RESPONSES OF ESTERASE 6 ALLELES OF *DROSOPHILA MELANOGASTER* AND *D. SIMULANS* TO SELECTION IN EXPERIMENTAL POPULATIONS¹

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THE existence of a protein polymorphism involving two forms of a nonspecific esterase has been established in *Drosophila melanogaster*. The two forms of the esterase, Esterase 6, are distinguishable by their different electrophoretic mobilities in starch gel, Esterase 6^F migrating more rapidly toward the anode than Esterase 6^S. The inheritance of these two forms is controlled by a pair of co-dominant alleles, Esterase 6^F (*Est* 6^F, 6^F, or *F*) and Esterase 6^S (*Est* 6^S, 6^S, or *S*), located at 36.8 ± on Chromosome 3. Individuals homozygous for the *Est* 6^F allele produce a strong, single Esterase-6 band which migrates faster than a similar, strong, single Esterase-6 band produced by individuals homozygous for the other allele, *Est* 6^S. Zymograms of heterozygotes, *Est* 6^F/*Est* 6^S, exhibit both the Esterase-6^F band and the Esterase-6^S band (WRIGHT 1963).

In addition, a gene-enzyme system homologous with Esterase 6 in *D. melanogaster* has been discovered in *D. simulans*. Along with *Est* 6^F and *Est* 6^S alleles, a third mutant allele, Esterase 6^I, which produces an enzyme that migrates to a position between those of Esterase 6^F and Esterase 6^S has been found in both laboratory stocks and natural populations of this species (WRIGHT and MACINTYRE 1963, 1965).

The study of electrophoretically determined gene-enzyme systems, such as Esterase 6, may have direct bearing on some current problems in population genetics. For example, it has often been postulated that most loci in the gene pool of an outbreeding population are occupied by heterozygous combinations of many slightly different but still "wild-type" alleles (WALLACE 1958a). However, very little specific information on the nature and the frequency of these so-called isoalleles (STERN and SCHAEFFER 1943) has been obtained (see MILKMAN 1960 and GIBSON and THODAY 1962). Consequently, the exact nature of the selective forces responsible for this suspected genetic heterogeneity in natural populations is poorly understood. Studies on the responses of genes to selection, usually conducted in population cages, have, with but few exceptions (HOCHMAN 1958, 1961; RASMUSON and RASMUSON 1962; VAN VALEN and LEVINE 1963), utilized

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morphologically visible, deleterious mutant alleles. These alleles are generally not present in natural populations, at least not in frequencies higher than those representing a balance between selection against the mutant and recurrent mutation of the wild-type allele. On the other hand, the flies of all the genotypes at the *Est 6* locus are morphologically indistinguishable in both *D. melanogaster* and *D. simulans*, and consequently, the "mutant" genes can be regarded as isoalleles. Furthermore, it is clear that at least some natural populations are polymorphic for the enzymes specified by the alleles at the *Est 6* locus. Although systematic sampling of wild populations of these two species has not yet been carried out, the frequencies of *Est 6^F* and *Est 6^S* alleles in *D. melanogaster* appear to be about equal in North Carolina and in the Baltimore area (KADEL, MACINTYRE and WRIGHT, unpublished). In addition, *Est 6^F*, *Est 6^S*, and *Est 6^I* alleles have been found in samples of *D. simulans* both from Baltimore (WRIGHT and MACINTYRE, 1963) and Hawaii (LARS BECKMAN, personal communication). Several polymorphic laboratory strains have also been found (WRIGHT 1963; WRIGHT and MACINTYRE 1963; MACINTYRE, unpublished). Thus, it is evident that a study of the forces responsible for these suspected polymorphisms of Esterase-6 enzymes should be undertaken.

The observations described below on the responses of *Est 6* alleles to selection resulting from intense competition and reduced environmental heterogeneity in population cages have been designed to answer, in the first place, the following question: What is the fate of Esterase 6 alleles from the same and different "populations" (laboratory strains) under these artificial conditions? Secondly, if stable polymorphisms of the Esterase 6 enzymes are established, what is the role of selection in the maintenance of the Esterase 6 "isoalleles" in population cages?

MATERIALS AND METHODS

Description of founder stocks: Population cages were grouped and studied with reference to the main questions asked above. First, are polymorphisms established and maintained in artificial populations (Cages 1 to 8)? Second, can it be determined if and how selection is responsible for these polymorphisms (Cages 9 to 18 and "A" Cages)?

With the exception of Cages 3 and 4, which were founded with stocks of *D. simulans*, the stocks are all *D. melanogaster*. The *Est 6^F* allele of *D. melanogaster* in all experimental populations was from the Ore-R CH stock (see WRIGHT 1963). The *Est 6^S* allele was from one of three stocks: Ore-R CH, Amherst Inbred or Lausanne-S. A brief description of the basic stocks, from which all of the founders were derived, follows:

Ore-R CH: *Est 6^S* (218): This stock, homozygous for *Est 6^S*, was derived from the polymorphic Ore-R CH stock by a single-pair mating.

Ore-R CH: *Est 6^F* (217) Screened: This stock was initially derived from the Ore-R CH stock by a single-pair mating. Subsequently, it was screened for lethals and made "isogenic" by the balanced lethal, crossover suppression technique.

