SELECTIVELY NEUTRAL ALLELES WITH SIGNIFICANT PHENOTYPIC EFFECTS: A STEADY-STATE MODEL

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Abstract: At least much allelic variation is probably expressed in the supramolecular phenotype, with effects on fitness; however, both mutation and molecular evolution are probably almost always effectively neutral selectively.

This apparent paradox can be resolved by a new way of looking at the genetic control of phenotypic evolution. Even strong fitness effects can be associated with selectively neutral alleles. In a polygenic system the background genotype interacts with the locus considered, in a positive-feedback manner, yet the selective effects per locus are probably so small that they are unimportant in determining the fate of individual alleles. A similar conclusion holds for effects of a fluctuating environment. Thus polygenic variation is not equilibrial but is in a steady state of continual turnover.

Phenotypic evolution occurs by changes in these flowing polygenic systems, not by selection of alleles from low frequency to fixation. Artificial directional selection is a poor analogy for natural selection, although pleiotropic interactions are important for both. The overall amount of natural selection may be unrelated to the rate of phenotypic change.

A variety of apparently unrelated or contradictory phenomena are readily explained by this way of looking at the shifting-balance approach.

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Introduction. The application of electrophoretic techniques and protein sequencing to questions of genetic variation (Hubby and Lewontin 1966) and evolution (Zuckerkandl and Pauling 1965), respectively, immediately made it apparent that the accepted neodarwinian paradigm could account neither for the observed high level of genic polymorphism (Lewontin and Hubby 1966) nor for the large and remarkable steady rate of molecular change at the gene level (Kimura 1968). Responses to this contradiction coalesced around two schools of thought and became known as the neutralist-selectionist controversy. The controversy has abated; selectionists have demonstrated the pervasive effects of natural selection, and neutralists have demonstrated the at least equally pervasive effects of stochastic factors in genetic evolution. A new paradigm has not emerged, however.

In the initial response, selectionists proposed that the logic or mathematics of the neodarwinian paradigm was repairable, and that population variation and molecular evolution could still be accounted for by balancing selection (e.g. King 1967, Milkman 1967) and by positive Darwinian selection (e.g. Sved 1968, Maynard Smith 1968, Stebbins and Lewontin 1972) respectively. After the neutralist hypothesis was proposed (Kimura 1968), neutralists proposed that most polymorphism and evolutionary change on the molecular level did not extend to other levels, and implied that the neodarwinian synthesis held unchanged for the populational variation and morphological change due to those genes that do affect phenotype and fitness. The presumption was that alleles affecting the gross phenotype could hardly be neutral, and that functionally important evolution occurred at a minority of loci against a background of extensive but essentially meaningless molecular change (King and Jukes 1969, Ohta and Kimura 1971). Only Ohta (1972a, 1976) began to explore a new paradigm of functional evolutionary change -- the evolutionary impact of "nearly-

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[+]Deceased, 1983. The abstract, figures, and specific references were supplied by the editor, who hopes that they are about the ones King wanted.

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neutral" mutations -- although she prefers to consider her work to be an extension of neutral theory.

The evidence for and against the neutral hypothesis has been equivocal, as is well known. On the one hand, mathematical analyses of population variation and patterns of molecular evolution usually appear to support the neutralist position. The strongest general support of the neutralist hypothesis has been the remarkably regular rate of change in given protein families and in total DNA and the corollary observation that molecular evolution appears to be thoroughly decoupled from morphological evolution. This is consistent with the notion that only a negligible
percentage of molecular changes are associated with adaptive evolution. The selectionist position, on the other hand, has always been strongly supported by the general observation that natural populations have abundant genetic variation in genes that affect the phenotype in almost any conceivable way; in particular, artificial-selection experiments unfailingly uncover ample underlying genetic variability for virtually any trait the experimenter may choose to select for (e.g. Wallace 1968; Milkman 1970).

