Cohen, *Biochim. Biophys. Acta* **31**, 378 (1959); M. H. Richmond, *J. Mol. Biol.* **6**, 284 (1963); O. M. Rennert and H. S. Anker, *Biochemistry* **2**, 471 (1962).
25. A. A. Gottlieb, Y. Fujita, S. Udenfried, B. Witkop, *Biochemistry* **4**, 2507 (1965).
26. R. L. Munier and G. Sarrazin, *Compt. Rend. Ser. C* **259**, 937 (1964); *ibid.* **262**, 1029 (1966).
27. E. Wecker and E. Schonne, *Proc. Nat. Acad. Sci. U.S.* **47**, 278 (1961); L. Leventow, M. M. Thorén, J. E. Darnell, Jr., L. L. Hooper, *Virology* **16**, 220 (1962).
28. S. Kang and A. Markovitz, *J. Bacteriol.* **93**, 584 (1967); *ibid.* **94**, 87 (1967).
29. N. K. Chaudhuri, B. J. Montag, C. Heidelberger, *Cancer Res.* **18**, 318 (1958); J. Horowitz and E. Chargaff, *Nature* **184**, 1213 (1959); M. P. Gordon and M. Staehelin, *Biochim. Biophys. Acta* **36**, 351 (1959); Y. Shimura and D. Nathans, *Biochem. Biophys. Res. Commun.* **16**, 116 (1964).
30. F. Gros, W. Gilbert, H. H. Hiatt, G. Attardi, P. F. Spahr, J. D. Watson, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 111 (1961).
31. R. J. Lowrie and P. L. Berquist, *Biochemistry* **7**, 1761 (1968).
32. Catabolite repression rather than fluorouracil incorporation into messenger RNA seems to be the explanation for inhibition of β-galactosidase synthesis; J. Horowitz and V. Kohlmeier, *Biochem. Biophys. Acta* **142**, 208 (1967).
33. S. P. Champe and S. Benzer, *Proc. Nat. Acad. Sci. U.S.* **48**, 532 (1962).
34. H. Bujard and C. Heidelberger, *Biochemistry* **5**, 3339 (1966).
34a. Y. Shimura, R. E. Moses, D. Nathans, *J. Mol. Biol.* **28**, 95 (1967); Y. Shimura, H. Kaizer, D. Nathans, *ibid.* **38**, 453 (1968).
35. O. M. Rennert, personal communication.
36. J. Fried, *Cancer* **10**, 752 (1957). I thank J. Fried for permission to reproduce his data in Table 2.
37. L. Sokoloff, in *New Research Techniques of Neuroanatomy*, W. Windle, Ed. (Thomas, Springfield, Ill., 1957), p. 54.
38. J. C. Krantz, Jr., and F. G. Rudo, in *Handbook of Experimental Pharmacology*, O. Eichler, A. Farah, H. Herken, A. D. Welch, Eds. (Springer-Verlag, New York, 1966), vol. 20, part 1, p. 501.
39. B. C. Saunders and G. J. Stacey, *J. Chem. Soc. London* **1948**, 1773 (1948); P. Mirosevic-Sorgo and B. C. Saunders, *Tetrahedron* **5**, 38 (1959).
40. G. M. K. Hughes and B. C. Saunders, *J. Chem. Soc. London* **1954**, 4630 (1954); B. C. Saunders, A. G. Holmes-Siedle, B. P. Stark, *Peroxidase* (Butterworths, London, 1964).
41. S. Kaufman, *Biochim. Biophys. Acta* **51**, 619 (1961); S. Kaufman, in *Oxygenases*, O. Hayaishi, Ed. (Academic Press, New York, 1962), p. 129.
42. In addition to the species of *Dichapetalum* mentioned, three other species of plants have been found to contain fluoroacetate; L. R. Murray, J. D. McConnell, J. H. Whittem, *Australian J. Sci.* **24**, 41 (1961); P. B. Oelrichs and T. McEwan, *Nature* **190**, 808 (1961); M. M. Oliveira, *Experientia* **19**, 586 (1963); T. McEwan, *Nature* **201**, 827 (1964).
43. P. Goldman, *J. Biol. Chem.* **240**, 3434 (1965).
44. N. Horiuchi, *Nippon Nogei Kagaku Kaishi* **35**, 870 (1961); K. Tonomura, F. Futai, O. Tanabe, T. Yamaoka, *Agr. Biol. Chem. Tokyo* **29**, 124 (1965); M. Kelly, *Nature* **208**, 809 (1965).
45. P. Goldman, G. W. A. Milne, D. B. Keister, *J. Biol. Chem.* **243**, 428 (1968).
46. P. Goldman and G. W. A. Milne, *ibid.* **241**, 5557 (1966).
47. S. Kaufman, W. F. Bridgers, F. Eisenberg, S. Friedman, *Biochem. Biophys. Res. Commun.* **9**, 497 (1962).
48. W. A. Cowdrey, E. D. Hughes, C. K. Ingold, *J. Chem. Soc. London* **1937**, 1208 (1937).
49. J. E. G. Barnett, W. T. S. Jarvis, N. K. A. Munday, *Biochem. J.* **103**, 699 (1967).
50. P. Henkart, G. Guidotti, J. T. Edsall, *J. Biol. Chem.* **243**, 2447 (1968).
51. P. Goldman, G. W. A. Milne, M. T. Pignataro, *Arch. Biochem. Biophys.* **118**, 178 (1967).
52. G. W. A. Milne, P. Goldman, J. L. Holtzman, *J. Biol. Chem.* **243**, 5374 (1968).
53. S. Kobayashi, S. Kuno, N. Itada, O. Hayaishi, S. Kozuka, S. Oae, *Biochem. Biophys. Res. Commun.* **16**, 556 (1964).
54. J. W. Daly, G. Guroff, S. Udenfriend, B. Witkop, *Biochem. Pharmacol.* **17**, 31 (1968); J. Renson, *Fed. Proc.* **23**, 325 (1964).
55. G. Guroff, K. Kondo, J. Daly, *Biochem. Biophys. Res. Commun.* **25**, 622 (1966); G. Guroff, J. W. Daly, D. M. Jerina, J. Renson, B. Witkop, S. Udenfriend, *Science* **157**, 1524 (1967).
56. R. A. Peters, L. R. Murray, M. Shorthouse, *Biochem. J.* **95**, 724 (1965); R. A. Peters, M. Shorthouse, P. F. V. Ward, *Life Sci.* **4**, 749 (1965).