Ore-R CH: *Est 6^S* Coisogenic: A stock made "isogenic" for chromosomes derived from the Ore-R CH *Est 6^S* (218) stock described above by means of the balanced lethal, crossover suppression technique. The multiply inverted balancers *FM4* (Chromosome 1), *SM1* (Chromosome 2) and *Ubx¹³⁰* (Chromosome 3) were used (see MACINTYRE 1964 for details). Difficulties encountered in the construction of this and the following stock are described in detail by MACINTYRE and WRIGHT (1965). Briefly, it was discovered that only three out of

every four wild-type Chromosomes 1 and 3 segregated from *FM4/+*; *SM1/+*; *Ubx¹³⁰/+* females without having been involved in a crossover, usually a multiple crossover, with the inverted, balancer chromosome. These crossovers were more or less evenly distributed along Chromosome 1 but were largely confined to the left arm of Chromosome 3, from the left tip to about 40.0 on the genetic map. Unfortunately, this area on Chromosome 3 includes the *Est 6* locus. Since the *Ubx¹³⁰* chromosome contains an *Est 6^S* allele of unknown origin, there is also a slight chance that this allele was introduced into the *Est 6^S* Coisogenic stock during its construction.

Ore-R CH: *Est 6^F* Coisogenic: This stock was constructed by, first, introducing the *Est 6^F* allele from *Est 6^F* (217) Screened into a Chromosome 3 marked with nine morphologically visible mutant genes distributed along its entire length. The *Est 6^F* allele was placed between hairy (26.0) on the left and thread (43.2) on the right. This allele in the marked chromosome was then "placed" by means of crossovers to either side of the locus between hairy and thread into the single wild-type Chromosome 3 from the Ore-R CH: *Est 6^S* Coisogenic stock which had contained an *Est 6^S* allele. The same wild-type Chromosome 3 thus contained an *Est 6^F* allele in one stock and an *Est 6^S* in a second. Chromosome 1 and Chromosome 2 of the stock containing the *Est 6^F* allele were then replaced by homologues from the Ore-R CH: *Est 6^S* Coisogenic stock, again using the *FM4* and *SM1* balancers. The procedure is diagrammed in MACINTYRE (1964).

When these two stocks described above are spoken of as coisogenic, it should be remembered that certain regions, notably Chromosome 4, parts of Chromosome 1 and some of the left arm of Chromosome 3, could not be rigidly controlled. Nevertheless, the two stocks are undoubtedly highly similar in allelic content.

Amherst Inbred: Stock # 1 from Amherst, Massachusetts. It had been maintained for 388 generations by sib matings up to the time of these investigations. It is homozygous for an *Est 6^S* allele.

Lausanne-S: From the Johns Hopkins University stock collection. It is monomorphic for Esterase 6^S.

Morro Bay: Wild-type *D. simulans* stock from the California Institute of Technology. It is monomorphic for Esterase 6^F.

New Orleans: Wild-type *D. simulans* stock from the Johns Hopkins University stock collection. It is monomorphic for Esterase 6^S.

In Table 1, the chromosome constitutions of the founders of all the cages are outlined. These derived stocks, like Ore-R CH: *Est 6^F* Coisogenic and *Est 6^S* Coisogenic, were constructed with complex mating schemes and special marker stocks which are depicted and described elsewhere (MACINTYRE 1964). The founders of Cages 15 to 18, which are referred to in Figures 8 and 9 as "Amherst Inbred Background" and "Lausanne-S Background" stocks, were constructed in such a way that Chromosome 3 of the Ore-R CH: *Est 6^F* (217) Screened stock was partially replaced by Chromosome 3 from either Amherst Inbred or Lausanne-S. The construction involved nine generations of backcrossing females heterozygous for the *Est 6* alleles to males from the *Est 6^S* stock. In the construction of the *Est 6^F* containing founders for Cages 15 to 18, it was, of course, impossible to know how many other genes from the Ore-R CH line besides the *Est 6^F* allele were introduced into the 3rd chromosomes of the Amherst Inbred and Lausanne-S stocks. However, the so-called "control" lines of MACINTYRE (1964) indicated that, even after nine generations of backcrossing, a rather large segment of Chromosome 3 including *Est 6^F* remained intact. This segment perhaps extended some 10-15 map units to either side of *Est 6^F*.

Initiation of the populations: The cages were run in matched pairs. Except for the "A" cages, the odd numbered cage had an initial *Est 6^F* frequency of 0.20 [$p(F) = 0.20$]. The $p(F)$ of the even numbered cages was, at the outset, 0.80. With the exception of the "A" cages, all the founders were homozygotes. Four hundred flies founded Cages 1, 2, 9 and 10. The other populations (except those of the "A" cages) were started with 200 flies.

The "A" cages contained populations derived from eight flies extracted from some of the populations listed in Table 1. A sample of larvae was taken from the "parental" population and

TABLE 1
Founder stocks of Cages 1 through 18

Cages	Cage designation in MacINTYRE (1964)	Esterase 6 allele of founders	Origin of chromosomes	
			Chromosome 1 and 2	Chromosome 3
1, 2	15, 16	6 ^F	Ore-R CH (217) Screened	Ore-R CH (217) Screened
		6 ^S	Ore-R CH (218)	Ore-R CH (218)
3, 4	1, 2	6 ^F	Morro Bay	Morro Bay
		6 ^S	New Orleans	New Orleans
5, 6	3, 4	6 ^F	Ore-R CH (217) Screened	Ore-R CH (217) Screened
		6 ^S	Amherst Inbred	Amherst Inbred
7, 8	9, 10	6 ^F	Ore-R CH (217) Screened	Ore-R CH (217) Screened
		6 ^S	Lausanne-S	Lausanne-S
9, 10	17, 18	6 ^F	Ore-R CH Coisogenic	Ore-R CH Coisogenic
		6 ^S	Ore-R CH Coisogenic	Ore-R CH Coisogenic
11, 12	5, 6	6 ^F	Amherst Inbred	Ore-R CH (217) Screened
		6 ^S	Amherst Inbred	Amherst Inbred
13, 14	11, 12	6 ^F	Lausanne-S	Ore-R CH (217) Screened
		6 ^S	Lausanne-S	Lausanne-S
15, 16	7, 8	6 ^F	Amherst Inbred	Ore-R CH (217) Screened and Amherst Inbred
		6 ^S	Amherst Inbred	Amherst Inbred
17, 18	13, 14	6 ^F	Lausanne-S	Ore-R CH (217) Screened and Lausanne-S
		6 ^S	Lausanne-S	Lausanne-S