Actually, neither classical newdarwinian theory nor neutral theory can adequately account for this general high level of functional genetic polymorphism. Selectionists have tended to explain this variability by citing the numerous deterministic models of balanced genetic equilibria, notably overdominance and frequency-dependent selection. For a variety of good reasons, neutralists have tended to reject deterministic equilibrium models, but leave unexplained the easily demonstrable existence of abundant, pervasive, and phenotypically effective genetic polymorphism.

I propose that both positions are essentially correct in their key premises. However, to the extent that both models of genetic evolution depend on the neodarwinian view of the emergence and fixation of rare beneficial alleles, the models may share inadequacies and errors. Thus I propose that much and possibly most molecular variation is effective in individual variation in phenotype as well as fitness; and that, at the same time, variation and evolutionary change at individual loci is almost entirely due to selectively neutral mutations and random genetic drift. These are not incompatible hypotheses. Selectively neutral alleles need not be without phenotypic effect.

A hypothetical example of a selectively neutral, phenotypically effective allele. In all populations for most polygenic traits nearly all the time, most natural selection is of the stabilizing type in which there is an optimal phenotype (or phenotypic range) in the neighborhood of the population mean, and natural selection selects against individuals toward either extreme of the distribution. The distribution of phenotypes can be represented by a normal distribution (Figure 1a) although any unimodal distribution would suffice. The curve of fitness as a function of phenotype can likewise have any shape so long as it is unimodal with the fitness optimum somewhere near the phenotypic mean. Variation in the phenotype typically has both genetic and environmental components.

Let us consider a single locus in this polygenic system. For simplicity's sake, since this is a hypothetical example, let us consider only two genotypes at this locus, either because the organism is haploid or because there is complete dominance. The genotypes are (-) and (+); the effect of the minus allele (or genotype) is to move the phenotype of an individual to the left, while the effect of (+) allele or genotype is to move the phenotype to the right. In Figure 1b, the two curves represent the phenotypic distributions of all (-) and (+) individuals respectively. The means of the distributions are displaced to the left and right of the fitness optimum, respectively, although both distributions greatly overlap the fitness optimum. The displacement is such that the mean fitness of each distribution is the same. The genotypes are selectively equivalent and the alleles are selectively neutral.

The alternate alleles of Figure 1b are not in any kind of an equilibrium. Their selective equivalence is not a direct function of their relative frequencies. If the fitness optimum and the distribution of background genetic and environmental effects are held constant (an unlikely assumption), the (-) and (+) alleles are free to increase or decrease in frequency through random genetic drift and recurrent mutation, exactly as

befits neutral genes. At the same time they each contribute to the geneitc component of phenotypic variance. The principal point of this hypothetical example is to demonstrate that phenotypic effects with strong fitness interactions are not logically imcompatible with selectively equivalent alleles. It would seem, in passing, that the term "neutral" has always meant "selectively equivalent," but seems to have been a poor choice of word in that it implies an absence of functional difference as well.

How realistic is this example? There are clearly some problems with it. First, the exact balance between alleles is unlikely. Second, the model does not apply fully to loci with three or more alleles, nor to loci in diploids with other than full phenotypic dominance. Third, neither the background genotype nor the fitness optimum are likely to remain stable; the genotype changes through selection and drift, and the fitness optimum will always be a function of a fluctuating environment. Fourth, loci active in one polygenic system generally are active in others: Sewall Wright, for one, has repeatedly stressed the generality of pleiotropy in genetic systems. On the other hand, and exact balance is not necessary. Kimura and Ohta (e.g. 1971) have shown that an allele behaves as if selectively equivalent if the difference in selection coefficient is within + $1/N_e$ of zero. With regard to the question of an exact balance, then, it seems likely that a substantial proportion of the genetic variability underlying polygenic systems under stabilizing selection may be due to mutation and drift in effectively neutral polymorphisms. This genetic variability is available to respond to artificial directional selection, at which time the phenotypic effects would cease to be selectively equivalent.

