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Stabilizing selection and metabolism

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This paper attempts to provide a biochemical explanation for selection against extreme phenotypes. From current ideas on the analysis of metabolic control, it is argued that, on average, most mutations will reduce flux through metabolic pathways and change substrate pool concentrations in an arbitrary direction. Currently available experimental data suggests that there may be a positive relationship between flux and fitness. It is also argued that there may be a relationship between phenotypic traits and substrate pool concentrations. Given these premises, it is shown that intrinsic selective constraints occur against the production of extreme phenotypic variants.

INTRODUCTION

It seems to be generally accepted that stabilizing selection is important in determining the amount of heritable variation within populations (Lande, 1976), and in explaining evolutionary stasis (Charlesworth *et al.*, 1982). The reason that stabilizing selection has such a pervasive hold on current thought may be due in part to its intuitive appeal and in part to its supposed ubiquity (Haldane, 1954). With respect to the latter point, the recent review by Endler (1986) is particularly interesting because it shows that while stabilizing selection is indeed very common in nature, disruptive selection is almost as common.

It is useful to distinguish between selection for an optimum that is independent of the mean and an optimum that depends on the mean (Mather, 1987). For example, consider the distribution of beak lengths in a population of birds. Beaks perform a function, and it is entirely reasonable to suppose that the ability to perform that function depends in some way on beak length. From this purely functional point of view we might suppose, for simplicity, that there is one optimal beak length. If the distribution of beak lengths does not encompass this optimum then directional selection will shift the mean until it coincides with the optimum. At this point individuals with extreme beak length will have lower fitness than those with intermediate beak lengths simply because their beaks do not function so well. Alternatively, we might regard the distribution of beak lengths merely as the outward manifestation of a maelstrom of epistatic and pleiotropic interactions amongst genes. Selection against extreme beak lengths, according to this view, may reflect selection against genotypes that are extreme in some other, quite different, respect, hidden from the observer. In the case of beak lengths, I imagine many would agree that the functional argument is of over-riding importance. But traits must be distributed on a spectrum of relative functional importance. Perhaps at the lower end of this spectrum we might expect to find such traits as bristle number or scutellum length. Selection that appears to act on variants of these traits, may, in fact, "see" correlated physiological differences rather than differences in function *per se*. Since they are less likely to converge, such traits are generally those of taxonomic importance. In what follows I would like to discuss in more detail the possible importance of such indirect effects, and in particular, how selection for the mean might arise.

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THE SUMMATION PROPERTY

It is becoming apparent that the phenotypic effect of even large alterations in enzyme activity caused

by mutation may be quite small (Kacser and Burns, 1981; Middleton and Kacser, 1983; Hartl *et al.*, 1985; Dykhuizen *et al.*, 1987). The theoretical basis for this observation is provided by the analysis of metabolic control (Kacser and Burns, 1973; Heinrich and Rapoport, 1974), a key prediction of which is the Summation Property: the sum of the "flux control coefficients" of enzymes in a metabolic pathway is equal to unity. A flux control coefficient is defined as $(dF/F)/(dE/E)$, where F is the steady state flux through the system and E is the enzyme activity. In other words the flux control coefficient of a particular enzyme-mediated reaction can be regarded roughly as the proportionate change in flux for a proportionate change in enzyme activity. A similar prediction can be made for the steady state concentration of intermediate substrates: the sum of their concentration control coefficients (proportionate change in substrate concentration per proportionate change in enzyme activity) is equal to zero. It inevitably follows from the Summation Property that in metabolic systems with many enzymes the expected value of the control coefficient of any enzyme mediated reaction will be small, and the relationship between flux and enzyme activity will be as shown in fig. 1.

The relevance of the Summation Property to the neutral theory, especially as modified by Ohta (1977) and Kimura (1979) to include slightly deleterious mutations, is clear. It has been a subject of discussion by many of the authors cited above. What is not so clear, however, is the phenotypic manifestation of variability at the biochemical level. In view of current attempts to show that the presence of a high degree of heritable variation is not incompatible with the segregation of alleles with only very slightly deleterious effects (Lande, 1976; Kimura, 1983, pp. 140–143), it is interesting to consider in more detail what the properties of metabolic pathways can tell us of the nature of the genotype-phenotype transformation.

