ENZYME VARIABILITY IN THE *DROSOPHILA WILLISTONI* GROUP. III. AMOUNTS OF VARIABILITY IN THE SUPERSPECIES, *D. PAULISTORUM*[1]

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ABSTRACT

The semispecies composing the superspecies, *Drosophila paulistorum*, have been analyzed for genetic variation at 17 enzyme loci. On the average a population of *D. paulistorum* is polymorphic for 55–67% of its loci and an average individual is heterozygous at 21% of its loci. The pattern of genetic variability found supports the hypothesis that allozyme variation is maintained in natural populations by some form of balancing selection. Evidence is presented which supports the hypothesis that glucose-metabolizing enzymes are less genetically variable than non-glucose-metabolizing enzymes. The known genetic relationships between the semispecies of *D. paulistorum* are discussed in the light of the frequencies of alleles at allozyme loci.

DROSOPHILA paulistorum is an example of a species in the process of diverging into several species (DOBZHANSKY and SPASSKY 1959). The species has a widespread distribution extending from Guatemala in Central America to the southern reaches of Brazil (see Figure 1, SPASSKY *et al.* 1971). Attempts to cross various strains of *D. paulistorum* from all parts of its range give these results: a) strains may cross easily and yield vigorous, fertile F_1 hybrids, b) hybrids may be formed but F_1 males are sterile, females fertile, c) no hybrids may be formed. Systematic intercrossing of strains has revealed the existence of five genetically homogeneous groups of strains termed semispecies. Crosses between semispecies or crosses in which the female parent carries the chromosomes of more than one semispecies usually produce sterile sons (EHRMAN 1960). The five semispecies would be effectively isolated from each other were it not for the existence of a heterogeneous grouping of strains some of which are capable of acting as genetic bridges between two semispecies. At present *D. paulistorum* is thought to consist of five semispecies which have been given the following names and abbreviations: Centroamerican (C), Andean–Brazilian (AB), Orinocan (O), Interior (I) and Amazonian (A) (SPASSKY *et al.* 1970). The sixth heterogeneous grouping of strains is termed the Transitional (T) (DOBZHANSKY, PAVLOVSKY and EHRMAN 1969). Some strains of the Transitional grouping are capable of

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giving fertile male progeny with both the Centroamerican and Andean–Brazilian semispecies.

Drosophila paulistorum presents geneticists with an unequaled opportunity to study the genetic structure of a species in the process of speciation. This report records the results of an analysis of enzyme variability in each of the semispecies.

MATERIALS AND METHODS

D. paulistorum from two sources was utilized during the course of this study: (1) stocks kept in Professor TH. DOBZHANSKY's laboratory at The Rockefeller University, and (2) freshly collected material. The laboratory stocks have been kept in culture for varying periods of time, in some few cases for more than 20 years. The number of single female lines which comprise each of these stocks is known in most cases. This material represents collections from over 70 localities in Central and South America. A complete listing of the localities is given in SPASSKY *et al.* (1971).

New collections of *D. paulistorum* were made in 22 localities during the course of this study. Material from 17 of these localities gave large enough sample sizes to be analyzed separately. The localities sampled are shown in Figure 1 and listed below. The numerals in Figure 1 refer to this list.

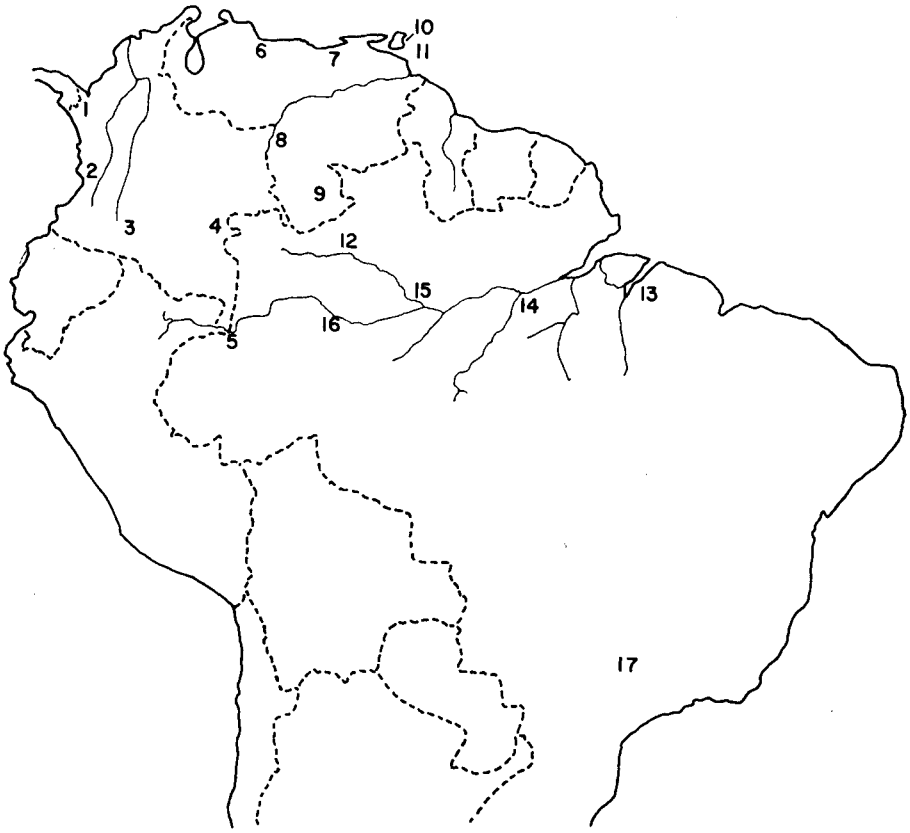

FIGURE 1.—Localities from which newly collected samples of *D. paulistorum* were studied. See the text for locality names.

Colombia: Teresita (1), Malaga Bay (2), Valparaiso (3), Mitu (4), Leticia (5). Venezuela: Rancho Grande (6), Guatopo (7), Puerto Ayacucho (8), Ocamo (9). Trinidad: Arima Valley (10), Mora Forest (11). Brazil: Tapuruquara (12), Belem (13), Santarem (14), Manaus (15), Tefe (16), Mirassol (17).

The field collection methodology and techniques used to classify *D. paulistorum* strains as to semispecies have been described in detail before (SPASSKY *et al.* 1971; DOBZHANSKY and PAVLOVSKY 1967).

The analysis of allozyme variation in *D. paulistorum* was made by the use of horizontal, starch gel electrophoresis followed by selective enzyme staining. Adult flies aged from 1–2 weeks at 25°C were used in all enzyme tests. No developmental differences in the enzyme patterns scored were observed in 1–2 week old individuals. Flies are individually homogenized in 0.01 ml of gel starch buffer (Table 1) using the method of JOHNSON (1964). The homogenate is absorbed into small rectangles (4×9 mm) of Whatman No. 1 filter paper (GORDON 1971). A vertical slit is cut lengthwise through a previously prepared gel 4.4 cm from one edge. The edges of the slit are carefully separated and the filter papers containing the homogenate are placed flush with one surface of the slit. The gels used are 17.4 cm wide × 19.7 cm long and 1.0 cm thick and can readily accommodate 27–30 samples. After the samples are inserted, the edges of the slit are pushed together and the surface of the gel is covered with Saran Wrap to prevent drying of the gel during electrophoresis. The gel is put into an electrophoresis chamber which is placed in a 5°C refrigerator for electrophoresis of the enzyme systems assayed.

Upon completion of electrophoresis the gel is sliced horizontally into 3–4 slices 1–2 mm thick. Each slice is placed in a box containing a specific enzyme staining solution. The gels are later fixed in a mixture of acetic acid, methanol and water (1:5:5). The composition of each of the enzyme-staining solutions utilized is listed below. Additional technical details can be found in RICHMOND (1971).

Acid phosphatase (SHAW and KOEN 1968): 100 ml of 0.05 M acetate buffer, pH 5.0; 100 mg Na-α-naphthyl acid phosphate; 100 mg Fast Garnet GBC diazonium salt. The gel is pre-soaked in 0.5 M Boric acid for one hour prior to staining.

Esterase (modified from JOHNSON *et al.* 1966): 100 ml of 0.1 M phosphate buffer, pH 6.5; 40 mg Fast Garnet GBC diazonium salt; 10 mg Black K diazonium salt; 1.5 ml of 2% (w/v) solution of α-naphthyl acetate in acetone:water (1:1); 1.5 ml of 2% (w/v) solution of β-naphthyl acetate in acetone.