Odd numbered cages had an initial $p(F)$ of .20. Even numbered cages had an initial $p(F)$ of .80. See text for further details. Cages 1 to 2 and 5 to 18 are *D. melanogaster*. Cages 3 to 4 are *D. simulans*.

allowed to develop under crowded conditions. The adults were pooled and mated in single pairs. Their *Est 6* genotypes were determined after it was certain the matings were successful. Before any imagoes appeared, the four vials whose parents' *Est 6* genotypes gave the desired founder gene-frequency were placed into a new cage. When adults appeared in these cages, fresh food vials were inserted, and the feeding cycle initiated. Egg samples were taken one to two weeks later to get supplementary estimates of the initial frequencies of the *Est 6^F* allele. The gene frequencies of the parents of the "A" cage populations and the supplementary estimates are embodied in Figures 2 to 4 and 9.

Maintenance of the cages: Two types of population cages were used in these experiments. Populations 1, 2, 9 and 10 were maintained in cages similar to those described in WRIGHT and DOBZHANSKY (1946). The other populations, 3-8 and 11-18, were maintained in so-called "Bennett Boxes." This type of cage has been described by FRYDENBERG (1962).

All cages were kept in incubators at $25 \pm 1^\circ\text{C}$. The relative humidity of the incubators was not determined. Further details on the initiation, maintenance and the transfer of these populations to new, clean cages can be found in MACINTYRE (1964).

Sampling: Egg samples were taken, as far as it was possible, every 24 days (or later, every 48 or 72 days). BARKER (1962) has shown that 24 days is a likely mean generation interval for populations of *D. melanogaster* in cages similar to those containing Populations 1, 2, 9 and 10. FRYDENBERG (1962) has demonstrated that the mean generation time in Bennet Boxes is 15 days.

Egg samples were obtained in the following manner. Cups and vials were filled with a cream-of-wheat, molasses medium. The vials were tilted so a slant formed as the food cooled. This slanted surface was kept at the very mouth of the vial; this is important for as FRYDENBERG found (1962), the composition of egg samples may be modified if the laying surface is far back inside the vial. Females in the population were allowed to oviposit for 20 hours. The cup (or vial) was then removed, and the eggs, easily seen against the dark medium, were randomly picked from the surface with a small spatula. 100 to 150 eggs were transferred to small squares of blotting paper soaked in a *Drosophila* Ringer solution. Each square was then placed in a heavily yeasted culture bottle containing about 60 ml of food. Thus, larval density (about two larvae to 1 ml of food) should have been such as to assure optimal developmental conditions. When the flies had hatched, they were sexed and frozen before being homogenized and electrophoresed.

In order to determine the gene frequency of the sample, individual flies were crushed on 5×7 mm pieces of moistened filter paper. These papers were then inserted into starch gels through which a current was subsequently run. The proteinaceous esterases, being charged molecules, will migrate to the pole opposite their own net charge. After electrophoresis, the gels were removed and placed in a solution consisting of a substrate and a suitable dye which precipitates after, and at the site of, enzyme activity. Details concerning the electrophoretic and staining procedures are described elsewhere (WRIGHT 1963; WRIGHT and MACINTYRE 1963).

As often as possible, the sample size consisted of 288 genes or 144 flies, 72 of each sex.

RESULTS

Each egg sample was analyzed to provide the following information: the gene frequency estimates of *Est 6^F* of the males and females as well as the males and females combined (total sample), the statistical significance of the difference between the *Est 6^F* frequencies of the males and females, the 95% confidence limits of the *Est 6^F* frequency of the total sample, and finally, the statistical significance of the difference between the observed and the expected numbers of the three genotypes (as predicted from the *Est 6^F* frequency) in the total sample. The methods of analysis can be found in any basic statistics text, and the results are reported in detail in MACINTYRE (1964). The small number of significant differences between the *Est 6^F* frequencies of males and females in the samples was quite compatible with that expected in random sampling procedures. When the observed and expected numbers of genotypes of the adults derived from the egg samples were compared, again, approximately only one in 20 chi-square values were significant at the .05 level. However, it was noted that there was an excess of cases in which the observed number of heterozygotes exceeded the expected number. These cases were found in the samples from all the cages. When all the samples were grouped together, the average magnitude of this excess was found to be just three more heterozygotes per sample than expected. By empirical tests, it was found that the reliability of the sampling method was not significantly affected (see MACINTYRE 1964 for details). Moreover, the changes in gene frequency during the tenure of an experimental population, which are of paramount importance in interpreting the results of these experiments, were not affected by this slight and consistent difference between the observed and expected numbers of heterozygotes in the samples. Thus, in the present communication, for each sample, only the *Est 6^F* frequency and its

95% confidence interval are reported. These are embodied in the graphs of Figures 1 to 9.