Genetic Load and Its Varieties

The term "genetic load" has been used to describe various situations, some harmful, some beneficial.

Alice M. Brues

In the aftermath of World War II, when the widespread dispersal of artificially produced radioactivity was a cause of serious concern, Muller discussed the problems of past and future damage to the genetic material of man in a paper entitled "Our load of mutations" (*1*). The term *genetic load* has been used since then to mean the abnormalities, deformities, and deaths produced in every generation by defective genetic material carried in the gene pool of man. Since the results of a mutation may not become evident until a number of generations later, it was feared at that time that radiation-induced mutations might reach dangerously high levels before the extent of the threat was appreciated.

The normal genetic load—the load that existed before the production of ionizing radiation by artificial means—consists of changes in the genetic material which occur at a very low but consistent rate and are referred to as "spontaneous" mutations, although numerous possible causes are known, including natural background radiation. It is sometimes stated that "all mutations are harmful." This is not strictly true: evolutionary progress has depended on mutations that were advantageous. But it is generally true, and two types of explanation are offered: (i) that in a complex and interdependent system such as the genetic configuration of a living organism, any change is more likely to disrupt function than to improve it, and (ii) that "good" mutations, when they appear, replace their precursors so rapidly that we rarely witness the process. In any case, the mutations of which we are most aware are those which result in impaired form or function. These tend, after appearing, to be eliminated as a result of shortened life span or lessened reproductive capacity of the individuals carrying the mutant genes. Under stable conditions, with the mutation rate and the intensity of selective elimination both constant, a particular mutant gene remains constant in numbers. Either an increase in mutation rate or a relaxation of selection will establish a new equilibrium at a higher level. (The effect of relaxed selection, resulting from medical alleviation of hereditary ailments, had been a cause of concern long before the possibility of increased mutation rates was envisaged, and was extensively discussed by Muller.) When an equilibrium has been established, the number of mutant genes eliminated per generation equals the number newly produced by mutation. However, if the equilibrium is disturbed, this is not immediately true; since many harmful mutants may remain in the gene pool for several or many generations before being eliminated, the full impact of genetic load will not be apparent until some time

The author is professor of physical anthropology and chairman in the department of anthropology of the University of Colorado, Boulder.

after the increase in mutation rate has occurred (*2*).

Mutation rates are commonly expressed by fractions representing the number of normal genes which, in the course of one generation, are found to have changed to mutant form. Selective effects are expressed in various ways. A useful way to express selection would be as the fraction of a particular class of individuals conceived who lived to reproduce, due allowance being made for reproductive spans cut short and for any degree of infertility. One would thus take into account the extent of random hazard to members of the species, which is hard to ascertain. However, it is easier to express the survival-reproduction capacities of various classes of individuals by parameters which express their *relative* biological success, without attempting to set absolute values. Such relative selection coefficients are of two general kinds. (i) "Loss" or "gain" parameters may be given, indicating differential selection with reference to a base level of 1 (this measure is preferred for algebraic demonstrations, in which parameters of the form "$1 - x$" often appear). (ii) The assumed base level may be incorporated in the coefficient, which then ranges from zero (which indicates total inability to survive or to reproduce) upward, not necessarily with an upper limit of 1, and represents a relative survival-reproduction index by which the number of individuals conceived may be multiplied to represent their contribution to the next generation (this measure is more convenient for numerical demonstrations and computer use). The latter measure is often called "fitness." In either case the base level of 1 may indicate any of various things: average survival, optimum survival of the most successful class of individuals, the amount of survival required to keep population levels constant, and so on. This is the choice of the individual researcher and leads to endless confusion if the relative nature of the selection coefficients is not understood. The assumption that plus and minus coefficients, as used for purposes of calculating changes in gene frequency, represent overall population gain or loss must be carefully avoided. Further confusion is caused by the fact that, in mathematical treatments of selection, the "surviving genes" left after the selection process has been simulated are usually converted to a percentage form, so that only relative gene frequency is represented and the possibility of a change in population size is studiously ignored (*3*).