Leucine aminopeptidase (modified from BECKMAN and JOHNSON 1964): 100 ml of 0.12 M maleate buffer, pH 5.0; 70 mg L-leucyl-β-naphthylamide-HCl; 50 mg Black K diazonium salt.

Tetrazolium oxidase (RICHMOND and POWELL 1970): 100 ml of 0.05 M Tris-HCl buffer, pH 8.5; 20 mg Nitro blue tetrazolium salt; 25 mg β-NAD+; 5 mg Phenazine methosulfate. Gel and stain exposed to light for 1–1½ hr then fixed.

Malate dehydrogenase, β-NAD+ dependent (modified from GILLESPIE and KOJIMA 1968): 100 ml of 0.05 M Tris-HCl buffer, pH 8.5; 20 mg Nitro blue tetrazolium salt; 25 mg β-NAD+, 50 mg L-malic acid, 5 mg Phenazine methosulfate added after 1 hr of incubation at 37°C. Gel is fixed when bands are sufficiently stained.

Malate dehydrogenase, β-NADP+ dependent (modified from GILLESPIE and KOJIMA 1968): Staining procedure identical to β-NAD+ dependent except that β-NADP+ is substituted for β-NAD+.

Alcohol dehydrogenase (modified from JOHNSON and DENNISTON 1964): 10 mg β-NAD+/400 ml gel buffer should be added to molten gel before degassing and pouring; 100 ml 0.05 M Tris-HCl, pH 8.5; 20 mg Nitro blue tetrazolium, 25 mg β-NAD+; 4.5 ml isopropyl alcohol; 5 mg Phenazine methosulfate is added after 1 hr of incubation at 37°C. Gel is stained when bands are sufficiently developed.

Octanol dehydrogenase (modified from COURTRIGHT *et al.* 1966): Same solution as alcohol dehydrogenase but substitute 0.5 ml octanol for isopropyl alcohol.

α-Glycerophosphate dehydrogenase (modified from GILLESPIE and KOJIMA 1968): 100 ml 0.05 M Tris-HCl, pH 8.5; 20 mg Nitro blue tetrazolium salt; 25 mg NAD+, 800 mg Na-α-

glycerophosphate; 180 mg EDTA, 5 mg Phenazine methosulfate added as for alcohol dehydrogenase.

Phosphoglucomutase (modified from SHAW and KOEN 1968): 100 ml of 0.05 M Tris-HCl, pH 8.5; 600 mg glucose-1-phosphate·$4H_2O$; 200 mg $MgCl_2 \cdot 6H_2O$; 10 mg β-$NADP^+$; 80 units glucose-6-phosphate dehydrogenase; 20 mg Nitro blue tetrazolium salt; 1 mg Phenazine methosulfate.

Triose phosphate isomerase (modified from SHAW and KOEN 1968): Substrate solution: 100 ml 0.02 M Tris-HCl, pH 8.0; 2.2 g Na-α-glycerophosphate; 1.1 g Na-pyruvate; 50 mg β-NAD^+; 200 mg α-glycerophosphate dehydrogenase, 200 mg Lactate dehydrogenase. This solution is incubated at 37°C for two hours and then adjusted to pH 2.0 with 1 N HCl. Add 1 M Tris to readjust to pH 7.3. Stain solution: 100 ml substrate solution; 60 mg β-NAD^+; 2 mg Phenazine methosulfate; 10 mg Phosphoglyceraldehyde dehydrogenase.

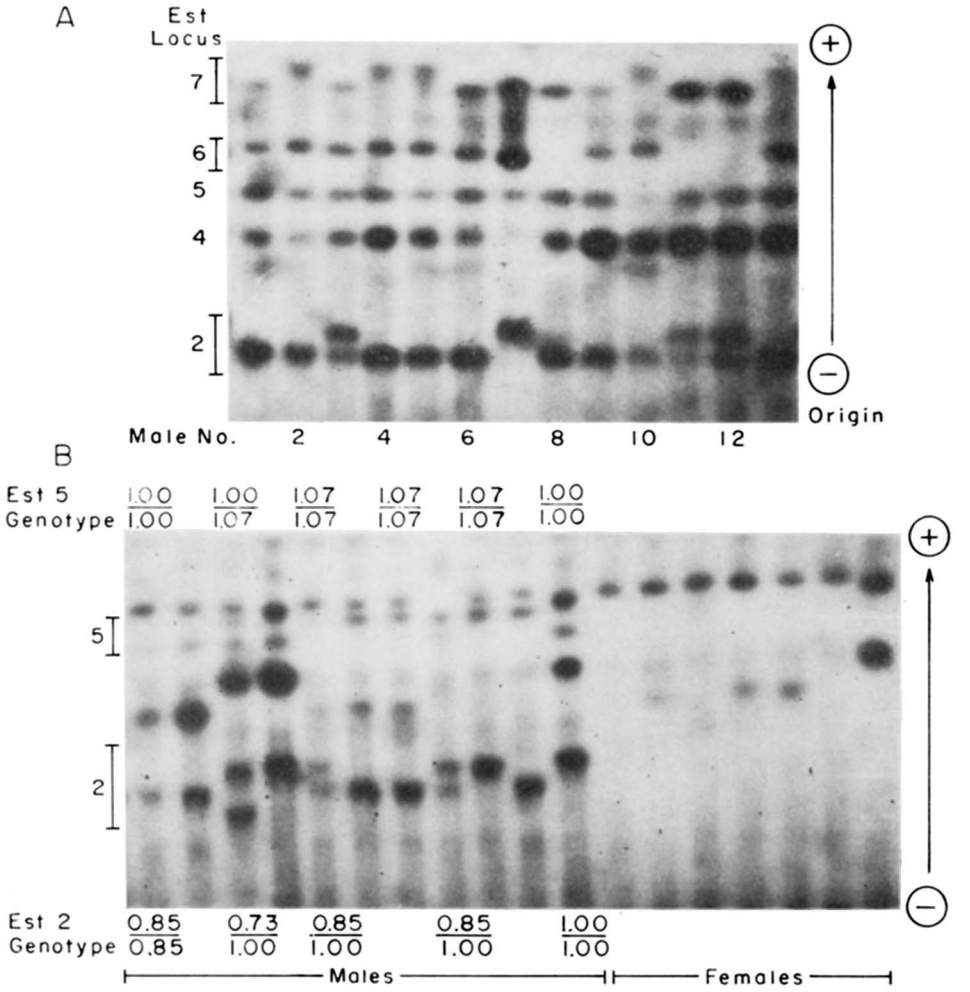

FIGURE 2.—A. Esterase zymogram of adult male *D. paulistorum*. The regions of esterase variability and the esterase locus controlling each region are indicated to the left of the zymogram. B. Esterase zymogram of both sexes. The genotypes of individuals for the *Est-2* and *Est-5* loci are given.

Each gel run contained a minimum of three control flies (1 ♀, 2 ♂ ♂). The control is a strain of the Amazonian semispecies collected in Perija, Venezuela and is homozygous for a single allele at each of the loci scored. Allozyme variants were named in reference to the control allozyme which is assigned a mobility of 1.00. The mobility of an allozyme different from the control is determined as a relative mobility of the control allozyme. When two or more loci with identical substrate specificities are found, each locus is numbered in ascending order according to the distance of migration from the origin. The nomenclature used here follows that of PRAKASH, LEWONTIN and HUBBY (1969) and others. Each isozyme has been given an abbreviation listed in Table 1. Multiple isozyme loci are indicated by a hyphenated numeral following the abbreviation, *i.e.*, *Lap-4*, *Lap-5*. Allozymes of a specific isozyme locus are indicated as a superscript to the locus notation. Thus *Lap-4*$^{1.00}$ and *Lap-4*$^{1.09}$ are two alleles at the *Lap-4* locus.

