultaneously on mutational and standing variation under the infinitesimal model have been obtained by Wei et al. (1996).

Under models of mutations with large effects, there is no simple formula for the cumulative selection response to generation t , but predictions have been made by transition matrix iteration for single genes (Hill 1982b; Hill and Rasbash 1986) or by Monte Carlo simulation for linked genes (Keightley and Hill 1983, 1987; Pamilo et al. 1987). In general, the asymptotic response (2) is reached much earlier than under the infinitesimal model because large effect mutations that appear early and become selected can contribute much variance and response during their sweep to fixation. The variance of the selection response under such models is therefore also much higher than under the infinitesimal model, since it depends on the chance appearance and fixation of large effect mutants. Importantly, the response is predicted to be proportional to N , because the total number of mutations appearing in the population is proportional to T , the number of progeny, and fixation probability is proportional to N/T . In random mating populations the contribution to the overall response from fully recessive mutations is expected to be small because they contribute negligible variance while at low frequencies (Hill 1982b). The strategy of partial inbreeding while selecting could therefore more effectively utilise recessive mutations, but this can severely reduce the effective population size, and reduces the response from additive genes as a consequence (Caballero and Santiago 1995). Selection responses from multiple mutations segregating together are reduced due to the "Bulmer effect" (Bulmer 1985) and due to genetic linkage (Hill and Robertson 1966; Birky and Walsh 1988; Barton 1995), although the effect of the latter in artificially selected populations is likely to be small (Keightley and Hill 1987).

Models of natural selection, including stabilizing selection and pleiotropic models of selection have also been studied. Zeng and Hill (1986) modeled the interaction between mutation, directional selection and pure stabilizing selection, which can result in a selection limit, and quantified the genetic variance expected at this limit. A pleiotropic model may be more plausible for the interaction between mutation and selection during artificial selection. Individuals carrying mutations are assumed to be less fit not because of direct effects of natural selection

on the trait, but rather because there is a correlation between effects on the trait and on fitness. An unfavourable correlation of the trait with natural selection leads to a reduced response and genetic variance, the proportional effect on the former being greater than the latter, or a selection limit can be reached if pleiotropic effects dominate (Tanaka 1998). There is also predicted to be a gradual drop in fitness, especially from mutations whose contribution to the net selective value is greater for the trait than for fitness. Detailed predictions depend on the properties of the joint distribution of mutation effects on the trait and fitness (Hill and Keightley 1988).

IV. ARTIFICIAL SELECTION EXPERIMENTS IN INBRED LINES

The earliest reports of artificial selection experiments using inbreds or pure-bred lines did not show evidence of significant selection responses (Johannsen 1909; Lindstrom 1941). However, East (1935) reported heritable variation for various traits in pure-bred lines of *Nicotiana*, and Castle (1905) artificially selected a new mutation for polydactyly in guinea pigs. This experiment was the first clear demonstration of the effectiveness of artificial selection in producing genetic change (Hill 1984b).

Mather and Wigan's (1942) classic experiment is a fore-runner of several more detailed experiments to study the impact of new mutations on artificial selection response that followed half a century later. As their base population, Mather and Wigan used a long-term inbred line of *Drosophila* that had been maintained by brother-sister mating for 78 generations. The experiment was of long duration, a total of 53 generations of selection on abdominal bristle number and 21 on sternopleural bristle number. Selection led to striking responses, particularly in the abdominal lines, for which the final divergence was about 3 bristles, or more than 1 phenotypic standard deviation, although the interpretation is somewhat complicated because lines were subdivided (Fig. 10.1). A feature of the abdominal selection lines was the presence of jumps in the selection response of *c.*5 generations duration. Interesting parallels can be made between Mather and Wigan's discussion of the mechanisms for the jumps with the arguments earlier in the century between "Mendelians" and "Biometricians" over the nature of genetic change under adaptation (see Hill 1984a, pp. 8–23). Mather and Wigan concluded that the jumps originated from mutation, but that their immediate cause was a release of genetic variation brought about by recombination. This required that mutations tend to build up balanced combinations of polygenic factors, so mutations would need to be of

