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INBRED GENETIC LOADS AND THE DETERMINATION OF POPULATION STRUCTURE

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Morton, Crow, and Muller, in a pioneering paper,[1] gave a method for determining from inbred and outbred individuals whether the genetic load in a population was due mainly to deleterious genes maintained by mutation pressure (mutational load) or to genes maintained because the heterozygote was superior to the homozygotes (the segregational load of Crow[2] or the balanced load of Dobzhansky[3]). Morton, Crow, and Muller applied their method to some human data from France and the United States, and concluded that the load due to inbreeding was mainly mutational. The method was subsequently applied by many authors. Perhaps the best and most extensive human data is that of Neel and Schull[4] from Japan, while the best nonhuman data so far published is that of Dobzhansky, Spassky, and Tidwell[5] on Drosophila. These two studies did not rule out a major role for overdominant loci. Recently there has been some controversy over the validity of the approach of Morton, Crow, and Muller, and it has been criticized on mathematical and theoretical grounds by Li[6, 7] and Sanghvi[8] and defended by Crow.[9] The present paper is devoted exclusively to continuing this discussion and to an examination of whether or not population structure can be determined by this method in practice. The question of whether, in Dobzhansky's terminology, the classical or the balanced theory is in fact more nearly correct can be attacked in many other ways, and will not be considered here.

Consider a single locus with two alleles. Under the assumption of random mating, zygotic frequencies will be p^2 AA:$2pq$ Aa:q^2 aa. Let the expected number of offspring one generation later of each of the three genotypes be w_1, w_2, w_3, respectively. The expected number of offspring of a random individual one genera-

tion later will be $\bar{w}_0 = p^2w_1 + 2pqw_2 + q^2w_3$. The population size will be decreasing, stable, or increasing, according as $\bar{w}_0$ is less than, equal to, or greater than one. However, as long as we are only interested in gene frequencies and not population size, the w's may all be multiplied by a common constant without altering anything, and in most experimental situations it is easier to find such relative w's rather than absolute ones. Crow[2] defined genetic load as $A = (w_i - \bar{w}_0)/w_i$, where w_i is the largest of the w's. The load as thus defined is independent of scale, it being the proportionate loss of average fitness due to the presence of the less fit genotypes. For convenience, Crow took the largest $w_i = 1$. This convention becomes a pitfall in the applications, as was seen but not clearly explained by Li.[6]

If we now suppose that all individuals are artificially made homozygous while the gene frequency, q, of a remains fixed, $\bar{w}$ becomes $\bar{w}_1 = pw_1 + qw_3$, and the load becomes $B + A = (w_i - \bar{w}_1)/w_i$. The ratio of the random load to the homozygous load is $(B + A)/A = (w_i - \bar{w}_1)/(w_i - \bar{w}_0)$ and is invariant when all the w's are added to or multiplied by a constant. Now let us suppose that the AA homozygote is the most fit. It is convenient to let $w_1 = 1$, $w_2 = 1 - hs$, and $w_3 = 1 - s$, where h and s are positive. Then $B + A = qs$ and $A = 2q(1 - q)hs + q^2s$, and

$$\frac{B + A}{A} = \frac{qs}{2q(1 - q)hs + q^2s} = \frac{1}{2h(1 - q) + q}, \tag{1}$$

which is independent of s. If $h = 0$, $(B + A)/A = 1/q$, while if $h > 0$, $(B + A)/A \to 1/(2h)$ as $q \to 0$, and, whenever q is small compared to $2h$, we will have approximately

$$\frac{B + A}{A} = \frac{1}{2h}. \tag{2}$$

Further, let A mutate to a at rate $m = 2\mu$, where μ is the probability of mutation in a gamete and m the expected number of mutations per zygote. Then there will be an equilibrium established, with equilibrium gene frequency $\bar{q} = \sqrt{m/(2s)}$ if $h = 0$ and a is completely recessive, or approximately $\bar{q} = m/(2hs)$ if $h > 0$ and hs is much larger than m, as is usually the case. Also under these conditions $\bar{q}$ will be small, and if $\bar{q}$ is small compared to $2h$, which will be true if m is small compared to $4sh^2$, then q may also be expected to be small, and equation (2) will be approximately correct. This equation was obtained by Crow[2] by a different argument, using the same assumptions. Actually, this result is independent of m, $\bar{q}$, s, or of any assumptions on h except $h > 0$, and holds if and only if the actual q is small compared to $2h$.