RESULTS

The eleven enzyme systems listed in Table 1 are coded for by a minimum of 17 loci. Figures 2 and 3 show variation at some esterase loci and the α-glycerophosphate dehydrogenase locus. The genetic basis for variation of 12 of the loci have been described in detail (RICHMOND 1971). Phenotypic variation at all loci studied is controlled by codominant alleles. Homozygous individuals show a single band of enzyme activity whereas heterozygotes exhibit either two or three bands of activity. Silent or null alleles which result in no detectable enzyme activity have been found at two loci—*Est-6* and *Est-7*. The genetic bases of five of these systems have not been worked out either because no electrophoretic variants were detected (*Lap-6*, *Me*), or the system was developed too late in the study to carry out genetic tests (*Odh*, *Pgm*, *Tpi*). These loci are included because their modes of inheritance are known in other closely related Drosophila species (AYALA *et al.* 1971b) and can be extrapolated to fit the phenotypes observed in *D. paulistorum*. In any event, the major portion of these investigations is based on data gathered from loci which have been characterized genetically for *D. paulistorum*. The known linkage relationships and other characteristics of the loci are given in the headings to Tables 2–17.

The calculation of gene frequencies in each of the populations studied was performed in two ways depending upon the type of material analyzed. When wild-collected individuals were available, two F_1 progeny (1 female, 1 male) of each wild female were studied. Wild males were generally not studied since there are no diagnostic, morphological differences between semispecies (PASTEUR 1970)

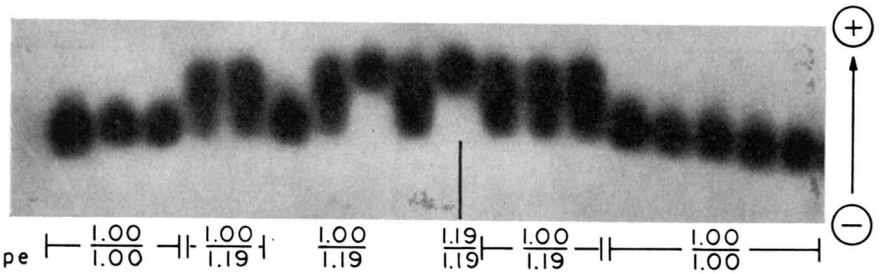

FIGURE 3.—α-Glycerophosphate dehydrogenase zymogram of *D. paulistorum* adults. Individuals homozygous and heterozygous for two alleles are shown.

TABLE 1

Conditions used for the electrophoresis of the enzyme systems analyzed

Enzyme systems	Abbreviation	Starch*	Buffers† Electrode	Gel	Running‡ potential	Running time (hours)
Hydrolases and lyases						
Acid phosphatase	Acph	Sigma	1A	IB	6–12 v/cm	3–5§
Esterases	Est	Sigma	1A	IB	6–12 v/cm	3–5
Leucine aminopeptidase	Lap	Sigma	1A	IB	6–12 v/cm	3–5
Oxidases						
Tetrazolium oxidase	To	Electro-188	2	2	12–25 v/cm	6–7
Dehydrogenases						
Malate dehydrogenase-NAD	Mdh	Electro-188	2	2	12–25 v/cm	6–7
Malate dehydrogenase-NADP	Me	Electro-188	2	2	12–25 v/cm	6–7
Alcohol dehydrogenase	Adh	Electro-188	2	2	12–25 v/cm	6–7
Octanol dehydrogenase	Odh	Electro-188	2	2	12–25 v/cm	6–7
α-glycerophosphate dehydrogenase	αGpdh	Electro-188	2	2	12–25 v/cm	6–7
Transferases						
Phosphoglucomutase	Pgm	Electro-188	3A	3B	6–10 v/cm	4–6
Isomerases						
Triosephosphate isomerase	Tpi	Electro-188	3A	3B	6–10 v/cm	4–6

* Sigma = Sigma Chemial Co., Hydrolyzed starch for electrophoresis; Electro-188 = Otto Hiller Co., Electro-starch Lot No. 188.

† 1A = 0.30 M boric acid, 0.05 M sodium hydroxide; pH 8.2. 1B = 0.076 M Tris-(hydroxymethyl)-aminomethane (Tris), 0.005 M citric acid; pH 8.65 (POULIK 1957). 2 = 0.087 M Tris, 0.0087 M boric acid, 2.5 mM ethylenediaminetetracetic acid (EDTA); pH 8.9. 3A = 0.135 M Tris, 0.043 M citrate; pH 7.0 (SHAW and KOEN 1968). 3B = 0.009 M Tris, 0.0029 M citrate; pH 7.0 (SHAW and KOEN 1968).

‡ The power supplies (Heathkit IP-17) were adjusted to a constant current of 50 ma. per gel until the upper limit of the potential gradient was reached.

§ The runs for hydrolases and lyases were terminated when the visible buffer front (POULIK 1957) had migrated 8.0 cm from the origin.

and in many localities two or three semispecies are sympatric. On the average, the analysis of two progeny of wild females provides information about three of the possible four genomes carried by the female. If both individuals analyzed were homozygous for one allele, the wild female was assumed to have carried three genomes having that allele. If two alleles were detected in the two flies analyzed, 1.5 genomes were assigned to each allele. If three alleles were present, each allele was assigned one genome and similarly for those rare cases in which both flies were heterozygous each for two different alleles.

The number of individuals analyzed from stocks which had been kept in culture beyond one generation removed from a wild population was determined as a function of the maximum number of wild genomes assumed to be present within the stock. Whenever possible one individual of each sex has been assayed for every wild genome thought to be present within a stock. The number of genomes in each such stock was assumed to be twice the number of isofemale strains used to initiate the stock. Since each isofemale strain initially contained four wild genomes, this calculation assumes the loss of 50% of the genomes originally

TABLE 2

Allele frequencies at the Est-2 *locus in* D. paulistorum *chromosome II, male-limited*

Semi-species	Locality	Genomes sampled	Allele 0.73	0.85	1.00	1.17	Proportion of heterozygous individuals
C	Lab Stocks	30	..	1.00	..	..	0 (1)*
T	Malaga Bay, Col.	94	..	.99	.01	..	.020
T	Rancho Grande, Ven.	196	.02	.88	.10	..	.215
T	Guatopo, Ven.	14	.07	.64	.29	..	.501
T	Lab Stocks	98	.02	.97	.01	..	.070(1)
AB	Mirassol, Brazil	262	..	.86	.14	..	.241
AB	Leticia, Col.	83	..	.91	.09	..	.164
AB	Valparaiso, Col.	42	..	.76	.19	.05	.384
AB	Pt. Ayacucho, Ven.	26	..	.89	.12	..	.214
AB	Lab Stocks	295	..	.89	.10	.01	.180(5)
O	Lab Stocks	109	.05	.42	.53	..	.390(4)
I	Mitu, Col.	102	.01	.37	.57	.05	.536
I	Valparaiso, Col.	92	..	.37	.60	.03	.502
I	Leticia, Col.	136	..	.36	.61	.03	.497
I	Pt. Ayacucho, Ven.	10	..	.40	.60	..	.480
I	Ocamo, Ven.	38	..	.47	.53	..	.498
I	Tapuruquara, Brazil	323	.01	.47	.51	.01	.519
I	Tefe, Brazil	46	..	.57	.43	..	.490
A	Teresita, Col.	28	..	.07	.82	.11	.311
A	Mitu, Col.	130	..	.23	.70	.07	.452
A	Rancho Grande, Ven.	15	..	.13	.87	..	.226
A	Guatopo, Ven.	276	..	.04	.71	.25	.432
A	Pt. Ayacucho, Ven.	18	..	.33	.67	..	.442
A	Arima Valley, Trin.	506	..	.01	.70	.29	0.426
A	Mora Forest, Trin.	22	..	..	.77	.23	.354
A	Tapuruquara, Brazil	308	..	.14	.75	.11	.406
A	Manaus, Brazil	60	..	.23	.62	.15	.540
A	Santarem, Brazil	14	..	..	1.00	..	0
A	Belem, Brazil	346	+	.23	.67	.10	.488
A	Lab Stocks	190	..	.06	.92	.02	.187(6)

* Figure in parentheses indicates the number of separate stock cultures used to estimate heterozygosity. See text for further details.

present in any stock due to sampling errors and selection in laboratory cultures. Nevertheless, this is probably an overestimate of the number of genomes present in each population considering the severe reductions in population size which laboratory cultures often suffer. Allele frequencies in these cultures were calculated by totaling the number of genomes per allele in the analyzed individuals and then correcting the total number of genomes analyzed to that assumed to be present in the stock.

