GENIC HETEROGENEITY WITHIN ELECTROPHORETIC "ALLELES" AND THE PATTERN OF VARIATION AMONG LOCI IN *DROSOPHILA PSEUDOOBSCURA*

RAMA S. SINGH

Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1

Manuscript received September 22, 1978
Revised copy received April 9, 1979

ABSTRACT

An investigation, similar to our previously reported xanthine dehydrogenase study, was undertaken to examine the extent of hidden genic variation at nine loci (five larval proteins, three esterases and one aldehyde oxidase) by sequential application of various electrophoretic criteria employing pH, gel concentration and buffer variation. Polymorphic loci appear to fall into two distinct groups: weakly polymorphic, including larval protein 6, 7, 8, 10 and 13 and esterase-1 and -6; and highly polymorphic, including esterase-5, Xdh and possibly Ao. Monomorphic loci may belong to a third group different from all polymorphic loci. Bogota, a geographical isolate that is reproductively isolated from the mainland population, was found to be genetically distinct at four of the ten loci examined in detail so far, including *Xdh*, whereas previously it was found to be genetically distinct at none. These results are discussed in the light of balancing selection, neutral and mutation-selection hypotheses of genic variation in natural populations.

IN population genetics, there are basically two approaches for evaluating the selective significance of genic polymorphisms in natural populations. One approach, experimental, involves the search for the selective forces, total or partial, working at specific polymorphic loci in laboratory or natural populations, which might indicate if some sort of balancing selection is operating at the locus. This approach has been widely used with morphological markers, chromosomal polymorphisms and lethal or semi-lethal genes (all involving genes or blocks of genes with large effects) with moderate success (see LEWONTIN 1974 for a review). Use of this approach with allozyme variants, however, has produced mixed results—some positive, some negative and more often, ambiguous (see LEWONTIN 1974; and HEDRICK, GINEVAN and EWING 1976 for reviews).

The second approach, theoretical, makes use of the specific predictions of allelic frequency distribution implied by the neutral theory and its comparison with the observed allele frequency distribution within and among populations. This is the approach that has been more widely used with electrophoretic data, as it was realized very early that to maintain the large amount of newly discovered genetic polymorphisms by balancing selection, individual polymorphic

loci could not be expected to experience selection of the order of those that can successfully be measured in any reasonable experimental scheme. When applied to electrophoretic data, this approach, too, has produced results that are at variance in one way or another with both the selectionist and neutralist hypotheses (LEWONTIN 1974; EWENS and FELDMAN 1976).

One of the reasons for such contradictory results might be that the observed allelic frequency distributions are simply wrong. For example, if alleles were being lumped into discrete electrophoretic classes ("electromorphs" of KING and OHTA 1975) producing a distorted allele frequency distribution, then the statistical predictions using these allele frequency distributions would be far off from the observed facts, and in selection experiments, using heterogeneous classes of alleles, any measurement of selective values would be spurious. The problem of underestimation of allelic diversity was considered by LEWONTIN and HUBBY (1966) in their seminal paper and was seriously raised again by BERNSTEIN, THROCKMORTON and HUBBY (1973) and by SINGH, HUBBY and THROCKMORTON (1975) with their heat-sensitivity work on Xanthine dehydrogenase and Octonol dehydrogenase, respectively, in the *Drosophila virilis* group. Studies by other workers have shown it to be a rather general problem (JOHNSON 1976, 1977; COBBS 1976; MILKMAN 1976; THORIG, SCHOONE and SCHARLOO 1975; MILKMAN and KOEHLER 1976; COCHRANE 1976; TRIPPA, LOVERRE and CATAMO 1976). But the seriousness of the problem was really not fully realized until SINGH, LEWONTIN and FELTON (1976) and COYNE (1976) reported their work on *Xdh* locus in *D. pseudoobscura* and *D. persimilis*, respectively, using a number of electrophoretic criteria. SINGH, LEWONTIN and FELTON found 37 alleles at the *Xdh* locus among only 146 genome samples from the entire range of *D. pseudoobscura*. Using four of the same criteria, COYNE (1976) reported 23 alleles among only 60 genome samples in *D. persimilis*. Of these, only three were indistinguishable from those in *D. pseudoobscura*. Thus, a total of 57 alleles were found to be segregating within the two sibling species among only 206 genomes. Previously, only six alleles had been reported in *D. pseudoobscura* and only three in *D. persimilis*, of which two were in common with those in *D. pseudoobscura*. Also Bogota, a geographically isolated population of *D. pseudoobscura*, which is partially reproductively isolated as well (PRAKASH 1972), had been found (PRAKASH, LEWONTIN and HUBBY 1969) to be monomorphic for the most common allele in the rest of the species' distribution. In SINGH, LEWONTIN and FELTON's (1976) study, it was highly polymorphic with five alleles in only 12 genomes. Moreover, nine out of 12 lines carry alleles that are unique to Bogota and two other lines have an allele that was found only once in the rest of the population. Thus, Bogota was found to be virtually distinct genetically at this locus.

The results on *Xdh* locus are so much at variance with the previously reported data that we had to ask: is xanthine dehydrogenase typical? *Xdh* in Drosophila is an unusually large molecule with a subunit molecular weight of 140,000 corresponding to about 1300 amino acids (CANDIDO, BAILLIE and CHOVNICK 1974; SEYBOLD 1974). The neutral mutation theory of genetic polymorphism predicts a positive correlation between the number of alleles and the size of the subunit,

and a positive correlation between heterozygosity and molecular weight has been reported in Drosophila species (KOEHN and EANES 1977), but not in man (HARRIS, HOPKINSON and EDWARDS 1977). To answer if the *Xdh* is unusually highly polymorphic or is typical of all loci, a similar survey of a representative sample of loci has to be undertaken. As we have pointed out (SINGH, LEWONTIN and FELTON 1976), such a sample should contain loci that are monomorphic as well as polymorphic, monomeric and dimeric, and loci with a range of molecular weight subunits.

In this report, we present data on nine loci (three esterases, five larval proteins and an aldehyde oxidase) that have been surveyed with different criteria employing pH variation, different gel concentrations and different buffers. These loci are not as diverse a sample of enzymes as we would have liked, but they do seem to fit the criteria mentioned above except for the fact that they all belong basically to three enzyme (or protein) systems. Their selection was mainly to enable us to score many loci simultaneously on the same gel. This study, along with the previous one on *Xdh*, shows that the previously described polymorphic loci become even more polymorphic when extensive search for genic variation is made. The results of the Bogota population bearing on the extent of genetic differentiation during species formation will be discussed elsewhere.

MATERIALS AND METHODS

Drosophila strains: We have examined 110 iso-female lines from seven different populations of *Drosophila pseudoobscura*. These populations are: Strawberry Canyon, Berkeley, California (SC); Mount Charleston, Nevada (CH); Cerbat Mountains, Nevada (CE); Mesa Verde, Colorado (MV); Austin, Texas (AU); and Bogota, Columbia (BO). These lines are the same as studied by PRAKASH, LEWONTIN and HUBBY (1969) and PRAKASH, LEWONTIN and CRUMPACKER 1974) and were obtained from R. C. LEWONTIN. All lines are kept on Carpenter's Medium at 18°. (Carpenter's medium = 15 g Agar; 100 g sucrose; 50 g Brewers yeast, 1 g potassium phosphate, 8 g potassium sodium tartrate, 0.5 g calcium chloride, 0.5 g sodium chloride, 0.5 g manganous chloride, 0.5 g ferrous chloride and 5.5 ml propionic acid in 1 l of water).

Gel electrophoresis: Electrophoresis on acrylamide slab gels was carried out by the standard method as described in PRAKASH, LEWONTIN and HUBBY (1969) and SINGH, HUBBY and LEWONTIN (1974). Of the various methods of detecting allelic substitution in a polypeptide (see SINGH, LEWONTIN and FELTON 1976), in this study we have employed three, namely, pH variation, different buffers and different gel concentrations. These different criteria (combination of gel concentration, buffers and pH), used in this study are given in Table 1. For each criterion the voltage and running time were adjusted to get a roughly constant current of 75 ma and maximum possible enzyme (or protein) migration on the gels. Our gels are pre-run overnight at 100 volts before the sample is applied for electrophoresis. Different criteria used were found by trial and error, as all enzymes (or proteins) do not behave identically with changes in pH and gel concentration. Reproduction of a relatively sharp band was a prerequisite for a criterion to be acceptable for use in this investigation.