Selection and Dominance

In a diploid organism, selection coefficients cannot be properly assigned to genes as such, except in the case of the unpaired sex chromosome genes in the male. The paired nature of ordinary somatic chromosomes requires consideration of the joint effect of the paired allelic genes. In respect to any one locus, there are three genotypes, and each may have its own selection coefficient. (Actually, interactions between genes at different loci may be important also.)

Mutations are conventionally classified as dominant or recessive, though such classification becomes increasingly difficult as our knowledge increases. Dominance in the ordinary sense results from our inability to determine genotype accurately through examination of the phenotype. If the heterozygote, which contains one normal and one mutant gene, entirely resembles, as far as we can tell with the available means of observation, the homozygote with two normal genes, the normal gene is considered to be dominant and the mutant recessive. If closer examination or an improved technique reveals that the heterozygote is to some degree intermediate, we say that dominance is incomplete. If we are concerned with selective advantage and disadvantage only, dominance must be defined in terms of relative selection coefficients. If the heterozygote entirely resembles the normal homozygote in net survival and reproduction capacity (that is, if the two have identical selection coefficients), the normal allele is considered fully dominant in respect to selection. If the selection coefficient of the heterozygote is merely closer to that of the normal homozygote than to that of a mutant homozygote, but not identical to it, the normal allele is considered incompletely dominant. In a corresponding way, complete or partial recessiveness, respectively, would be demonstrated if the heterozygote were identical to, or approximated, the mutant homozygote in respect to selection coefficient.

In the case of genes which have both a visible effect and a selective effect, caution should be observed in assuming that dominance in respect to visible trait is the same as dominance in respect to selection, except in such an obvious case as the reduced reproduction of achondroplastic dwarfs, which is a direct effect of their appearance and the way other people respond to it. This caution is particularly pertinent in cases where the heterozygote appears to have a higher selection coefficient (that is, appears to be more viable) than either homozygote, an interesting condition that is discussed below. This situation, called heterosis, has no obvious parallel in ordinary dominance of visible or serologic traits, unless we compare it to co-dominance, as of the A and B blood groups, which give a double reaction in the AB genotype. Population studies on the A-B-O blood groups, in fact, suggest that in this system all the heterozygotes have some selective advantages (*4*, *5*). If this is the case, those who have conducted clinical studies designed to reveal possible disease susceptibilities associated with the alleles of this system may have badly confounded their data by taking serologic phenotypes, such as "A," which includes both the homozygote AA and the heterozygote AO, as a basis for analysis, since selective effects on the homozygote and heterozygote may be quite divergent, contrary to what might be expected from the dominance of the alleles in regard to serologic phenotype.

As in the case of the harmfulness of mutants, we make a shaky generalization that most mutants are recessive. Apparently the actions of genes are mediated by the formation of enzymes within the cell, which in turn bring about specific biochemical reactions. Since enzymes are often required only in very small amounts, it is probable that in many cases a single normal gene can produce an adequate amount of enzyme, and therefore the biochemical function of the heterozygote is not detectably different from that of the homozygote. This suggests that often the mutant gene is so changed as to become functionally inert. A dominant mutation may be interpreted as a rarer sort of event in which the altered gene still functions but in a different and often disruptive way. Heterozygote advantage may represent a situation in which the mutant gene and its precursor are useful in slightly different ways, so that the presence of both genes in the same individual results in greater biochemical efficiency or versatility (*6*).

Genetic Equilibrium

The establishment of equilibrium levels of mutants is important in determining genetic load. The mutation rate interacts with the selection coefficients of all three genotypes in the establishment of equilibrium. The simplest case is that of a mutant which continues to appear at a low constant rate but is consistently eliminated due to a disadvantage which it confers on those carrying it. If the mutation is lethal and strictly dominant—that is, incompatible with life or reproduction even in the heterozygous form—it immediately exterminates itself. In this case, if the individuals in whom the mutant appears live long enough to be counted, the number of recorded cases in any generation equals the number of mutations. If adverse selection is less than complete, mutant genes may accumulate to some extent.

If, instead, the mutation is lethal and strictly recessive—that is, if it affects survival or reproduction in the homozygous form but not in the heterozygote—a long time may elapse before two of the mutant genes meet in the same individual, manifest their effect, and are eliminated. In a large randomly breeding population the fraction of homozygous individuals produced equals the square of the gene frequency. This is an extremely small fraction if the gene frequency of the mutant is 0.01 or less. At a low frequency, therefore, the recessive lethal is virtually unaffected by adverse selection against the homozygote. In such case the gene frequency slowly increases as a result of repeated mutation. The equilibrium point is reached when the gene frequency is sufficiently high to produce enough homozygotes so that the selection against them removes genes as fast as they are being produced by mutation. If a mutation rate is 0.0001 (a value which is considered high as mutation rates go), 1 out of 10,000 normal genes is converted into a mutant in every generation, and the frequency of the mutant can climb to 0.01 in the case of an entirely lethal recessive. At this point 1/10,000 (0.01^2) recessive individuals will be produced in an average generation, and the loss, through death, of their genes will just balance the production of new ones by mutation. If the lethality of the recessive mutation is only partial, the equilibrium will occur at an even higher gene frequency. Thus the point of equilibrium depends on the mutation rate, the amount of adverse selection, and the degree of recessiveness, for if the heterozygote participates to some degree in the adverse selection (a condition known as incomplete recessiveness), selective loss is greater, most markedly so at low gene frequencies.