Material from both laboratory cultures and wild-collected females was available for the Transitional, Andean–Brazilian, and Amazonian semispecies. The Interior semispecies samples are all from freshly collected material. Only labora-

TABLE 3

Allele frequencies at the Est-4 *locus in* D. paulistorum *chromosome II*

Semi-species	Locality	Genomes sampled	Allele						Proportion of heterozygous individuals
			0.77	*0.85*	*0.89*	*1.00*	*1.05*	*1.10*	
C	Lab Stocks	30	..	.48	..	.53	..	..	.304(1)
T	Malaga Bay, Col.	88	..	1.00	..	..	..	..	0
T	Rancho Grande, Ven.	138	.12	.83	.03	.03	..	..	.315
T	Guatopo, Ven.	21	.21	.50	..	.29	..	..	.622
T	Lab Stocks	108	.09	.91	..	+	..	..	.152(1)
AB	Mirassol, Brazil	399	+	.31	..	.69	..	..	.430
AB	Leticia, Col.	68	..	.28	..	.72	..	..	.403
AB	Valparaiso, Col.	16	..	.38	.45	.17	..	..	.624
AB	Ocamo, Ven.	45	.04	.46	..	.50	..	..	.537
AB	Lab Stocks	249	.09	.33	.10	.48	..	..	.180(5)
O	Lab Stocks	110	..	..	..	.86	.14	..	.241(4)
I	Mitu, Col.	100	..	..	..	1.00	..	..	0
I	Valparaiso, Col.	110	..	..	..	1.00	..	..	0
I	Leticia, Col.	19	..	..	..	1.00	..	..	0
I	Tapuruquara, Brazil	241	..	.02	..	.98	..	..	.039
I	Tefe, Brazil	75	..	..	..	1.00	..	..	0
A	Teresita, Col.	16	..	..	..	1.00	..	..	0
A	Guatopo, Col.	212	..	..	..	1.00	..	..	0
A	Tapuruquara, Brazil	138	..	.06	..	.94	..	..	.113
A	Santarem, Brazil	24	..	.06	..	.94	..	..	.113
A	Belem, Brazil	465	..	.04	..	.96	..	..	.077
A	Lab Stocks	175	+	.01	..	.99	+	..	.026(5)

tory stocks were used for the analysis of gene frequencies in the Centroamerican and Orinocan semispecies. Tables 2–17 give allele frequencies in newly collected material and laboratory stocks for 16 of the 17 loci studied. The semispecies abbreviation, sample locality, number of genomes sampled and the proportion of individuals expected to be heterozygous assuming Hardy-Weinberg equilibrium are also included in each table. Samples of new material of 10 or more genomes have been included as a separate entry in these tables. All laboratory stocks and small samples (<10 genomes) of new material have been combined under the entries "Lab stocks." Allele frequencies in lab stock samples are averages weighted for the total number of genomes present in each stock culture of that semispecies. Alleles having a frequency of less than 0.005 have been recorded in the tables as a "+." The proportion of heterozygous individuals in lab stocks is calculated as an unweighted average of the heterozygosities of each stock culture which contained a minimum of 10 genomes. The number of such cultures used to estimate the average heterozygosity is given in parentheses following the value for each lab stock entry. Allele frequencies at the *Lap-6* locus are not included in these tables since only a single allele was found among 394 genomes of the three semispecies (C, AB, I) surveyed. Eleven loci (*Est-2*, *Est-4*, *Est-5*, *Est-6*, *Est-7*, *Lap-4*, *Lap-5*, *Acph*, *Adh*, *αGpdh*, *To*) were studied in all semispecies.

TABLE 4

Allele frequencies at the Est-5 *locus in* D. paulistorum *chromosome II, male-limited*

Semi-species	Locality	Genomes sampled	Allele				Proportion of heterozygous individuals
			0.90	*1.00*	*1.07*	*1.14*	
C	Lab Stocks	30	..	.81	.19	..	0 (1)
T	Malaga Bay, Col.	106	.03	.75	.22	.01	.408
T	Rancho Grande, Ven.	178	.01	.43	.43	.13	.613
T	Guatopo, Ven.	23	..	.64	.36	..	.461
T	Lab Stocks	99	.12	.80	.08	+	.341 (1)
AB	Mirassol, Brazil	254	.02	.89	.09	..	.199
AB	Leticia, Col.	70	..	.93	.07	..	.130
AB	Valparaiso, Col.	32	.09	.88	.03	..	.217
AB	Pt. Ayacucho, Ven.	26	..	1.00	..	..	0
AB	Lab Stocks	266	.01	.97	.02	..	.114 (6)
O	Lab Stocks	116	..	.97	.03	..	.021 (4)
I	Mitu, Col.	90	.02	.77	.21	..	.363
I	Valparaiso, Col.	82	.01	.83	.16	..	.285
I	Leticia, Col.	128	.02	.81	.17	..	.315
I	Pt. Ayacucho, Ven.	10	..	.90	.10	..	.180
I	Ocamo, Ven.	42	..	.86	.14	..	.241
I	Tapuruquara, Brazil	326	.02	.79	.20	..	.356
I	Tefe, Brazil	50	..	.78	.22	..	.343
A	Teresita, Col.	28	..	.89	.11	..	.196
A	Mitu, Col.	122	.03	.86	.11	..	.247
A	Guatopo, Ven.	250	.04	.94	.02	..	.114
A	Pt. Ayacucho, Ven.	16	.19	.81	..	..	.308
A	Arima Valley, Trin.	490	.13	.87	..	..	.226
A	Mora Forest, Trin.	18	.06	.94	..	..	.113
A	Tapuruquara, Brazil	288	.01	.92	.07	.01	.169
A	Manaus, Brazil	60	.03	.88	.08	..	.198
A	Santarem, Brazil	12	..	1.00	..	..	0
A	Belem, Brazil	316	.01	.93	.06	..	.131
A	Lab Stocks	183	+	.99	.01	..	.025 (6)

Mdh was studied in all but the Orinocan semispecies, and *Tpi* in all but the Centroamerican and Orinocan semispecies. *Odh*, *Pgm* and *Me* loci were studied in two semispecies each (Tables 15–17). The latter five loci were not studied in all semispecies due to restrictions in time and the expense of assay materials.

The patterns of allelic variation over the 17 loci studied in *D. paulistorum* can be divided into three classes based upon the extent of polymorphism in the semispecies surveyed. A locus is considered to be polymorphic in a semispecies if at least two alleles have a frequency of 5% or greater (KOJIMA, GILLESPIE and TOBARI 1970). (1) Five loci (*Est-2*, *Est-5*, *Est-6*, *Est-7*, *Lap-4*) are polymorphic in five or more semispecies. (2) Six loci (*Est-4*, *Lap-5*, *Acph*, *To*, *Me*, *Odh*) are polymorphic in at least one but less than five semispecies. (3) Six loci (*Lap-6*, *Adh*, *Mdh*, *αGpdh*, *Tpi*, *Pgm*) are monomorphic in all semispecies studied.

If a locus is polymorphic in *D. paulistorum*, the data of Tables 2–17 show that

TABLE 5

Allele frequencies at the Est-6 *locus in* D. paulistorum X *chromosome*

Semi-species	Locality	Genomes sampled	Allene							Proportion of heterozygous females
			Null	*0.93*	*0.95*	*0.97*	*1.00*	*1.02*	*1.03*	
C	Lab Stocks	30	.60	..	..	..	..	.40	..	0 (1)
T	Malaga Bay, Col.	47	..	..	..	..	.13	.87	..	.226
T	Rancho Grande, Ven.	37	..	..	.03	..	..	.81	.16	.317
T	Lab Stocks	45	.07	..	..	.10	.04	.74	.05	.350(1)
AB	Mirassol, Brazil	137	.03	..	..	..	.04	.58	.36	.552
AB	Leticia, Col.	36	..	..	..	..	..	.67	.33	.442
AB	Valparaiso, Col.	18	..	..	..	..	.11	.64	.25	.516
AB	Lab Stocks	147	.10	..	..	.01	.08	.52	.29	.339(6)
O	Lab Stocks	62	.27	..	.02	.24	.15	.30	.03	.625(1)
I	Mitu, Col.	21	.24	..	..	..	.14	.52	.09	.624
I	Valparaiso, Col.	21	.10	..	..	..	.10	.81	..	.344
I	Leticia, Col.	31	.10	..	.10	..	..	.65	.16	.552
I	Tapuruquara, Brazil	130	.18	..	.01	.02	.11	.64	.05	.563
I	Tefe, Brazil	25	.12	..	..	..	..	.68	.20	.483
A	Teresita, Col.	24	..	..	..	.33	..	.67	..	.442
A	Mitu, Col.	41	.24	..	.02	.07	.44	.17	.05	.692
A	Guatopo, Ven.	79	.06	..	..	.10	.80	.04	..	.345
A	Arima Valley, Trin.	130	.12	.02	.39	.05	.42	..	..	.652
A	Tapuruquara, Brazil	140	.15	..	.05	.13	.42	.25	..	.719
A	Manaus, Brazil	33	.27	..	.03	.12	.42	.15	..	.693
A	Belem, Brazil	143	.13	.01	.01	.25	.50	.07	.02	.645
A	Lab Stocks	149	.17	.01	.04	.06	.69	.03	.01	.189(5)