Partial phenotypic dominance in a polygenic system leads to overdominance in fitness. If the organism is diploid, and if the two homozygous genotypes straddle the fitness optimum, the phenotypic distribution of a heterozygote with partial dominance would more closely approach the optimum and the heterozygous genotype would have the highest fitness. Thus there would be component of overdominance in fitness, and its corollary of inbreeding depression. Unless the effects are large, however, such overdominance does not lead to stable allele frequencies in polygenic systems, as has been shown by Roberston (1956). If the effects are very small, the alleles will of course drift as if neutral. Somewhat larger selection coefficients will be destabilized by induced changes in the genetic background and by mutation to new alleles with yet more intermediate effects.

Changes in the genetic background. In a purely deterministic model with a stable fitness optimum, and stabilizing selection, polymorphisms do not remain selectively equivalent but become unstable and move to fixation. Let us again suppose that the (-) and (+) genotypes of Figure 1b were in perfect balance. If one of the genotypes were more common that the other, however, the population mean and the fitness optimum would not match exactly. Suppose that, through drift, the (+) genotype became more common than the (-) genotype. At this point, as we have seen, the (-) and (+) alleles would still be selectively equivalent. But the population as a whole would be shifted to the right of the distribution, which would result in changing selection coefficients of all other loci in the system -- since the locus in question is part of the genetic background of all the other loci. The expected deterministic response, then, would be a shift in the genetic background toward the left. This would mean that the phenotypic distributions of both (-) and (+) genotypes would be shifted to the left (Figure 2). Since the mean of the (+) distribution is moved toward the fitness optimum, the (-) and (+) alleles would no longer be selectively equivalent; (+) would continue to increase in response. Thus the carefully balanced situation found in Figure 1b is in fact an equilibrium if the background genotypic distribution is taken into account --- but it is an unstable equilibrium, leading to the selective fixation of one allele or another. All the same, such selective effects are likely to be extremely small, of the order of the mutation rate or the reciprocal of the effective population size, and it might still be expected that mutation and drift would be the predominant factors determining the fate of the alleles.

Fluctuating fitness optima. The population mean and the fitness optimum rarely coincide exactly. Drift and mutation will cause the population mean to vary, but more importantly, temporal fluctuations in the environment will favor first one phenotype, then

another, so that the optimum phenotype is by no means constant. Natural selection, of course, will move the phenotypic mean toward the fitness optimum, so that the two values will fluctuate around one another. The population mean may be above the fitness optimum one year, below it the next; generally above it for most of a century or a sun-spot cycle, below it for most of the next, and so on.

Selection coefficients of alleles in polygenic systems follow the same fluctuating, irregular cycle. At times when the population mean is below the fitness optimum, all (+) alleles will be selectively favored over their (-) counterparts, providing the effect of the allele substitution is less than twice the current difference between the mean and the optimum. At a later point in time, when the population mean is above the fitness optimum, all (-) alleles will be favored and all (+) alleles selected against. Over a long period, the phenotypic mean will tend to track the elusive fitness optimum, but will vary symmetrically around it. The selection coefficients of the various alleles in the polygenic systems will similarly fluctuate in time, negative and then positive, with a mean near zero. An allele with a selection coefficient that fluctuates randomly around a mean of zero is effectively selectively neutral with regard to polymorphism, fixation, and the effects of mutation and drift (Ohta 1972b).

When selection does act in a polygenic system, the effect is unpredictable. A mutation anywhere in the genome may be (temporarily) selectively advantageous if it tends to push a population phenotypic distribution closer to the current fitness optimum, and may increase in frequency. That same allele is a part of the genetic background of other gene loci; when the fitness optimum shifts, and the phenotype responds to natural selection the newly introduced allele may not be among those lost to selection but may increase, possibly to fixation, through the kind of positive-feedback instability described above.

Genetic variability in a polygenic system, then, does not have the properties of a stable equilibrium. Rather it has the properties of a steady-state system, in which new genetic variability is constantly being input through mutation as older genetic variability is lost through chance fixation or loss (drift).

