Enzyme activity levels in individuals: Selective value, over-reaction and conditional neutrality

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ABSTRACT: Several alternative pathways probably exist in an organism for every essential biochemical process. Therefore, the much-debated selective value of replacement of one allozyme by another may be less important than the value of total activity of all allozymes of the relevant enzyme.

However, a review of available data on activity levels in 3 insect enzymes (esterases, alcohol dehydrogenase and amylase) reveals that correlation of activity levels with fitness characters is often weak, absent or negative, although in some cases high activity confers a selective advantage. I suggest that the enzymes may be produced, when stimulated, in great excess over the level necessary for the use of the available substrate. As a result of this over-reaction, no correlation need be expected between activity and fitness except in the limiting case when the animal cannot produce the minimal amount of the enzyme necessary for survival.

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Introduction

The discovery of large amounts of electrophoretic variation in natural populations triggered the controversy over its evolutionary (selective) value. The issue was whether the replacement of one allozyme (electrophoretic allele) by another affects the survival and reproductive potential of an individual.

In the heat of the selectionist/neutralist argument, little attention was paid to another aspect of enzyme variation in populations, namely, variation in enzyme activity levels.

In many organisms, staining a gel for any enzyme system reveals a number of isozymes (bands). They are detected when the gel is immersed in a single substrate/stain combination. The in vivo substrate of the enzymes is generally unknown, so as a "null hypothesis" we must assume that all these isozymes are able to break down the same or similar compounds also in vivo, although perhaps not with the same efficiency. The selective value (if any) of the enzyme system may be determined by the total activity of all isozymes combined and not the presence or absence of any one isozyme. I wish to offer the following 5 propositions:

(1) As long as at least one isozyme is present in the individual, it will be able to use the available substrate and will survive and reproduce.

(2) If total activity is selectively important, we expect to find positive correlations between high enzyme activity and fitness-related characters.

(3) However, although some positive correlations were found, in most cases the correlations are weak, absent or even negative. Alternative biochemical pathways overcome the absence of enzyme activity ("null" alleles).

(4) Enzymes may be generally produced in great excess over the amount necessary for the available substrate.

(5) the critical issue may be the production of a sufficient minimum enzyme activity under the worst conditions. Overproduction under good conditions may not be harmful: materials may be recycled and the energy expenditure negligible. Overproduction under bad conditions may be deleterious.

I shall discuss in detail data on three enzyme systems: Esterases in mosquitoes and aphids, Alcohol dehydrogenase in Drosophila, and Amylase in Drosophila and flour beetles (Tribolium).

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A. Esterases in mosquitoes and aphids resistant to organophosphorous insecticides

Insecticide resistance is one of the most prominent cases of an extant evolutionary process. It leads to the adaptation of the insect population to a new environmental stress. Mechanisms conferring insecticide resistance then certainly have a selective value in an insecticide-treated environment.

There is convincing evidence that esterases, particularly carboxyesterases, are involved in conferring organophosphate resistance to individuals by metabolizing the pesticide.

Georghiou and Pasteur (1978) studied the electrophoretic profile of three species of Culex mosquitoes. A number of isozymes were present in each strain and species, and at least one highly active esterase was present in every organophosphate-resistant strain (in this study, activity was described qualitatively as "strong" by inspection of the gels). Their fig. 1 illustrates that the strongly-staining isozymes were not the same in the different resistant strains. The authors suggest that more than one gene may be involved in resistance. In a study of genetic crosses of field-collected mosquitoes in one species of Culex, Georghiou and Pasteur (1980) found that the most active esterase (2c) is always associated with organophosphate-resistant genotypes.

The association of strong esterase activity with OP resistance enabled the authors to devise a test procedure based on total esterase activity for detecting resistance of mosquitoes in the field: Individual insects are crushed on filter paper which is then immersed in a solution containing standard in vitro substrate (- Naphthyl acetate) and the stain (fast blue RR). Resistant individuals are detected by the strong staining reaction (Pasteur and Georghiou, 1980).