Loci analyzed: We have examined three esterases (*Est-1,5* and *6*), five larval proteins (*Pt-6,7,8,10* and *13*) and an aldehyde oxidase (*Ao*). *Est-1* and *Pt-6* are new loci and are being reported here for the first time. We deliberately chose esterases and larval proteins, as all esterases (or all larval proteins) could be examined on the same gel simultaneously, thus reducing the amount of work involved. These nine loci, along with *Xdh*, include loci that are monomorphic or polymorphic, monomeric (larval proteins and possibly *Est-1*) or dimeric (*Est-5*, *Xdh* and *Ao*) and have a range of subunit molecular weights from about 20,000 to 140,000.

TABLE 1

Criteria used in sequential analysis of the electrophoretic variation at ten loci in D. pseudoobscura

Locus	Criterion used in sequence 1	2	3	4	5
Ao	TBE 8.9, 4%	TBE 8.9, 6.5%	—	—	—
Est-1	TG 8.5, 8%	—	—	—	—
Est-5	TBE 8.0, 8%	TBE 8.0, 10% read dimer	same as 2 read monomer	TGA 8.0, 4%	—
Est-6	TBE 8.9, 5%	TBE 8.0, 8%		—	—
Pt-6	TGA 8.0, 6%	—	—	—	—
Pt-7,8,13	TBE 8.9, 5%	TBE 8.9, 4%	TBE 8.9, 8%	—	—
Pt-10	TBE 8.9, 5%	TBE 8.9, 4%	—	—	—
Xdh	TBE 8.9, 5%	TBE 8.9, 7%	TBE 7.1, 5%	TBE 7.1, 7%	Heat Denaturation

Electrophoretic criteria that produced no additional new alleles:
Aldehyde oxidase: Criterion 3. TBE, 8.0, 6% gel.
Criterion 4. TGA, 8.0, 8% gel.
All larval proteins: Criterion 4. TBE 8.0, 6% gel.
Criterion 5. TGA 8.0, 7% gel.
Esterase-5: Criterion 5. TGA, 8.0, 10% gel.
Criterion 6. TBE 8.9, 6% gel.

Running Buffers:
1. TBE, pH 8.9 = 9.825 gm. Tris + 0.765 gm. boric acid + 0.555 gm. EDTA per l.
2. TBE, pH 8.0 = 12.12 gm. Tris + 8 gm. boric acid + 1.2 gm. EDTA per l.
3. TBE, pH 7.1 = same as (1) plus enough boric acid to bring pH down to 7.1. This buffer at this pH is unstable and, thus, was discarded after each use.
4. TG, pH 8.5 = Tris-glycine (20 gm. glycine + 8 gm. Trizma per 4 ls).
5. TGA, pH 8.0 = same as (4) + 2 ml mercaptoacetic acid per 4 l 0.1 M TG buffer.

Gel Buffers:
In all cases except (5) the gel and running buffer are the same. In (5), the running buffer was TGA, pH 8.0, but gel buffer was TG, pH 8.5, same as (4).

Allelic designation: In this study, like that on *Xdh*, different criteria were used in sequence, and all the lines were grouped by a hierarchical procedure, each criterion successively breaking down previously homogeneous classes. Our method of allelic designation is the same as used in our *Xdh* study, except for the first criterion where relative mobility is given. For subsequent criteria, each allele is identified by a string of digits, each digit position in order representing a successive criterion applied within the previous criterion. The numerical value at a digit position is the allelic designation for that criterion and for electrophoresis, the number represents increasing electrophoretic mobility. For example, at *Pt-7*, four alleles with relative mobilities of 0.68, 0.73, 0.75 and 0.83 have been reported. Within the 0.75 allelic class the second criterion did not produce any new alleles, but the third criterion did produce two new alleles, which were designated as 0.75-00 and 0.75-01. For these loci, we would be able to make a comparative study of each allelic class previously reported and the extent of allelic diversity contained in them. We could not do this for *Est-5* (nor for *Xdh* in our previous study), as we did not have the previous information on the allelic content of these lines. At *Est-5*, in one case the presence or absence of monomer (M+ *vs.* M-) was noted, probably indicating the stability of the dimer molecule under that criterion (see APPENDIX for list of all alleles observed at each locus).

In this study we have not used heat sensitivity as a criterion, for two reasons. First, it is

necessary to use isogenic lines. And secondly, it requires the generation of heat-stability curves for each line and for each enzyme. In our study on *Xdh*, all isogenic lines were examined at least twice for their heat sensitivity at 66° for five, ten and 15 min, but the variation around each point was still large. For this reason, we reported only the number of heat-sensitivity alleles with large and repeatable differences, but not their frequency in populations. It seems that heat treatment for a constant time at several temperatures produces more uniform and repeatable results than heat treatment for variable times at a constant temperature and can successfully be used in polymorphism studies (SELANDER, personal communication). Heat sensitivity as a criterion has successfully been used by other workers (MILKMAN 1976; TRIPPA, LOVERRE and CATAMO 1976; SAMPSELL 1977; COCHRANE 1976; COYNE, FELTON and LEWONTIN 1978).

Genetic analysis: Since many of the newly found electrophoretic alleles differ by less than 1% in their relative mobilities, it is important to be sure that these differences are at the structural gene locus and are not the result of post-translational modification as reported by COCHRANE and RICHMOND (1979) for esterase and by FINNERTY and JOHNSON (1979) for *Xdh* in *D. melanogaster*. We have done four kinds of experiments to prove that the newly found alleles are at the structural locus. (1) Some of the iso-female lines used in our study are heterozygous and segregating for two alleles. We have examined single individuals from these lines and have found all the expected genotypes. (2) For *Ao* and *Est-5*, two loci with large numbers of alleles, genetic crosses were made between newly found alleles, and single individuals from the F_2 or backcross generation were examined for the presence of expected genotypes. This was done for four alleles at each of these two loci. (3) Sixteen alleles of *Ao* and 16 alleles of *Est-5* were crossed to an allele of different electrophoretic mobility at the respective locus and F_1 progeny were examined to see if the relative mobility of different alleles had changed in any way. No such change was found. (4) To see how often relative mobility of an allele may be changed by putting it into different genetic background, we extended the experiment described in (3) as follows: we crossed each of 16 lines, chosen deliberately to represent alleles of several mobilities, separately to four alleles of different electrophoretic mobility. Lines were chosen in such a way that same crosses could be examined for both *Ao* and *Est-5*. Thus, for each of these two loci, we had four sets of 16 different F_1's, and each set had one allele in common whose electrophoretic mobility was of interest to us. Thus, we examined four alleles in a total of 64 different genetic backgrounds and have not found a single case of altered electrophoretic mobility for either *Ao* or *Est-5* (SINGH 1979). These results are in agreement with those of COYNE, EANES and LEWONTIN (1979) and COCHRANE and RICHMOND (1979), who have reported that post-translational modification as a source of electrophoretic variation is very rare. Thus, data from all our genetic studies show that the newly found alleles are at the structural locus; we have no data to believe otherwise.

RESULTS

The basic data from this study are summarized and presented in Tables 2 and 3. Table 2 shows the genetic parameters for all nine loci and Table 3, the frequencies of most common alleles observed at each locus. For comparison, also vided in Table 3 are the corresponding allele frequencies from previous studies (PRAKASH, LEWONTIN and CRUMPACKER 1974; PRAKASH 1977). A list of the alleles observed at each locus is given in the APPENDIX. For calculation of gene frequencies, we have assumed two genes per iso-female line.

