(*9*) and Australian aborigines (*10*) the melanosomes are dispersed singly within the cytoplasm of epidermal cells, whereas in Caucasoids they are packed into so-called melanosome complexes (*9*). Our model system demonstrates that the mode of uptake of particles resembling melanosomes is size-dependent, with the size of the individual particle determining whether particles are taken up collectively or individually, and thus whether they are packed into complexes or dispersed. We propose that the differing distribution patterns of melanosomes in the Caucasoid and Negroid races reflect differences in melanosome size, and recent observations support the validity of this proposal. Toda *et al.* (*11*) treated Caucasoid volunteers with Psoralen and long-wave ultraviolet light and noted that, in the exposed skin, the keratinocytes contained melanosomes dispersed singly. These melanosomes were remarkably large (1.1 by 0.6 μm) compared to melanosomes of the controls (0.6 by 0.3 μm), which were packed into melanosome complexes. Obviously, melanosomes may occur singly even in Caucasoid keratinocytes provided their dimensions exceed a certain limit. This is in agreement with the conclusions derived from the model system; by inference it may be assumed that the crucial size above which individual uptake occurs is somewhere between 0.6 and 0.8 μm.

Comparative studies of melanosome size in Negroids and Caucasoids have not been performed so far, but Szabo *et al.* (*9*) mention that Negroid melanosomes are "larger and wider" than those of other racial groups. From our findings we predict that exact measurements of these organelles will disclose that they are larger in Negroid than in Caucasoid skin. Accordingly, the genetically determined distribution patterns of melanosomes, and thus the skin color, in the two races would primarily depend on the melanocyte, which produces the melanosomes and determines their size, and not on the keratinocyte, which receives and packs these granules.

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Genetic Polymorphisms in Varied Environments

Abstract. *Thirteen experimental populations of* Drosophila willistoni *were maintained in cages, in some of which the environments were relatively constant and in others varied. After 45 weeks, the populations were assayed by gel electrophoresis for polymorphisms at 22 protein loci. The average heterozygosity per individual and the average number of alleles per locus were higher in populations maintained in heterogeneous environments than in populations in more constant environments.*

Studies by means of gel electrophoresis have disclosed a large amount of genetic variation in populations of organisms as diverse as man, drosophila flies, and oats (*1–3*). What mechanisms are operating to maintain this variation is an open question. According to classical population models, if all protein polymorphisms in a population were maintained by heterosis, this would lead to an unbearable genetic load (*4*). Some investigators have devised alternative models of population fitness which will allow many polymorphisms to be maintained by heterosis (*5*). Others have escaped the problem of genetic load by postulating that most protein variants are neutral to natural selection and thus neither contribute to nor lessen the fitness of a population (*6*). Experimental evidence has not supported the neutrality hypothesis (*7*).

If a form of balancing selection is maintaining these protein polymorphisms, several kinds of balancing selection are possible. There is evidence for at least two types, heterosis (*8*) and frequency-dependence (*9*). In this report evidence is presented for a multiple niche polymorphism (*10, 11*).

Thirteen population cages were started each with 500 *Drosophila willistoni* from a collection made at Mirassol, Brazil. This collection consisted of more than 500 single female lines that were combined to begin the cages.

Table 1 indicates the conditions in each cage. The cages used were those described by Ayala (*12*) in which eight food cups are available to the flies. In Table 1, y_1 means all eight food cups were heavily seeded with bakers' yeast (Fleischmann's), y_2 means that all eight food cups were heavily seeded with brewers' yeast (Budweiser), while y_1/y_2 means that four cups were seeded with bakers' and four with brewers' yeast. Similarly f_1 (Carolina instant

Table 1. Genetic variability in populations of *Drosophila willistoni* that were maintained in laboratory cages under differing conditions. In cages 1, 2, 3, 6, and 9, only one type of yeast, medium, and temperature was present. In cages 4, 5, 7, 8, 10, and 11, one factor was varied. In cages 12 and 13, all three factors were varied. Symbols are explained in the text.

Cage	Conditions	Heterozygosity per individual ⟨% ± S.D.⟩	Alleles per locus ⟨No.⟩
	Constant environment		
1	y_1f_125	7.29 ± .83	1.68
2	y_1f_125	7.80 ± .82	1.68
3	y_2f_125	8.04 ± .81	1.68
6	y_1f_225	7.96 ± .85	1.68
9	y_1f_119	7.98 ± .84	1.64
	One variable		
4	y_1/y_2f_125	11.09 ± .89	1.91
5	y_1/y_2f_125	9.06 ± .84	2.00
7	y_1f_1/f_225	10.16 ± .88	2.05
8	y_1f_1/f_225	9.33 ± .88	1.91
10	$y_1f_119/25$	8.86 ± .80	1.95
11	$y_1f_119/25$	9.21 ± .88	1.68
	Three variables		
12	$y_1/y_2f_1/f_219/25$	13.90 ± .98	2.00
13	$y_1/y_2f_1/f_219/25$	12.81 ± .92	2.23

Table 2. Average genetic variability for all populations kept in constant environments, in which one factor varied, and in environments in which three factors varied.

