GENETIC BACKGROUND AND THE FITNESS OF ALLOZYMES

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ABSTRACT

Experimental perturbations of gene frequency at the esterase-5 locus in *Drosophila pseudoobscura* were carried out in a series of population cages started with differing numbers of founder chromosomes. Cages founded with few chromosomes showed changes in gene frequency at the allozyme locus. Such changes were less marked in cages founded with a larger sample of chromosomes. These experiments show the importance of linkage disequilibrium in affecting allozyme frequencies, and emphasize the necessity of careful experimental design when studying fitness differences between allozymes in laboratory populations.

SINCE the discovery of widespread protein polymorphism in a variety of organisms (LEWONTIN and HUBBY 1966; HARRIS and HOPKINSON 1972) opinion has been divided on the relative importance of natural selection and of random processes in controlling the frequency of allozymes in natural populations (KIMURA 1968; CLARKE 1970). One method of investigating this problem is to examine alterations in allozyme frequencies in laboratory populations subjected to a variety of experimental treatments. BIJLSMA-NEELES and VAN DELDEN (1974), for example, describe changes in the frequencies of alleles at the alcohol dehydrogenase (*adh*) locus in experimental populations of *Drosophila melanogaster* placed in a number of different environments. They suggest that these changes are probably due to the action of natural selection at the *adh* locus. The relative simplicity of such experimental techniques and their apparent ability to provide direct evidence on fitness differences between alleles at enzyme loci may be expected to lead to similar investigations being carried out in the future using new enzymes and other experimental animals.

We wish to point out that such changes in allozyme frequency in laboratory populations founded with a relatively small sample of a natural population (BIJLSMA-NEELES and VAN DELDEN 1974; MACINTYRE and WRIGHT 1966; YARBROUGH and KOJIMA 1967) or subjected to many generations of inbreeding (WILLS and NICHOLS 1971, 1972) are not necessarily due to the action of selection at the single locus under consideration. These experimental techniques will produce linkage disequilibrium between the allozyme locus and other loci which are themselves likely to be subject to selection. Apparent selective responses at the allozymes locus may therefore be due to selection at associated loci and not to selective differences between the allozymes themselves.

YAMAZAKI (1971), using a technique designed to avoid the problems of linkage disequilibrium by utilizing a large number of independent homozygous lines as founders of his experimental populations, could demonstrate no differences in fitness between alleles at the esterase-5 (*est-5*) locus in *D. pseudoobscura*. We describe here an experiment using these alleles which shows how inadequate sampling of a natural population may give rise to a spurious appearance of fitness differences between allozymes.

MATERIALS AND METHODS

A number of population cages were set up on cornmeal food at 25° using different numbers of homozygous lines as the base population. The lines used were selected at random from those utilized by YAMAZAKI (1971), and experimental techniques were generally similar to those described by him. In YAMAZAKI's experiments, cages were set up using 22 independent lines homozygous for *est-5*$^{1.00}$ and 22 lines homozygous for *est-5*$^{1.12}$ as a base population. Initial frequencies in these 44-line cages were .909 *est-5*$^{1.00}$ or .091 *est-5*$^{1.00}$. No systematic changes in gene frequency took place in his cages, and an extensive series of fitness estimations revealed no selective differences between the *1.00* and *1.12* alleles at this locus. The present experiments used two homozygous lines (cages 1, 2, 3, 4), ten lines (cages 5 and 6), or twenty lines (cages 7 and 8) as the founding stock. Different pairs of homozygous lines from those used in cages 1 and 2 were used to found cages 3 and 4. In each of cages 1–4 one generation of backcrossing was carried out between lines to increase the overall homozygosity of the founding population. Initial frequencies of *est-5*$^{1.00}$ were .909 (cages 1, 3, 5, 7) or .091 (cages 2, 4, 6, 8). Cage 8 was contaminated by *D. melanogaster* and was discarded.

RESULTS AND DISCUSSION

The frequencies of the *est-5* alleles in the surviving cages behave in a manner which is clearly related to the number of founding homozygous lines and therefore to the amount of linkage disequilibrium due to sampling effects in the base population (Figure 1). Cages founded with only two homozygous lines show marked temporal changes in allele frequency. In cage 1 the frequency of *est-5*$^{1.00}$ rose from .909 to 1.00 within a few months, while in cage 3 it fell to a new equilibrium of approximately 0.80. Similarly, in cage 2 (two founding homozygous lines; initial frequency of *est-5*$^{1.00}$ = .091) a new equilibrium of *est-5*$^{1.00}$ = 0.55 was attained, while in cage 4 (founded with the same number of homozygous lines and initial *est-5*$^{1.00}$ frequency) the frequency of this allele fluctuated around 0.20. Cages founded with ten or twenty homozygous lines (cage 5, 6 and 7) showed less marked changes in allele frequency, while in those containing forty-four lines allele frequencies were almost stable.

