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SHORT PAPER

Accumulation of mutations affecting body weight in inbred mouse lines

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Summary

The variation from spontaneous mutations for 6-week body weight in the mouse was estimated by selection from a cross of two inbred sublines, C57BL/6 and C57BL/10, separated about 50 years previously from the same inbred line. Selection was practised high and low for 12 generations from the F_2, followed by one generation of relaxation. The lines diverged by approximately 1·7 g or 0·7 sd. The additive genetic variance was estimated in the F_2 by restricted maximum likelihood and from the selection response, and from this variance the mutational heritability h_M^2 was estimated using the number of generations since divergence. Estimates of h_M^2 range from 0·08 to 0·10% depending on the method of analysis. These estimates are similar to those found for other species, but lower than other estimates for the mouse. It is concluded that substantial natural and, perhaps, artificial selection operated during the maintenance of the sublines.

1. Introduction

Spontaneous mutation is the source of variation in quantitative as in other traits. While some factors such as heterozygote superiority or spatial and temporal differences in fitness can help to retain variation, most forces, including random drift, directional and stabilizing selection and pleiotropic effects on fitness of genes affecting the metric trait, lead to loss of variability. Hence knowledge of the magnitude of the variation arising *de novo* in quantitative traits from mutation is important if we are to understand what maintains the levels of variation that are seen in natural and domestic populations. Further, mutations give the opportunity for continued evolutionary change and for response to artificial selection in laboratory experiments and breeding programmes, so the rate of increase of variation from mutation is critical in determining the long-term rates of change.

Rates of mutation for quantitative traits are conveniently expressed in terms of the increment in genetic variation per generation, V_M, and the 'mutational heritability', $h_M^2 = V_M/V_E$, where V_E is the environmental variance. Estimates of these parameters have been summarized by Lynch (1988). Most data have been collected on *Drosophila*, particularly for bristle number, for which published values of h_M^2 are typically about 0·001, i.e. an increment in heritability of 0·1% per generation. Estimates of mutation rates for quantitative traits in mammals are few. Using data from divergence among inbred sublines of mice, Lynch (1988) obtained values of mutational heritability of about 1% for a range of skeletal traits. For example, Bailey (1959) provided such data on length of the radius and ulna bones in the leg, from which Lynch estimated the mutational heritability to be 1·6 and 3·1%, respectively. Using selected high and low lines founded from an inbred base population, the mutational heritability for 6-week body weight in the mouse was estimated to be 1·0% (Keightley & Hill, 1992), although recent estimates from that experiment are about half this value (see below). Thus the mutational heritability for body weight in the mouse appears to be substantially higher than the 'typical' values of 0·1% found for bristle number in *Drosophila*.

Estimates are, however, likely to depend not only on the species and the trait (and perhaps population), but also on the method of estimation. Mutants affecting the metric trait under examination are also likely to have a pleiotropic effect, usually deleterious, on fitness (Hill & Keightley, 1988). Therefore the opportunity for mutations to be lost before their effect on the metric trait is recorded in some way is likely to affect the estimate of mutational heritability. Based on

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these arguments and information on the joint distribution of effects on bristle number and viability, Keightley, Mackay & Caballero (1993) found that the assumption of a model of many additive genes of small effect, neutral with respect to fitness (the infinitesimal model), may lead to an underestimation of the mutational heritability of bristle number in *Drosophila* by a factor of about three. The present experiment was designed to obtain an independent estimate of mutation rate for body weight in the mouse from that obtained previously (Keightley & Hill, 1992). The method was to take two long separated inbred sublines of an old inbred; use selection to estimate the genetic variance present in the F_2 of their cross; and from that infer the rate of accumulation of mutations influencing the quantitative trait. This was preferred to estimating the variance among such inbred sublines, for such estimates have one (for two sublines) or few degrees of freedom. The present estimate and that of Keightley and Hill are considered together in an attempt to make inferences about the effects of natural selection. A preliminary report of this experiment was given previously (Hill, Caballero & Keightley, 1994). Further information and a new analysis are given here.

2. Material and methods

Males and females of the inbred sublines C57BL/6 and C57BL/10 were obtained from Bantin and Kingman Ltd, England, in 1989. The company had obtained these sublines from the Jackson Laboratory in 1977. These had been derived prior to 1937 from the parental inbred C57BL, which had already undergone many generations of brother–sister mating; and the sublines were subsequently maintained that way for about 150 generations (Lyon & Searle, 1989, p. 639), a more precise figure than the 160 generations previously used (Hill *et al.* 1994). The animals brought to our laboratory were crossed reciprocally and the F_1 subsequently crossed to give the F_2, which formed generation 0 of this experiment. The identities of the inbred sublines were verified by checking for the presence of an endogenous non-ecotropic retrovirus, which differs between C57BL/6 and C57BL/10 (Stoye & Coffin, 1988), by Southern blotting with a flanking probe.