Aldehyde oxidase

This locus has been analyzed in three independent studies, and here are presented all the results to show how this locus has become more polymorphic as more exhaustive search for hidden allelic variation has been made. PRAKASH

TABLE 2

Genetic parameters of nine loci studied by new electrophoretic techniques in D. pseudoobscura

Genetic parameter	Population SC	CH	CE	MV	AU	BO
			Aldehyde oxidase			
No. of genes sampled	30	58	30	30	20	25
Observed no. of alleles	7	6	8	4	1	4
Effective no. of alleles	3.7	2.2	4.0	2.6	1.0	3.1
No. of unique alleles	1	1	4	1	0	2
Heterozygosity	0.73	0.55	0.75	0.62	0	0.68
$\bar{H}^* = 0.56$						
			Larval protein-6			
No. of genes sampled	28	58	34	30	20	24
Observed no. of alleles	2	1	1	1	1	1
Effective no. of alleles	1.2	1.0	1.0	1.0	1.0	1.0
No. of unique alleles	1	0	0	0	0	0
Heterozygosity	0.192	0	0	0	0	0
$\bar{H} = 0.04$						
			Larval protein-7			
No. of genes sampled	32	58	34	30	20	29
Observed no. of alleles	3	2	1	2	1	3
Effective no. of alleles	1.3	1.1	1.0	1.1	1.0	1.3
No. of unique alleles	1	0	0	1	0	1
Heterozygosity	0.23	0.07	0	0.13	0	0.25
$\bar{H} = 0.09$						
			Larval protein-8			
No. of genes sampled	32	56	34	30	20	27
Observed no. of alleles	3	3	3	2	4	3
Effective no. of alleles	2.2	2.2	2.2	1.9	2.7	1.3
No. of unique alleles	1	0	1	0	1	3
Heterozygosity	0.55	0.55	0.55	0.48	0.63	0.20
$\bar{H} = 0.55$						
			Larval protein-10			
No. of genes sampled	29	58	34	28	20	24
Observed no. of alleles	4	2	1	1	1	2
Effective no. of alleles	1.5	1.1	1.0	1.0	1.0	1.4
No. of unique alleles	2	1	0	0	0	1
Heterozygosity	0.35	0.07	0	0	0	0.28
$\bar{H} = 0.08$						
			Larval protein-13			
No. of genes sampled	28	56	32	30	24	24
Observed no. of alleles	4	3	3	3	3	4
Effective no of alleles	1.6	1.4	1.9	1.2	2.0	2.7
No. of unique alleles	2	0	0	0	0	2
Heterozygosity	0.37	0.30	0.46	0.24	0.49	0.61
$\bar{H} = 0.36$						

TABLE 2—Continued

Genetic parameter	Population SC	CH	CE	MV	AU	BO
			Esterase-1			
No. of genes sampled	28	56	32	30	20	24
Observed no of alleles	6	6	3	4	5	1
Effective no. of alleles	5.0	2.6	2.4	3.3	4.5	1.0
No. of unique alleles	1	1	0	0	0	0
Heterozygosity	0.80	0.62	0.59	0.70	0.78	0
$\bar{H} = 0.68$						
			Esterase-5			
No. of genes sampled	28	54	32	30	20	24
Observed no. of alleles	9	15	13	11	7	1
Effective no. of alleles	5.6	7.1	7.1	8.3	5.6	1.0
No. of unique alleles	1	9	5	4	2	0
Heterozygosity	0.82	0.86	0.86	0.88	0.82	0
$\bar{H} = 0.85$						
			Esterase-6			
No. of genes sampled	28	58	32	28	20	22
Observed no of alleles	3	5	4	3	3	2
Effective no. of alleles	1.2	2.0	1.5	2.0	1.5	1.4
No. of unique alleles	1	1	0	1	1	0
Heterozygosity	0.14	0.50	0.33	0.50	0.34	0.30
$\bar{H} = 0.39$						

* $\bar{H}$ = Average heterozygosity of all populations, excluding Bogota.

LEWONTIN and CRUMPACKER (1974) studied 12 populations of *D. pseudoobscura* and found only four alleles at this locus. Only Strawberry Canyon and Bogota were polymorphic, segregating for four and three alleles, respectively, the rest of the populations being monomorphic for the most common allele $Ao^{1.00}$. This locus was reanalyzed in 12 populations including nine of the original populations studied by PRAKASH, LEWONTIN and CRUMPACKER (1974) and, surprisingly, a total of seven alleles in eight of the same 12 populations were found (SINGH 1976). Of the 12 populations studied, all but two (Austin and Guatemala) were polymorphic, segregating from three to five alleles each. There still was a most common allelic class in all the populations ($Ao^{1.00}$, with an average frequency of 69%) except in Bogota where the most common allelic class ($Ao^{1.03}$ with a frequency of 42%) was the second most common allele in the "mainland" populations. In this study *Ao* was named in a nonspecific way as *Adh-6*, as it was studied by using various alcohols as substrates, several of which are readily accepted by *Ao*.

In the present study, I have reanalyzed six of the same populations with two criteria and have found a total of 16 alleles including, again, a null. Austin remains monomorphic for the most common allele in the "mainland" populations, but the other four populations are polymorphic, segregating for four to eight alleles each. The most common allele in the "mainland" populations has

TABLE 3

Frequencies of most common alleles at nine loci in the present and previous studies of D. pseudoobscura

Locus	Allele	Present study SC	CH	CE	MV	AU	BO	Previous studies* Mainland populations	BO
Ao	*0.95–0*	—	—	—	—	—	0.40		
	1.00–1	0.40	0.64	0.43	0.40	1.00	—	0.98(0.94–1.0)	0.83
	1.01–1	—	—	—	—	—	0.40		
	1.03–1	0.30	—	—	0.47	—	—		
Pt-6	*1.00*	0.89	1.00	1.00	1.00	1.00	—	NA†	NA
	1.04	—	—	—	—	—	1.00		
Pt-7	*0.75–00*	0.88	0.97	1.00	0.93	1.00	0.86	0.97(0.95–1.00)	0.93
Pt-8	*0.80–10*	—	—	—	—	—	0.89	0.01(0–0.03)	0.87
	0.81–11	0.41	0.43	0.47	0.60	0.40	—	0.51(0.41–0.63)	0.09
	0.83–00	0.53	0.52	0.47	0.40	0.45	—	0.47(0.38–0.58)	0.04
Pt-10	*1.04–1*	0.79	0.97	1.00	1.00	1.00	—	0.73(0–0.99)	—
	1.06–2	0.10	—	—	—	—	0.83	0.26(0–1.00)	1.00
Pt-13	*1.30–11*	0.79	0.82	0.25	0.87	0.67	—	0.97(0.92–1.00)	0.73
	1.30–20	—	0.14	0.69	—	0.25	0.29		
	1.37–00	—	—	—	—	—	0.54	0.01(0–0.04)	0.27
Est-1	Null	0.21	0.18	0.56	0.27	0.30	1.00	NA	NA
	1.00	0.56	0.57	0.25	0.37	0.20	—		
Est-5	*0.98–000*	—	—	—	—	—	1.00	‡	‡
	1.00–1M+0	—	0.26	0.28	0.10	—	—		
	1.00–210	0.07	—	—	—	0.30	—		
	1.04–000	—	0.20	—	—	—	—		
	1.05–001	0.36	0.04	0.03	0.23	0.10	0		
Est-6	*1.00–1*	0.93	0.69	0.81	0.64	0.80	—	0.60(0.30–0.85)	—
	1.10–2	—	0.07	0.06	—	—	0.82	0.18(0.09–0.30)	1.00

* Prakash, Lewontin and Crumpacker, from Lewontin (1974); and Prakash (1977).
† NA = Not analyzed.
‡ = Since there were already many alleles at this locus, we do not have data on previous allelic classification of these lines to compare with the new ones.

a weighted-mean allele frequency of 52%, the second most common allele, 12% (Table 3) and the rest segregating with gene frequencies from 1 to 5%.

There also appears to be some population differentiation in allele frequencies. Mesa Verde has two equally common alleles, one of which (*1.03–1* with frequency of 47%) is shared by Strawberry Canyon with 30% gene frequency, but found only once in Cerbat, and which is absent in the rest of the populations. The Bogota result is again surprising. Bogota is segregating for four alleles, of which two are equally common, each contributing 40% of the gene frequency and both are unique to Bogota. The null allele in Bogota contributes 16% of the gene frequency and is shared by Strawberry Canyon and Mount Charleston with similar gene frequencies. Considering the small samples, it is remarkable that all polymorphic populations are highly heterozygous, with heterozygosity values ranging from 55 to 75%, and a weighted mean heterozygosity $\overline{H} = 56\%$.

