Letter to the Editor

Average Dominance for Polygenes: Drawbacks of Regression Estimates

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THE dominance of genes controlling fitness components and other quantitative characters is important for theoretical predictions concerning the maintenance of genetic variability (see *e.g.*, CABALLERO and KEIGHTLEY 1994). Consider, for instance, mutation-selection balance at a diallelic autosomal locus with mutation rate μ to the deleterious allele and fitnesses 1, $1 - sh$, and $1 - s$, with $s > 0$ and $0 < h < 1$. Assuming that $\mu \ll sh$, the equilibrium mean fitness is approximately $1 - 2\mu$, independent of h, but the additive (and total) genetic variance for fitness is approximately $2\mu sh$ (MUKAI *et al.* 1974), which is directly proportional to h. Moreover, if these genotypes have pleiotropic effects on a quantitative trait, with average effects a, d, and $-a$, the additive variance for the trait contributed by this locus depends on the deviation d of the heterozygote from the average of the two homozygotes, *i.e.*, on the coefficient of dominance for the effects on the trait (FALCONER and MACKAY 1996, Chapter 8).

In Drosophila, estimates of the average dominance for newly arising spontaneous mutations (*e.g.*, MUKAI and YAMAZAKI 1968), *P*-element insertion mutations (MACKAY *et al.* 1992; LYMAN *et al.* 1996), and chromosomes extracted from segregating laboratory or natural populations (*e.g.*, MUKAI *et al.* 1972; MUKAI and YAMAGUCHI 1974; WATANABE *et al.* 1976; EANES *et al.* 1985; HUGHES 1995) have been obtained by regressing the heterozygous effect of the chromosome or line on the sum of the two homozygous effects of the two parental lines (or the homozygous effect of the insertion line only for *P* elements). Estimates of $\bar{h}$ also feature prominently in an estimation method for genomic mutation rates for fitness traits based on the performance of selfed and outbred progeny in populations assumed to be at mutation-selection balance (CHARLESWORTH *et al.* 1990; JOHNSTON and SCHOEN 1995). These and other methods for estimating average degrees of dominance are discussed by LYNCH and WALSH (1997, Chapters 10 and 20).

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Consider a diallelic locus i with genotypes AA, Aa, and aa, relative fitness values 1, $1 - s_i h_i$, and $1 - s_i$, as above, and genotypic frequencies p_i^2, $2p_iq_i$, and q_i^2, respectively. In their analysis of viability polygenes segregating on chromosomes sampled from nature, MUKAI *et al.* (1972) and MUKAI and YAMAGUCHI (1974) showed that the regression coefficient of the heterozygous effect on the sum of the two homozygous effects is equal to a weighted average of the coefficient of dominance over loci ($\bar{h}$),

$$\bar{h} = \frac{\sum p_i q_i s_i^2 [h_i + q_i (1 - 2h_i)]}{\sum p_i q_i s_i^2 (1 + 2q_i)} \approx \frac{\sum p_i q_i s_i^2 h_i}{\sum p_i q_i s_i^2}, \quad (1)$$

where the summation is over loci, and the right hand side approximation assumes small values of q_i. The reliability of estimates from the regression coefficient depends on two assumptions: (1) that the homozygous effects are known precisely and (2) that the dominance of alleles are uncorrelated with their homozygous effects. The second assumption was repeatedly emphasized by MUKAI and his co-workers (*e.g.*, MUKAI and YAMAZAKI 1968, p. 525). The first was noted by MUKAI (1969, p. 475) but it seems not to have received comparable attention. This assumption can be ignored if $\bar{h}$ is calculated as the ratio of the genetic covariance between homozygous and heterozygous effects to the genetic variance of homozygous effects, both estimated from ANOVA (*e.g.*, MUKAI and YAMAZAKI 1968; MUKAI *et al.* 1972). It cannot be ignored, however, if $\bar{h}$ is obtained from the regression of heterozygous phenotypic effects on homozygous phenotypic effects (*e.g.*, EANES *et al.* 1985; MACKAY *et al.* 1992; HUGHES 1995; LYMAN *et al.* 1996).