There is as much natural selection occurring (that is, as much genotype-associated differences in fitness) with stabilizing selection as with directional selection; the phenotype may remain stable, or may fluctuate symmetrically around a long-term average value, while the underlying allelic determinants undergo constant origin, fluctuation and turnover.

Pleiotropic alleles in polygenic systems. "Polygenes" are presumably ordinary genes, or ordinary controlling genes, that have specific functions. Allelic variation will tend to have minor effects in any of a number of various morphological, physiological or behavioral categories. Focusing on polygenic variation of specific systems obscures both the primary functions of the genes involved and the secondary, polygenic effects these same allelic variants have on other polygenic systems not being monitored. Natural selection, however, monitors all systems. This complexity allows an opportunity for alleles having significant effects in one system to be balanced, in terms of net selection, by contrary effects in other systems. Most mutations would not be expected to be so balanced and would not be selectively neutral -- molecular-evolution studies are clear in showing that most molecular changes are indeed not neutral but are selected against (King and Jukes 1969). Pleiotropy, however, allows for the possibility of a new mutation being distinctly deleterious in one system and distinctly favorable in another --- with regard to its net effect on fitness, such a mutation may often be within the allowed limits of selective neutrality and may become established and perhaps fixed as a selectively equivalent allelic change. It is in fact likely that most beneficial mutations have unfavorable effects in some systems, effects which nonetheless can be rectified by responding frequency changes at other gene loci.

Stabilizing selection and directional selection: populations in stasis and in transitions. The paleontological record indicates that most species are morphologically stable over most periods of time, and that long periods of stability are interspersed

randomly with much shorter periods of comparatively rapid phenotypic change. Through most evolutionary time, then, natural selection will be primarily of two kinds; cleansing selection that removes unambiguously deleterious mutations, and stabilizing selection that acts against the ends of phenotypic distribution. Evolutionarily the most interesting adaptations occur, of course, not during stasis but during periods of transition. During such intervals alleles that formerly had negligible net effects of fitness may become adaptive, or maladaptive, and change frequency.

The neodarwinian synthesis has tended to make a strong analogy between progressive evolutionary change and the observed response to unidirectional artificial selection. Indeed, Darwin based his theory on just this analogy. Progressive evolution has been seen, then, as a process of bringing favorable alleles to fixation, either by depending on the genetic variation already present in the population, as in artificial selection, or by using the spontaneous occurrence of rare favorable mutations. I doubt that evolution has often occurred this way (although a good case might be made for rare favorable gene duplications).

Directional selection of the type operating in artificial selection experiments involves the reproductive success of one extreme of a distribution, and is thus clearly differentiated from stabilizing selection. I doubt that this situation ever occurs in nature. Rather, the current selective optimum may be displaced from the phenotypic mean, but hardly to the extreme of the distribution. At any point in time the population is likely to be close to its optimum, including times of geologically rapid transition. Throughout the famous evolutionary lengthening of the giraffe's neck, for instance, natural selection was probably very nearly as intense against both extremes of whatever was the current distribution. There may well have been times during this period when the net effect of selection was against long-neck alleles, as the environmental demands fluctuated. The set of alleles affecting variability in neck length, and even the set of gene loci affecting variability in neck length, may well have been different at the beginning of the process and toward the end, as new variability was introduced by mutation and older variability lost by drift -- or natural selection. During transition, the fitness optimum and the population mean would continue to fluctuate around one another, but instead of this fluctuation being symmetrical, as in periods of stasis, it would be asymmetrical over a long period. The fitness optimum might exceed the population mean not 50% of the time, but 60 percent of the time; or 55 percent, or 50.5 percent of the time; or 80 percent of the time, but at no time would the extremes of the phenotypic distribution be favored. Normalizing selection continues to occur in the presence of net phenotypic change, and the alleles involved may remain very close to being selectively neutral.