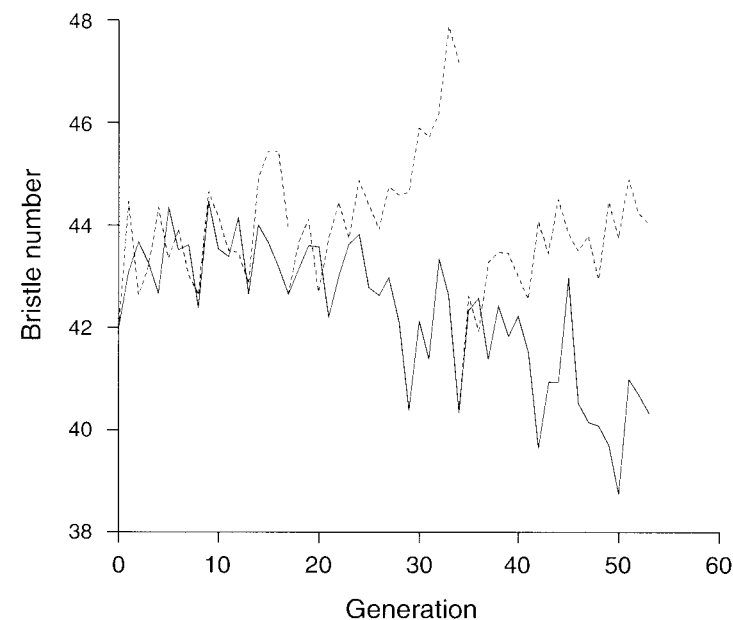


Fig. 10.1. Mather and Wigan's (1942) selection experiment for abdominal bristles starting from an inbred line. Responses in females to downward (solid line) and upward (dotted line) selection on abdominal bristle number are plotted from the data in the paper. The upward selection line was terminated at generation 17, and reversed selection (dotted lines) started from the low line at generations 17 and 34.

small, approximately equal effects. This hypothesis seems implausible, and has not been substantiated in simulations in which mutations appear uniformly along chromosomes of similar map length to *Drosophila* (Keightley and Hill 1983). Selection of large effect mutations is much more likely to lead to jumps in the phenotypic mean, whereas models in which there are many small effect mutations tend to lead to smoother responses.

V. PROPERTIES OF SELECTION RESPONSE IN INBRED LINES AND THE NATURE OF MUTATIONAL VARIATION

A. Magnitude of Response

Table 10.1 shows selection responses, expressed as the mean phenotypic divergence between high and low selection lines in standard deviation units, at 5 time points in several long-running selection experiments that

Table 10.1. Selection responses observed in long-term experiments starting from inbred strains.^a

Trait	Divergence (σ_p) to generation					Reference
	10	20	30	40	50	
<i>D. melanogaster</i> , abdominal bristles, $N = 10$ parents	0.1	0.2	0.5	0.7	—	López & López-Fanjul 1993
<i>D. melanogaster</i> , abdominal bristles, $N = 50$ parents	0.2	0.8	1.3	2.4	—	López & López-Fanjul 1993
<i>D. melanogaster</i> , abdominal bristles	2.0	2.5	3.1	3.4	5.4	Mackay et al. 1994
<i>D. melanogaster</i> , sternopleural bristles	0.7	2.1	2.9	3.4	4.2	Mackay et al. 1994
Mouse, body mass	0.2	0.0	1.7	4.0	3.4	Keightley 1998
<i>C. elegans</i> , body volume	0.3	1.3	2.4	3.3	4.6	Azevedo et al. 2002