In this note we show how violation of each of the regression method's assumptions leads to biased or misleading estimates of $\bar{h}$. First, error in the estimation of homozygous effects biases the regression slope toward zero (*i.e.*, toward recessivity). Second, the assumption that dominance coefficients and homozygous effects are uncorrelated is unlikely to hold. Mutations affecting viability in Drosophila show a negative correlation between s and h (*e.g.*, SIMMONS and CROW 1977), and

loss-of-function mutations at loci coding for enzymes acting in metabolic pathways will often be more recessive than mutations with small effects (KACSER and BURNS 1981). Hence, mutations or chromosomes with extreme effects have the greatest impact on the regression slope and can produce a misleading picture of gene action if they are included in the analysis. Finally, even if the assumptions of Equation 1 are met, the weights in Equation 1 are proportional to the relative contribution that each locus would make to genetic variance if only homozygotes were present in the population at frequencies p_i and q_i. This weighting has no clear biological justification. When q_i is small, the rare allele will be found almost exclusively in heterozygotes, so its homozygous effect would be largely irrelevant to segregating variance. If, on the other hand, allele frequencies at all loci are equal to one half, it turns out that $\bar{h} = 0.25$ (see left-hand side of Equation 1), irrespective of the distribution of h values (CHARLESWORTH and HUGHES 1997). Each of these topics will be discussed in turn.

Bias due to errors of estimation of homozygous effects: As discussed by SNEDECOR and COCHRAN (1989), when the independent variable is measured with error, regression coefficients are biased towards zero by a factor k, the reliability ratio, whose magnitude depends on the error variance of the independent variable, V_u, relative to its true variance in the reference population. This error in the predictor variable may occur because of measurement error, sampling error, or background genetic or environmental variation in the trait. In the context of regressing heterozygous effects on the sum of the homozygous effects, for the case of zero environmental covariance between the effects, the reliability ratio is

$$k = \frac{V_G}{V_G + V_u}, \qquad (2)$$

where V_u is the error variance for the individual estimates of homozygous effects and V_G is the true variance of homozygous effects. [See RISKA (1991) for a discussion of this same problem in the context of evolutionary allometry.] If extreme lines are eliminated from the analysis, V_G will become smaller and this bias becomes even larger, assuming that the error variance in estimates of homozygous effects is unchanged. Note, however, that when the estimate of $\bar{h}$ is obtained from the ratio of genetic components of variance estimated in the analysis (*e.g.*, MUKAI and YAMAZAKI 1968; MUKAI *et al.* 1972), this source of bias does not occur.

To illustrate this bias, consider the analysis by HUGHES (1995), who extracted chromosome lines from a laboratory population and estimated homozygous and heterozygous effects for several male characters. The experimental procedure used a balancer chromosome to generate lines homozygous for the same chromosome or heterozygous for different chromosomes. A diallel crossing scheme was used to obtain the heterozygotes. The average of $\bar{h}$ across traits obtained with the regression method was 0.158 (excluding male fertility; the value is 0.113 if male fertility is included), and the average with an indirect method based on the assumption that genes were segregating at mutation-selection equilibrium frequencies was 0.362. At least part of this difference could be explained by sampling errors in the estimation of homozygous effects. For example, from the data in her Table 3, we can infer the bias in the estimates. Take the trait body mass for illustration. The ANOVA estimates of V_G and V_E are 0.0017 and 0.0022, respectively. The "effective" number of observations per homozygous line is 1.76 (not an integer because the analysis was unbalanced), so we can assume that the true genetic variance among homozygous lines is $V_G = 0.0017$, while the error variance for the individual estimates of homozygous effects is $V_u = V_E/1.76 = 0.00125$. The reliability ratio from Equation 2 is then $k = 0.0017/(0.0017 + 0.00125) = 0.58$, and the estimated $\bar{h}$ (0.080) has to be corrected by a factor $1/0.58$, so the unbiased $\bar{h}$ would be 0.139. When analogous corrections are made to each of the traits analyzed by HUGHES (1995), the average corrected h is 0.317 (excluding male fertility), which is in good agreement with the estimate 0.362 from the indirect method. Alternatively, estimation of $\bar{h}$ using the ratio of genetic variance components from ANOVA would be free of this bias but the results are very sensitive to negative variance estimates and/or variance estimates very close to zero if the sample sizes are small (K. HUGHES, personal communication).