Populations initiated with founders from the same genetic stock: The results from Cages 1 and 2 (Figure 1) show that gene pools of populations founded by flies from stocks *Est 6^F* (217) Screened and *Est 6^S* (218) (both originally from the same Ore-R CH stock) exhibit an Esterase-6 polymorphism with nearly equal proportions of the two alleles at equilibrium.

Populations initiated with founders from different genetic stocks: In Cages 3 and 4 (*D. simulans*), there was a rapid approach to a $p(F)$ of about .45 (Figure 2). This was accomplished in but five generations. In Cage 3, the frequency of *Est 6^F* then rose to about .55 where it remained for the duration of the experi-

FIGURE 1.—Changes in frequencies of *Est 6^F* in Cages 1 and 2. For Figures 1 to 9 the source of founders carrying the *Est 6^F* alleles and those carrying the *Est 6^S* alleles are indicated at the top of each figure and in Table 1. Brackets in Figures 1 to 9 include the 95% confidence interval for each estimate.

FIGURE 2.—Changes in frequencies of *Est 6^F* in Cages 3, 3A, 4 and 4A. The question marks in Figures 2, 3, 4 and 9 point out that the gene frequency of the founders of each of the "A" cages was not estimated from an egg sample, but rather from the genotypes of the eight parents whose offspring comprised the first generation of flies within each "A" cage.

FIGURE 3.—Changes in frequencies of *Est* 6^F in Cages 5, 5A, 6 and 6A.

FIGURE 4.—Changes in frequencies of *Est* 6^F in Cages 7, 7A, 8 and 8A.

ment. The frequency of *Est* 6^F in Cage 4 ultimately stabilized at the same level, but did not rise until several more generations had elapsed. The overall picture presented by Cages 7 and 8 (Figure 4) is very similar. The gene-frequency estimates of *Est* 6^F from the two cages are essentially identical for the first ten generations. Then they started to fluctuate in an erratic fashion, at least until the 30th generation, while still maintaining an overall gene frequency of approximately .38. The interpretation of the results from Cages 5 and 6 (Figure 3) is complicated by the near elimination of the *Est* 6^S alleles in the first two generations. This was probably a result of the comparatively poor fitness of the Amherst Inbred stock. Upon sufficient hybridization between the two founder stocks, the frequency of *Est* 6^F in both cages began to decline until about the 20th generation. Then in Cage 5, the frequency of that allele stabilized at about .43. In Cage 6, the decline continued until nearly the termination of the experiment.

The "A" cages derived from Populations 3 to 8 fall into two groups with respect to the behavior of the Esterase 6 alleles. In the first group (Cages 5A, 6A

and 7A), the frequency of the *Est 6^F* allele returned or tended to return to the equilibrium level of the base population. The difference between the *Est 6^F* frequency estimates of these A cages at the first and last samples are statistically significant, and the changes in gene frequency were directional in each case (Figures 3, 4). It is noteworthy that these progressive changes in the proportions of the *Est 6* alleles in the A cages toward the equilibrium levels of the base populations were much slower than were the initial approaches to equilibrium in the base populations. For example, Cage 7 reached an apparent *Est 6* equilibrium in just three generations, but it took Cage 7A some 14 generations to reach the same, apparently stable, proportion of *Est 6* alleles.

In the second group of A cages, including 3A, 4A and 8A, there were apparently no significant changes in the frequencies of the *Est 6* alleles either toward or away from the equilibrium levels of the base populations. In other words, in these instances, the *Est 6* alleles acted as if they were neutral in selective value. Cages 3A and 4A were sampled for only five generations so it is difficult to say definitely if the apparent stability in the frequencies of the *Est 6* alleles is real. However, there was still no apparent change in the frequency of *Est 6^F* in Cage 8A after 17 generations.

Populations initiated with founders characterized by high levels of genetic homozygosity: The genetic background of the founders of Cages 9 and 10 is ultimately from the same stock which provided the founders for Cages 1 and 2. However, in Cages 9 and 10, owing to the way in which the genomes of these founders were manipulated, there should be a much higher proportion of loci homozygous for alleles characteristic of the *Est 6^S* founders of Cages 1 and 2. Clearly, the results from Cages 9 and 10 (Figure 5) do not resemble those from Cages 1 and 2 (Figure 1). The genetic background obviously influences the equilibrium levels of the *Est 6* alleles. However, it is necessary to assume that the *Est 6^S* allele from the *Ubx¹³⁰* chromosome (see MACINTYRE and WRIGHT 1965) is either not present or is not a complicating factor. Each cage may be unique with respect to the behavior of the *Est 6* alleles, or the adaptive values of the three

FIGURES 5.—Changes in frequencies of *Est 6^F* in Cages 9 and 10.

genotypes in both cages may be such that there is an equilibrium frequency of the *Est 6^F* allele at approximately .30.

Populations initiated with founders having the genetic background of the Est 6^S founder stock: In Cages 11 to 14 the genetic background of the *Est 6^F* containing founder is that of the *Est 6^S* stock, except for the entire 3rd chromosome. Examination of Figures 6 and 7 shows that when Cages 11 and 12 are compared with 5 and 6 and when 13 and 14 are compared with 7 and 8, the correspondence between gene frequencies at the 20th generations and between general changes in allele proportions after the first few generations are quite close.