If, on the other hand, the heterozygote is superior, it will be convenient to let $w_1 = 1 - kt$, $w_2 = 1$, and $w_3 = 1 - t$, where k and t are positive.[10] Then there will be an equilibrium with $q = \bar{q} = kt/(kt + t) = k/(1 + k)$, independent of the value of t, which measures the strength of the selection. It is also convenient, though not essential, to let aa represent the poorer homozygote, so that $k \leq 1$. Then $B + A = qt + (1 - q)kt$, and $A = q^2t + (1 - q)^2kt$, giving

$$\frac{B + A}{A} = \frac{qt + (1 - q)kt}{q^2t + (1 - q)^2kt} = \frac{q + (1 - q)k}{q^2 + (1 - q)^2k}, \tag{3}$$

which is independent of t. Considering $(B + A)/A$ as a function of q for fixed k,

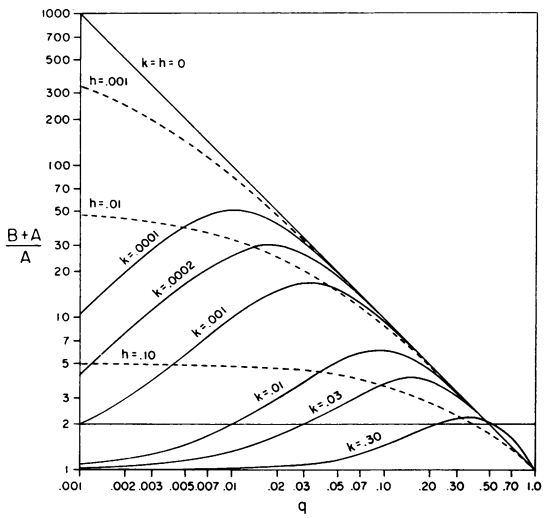

FIG. 1.—Value of $(B+A)/A$ as a function of gene frequency q on logarithmic coordinates for a semidominant locus with various h (dashed lines), and for a heterotic locus with various k (solid lines). See text for further explanation.

it is easy to show that it takes the value 1 for $q = 0$ and $q = 1$, the value 2 for $q = \bar{q} = k/(1 + k)$ and for $q = 1/2$, and attains a maximum value of $(1 + \sqrt{k})^2/(2\sqrt{k})$ when $q = (-k + \sqrt{k})/(1 - k)$. This last value is between $\bar{q}$ and $1/2$. Figure 1 gives $(B + A)/A$ as a function of q for various values of k, using equation (3), and for various values of h, using equation (1).

Morton, Crow, and Muller[1] and Crow[2] assumed that the population was at equilibrium with $q = \bar{q}$, and thus $(B + A)/A$ would be 2, compared to a much larger value for a mutational load, where they felt that $(B + A)/A = 1/2h$ would be of the order of 25. Crow extended the results for the segregational case to n alleles and showed that *at equilibrium* $(B + A)/A \leq n$, taking this maximum value only if all heterozygotes had the same value of w. Since it was felt that in general there would not be many equally fit heterozygotes, the basic method was to estimate $(B + A)/A$ (or for technical reasons B/A), and if this was large (say greater than 10) decide that the load was largely mutational. However, two further extensions had to be made by Morton, Crow, and Muller[1] before the method could be applied. First, it could be extended without change to many loci acting together provided their effects were independent, or not "synergistic." This is nearly, but not quite, the same as assuming additive effects or no epistasis. Nonsynergistic environmental effects were not explicitly included, but it was shown they would tend to reduce the value of B/A. Second, $B + A$, the load for complete homozygosis, corresponds to an inbreeding coefficient, $F = 1$, and normally cannot be observed. However, it could be estimated from the load in individuals who resulted from the mating of close relatives, which happens by chance in a finite population with small effective population size.