A mechanism is possible whereby even higher equilibrium levels might be attained (*7–9*). If different loci interact in such a way that their disadvantageous effect is fully expressed only when there is a coincidence of recessive conditions at several loci, the "affected individuals" will appear very sporadically indeed. Such a situation would exaggerate the effect of single-locus recessiveness by permitting higher equilibrium levels of each of the individual genes involved, and is, in effect, a "superrecessiveness."

In the long run, provided the processes outlined above are the only ones acting, deleterious genes are eliminated at the same rate at which they are produced by mutation. The frequency of a particular mutant in the gene pool at any one time is proportionate to the average length of time that elapses between its production and its elimination (*1*). The more recessive and the less deleterious the gene, the longer this time and, therefore, the higher the equilibrium frequency.

Heterosis

The type of selection in which the heterozygote has a higher selection coefficient than either homozygote leads to interesting and paradoxical results. In this case both genes are, in effect, advantageous when they are combined in the heterozygote but either one is disadvantageous when it is present in a homozygous individual. The balance of selective advantage between the genes therefore varies with the gene frequency, each gene becoming relatively undesirable as it becomes too common. Equilibrium is arrived at when a frequency is attained at which each generation's loss of homozygotes, at their respective rates of selection, is such as to leave the gene frequency the same. If the two homozygotes have equal selection coefficients, the gene frequency will stabilize when the two genes are equal in numbers, each 50 percent; if the selection coefficients are unequal, there is an excess of the gene which has the most viable homozygote when equilibrium is attained. In these cases the mutation rate can be largely ignored, since even the most rarely occurring mutation, if favored by heterozygote advantage, will multiply until it reaches the equilibrium determined by selection.

If the heterozygote is the most fit of the three genotypes, the gene of which the homozygote is the least fit may reach much higher equilibrium levels than it would if it were a simple disadvantaged recessive. In fact, such a gene may be completely lethal in the homozygous form and still be maintained at a substantial level in the population. A classic case is that of the sickle-cell gene, which results, in the homozygote, in a severe and generally fatal anemia, so that the gene appears to be a recessive lethal. Considerable mystery attached to the fact that this gene occurred at quite high levels (to nearly 20 percent) in certain areas of Africa and other parts of the Old World tropics. Since this gene is virtually completely lethal, such a situation seemed inexplicable unless there were an extraordinary and geographically limited mutation rate. The notion of a specific mutation rate's being affected by environment in this fashion is entirely at odds with our knowledge of mutation. In this case the heterozygotes can be identified by the fact that their red blood corpuscles, though not likely to shrivel spontaneously as those of the anemic homozygotes do, can be induced to do so if they are suitably abused in the laboratory. This facilitated investigations which now have demonstrated that a heterozygote advantage exists, due to the superior resistance of the heterozygote to tertian malaria, the geographical distribution of the sickling gene being in fact closely correlated with prevalence of the malaria parasite (*10*). A moderate selective advantage of the heterozygote (which is eight times as common as the homozygote, at the gene frequency of 0.2) is sufficient to counteract homozygote lethality and maintain the gene frequency in equilibrium.

The prevalence of heterozygote advantage is hard to evaluate, though it probably underlies the maintenance of many genetic polymorphisms—that is, balanced conditions where two or more genes at the same locus appear to coexist in many populations and presumably for long periods. Such polymorphisms are known to exist in a great variety of animals, with respect not only to visible variation but to many serologic traits, of which the

blood groups in man are only one example. Also, heterozygote advantage undoubtedly is responsible for the phenomenon of hybrid vigor, which is sufficiently marked in many species to have valuable commercial application.

Heterozygote advantage introduces complications into the relation of mutation rate to elimination of mutants, since an advantage on the part of the heterozygote will cause the mutant gene to increase its numbers far more rapidly than it would by mere recurrence of mutation: all the sickle-cell genes in the world, in fact, may be descendents of one or a very few mutations. In this case the number of genes eventually eliminated by the deaths of homozygotes is not simply equivalent to the number produced by mutation but an inflated number, due to a disproportionate multiplication of the mutant genes within the gene pool. There are thus, obviously, far more deaths per generation from sickle-cell anemia than would be the case if the gene were a simple deleterious recessive.

The Measurement of Load

Muller, in his original paper, defined genetic load in terms of "either the proportion of the population suffering genetic elimination or the amount of disability suffered by the average individual." The actual measure of load is the number of "genetic deaths" per unit population, the average disability being a derived figure. *Genetic death* is a composite term which includes literal death due to genetic disability and also any degree of failure to reproduce in normal amount. The number of genetic deaths bears a simple relation to mutation rate and dominance, if the mutation rates are constant for a length of time sufficient to allow the mutation to reach equilibrium. Either one or two mutant genes are lost per genetic death in the case of one-locus systems. Death of a strict recessive does the job most efficiently, since the elimination of each homozygote results in the loss of two mutant genes; if a mutant is dominant, each death destroys only one mutant gene (*1*).