In the F_2 and subsequent generations body weight was recorded at 6 weeks of age. Twelve families were maintained in each of unreplicated High and Low lines, in which selection of males and females was practised within families for high and low body weight respectively, taking two litters from each pair to increase family size and selection intensity. Parents of generation 12 were selected at random, and after their offspring were recorded the experiment was terminated; thus there were 12 generations of directional and one of relaxed selection. Mating within lines was according to the scheme of Falconer (1973).

Estimation of the additive genetic variance within the F_2 population was undertaken in a number of ways:

(*a*) From a linear regression of response between generations 0 and 12, estimated as the High–Low divergence each generation, on cumulative selection differential, assuming many loci affect the trait, i.e. the infinitesimal model.

(*b*) From the average divergence between lines after generation 5, assuming it had reached a plateau due to fixation of a single segregating locus affecting the trait.

(*c*) By restricted maximum likelihood (REML) with an animal model using Meyer's (1989) DFREML package. This also assumes an infinitesimal model, and incorporates information both from the selection response and from other correlations among relatives. The model included fixed effects for sex, litter size (6 levels), parity (2 levels) and generation, and variance components for additive genetic (V_A) and environmental (V_E) effects. The latter comprises variance of common litter effects (V_{Ec}), variance of individual effects (V_{Ew}) and environmental covariance of body size of mother and offspring (Cov_{Em}) (Keightley & Hill, 1992).

The mutational variance and heritability were in turn estimated from each of the above estimates of additive genetic variance in the F_2. From Lynch & Hill (1986) the variance between lines after t generations due to fixation of neutral mutations for a quantitative trait approaches $2tV_M$, so the variance of the divergence between a pair of lines approaches $4tV_M$. Assuming different mutations are fixed in different sublines and that genes are additive, the additive genetic variance contributed by a gene of effect a (as the difference between homozygotes) to the F_2 is $a^2/8$, i.e. one-eighth of the variance it contributes to the difference between line means. Hence $V_M = 8V_A/4t$. The lines have been separated for about $t = 150$ generations and, therefore, the estimate is $V_M = V_A/75$.

3. Results

Line means are given in Fig. 1, which shows that there was a decline in mean weight in both High and Low lines, associated with severe health problems in these lines. They had previously been maintained in a minimal disease facility, and presumably contracted disease in our formerly open animal house. Nevertheless, a clear divergence of almost 1·7 g or 0·7 phenotypic standard deviations between the lines was soon obtained (Fig. 2, which corrects fig. 2 of Hill *et al.* [1994]). There was, however, little apparent additional response after about generation 5. Following relaxation of selection for the last generation, divergence was reduced by 0·61 g (almost 40%). Although this reduction has a large standard error

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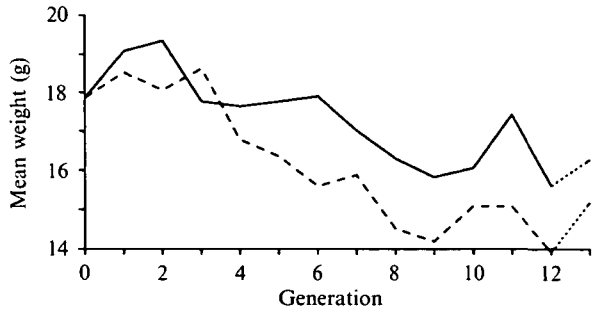

Fig. 1. Mean 6-week body weight in High (——) and Low (– – –) selection lines (selection relaxed for generation 13).

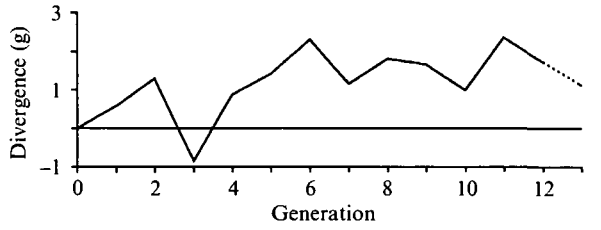

Fig. 2. Divergence in 6-week body weight between High and Low selected lines.

and could be explained by natural selection, it also suggests that a fraction of the divergence might be due to maternal environmental effects, i.e. to the fact that large mothers tend to produce large offspring and vice versa (Falconer, 1965; Kirkpatrick & Lande, 1989).