The genetic background of the founders of Cages 15 to 18 must have contained more genes on Chromosomes 3 characteristic of the original *Est 6^S* stock, i.e. Amherst Inbred or Lausanne-S, than did the founders of Cages 11 to 14. This replacement of 3rd chromosome alleles has definitely altered the final relative proportions of the *Est 6* alleles and their changes with time in Cages 15 to 18. In none of the latter cages is there an initial and rapid approach to an inter-

FIGURE 6.—Changes in frequencies of *Est 6^F* in Cages 11 and 12.

FIGURE 7.—Changes in frequencies of *Est 6^F* in Cages 13 and 14.

FIGURE 8.—Changes in frequencies of *Est* 6^F in Cages 15 and 16.

FIGURE 9.—Changes in frequencies of *Est* 6^F in Cages 17, 17A, 18 and 18A.

mediate equilibrium, as is found in Cages 11 to 14. Rather, and especially in the early generations of Cages 15 to 18, there appear to be no significant changes in the frequencies of the *Est* 6^F alleles (Figures 8, 9). In later generations, there seems to be no general trend in the behavior of the *Est* 6 alleles in the four cages. There is a significant increase in the frequency of *Est* 6^F beginning at Generation 5 in Cage 15, but a decrease starting at Generation 15 in Cage 16 (Figure 8). On the other hand, no significant changes occurred in either Cage 17 from its initiation or in Cage 18 after the 3rd generation. Cages 17A and 18A appear to be mimicking their respective base populations (Figure 9).

DISCUSSION

Esterase 6 alleles in artificial populations: In the cages in which no attempt was made to control the genetic background of the founders (Cages 1 to 8), the Esterase 6 alleles established intermediate equilibrium frequencies in less than ten generations. This was true when both the founders were from the same (Cages

1, 2) and different (Cages 3 to 8) genetic stocks. In only one case (Cage 6) is there a suggestion that both alleles may not remain at or very near those initially established proportions. In other words, the *Est 6* equilibrium frequencies, once established in these artificial populations, appear to be quite stable. This would suggest, but certainly not prove, that the Esterase 6 polymorphisms found in natural populations are not transient.

It is interesting to compare the above results with those from cages where changes in the frequencies of morphologically visible and, probably, deleterious mutant alleles were followed. The *Est 6* alleles persist at apparently stable levels in some instances for at least 35 generations. In many cases this is not true of mutant and wild-type alleles in cages under similar conditions (see especially BUZZATI-TRAVERSO 1955; HEXTER 1955; MUKAI and BURDICK 1960a; POLIVANOV 1964; MERRELL 1965). Occasionally in such studies, apparent equilibria between mutant and wild-type alleles may be initially established, but after several generations the mutant genes generally begin to decline in frequency (see MERRELL 1965 for a review). However, it should be stressed that this is not always the case. Some mutant genes have remained at equilibrium in artificial populations at least as long as the *Est 6* alleles (CANNON 1963). Some have even increased in frequency (CHIGUSA and MUKAI 1964). Therefore, before one can definitely say that *Est 6* "isoalleles" behave differently from morphologically visible mutant genes in artificial populations, cages containing these "isoalleles" should be examined for longer periods of time, perhaps for 100 generations or more.

The role of selection in the establishment and maintenance of the Esterase 6 polymorphisms: In the present studies, changes in gene frequencies were followed and used as indications of the presence or absence of an effect of selection on the *Est 6* alleles. Genotype frequencies at or before gene-frequency equilibria were not determined since adults, once scored for Esterase 6 phenotypes, obviously could not be returned to the population. Also, genotype sampling from a population, even if gene frequencies are stable (see WALLACE 1958b), does not always confirm heterotic selection (see DOBZHANSKY 1954, p. 440). More comprehensive analyses of fitness components of flies extracted from the cages are necessary (BUZZATI-TRAVERSO 1955; MERRELL 1965). These analyses are outside the scope of the present investigations.

Although other mechanisms may occasionally play a role, genetic polymorphisms in natural populations are most commonly maintained by the superiority of the heterozygous genotype in one or more of the components of fitness, i.e. viability, fecundity, etc. (DOBZHANSKY 1951). In population cages, which are designed to reduce environmental heterogeneity, it is highly likely that the establishment and maintenance of gene frequency equilibria is due to the selective superiority of the heterozygote (see FRYDENBERG 1963, for other, less likely explanations). However, the establishment of gene-frequency equilibria in the first few generations of experimental populations does not necessarily mean that the particular alleles under study are themselves functional parts of selectively maintained interacting gene complexes. Especially when the founders carrying the different alleles were derived from separate geographic strains (Cages 3 to 8) or

include only a small portion of the gene pool of an originally polymorphic population (Cages 1 and 2), the interactions may involve the gene products of other, linked loci. These interactions may involve dominant genes (MUKAI and BURDICK 1960b) or what appear to be overdominant gene complexes (see WALLACE and VETUKHIV 1955). The latter phenomenon has been variously called "pseudo-overdominance" (LERNER 1958), "multilocus heterosis" (HEXTER 1955) and "associative overdominance" (FRYDENBERG 1963). And, in fact, several instances have been recorded in which apparent overdominant interactions between mutant and wild-type alleles were or would be lost upon recombination with other genes or inversions along the chromosome (HEXTER 1955; CHIGUSA and MUKAI 1964; POLIVANOV 1964; FRYDENBERG 1964a). In addition, CARSON and his students have shown that intact blocks of genes persist in early generations of experimental populations of hybrid origin and contribute substantially, if not totally, to a rather prolonged improvement in fitness. He has referred to this phenomenon as "captured luxuriance" (CARSON 1961). Of interest here is the fact that supposedly deleterious mutant alleles, by being included within these larger integrated blocks of genes, can reach high, apparently stable frequencies (CARSON 1961; CANNON 1963).