I have referred above to interaction effects which, by allowing equilibrium levels of mutants to rise, constitute a "superrecessiveness." These effects also extend the effects of recessiveness in respect to efficiency of mutant gene elimination (*6*, *7*). If different loci interact in such a way that the selective disadvantage of a combination of deleterious genes at more than one locus is more than the simple sum of the effects of each locus separately, the individuals affected will be disproportionally drawn from those having deleterious genes at more than one locus. Elimination of such an individual from the breeding population will withdraw a number of deleterious alleles from the gene pool simultaneously; thus, whereas a genetic death due to a dominant eliminates one mutant gene, and a genetic death due to a recessive eliminates two mutant genes, in the interactional system more than two genes are likely to be removed simultaneously. In the case, for instance, where an adverse effect is apparent only when there is coincidence of mutant homozygosity at each of two loci, four mutant genes would be involved in each genetic death. Much remains to be learned about the effects of such complex systems, the extent to which they actually exist, and whether, as has been suggested, they can account for levels of gene frequency commonly attributed to heterosis. Partial dominance results in an intermediate degree of efficiency. To the parent or breeder, the recessive is more dreaded, since its phenotype appears unexpectedly; genetically, it causes less damage per mutation. If genetic disadvantage is not total, gene elimination does not occur every time the disadvantaged genotype appears; in this case the gene or genes remain in the gene pool and the ultimate loss is merely delayed until a later generation.

A useful concept in the measurement of genetic load is that of lethal equivalents suggested by Morton, Crow, and Muller in 1956 (*11*). A lethal unit may be a single recessive gene which would be completely lethal if manifested in the homozygous form, or it may represent a sum of recessive genes not fully lethal, having detrimental effects such that the likelihood of their producing death in the homozygote adds up to 1.0. Thus a measure of total genetic risk is arrived at which subsumes all degrees of lethality.

The average number of lethal equivalents per individual in a population can be investigated through the study of inbreeding effects. A lethal or detrimental gene which is rare (as most obviously are) will, in a randomly mating population, meet with its counterpart and thus produce a homozygous individual only very rarely indeed. However, if there is any common ancestry between parents, the possibility of a lethal gene's meeting an identical lethal gene (derived from the same common ancestor) is greatly increased. The belief that there is a greater likelihood of defective offspring when the parents are related by blood is very old, long antedating knowledge of its genetic basis. Experimental inbreeding, therefore, or the searching out of consanguineous marriages in man, gives valuable information about the frequency of lethal genes. An overall estimate can be made without dividing the various genetic deaths into separate clinical classes. On this basis Morton, Crow, and Muller, counting all deaths from late fetal to early adult life as probably genetic, estimated that the average person carries between three and five lethal-gene equivalents (*11*). They considered this an underestimate, due to the fact that early fetal deaths and later adult deaths were not included. At about the same time, Dobzhansky calculated that, in the fruit fly, a fourth to a third of all chromosomes contained at least one lethal or semilethal gene (*12*). This is an interestingly large figure for a species which is totally exposed to natural selection and which has a low rate of individual survival but is conspicuously *not* a threatened species.

Segregational Load

An interesting question which arises in the evaluation of genetic load is the effect of heterozygous advantage on the total load of a population. A distinction has thus been made between "mutational load"—that portion of the genetic load which represents deleterious genes produced by mutation—and "segregational load"—that portion which represents an excess over and above the number of mutations, which has accumulated within the gene pool as the result of selection favoring heterozygotes. This distinction is of practical importance in estimating the probable effect of increased mutation rates, for if a considerable portion of man's genetic load is due to heterozygote advantage, the total mutability of human genes may be less than we would otherwise estimate it to be. In 1958 Crow presented a theorem which compared the amount of load apparent in a randomly breeding population with that in an inbred population for the two cases: (i) the mutant maintained by recurrent mutation alone, and (ii)

the mutant maintained by heterozygous advantage (*13*). He showed that the increase in incidence of genetic deaths which followed inbreeding was much greater in the case of "mutational load" than in that of "segregational load," the increase in the latter case being limited to doubling of the amount.

This theorem has raised some questions in regard to the base level from which load should be measured (*14, 15*). In the case of a deleterious mutant which is partially dominant—that is, disadvantaged in the heterozygous as well as the homozygous form, or, at most, neutral in the heterozygote—the normal homozygote is generally considered fully fit (fitness, 1.0; associated load, 0). The other two genotypes are assigned fitnesses of 1 or less. When the heterozygote is the most fit genotype, it has seemed logical to some people to rate it fully fit and assign a load to both homozygotes (*13*). To others this does not seem logical at all, since the heterozygote cannot be established as the sole genotype of any population, except in special circumstances not applicable to man or higher animals (*16*). Some dissenters have suggested that the most fit *homozygote* be assigned the rating of 1.0. This necessitates a "negative load" for the heterozygote, for which fitness then becomes greater than 1.0. An "average fitness" base level has also been suggested; this, too, involves negative loads (*14, 15*). Sanghvi has pointed out (*15*) that if both homozygotes, the common normal one and the rare mutant, are considered to be inferior, a large part of the total load in a randomly mating population is made up of a very low risk of death to the more abundant normal homozygotes, the remainder of the load being contributed by a higher risk to the rare mutant homozygotes. Under this system, at least half of the population must be subject to some degree of load. This brings the total load of the randomly breeding population to such a high level that it cannot be more than doubled by total inbreeding (inbreeding of a type not feasible in man, resulting in homozygosity of *all* individuals).