Similar corrections can be applied to the estimates of $\bar{h}$ obtained by MACKAY *et al.* (1992) and LYMAN *et al.* (1996) for effects of artificially induced *P*-element insertions on bristle traits in which trait values were estimated independently for homozygous or heterozygous *P*-element-bearing chromosomes. For example, from MACKAY *et al.* (1992, Table 1), the variance among control (unmutated) lines was 0.061 for abdominal bristle number. The variance among homozygous insert lines was 0.382. Therefore, we can assume that the genetic variance among homozygous insert lines was $V_G = 0.382 - 0.061 = 0.321$, and the bias factor would be $0.321/0.382 = 0.84$. Their original estimate of $\bar{h}$ from regression of heterozygous on homozygous bristle score was 0.144, which is increased to $0.144/0.84 = 0.171$ after correction. The analogous corrected value for sternopleural bristle number is 0.108, 1.4 times larger than the original estimate. Similar corrections for the single *P*-element insertions (LYMAN *et al.* 1996) show biases of the same order, with corrected estimates of $\bar{h}$ about 1.4 times larger than estimated values.

An alternative method for estimating the dominance of polygenes was described by COMSTOCK and ROBINSON (1952). Unlike the regression method, theirs was based on ANOVA estimates of variance components and provides an estimate of the square of the dominance coefficient (measured as a scaled deviation of

the mean heterozygote phenotype from the arithmetic mean of the two homozygote values). Although this method avoids the assumption that homozygous effects are known precisely, it implicitly assumes that the dominance coefficients are uncorrelated with homozygous effects. Thus, like the regression method, it also suffers the bias discussed next.

Bias due to pooling heterogeneous mutation effects: This second source of bias occurs if mutants or chromosomes of large effect tend to be recessive, thereby creating a negative covariance between homozygous effects and dominance coefficients. This effect leads to a nonlinear relationship between s and h and also tends to bias estimates of $\bar{h}$ downward if a linear regression is used. This problem may have occurred in several analyses of homozygous and heterozygous viabilities of chromosomes extracted from natural populations, where lethal chromosomes were included in the calculations (see SIMMONS and CROW [1977], p. 61 for references) and in analyses of *P*-element-induced mutation lines (MACKAY *et al.* 1992; LYMAN *et al.* 1996). Let us consider the latter for illustration.

Estimates of $\bar{h}$ for the effects of single or multiple *P*-element insertions on viability and bristle traits in *Drosophila melanogaster* were obtained by the regression method. Although the following argument applies in general, the effect is most pronounced for viability mutations that show systematically different patterns of dominance for mutations of different severity. Using the regression slope of heterozygous score on homozygous score for all insertion lines and giving each line equal weight, the estimated $\bar{h}$ was 0.01 for the chromosome III multiple insertion lines (MACKAY *et al.* 1992) and 0.002 and −0.01 for the chromosome II and III single insertion lines, respectively (LYMAN *et al.* 1996). The results, therefore, imply nearly complete recessivity for the effects of insertions on viability. It was also found that the ANOVA genetic estimates of heterozygous viabilities showed no significant effect of line for the chromosome III multiple insertion lines of MACKAY *et al.* (1992; T. F. C. MACKAY, personal communication) and for the chromosome II single insertion lines (LYMAN *et al.* 1996), pointing again toward complete recessivity. However, as noted by CABALLERO and KEIGHTLEY (1994) and LYMAN *et al.* (1996), these estimates are biased if there is a negative correlation between homozygous effects and h. The reason is easy to see from Equation 1. When all lines are equally weighted, this equation can be rewritten as

$$\bar{h} = \frac{\mathrm{E}(s^2h)}{\mathrm{E}(s^2)} = \mathrm{E}(h)\,[1 + \frac{\mathrm{Cov}\,(s^2, h)}{\mathrm{E}(s^2)\,\mathrm{E}(h)}], \qquad (3)$$

so that the "average" h can be negative, even when all of the individual values are positive, if the covariance between s^2 and h is negative. A negative correlation has been repeatedly observed, as mutants with large effects on viability and other quantitative traits tend to be partially or completely recessive, while mutants of small effect appear to have variable gene action that is, on average, roughly additive (see review by CABALLERO and KEIGHTLEY 1994). In this case, the regression method yields estimates of $\bar{h}$ biased downward even when the vast majority of mutants have minor effects and are additive, on average.

