*GENETIC VARIATION IN NATURAL ISLAND POPULATIONS OF MEMBERS OF THE DROSOPHILA NASUTA AND DROSOPHILA ANANASSAE SUBGROUPS**

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The patterns and amounts of genetic variations in *Drosophila* populations have been of extreme interest to investigators in the area of population and evolutionary genetics, and our knowledge in this area is continuously expanding.[1-3] Recent advances using gel electrophoresis of single flies has added another dimension to the study of genetic variations and their selective mechanisms in populations.[4-6] The enzyme variation measured by this technique can be used to estimate genetic variation in natural populations. Such studies carried out with *Drosophila ananassae*,[5] *D. pseudoobscura*,[6] *D. aldrichi*, and *D. mulleri*,[8, 9] and small samples of a number of other *Drosophila* species[7] indicated that most natural populations of *Drosophila* were highly polymorphic in this regard. In the previous studies, however, either the samples were not taken freshly from natural populations, sample size was not large enough, or the enzymes assayed were few. The present investigation measures the variation found in a number of enzymes in relatively large samples of wild-caught flies of two species.

Materials and Methods.—Population samples were collected during the winter (July and August) of 1967 from fallen papaya (unless otherwise indicated) on three Samoan and two Fijian islands: (1) Tutuila, American Samoa; Iliili farm; (2) Upolu, Western Samoa; Alifua College outside Apia; (3) Savaii, Western Samoa; farms outside Aopo (fallen papaya, banana, and breadfruit); (4) Viti Levu, Fiji; about half at Nandaruloulou, Suva region (on drying and fermenting cacao pods); the others (on papaya) in Suva; (5) Viti Levu, Fiji; Lautoka area in the mountains near Nandarivatu (on fermenting coffee berries); (6) Vanua Levu, Fiji; Lambasa region near Savusavuitangga. The living *Drosophila* were sent to Austin as rapidly as possible.

We studied one member of the *ananassae* subgroup, presently designated *ananassae* dark, and one of the species of the *nasuta* subgroup. The latter species is found from Hawaii to Fiji and Tonga. Definitive naming will have to await necessary taxonomic (and genetic) information on the original and other earlier-named forms. The material of the *nasuta* subgroup was cytologically homozygous. The *ananassae* might have contained some inversions, but the relations between the enzyme systems and inversions are not known.

Wild-caught flies were homogenized individually in 0.01 ml of deionized water. Starch gel electrophoresis was carried out horizontally using Poulik's discontinuous system of buffers.[10] After electrophoresis, the gels were cut into three replicate slices and stained for esterase (EST),[7] acid phosphatase (ACPH),[7] and alcohol/octanol dehydrogenase (A/ODH) activity. In slices stained for ACPH some bands of alkaline phosphatase (APH) activity also appear. The

TABLE 1. *Esterase F alleles in populations of a species of the Drosophila nasuta subgroup.*

Collecting area	Phenotype Classes 2-2	2-4	4-4	Others	Total	Allele Frequency 2	4	8	1
Samoa									
T	25*	58	29	0	112	0.482	0.518		
	26.0†	56.0	30.0	0					
U	38	36	28	0	102	0.549	0.451		
	30.7	50.5	20.8	0					
S	68	84	63	0	215	0.512	0.488		
	56.3	107.4	51.3	0					
Fiji									
V	3	18	142	1	164	0.073	0.924	0.003	
	0.9	22.1	140.0	1.0					
N	3	59	305	11	378	0.089	0.896	0.013	0.001
	3.0	60.3	303.5	11.2					
A	3	77	178	1	259	0.160	0.838	0.002	
	6.6	69.5	181.9	1.0					

* Observed numbers.
† Expected numbers.
T = Tutuila; U = Upolu; S = Savaii; V = Viti Levu, near Suva; N = Viti Levu, Nandarivatu; A = Vanua Levu.

APH bands are identified by a stronger reaction in alkaline buffers and a slightly different color from ACPH bands in the ACPH gels. Dehydrogenase activity was detected with a stain containing 5 mg phenazine methosulfate, 25 mg nitro-blue tetrazolium, and 25 mg diphosphopyridine nucleotide in 100 ml Tris(hydroxymethyl)aminomethane buffer (0.1 ml, pH 8.5), with 5 ml n-octanol and 5 ml isopropanol added for simultaneous display of alcohol dehydrogenase (ADH)[11, 12] and octanol dehydrogenase (ODH).[12] The two enzymes are controlled by two separate loci.