The estimates of parameters in the F_2 generation were as follows:

(*a*) *Response by linear regression*
Regression of divergence between High and Low lines on cumulative selection differential: h^2 in $F_2 = 0{\cdot}073$
From REML analysis, $V_E = 5{\cdot}34\ g^2$
Thus V_A in $F_2 = 0{\cdot}42\ g^2$
Hence $h_M^2 = 0{\cdot}0010$.

(*b*) *Response due to a single gene*
Divergence between lines, average over generations 5–13: $a = 1{\cdot}60$ g
Assuming single gene, in F2: $V_A = a^2/8 = 0{\cdot}32\ g^2$
Hence $h_M^2 = 0{\cdot}0008$.

(*c*) *REML analysis:*
$V_A = 0{\cdot}33\ g^2$, $V_{Ec} = 2{\cdot}30\ g^2$, $V_{Ew} = 2{\cdot}55\ g^2$, $Cov_{Em} = 0{\cdot}49\ g^2$; then $V_E = 5{\cdot}34\ g^2$, $V_p = 5{\cdot}67\ g^2$.
Hence $h_M^2 = 0{\cdot}0008$.

The point estimates of h_M^2 are thus in the range 0·08–0·10% according to the method of analysis. From REML, support (i.e. approximate 95% confidence) limits for h^2 in the F_2 are 0·03–0·17, and thus support limits for h_M^2 are 0·04–0·27%. If, as suggested by the pattern of divergence almost all the response was due to a single locus, then the standard error is much greater than the estimate itself, for it is an entirely hit-or-miss process. Nevertheless, the occurrence of only one 'useful' mutant over the 2×150 generations the sublines were separated is somewhat implausible. It could be checked by observation of the sublines themselves, for they should differ by 1·6 g or so if one gene is responsible, but the lines are not kept together by the supplier to avoid contamination. We have, however, limited records of both lines obtained in our laboratory at the beginning of the experiment; the difference was $1{\cdot}4 \pm 0{\cdot}9$ g.

The maternal covariance component estimate by REML analysis was 9% of the phenotypic variance. This was highly significant, with a 100 times higher likelihood than a zero covariance. The result is in agreement with that obtained by Keightley & Hill (1992) where a maternal covariance of 10% of V_p was found. The point of intercept of the regression line was at 0·48 g, consistent with the reduction of divergence after relaxation (0·61 g), and also suggesting the presence of maternal effects. Thus, estimates of h_M^2 are about 30% smaller than those obtained by Hill *et al.* (1994), where maternal effects were not considered.

4. Discussion

Although a response in body weight clearly occurred, it rapidly seemed to reach a plateau. One interpretation is that this was just chance and the observed means masked an underlying steady genetic change. On that basis the analysis assuming the infinitesimal model by REML and selection response were undertaken, and gave similar results. A real plateau in five generations or so is unlikely to be achieved unless almost all genetic variation in the F_2 is due to a single locus with very large effect and hence selective value. A gene of effect 0·7 sd would, however, with the rather weak selection pressure practised since family sizes were small, give an initial change of gene frequency up and down from the F_2 of approximately 0·045 per generation, or 0·22 over five generations, obtained by equating expected response to change of gene frequency. This result would be approximately the same for any dominance coefficient of the gene as the initial frequency is assumed to be 0·5. As it happens, however, the estimates of mutational heritability are similar (range 0·08–0·10%) whichever model or assumption is adopted.

These estimates are very much lower than the value of about 1·0% initially quoted by Keightley & Hill (1992) for the same trait in the same laboratory. Selection was practised from a C3H/He inbred line base high and low for body weight, with similar numbers of animals per line and generation. Results for the first 25 and 34 generations were reported by Keightley & Hill (1992) and Hill *et al.* (1994), respectively. Analyses of 36 generations of selection

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Table 1. *Estimates of mutational variance for 6-week weight in the mouse*

Experiment and analytical method		$V_M/V_E \times 10^{-3}$	Confidence Interval
1. Inbred base (Keightley & Hill, 1992)			
Selection response:	infinitesimal model	2·1	
	genes of large effect	0·8	
REML:	utilizing response	4·2	2·5–6·5
	ignoring response	6·4	3·2–11
2. Inbred subline cross base (this experiment)			
Selection response:	infinitesimal model	1·0	
	single locus	0·8	
REML:	utilizing response	0·8	0·4–2·7