In England, esterase activity is used in routine screening for resistance in the aphid, Myzus persicae (e.g. Baker, 1977). The field procedure is based on laboratory work by Needham and Sawicki (1971), Devonshire (1975, 1977) and others who measured esterase activity quantitatively in single individuals from susceptible and resistant clones. The OP resistant strains had a much higher esterase activity than the susceptible strains (see also Wool, Bunting and van Emden, 1978). Total esterase activity correlated well with the resistance level, although the activity of one particular esterase isozyme contributes most of the effect (see Devonshire & Sawicki, 1979; Bunting & van Emden, 1982). Evidence that a particular esterase (Est-2) is identical with the organophosphate-hydrolyzing enzyme was presented by Beranek and Oppenoorth (1977), who recovered that isozyme from the gels after electrophoresis. Blackman, Devonshire and Sawicki (1977) showed that esterase activity in M. persicae is co-inherited with OP resistance.

The data on esterase activity in mosquitoes and aphids indicate that in an insecticide-polluted environment, high esterase activity in these organisms may confer a selective advantage. However, data on other organisms do not agree with this interpretation. In Tribolium, resistant strains have lower esterase activity than susceptible ones (Wool, Noiman, Manheim and Cohen, 1982). In ticks, Stone (1968) concluded that low brain acetyl-choline esterase levels and resistance to organophosphorous compounds are pleiotropic effects of the same gene or are closely linked.

B. Alcohol dehydrogenase in Drosophila

Research on alcohol dehydrogenase (ADH) isozymes in natural and laboratory populations of several Drosophila species, in particular D. melanogaster, was recently reviewed by van Delden (1982), and by Laurie-Ahlberg (1985). Most of the published work was concerned with frequencies of different electrophoretic alleles, and is outside the scope of this presentation. However, "Fast" (F) alleles are generally more active than "Slow" (S) alleles. F- and S- proteins differ in only a

single amino acid substitution. Most authors claim that the higher activity in FF individuals results from production of more enzyme molecules (references in van Delden, 1982). The literature on the regulation of ADH activity levels is thoroughly reviewed by Laurie-Ahlberg (1985).

Alcohol dehydrogenase oxidizes alcohols to aldehydes and ketones. Drosophila larvae live in fermenting environments which contain various amounts of alcohols. It was therefore assumed that individuals with higher levels of ADH may survive better in the presence of environmental alcohol.

This assumption was tested by a number of investigators. Different strains, homozygous for FF and for SS, were shown to vary in ADH activity, and genetic crosses suggested that activity was regulated by elements located outside the structural gene, (e.g. Ward 1975, McDonald and Ayala 1978). The variation in activity is, at least in part, heritable, and can be increased by artificial selection (van Delden 1972, van Delden and Kamping 1983). Survival of high-activity strains on food media supplemented with alcohols, is generally higher than that of low-activity strains. (e.g. Kamping and van Delden 1978; Thompson and Kaizer 1977). An ADH-null strain, synthesized in the laboratory from field-collected heterozygotes, was far less tolerant to environmental alcohol than ADH-carrying strains (David et al. 1976).

There is evidence that FF adults may metabolize ethanol vapor and use it for energy production (Daly & Clarke, 1981). Longevity of starved , ADH-active adults is considerably prolonged in the presence of low concentration of ethanol vapor, and this character may be increased by selection for ethanol tolerance in ADH-active, but not in "null" strains (David et al. 1979; Van Herrewege and David 1980). All this information seems to support a positive role to high ADH activity in adaptation to alcohol-containing environments.