GENOTYPE AND PHENOTYPE

The idea that flux and fitness are in some way related is well argued in the literature (Kacser and

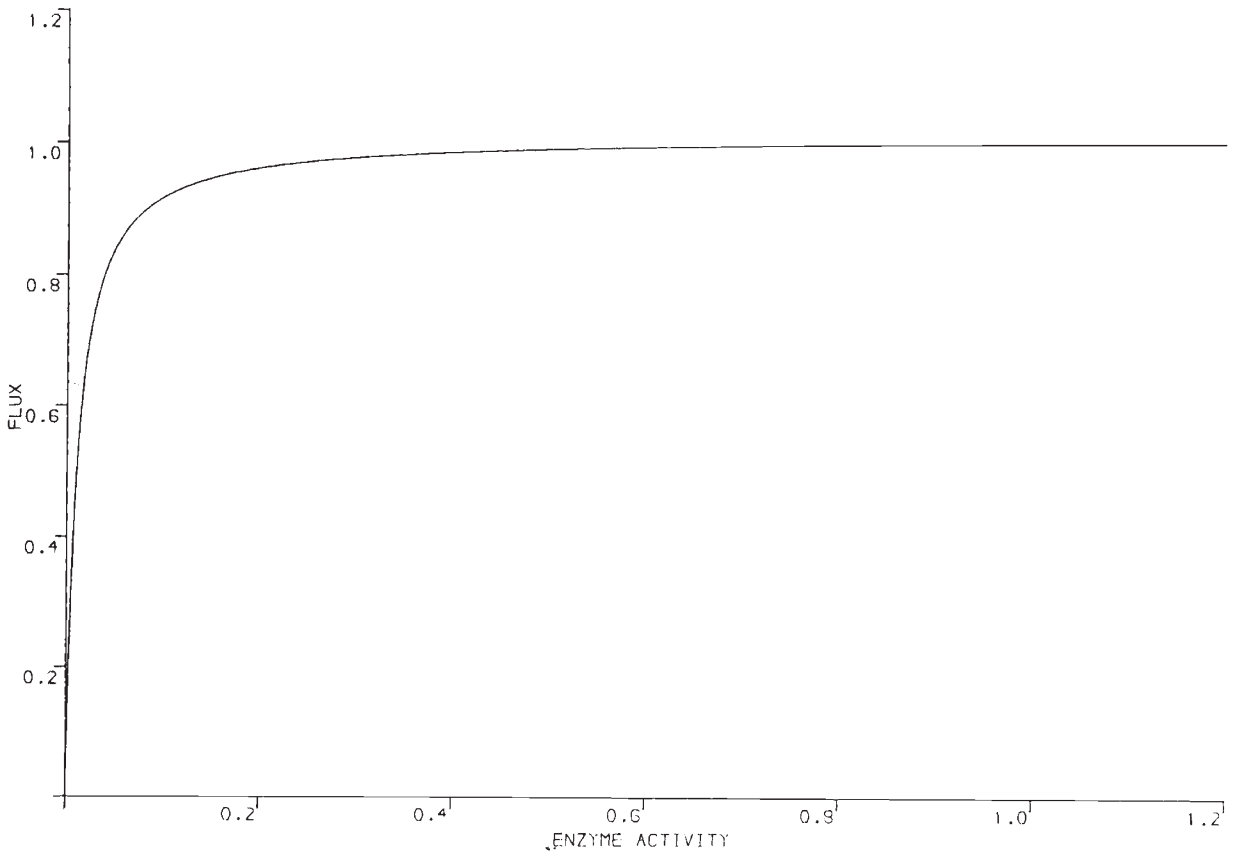

Figure 1 A plot of the steady state flux through a pathway of 100 enzymes against enzyme activity. The curve has been derived from equation 1 by setting the value of R to 1, for 99 enzymes, and allowing the value of R of the remaining enzyme to vary between 0 and 1·2.