Results.—In both species, systems of alleles were identified by electrophoretic mobility. Mobility positions were arbitrarily numbered in decreasing order of mobility from the anode. A homozygous condition of an allele results in a single band or a set of bands basic to that allele; heterozygotes display bands of both alleles and, sometimes, additional bands which are not basic to either allele. While in some instances genotype and phenotype may be equated, in other cases there are probably several genotypes which produce a similar phenotype. In *ananassae* ACPH some gels showed evidence of there being two *Acph 3* alleles. Slight differences in mobility were observed, but they could not be routinely scored with any confidence.

In addition to the variability treated quantitatively in this study a number of zones were seen to vary; but because of unclear patterns and/or faintness of stained bands, reliable scoring could not be made.

The inheritance of the alternate enzyme forms as allelic products has been partially determined for both *ananassae* dark[7] and the *nasuta* subgroup species.[13] Additional data are being accumulated and will be presented in later reports. There is presently little reason to doubt that the phenotypes for the enzyme systems described represent at least as many genotypes controlled by multiple alleles at enzyme structural loci.

Genotype frequencies: The observed numbers of phenotypes (which also

TABLE 2. *Octanol dehydrogenase alleles in populations of a species of the Drosophila nasuta subgroup.*

Collecting area	Phenotype Classes 3-3	3-4	4-4	Others	Total	Allele Frequency 3	4	1
Samoa								
T	60	42	10	0	112	0.723	0.277	
	58.5	44.9	8.6	0				
U	56	31	8	0	95	0.753	0.247	
	53.9	35.3	5.8	0				
S	111	86	16	1	214	0.722	0.276	0.002
	111.6	85.3	16.3	0.8				
Fiji								
V	97	23	0	1	121	0.901	0.095	0.004
	98.2	20.7	1.1	1.0				
N	294	59	5	3	361	0.900	0.096	0.004
	292.4	62.4	3.3	2.9				
A	201	52	3	1	257	0.885	0.113	0.002
	201.3	51.4	3.3	1.0				

represent genotypes in the present study) are summarized in Tables 1 through 7 for three systems in *nasuta* and four systems in *ananassae*. These tables also contain the allele frequencies calculated at individual loci, and the numbers of genotypes expected on the basis of Hardy-Weinberg ratios computed from the allele frequencies. The agreement of the observed numbers with the Hardy-Weinberg expectations is extremely good in general. Statistically significant excess of heterozygote frequency was detected only for the *Est-F* alleles in Vanua Levu population of *nasuta*, while significant deficiency was found for the same enzyme system in Upolu and Savaii populations.

Allele frequencies: From the standpoint of selection for different local conditions, it is significant that the same allele of a given system is found to be most common in all locations except a few cases of *Est-F* in *nasuta*. However, actual frequencies of common alleles vary considerably over locations. For this reason, a nested analysis of variance on arcsin-transformed frequencies of the majority alleles was performed, omitting *ananassae* ODH and ADH for which minority

TABLE 3. *Acid phosphatase alleles in populations of a species of the Drosophila nasuta subgroup.*

Collecting area	Phenotype Classes 3-3	3-5	5-5	Others	Total	Allele Frequency 3	5	2	4
Samoa									
T	71	33	6	0	110	0.795	0.205		
	69.5	35.9	4.6	0					
U	60	36	4	2	102	0.770	0.221	0.009	
	60.5	34.7	5.0	1.8					
S	151	56	6	2	215	0.837	0.158	0.005	
	150.6	56.9	5.4	2.1					
Fiji									
V	83	60	20	0	163	0.693	0.307		
	78.3	69.4	15.4	0					
N	190	153	34	0	377	0.707	0.293		
	188.4	156.2	32.4	0					
A	131	109	17	1	258	0.720	0.278		0.002
	133.7	103.3	19.9	1.1					

TABLE 4. *Esterase C alleles in populations of the species now designated as Drosophila ananassae dark, a member of the Drosophila ananassae subgroup.*

Collecting area	Phenotype Classes								Allele Frequency				
	3-3	3-4	4-4	3-5	3-1	4-5	Others	Total	3	4	5	Others	1
Samoa													
T	211	36	6	22	4	0	3	282	0.862	0.087	0.041	0.003	0.007
	209.5	42.3	2.1	19.9	3.4	2.0	2.8		(0.84)*	(0.14)	(0.02)		
U	283	24	2	26	8	0	1	344	0.907	0.041	0.039		0.013
	283.0	25.6	0.6	13.7	8.1	1.1	11.9		(0.67)	(0.33)	(0.00)		
S	114	9	2	4	4	0	1	134	0.914	0.052	0.015	0.004	0.016
	111.9	12.7	0.4	3.7	3.7	0.2	5.1						
Fiji													
V	42	31	23	17	4	9	7	133	0.519	0.338	0.102	0.015	0.026
	35.8	46.7	15.2	14.1	3.6	9.2	8.4						

* The gene frequencies given in parentheses were determined from *Drosophila* collected in July 1965.