Therefore, in order to determine if the rapidly established *Est 6* gene frequency equilibrium characteristic of Cages 1 to 8 might be due to selection for the overall F_1 hybrid genotype and to better understand the responses to selection of the *Est 6* alleles apart from the possible complicating effects of nonrandomly linked dominant or overdominant alleles, new cages were initiated. These cages included populations in which the initial genetic differences between the founders were, hopefully, either randomized (A Cages), minimized (Cages 9 and 10) or controlled in more or less specific ways (Cages 11 to 18). The results will be discussed in that order.

Populations with "randomized" genetic backgrounds: It seems clear, that in the gene pools of populations initiated with founders from different geographic strains, the initial selective preservation of the F_1 hybrid genotype does not persist indefinitely (CANNON 1963). As the "luxuriant" gene blocks are broken down by recombination, new adaptive, but not necessarily predictable, gene combinations are formed (see especially DOBZHANSKY 1954; BUZZATI-TRAVERSO 1955; CHIGUSA and MUKAI 1964). It was pointed out that either the *Est 6* frequencies in the A cages slowly returned to the equilibrium levels of the base populations, or did not significantly change at all. This suggests that the rapid establishment of *Est 6* equilibrium frequencies in the early generations of the base populations did indeed result from selection for flies heterozygous for intact blocks of genes from parental strains.

The genetic backgrounds of the founders of the A cages surely must have been different from those of the founders of the base populations. However, there is no way of knowing to what extent recombination had randomized linkage relationships within the gene pools of the base populations at the time those A-cage founders were extracted. For this reason conclusions about the selective value of *Est 6* alleles in the A cages can only be tentative. If the "luxuriant" gene blocks

were completely broken down by recombination in all the base populations when the A-cage founders were extracted, then in some gene pools, the *Est 6^F/Est 6^S* heterozygote may have been selectively advantageous and the gene frequencies would return to a predetermined equilibrium, namely that established in later generations by the base populations. The fact that the *Est 6* gene frequencies did not change in some of the A cages might mean that the alleles in other genetic backgrounds are neutral or very nearly neutral in selective value. Or, the two types of response to selection in the A cages by the *Est 6* alleles may reflect the possibility that in some base populations recombination had operated but linkage equilibrium was still not complete. In A cages extracted from those populations, the *Est 6^F* gene frequencies would return to the equilibria of the base populations but at comparatively slower rates. Thus, according to the second interpretation, it is possible that in a randomized genetic background the *Est 6* alleles are neutral in selective value. This may be indicated, then, by the lack of change in *Est 6* gene frequencies both in some A cages and in the later generations of nearly all the base populations. Lacking more detailed knowledge of the differences in the gene pools of the base populations between the initial generations and the generations when the A-cage founders were extracted, it is not possible to decide which of these interpretations is more likely to be correct.

Populations with largely homozygous genetic backgrounds: In Cages 1 and 2, whose founders were taken from the same polymorphic population, intermediate *Est 6* equilibria were quickly established. However, the founders contained, at most, only five different 1st, 2nd and 3rd chromosomes (since Ore-R CH stock *Est 6^F* (217) Screened was isogenic and Ore-R CH stock *Est 6^S* (218) was derived from a single-pair mating). POLIVANOV (1964) has pointed out that an apparent but spurious overdominant relationship between alleles at a single locus can accompany the interaction of "linked polygenic blocks" or "supergenes" (equivalent to "captured luxuriance" in the sense of CARSON 1961) in artificial populations. POLIVANOV has also shown that this spurious overdominant effect will usually take place in populations started with stocks containing one or a few nonmutant chromosomes. It was suspected, then, that selection for "linked polygenic blocks" could explain the *Est 6* equilibria in Cages 1 and 2.

Since the interaction of linked "supergenes" necessarily implies genic heterozygosity (see BODMER and PARSONS 1962), the founders of Cages 9 and 10 were made as coisogenic as possible. Therefore, by preventing selection from acting on the effects of linked interacting genes, it was hoped that the relationship between selection and the *Est 6* alleles in population cages derived from founders of the same genetic stock could be made more evident. A similar approach was taken by HOCHMAN (1958, 1961) in the study of selection and *ci* isoalleles. He found no fundamental difference between the action of selection in populations with homozygous and heterozygous genetic backgrounds.

However, it is impossible to unequivocally interpret the gene-frequency changes in Cages 9 and 10. The fact that equilibrium frequencies around .50 were not established in these populations may mean that the Esterase 6 polymorphisms in Cages 1 and 2 were due to the overriding effect of selection for

linked interacting gene complexes. In Cage 9, the absence of any significant change in the frequency of *Est 6^F* would, by itself, imply that in genetic backgrounds characterized by high levels of homozygosity, the *Est 6* alleles are neutral in selective value. On the other hand, the decline in the frequency of *Est 6^F* in Cage 10 to about .30 may indicate that a selectively maintained gene-frequency equilibrium at about that level characterizes populations founded by the "coisogenic" stocks. The difficulty in interpreting these results may stem from the fact that the loci closely linked to *Est 6* could not be more precisely controlled in the construction of the "coisogenic" stocks (see MATERIALS AND METHODS and MACINTYRE and WRIGHT 1965).