However, even in this well-known enzyme system and after 15 years of research, the adaptive role of ADH activity is not clear (nor, in fact, is its exact in vivo function). Under some circumstances, toxic aldehydes and ketones may accumulate and reduce the survival of high-ADH individuals; under these conditions, ADH-null individuals will survive better. ADH-null individuals with total absence of ADH activity do occur in nature at low frequencies (about 0.001) and perform well in laboratory cultures (van Delden, 1982). Such strains do not compete well with ADH-active strains (even in the absence of alcohol) but there is no evidence that the low competitive ability is related to the absence of ADH. Disturbing results were obtained in Australia in studies of F -and S- allele frequencies in "natural" Drosophila populations, in wineries, where the level of alcohols in the larval environment could be directly measured. (e.g. McKenzie and Parsons 1974). In one recent study, larvae were collected in habitats containing 0.07% to 10.8% ethanol, but there was no significant association of the high-activity, F-allele frequencies with ethanol level (Gibson, May and Wilks 1981). The authors conclude that there was "no evidence, within wineries or between winery-non winery sites, that the frequency of ADH-F is adjusted by natural selection to track the environment."

Successful selection for alcohol tolerance, which increased activity of ADH in both FF and SS strains, resulted also in an increase in body weight of the emerging adults. It is possible that the increased ADH activity is due to the increase in body weight (van Delden and Kamping 1983). And some evidence suggests that the measured activity of ADH in adult flies may be determined to a considerable degree by environmental factors such as yeast levels and microorganisms in the larval medium. (Clarke, Camfield, Galvin and Pitts 1979).

Finally, the activity of ADH in vivo cannot be dissociated from other metabolic systems. The first stage in ethanol metabolism is conversion to acetaldehyde, which is toxic for Drosophila. It must then be further converted to acetate by Aldehyde oxidase, an enzyme coded for by the aldox locus. However, two mutant strains which do not produce this enzyme, were nonetheless ethanol tolerant (David et al. 1978).

Detailed analysis revealed another isozyme, with minor activity, which is able to metabolize the same substrate. Some aldehyde dehydrogenase activity is present also in ADH itself, and it is unclear whether acetaldehyde in fact accumulates in vivo (Heinstra et al., 1983; Moxon et al., 1985).

C. Amylase

Amylases hydrolyze starch to disaccharides (mostly to maltose) and, most probably, perform in vivo the same function on the same substrate as in the test tube. Considerable physiological and biochemical work was done on mammalian amylase, which is produced in two locations - the pancreas and the salivary glands. Recent research in molecular genetics revealed that the genes coding for amylase from the two sources may be related. Several duplicate genes code for the enzyme in the mouse and in the rat (Thomsen, Hjorth and Nielsen 1984). However, mammalian geneticists have so far not discussed the evolutionary value of variation in amylase activity.

Most of the information on amylase in insects comes from Drosophila - in which starch is not an essential component of the diet (review by Laurie-Ahlberg 1985). A number of amylase isozymes were discovered in Drosophila (Kikkawa, 1964), some of which also differ in activity levels. In D. melanogaster and D. pseudoobscura, a series of "temporal genes" regulate the activity of the enzyme in different parts of the digestive tract (Doane, 1977; Powell and Lichtenfels 1979).

Detailed investigation of Drosophila amylase revealed two facts which are important in connection with the present discussion. First, larvae reared on a starch-containing medium have higher amylase levels than on a starch-free medium (Hickey 1977, 1981; Yamazaki and Matsuo 1983). (But Hickey and Benkel (1982) have shown that this results from amylase repression by glucose rather then stimulation by starch). After rearing for one year on the two media, amylase activity increased on the starch medium (Yamazaki and Matsuo 1983), indicating that high activity may be associated with fitness and can be selected for. Positive correlations of amylase "inducibility" with productivity in 44 isofemale lines was reported by Yamazaki & Matsuo (1984) and Matsuo & Yamazaki (1984). Second, De Jong and Scharloo (1976) discovered that a high activity (Amy 4.6) mutant had a selective advantage over a low-activity strain (Amy 1) only when yeast levels in the medium were low, and when the quantity of starch was limiting. No difference was detected otherwise.