This locus has also been recently analyzed by Coyne and Felton (1977)

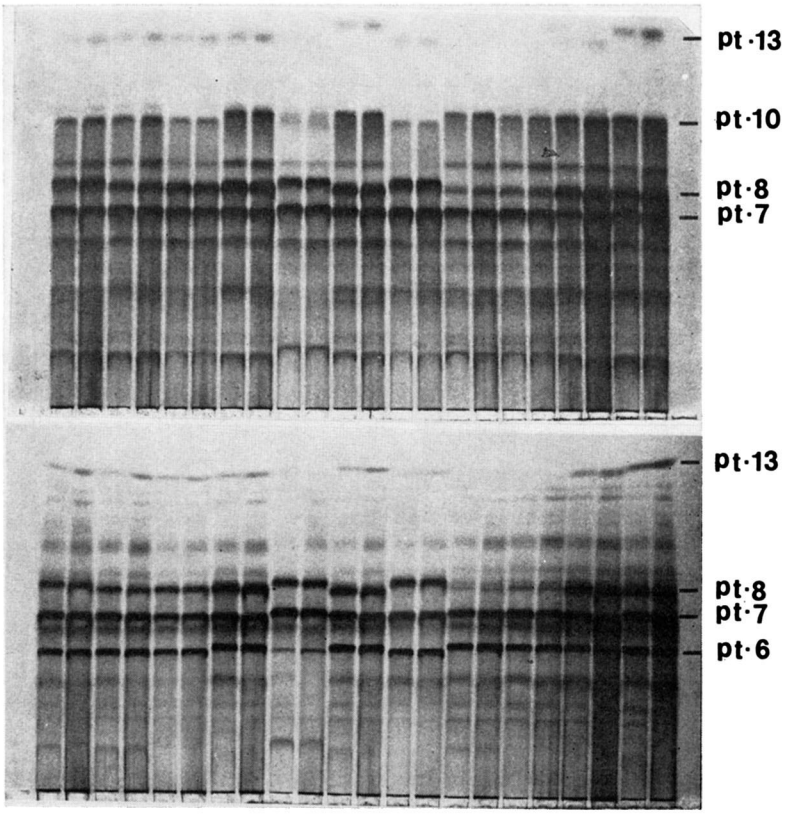

FIGURE 1.—Two gels showing larval proteins. Top gel = 0.1 M Tris-borate EDTA, pH 8.9 and 6% acrylamide; Bottom gel = 0.05 M Tris-glycine-mercaptoacetic acid, pH 8.0 and 6% acrylamide. Each line is run in duplicate pockets and both gels have identical samples.

and rather remarkable similar results have been obtained. Small discrepancies in gene frequencies can be explained by the fact that they have used isogenic lines, whereas I have used iso-female lines and hence my sample size per population is larger than theirs.

Larval proteins

Protein-6: First, we report a new larval protein that has been designated *Pt-6* (Figure 1). It appears with TGA buffer, but not with TBE, which is good for *Pt-7*, *8*, *10* and *13*. All mainland populations except Strawberry Canyon are monomorphic for the same allele ($Pt\text{-}6^{1.00}$). Strawberry Canyon has an additional allele with a frequency of about 11%. Both of these alleles are absent in Bogota, which is monomorphic for an allele found nowhere else in the rest of the populations. Thus, Bogota is genetically differentiated from the mainland populations at this locus as well.

Protein-7: PRAKASH, LEWONTIN and CRUMPACKER (1974) found four alleles (*0.68*, *0.73*, *0.75*, *0.77*) at this locus, of which $Pt\text{-}7^{0.75}$ contributed 97% of the allele frequency with little variation among populations. Bogota was found to

be remarkably similar to the mainland populations, segregating for three alleles with gene frequencies of 0.05, 0.925 and 0.025. In this study, I have detected five new alleles within the *Pt-7*$^{0.75}$ mobility class. The most common allelic class in the species as a whole still remains predominant, with an allele frequency of about 93%. The other four alleles are rare, with gene frequencies of 1 to 2% each. There were no lines containing allele *Pt-7*$^{0.68}$ and *Pt-7*$^{0.73}$ to examine. The Bogota population also remains similar to mainland populations and shares the same most common allele with a frequency of about 86%. The heterozygosity within populations is rather low and variable among populations.

Protein-8: PRAKASH, LEWONTIN and CRUMPACKER (1974) discovered four alleles (*0.80*, *0.81*, *0.83* and *0.85*), of which *Pt-8*$^{0.81}$ and *Pt-8*$^{0.83}$ were the most common, with average gene frequencies of 51 and 47%, respectively, in the mainland populations; the other two alleles were rare. Bogota had three alleles, of which the most common one (*Pt-8*$^{0.80}$), with a frequency of about 87%, was rare in mainland populations. I have found a total of nine alleles at this locus, but the main pattern of genetic variation remains essentially unchanged. The two most common alleles still make up about 82% of the total allele frequency. In Bogota, two new alleles, which are rare but unique to Bogota, were found. The most common allele in Bogota makes up about 89% of the gene frequency. This locus has a very similar heterozygosity in all mainland populations, ranging from 48% in Mesa Verde to 65% in Austin and an overall weighted heterozygosity of 55%. Bogota has only 20% heterozygosity, but is completely differentiated at this locus.

Protein-10: In previous studies there were only three alleles, *1.02*, *1.04* and *1.06*, having average gene frequencies of 1%, 73% and 26%, respectively. Bogota was monomorphic for *Pt-10*$^{1.06}$, the second most common allele in the mainland population. With an additional criterion, I have discovered a total of six alleles. TBE, 8.9, 8% gel, the third criterion for *pt-7*, *8* and *13*, could not be used for *Pt-10*, as its expression was too poor under this condition to be scored reliably. The polymorphism pattern of this locus has remained essentially unchanged except for the discovery of a few rare alleles. The most common allele still contributes 95% of the gene frequency. Bogota has two alleles, and the most common one contributes 83% of the gene frequency and is shared by only Strawberry Canyon with low frequency. Thus, Bogota is quantitatively differentiated in terms of its gene frequency from the rest of the populations. Appreciable heterozygosity is found only in Strawberry Canyon and Bogota. Cerbat, Mesa Verde and Austin are monomorphic.

Protein-13: Previously, there were three alleles at this locus, and the most common alleles contributed 97% of the gene frequency, with very little variation in gene frequency among populations. Bogota had two alleles with allele frequencies of 0.725 and 0.275. In this study, with three criteria I have discovered a total of ten alleles at this locus. Mainland populations are segregating for two to four alleles each, with the most common alleles making up, on the average, 62% of the gene frequency. All new alleles are shared among populations, and only Strawberry Canyon and Bogota have two unique alleles each. Bogota is

segregating for a total of four alleles, of which two, including the most common one, are unique to Bogota. The heterozygosity in the mainland populations ranges from 24 to 49% with a weighted mean heterozygosity of 36%. Bogota is the most heterozygous population with a heterozygosity value of 61%. The main pattern of genetic variation has remained unchanged except that the most common allele in Cerbat (*1.30–20*) is different from that in other populations (*1.30–11*). The mean heterozygosity as a whole has increased from 7 to 36%.

Esterases

Esterase-1: This is a new locus being reported here for the first time (Figure 2). It appears as a single band with Tris-glycine buffer (pH 8.5). A total of nine alleles have been discovered, and the three most common alleles have gene frequencies of 36% (null), 32% (allele *6*) and 11% (allele 7) in the species as a whole. All mainland populations are similar in their gene frequency distribution and are highly heterozygous, with a weighted mean heterozygosity of about 68%. Bogota is fixed for the null allele, as no lines showed any trace of activity on repeated runs.

Esterase-5: In PRAKASH and LEWONTIN's study, this locus was the most polymorphic of all loci studied, with 13 alleles, including a null. All mainland populations had the same most common allele and also the same second most common allele with gene frequencies of 0.292 to 0.579 and 0.054 to 0.273, respectively. The heterozygosity of this locus was high, with a mean heterozygosity of 73%. On the other hand, Bogota was found to be segregating for only two alleles with frequencies of 0.974 and 0.026, and the most common allele was the same as in the rest of the populations.