^aThe responses are shown as average divergences between high and low line replicates, and are converted to phenotypic standard deviation units. The phenotypic standard deviations of the traits are as follows: López & López-Fanjul (1993): 2.1 bristles; Mackay et al. (1994): 1.7 bristles (abdominals), 1.0 bristles (sternopleurals); Keightley (1998): 1.46 g; Azevedo et al. (2002): 0.19 ($\text{mm}^3 \times 10^3$). The measurements of the latter experiment were taken at generations 12, 24, 36, and 48, and estimates for the generations in the table were made by linear interpolation or extrapolation.

started from inbred base populations. The rates of response are remarkably similar, considering the range of traits and organisms. Response rates in generations 0–20 and 30–50 average at $0.06\sigma_p$ and $0.08\sigma_p$ per generation, respectively. These mutational responses are a good deal lower than responses in the early generations of selection lines derived from outbred populations, but are nonetheless important. For example, the rates of divergence between generations 10 and 20 of selection for 6-week body weight in outbred mice were $0.14\sigma_p$ per generation (Falconer 1973) and $0.23\sigma_p$ per generation (Roberts 1966). For bristle number in *Drosophila*, a typical divergence rate is $0.85\sigma_p$ per generation for sternopleurals (Gurganus et al. 1999). For abdominals, rates of $0.62\sigma_p$ per generation (Clayton and Robertson 1957) and $0.44\sigma_p$ per generation (Long et al. 1995) have been observed.

B. Dependency of Response on Population Size

The asymptotic response to selection from new mutations with additive effects is proportional to N , independent of the magnitude of mutational effects (Hill 1982b, Equation 2). Cumulative responses to time t under

the infinitesimal model are not strongly influenced by population size until $t/2N$ becomes large [equation (1)]. However, if the mutational variance is due to large additive gene effects the asymptotic response can be reached very quickly, so the response in early generations could potentially be proportional to N .

An experimental test of the dependency of the response on N was carried out by Caballero et al. (1991), and continued by López and López-Fanjul (1993a), by selecting divergently on abdominal bristle number in initially isogenic lines of *Drosophila melanogaster* at two different population sizes (5 or 25 pairs of parents selected from 25 or 125 flies, respectively, from each sex). The selection lines were replicated at 16 small lines and 4 large lines in each direction. This experiment, together with Mackay et al.'s (1994) selection experiments with inbred *Drosophila*, is 1–2 orders of magnitude larger in scale than others involving selection in inbreds, so provide unique information. López and López-Fanjul's (1993a) responses to generation 47 seem to have been heavily influenced by the chance appearance of mutations with large effects (López and López-Fanjul 1993b), but the high line–low line divergences are nearly precisely proportional to population size over the course of the experiment (Fig. 10.2). This is a remarkable confirmation of Hill's (1982a,b) prediction of the dependency of the response on N , and also provides indirect evidence for the contribution of mutations with large additive effects to mutational selection response. Note that response from standing variation has also been shown to increase with increasing N , but the effect is not as dramatic (Jones et al. 1968; Weber 1990; Weber and Diggins 1990).

C. Predictability of Response

In selection lines of small effective size, and up to the first 20 generations or so of selection, theory predicts little response from new spontaneous mutations for a wide range of parameter values (Hill 1982b), and this was largely born out in practice (Clayton and Robertson 1955; Kitagawa 1967; Hollingdale and Barker 1971). All these experiments were controls for experiments to assess the impact of X-ray mutagenesis on selection response. However, a lack of response in a particular experiment could be a matter of chance, since the random appearance of rare mutations with large effects can be crucial.