Populations with controlled genetic backgrounds: It has already been stressed that the role of selection in the maintenance of the *Est 6* alleles in the populations of hybrid origin (Cages 3 to 8) could be obscured by the interstrain or "luxuriant" heterosis of the F_1 hybrid genotype in the early generations. In Cages 11 to 18, *Est 6^F* and *Est 6^S* founder strains were constructed in such a way that the interstrain genetic differences of the founders of Cages 3 to 8 were restricted to either the entire 3rd chromosome (Cages 11 to 14) or to some fractional part of the 3rd chromosome including the *Est 6* locus and an undetermined number of genes to either side (Cages 15 to 18).

In Cages 11 to 14, the *Est 6* alleles quickly reached apparently stable, intermediate frequencies very much like those of Cages 3 to 8. However, when interstrain genetic differences in the 3rd chromosomes of the founders were reduced, the *Est 6* alleles of those populations exhibited somewhat different responses to selection. Changes in allele frequencies were much slower in Cages 15 to 18. Thus, the suspicion seems well founded that the initially established *Est 6* gene-frequency equilibria in Cages 3 to 8 and 11 to 14 are spurious by-products of selection for heterozygotes containing integrated gene blocks or "supergenes" (in the sense of POLIVANOV 1964) from each geographic strain.

The results from the early generations of Cages 15 to 18 also suggest that, once separated from these interacting gene complexes, the *Est 6* alleles could be neutral or nearly neutral in selective value. The rather indeterminate changes in *Est 6* allele frequencies in the later generations may also be spurious. For example, a change in the frequency of the *Est 6^F* allele may simply result from a change in frequency of a linked gene during its inclusion in a newly formed adaptive gene complex (see CHIGUSA and MUKAI 1964). On the other hand, it is possible that the *Est 6* alleles, even though previously neutral in selective value, could themselves eventually become functional parts of interacting gene combinations such as that described by GIBSON and THODAY (1962). At present, there is no way to decide which is the more likely alternative.

When considered together, the results from the A cages and Cages 9 to 18 all suggest that the selective retention of interacting blocks of linked genes ("captured luxuriance") in the early generations of Cages 1 to 8 was responsible for the rapidly established intermediate gene-frequency equilibria in those populations. Whether or not the *Est 6* alleles were functional parts of the "luxuriantly" interacting gene complexes is not known.

At any rate, when the interstrain genetic differences between the founders of the populations were reduced or controlled, the *Est 6* alleles in the subsequent gene pools did not appear to be "intrinsically" overdominant (in the sense of FRYDENBERG 1963). In other words, there is no evidence for "single gene heterosis" at this locus. This is not surprising, for the complexity inherent in the development, physiology and ecology of the organism ought to make this a highly unlikely form of gene interaction.

It was pointed out that in some randomized genetic backgrounds the *Est 6* alleles appear to either be neutral in selective value or have very small selection coefficients. FORD (1957) has cogently argued that cases in which alleles remain selectively neutral for any extended period of time must be very rare. However, at the protein level of gene action, not enough is known concerning the relationship between primary and tertiary structures to say that an amino acid change in a functional enzyme will always affect its selective value. The Esterase 6^F and Esterase 6^S enzymes must have at least one different amino acid, yet there is no noticeable difference in enzyme activity. They do differ in heat stability, however (WRIGHT and MACINTYRE 1965), but it is doubtful that this is significant at the temperature of the population cage environment. In fact, the importance of this gene-enzyme system itself to the organism is not known. A strain which lacks detectable Esterase 6 activity has been found. There are also reports of homozygous esterase deficiencies from *D. melanogaster* (JOHNSON 1964) and *Musca domestica* (VELTHUIS and VAN ASPEREN 1963) which do not noticeably affect the viability of the flies.

Therefore, although both *Est 6^F* and *Est 6^S* alleles tend to remain in artificial populations, the role of selection in the maintenance of the polymorphisms is not clear. Even selective neutrality of these alleles cannot be ruled out. The fact that these alleles may be selectively neutral in some genetic backgrounds does not preclude the possibility that they can also become functional parts of adaptive gene combinations in other gene pools. Studies are now being conducted on the responses of *Est 6* alleles to selection in cages maintained for much longer periods of time. Other populations founded with flies whose genetic backgrounds have been very specifically controlled are being initiated and observed. It is hoped that from these results a more precise explanation of the relationship between selection and the Esterase 6 polymorphisms can be obtained.

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SUMMARY

Esterase 6^F and Esterase 6^S alleles from the same polymorphic stock of *D. melanogaster* reached intermediate and apparently stable equilibrium frequencies in experimental populations whose founders came from that same Ore-R stock. Very similar results were obtained in populations of both *D. melanogaster* and

D. simulans founded with flies from different geographic strains. However, studies were also made of populations in which the initial genetic differences between the founders were either more randomly distributed, reduced to a small number of loci, or controlled in more or less specific ways with respect to the 3rd chromosome. It was concluded that in the cages in which no attempts were made to control the genetic backgrounds of the founders, the initial rapidly established *Est 6* gene frequency equilibria were by-products of selection for linked, interacting gene complexes. Nevertheless, both the alleles persisted at rather stable relative proportions in most of these populations. It cannot be determined from these studies if the *Est 6* alleles became functional parts of selectively established gene combinations or were neutral, or very nearly neutral, in selective value.