If genetic load pertains only to one homozygote, and that the one which is extremely rare, the load under random breeding is very low and is much more conspicuously increased by inbreeding. We have no assurance, however, that in the segregational situation the mild deficiency of fitness in the normal homozygote will manifest itself in a way that is comparable to the manifestation of the severe deficit of the mutant homozygote. A real lethal gene, at a load of 100 percent, should kill all its phenotypes before they reach puberty. If a genotype with a load of 5 percent showed its deficit by death before puberty of 1 out of 20 individuals, the other 19 being quite normal and healthy, the comparison would be simple. However, the mild deficit, if it shows itself by a moderate lack of energy and resistance in adult years, or by lowered fertility, would not be adequately detected by surveys designed to identify serious genetic defects (*15–17*). If, as a result of this difficulty, only the major defects due to the rare mutant homozygote were fully counted, the inbreeding effect detected for the segregating locus would approximate that for the nonsegregating locus. Not surprisingly, the application of this load ratio has given contradictory results, and the original hopes that it would give an unequivocal estimate of the amount of load maintained by heterozygote advantage, as compared to that arising directly from mutation, have been disappointed (*11, 18*). (A point not generally considered in the effect of inbreeding on load is that the increase in homozygosity of the inbred population increases the number of adversely affected individuals only if the deleterious alleles are recessive—which, to be sure, they commonly are. A dominant defect, such as achondroplastic dwarfism, will become rarer if homozygosity is increased by a generation of inbreeding.)

Actually, one may seriously question whether the segregational load is a load at all in the sense that mutational load is. This again involves the question of the base level from which load is measured. Obviously the maximum load will be obtained when the optimum genotype is used as a base and all others are declared deficient. The fact that the optimum genotype, if a heterozygote, cannot be established in any entire population of a higher animal is perhaps not the most important point. If we are interested in relative load as a means of comparing the success of two populations with different genetic equipment, the comparison cannot be fairly made unless the standards of fitness applied to the two are the same. If we use a selection coefficient of less than 1 for a homozygote in a population in which a superior heterozygote is present, we should use the same coefficient for the same homozygote in a population in which the heterozygote is not present, if we wish to compare the fitness of the two populations. Only if our interests are limited to determining gene-frequency change or equilibrium point within a single population are we justified in establishing our selection coefficients on a basis unique to that population.

A meaningful way to evaluate the benefit or detriment which a gene confers on a population is to compare, using some consistent set of selection coefficients, the present state of the population with what its state would be if, given the same environment, the gene in question had never been introduced into it. The classical situation of sickle-cell anemia can provide an example—that is, a comparison between a population with the sickling gene present at a moderate frequency and a population homozygous for normal hemoglobin, both living in a malarial environment.

One possible equilibrium condition would be a frequency of the sickling gene of 0.1, a frequency of the normal allele of 0.9, and selection coefficients of 1.0 for the normal homozygote, 1.125 for the heterozygote, and 0 (complete lethality) for the sickling homozygote. This is actually a lower frequency of the sickling gene than occurs in many populations, but it will serve as an example. These parameters give a fitness of 1.0 for the control (normal homozygote) population.

The population containing the sickling gene will produce offspring in the proportion of 0.81 homozygotes for the normal gene, 0.18 heterozygotes, and 0.01 homozygotes for sickling. If all other circumstances are the same as in the control population, the surviving normal homozygotes in the affected population will equal 0.81 of the parent population; the 0.01 sickling homozygotes will die, each death eliminating two sickling genes; and 2¼ heterozygous individuals per 100 individuals will be added, this increase representing individuals spared from the malarial death to which homozygotes in the same environment are maximally subject. The relative frequency of sickling genes remains the same (since we purposely chose equilibrium parameters), and the population size has increased by 1¼ percent as compared with the control population. Various other absolute values might be chosen for the selection coefficients, but if the proportionate survival of the several genotypes remains the same and

the same values are assigned to the same genotypes in both the control and the affected population, the affected population will show a relative population increase when the gene frequencies are at selective equilibrium, and, in fact, at a considerable range of gene frequencies on either side of equilibrium.

How is it, then, that "segregation load" has been taken to indicate a detriment to a population? Because, by the rule that the most viable genotype in a population should be taken as the basis for assigning selection coefficients, different sets of coefficients were used for the control and the affected populations.

One author writes (*6*), "It is even doubtful if the very considerable increase of resistance to malaria postulated for heterozygotes for the sickling gene is sufficient to balance the misery and early death of homozygotes with the full disease picture." This is a somewhat unimaginative point of view, for it overlooks the fact that the "increased resistance," translated into concrete terms, means that 2¼ individuals are spared from misery and early death by malaria for every one that succumbs to sickle-cell anemia. Given the particular environment, the sickle-cell gene is evidently an asset to the population, despite its highly undesirable "side effects." It is the malaria-ridden environment which has imposed a load; the genetic makeup of the population is, in an imperfect way, alleviating it.