It is impossible to say whether there is more selection during periods of transition than during periods of stasis. Stabilizing selection can be transformed into progressive selection equally by selecting more heavily against one end of the distribution, or by selecting less heavily against the other. In general I should expect that there is almost no relationship between the total amount of natural selection and the net amount of phenotypic change occurring during a specified period of time; nor is there likely to be measurably more net allelic turnover during a period of stasis or cyclic selection than during a geological period of net phenotypic change. The lack of coupling between rates of molecular evolution and rates of phenotypic evolution ought then to be expected.

Conclusion. Much of the conceptual difficulty with this view of the roles of natural selection, mutation and drift is on the level of semantics. Not only is "neutral" an unfortunate word for allelic differences that may affect not only phenotype but fitness, but "selectively equivalent" is an inadequate term for the allelic variation that may be the principal stuff of evolutionary change under natural selection. Aside from the words, the basic idea is that a lot of the genetic variability underlying measurable phenotypic variance, and responsible for progressive evolutionary change, is due to allelic differences for which the net effect on fitness differences is of such a small magnitude that mutation and drift have greater individual effects than does natural selection. Ohta's term "nearly neutral alleles" comes the closest to describing such allelic effects; howe-

ver, she has used that term to describe genetic effects in which the effects of natural selection are of approximately equal importance with those of mutation and drift.

In sum, the dynamics of genic change at the molecular level are those of the diffusion equations as exemplified by the works of Kimura (Kimura and Ohta 1971). That is, it is powered by mutation and drift. This does not mean that the alleles themselves are necessarily ineffective in phenotypic expression or adaptive change. The dynamics of adaptive phenotypic change, on the other hand, operate on a different level. In a sense they operate on the level of the quantitative genetics and heritability of more successful or less successful phenotypes, uncoupled form the underlying genic turmoil.

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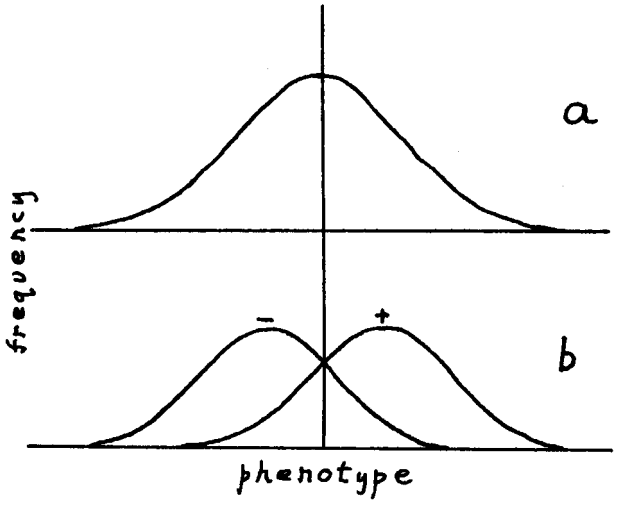

Figure 1. Phenotype distributions: (a) entire population; (b) for (+) and (-) alleles separately. The vertical line is the phenotype of maximum fitness. See text.

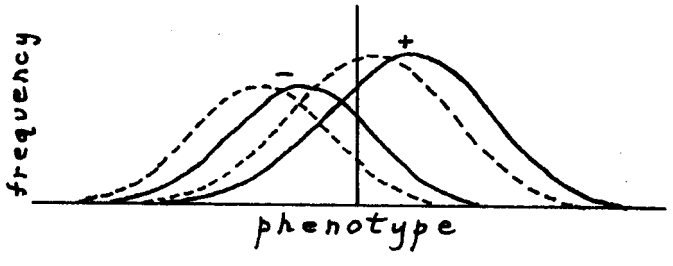

Figure 2. The (+) allele has increased in frequency by drift. The solid lines represent the phenotypic frequencies before (or without) response by the background genotype; the dotted lines represent the distributions after such response. The vertical line is again the phenotype of maximum fitness.