Burns, 1981; Middleton and Kacser, 1983; Hartl *et al.*, 1985), but it has not been thoroughly tested. Growth rate, probably a major component of fitness, may well depend on the rate at which an organism processes resources and lays down its structures. This in turn may well depend on the flux through metabolic pathways. For simple substrate limited cultures this idea has some experimental support (Dykhuizen *et al.*, 1987).

The relationship between the properties of metabolic systems and those of morphological traits remains more obscure. Embryogenesis is often regarded as an orderly series of changes in the expression of structural genes mediated by *cis*- and *trans*-acting regulatory genes. At first sight it is difficult to see the relevance to development of any theory dealing with the control of intermediary metabolism. For example, Kacser and Burns (1981) have suggested that the integral of flux through a pathway over time may correspond to some morphological character. But time itself is a variable (Slatkin, 1987), and is probably determined by the action of regulatory genes. If, however, we admit the importance of simple metabolites acting as co-repressors or inducers in regulation, as suggested by Kacser and Burns (1981), then the distinction between *trans*-acting regulatory genes and the structural genes themselves becomes far less clear-cut. The very recent identification of a gradient in retinoic acid concentration in the chick limb bud, which may be partly responsible for the observed pattern of morphogenesis (Thaller and Eichelle, 1987), and the positive identification of a "retinoic acid-inducible *trans*-acting enhancer factor" (Petkovich *et al.*, 1987) in humans may be relevant to this idea. Similarly the study of MacDonald *et al.*, (1988) shows that mannose-6-phosphate specifically doubles the apparent affinity of the purified rat placental receptor for insulin-like growth factor II, whereas other sugar derivatives have no such effect. If these phenomena are quite general, we might conjecture that the fitness of an animal is positively related to flux through a given pathway and that the dimension of a character may be determined in part by the concentration of substrate pools in a pathway.

Using this idea I describe below a feature of metabolic pathways that, to my knowledge, has not previously been noted. For simplicity, I will give a concrete numerical example, the results of which will be extrapolated in an intuitive way.

A MODEL

A linear chain of enzymes is considered, where 100 enzymes process substrates from an initial pool with a constant concentration of 100 units to a final sink with a constant concentration of 0 units, via 99 intermediate pools of substrates. For simplicity the equilibrium constant for each reaction is set to unity and therefore the system is driven by the concentration gradient. At steady state, assuming Michaelis-Menten dynamics and unsaturated enzymes, the flux and pool concentrations are given by

$$F = 100 \Big/ \sum_{i=1}^{100} \frac{1}{R_i} \tag{1}$$

$$P_i = 100 \sum_{j=i+1}^{100} \frac{1}{R_j} \Big/ \sum_{j=1}^{100} \frac{1}{R_j} \tag{2}$$

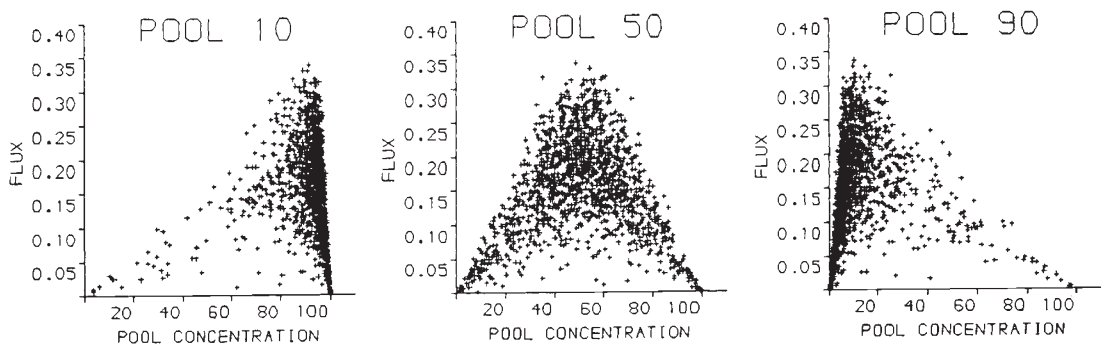

Figure 2 A plot of flux against substrate pool concentration of the 10th, 50th, and 90th substrate pools. Each point represents the value of the flux and respective substrate pool concentration in 1500 randomly generated enzyme pathways. Each pathway has been generated by giving each of the 100 enzymes in the pathway an activity drawn uniformly randomly from the range 0-1. Thus the same fluxes are plotted in each graph but against the concentrations of different substrate pools. The expected mean value of R for each pathway is 0·5.

where F is the flux, P_i is the steady state concentration of the ith pool and R_i is the activity of ith enzyme defined (in the present context) as the ratio of the maximal velocity to the Michaelis constant (see Kacser and Burns, 1981). Thus, if we consider initial values of 1 for each value of R, the flux through the system is equal to 1 and the substrate pool concentrations vary from 99 down to 1 in decrements of one unit.