A feature of almost all long-term selection experiments in inbred lines is unpredictable jumps in selection response, first noted by Mather and Wigan (1942; see Fig. 10.1). In later experiments in *Drosophila* these were found to be associated with large rises in the phenotypic variance,

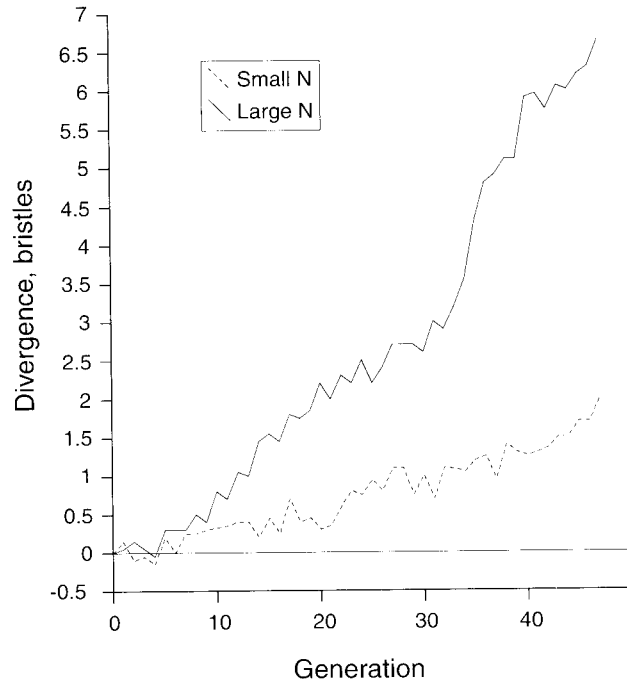


Fig. 10.2. López and López-Fanjul's (1993a) selection experiment over 47 generations for abdominal bristle number starting from an isogenic line. The divergences in mean bristle number in lines of small (5 pairs) and large (25 pairs) numbers of parents are plotted from the original source.

and are highly likely to be due to the selection of new mutations with large effects. In López and López-Fanjul's (1993a) selection experiment, a jump in the response of *c.*2 bristles between generations 32 and 36 is apparent even in the mean divergence of the large selection lines (Fig. 10.2); this is associated with jumps of *c.*4 bristles at about the same time in 2 of the 4 high line replicates. Mackay et al. (1994) [see also Mackay (1995); Mackay and Fry (1996)] describe the longest duration (200 generations) experiment involving selection in an inbred. There were 3 replicates in each direction with 20 parents selected from 80 or 40 scored for either abdominal and sternopleural bristle number. Response was negligible for about the first 10 generations, then spectacular and irregular responses occurred in many replicates. The jumps in response were extremely obvious in the low abdominal and high sternopleural lines, but much smoother and smaller responses occurred in the opposite direction for these traits. Curious reversals in the selection response

occurred, on occasion of up to 5 bristles, and these are difficult to explain. One possibility is that these were associated with the appearance of mutations having large effects on bristles plus negative pleiotropic effects on fitness that were selected to high frequency. A subtle change in the environment could increase the fitness effect (fitness is more sensitive to environmental fluctuations than bristles), leading to a net disadvantage of the mutant and a tendency for it to be reduced in frequency. Bursts of response also occurred in the *Drosophila* abdominal bristle number selection experiments of Frankham (1980) and Merchante et al. (1995), and in a mouse body weight selection experiment (Keightley 1998; Fig. 10.3).

D. Symmetry of Response

Chance asymmetry often arises due to the involvement of rare mutations with large effects, but there are also consistent patterns between traits. Abdominal and sternopleural bristle number of *Drosophila* are by far the best-studied traits. Frankham (1980) and Mackay et al. (1994) observed much larger responses to downwards selection than upwards selection for abdominal bristle number, whereas Mackay et al. (1994) observed the opposite direction of asymmetry for the response to selection on sternopleural bristles. For abdominals, the asymmetrical response is so striking that it is hard to believe that the same trait is under selection in each direction. There is other evidence for asymmetrical distributions of mutational effects for *Drosophila* bristle number. The distribution of effects of *P* element insertional mutations on abdominal and sternopleural bristles are skewed in the same directions as inferred from the selection experiments (Mackay et al. 1992; Lyman et al. 1996), as are the effects of ethylmethane sulphonate (EMS)-induced mutations on abdominal bristles (Keightley and Ohnishi 1998). In contrast, the selection experiments of López and López-Fanjul (1993a) and Merchante et al. (1995), which started with the same isogenic strain, produced somewhat more symmetrical responses to selection on abdominal bristle number, suggesting that there could be strain-specific differences in the symmetry of the distribution of mutational effects. It is difficult to understand why distributions of mutational effects for traits such as bristle number should frequently be strongly asymmetrical, given that responses from standing variation are fairly symmetrical. One possibility is that mutations with large effects are responsible for the asymmetry, but these contribute little to standing variation. In contrast, mutations with small effects could have a symmetrical distribution. Another possibility is that mechanisms other than mutation-selection