We have analyzed this locus with four different criteria and have found a total of 32 alleles! The third electrophoretic criterion is the same as the second except that this time we read the mobility of the monomer. There were cases when several lines with the same main band (dimer) produced monomeric bands of different mobility (see COBBS and PRAKASH 1977). All mainland populations are segregating for seven to 15 alleles each, and several of them have as many

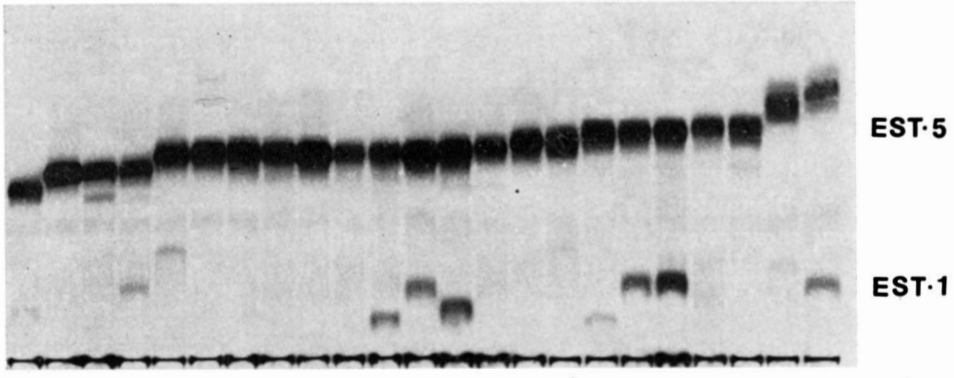

FIGURE 2.—A gel showing zones of *Est-1* and *Est-5* activity. The gel was run on 8% acrylamide with 0.05 M Tris-Glycine, pH 8.5 buffer. Black pupae were used as sample.

unique alleles as nine in Mount Charleston, five in Cerbat and four in Mesa Verde. There does not appear to be a single common allelic class shared by all populations. The most common allele in Strawberry Canyon is *1.05–001* and the allelic frequency distribution is J-shaped. In Mount Charleston, there are two equally common alleles making up 26% and 20% of the gene frequency. Cerbat shared its common allele only with Mount Charleston and Mesa Verde shares its most common allele only with Strawberry Canyon. Bogota has remained monomorphic for allele *0.98–000*, which is unique to Bogota. It should be pointed out that PRAKASH and LEWONTIN had found another allele in Bogota with a frequency of 0.026, but it is lost from our lines. Thus, there is some polymorphism for *Est-5* in Bogota, but not much. The heterozygosity in all mainland populations is very high and remarkably similar, ranging from 82–88%, with a weighted-mean heterozygosity of 85%. These *Est-5* data compare remarkably well with those by COYNE, FELTON and LEWONTIN (1978), where a total of 30 alleles were shown to exist at this locus.

Esterase-6: This locus has been studied by PRAKASH (1977), who reported three alleles, *0.90*, *1.0* and *1.10*, with gene frequencies of 0.227, 0.595 and 0.178, respectively. Bogota was monomorphic. With the application of two criteria used in this study, I have found a total of nine alleles and several of the new alleles are shared among populations. All mainland populations have the same most common allele, *1.00–1*, with a mean frequency of 69%, and the heterozygosity ranges from 15% in Mount Charleston to 50% in Mesa Verde. Bogota is segregating for two alleles, *1.10–1* and *1.10–2*, with frequencies of 82% and 18%, respectively. Both of these alleles are shared by the mainland populations. Thus, Bogota has no unique *Est-6* alleles, but is quantitatively differentiated (in terms of gene frequency) from the rest of the populations.

DISCUSSION

The main features of the results, summarized in Table 4, are as follows: (1) different loci have yielded differentially to our search for allelic variation. One group of loci, consisting of larval proteins and esterase-1 and 1–6, have a low level of polymorphism, with an average observed number of alleles of about eight per locus. A second group, consisting of *Est-5*, *Xdh* and *Ao*, have high levels of polymorphism, with an average number of alleles of about 29. The relative increase in observed number of alleles in these two groups of loci has been 2.4- and 3.7-fold, respectively. (2) The average number of alleles in mainland populations has increased from 3.2 to 4.13 and in Bogota, from 2.0 to 2.6, a very slight increase indeed. But this is because most of the newly discovered alleles are unique to individual populations and consequently do not show up in these averages. In fact, the average number of alleles per locus in the species as a whole has increased from 5.0 to 13.9! (3) The mean heterozygosity in mainland populations has increased from 33 to 42%, whereas in Bogota it has more than doubled, from 14% to 30%.

Now, we shall discuss these results in the light of selectionist and neutralist hypotheses of interlocus variation in genetic polymorphisms, patterns of varia-

TABLE 4

A comparison of mean number of alleles and mean heterozygosity between Bogota and mainland populations of D. pseudoobscura

	Previous study*					Present study				
	Ten mainland populations		Bogota			Five mainland populations		Bogota		
Locus	No. alleles	Het.	No. alleles	Het.	Total alleles in species	No. alleles	Het.	No. alleles	Het.	Total† alleles in species
Ao	1.3	0.01	3	0.29	4	5.2	0.56	4	0.68	16
Est-6‡	3.0	0.50	1	0.00	3	3.6	0.36	2	0.30	9
Pt-6	NA§	NA	NA	NA	—	1.2	0.04	1	0.00	3
Pt-7	2.5	0.06	3	0.14	4	1.8	0.09	3	0.25	8
Pt-8	3.0	0.51	3	0.23	4	3.0	0.55	3	0.20	9
Pt-10	2.5	0.23	1	0.00	3	1.8	0.08	2	0.28	6
Pt-13	1.9	0.07	2	0.40	3	3.2	0.36	4	0.61	9
Xdh¶	4.4	0.49	1	0.00	6	5.9	0.64	5	0.68	37
Est-1	NA	NA	NA	NA	—	4.8	0.70	1	0	9
Est-5	7.2	0.73	2	0.05	13	10.8	0.85	1	0	33
Average	3.2	0.33	2.0	0.14	5.0	4.13	0.42	2.6	0.30	13.9

* Data of PRAKASH, LEWONTIN and CRUMPACKER, taken from LEWONTIN (1974).
† Total no of alleles refers to the total number observed from both previous and present studies.
‡ Previous data from PRAKASH (1977) based on four populations.
§ NA = Not analyzed.
¶ Present study is also based on ten populations. Total number of alleles (37) includes ten heat-sensitivity alleles.

tion within and among populations and causal mechanisms for their maintenance in natural populations.

Molecular structural and functional component of genic variation

This study has shown that different loci have different amounts of genic variation, and the differences may, in part, be due to molecular structural and functional differences that control the observable amount of genic variation in natural populations. These molecular structural and functional constraints affecting the level of genic variation at the molecular level are as follows:

Molecular weight: KOEHN and EANES (1977) have shown a positive correlation between subunit size, average heterozygosity and average number of observed alleles per locus in Drosophila. HARRIS, HOPKINSON and EDWARDS (1977), on the other hand, have found no difference between the average subunit size of monomorphic and polymorphic enzymes, but they have not considered the effect of subunit size on the degree of polymorphism on loci within the polymorphic class, as done in KOEHN and EANES' (1977) study. They did, however, find a negative correlation between the number of subunits and the level of polymorphisms. They have shown that monomeric enzymes are more polymorphic than the dimeric enzymes, dimeric more polymorphic than trimeric, and so on. This effect of subunit number on genic variation has also been found in other animals and plants (ZOUROS 1976; WARD 1977). While to ZOUROS this finding means that heterosis is not a causal mechanism in the maintenance of genic variation, HARRIS, HOPKINSON and EDWARDS (1977) have interpreted this result

as evidence in favor of the neutral hypothesis, which predicts a higher rate of mutation to neutral alleles for monomeric proteins than for multimeric proteins, which are under structural constraints to maintain quaternary structure (WARD 1977).

Another factor that may account for the differences in the amount of genic variation at different loci is the differential sensitivity of these loci to electrophoretic techniques. Change in electrophoretic mobility is the result of changes in net charge, which in turn depends on the total charge of the protein. A charge change in a small protein will have a larger effect on the net charge than the same charge change in a large protein. This means that, for smaller proteins, a proportionately larger fraction of genic variants has already been detected. This explains why monomeric proteins, which are generally of smaller size than dimeric ones, should show more variation. Thus, KOEHN and EANES' prediction of more variability in large-subunit enzymes may be true, not only because of the observed correlation between the subunit size and the genic variation, but also because electrophoretic techniques, as applied in the past, have unintentionally been more efficient in detecting genic variants of smaller molecular weight proteins.