Next, consider the case where the activities, R, of all the 100 enzymes are simultaneously "mutated" uniformly and randomly between 0 and 1. The flux and pool values are then calculated, and the simulation is replicated many times. The purpose of this simulation is to examine the *average* effect of a change in enzyme activity on flux and substrate pool concentrations. In fig. 2 the flux through each of these randomly generated pathways is plotted against substrate pool concentration in the 10th, 50th, and 90th substrate pools in each pathway. The figure is intended to show *only* the *relationship* between flux and substrate pool concentration as might be measured in a population of organisms. The degree of genetic variability that can be maintained by mutation/selection balance may be much less. Preliminary simulations have shown that reasonable amounts of heterozygosity and a small but significant variance in fitness can be maintained for realistic mutation rates and effective population sizes, when fitness is made a function of flux. This aspect remains to be studied in detail and will be the subject of a future publication.

The relationship seen in fig. 2 can be explained by referring, once more, to fig. 1. It can be seen that a large proportion of mutations have little effect on flux or on any of the substrate pool concentrations. All mutations of large effect will reduce flux and therefore, on average, deviations in the pool concentrations away from the mean will be associated with a reduced flux through the pathway, as can be seen in fig. 2. For variation in enzyme activity before a given pool (*i.e.* upstream of it in the pathway) there is a positive relationship between flux and pool concentration, whereas for variation in enzyme activity after the pool (*i.e.* downstream of it in the pathway) there is a negative relationship; hence the angular profiles observed in fig. 2. In the simple pathway considered here, substrate pools near either the source or the sink show a skewed relationship because the pool concentrations are constrained to vary between the source and sink concentrations. In reality the equilibrium constants will differ for each reaction. If there is an electrochemical potential difference between source and sink such that there is a flux from source to sink, the maximum substrate pool concentration will be constrained to be that of the source, scaled by the equilibrium constant for the series of reactions from the source to that substrate pool (*i.e.*, the product of all the equilibrium constants of the reactions upstream of the substrate pool). Similarly the minimum substrate pool concentration will be constrained to be that of the sink scaled by the appropriate equilibrium constants. The steady state substrate pool concentration obtained when none of the enzyme activities (scaled by the appropriate equilibrium constants) are very much less than the mean (*i.e.*, when the flux is at a maximum) lies somewhere within this range. Depending on the particular values of the equilibrium constants for each reaction, this "optimal" substrate pool concentration will be nearer or further from these two limits, and hence the relationship between flux and substrate pool concentration will be more or less skewed. But there is no obvious biochemical reason to suppose that this "optimum" will always be near one of the limits for any randomly chosen substrate pool. Furthermore, if a phenotypic character is a function of a number of substrate pool concentrations, then, clearly, the effect of a mutation on that character will become more symmetrical.

Having discussed this specific example, it is interesting to consider the more general case. Imagine an enzyme embedded in a complex metabolic network with branching pathways and feedback loops. What will be the expected effect of a change in the activity of this enzyme on a randomly chosen flux and on the concentration of a randomly chosen substrate pool? From the Summation Property it is clear that, in general, an increase in enzyme activity will have a negligible effect on either the fluxes or substrate pool concentrations. Most changes that have an appreciable effect must come about from reduced enzyme activity. Reduced enzyme activity must, on average, be correlated with reduced flux. It might be thought that in branched pathways there will be negative correlations between changes in flux in one pathway and changes in another, but, as Kacser and Burns (1981) have emphasized, most divided pathways will rejoin via some common pool. There will be negative correlations only between pathways that exit the system. Reduced enzyme activity may increase some fluxes through allosteric effects; but these indirect interactions are just as likely to reduce fluxes. Indeed it is likely that indirect interactions that reduce flux will have a larger effect than those that increase flux. Therefore, overall,