TABLE 5. *Acid phosphatase alleles in Drosophila ananassae dark.*

Collecting area	Phenotype Classes							Allele Frequency				
	2-2	2-3	3-3	3-5	3-4	Others	Total	2	3	4	5	Others
Samoa												
T	2	28	209	27	2	3	271	0.061	0.878	0.006	0.054	0.001
	1.0	29.0	208.9	25.7	2.9	3.5						
U	4	58	246	25	4	12	349	0.102	0.835	0.007	0.047	0.009
	3.6	59.4	243.3	27.4	4.1	11.2						
S	0	25	91	14	2	3	135	0.093	0.833	0.007	0.059	0.008
	1.2	20.9	93.7	13.3	1.6	4.3						
Fiji												
V	18	54	86	0	0	0	158	0.285	0.715			
	12.8	64.4	80.8	0	0	0						

TABLE 6. *Alcohol dehydrogenase alleles in Drosophila ananassae dark.*

Collecting area	Phenotype Classes 6-6	6-8	Others	Total	Allele Frequency 6	8	Other
Samoa							
T	280	2	0	282	0.996	0.004	
U	343	1	1	345	0.998	0.001	0.001
S	135	0	0	135	1.000		
Fiji							
V	131	1	1	133	0.992	0.004	0.004

alleles were extremely low in their frequencies. The test of significance for the diversity between the two regions was made by the F-test of between versus within variances, while that for within-region variations was carried out by a multiple range test using expected variances as the source of error variances. The results are given in Table 8.

Evidently, significant diversity in the majority allele frequencies exists between the Fiji and Samoa populations of the *nasuta* species, but the variation among individual island populations within regions does not seem to differ significantly except *Est-F* in the *nasuta*. This situation might be caused by simple geographic divergence due to distance. However, an appealing alternative interpretation is that such situations have been evolved through different adaptive conditions existing between Samoa and Fiji environments while much more nearly similar conditions prevail within each region.

In the case of *ananassae* populations, there appears to be relatively little divergence between the two regions. Out of four enzyme systems examined, the EST C is the only system with some degree of significant divergence. The variation among islands within regions can be evaluated only for the Samoa region for *ananassae*, and these islands are not significantly different from one another.

The frequency of minority alleles in the ODH and ADH systems in *ananassae* is no more than that expected from the theory of mutation-selection balance at the gene locus where minority alleles are recessive in determining fitness. Since the majority allele in either of these two systems is the same for all four populations of *ananassae* investigated, it is clear that the selective mechanisms and adaptive significance of these alleles are different from those for the majority alleles in the other enzyme systems.

Some points are not obvious in Tables 1 through 7. In these tables one may notice apparent lack of null alleles whose products do not display *in vitro* catalytic activity. If null allele homozygotes are viable and the frequency of a null allele is high in a given population, individuals lacking such zones are expected.

TABLE 7. *Octanol dehydrogenase alleles in Drosophila ananassae dark.*

Collecting area	Phenotype Classes 3-3	1-3	3-5	Total	Allele Frequency 3	1	5
Samoa							
T	251	1	2	254	0.994	0.002	0.004
U	344	2	3	349	0.993	0.003	0.004
S	132	0	2	134	0.993		0.007
Fiji							
V	109	2	6	117	0.966	0.009	0.025

TABLE 8. *Results of the analysis of divergences among island populations using the majority allele frequency.*

	Fiji vs. Samoa	Within regions
nasuta		
EST-F	$P < 0.005$	A
ODH	$P < 0.005$	N
ACPH	$0.010 < P < 0.025$	N
ananassae		
EST-C	$0.010 < P < 0.025$	N
ACPH	$0.100 < P < 0.050$	N

A = Some islands were significantly different at $P = 0.05$.
N = No significant differences among islands.