a single allele is usually predominant in all populations of a semispecies studied. This is true with a very few exceptions for all polymorphic loci in classes 1 and 2 except for *Lap-4* (Table 7). It is not, however, the case that a single allele is most common in all populations of *D. paulistorum* for all polymorphic loci. The C, T, and AB semispecies are similar in that a single allele, *Est-2*$^{0.85}$, is the most frequent allele found in populations of these semispecies at this locus (Table 2). However the O, I, and A semispecies are characterized by having *Est-2*$^{1.00}$, as their most common allele. Both the *Est-4* locus (Table 3) and the *Est-6* locus (Table 5) fit this pattern of having two predominant alleles one of which is the most frequent allele in some semispecies and the second allele the most frequent in the other semispecies. At the *Est-4 locus* (Table 3), allele, *Est-4*$^{0.85}$, *is the* most frequent allele in populations of the C and T semispecies, but allele, *Est-4*$^{1.00}$, is the most frequent allele in populations of the AB, O, I and A semispecies. At the *Est-6* locus (Table 5) all of the semispeces but the Amazonian have a single most common allele. The *Lap-4* locus (Table 7) is polymorphic in all the semispecies. With the exception of the populations of the A semispecies which all have a single most frequent allele, *Lap-4*$^{1.09}$, variation at this locus does not appear to follow any systematic pattern.

TABLE 6

Allele frequencies at the Est-7 *locus in* D. paulistorum X *chromosome*

Semi-species	Locality	Genomes sampled	Allele						Proportion of heterozygous females
			Null	*0.93*	*0.96*	*0.97*	*1.00*	*1.02*	
C	Lab Stocks	30	.07	..	..	..	.87	.07	.278(1)
T	Malaga Bay, Col.	48	..	..	.13	..	.88	..	.219
T	Rancho Grande, Ven.	37	.03	..	..	..	.81	.16	.317
T	Lab Stocks	45	.08	..	.04	.03	.80	.05	.350(1)
AB	Mirassol, Brazil	135	.02	..	.02	..	.62	.36	.486
AB	Leticia, Col.	36	..	..	..	..	.67	.33	.442
AB	Valparaiso, Col.	18	..	..	..	..	.75	.25	.375
AB	Lab Stocks	147	.05	..	.01	..	.61	.33	.169(5)
O	Lab Stocks	61	..	.02	.50	..	.45	.03	.420(1)
I	Mitu, Col.	28	.14	..	..	..	.82	.04	.306
I	Valparaiso, Col.	19	..	..	.11	..	.90	..	.188
I	Leticia, Col.	33	.12	..	..	..	.67	.21	.493
I	Tapuruquara, Brazil	84	.21	..	.04	..	.66	.10	.520
I	Tefe, Brazil	25	.24	..	..	..	.68	.08	.474
A	Teresita, Col.	24	..	..	.33	..	.67	..	.442
A	Mitu, Col.	45	.11	..	.11	..	.71	.07	.467
A	Guatopo, Ven.	46	.07	..	.07	..	.87	..	.235
A	Arima Valley, Trin.	89	.51	..	.07	..	.43	..	.560
A	Tapuruquara, Brazil	132	.15	..	.14	.01	.58	.12	.607
A	Manaus, Brazil	29	.24	..	.07	..	.52	.17	.638
A	Belem, Brazil	120	.24	..	.08	..	.63	.05	.600
A	Lab Stocks	152	.08	.02	.09	..	.80	.01	.251(5)

At the remainder of the loci falling in classes 1 and 2, a single allele is predominant in all populations of the species regardless of semispecific barriers.

There is evidence for differentiation of gene frequencies both between semispecies and among the populations of single semispecies. As discussed above, the *Est-2*, *Est-4* and *Est-6* loci (Tables 2,3,5) show gene frequencies which differ between semispecies or groups of semispecies. In addition, the *Est-2* and *Est-6* loci give evidence of differentiation of gene frequencies between populations within semispecies. In the T semispecies, allele, *Est-2*$^{0.85}$, is found in high frequency (99%) in Malaga Bay along the west coast of Colombia. The frequency of this allele decreases to 88 and 64% respectively along the northern coast of Venezuela in Rancho Grande and Guatopo (Table 2). In the I semispecies this same allele varies in frequency from 36–37% in the samples from southern Colombia to 57% in the sample from Tefe on the Amazon River in western Brazil. Similar variation in allele frequencies within semispecies can be found at a number of other loci. While the range of frequencies is not so great as that at the *Est-2* locus, evidence for differentiation of gene frequencies can be found at the *Est-5*, *Est-6*, *Est-7*, *Lap-5* and *To* loci (Tables 4, 5, 6, 8, 14). Only the Ab semispecies shows a substantial degree of polymorphism at the *To* locus, but the frequency of allele

TABLE 7

Allele frequencies at the Lap-4 *locus in* D. paulistorum *unmapped, only females variable*

Semi-species	Locality	Genomes sampled	Allele		Proportion of heterozygous individuals
			1.00	*1.09*	
C	Lab Stocks	18	.29	.71	.459(1)
T	Malaga Bay, Col.	42	.14	.86	.241
T	Rancho Grande, Ven.	26	.81	.19	.308
T	Lab Stocks	32	.75	.25	0 (1)
AB	Mirassol, Brazil	100	.46	.54	.497
AB	Leticia, Col.	52	.62	.39	.484
AB	Valparaiso, Col.	36	.34	.66	.449
AB	Lab Stocks	106	.69	.31	.362(3)
O	Lab Stocks	54	.29	.71	.280(2)
I	Mitu, Col.	56	.77	.23	.354
I	Valparaiso, Col.	62	.68	.32	.435
I	Leticia, Col.	56	.87	.13	.226
I	Tapuruquara, Brazil	110	.15	.85	.255
I	Tefe, Brazil	38	.13	.87	.226
A	Teresita, Col.	18	..	1.00	0
A	Rancho Grande, Ven.	12	.33	.67	.442
A	Guatopo, Ven.	104	.26	.74	.385
A	Arima Valley, Trin.	36	.11	.89	.196
A	Tapuruquara, Brazil	124	.22	.78	.343
A	Manaus, Brazil	64	.14	.86	.241
A	Santarem, Brazil	14	.14	.86	.241
A	Belem, Brazil	134	.38	.62	.471
A	Lab Stocks	64	.42	.58	.498(1)

$To^{1.00}$ varies from approximately 60% in populations from southern Brazil and Colombia to 88% in an south, central Colombia population.

Among the six loci falling into class 3 (Monomorphic loci) only a single locus, *Lap-6*, failed to yield more than one allozyme variant. At each of the remaining five loci a minimum of two to six alleles were found. However, the majority of populations sampled for these loci are fixed for a single allele (Tables 10, 11, 12, 14, 17). Occasional samples do occur which suggest that some of these loci may be polymorphic in some populations. Bearing in mind the rather small sample of 24 genomes, some variant alleles from a population in Mora Forest, on the island of Trinidad reach a frequency of 2–13% at the *Adh*, *Mdh*, *aGpdh*, and *Tpi* loci. Although the general pattern of monomorphism at these loci is clear, it is of interest to note that the majority of allelic variants detected in class 3 loci have been found in populations of the A semispecies.