are now available, and these are given in Table 1, along with results from the present experiment. Estimates for the C3H inbred base range from 0·08 to 0·64% depending on the method of estimation, those based on selection response being lower than the REML estimates which also utilize other correlations among relatives. All estimates are lower than previously reported (Hill *et al.* 1994) because the magnitude of divergence was much less in the last two generations, and in the case of the selection response because a correction was made to equations (1) and (2) of Keightley & Hill (1992). (The term $1/n$, where n is the number of offspring per sex and family, should be removed, as it is included in the selection intensity.) Two REML estimates are given: in one, generation effects are fitted, and in the other, generation × line effects, and both assume that the base population genetic variance is $4V_M$. Thus in the former estimate (0·42%), response to selection is incorporated in the analysis, whereas in the latter (0·64%) it is not. Estimates presented earlier in the experiment make relatively more use of the correlation among relatives as the information from responses increases non-linearly (Juga & Thompson, 1989). This suggests that covariances between relatives are being observed and attributed in the analysis to additive genetic variance, but not being realized in the selection responses. Although the estimate from selection response assuming genes of large effect is of the same order of those in the present experiment, estimates with the infinitesimal model are several times larger. Confounding due to non-additive genetic factors could occur, but maternal and sib environmental covariances were fitted in the model (Keightley & Hill, 1992) and such confounding may not explain the discrepancy between the estimates from the two experiments.

There are other possible and contributory explanations for the discrepancy, however. They were made on different populations, which for some reason may have real differences in mutation rates. The Peru strain of mouse, for example, may have an elevated rate of spontaneous mutation (Wallace, 1985). A more appealing explanation which accords with analyses and models to explain data on *Drosophila* is that many of the mutations affecting body size also have substantial pleiotropic effects on fitness (Keightley *et al.* 1993). After they have occurred, such mutants can segregate in the population for some generations contributing to the covariance among relatives, but they are eventually lost and do not contribute to the long-term response. Although the inbred sub-lines were bred with only one pair of parents each generation, such that even deleterious mutations would seem likely to be fixed, that may not have been the case. Inbred lines are usually maintained with a family substructure of grandparents, parents and offspring present each generation to reduce risks of loss of the line. Although there is then culling back to a single family, it is standard practice to choose the most fertile. Hence at least some natural selection occurs. Further one can speculate that some artificial, perhaps stabilizing, selection is practised to maintain the established phenotype of the well-known inbred line. In analyses in *Drosophila*, López & López-Fanjul (1993) found that, in lines selected from an isogenic base for abdominal bristle number, almost half the mutations detected which affected bristle number were lethal as homozygotes, whereas in inbred lines established from the same base, no lethals were found and many mutations affecting bristle number were neutral with respect to fitness or nearly so. In other selection lines, estimates of mutational heritability from response to selection and REML analysis were of the same order of magnitude (about 0·1%) in lines where most of the response was due to non-lethal mutants, whereas REML estimates were about ten times larger in lines where the response was mostly due to genes with large effect on bristle score in the heterozygote, but lethal as homozygote (M. Merchante, A. Caballero & C. López-Fanjul, unpublished). This suggests that deleterious mutations contribute much variation to the REML estimates but not those from selection response. Thus the two-fold difference between such estimates for the lines of mice selected from an inbred base (Keightley & Hill, 1992; this Table 1) and the similarity of the estimates in the

present experiment indirectly support the hypothesis that elimination of deleterious mutations has occurred in the inbred sublines. Finally, in this experiment the estimate of the heritability of 6-week body weight in the F_2 population founded from the inbred subline cross was about 6%, which corresponds to a mutational heritability of 0·08%. The heritability of this trait in mice is typically around 35% in laboratory populations (Falconer, 1973). Thus, even if the heritability in our F_2 had been that high, the estimates of mutational heritability would have been about 0·5%. This either suggests that the mutational heritability is very substantially less than 0·5% or confirms the suggestion that considerable natural and artificial, perhaps stabilizing, selection is practiced in laboratory inbred lines which substantially reduces the rate of fixation of genes affecting body weight and, presumably, other quantitative traits.

Estimates of mutational heritability for skeletal traits in mice (summarized by Lynch, 1988) are in the range 0·5–3%, and so tend to be higher than the present estimates for body weight. One possible explanation is that body size and particularly weight are more strongly influenced by environmental effects (i.e. V_E is lower for skeletal traits). It is also possible to question the reliability of estimates of genetic differences among the sub-lines on which the estimates of h_M^2 for skeletal traits are based. For example, for skull and mandible measures, most estimates were based on the divergence among members of the C57BL/Gr subline set, samples from individual sublines of which were taken in different environments over a period of up to 20 years.

In conclusion, we are not able to give an unequivocal estimate of mutational heritability for body weight in the mouse. It seems likely, however, that the total mutational heritability, including genes conferring significant fitness reduction, is at least 0·5% per generation, but that which is contributed by genes which are neutral or nearly neutral with respect to fitness is around 0·1%. Nevertheless, the results show that mutation rates for body weight in mammals are at least as large as for bristle number in *Drosophila* and sufficient to contribute to long-term selection response.

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