Variables	Heterozygosity per individual (% ± S.E.)	Alleles per locus (No.)
0	7.81 ± 0.31	1.67 ± 0.02
1	9.62 ± 0.84	1.92 ± 0.13
3	13.36 ± 0.76	2.12 ± 0.17

drosophila medium) and f_2 [Spassky's medium (*13*)] indicate the type of medium each cup contained. Cages were kept at 25°C, 19°C, or on alternate weeks at 25°C and 19°C (symbolized 19/25). Thus five cages, 1, 2, 3, 6, and 9, had constant environments; six cages, 4, 5, 7, 8, 10, and 11, had one condition varied; two cages, 12 and 13, had all three conditions varied.

After the populations were maintained for 45 weeks, or about 15 generations, samples of adult individuals were assayed for genetic variability by starch-gel electrophoresis, with the use of techniques already described (*2*). Twenty-two enzyme loci were studied in each population (*14*). Fifty individuals from each population were assayed for each enzyme. Therefore, except for two sex-linked loci for which males were assayed, 100 genomes were studied at each locus in each population.

Two measures of genetic diversity are given in Table 1. The first is the percentage of average heterozygosity per individual, as calculated by Lewontin and Hubby (*1*) except that actually observed heterozygotes are used instead of assuming Hardy-Weinberg equilibria. (The Hardy-Weinberg assumption had to be used for the two sex-linked loci for which males were assayed.) The standard deviations for these figures were calculated by averaging the binomial variance of the frequency of observed heterozygotes at each locus (*15*). The second measure of diversity is the average number of alleles maintained at a locus. Since the sample size was the same in all cages, this meant that an allele had to be present at a frequency of 1 percent or greater to be included in this measurement.

As is shown in Table 1, populations in more variable environments have maintained more genetic variability. This is true for both those factors that are available to the population at the same time (spatial heterogeneity) and for the factor that varies with respect to time (temporal heterogeneity). Combining all three factors—yeast, medium, and temperature—increases genetic variability more than any single factor alone.

Table 2 gives the average genetic variability for all populations kept in constant, one-variable, and three-variable environments. Again, more variable environments maintain more genetic heterogeneity by either measure.

Using the technique of gel electrophoresis to determine total genetic variability in a population is subject to several biases (*1*). However, in my study, it is not so important to know the total genetic variability of the population, but it is sufficient to have shown that the type of variability detected by electrophoresis can be affected by varying the environment of the population.

The results show that at least some of the protein polymorphisms in these experimental populations are maintained by environmental heterogeneity and are not neutral to natural selection. In the natural population from which these experimental populations were begun, the average heterozygosity per individual was about 19 percent (*2*). In the experimental populations the average heterozygosity per individual ranged from about 8 percent in the most constant environments to about 13 percent in the most varied. As *D. willistoni* is native to Neotropical rain forests, it undoubtedly meets a more complex and diversified environment than can be easily created in the laboratory.

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15. Whenever the frequency of observed heterozygotes was less than .05, the variance was calculated as if its frequency were .05. This would cause overestimation of the variance. The variances were averaged over the 22 loci and the square root was taken as the standard deviation.
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Barbiturates: Radioimmunoassay

Abstract. *The development of a radioimmunoassay for barbiturate is described. The barbiturate is made antigenic by coupling it to a protein, bovine gamma globulin. The radioimmunoassay can measure as little as 5 nanograms of barbiturate.*

For study of the metabolism of barbiturates, quantitative assays that are rapid, sensitive, specific, and reliable even for small amounts of barbiturates would be most advantageous. Methods now available are suited for qualitative and quantitative analysis only if an adequate sample of biological tissue, 1 to 5 ml, is available (*1*). In addition, these methods require solvent extraction (*1*) and, in some instances, filtration and evaporation (*2*). Gas chromatography and spectrophotometry are sensitive to 10 μg/ml but require solvent extraction (*3*). Immunologic methods for assaying polypeptides, hormones, and drugs have been reported (*4*). We have conjugated a barbiturate to bovine