There are a number of difficulties in applying this method. First, most applications so far have been based on mortality (or survival) between two points in the life cycle, e.g., stillbirths, neonatal deaths, and juvenile deaths (Morton, Crow,

and Muller[1]), and survival from egg to imago (Dobzhansky, Spassky, and Tidwell[5]). Here, assuming that all environmental or accidental causes of death could be eliminated, the ideal genotype would have 100 per cent survival. If the probability of survival of genotype i is v_i, then $w_i = v_i z_i$, where z_i includes differential survival at other stages of the life cycle, differential fertility, and other factors affecting over-all fitness. The data used estimates only v, and the assumption that the actual $\bar{q}$ based on w,$\bar{q}_w$ will be close to the $\bar{q}$ calculated from v, $\bar{q}_v$ is unwarranted. Thus, even if q is close to the true $\bar{q}_w$, it may be quite different from the ostensible $\bar{q}_v$ based only on v; and it is only when q equals $\bar{q}_v$ that $(B + A)/A$, calculated from survival data, will equal 2 for a heterotic locus. Examination of Figure 1 will show how much the $(B + A)/A$ ratio can depart from the theoretical, when $q \neq \bar{q}$. For example, gene a may be kept at a frequency of 0.03 by over-all heterozygous advantage, but behave like a deleterious semidominant with $h = 0.01$ when only survival is considered, and under those conditions will give an ostensible $(B + A)/A = 20$. Thus, studies based only on mortality are of doubtful value at best for determining the nature of the genetic load.

On the other hand, studies involving all components of fitness over an entire generation on a population in genetic equilibrium are extraordinarily difficult and, as far as I know, have never been carried out even for Drosophila. Even if they could be carried out, another difficulty would arise. Dobzhansky[11, 12] and others have pointed out that a best genotype is pretty much of a fiction. Given a large number of loci, the number of possible genotypes is so large that even if there is a best it probably will occur at most in one individual in an occasional generation. Even under the "classical" theory, with the best genotype homozygous for all the "normal" alleles, Muller[13] has estimated that the average human is heterozygous for at least 8 deleterious genes. The probability that an individual carries none would then be of the order of magnitude of $e^{-8} = 0.0003$, and the ideal would still be too rare to be usable experimentally. Furthermore, one cannot now define a perfect genotype on *a priori* grounds; for example, the number of offspring of an active human male could be in the hundreds, but no such person would be detected by a genetic survey.

In the literature, the largest w is taken as one, and thus in practice the load is always given as $1 - \bar{w}$, although in his text Crow points out that this is a convention. Li[6] proceeded to criticize the use of $1 - \bar{w}$ as making the "load" depend on the notation one used. This criticism is perhaps misdirected, but nevertheless Li has performed a useful service in pointing out how extremely sensitive the $(B + A)/A$ ratio is to the quantity from which one subtracts $\bar{w}$. The conventional setting of the maximum w equal to one has helped to sweep under the carpet the very real difficulty of finding that maximum—a difficulty that is not ameliorated by the fact that we may divide all the other w's by this maximum once we have found it. The fact that a unique beneficial mutation creating a new and larger maximum w will instantly, and before it is increased in frequency by selection, increase the load in the population is a semantic problem and irrelevant for the present purpose.

The two difficulties discussed above are so great that it seems doubtful if the calculation of B/A ratios can shed any real light on the nature of the genetic load. However, they by no means exhaust the difficulties. All evidence suggests that w

values vary considerably from one generation to another, so that no population is in genetic equilibrium for its present adaptive values. We have pointed out the difficulties caused by such a departure from equilibrium. Likewise, if a population occupies several ecological niches, as most populations probably do, the theory is upset in ways that have not as yet been explored.