Average individual heterozygosities for the loci studied are highest among the *Est* and *Lap* loci (Table 18). At the *Est-6* locus, 50% or more of the individuals in a population are expected to be heterozygous. Heterozygosities among the class 2 and 3 loci are low and rarely exceed 12% with the exception of the *Lap-4* and *Est-4* loci.

TABLE 8

Allele frequencies at the Lap-5 *locus in* D. paulistorum *autosomal*

Semi-species	Locality	Genomes sampled	Allele								Proportion of heterozygous individuals
			0.80	*0.92*	*0.95*	*0.97*	*0.98*	*0.99*	*1.00*	*1.02*	
C	Lab Stocks	30	..	..	..	..	..	..	1.00	..	0 (1)
T	Malaga Bay, Col.	132	..	..	..	.01	.21	.06	.69	.03	.475
T	Rancho Grande, Ven.	108	..	..	..	.18	..	..	.82	..	.295
T	Guatopo, Ven.	10	..	..	..	..	.20	.10	.70	..	.460
T	Lab Stocks	108	..	..	..	.33	..	..	.67	..	.444(1)
AB	Mirassol, Brazil	336	..	..	..	..	..	..	1.00	..	0
AB	Leticia, Col.	66	..	..	..	..	..	..	1.00	..	0
AB	Valparaiso, Col.	36	..	..	..	..	..	..	1.00	..	0
AB	Lab Stocks	249	..	..	..	..	..	..	1.00	+	0 (5)
O	Lab Stocks	108	..	..	.03	..	.55	.09	.30	.04	.622(3)
I	Mitu, Col.	56	..	.04	..	..	..	..	.93	.04	.113
I	Valparaiso, Col.	64	..	.13	.03	..	..	..	.84	..	.277
I	Leticia, Col.	50	..	..	..	..	..	..	1.00	..	0
I	Tapuruquara, Brazil	426	..	..	..	..	..	+	.95	.04	.088
I	Tefe, Brazil	75	..	..	..	..	..	..	.74	.26	.385
A	Teresita, Col.	30	..	..	..	..	.03	..	.97	..	.058
A	Rancho Grande, Ven.	12	..	..	..	..	.17	..	.83	..	.282
A	Guatopo, Ven.	408	.09	.07	..	..	..	..	.79	.05	.360
A	Tapuruquara, Brazil	249	..	..	..	..	.05	..	.85	.10	.265
A	Manaus, Brazil	30	..	..	..	..	.10	..	.90	..	.180
A	Belem, Brazil	147	..	..	..	..	.03	..	.92	.06	.159
A	Lab Stocks	166	..	..	..	..	.09	..	.79	.12	.300(4)

TABLE 9

Allele frequencies at the Acph *locus in* D. paulistorum *autosomal*

Semi-species	Locality	Genomes sampled	Allele						Proportion of heterozygous individuals
			0.91	*0.94*	*1.00*	*1.04*	*1.08*	*1.24*	
C	Lab Stocks	30	..	..	1.00	..	..	..	0 (1)
T	Malaga Bay, Col.	168	..	.07	.78	.04	..	.11	.373
T	Rancho Grande, Ven.	108	..	..	1.00	..	..	..	0
T	Lab Stocks	108	..	..	.95	.05	..	..	.116(1)
AB	Mirassol, Brazil	237	..	.04	.74	.22	..	..	.403
AB	Leticia, Col.	68	..	..	.98	..	.02	..	.039
AB	Valparaiso, Col.	36	..	..	1.00	..	..	..	0
AB	Lab Stocks	277	..	.08	.89	.02	+	..	.115(5)
O	Lab Stocks	110	..	..	.99	.01	..	..	.073(3)
I	Valparaiso, Col.	28	..	.02	.98	..	..	..	.039
I	Leticia, Col.	16	..	..	1.00	..	..	..	0
A	Teresita, Col.	30	..	.09	.89	.02	..	..	.199
A	Guatopo, Ven.	140	..	.10	.89	.01	..	..	.198
A	Lab Stocks	170	.07	..	.93	..	..	..	.067(5)

TABLE 10

Allele frequencies at the Adh *locus in* D. paulistorum *chromosome II*

Semi-species	Locality	Genomes sampled	Allele				Proportion of heterozygous individuals
			—0.39	*1.00*	*1.12*	*2.81*	
C	Lab Stocks	30	..	1.00	..	..	0 (1)
T	Malaga Bay, Col.	135	..	1.00	..	..	0 (1)
T	Guatopo, Ven.	14	..	1.00	..	..	0
T	Lab Stocks	94	..	1.00	..	..	0
AB	Mirassol, Brazil	420	..	1.00	..	..	0
AB	Leticia, Col.	12	..	1.00	..	..	0
AB	Valparaiso, Col.	36	..	1.00	..	..	0
AB	Ayacucho, Ven.	45	..	1.00	..	..	0
AB	Lab Stocks	142	..	1.00	..	..	0 (4)
O	Lab Stocks	68	..	1.00	..	..	..
I	Mitu, Col.	90	.03	.97	..	..	.058
I	Valparaiso, Col.	110	..	1.00	..	..	0
I	Leticia, Col.	118	..	.98	.02	..	.039
I	Tapuruquara, Brazil	552	+	.99	..	.01	.028
I	Tefe, Brazil	75	.02	.96	..	.02	.078
A	Teresita, Col.	30	..	1.00	..	..	0
A	Guatopo, Ven.	474	..	1.00	..	+	.004
A	Arima Valley, Trin.	312	.01	.99	..	..	.020
A	Mora Forest, Trin.	24	.04	.96	..	..	.077
A	Tapuruquara, Brazil	498	+	.99	..	+	.006
A	Manaus, Brazil	75	..	1.00	..	..	0
A	Santarem, Brazil	24	..	1.00	..	..	0
A	Belem, Brazil	541	..	.99	..	.01	.020
A	Lab Stocks	84	..	1.00	..	..	0 (3)

In summary enzyme variation in *D. paulistorum* falls into three categories: loci which are polymorphic in all semispecies, loci which are polymorphic in at least one but less than six semispecies, loci which are monomorphic in all semispecies. The polymorphic loci can be divided into two subclasses: loci with a single allele predominant in all populations of the species, loci with two predominant alleles each in different groups of semispecies. At least six of the loci which are polymorphic in one or more semispecies provide evidence for differentiation of gene frequencies between populations of a semispecies.

DISCUSSION

The data of Tables 2–17 have been summarized in Tables 18 and 19. Table 18 records for each of the 17 loci studied the total number of genomes sampled, the number of samples, the proportion of individuals expected to be heterozygous at each locus, and the standard error of the estimate of heterozygosity. Laboratory stock samples have been counted as a single sample for this analysis. Average proportions of heterozygosity in lab stock samples have been calculated as an

TABLE 11

Allele frequencies at the Mdh *locus in* D. paulistorum *chromosome III*

Semi-species	Locality	Genomes sampled	Allele				Proportion of heterozygous individuals
			0.46	*0.63*	*1.00*	*1.22*	
C	Lab Stocks	26	..	..	1.00	..	..
T	Malaga Bay, Col.	135	..	..	1.00	..	0
T	Guatopo, Ven.	14	..	..	1.00	..	0
AB	Mirassol, Brazil	408	..	..	1.00	..	0
AB	Leticia, Col.	46	..	..	1.00	..	0
AB	Valparaiso, Col.	36	..	..	1.00	..	0
AB	Ayacucho, Ven.	44	..	..	1.00	..	0
AB	Lab Stocks	42	..	..	1.00	..	0
I	Mitu, Col.	90	..	..	1.00	..	0
I	Valparaiso, Col.	110	..	..	1.00	..	0
I	Leticia, Col.	118	..	..	1.00	..	0
I	Tapuruquara, Brazil	552	..	..	1.00	+	.006
I	Tefe, Brazil	75	..	..	1.00	..	0
A	Teresita, Col.	30	..	..	1.00	..	0
A	Guatopo, Ven.	474	..	..	1.00	..	0
A	Arima Valley, Trin.	292	..	+	.99	.01	.020
A	Mora Forest, Trin.	24	..	..	.96	.04	.077
A	Tapuruquara, Brazil	498	..	..	.99	.01	.020
A	Manaus, Brazil	108	.01	..	.99	..	.020
A	Santarem, Brazil	24	..	..	1.00	..	0
A	Belem, Brazil	539	..	..	.99	.01	.020

unweighted average of the heterozygosities of each stock culture having a minimum of 10 genomes. Since two criteria for polymorphic loci are often found in the literature (AYALA *et al.* 1971b), two proportions of polymorphic loci are given in Table 18. The "1% Criterion" gives the proportion of samples which have at least two alleles, each having a frequency of 0.01 or greater, and the "5% Criterion" gives the proportion of samples which have at least two alleles each having a frequency of 0.05 or greater. The total number of genomes and samples studied, the proportion of all samples which were polymorphic, the unweighted average proportion of heterozygous individuals in all samples, and the standard error of this latter estimate are given in the last row of entries for each locus.