As an illustration of this, consider the following simple numerical example. Assume that the mean number of progeny of a nonmutant genotype is 2.0 and we have four mutant lines with (precisely known) homozygous (*Hom*) effects 0, 1.8, 1.6, and 1.4 and corresponding heterozygous (*Het*) effects 2, 1.9, 1.8, and 1.7. The corresponding coefficients of dominance (estimated as $h = [Het/2 - 1]/[Hom/2 - 1]$) are 0, 0.5, 0.5, and 0.5. Therefore, the first line carries a recessive lethal mutation, and the last three carry detrimental additive mutations. The regression of heterozygous score on homozygous score is 0.5 if the recessive lethal line is excluded, but it is −0.1, suggesting overdominance, if it is included. The recessive lethal dominates the regression slope, making the average h negative. This example also illustrates that with a negative covariance between s and h, there may be a large amount of variation among homozygous effects and little variation among heterozygous effects (as observed by MACKAY *et al.* [1992] and LYMAN *et al.* [1996]). The problem can also be exacerbated when there are many lines with mutants that have no effect on the trait. This class of mutants along with the lethal recessives would tend to push the regression slope toward zero even though most detrimental mutants may be nearly additive in their effects. As noted above, environmental error in the measurements of homozygous effects produces an additional push towards zero.

The arithmetic mean of h in the simple numerical example above is 0.375, and the mean h weighted by the selective value in the homozygote is 0.188. Neither of these averages accurately represents the data. Perhaps, a more illustrative procedure is to subdivide the data into ranges according to the homozygous effect of different lines. For example, the average h for homozygous viability effects s between 0.3 and 0.6 for the multiple insertion lines of MACKAY *et al.* (1992) was 0.53 ± 0.11 and for more deleterious effects, the average h was 0.17 ± 0.04 (CABALLERO and KEIGHTLEY 1994).

Inappropriate weighting of *h* values: Using chromosomes or lines extracted from natural or segregating populations, the regression of heterozygous effects on the sum of the two homozygous parental lines (Equation 1) estimates the average value of h for mutations segregating in the population. In this average, the h values are weighted by the genetic variance that would be contributed by each locus if the population consisted only of the two homozygous types at frequencies p and q, *i.e.*, $V_G = 2pqs^2$ (MUKAI *et al.* 1972). This weighting seems to have little biological justification, at least at mutation-selection balance, because it is expected that most of the contribution of the segregating deleterious

mutants to the genetic variance is through heterozygotes (with a contributed variance of $2\mu sh$).

Equation 1 is also interpreted as giving an estimate of the harmonic mean of newly arisen mutations weighted by their frequency of origin and their effect (*e.g.*, SIMMONS and CROW 1977). At mutation-selection balance, the frequency of the deleterious allele is approximately $q = \mu/sh$, and Equation 1 becomes $\bar{h} = \Sigma\mu s/\Sigma(\mu s/h)$. Because the harmonic mean is always smaller than the arithmetic mean, this is consistent with the expectation that the average value of h for segregating deleterious mutations (Equation 1) should be smaller than that for newly arisen mutations (MORTON *et al.* 1956). But, again, the harmonic mean of h for newly arisen mutations weighted by their effects will give most weight to those mutants with small h and large s, which seems to be of dubious value. An indirect estimate of the inverse of the arithmetic mean of h values for newly arisen mutations can also be obtained from the regression of the sum of homozygous scores on the heterozygous scores (MUKAI and YAMAGUCHI 1974).

By using chromosomes or lines that have accumulated spontaneous or induced mutations, the average degree of dominance for newly arisen mutations can be estimated directly by the regression of heterozygous on homozygous scores (*e.g.*, MUKAI and YAMAZAKI 1968; MUKAI 1969; MACKAY *et al.* 1992; LYMAN *et al.* 1996). In this case, there is not a problem about gene frequency weightings, and the regression method would give appropriate estimates, except for the other sources of bias discussed in this article.

When considering the sources of variation in outbred populations, we are probably more interested in the dominance coefficients of mildly detrimental mutants than highly deleterious ones (which occur much less frequently), but Equation 1 disproportionately weights very recessive mutations of large effect. If highly deleterious mutants are disregarded in the regression analysis, this problem is reduced. A single summary statistic may be useful in giving a lower bound estimate, but it is of dubious value without more information on the relation between s and h for slightly detrimental alleles. For instance, to describe fully the consequences of *P*-element-induced mutations, the characteristics of the joint distribution of s and h are required (CABALLERO and KEIGHTLEY 1994). By extending methods used to infer univariate distributions of mutation effects (KEIGHTLEY 1994) to bivariate distributions, progress toward this goal might be possible (R. G. SHAW and P. D. KEIGHTLEY, unpublished results). A yet more difficult problem will be inferring the joint distribution of s, h, and q from crosses among individuals sampled from natural populations.

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