The Drosophila results prompted us to investigate amylase activity in the flour beetle, Tribolium. In this organism, in contrast to Drosophila, amylase must be an essential enzyme for survival since starch is their major source of carbohydrates. Amylase is in fact, by far the most active carbohydrase in the digestive tract of the beetles (Krishna and Saxena 1962).

In a study of 28 strains belonging to 4 Tribolium species, no electrophoretic variation was detected within species - each species had a monomorphic, distinct pattern of 1-3 isozymes (Wool 1982). However, we found considerable variation in enzyme activity among individuals within each strain of T. confusum, spanning an order of magnitude (Wool and Noiman 1980; Wool and Shirtz 1984). Successful selection for high activity in two strains demonstrates that activity is partly under genetic control (Wool and Shirtz 1984), but a line selected for high amylase activity had lower fitness than a line selected in the opposite direction (Wool 1984). Most importantly, we discovered that individual amylase activity level is strongly affected by the diet (Wool, Namir and Bergerson, 1986). Tribolium is a pest of a wide range of stored products, and we thought that the level of amylase activity may be one determinant of the ability to colonize new niches. It now seems likely, however, that the observed level of activity may be the result, rather than the determinant, of feeding on a given diet.

D. Enzyme activity regulation

High activity, in some cases, was shown to result from the production of more enzyme molecules - perhaps through the control of the structural gene by a regulatory element (Hickey, 1981 in Drosophila amylase; McDonald and Ayala (1978), Wilkinson, Rowan and Brennan (1984) and others, in Drosophila ADH) - but other mechanisms, such as gene amplification during selection (in Myzus esterases, Bunting and van Emden 1982) or differences in the catalytic properties of the enzymes (Day, Hillier and Clarke, 1974a,b) have been suggested.

Activity variation among individuals within strains was reported for all three enzyme systems described here. The variation often seems continuous (Ward 1975; Wool and Shirtz 1984; Wool 1984). This may result from the action of a regulatory locus, in particular if pleiotropic or epistatic gene action and environmental factors influence the final phenotype (activity). In Drosophila and Tribolium body weight may be one factor (Clarke, Camfield, Galvin and Pitts, 1979; Wool, 1984); the larval or adult diet may be another. Wool, Namir and Bergerson (1986) found a 2-4-fold increase in amylase activity when a pure starch diet was supplemented with brewers' yeast or casein; Clarke, Camfield, Galvin and Pitts (1979) found that by increasing yeast level from 0 to 50g per liter medium, they more than doubled ADH activity in the emerging Drosophila adults (weight increased by 30%).

Activation of digestive enzymes by dietary components is not a new phenomenon. In mosquitoes, the stretching of the female midgut (after a blood meal, but also when air is injected into the gut) stimulates the activity of esterases, although not of other enzymes (Geering and Freyvogel, 1975), while the presence of blood in the gut triggers the secretion of proteases (Briegel 1975). When protein was injected directly into the gut, to avoid stimulating the sensory cells responsible for the feeding response, only little effect was detected. Briegel and Lea (1975) suggest that only proteins with special chemical properties can stimulate proteolytic activity. The effect of protein concentration on proteolytic activity is known also in carpet beetles (Baker 1977). Dietary stimulation of amylase activity was reported in a number of organisms. In the moth Spodoptera littoralis amylase activity was strongly stimulated by feeding with the disaccharides sucrose, maltose, raffinose and melezzitose relative to the control (Ishaaya and Meisner 1973); and by increasing the protein level in an artificial diet (Ishaaya, Moore and Joseph (1971). The possible role of hormones in the control of amylolytic activity was suggested by some results with the flour beetle, Tenebrio molitor (Jankovic-Hladni et al., 1978).