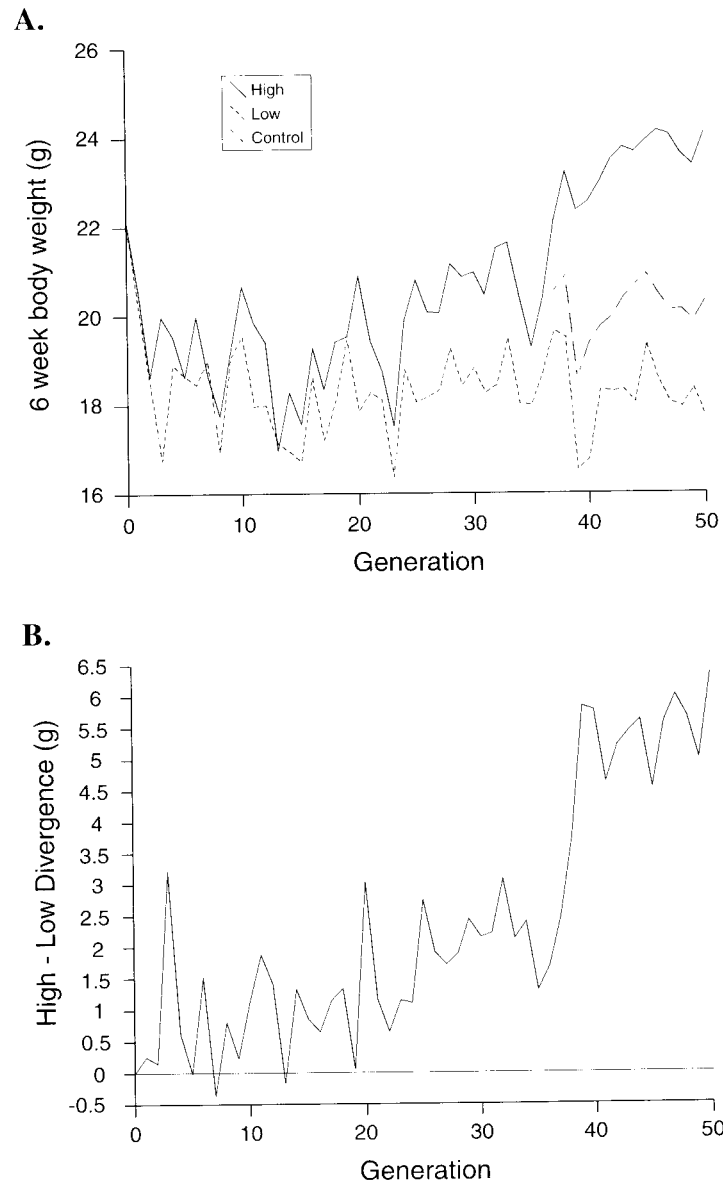


Fig. 10.3. Response (A) and divergence (B) from 50 generations of selection on 6-week body weight in an initially inbred line of mice of the C3H/He strain (from Keightley 1998). An unselected control line, which had been maintained by brother-sister mating by the supplier of the original inbred line, was maintained in parallel with the selection lines from generation 37.

balance lead to the maintenance of standing variation; for example, heterozygote superiority would lead to intermediate allele frequencies and a symmetrical response to artificial selection.