The *Xdh* data with 37 alleles and a molecular weight of 140,000 is certainly in good agreement with KOEHN and EANES' prediction. On the other hand, the *Est-5* data do not seem to fit their prediction very well. *Est-5* is highly polymorphic with 32 alleles, but its molecular weight has been shown to be about 100,000 (NARISE and HUBBY 1966; COBBS 1976). It appears that besides molecular weight, there is another aspect of the molecular structure that may explain its highly polymorphic nature (see below). Aldehyde oxidase has a subunit molecular weight of 132,000 (COURTRIGHT 1967), and this puts it in the same range as *Xdh*. Although the present results show that *Ao* is highly polymorphic, it is still not nearly as polymorphic as *Xdh*. Two independent studies, this one and that of COYNE and FELTON (1977), have come up with the same level of polymorphism. Previous reports that this locus is weakly polymorphic (PRAKASH, LEWONTIN and CRUMPACKER 1974) are certainly anomalous. Similarly, the extent of genic variation for larval proteins does not appear to depend entirely on molecular weight. JOHN RAMSHAW (personal communication) has found the subunit weight of *Pt-7*, *8*, *10* and *13* to be about 85,000, 85,000, 80,000 and 20,000, respectively. (He thinks that these values may have large errors, but that the order of magnitude is certainly correct.) *Pt-7* and *8* have similar subunit weights, but they differ significantly in the amount of genic variation.

Another aspect of genic variation concerns the presence of too many rare alleles at many loci. It has been suggested that intragenic recombination is responsible for these low-frequency variants (KOEHN and EANES 1976; STROBECK and MORGAN 1978; WATT 1972). Loci like *Est-5*, with several common alleles, are the prime candidates for intragenic recombination to have a significant effect on the total observed alleles at a locus.

Amino acid composition: Since mutation of charged residues are more likely to change the charge and, consequently result in detection by gel electrophoresis,

a positive correlation between the proportion of charged residues in a protein and the genic variability is expected. It appears that *Est-5* is unusually high in charged residues, with 38.3% (WALLACE and LEWONTIN, personal communications) compared to only 20.9% for five enzymes in eukaryotes taken from Dayhoff's Atlas. Like *Est-5*, butyrylcholinesterase in the pig and Acetylcholinesterase in the electric eel are high in charged residues, with 32.6% and 26.9%, respectively (SILVER 1974). We think that, besides molecular weight, amino acid composition is also a factor affecting genic variation at *Est-5* locus. Also, in comparison with *Xdh* data where the electrophoretic variants are tightly packed in their mobility, *Est-5* variants are more widely spaced, suggesting that a large proportion of these are the result of charge changes. Likewise, under the neutral hypothesis, enzymes and proteins whose total number of residues are evenly distributed among amino acids with similar physico-chemical properties should allow more variation than those having predominantly dissimilar amino acids. Once we know more about the amino acid composition of enzymes and proteins whose polymorphism has been studied, this relationship can be critically examined.

Functional constraint: In an earlier paper (SINGH 1976), I pointed out that another factor likely to affect genic variation is the functional constraint imposed on the gene. From comparative evolution of various enzymes and proteins, it is generally believed that genic variation at a locus is, among other things, affected by the sensitivity of its physiological function to mutational changes. *Ipso facto*, proteins with less genic variation like cytochrome *c*, are said to be under more functional constraints than highly variable ones like fibrinopeptide. Using this concept, it was suggested that enzyme systems with isozyme loci like esterases (loci with functional similarity) have fewer physiological constraints, and this in part was substantiated by the observation than isozyme loci are more prone to having null alleles than single-locus enzyme systems (SINGH 1976). This factor may, in part, be responsible for our recent observation that acetylcholine esterase has very little genic variation (MORTON and SINGH 1980, in press) despite the fact that its amino acid composition is similar to that of other esterases.

Pattern of genetic variation within and among populations

One of the two most common observations, the other being the fact that the genic variation in natural populations is ubiquitous, has been the similarity of allele frequencies among populations, with exception to populations involving nonrandom mating systems. This observation has been the most prominent argument in favor of balancing selection. Any appeal to local adaptation and the role of environmental heterogeneity has to recognize this factual observation.

Now, it becomes important to see if this observation still holds after the hidden allelic variation has been detected. The allele frequency distribution at the *Xdh* locus is very similar among populations and shows no evidence of local differentiation, except in Bogota. However, there are a large number of *Xdh* alleles, and even in these small samples there is on the average one unique allele per

population. However, *Est-5*, a locus with as much genic variation as that of *Xdh*, has a very different pattern. There is some evidence of gene frequency differentiation among populations besides Bogota. The most common class does not appear to be the same in all populations, and there are, on the average, three unique alleles per population. Bogota, which is polymorphic for many loci examined here, has only two alleles at this locus, and the most common allele in Bogota is also unique. The second most common allele in Mount Charleston is also unique. In mainland populations, four different alleles ranging in gene frequency from 20 to 36%, can be identified as the most common allele. The rest of the alleles occur with gene frequencies from 0.5 to 4%. Larval proteins and aldehyde oxidase are not differentiated among populations, with exception of Bogota. If more loci, like *Est-5* and *Xdh*, show different patterns among populations, then this kind of locus-specific pattern of genic variation will be useful in establishing the role of selective *vs.* nonselective forces operating in natural populations, as demonstrated by CHRISTIANSEN and FRYDENBERG (1974) in Zoarces in which two unlinked genes have parallel clines, while two other unlinked genes have similar allele frequencies among populations.

Genic variation within different "electromorphs"

The observed allele frequency distributions at enzyme loci have been subjected to a great deal of theoretical analysis under the neutral hypothesis. The observation of fewer effective alleles than expected under KIMURA and CROW's (1964) "infinite allele" model led to the development of the "step-allele" model (OHTA and KIMURA 1973), which reduced, but did not eliminate altogether, the dependence of effective numbers of alleles on population size.

KING (1974) proposed a model with a restricted number of variable sites, thus making the effective number of alleles independent of population size in very large populations. In KING's (1974) model, electrophoretic alleles were thought of as "electromorphs" consisting of many alleles, having similar net charge. With the apparent solution of the problem of the number of alleles, the remaining problem was the presence of too many rare alleles under the strict neutral hypothesis. By the very fact that alleles were rare, OHTA (1973) proposed that they are slightly deleterious and are selected against. OHTA and KIMURA (1975) and KING and OHTA (1975) have considered several selection models assuming one or two type (normal) alleles and multiple mutant alleles with deleterious effects of constant or increasing magnitude in proportion to their charge distance from the type allele. Both neutral models and the new models of deleterious mutations predict a unimodal distribution of equilibrium electromorph frequencies, with the type allele flanked by progressively less common electromorphs (OHTA and KIMURA 1975; KING and OHTA 1975; NEI and CHAKRABORTY 1976). This pattern, more or less, fits a large amount of early data on enzyme variation. This model also predicts a relatively larger proportion of hidden variation in the common "electromorphs" than in the rare ones (OHTA and KIMURA 1975; KING and OHTA 1975; NEI and CHAKRABORTY 1976).

In the present study, we can test this prediction. In Table 5, are summarized

TABLE 5

Allelic variation within "electromorphs" as five loci in D. pseudoobscura

Locus	From Prakash's study Allele	Frequency	From present study No. of genes examined	No. of alleles found	No. of alleles per 100 genes examined
Pt-8	*0.80*	0.870 Bogota	27	2	7.41
	0.81	0.509 Mainland	98	5	5.10
	0.83	0.412 Mainland	83	1	1.20
Pt-10	*1.04*	0.733 Mainland	173	2	1.16
	1.06	0.260 Mainland 1.000 Bogota	31	2	6.45
Pt-13	*1.30*	0.967 Mainland	191	7	3.66
	1.37	0.005 Mainland 0.275 Bogota	15	2	13.33
Est-6	*0.90*	0.227 Mainland	11	1	9.09
	1.00	0.598 Mainland	145	3	2.07
	1.10	0.178 Mainland	44	5	11.36
Xdh	*0.99*	0.232 Mainland	19	6	31.58
	1.00	0.696 Mainland and Bogota	103	9	8.74

the data showing alleles (electromorphs) and their frequencies from the previous study of PRAKASH, LEWONTIN and CRUMPACKER (1974) and the number of genes examined and the number of alleles found from the present study. It can be seen that more common electromorphs do not always contain more alleles, as expected under the "step-allele" model of neutral or deleterious mutations. This means that all alleles within an electromorph are not equally fit, and some selection must be occurring regardless of the charge. Also, our *Xdh* and *Est-5* data do not seem to support the idea of restricted variable sites proposed by KING (1974).