Table 19 provides a summary of genetic variability in the semispecies of *D. paulistorum*. The table lists the number of loci studied in each semispecies, the average number of genomes analyzed per locus, the proportion of polymorphic loci in each species using both criteria discussed above, and the average portion of an individual's genome expected to be heterozygous averaging over all samples in the semispecies and averaging over freshly collected samples. The means and standard errors of these estimates for *D. paulistorum* as a whole are given in the last two rows.

LEWONTIN and HUBBY (1966; see also PRAKASH *et al.* 1969) initially raised the question of the degree of polymorphism to be expected at enzyme loci in

TABLE 12

Allele frequencies at the αGpdh *locus in* D. paulistorum *chromosome II*

Semi-species	Locality	Genomes sampled	Allele						Proportion of heterozygous individuals
			0.30	*0.64*	*0.77*	*1.00*	*1.09*	*1.19*	
C	Lab Stocks	30	..	..	..	1.00	..	..	0 (1)
T	Malaga Bay, Col.	135	..	.01	..	.97	.01	.01	.059
T	Guatopo, Ven.	14	..	..	..	1.00	..	..	0
T	Lab Stocks	90	..	..	..	1.00	..	..	0 (1)
AB	Mirassol, Brazil	420	..	..	..	1.00	..	..	0
AB	Leticia, Col.	12	..	..	..	1.00	..	..	0
AB	Valparaiso, Col.	36	..	..	..	1.00	..	..	0
AB	Ayacucho, Ven.	45	..	..	..	1.00	..	..	0
AB	Lab Stocks	145	..	..	..	1.00	..	..	0 (4)
O	Lab Stocks	68	..	..	..	1.00	..	..	..
I	Mitu, Col.	90	..	..	..	.99	..	.01	.020
I	Valparaiso, Col.	110	..	..	..	1.00	..	..	0
I	Leticia, Col.	118	..	..	..	1.00	..	..	0
I	Tapuruquara, Brazil	552	..	..	..	1.00	..	..	0
I	Tefe, Brazil	75	..	..	..	1.00	..	..	0
A	Teresita, Col.	30	..	..	..	1.00	..	..	0
A	Guatopo, Ven.	474	..	..	..	1.00	..	..	0
A	Arima Valley, Trin.	296	..	..	..	.99	..	.01	.020
A	Mora Forest, Trin.	24	..	..	..	.96	..	.04	.077
A	Tapuruquara, Brazil	498	+	+	..	.99	..	+	.018
A	Manaus, Brazil	108	..	..	..	1.00	..	..	0
A	Santarem, Brazil	24	..	..	..	1.00	..	..	0
A	Belem, Brazil	539	..	.01	.01	.99	..	..	.028
A	Lab Stocks	68	..	..	..	.99	.01	..	0 (3)

natural populations. Since this investigation, a number of estimates of the proportion of polymorphic loci and the proportion of an individuals genome expected to be heterozygous have been obtained (SELANDER *et al.* 1970, Table 3). Table 19 shows that an average population of *D. paulistorum* is polymorphic for some 55–67% of its loci depending upon the criterion of polymorphism used. These figures are substantially higher than the 42% obtained for *D. pseudoobscura* (PRAKASH *et al.* 1969) or the average of 40.3% obtained for four Drosophila species collected in the United States and Japan (KOJIMA *et al.* 1970). The figure for *D. paulistorum* is in agreement with the figure of 54–83% found for the closely related species, *D. willistoni* (AYALA *et al.* 1971b). F. J. AYALA (pers. comm.) reports that the proportion of polymorphic loci in two of the other sibling species of *willistoni* group is in agreement with that found in *D. paulistorum* and *willistoni*. The difference in the amounts of genetic variability found in the tropical *willistoni* group species compared to the temperate *obscura* group and *melanogaster* group species agrees well with information derived from studies of chromosomal inversion polymorphisms. *D. paulistorum* and *willistoni* are among the most chromosomally polymorphic Drosophila species known (DOBZHANSKY

TABLE 13

Allele frequencies at the To *locus in* D. paulistorum X *chromosome*

Semi-species	Locality	Genomes sampled	Allele 0.59	0.88	0.98	1.00	1.06	Proportion of heterozygous individuals
C	Lab Stocks	30	..	..	.13	.87	..	0 (1)
T	Malaga Bay, Col.	58	..	..	..	.94	.06	.113
T	Rancho Grande, Ven.	108	..	..	..	1.00	..	0
T	Lab Stocks	108	..	..	..	.99	.01	.022(1)
AB	Mirassol, Brazil	398	.41	..	..	.59	..	.484
AB	Leticia, Col.	34	.12	..	..	.88	..	.211
AB	Valparaiso, Col.	36	.38	..	..	.60	.02	.495
AB	Lab Stocks	257	.07	..	..	.88	.05	.134(5)
O	Lab Stocks	86	..	..	1.00	..	..	0 (2)
I	Mitu, Col.	70	..	.03	..	.97	..	.058
I	Valparaiso, Col.	88	..	..	..	1.00	..	0
I	Leticia, Col.	92	..	.01	..	.99	..	.020
I	Tapuruquara, Brazil	365	..	.01	..	.98	.01	.039
I	Tefe, Brazil	50	..	.04	..	.92	.04	.150
A	Teresita, Col.	24	..	..	..	1.00	..	0
A	Arima Valley, Trin.	292	..	..	..	1.00	..	0
A	Mora Forest, Trin.	24	..	..	..	1.00	..	0
A	Tapuruquara, Brazil	332	+	..	..	1.00	..	.006
A	Manaus, Brazil	72	.01	..	..	.99	..	.020
A	Santarem, Brazil	16	..	..	..	1.00	..	0
A	Belem, Brazil	318	..	..	..	1.00	..	0
A	Lab Stocks	171	..	..	..	1.00	..	0 (5)

TABLE 14

Allele frequencies at the Tpi *locus in* D. paulistorum *autosomal*

Semi-species	Locality	Genomes sampled	Allele 1.00	1.18	Proportion of heterozygous individuals
T	Malaga Bay, Col.	132	1.00	..	0
AB	Leticia, Col.	46	1.00	..	0
I	Leticia, Col.	140	1.00	..	0
A	Teresita, Col.	30	1.00	..	0
A	Guatopo, Ven.	68	1.00	..	0
A	Mora Forest, Trin.	24	.87	.13	.113

TABLE 15

Allele frequencies at the Odh *locus in* D. paulistorum *autosomal*

Semi-species	Locality	Genomes sampled	Alleles 0.89	1.00	1.01	Proportion of heterozygous individuals
T	Malaga Bay, Col.	32	.03	.97	..	.058
AB	Mirassol, Brazil	135	.08	.91	.01	.165

TABLE 16

Allele frequencies at the Pgm *locus in* D. paulistorum *unmapped*

Semi-species	Locality	Genomes sampled	Allele				Proportion of heterozygous individuals
			0.83	*0.86*	*1.00*	*1.59*	
T	Malaga Bay, Col.	135	.01	. .	.97	.01	.055
A	Guatopo, Ven.	68	.01	.01	.98	. .	.039

TABLE 17

Allele frequencies at the Me *locus in* D. paulistorum *unmapped*

Semi-species	Locality	Genomes sampled	Allele		Proportion of heterozygous individuals
			0.93	*1.00*	
AB	Leticia, Col.	46	.06	.94	.113
I	Leticia, Col.	140	. .	1.00	0

TABLE 18

Proportion of polymorphic samples and the average proportion of heterozygous individuals at each of 17 loci in the semispecies of D. paulistorum