Direct evidence on other morphological traits is sparse. Azevedo et al. (2002) report a strongly asymmetrical response to artificial selection for body volume in *C. elegans*, which implies that only 10–20% of mutations increase the trait (Fig. 10.4). This estimate is consistent with the changes in the distribution of mean body volume between *C. elegans* MA lines and their controls in two independent MA experiments (Azevedo et al. 2002). The distributions of effects of spontaneous mutations affecting wing length and wing width in *Drosophila* (traits strongly correlated with body size) are also skewed downwards (Santiago et al. 1992), as is the distribution of EMS-induced mutations on body size in *Drosophila* (Keightley and Ohnishi 1998). Surprisingly, the mutational response to selection on body weight in mice was higher in the upwards direction than downwards, but this was probably due to the selection of one or two major mutations (Fig. 10.3).

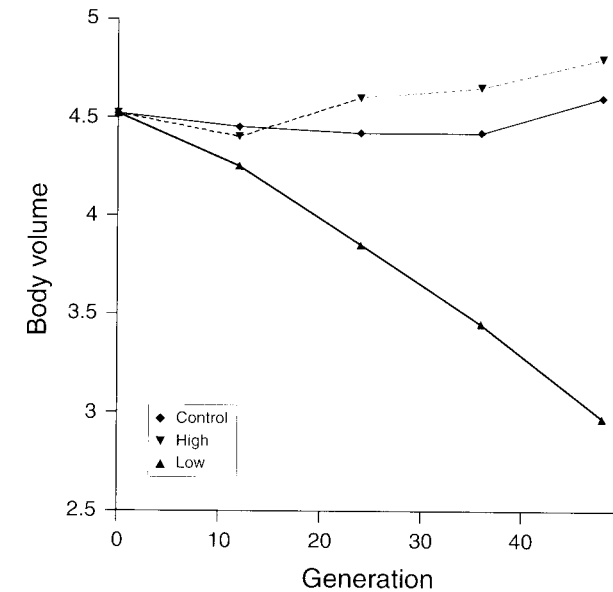


Fig. 10.4. Response to selection on body volume (× 10⁴ mm³) in the N2 strain of *C. elegans*, a naturally inbred strain (from Azevedo et al. 2002). Frozen stocks of worms from the high, low, and control lines of the selection experiment were revived and assayed in parallel at generations 12, 24, 36, and 48.

A large body of evidence supports the idea that the overwhelming majority of mutations affecting life history traits are deleterious, presumably reflecting the past action of natural selection on these traits. This is seen in downwardly skewed distributions of life history trait values in spontaneous MA lines of *Drosophila* (reviewed by Fry 2001), *C. elegans* (Keightley and Caballero 1997; Vassilieva et al. 2000), *Arabidopsis* (Schultz et al. 1999), and wheat (T. Bataillon pers. comm.), the effect of EMS mutagenesis on life history trait means in *Drosophila* (Mukai 1970; Ohnishi 1977; Keightley and Ohnishi 1998; Yang et al. 2001) and in *C. elegans* (Davies et al. 1999), and asymmetrical effects of transposable element mutagenesis on *Drosophila* life history traits (Eanes et al. 1988; Mackay et al. 1992; Lyman et al. 1996). Skewed distributions of mutational effects manifest themselves in the asymmetrical responses to artificial selection on life history traits that are usually observed in outbreeds (Frankham 1990). A recent claim that spontaneous mutational effects for life history traits in *Arabidopsis* have an approximately symmetrical distribution is therefore at odds with these observations (Shaw et al. 2000; Shaw et al. 2002), although the duration of this MA experiment was short and the amount of among-MA line mutational variation small.

Taking all sources of information into consideration, it is safe to infer that mutational responses to selection on morphological traits will usually be asymmetrical, and that responses to selection for life history traits invariably will be strongly asymmetrical. The mutational variation available for selection in a desired direction could therefore be a small proportion of the overall mutational variation for the trait.