Much remains to be learned about the frequency and importance of loci with heterozygote advantage. Surprisingly, in man, several other loci are known in which, as in the case of sickle-cell, the advantage of the heterozygote is due to resistance to malaria. Loci with respect to which neither homozygote is seriously disadvantaged do not lend themselves readily to study except insofar as we infer heterosis from the existence of balanced equilibriums of two or more alleles (*4*). Some experimental studies have shown that, in general, the so-called recessive detrimental genes are incompletely recessive—that is, the heterozygotes are somewhat selected against also (*19, 20*). However, this effect is an average for a wide range, from clear disadvantage of the heterozygote to definite heterosis. Even though the loci in which the heterozygote has superior fitness may be fewer than those in which it is disadvantaged, selection ensures that the genes with heterozygote advantage will be maintained at much higher frequencies in any natural population, the incompletely recessive lethal being eliminated even faster than the complete recessive, and the heterotic gene reaching a higher equilibrium level than any simple lethal gene (*21*). However, until much more work is done on this aspect of genetic disability, the very healthy individual will remain an enigma: we do not know to what extent he owes his vigor to an unusual freedom from incompletely recessive detrimental genes in heterozygous condition and to what extent to an unusual wealth of heterotic genes in heterozygous condition. Probably a fortunate combination of the two is required.

Substitutional Load

Another problem related to that of genetic load was discussed extensively by Haldane in 1957 under the title "The cost of natural selection" (*22*). The "cost" is the number of deaths required to eliminate a gene which is in process of being replaced by another allele. He calculated that the number of deaths needed is fairly constant, a greater number of deaths per generation resulting in a more rapid completion of the replacement, and that the total number of deaths required amounted to 10 to 20 times the number of individuals living at any one time. This figure was based on an initial frequency of the favored phenotype of 1/10,000. Since the replacement process is slow at first, and the mortality most severe at this stage, the number of genetic deaths is considerably reduced if the favored gene is present in larger numbers when the process begins. Haldane was concerned with the problem of how rapidly evolution could take place at all if such a large amount of mortality was involved in gene substitution. Pertinent to this is the suggestion that, to some degree, selective effects may be enhanced by the interaction of genes, so that one genetic death may eliminate more than the expected number of deleterious mutations. It is interesting to note that the interaction effect has been referred to as a "component of the genetic load" (*7, 8*), although in an equilibrium situation this effect would lead to greater population fitness per specific mutation rate because of the smaller number of genetic deaths required for the elimination of deleterious alleles. It is true that, if an interactional system were to be put into effect suddenly in a population which had been operating under other rules, an extra burden would be imposed, but this is an event possible only on paper. Kimura referred to Haldane's work in a later paper (*23*), in which he added to mutational load and segregational load a third type of load—"substitutional or evolutional load." He states in reference to the latter, "The process of substituting one allele for another through natural selection involves lowering of population fitness and thus creates a genetic load."

The concept of substitutional load involves us again in the thorny question of base levels of fitness. In a situation where gene replacement occurs, it would seem a truism that the new gene which is increasing in numbers does so because it confers a greater fitness on its possessors. The genetic load involved in the substitutional situation is in fact an artifact of the change in base level which we must make if we adhere to the definition of fitness in terms of the optimum genotype. The appearance of a new advantageous gene in even the smallest numbers creates a new optimum genotype in relation to which the formerly optimum genotype is demoted, accused of contributing a large amount of load, and blamed for a loss of population fitness. Actually, the appearance and multiplication of a new and advantageous gene leads to an increase in population fitness (*24*).

The expected course of events would be an increase in numbers of the new gene, but not at the expense of the old one (that is, with some increase in total population size) until such time as the limits of subsistence produced mortality from overcrowding (*24*). Then, of course, excess deaths would occur. However, at no time would the species be threatened by sheer loss of numbers, as would be implied by use of the term *load*. If a new gene increases the ability of the species to exploit its environment, or opens more ecological niches to it, the increase in population may be unpredictably large.

A closer look at the example given by Haldane reveals that he pictured the gene replacement as being a forced one, due to a change of the environment which caused excessive mortality of the original genotype, and cited as an example that eminent British moth *Biston betularia*, whose response to a sooty environment has become a classic of natural selection. In

this case "load" is certainly present, but it can hardly be called genetic, since it is strictly external in origin. In fact, if no alternative gene existed and no replacement occurred, the population might well be faced with extinction (*25*). The replacement process offers an escape: if the replacement is rapid enough, the gradual decrease in environmentally caused deaths will enable the population to pull through, in contrast to a control population which finds itself in the same situation without the alternative gene which is able to cope with the environmental change. To evaluate this situation properly, we must observe the same caution that is needed in estimating segregational load. The comparison must be made between populations in the same external situation, one with and one without a particular gene, and whatever standards of fitness are used must be the same for the two populations. It is not correct to compare the fitness of the struggling population in the deteriorated environment with its fitness in the former environment, and it is not correct to rate a population of uniform inadequate genotype as maximally fit because no individual is worse off than any other, and to rate another population as subject to load because a few individuals are functioning adequately. For purposes of algebraic and mathematical handling, the substitutional load can be placed in the same framework as mutational load; in its significance to the welfare of the species, it differs.

Good and Bad Loads

It seems apparent that some of the confusion and controversy surrounding the concept of genetic load is due to the fact that the parameters used do not reflect the realities of species success. The gain and loss attributed to the various genotypes are relative and tell us nothing about whether total population size is being maintained, increased, or decreased from one generation to another. Neither do they tell us the actual probability that an individual of the species will survive to complete his reproductive activity. The mathematical convenience of the load calculations is due to the elimination of these considerations, which are not pertinent to the more limited question of stability or change of gene frequencies.