reduced enzyme activity must, on average, correlate with reduced flux. On the other hand, the effect of reduced enzyme activity on the concentration of a randomly chosen substrate pool will be unpredictable. There will be *some* effect, but, for a randomly chosen pool, it is as equally likely to be up as down. Therefore, it can be seen that there is a fundamental asymmetry in the average effect of a change in enzyme activity on flux and on substrate pool concentration. If fitness is related to flux, this gives rise to the stabilizing selection effect. Furthermore, the converse is not true: directional selection on substrate pool concentrations will not, in general, give rise to stabilizing selection on flux.

DISCUSSION

Given the assumed relationship between flux and fitness and between pool concentrations and morphological characters, stabilizing selection arises as a simple consequence of the Summation Property. The Summation Property predicts that all mutations of appreciable effect reduce metabolic flux. These mutations also alter substrate pool concentrations. It is the thesis of this paper that changes in substrate pool concentrations themselves change the magnitude of morphological traits in unpredictable ways, but, overall, mutations that have a large effect on flux also produce extreme phenotypes.

The model gives us an insight into how intrinsic selective constraints might arise. Most selective changes in the mean phenotype must occur primarily through a change in the frequency of genes that have the largest effect on phenotypic value. From the Summation Property, mutations of large effect will be those that reduce enzyme activity and hence reduce flux. Therefore selection on a phenotypic trait correlated with a number of substrate pool concentrations will, on average, reduce flux. Thus if fitness is related to flux, selection on a phenotypic trait will be antagonized by selection on flux. Of course this neglects other genes outside the system considered here, namely those regulatory genes that map pool concentrations onto the phenotype. It is to be expected that these loci will be relatively rarer than those loci that control the concentration of a particular metabolite, changes in the concentration of which will be mediated to a greater or lesser extent by variation in the activity of a myriad enzymes. Thus it is possible to ascribe a component of additive genetic variance to three classes of segregating alleles: those alleles segregating at loci controlling enzyme activity that correlate with reduced enzyme activity; those alleles segregating at loci controlling enzyme activity that correlate with increased enzyme activity; and those alleles segregating at loci that map substrate pool concentrations onto the phenotype. The genetic variance associated with the first group may be quite high because they are very common and their effect may be moderately large. The genetic variance associated with the second group will be low because, although these alleles may be common, their effects will be very small. The genetic variance associated with the third group may also be quite small because, although their effects may be very large, they will be relatively rare. The initial response to selection on the phenotypic trait may involve changes in the frequency of the first group of alleles, and may be reversed if selection on the phenotypic trait is relaxed.

Although the transformation from genotype to phenotype remains as ever a "black box", the fluxes and pool concentrations are at least measurable (see *e.g.*, Salter *et al.*, 1986). Perhaps the measurement of such variables will form a useful point of departure in studies of the differences in morphology among individuals or, more practically, isogenic lines.

Returning to the considerations that prompted this study, it is worth asking whether the environment is so invariant, or the population's ability to track its changes is so good, that the range of a morphological trait is in general expected to encompass an environmentally determined optimum at the time an observer actually makes the appropriate measurements. If most observed cases of stabilizing selection arise through pleiotropy, the rôle of the environment in determining the mean value of a trait may be overestimated in current evolutionary theory. Furthermore, on a slightly different point, even for traits that are regarded as functionally important, such as beak length, the optimum will depend both on environmental factors such as the distribution of food sizes, and internal factors such as the metabolic cost of producing larger beaks, quite apart from the so-called "developmental constraints". "Shifts in the environment" will compete with "quite unrelated and fortuitous changes in physiology" as alternative explanations for the adaptive significance of changes in the mean value of a trait through time, as might be seen in the fossil record.

In conclusion, the idea of the "coadapted genotype" has caught the imagination of many evolutionary biologists, but has provoked frequent

criticism for its lack of a quantitative theoretical framework. Perhaps the arguments outlined here herald such a framework, which is yet to come.

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