This brings us to the final question: It there any evidence in the new data to discriminate between the balancing selection and neutral or deleterious mutation selection hypotheses of genic variation? The new body of data, as presented here, is not yet large enough to answer this question definitely. Even with the limited studies reported, those with the most extensive search for hidden genic variation have been made on rather small sample sizes (SINGH, LEWONTIN and FELTON 1976; COYNE 1976; COYNE and FELTON 1977; COYNE, FELTON and LEWONTIN 1978, and the present study), and those with large sample sizes have been carried out with one or two criteria only (MILKMAN 1976; JOHNSON 1976, 1977, THORIG, SCHOONE and SCHARLOO 1975; TRIPPA, LOVERRE and CATAMO 1976; COCHRANE 1976; SAMPSELL 1977). Nevertheless, some of the new results have been remarkable as large numbers of genic variants have been found at loci that were already polymorphic. These results and those from studies on the effect of molecular structure (KOEHN and EANES 1977; HARRIS, HOPKINSON and EDWARDS 1977) and function (OHTA 1973; OHTA and KIMURA 1975; KING and

OHTA 1975; SINGH 1976), enable us to draw some conclusions about the causes of genic variation in natural populations:

(1) The molecular structure (subunit size and subunit number of an enzyme is certainly a causal factor of genic variation, as shown by KOEHN and EANES (1977). HARRIS, HOPKINSON and EDWARDS' (1977) result is not negative, as they have not shown that the subunit size has no effect on genic variation at loci within the polymorphic group; they have shown only that polymorphic and monomorphic loci are not different in their mean subunit size. However, subunit size cannot explain all the results, as many loci with large subunits are known to be monomorphic. For example, *Odh* with a subunit size of 50,000 and *Hex-1* with a subunit size of 45,000 have been studied thoroughly, but no additional variation has been discovered (COYNE and FELTON 1977; BECKENBACH and PRAKASH 1977).

(2) The presence of truly monomorphic loci and the observation (HARRIS, HOPKINSON and EDWARDS 1977) that monomorphic and polymorphic loci do not differ in their subunit size, suggest that monomorphic loci form a separate group and are not just the tail of the same distribution covering the polymorphic loci. The causes of monomorphism may be as much revealing as the causes of polymorphism.

(3) In our study, some loci have shown differentiation among populations, while others have maintained a uniform distribution. This kind of locus-specific pattern of genic variation can help discriminate between balancing selection and neutral or deleterious mutation selection hypotheses of genic variation. We think that present figures on genic similarity among populations are overestimates.

(4) LEWONTIN (1974) observed that, while various species ranging from *E. coli* to man have population sizes of different orders, their genic heterozygosity falls in a very narrow range. This observation is incompatible with the neutral theory, which predicts an increase in heterozygosity with population size. One solution to this problem has been the proposition that vertebrates, with low heterozygosity, have not existed long enough to build up the genic variation expected under the neutral hypothesis. Even under the new deleterious mutation selection model of OHTA and KIMURA (1975), this observation cannot be explained except by invoking the limited time span.

We think that observations of rather similar heterozygosity in species with population sizes of different orders of magnitude may be incorrect. Studies with Drosophila have shown an increase in genic variation, whereas a similar study by SELANDER (personal communication) with mice has produced only a limited number of new variants. The overall picture of heterozygosity between different groups of organisms may be changed when more studies are done. On the other hand, population size is a problem only in the neutral model. The problem disappears in the new deleterious mutation selection model.

(5) Regardless of what the true estimates of genic heterozygosity in vertebrates may be, the role of interlocus genic variation produced by isozyme loci (with or without the ability to form interlocus hybrid molecules) as an alternative to intralocus genic variation for adaptation should be considered (HARRIS,

HOPKINSON and EDWARDS 1977; MYERS 1978). Do vertebrates, with generally low genic heterozygosity, have more isozyme variation than invertebrates, with generally high genic heterozygosity? In the long run, the isozyme variation will be depleted as isozyme loci become nonfunctional or take up new functions. If in the past gene duplications producing isozyme variation have occurred, then some of this isozyme variation might have been preserved, which would provide the population with a constant source of genetic homeostasis without the problem of segregation.

(6) In the deleterious mutation selection model (KING and OHTA 1975), one optimal type allele is considered at each locus, and this is said to be heterogeneous with respect to sequence, function and fitness (KING and OHTA 1975). But the kind of selection considered to be overriding is the one where the relative fitness decreases with the increasing number of mutational differences. Contrary to this prediction, we have shown that selection does occur among alleles within an electromorph. In KING and OHTA's model, no local adaptive peak is allowed within a population, but they may occur between populations giving rise to new species. It is a very peculiar model where positive selection is allowed between species but not within species, even though the targets of selection are the same gene loci in both situations.

I wish to express my thanks to S. PRAKASH, R. C. LEWONTIN, J. L. HUBBY and D. W. CRUMPACKER, without whose pioneering works on *Drosophila pseudoobscura*, the present study would be incomplete in telling the whose story about the nature of genic variation in this species. Mrs. S. THOMAS provided competent technical assistance in this investigation.

LITERATURE CITED

BECKENBACH, A. T. and S. PRAKASH, 1977 Examination of allelic variation at the hexokinase loci of *Drosophila pseudoobscura* and *D. persimilis* by different methods. Genetics **87**: 743–761.

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.

CANDIDO, E. P. M., D. L. BAILLIE and A. CHOVNICK, 1974 A rapid purification of xanthine dehydrogenase for genetic studies. Genetics **77**: s9 (abstract).

CHRISTIANSEN, F. B. and O. FRYDENBERG, 1974 Geographical patterns of four polymorphisms in *Zoarces viviparus* as evidence of selection. Genetics **77**: 765–770.

COBBS, G., 1976 Polymorphism for dimerizing ability at the esterase-5 locus in *Drosophila pseudoobscura*. Genetics **82**: 53–62.

COBBS, G. and S. PRAKASH, 1977 A comparative study of esterase-5 locus in *Drosophila pseudoobscura*, *D. persimilis* and *D. miranda*. Genetics **85**: 697–711.

COCHRANE, B. J., 1976 Heat stability variants of esterase-6 in *Drosophila melanogaster*. Nature **261**: 131–132.

COCHRANE, B. J. and R. C. RICHMOND, 1979 Studies of esterase-6 in *Drosophila melanogaster*. I. The genetics of a post-translational modification. Biochem. Genet. **17**: 167–184.

COURTRIGHT, J. B., 1967 Polygenic control of aldehyde oxidase in Drosophila. Genetics **57**: 25–39.

COYNE, J. A., 1976 Lack of genetic similarity between two sibling species as revealed by varied techniques. Genetics **84**: 593–607.

COYNE, J. A. and A. A. FELTON, 1977 Genic heterogeneity at two alcohol dehydrogenase loci in *Drosophila pseudoobscura* and *Drosophila persimilis*. Genetics **87**: 285–304.

COYNE, J. A., W. F. EANES and R. C. LEWONTIN, 1979 Letter to the editor. Genetics **92**: 353–356.

COYNE, J. A., A. A. FELTON and R. C. LEWONTIN, 1978 Extent of genetic variation at a highly polymorphic esterase locus in *Drosophila pseudoobscura*. Proc. Natl. Acad. Sci. U.S. **75**: 5090–5093.

EWENS, W. J. and M. W. FELDMAN, 1976 The theoretical assessment of selective neutrality. pp. 303–337. In: *Population Genetics and Ecology*. Edited by S. KARLIN and E. NEVO. Academic Press, New York.

FINNERTY, V. and G. B. JOHNSON, 1979 Post-translational modification as a potential explanation of high levels of enzyme polymorphism. I. Modification of Xanthine dehydrogenase and aldehyde oxidase in *Drosophila melanogaster*. Genetics **91**: 695–722.

HARRIS, H., D. A. HOPKINSON and Y. H. EDWARDS, 1977 Polymorphism and the subunit structure of enzymes: A contribution to the neutralist-selectionist controversy. Proc. Natl. Acad. Sci. U.S. **74**: 698–701.