If we examine the different types of situation in which "load" has been described, we see that, in spite of the fact that the same form of calculation may be applied to them, their significance for the welfare of the species differs. Mutational load—the original Mullerian load—we can concede to be all bad. It is the result of actual deterioration of the genetic material, in which there is no profit. The elimination of the mutants by genetic death is a tedious maintenance job which, at best, keeps the genetic structure of the population in working condition. Segregational load presents a paradox. It can result in clearly identifiable genetic deaths, yet its overall effect on the population is a favorable one, and the species is not, as it might appear to be, betrayed by natural selection. Perhaps it is offensive to our sense of fairness: it would be more democratic to have everyone slightly sickly than to have a few die outright (*6*). In the case of substitutional load, the effect on the population is definitely favorable, even though the mathematical expression of it can be made to simulate mutational load. If substitutional load occurs in response to an environmental crisis, it may coincide with increased mortality, but it is not the cause of it.

As we follow the history of the word *load* and of its corollary *fitness*, we see that, at the time of the definition of segregational load and, again, of substitutional load, as contrasted with the original mutational load, the terms ceased to have their vernacular connotations and became, instead, technical terms for mathematical constructs. It would seem advisable, in the interests of good sense and adequate communication, that when this change takes place, as it frequently does in scientific work, the vernacular words should be replaced by obviously specialized ones which will mislead no one into believing that he understands their meaning when in fact he does not. Such a disentanglement of everyday and technical meaning would certainly relieve controversy and allay misunderstanding. At the time of Muller's original paper, in 1950, the use of a familiar word with an obvious emotional impact was appropriate: one need only reread his references to some of the pre-1950 radiological practices, now quite incredible, to realize that the situation called for a reformer's zeal and oratorical skill. But at the present time the remnant of this emotional charge, still attached to the concept of "load," a term now used with a variety of meanings, leads only to confusion.

Examination of the various situations in which genetic load has been described shows that they have one thing in common: the populations under consideration are polymorphic with respect to viability. Do they have anything else in common? If not, then perhaps *viability polymorphism* would be a suitable neutral term which could be applied to these situations without prejudice to the evaluation of the ultimate effects, whether favorable or unfavorable, on the welfare of the species. Not a few of the difficulties of the load concept are due to the fact that it has attempted to wrap up in one measure both the mean and the variance of a population's viability, by a rule which implied that increased heterogeneity must be associated with decreased mean fitness. It would be advantageous to keep the mean and the variance conceptually distinct, recognizing that the *mean* fitness of a population involves biological and ecological factors of considerable complexity. The *variance* of fitness, with its specific effects on gene frequency through time, can be dealt with mathematically in an unambiguous way if the difficult question of overall population success is abstracted from it. According to Muller's original definition of "mutational load," increased heterogeneity always indicated lowered fitness; in this particular case the term *load* might appropriately be retained. For the other types of "load," however, the substitution of a neutral term such as *viability polymorphism* would facilitate understanding.

References and Notes

1. H. H. Muller, *Amer. J. Human Genet.* **2**, 111 (1950).
2. J. F. Crow, *Eugenics Quart.* **4**, 67 (1957).
3. W. Feller, *Genet. Res.* **9**, 1 (1967).
4. A. M. Brues, *Amer. J. Phys. Anthropol.* **21**, 287 (1963).
5. S. P. H. Mandel, *Heredity* **13**, 289 (1959).
6. G. R. Fraser, *Ann. Human Genet.* **25**, 387 (1962).
7. J. L. King, *Genetics* **53**, 403 (1966).
8. J. M. Smith, *Nature* **219**, 1114 (1968).
9. J. A. Sved, T. E. Reed, W. F. Bodmer, *Genetics* **55**, 469 (1967).
10. A. C. Allison, *Brit. Med. J.* **1954-I**, 290 (1954).
11. N. E. Morton, J. F. Crow, H. J. Muller, *Proc. Nat. Acad. Sci. U.S.* **42**, 855 (1956).
12. T. Dobzhansky, *Science* **126**, 191 (1957).
13. J. F. Crow, *Human Biol.* **30**, 1 (1958).
14. C. C. Li, *Amer. J. Human Genet.* **15**, 316 (1963).
15. L. D. Sanghvi, *ibid.*, p. 298.
16. J. F. Crow, *ibid.*, p. 310.
17. N. E. Morton, *ibid.* **12**, 348 (1960).
18. J. V. Neel and W. J. Schull, *Proc. Nat. Acad. Sci. U.S.* **48**, 573 (1962).
19. J. F. Crow and R. G. Temin, *Amer. Naturalist* **48**, 21 (1964).
20. Y. Hiraizumi and J. F. Crow, *Genetics* **45**, 1071 (1960).
21. T. Dobzhansky, *Amer. Naturalist* **48**, 151 (1964).
22. J. B. S. Haldane, *J. Genet.* **55**, 511 (1957).
23. M. Kimura, *ibid.* **57**, 21 (1960).
24. L. Van Valen, *Amer. Naturalist* **47**, 185 (1963).
25. A. M. Brues, *Evolution* **18**, 379 (1964).

26. I thank the National Science Foundation for support under grant GB5169.