Stimulation of activity may not depend on the presence of a suitable substrate. Krieger et al (1971), suggested that in Lepidopterous larvae high activity of mixed-function oxidases (MFO) is an adaptation to the presence of a range of alkaloids, in the host plants. Research by Brattsten et al. (1977) indicates that activation of MFO occurs by compounds which are not substrates for the enzymes in question, while compounds which are, do not stimulate activity (Gould, 1984).

E.General discussion and speculations: over-reaction and conditional neutrality.

In discussions of the adaptive value of enzyme activity, we should keep in mind that the activities measured in vitro may be poor predictors of the importance of the reaction in the living organism. Complex interactions with other systems may provide a buffering effect - which will be absent in the test tube (Middleton and Kacser, 1983). However, so long as we do not know the entire system, or the in vivo functions of the enzymes, we can only look at the end result in the test tube.

The published literature provides some evidence that high enzyme activity may have a selective value under some circumstances, but this is difficult to prove even in cases which seem obvious. Perhaps more important is the fact that low activity -

or even no activity, as in the Drosophila ADH-null strains - is not neccesarily deleterious or lethal: the presence of other isozymes or alternative biochemical pathways may compensate for the loss of activity at a mutated locus, as in the case of the aldox locus in Drosophila (David et al. 1978).

These properties suggest that a high enzyme activity level is conditionally neutral. It may become advantageous under some restrictive conditions: high amylase activity when starch is a limiting factor in Drosophila, and prolonged survival of high-ADH Drosophila adults in the presence of ethanol - under starvation, may be examples. When conditions are less extreme enzymes may be produced in much larger quantities than needed and quantitative differences among individuals may have no adaptive significance.

At least in three enzymes discussed here, it does not seem that the production of enzymes in individuals reflects, or quantitatively follows, the need to break down substrates. It seems to me that the system over-reacts when stimulated to produce the enzyme: much more is secreted than is actually needed for the substrate. David et al. (1978) suggested that this is so in the Aldox locus in Drosophila. This is the impression from our amylase work. Feeding on yeast or casein, which contain no starch (but certainly do contain some stimulatory compound - perhaps a free amino acid - see Hori, 1969), stimulates the production of much more enzyme than feeding on starch or wheat flour (Wool, Namir and Bergerson, 1986).

Over-reaction may not be maladaptive if the cost of producing excess enzyme is low, either because the enzyme is easy to produce or when the effort is required for only a short time and substrate abundance follows. We do not know what is the energetic cost to the organism of producing an enzyme. Most discussions on the subject are based on beliefs rather than on real data. One attempt to actually measure the cost to bacteria of producing Tryptophan, suggested that it may be trivial (Dykhuisen, 1978).

My own suggestion for the existence of over-reaction in enzyme production is that "flooding" of the blood stream or the digestive tract with enzyme molecules, when stimulated by some chemical or mechanism, evolved because it may be far more damaging (costly) to the organism not to have enough enzyme in time of need, than to have too much when not in need. The excess enzyme may be recycled or absorbed and the materials may not be entirely lost. The energy required may be minimal. But not having the enzyme may be crucial (e.g. when esterases are needed in an insecticide-treated organism). Perhaps a very quick-responding feedback system is difficult to make - and it seems not to have evolved.

Therefore, we should perhaps not expect to find a neat correlation between enzyme activity and fitness (let alone isozyme frequency and fitness), except when the organism cannot produce the minimal quantity required for survival. The genetic regulatory mechanisms will be adaptive under these limiting conditions, and neutral otherwise.

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NOTE ADDED IN PRINT: In a recent paper in Genetics (111:655-674, November 1985), Hartl, Dykhuizen and Dean present a theory which explains how enzyme activity may become "neutral" in evolution. They reason that enzyme activity effects on fitness follow a saturation curve, so that over much of the activity range, further increase in activity has little or no effect on fitness. Their theory, and the examples they cite, provide support for the suggestions I have made in the present paper.