These experiments show that gene frequency changes at an allozyme locus in experimental populations may be due to the effects of the genetic background of the founding population rather than to the intrinsic properties of the allozymes themselves. The experimental populations used by BIJLSMA-NEELES and VAN DELDEN (1974) originated from five homozygous *adh*^{S} and five *adh*^{F} lines taken from a three-year-old population cage founded with "several" wild-caught inseminated females. Linkage disequilibria between the *adh* locus and other loci may therefore have arisen on both sampling occasions. MACINTYRE and WRIGHT

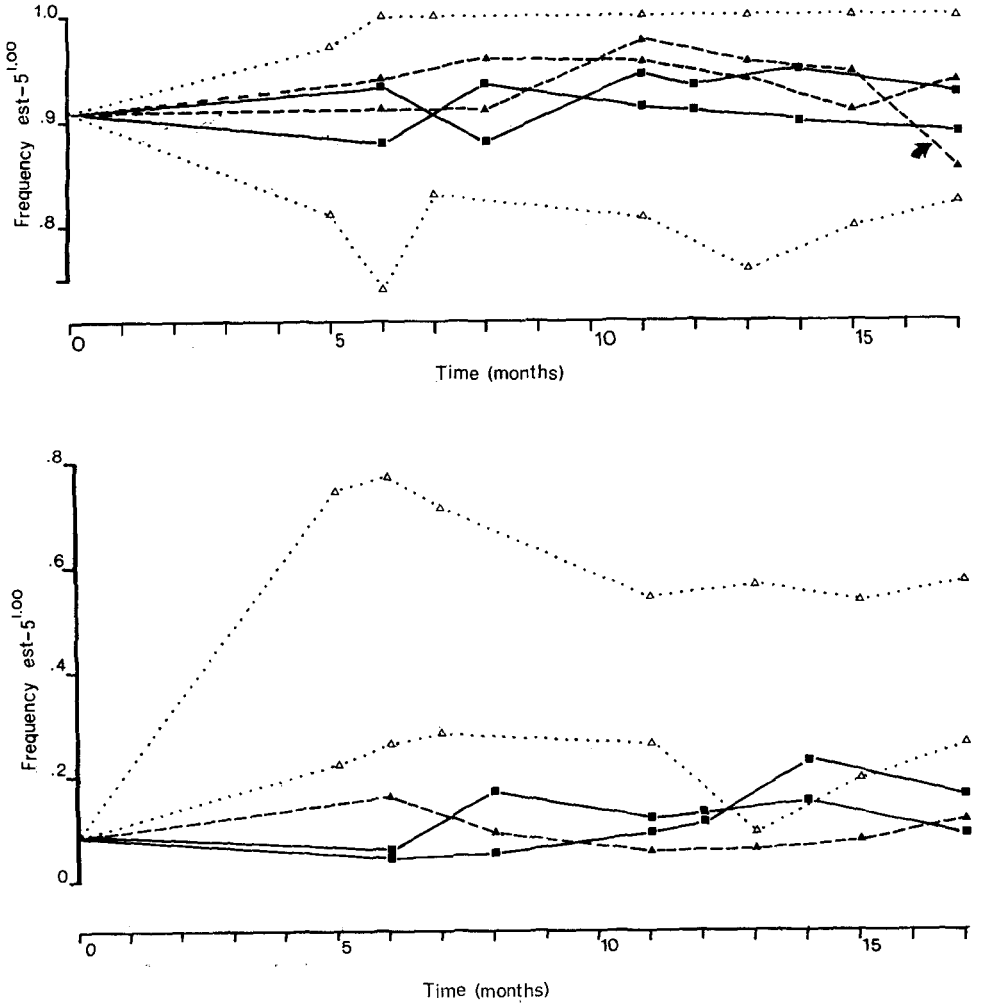

FIGURE 1.—Changes in the frequency of *est-5*$^{1.00}$ in cages containing different numbers of founding homozygous lines. △ · · · · · △ 2 founding lines; ▲ - - - - - ▲ 10 or 20 founding lines (20-line cage arrowed); ■ —— ■ 44 founding lines.