LITERATURE CITED

- BARKER, J. S. F., 1962 The estimation of generation interval in experimental populations of *Drosophila*. *Genet. Res.* **3**: 388-404.
- BODMER, W. F., and P. A. PARSONS, 1962 Linkage and recombination in evolution. *Advan. Genet.* **11**: 1-100.
- BUZZATI-TRAVERSO, A. A., 1955 Evolutionary changes in components of fitness and other polygenic traits in *Drosophila melanogaster* populations. *Heredity* **9**: 153-186.
- CANNON, G. B., 1963 The effects of natural selection on linkage disequilibrium in experimental populations of *Drosophila melanogaster*. *Genetics* **48**: 1201-1216.
- CARSON, H. L., 1961 Heterosis and fitness in experimental populations of *Drosophila melanogaster*. *Evolution* **15**: 496-509.
- CHIGUSA, S., and T. MUKAI, 1964 Linkage disequilibrium and heterosis in experimental populations of *Drosophila melanogaster* with particular reference to the sepia gene. *Japan. J. Genet.* **39**: 289-305.
- DOBZHANSKY, TH., 1951 *Genetics and the Origin of Species*, 3rd ed. Columbia University Press, New York. — 1954 Evolution as a creative process. *Proc. 9th Intern. Congr. Genet. Cytology* **6** (Suppl.): 435-449.
- FORD, E. B., 1957 Polymorphism in plants, animals and man. *Nature* **180**: 1315-1319.
- FRYDENBERG, O., 1962 Estimation of some genetical and vital statistics of Bennett populations of *Drosophila melanogaster*. *Hereditas* **48**: 83-104. — 1963 Population studies of a lethal mutant of *Drosophila melanogaster*. I. Behavior in populations with discrete generations. *Hereditas* **50**: 89-116. — 1964 Population studies of a lethal mutant in *Drosophila melanogaster*. II. Behavior in populations with overlapping generations. *Hereditas* **51**: 31-66.
- GIBSON, J. B., and J. M. THODAY, 1962 Effects of disruptive selection. IV. A second chromosome polymorphism. *Heredity* **17**: 1-26.
- HEXTER, W. M., 1955 A population analysis of heterozygote frequencies in *Drosophila melanogaster*. *Genetics* **40**: 444-459.
- HOCHMAN, B., 1958 Competition between wild type isoalleles in experimental populations of *Drosophila melanogaster*. *Genetics* **43**: 101-121. — 1961 Isoallelic competition in populations of *Drosophila melanogaster* containing a genetically heterogeneous background. *Evolution* **15**: 239-246.
- JOHNSON, F. M., 1964 A recessive esterase deficiency in *Drosophila melanogaster*. *J. Heredity* **55**: 76-78.

- MACINTYRE, R. J., 1964 Responses of Esterase 6 alleles of *Drosophila melanogaster* and *D. simulans* to selection in experimental populations. Ph.D. Thesis. The Johns Hopkins University, Baltimore, Maryland.
- MACINTYRE, R. J., and T. R. F. WRIGHT, 1965 Recombination within multiply inverted "balancer" chromosomes. *Drosophila Inform. Serv.* **41**: 141-143.
- MERRELL, D. J., 1965 Competition involving dominant mutants in experimental populations of *Drosophila melanogaster*. *Genetics* **52**: 165-189.
- MILKMAN, R. D., 1960 The genetic basis of natural variation. II. Analysis of a polygenic system in *Drosophila melanogaster*. *Genetics* **45**: 377-391.
- MUKAI, T., and A. B. BURDICK, 1960a Concerning equilibria of heterotic lethals in random mating populations with particular reference to *l(2)55i* in *Drosophila melanogaster*. *Genetics* **45**: 1581-1594. — 1960b Examination of the closely linked dominant adaptive gene hypothesis as an alternative to single gene heterosis associated with *l(2)55i* in *Drosophila melanogaster*. *Japan. J. Genet.* **36**: 97-104.
- POLIVANOV, S., 1964 Selection in experimental populations of *Drosophila melanogaster* with different genetic backgrounds. *Genetics* **50**: 81-100.
- RASMUSON, M., and B. RASMUSON, 1962 Competition experiments with isoalleles. *Nature* **194**: 997-998.
- STERN, C., and E. W. SCHAEFFER, 1943 On wild type isoalleles in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.* **39**: 361-367.
- VAN VALEN, L., and L. LEVINE, 1963 Natural selection on an isoallele. *Nature* **200**: 1236-1237.
- VELTHUIS, H. H. W., and K. VAN ASPEREN, 1963 Occurrence and inheritance of esterases in *Musca domestica*. *Entomol. Exp. Appl.* **6**: 79-87.
- WALLACE, B., 1958a The role of heterozygosity in *Drosophila* populations. *Proc. 10th Intern. Congr. Genet.* **1**: 408-419. — 1958b Comparison of observed and calculated zygotic frequencies. *Evolution* **12**: 113-115.
- WALLACE, B., and M. VETUKHIV, 1955 Adaptive organization of the gene pool of *Drosophila* populations. *Cold Spring Harbor Symp. Quant. Biol.* **20**: 303-313.
- WRIGHT, S., and TH. DOBZHANSKY, 1946 Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. *Genetics* **31**: 125-156.
- WRIGHT, T. R. F., 1963 The genetics of an esterase in *Drosophila melanogaster*. *Genetics* **48**: 787-801.
- WRIGHT, T. R. F., and R. J. MACINTYRE, 1963 A homologous gene-enzyme system, Esterase 6, in *Drosophila melanogaster* and *D. simulans*. *Genetics* **48**: 1717-1728. — 1965 Heat stable and heat labile Esterase 6^F enzymes in *Drosophila melanogaster* produced by different *Est 6^F* alleles. *J. Elisha Mitchell Sci. Soc.* **81**: 17-19.