HEDRICK, P. W., M. E. GINEVAN and E. P. EWING, 1976 Genetic polymorphism in heterogeneous environments. Ann. Rev. Ecol. Syst. **7**: 1–32.

JOHNSON, G. B., 1976 Hidden alleles at the α-glycerophosphate dehydrogenase locus in Colias butterflies. Genetics **83**: 149–167. ——, 1977 Characterization of electrophoretically cryptic variation in the alpine butterfly, *Colias meadii*. Biochem. Genet. **15**: 665–693.

KING, J. L., 1974 Isoallele frequencies in very large populations. Genetics **76**: 607–613.

KING, J. L. and T. OHTA, 1975 Polyallelic mutational equilibrium. Genetics **79**: 681–691.

KIMURA, M. and J. F. CROW, 1964 The number of alleles that can be maintained in a finite population. Genetics **49**: 725–738.

KOEHN, R. K. and W. F. EANES, 1976 An analysis of allelic diversity in natural populations of Drosophila: The correlation of rare alleles with heterozygosity. pp. 377–390. In: *Population Genetics and Ecology*. Edited by S. KARLIN and E. NEVO. Academic Press, New York.

KOEHN, R. K. and W. F. EANES, 1977 Subunit size and genetic variation of enzymes in natural populations of *Drosophila*. Theoret. Popul. Biol. **11**: 330–341.

LEWONTIN, R. C., 1974 *The Genetic Basis of Evolutionary Change*. Columbia Univ. Press, New York & London.

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.

MILKMAN, R., 1976 Further evidence of thermostability variation within electrophoretic mobility classes of enzymes. Biochem. Genet. **14**: 383–387.

MILKMAN, R. and R. KOEHLER, 1976 Isoelectric focusing of MDH and 6-PGDH from *Escherichia coli* of diverse natural origins. Biochem. Genet. **14**: 517–522.

MORTON, R. A. and R. S. SINGH, 1980 Variation in Drosophila acetylcholinesterase. Biochem. Genet. (in press).

MYERS, J. H., 1978 Isozymes and allozymes: Alternate forms of protein adaptation. Can. J. Genet. Cytol. **20**: 187–192.

NARISE, S. and J. L. HUBBY, 1966 Purification of esterase-5 from *Drosophila pseudoobscura*. Biochim. Biophys. Acta **122**: 281.

NEI, M. and R. CHAKRABORTY, 1976 Electrophoretically silent alleles in a finite population. J. Mol. Evol. **8**: 381–385.

OHTA, T., 1973 Slightly deleterious mutant substitutions in evolution. Nature **246**: 96.

OHTA, T. and M. KIMURA, 1973 A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genet. Res. **22**: 201–204. ——, 1975 Theoretical analysis of electrophoretically detectable polymorphisms: Models of very slightly deleterious mutations. Am. Naturalist **109**: 137–145.

PRAKASH, S., 1972 Origin of reproductive isolation in the absence of apparent genic differentiation in a geographic isolate of *Drosophila pseudoobscura*. Genetics **72**: 143–155. ——, 1977 Further studies on gene polymorphism in the mainbody and geographically isolated populations of *Drosophila pseudoobscura*. Genetics **85**: 713–719.

PRAKASH, S., R. C. LEWONTIN and D. W. CRUMPACKER, 1974 A molecular approach to the study of genic heterozygosity. VI. The remarkable genetic similarity of geographical populations of *D. pseudoobscura*. pp. 138–139. In: *The Genetic Basis of Evolutionary Change*. Edited by R. C. LEWONTIN, Columbia Univ. Press, New York.

PRAKASH, S., R. C. LEWONTIN and J. L. HUBBY, 1969 A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. Genetics **61**: 841–858.

SAMPSELL, B., 1977 Isolation and genetic characterization of alcohol dehydrogenase thermostability variants occurring in natural populations of *Drosophila melanogaster*. Biochem. Genet. **15**: 971–988.

SEYBOLD, W. D., 1974 Purification and partial characterization of xanthine dehydrogenase from *Drosophila melanogaster*. Biochim. Biophys. Acta **334**: 266–271.

SILVER, A., 1974 *The Biology of Cholinesterases*. American Elsevier, New York.

SINGH, R. S., 1976 Substrate-specific enzyme variation in natural populations of *Drosophila pseudoobscura*. Genetics **82**: 507–526. ——, 1979 Letter to the editor. Genetics **92**: 679–684.

SINGH, R. S., J. L. HUBBY and R. C. LEWONTIN, 1974 Molecular heterosis for heat sensitive enzyme alleles. Proc. Natl. Acad. Sci. U.S. **71**: 1808–1810.

SINGH, R. S., J. L. HUBBY and L. THROCKMORTON, 1975 The study of genic variation by electrophoretic and heat denaturation technique at the octanol dehydrogenase locus in members of the *Drosophila virilis* group. Genetics **80**: 637–650.

SINGH, R. S., R. C. LEWONTIN and A. A. FELTON, 1976 Genic heterogeneity within electrophoretic "alleles" of xanthine dehydrogenase in *Drosophila pseudoobscura*. Genetics **84**: 609–629.

STROBECK, C. and K. MORGAN, 1978 The effect of intragenic recombination on the number of alleles in a finite population. Genetics **88**: 829–844.

THORIG, G. E. W., A. A. SCHOONE and W. SCHARLOO, 1975 Variation between electrophoretically identical alleles at the alcohol dehydrogenase locus in *Drosophila melanogaster*. Biochem. Genet. **13**: 721–731.

TRIPPA, G., A. LOVERRE and A. CATAMO, 1976 Thermostability studies for investigating non-electrophoretic polymorphic alleles in *Drosophila melanogaster*. Nature **260**: 42–44.

WARD, R. D., 1977 Relation between enzyme heterozygosity and quaternary structure. Biochem. Genet. **15**: 123–135.

WATT. W. B., 1972 Intragenic recombination as a source of population genetic variability. Am. Naturalist **106**: 737–753.

ZOUROS, E., 1976 Hybrid molecules and the superiority of the heterozygote. Nature **262**: 227–229.

Corresponding editor: W. W. ANDERSON

APPENDIX

A list of electrophoretic alleles observed. (Nomenclature explained in text.)

Locus								
Ao	*Pt-6*	*Pt-7*	*Pt-8*	*Pt-10*	*Pt-13*	*Est-1*	*Est-5*	*Est-6*
Null	*1.00*	*0.68–00*	*0.80–00*	*1.02–0*	*1.23–00*	Null	Null	*0.90–0*
0.95–0	*1.02*	*0.73–00*	*0.80–10*	*1.04–0*	*1.30–00*	*0.33*	*0.81–000*	*1.00–0*
0.97–0	*1.04*	*0.75–00*	*0.81–00*	*1.04–1*	*1.30–10*	*0.50*	*0.88–000*	*1.00–1*
0.98–0		*0.75–01*	*0.81–10*	*1.06–0*	*1.30–11*	*0.54*	*0.88–100*	*1.00–2*
0.99–0		*0.75–10*	*0.81–11*	*1.06–1*	*1.30–12*	*0.74*	*0.90–000*	*1.10–0*
0.99–1		*0.75–20*	*0.81–20*	*1.06–2*	*1.30–20*	*0.79*	*0.91–000*	*1.10–1*
1.00–0		*0.75–30*	*0.81–21*		*1.30–21*	*1.00*	*0.91–001*	*1.10–2*
1.00–1		*0.77–00*	*0.83–00*		*1.30–30*	*1.03*	*0.98–000*	*1.10–3*
1.00–2			*0.85–00*		*1.37–00*	*1.58*	*0.98–010*	*1.10–4*
1.01–0					*1.37–10*		*0.98–100*	
1.01–1							*0.98–101*	
1.02–0							*0.98–110*	
1.02–1							*0.99–000*	
1.03–0							*1.00–000*	
1.03–1							*1.00–1M⁻0*	
1.06–0							*1.00–1M⁺0*	
							1.00–200	
							1.00–210	
							1.01–000	
							1.02–000	
							1.02–010	
							1.04–000	
							1.05–000	
							1.05–001	
							1.07–000	
							1.09–000	
							1.10–000	
							1.11–000	
							1.11–001	
							1.12–000	
							1.12–010	
							*1.22–000**	
							*1.22–000**	

* Monomeric form.