E. Genetic Basis of Response

Most information on the nature of selection response from new mutations refers to the net effects of mutations that are present in individual selection line replicates. As noted previously, sudden changes in mean performance suggest that selection response frequently involves mutations with large effects. Mutations with large effects also seem to make substantial contributions to subline divergence under spontaneous mutation accumulation in *Drosophila*, *C. elegans*, and other species (Bataillon 2000). Several kinds of biometrical analysis have been carried out on the long-term *Drosophila* selection lines of Mackay et al. (1994). Analysis of line crosses to estimate the effective number of genetic factors suggests that few mutations (generally less than 5) explain most of the selection responses in most of the replicates (Fry et al. 1995),

although estimates of gene number based on biometrical analysis are minima (Lynch and Walsh 1998). López and López-Fanjul (1993b) and Nuzhdin et al. (1995), working on the long-term selection lines of Mackay et al. (1994), carried out experiments to analyse the net dominance effects of artificially selected mutations in *Drosophila* by intercrossing high and low selection lines or by crossing selection lines to a control line. In both experiments, the net effects of non-lethal mutations were close to additive, on average, but a lack of net dominance does not exclude the possibility that mutations have variable dominance effects. A small number of large effect mutations with approximately additive net effects also explained mutational selection responses for body weight in mice (Keightley 1998).

In long-term *Drosophila* bristle selection experiments from inbred base populations, mutations with effects on bristles in the heterozygote that are lethal in the homozygote have been shown to make an important contribution to the selection response (López and López-Fanjul 1993b; Fry et al. 1995; Merchante et al. 1995). For example, experiments in which balancer chromosomes have been used to assay for lethals in extracted selection line chromosomes have shown that as many as one-third of the selection line replicates contained a lethal mutation at high frequency by generation 47 (López and López-Fanjul 1993b). The segregation of lethal mutations with effects on the selected trait tends to reduce subsequent selection response by inflating the phenotypic variance and reducing the selection intensity. They are frequently present in long-term selection lines (e.g., Yoo 1980), and are thought to be common in populations that have reached a limit under artificial selection (García-Dorado and López-Fanjul 1983; Falconer and Mackay 1996), suggesting a role for new mutations in causing selection limits. Mutant alleles with major beneficial effects on traits, but having strongly deleterious side-effects have been picked up in commercial pig populations (the halothane stress susceptibility gene; Aalhus et al. 1991; Hanset et al. 1995) and in selection lines of mice (the high growth mutation; Bradford and Famula 1984; Cargill et al. 2000). Lethal mutations were not selected to high frequencies in a *Drosophila* bristle selection experiment in which half of the matings were between full sibs, the remainder between random individuals (Merchante et al. 1995). The pattern of selection response also differed markedly between partially full-sib mated lines and the randomly mated lines. The latter were more prone to bursts of selection response and concomitant increases in phenotypic variance, presumably associated with selection of lethal mutations with large heterozygous bristle effects. The cost of full-sib matings was a

lower overall selection response and lower effective population size, as expected from theory (Caballero and Santiago 1995). The fitness effects of non-lethal bearing selection lines have also been studied. López and López-Fanjul (1993b) did not detect net fitness effects for non-lethal lines; in contrast, a decline in competitive fitness of ~50% was detected in the longer-running selection lines of Mackay et al. (1994) (Nuzhdin et al. 1995).

In some cases the specific loci that have led to mutational selection response have been identified. Frankham (1980) and Gillings et al. (1987) reported evidence for the involvement of mutations at the *bobbed* locus of *Drosophila*, a locus consisting of arrays of rRNA genes on the X and Y chromosomes. This locus is hypermutable due to unequal exchange, and causes characteristic female-specific effects on bristle number, attributable to deletions in X-linked tandons, and these have been seen in several long-term selection experiments (e.g., Clayton and Robertson 1957). Frankham (1980) confirmed that the locus was involved by deletion mapping. A sex-limited response in 4 of 6 of the downward selection lines of Mackay et al.'s (1994) experiment is probably attributable to mutations in the Y-linked copy of this locus (Fry et al. 1995; Mackay and Fry 1996). The high mutation rate at *bobbed* is compatible with the frequency at which mutations are found at the locus in long-term bristle selection lines (Frankham 1980).