If the frequency is not sufficiently high, probably only heterozygotes of null and non-null alleles will be present in samples of limited size; and these heterozygotes are usually classified as non-null allele homozygotes. This will make an excess of apparent homozygotes and result in a deviation from Hardy-Weinberg ratios. Admittedly, the efficiency of detecting such deviations is very weak in the range of sample size used in this study; but it can be safely stated that even if null alleles were present in the populations, their frequencies were low for all the systems investigated.

Another point is the shift in allele frequency between the 1965 and 1967 data of EST C in the Samoa *ananassae* populations (Table 4). The 1965 data were taken not from original flies caught in the wild but from iso-female line cultures analyzed some generations after they were established. It is not clear what caused the rather marked shift between the frequencies of alleles 3 and 4 in the Upolu population. There was a severe typhoon in early 1966 in the Samoa region which reduced the size of *Drosophila* populations drastically. Such a reduction could have caused a "founder effect" in the population which sprang back from a small number of surviving *Drosophila*. Furthermore, selection probably was much more stringent and perhaps differed in the recovery period after the typhoon.

Proportion of polymorphic systems per population: The enzyme banding patterns observed fell into three major classes: nonvariable, variable with unclear patterns, and variable with classifiable patterns. The last class can be somewhat arbitrarily divided into polymorphic and nonpolymorphic systems by observing the magnitude of the major allele frequencies in individual populations. If polymorphism is defined as those cases where major allele frequency is less than 95 per cent, the following three figures are obtained for the estimate of proportion of polymorphic systems per population: 72 per cent if all unclear classes are polymorphic, 45 per cent if they are ignored, and 23 per cent if they are all monomorphic. It is impossible to arrive at a reasonable estimate of proportion of polymorphic gene loci from these figures, since there is no information concerning the number of loci involved in the banding patterns of nonvariable and variable with unclear patterns.

Discussion.—As we do not know the physiological adaptive significance of any of these enzyme systems, the present discussion must be limited to an aspect of polymorphisms which can be illuminated by the analysis of frequency data.

The analysis of the majority allele frequencies indicated that the same alleles are predominant in most enzyme systems in all populations, but there are variations of these allele frequencies which are correlated with the degree of geographic diversity among islands. This finding strongly supports the idea that some selective mechanisms are involved for the maintenance of majority alleles. The exact nature of selective mechanisms for individual systems is not known, but some speculations are in order. The widely held idea of heterotic selection is a possibility, provided that it is a type which does not give large deviations of adult genotype frequencies from those under Hardy-Weinberg expectations. This is possible if major selection exists in fertility and fecundity differentials and relatively weak selection exists in survival of fertilized eggs to adults.

Another alternative mode of selection is frequency-dependent selection with reversal of selective advantage between the allele frequency range above the point of equilibrium and that below the point. Existence of this type of selection was demonstrated with the Esterase 6 system in *D. melanogaster*.[4, 14] With this mode of selection, an allele frequency with a large deviation from its equilibrium value will be rapidly pushed back to the neighborhood of the equilibrium and selective differentials among genotypes become small near the equilibrium.

A likely mechanism for maintaining alleles with frequencies below a few per cent in a population is the balance between mutation and selection against the homozygotes of these alleles. If an allele homozygote is 5 per cent inferior to the heterozygote and to the other homozygote, the equilibrium frequency of this allele is expected to be about 3 per cent with a mutation rate of 5×10^{-5}.

Summary.—Enzyme variations were investigated using wild-caught flies of two *Drosophila* species from South Pacific islands. For the majority of the enzyme systems analyzed, there were two or three alleles with high frequencies (5% or more in some populations) which accounted for 99 per cent of the total, and the remainder was shared by one or more alleles with extremely low frequencies. However, there were systems for which either only one major allele existed with a series of low-frequency alleles or no variation was detected. The genetic diversity among the populations within and between the Samoa and Fiji island groups was investigated, using the frequencies of majority alleles in each species. The diversity was highly significant between the island groups, while there was only one enzyme system showing significant divergence of allele frequencies among islands within each group. The heterozygosity for the enzyme systems studied was determined. It ranged from 51 per cent to less than 1 per cent, with a few esterase bands showing no variation. The population divergence revealed by an analysis of variance of the degrees of heterozygosity paralleled the findings from the analysis of variance of major allele frequency, except that the within-region variation among islands was slightly greater with the heterozygosity analysis.

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