Finally, it seems to the present author that epistatic interactions are too common and important to be ignored. The implications have only been explored for one simple case. Consider two loci each with two alleles and fitnesses, as follows:

	BB	*Bb*	*bb*
AA	$1 - 2c - d$	$1 - c$	$1 - 2c - d$
Aa	$1 - c$	1	$1 - c$
aa	$1 - 2c - d$	$1 - c$	$1 - 2c - d$

We assume $c > 0$. If there is any equilibrium, by symmetry it is with A and B each having a frequency of $1/2$. For these gene frequencies

$$\frac{B + A}{\mathrm{A}} = 2 + \frac{2d}{4c + d}. \tag{4}$$

If $d = 0$, there is no epistatic interaction and $(B + A)/A = 2$. If $d > 0$, the double homozygote is less fit than would be expected under additivity, and $(B + A)/A$ is greater than 2, taking a maximum value of 4 when $d > 0$ and $c = 0$. If $d < 0$, $(B + A)/A$ is less than 2, taking a value of 4/3 when $d = -c$. Professor Crow has pointed out (personal communication) that if there is epistasis the load is no longer a linear function of F, but a quadratic. We can easily show that for the present example the load under partial inbreeding is $(c + 1/4d) + (c + 1/2d)F + (1/4d)F^2$. Setting $F = 0$ gives $A = c + 1/4d$, and $F = 1$ gives $B + A = 2c + d$, giving $(B + A)/A$ as above. However, if B is estimated from data based on small degrees of inbreeding, F^2 will be very small compared to F and can be ignored, giving a linear relationship for small values of F. The slope B will then be estimated as $c + 1/2d$, and the estimated $(B + A)/A$ ratio will be $(2c + 3/4d)/(c + 1/4d) = 2 + d/(4c + d)$, giving a value of 2 when $d = 0$ (as it should), but a maximum of 3 when $c = 0$ and $d > 0$. Thus, the $(B + A)/A$ ratio will still be inflated by epistasis, but to a lesser degree. It is clear that with larger numbers of loci the effects might be even larger. With $d > 0$, the graph of load against coefficient of inbreeding would curve upward for large F. There is some evidence from Dobzhansky, Spassky, and Tidwell[5] and from unpublished data that this actually happens for Drosophila for $F = 1/4$, and even more when a single chromosome is made completely homozygous, giving $F = 1$ for that part of the genome.

In closing, it may be noted that Li[6] and Sanghvi[8] have suggested using $\bar{w}_1/\bar{w}_0$ instead of $(B + A)/A$ as a measure of inbreeding depression, and that Dobzhansky[12, 14] has suggested taking $\bar{w}_0 = 1$ and measuring adaptedness of all genotypes as a departure, plus or minus, from this norm. While both these suggestions have value in discussing the biological aspects of adaptedness, inbreeding depression, and genetic norms, and Dobzhansky's standard is operationally simpler than Crow's, neither formulation is useful for determining the relative importance of the mutational and the balanced load. Thus, *for this purpose*, neither formulation is a rival for the $(B + A)/A$ ratio, which in theory, under Crow's assumptions, *will* make this determination. It is unfortunate that the difficulties discussed in the present

paper make it unlikely that the $(B + A)/A$ ratio will answer the question in practice.

This paper is dedicated to Dr. L. C. Dunn in appreciation of a cherished teacher, colleague, and friend.

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*VARIATIONAL BOUNDARY VALUE PROBLEMS FOR QUASI-LINEAR ELLIPTIC EQUATIONS, II**

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In a preceding note under a similar title,[1] which we shall refer to below as (I), we have presented a new form of the orthogonal projection method for solving variational boundary value problems for equations of the form

$$Au = \sum_{|\alpha|,|\beta| \leq m} D^{\beta}[a_{\alpha\beta}(x, u, \ldots, D^{m}u)D^{\alpha}u] = f. \tag{1}$$

It is our purpose in the present note to strengthen and generalize these results to equations of the form

$$Au = \sum_{|\alpha| \leq m} D^{\alpha}[A_{\alpha}(x,u,\ldots,D^{m}u)] = f \tag{2}$$

while replacing the positivity assumptions of (I) by semiboundedness assumptions of the type which appear in the study of linear elliptic operators of order greater than two. In order to treat differential operators of the form (2), we shall study below properties of nonlinear operators in Hilbert space satisfying even weaker continuity conditions than those considered in (I). To treat the case of semiboundedness rather than positivity, we consider the perturbation of such operators by compact operators.