Mackay and Fry (1996) tested for the involvement of 14 loci, including *bobbed*, in explaining long-term selection response for bristle number in *Drosophila* by using a version of the classical complementation test to probe for interactions between selection lines and candidate genes. In essence, they tested for a difference in the effect of a mutation at a candidate gene between the selection line background and a control line background. In about half of the cases, a significant interaction was found, implying a possible involvement of the candidate gene. The mutation rate at a candidate gene would need to be unusually high for specific mutations to be picked up in several different selection line replicates, but high mutation rates might be plausible for some loci if there are *P* element insertion hot spots (the line was Harwich, a strain containing active *P* elements). Support for the involvement of the same loci in different replicates comes from the lack of additional response to selection in lines derived from crosses between different replicates (Fry et al. 1995). An alternative explanation is the interaction between the candidate genes and other spontaneous mutations in the selection lines.

VI. CONCLUSIONS

More than 20 years ago, an important role for new mutations in contributing to artificial selection response was proposed by Frankham and Hill. We now have a substantial body of data from selection and MA experiments with inbred lines in animal models that confirms the need to consider the role of new mutations in sustaining long-term selection responses. In many ways, properties of the selection response arising from new mutations resemble the major features of long-term response in outbred populations (Falconer and Mackay 1996, chapt. 12), but departures from simple expectation based in the infinitesimal model are exaggerated. Thus, extremely asymmetrical mutational selection responses can consistently occur in a trait such as abdominal bristle number in *Drosophila*, whereas responses in outbreds are close to symmetrical. Responses from new mutations are erratic and unpredictable due to the infrequent random sampling of mutations with large effects, whereas responses from standing genetic variation tend to be fairly smooth. Problems of reduced fitness brought about by artificial selection of new mutations are probably more severe than for selection on standing variation, because much of the variance contributed by new mutations takes the form of lethals or severely detrimental genes with effects on the quantitative trait in the heterozygote. The evidence from studies of the net effects of mutations in selection line replicates suggested the following model (García-Dorado et al. 1999): Much mutational response is due to mutations with large, nearly additive effects, which are quasi-neutral with respect to fitness, or at least do not lead to a substantial fitness decline if fixed. There is also an important class of lethal mutations that have effects on quantitative traits in the heterozygote, selection on which leads to undesirable phenotypic effects and potentially to attenuation of the selection response.

How then should breeders make optimal use of new mutational variation? Two issues to consider are the efficient fixation of beneficial mutations, while avoiding the selection to high frequencies and fixation of detrimental alleles. As emphasised by Hill (1982a,b), large population size is predicted to give the greatest long-term response, since asymptotic response from mutations is proportional to N for additive genes. Modest selection intensity, while leading to smaller selection response in the short term, gives the largest total response from standing variation (Robertson 1960), and increases the opportunity for natural selection to eliminate mutations with undesirable fitness effects. Unfortunately, this

acts against short-term breeding objectives. Lethals with effects on the trait(s) in the heterozygote are perhaps not as serious a problem in domesticated populations, because selection on specific traits is usually not as strong as in selection experiments in *Drosophila* in the laboratory. However, the inclusion of selection on fitness traits in a selection index could mitigate their threat. "Dangerous" mutations that the breeder can do little about are those that have large effects on the target trait(s), but modest deleterious pleiotropic effects on fitness components. Only by reducing the selection intensity to (presumably) unacceptable levels would such mutants be prevented from fixing. The erosion in fitness traits that has been observed in intensively selected poultry species (Emmerson 1997) may well be due to selection of new mutations of this class that have arisen since organised artificial selection programmes were initiated.

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