(1966) and YARBROUGH and KOJIMA (1967) used even fewer lines to found their base populations. Our experiments on *est-5* in *D. pseudoobscura* show that the effects of linked loci may be important in affecting gene frequency at an allozyme locus when a small sample of the natural population is used as a founding population. A sample of ten or twenty homozygous lines in the founding population does not appear to lead to major deviations from apparent selective neutrality at the allozyme locus in our experiments. But we feel that to be absolutely certain of obviating the possibility of genetic interactions overcoming single-locus effects, at least forty independent lines should be used to found experimental populations used in estimating fitness differences at such loci.

Some of the apparent responses to a variety of selective regimes described by BIJLSMA-NEELES and VAN DELDEN (1974) and by other workers may therefore be due to the effects of linked loci. Some authors (MACINTYRE and WRIGHT 1966; YAMAZAKI 1972) have pointed out the equivocal nature of the results of fitness estimations based on limited samples from natural populations, or on artificially inbred populations. SING, BREWER and THIRTLE (1973) have carried out an extensive series of experiments which show how the selective coefficients of allozymes can be affected by linked loci. In number of investigations, however, this methodological difficulty has not been emphasized, and some publications (BERGER 1971) do not specify the exact size of the original population sample used in estimating fitness. The effects of genetic background are likely to be particularly important when examining species such as *D. willistoni* (POWELL 1973) in which there are extensive inversion polymorphisms which are known to be selectively important, and with which the allozyme loci are intimately associated. Experimental design should therefore be carefully controlled to avoid these effects. The recent discovery that apparently homogeneous electrophoretic classes can conceal several alleles with different biochemical properties (BERNSTEIN, THROCKMORTON and HUBBY 1973) casts further doubt on the value of investigations based on small population samples.

A number of theoretical treatments (OHTA and KIMURA 1970) have emphasized the importance of linked loci in controlling the frequency of allozymes in natural populations. Experiments using carefully constructed laboratory populations on the response of allozymes to a variety of environmental conditions are likely to provide valuable information on the existence, if any, of selective differences between allelic variants at enzyme loci, particularly when environments containing different concentrations of the enzyme's substrate are used. However, experiments which confound selective responses at many loci simultaneously can only give exaggerated and misleading estimates of fitness differences between allozymes.

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LITERATURE CITED

BERGER, E., 1971 A temporal survey of allelic variation in natural and laboratory populations of *Drosophila melanogaster*. Genetics **67**: 121–136.

BERNSTEIN, S. C., L. H. THROCKMORTON and J. L. HUBBY, 1973 Still more genetic variability in natural populations. Proc. Natl. Acad. Sci. U.S. **70**: 3928–3931.

BIJLSMA-NEELES, E. and W. VAN DELDEN, 1974 Intra- and interpopulation selection concerning the alcohol dehydrogenase locus in *Drosophila melanogaster*. Nature **247**: 369–371.

CLARKE, B., 1970 Darwinian evolution of proteins. Science **168**: 1009–1011.

HARRIS, H. and D. HOPKINSON, 1972 Average heterozygosity per locus in man; an estimate based on the incidence of enzyme polymorphisms. Ann. Hum. Genetics **36**: 9–20.

KIMURA, M., 1968 Evolutionary rate at the molecular level. Nature **217**: 624–626.

LEWONTIN, R. C. and J. L. HUBBY, 1966 A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics **54**: 595–609.

MACINTYRE, R. J. and T. R. F. WRIGHT, 1966 Response of esterase-6 alleles of *Drosophila melanogaster* and *D. simulans* to selection in experimental populations. Genetics **53**: 371–387.

OHTA, T. and M. KIMURA, 1970 Development of associative overdominance through linkage disequilibrium in small populations. Genet. Res. **16**: 165–177.

POWELL, J. R., 1973 Apparent selection of enzyme alleles in laboratory populations of Drosophila. Genetics **75**: 557–570.

SING, C. F., G. J. BREWER and B. THIRTLE, 1973 Inherited biochemical variation in *Drosophila melanogaster*. Noise or signal? I. Single-locus analyses. Genetics **75**: 381–404.

WILLS, C. and L. NICHOLS, 1971 Single gene heterosis in *Drosophila* revealed by inbreeding. Nature **233**: 123–125. ——, 1972 How genetic background masks single gene heterosis in *Drosophila*. Proc. Natl. Acad. Sci. U.S. **69**: 323–325.

YAMAZAKI, T., 1971 Measurement of fitness at the esterase-5 locus of *Drosophila pseudoobscura*. Genetics **67**: 579–603. ——, 1972 Detection of single gene effects by inbreeding. Nature New Biol. **240**: 53–54.

YARBROUGH, K. and K. KOJIMA, 1967 The mode of selection at the polymorphic esterase-6 locus in *Drosophila melanogaster*. Genetics **57**: 677–686.

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