Locus	Semi-species	Genomes sampled	Samples*	Proportion of polymorphic samples		Average proportion heterozygous individuals ± S.E.†
				1 percent criterion	5 percent criterion	
Est-2	C	30	1	0	0	———
	T	402	4	.75	1.00	.202 ± .108
	AB	708	5	1.00	1.00	.237 ± .039
	O	109	1	1.00	1.00	.390 ± .098
	I	747	7	1.00	1.00	.503 ± .007
	A	1,913	12	.92	.92	.355 ± .044
	TOTALS	3,909	30	.93	.87	.339 ± .032
Est-4	C	30	1	1.00	1.00	———
	T	355	4	.75	.75	.272 ± .133
	AB	777	5	1.00	1.00	.435 ± .075
	O	110	1	1.00	1.00	.241 ± .139
	I	545	5	.20	0	.008 ± .008
	A	1,030	6	.50	.33	.055 ± .022
	TOTALS	2,847	22	.64	.55	.189 ± .046
Est-5	C	30	1	.17	.17	———
	T	406	4	1.00	1.00	.456 ± .058
	AB	648	5	.80	.60	.132 ± .038
	O	116	1	1.00	0	.021 ± .014
	I	728	7	1.00	1.00	.298 ± .026
	A	1,783	11	.82	.82	.157 ± .028
	TOTALS	3,711	29	.70	.83	.218 ± .028

TABLE 18—Continued

Est-6	C	30	1	1.00	1.00	———
	T	129	3	1.00	1.00	.298±.037
	AB	338	4	1.00	1.00	.462±.047
	O	62	1	1.00	1.00	.625
	I	228	5	1.00	1.00	.513±.048
	A	739	8	1.00	1.00	.547±.070
	TOTALS	1,526	22	1.00	1.00	.469±.040
Est-7	C	30	1	1.00	1.00	———
	T	130	3	1.00	1.00	.295±.039
	AB	336	4	1.00	1.00	.368±.070
	O	61	1	1.00	1.00	.420
	I	189	5	1.00	1.00	.396±.064
	A	637	8	1.00	1.00	.475±.056
	TOTALS	1,383	22	1.00	1.00	.402±.030
Lap-4	C	18	1	1.00	1.00	———
	T	100	3	1.00	1.00	.183±.094
	AB	294	4	1.00	1.00	.448±.030
	O	54	1	1.00	1.00	.280±.061
	I	322	5	1.00	1.00	.299±.041
	A	540	9	.89	.89	.313±.053
	TOTALS	1,358	23	.96	.96	.321±.030
Lap-5	C	30	1	0	0	———
	T	358	4	1.00	1.00	.419±.042
	AB	687	4	0	0	0
	O	108	1	1.00	1.00	.622±.069
	I	671	5	.80	.80	.173±.070
	A	1,042	7	1.00	.86	.229±.039
	TOTALS	2,896	22	.73	.68	.217±.040
Lap-6	C	12	1	0	0	———
	AB	42	1	0	0	0
	I	336	3	0	0	0
	TOTALS	390	5	0	0	0
Acph	C	30	1	0	0	———
	T	384	3	.67	.67	.163±.110
	AB	618	4	.75	.50	.139±.091
	O	110	1	1.00	0	.073±.073
	I	44	2	.50	0	.020±.020
	A	340	3	1.00	1.00	.155±.044
	TOTALS	1,526	14	.71	.50	.116±.036
Adh	C	30	1	0	0	———
	T	243	3	0	0	0
	AB	655	5	0	0	0
	O	68	1	0	0	0
	I	945	5	.60	0	.041±.013
	A	2,062	9	.67	0	.014±.008
	TOTALS	4,003	24	.25	0	.014±.005

TABLE 18—Continued

Proportion of polymorphic samples and the average proportion of heterozygous individuals at each of 17 loci in the semispecies of D. paulistorum

Locus	Semi-species	Genomes sampled	Samples*	Proportion of polymorphic samples		Average proportion heterozygous individuals ± S.E.†
				1 percent criterion	5 percent criterion	
Odh	T	32	1	1.00	1.00	.058
	AB	135	1	1.00	1.00	.165
	TOTALS	167	2	1.00	1.00	.112 ± .054
Mdh	C	26	1	0	0	———
	T	149	2	0	0	0
	AB	576	5	0	0	0
	I	945	5	0	0	.001 ± .001
	A	1,989	8	.50	0	.020 ± .009
	TOTALS	3,685	21	.19	0	.005 ± .005
αGpdh	C	30	1	0	0	———
	T	239	3	.33	0	.020 ± .020
	AB	658	5	0	0	0
	O	68	1	0	0	———
	I	945	5	.20	0	.004 ± .004
	A	2,061	9	.33	0	.016 ± .009
	TOTALS	4,001	24	.21	0	.010 ± .005
To	C	30	1	1.00	1.00	———
	T	274	3	.67	.33	.045 ± .035
	AB	725	4	1.00	1.00	.331 ± .093
	O	86	1	0	0	———
	I	665	5	.80	.20	.053 ± .026
	A	1,249	8	.11	0	.003 ± .003
	TOTALS	3,029	22	.55	.27	.108 ± .075
Tpi	T	132	1	0	0	0
	AB	46	1	0	0	0
	I	140	1	0	0	0
	A	122	3	1.00	1.00	.113
	TOTALS	440	6	.17	.17	.028 ± .028
Pgm	T	135	1	1.00	0	.055
	A	68	1	1.00	0	.039
	TOTALS	203	2	1.00	0	.047 ± .008
Me	AB	46	1	1.00	1.00	.113
	I	140	1	0	0	0
	TOTALS	186	2	.50	.50	.057 ± .057

* Samples are the number of localities studied for each semispecies given in Tables 2–17. "Lab stocks" are considered to be a single sample.

† Standard error of the mean proportion of heterozygous individuals for samples of each semispecies given in Tables 2–17.

TABLE 19

Summary of genetic variability in the semispecies of D. paulistorum

Species	Number of loci	Mean Number of genomes per locus	Proportion* of loci polymorphic per		Proportion of genome heterozygous per individual	
			Semispecies	Population†	Semispecies	Population
C	13	27	.46(.46)	———	.08±.04	———
T	15	231	.73(.53)	.70(.61)	.16±.04	.24±.06
AB	16	456	.63(.56)	.64(.61)	.18±.05	.23±.03
O	11	87	.73(.55)	———	.30±.08	———
I	15	506	.60(.40)	.66(.50)	.15±.05	.19±.02
A	14	1,115	.93(.50)	.69(.49)	.18±.05	.18±.03
Mean	14	404	.68(.50)	.67(.55)	.18	.21
S.E.			.06(.03)	.01(.03)	.03	.02

* Figures in parentheses give proportions of polymorphic loci when at least two alleles have a frequency of 0.05 or greater. The first figure of each pair gives the proportion of polymorphic loci when at least two alleles have a frequency of 0.01 or greater.

† Only F_1 samples used.

1950). KASTRITSIS (1969) has described some 85 different inversions in *D. paulistorum* making it the most chromosomally polymorphic Drosophila species known. Many of the inversions in *paulistorum* were found in all the semispecies studied by KASTRITSIS. Whether or not these differences in the amounts of genetic variability found in tropical *vs.* temperate region species will hold true for other animal species must await the accumulation of more data. Nevertheless the information now available raises the intriguing possibility that the diversity of tropical species may be related to an increased amount of genetic variability in these forms.

The average portion of an individual's genome which is expected to be heterozygous in *D. paulistorum* is approximately 18–21% (Table 19). The latter figure gives an average for individuals from freshly collected material on a population basis. These figures correspond well with that of 17.6% obtained for *D. willistoni* (AYALA *et al.* 1971b), but are again somewhat higher than the 12–13% figure found for temperate region Drosophila species (PRAKASH *et al.* 1969; KOJIMA *et al.* 1970).

PRAKASH *et al.* (1969) have called attention to the observation that an isolated, marginal population of *D. pseudoobscura* has a level of genic heterogeneity not significantly below what is found in more central populations of the species. Populations of *D. paulistorum* from Teresita, Colombia (#1, Figure 1); Malaga Bay, Colombia (#2); Leticia, Colombia (#3); and Mora Forest, Trinidad (#11), have substantially lower degrees of individual heterozygosity than do the other populations sampled. The average for these four populations is 13.8% compared to 23.6% for the remaining populations. Only samples of greater than 20 genomes have been considered. The low amounts of heterozygosity in the Malaga Bay and Teresita samples agrees with a similar finding of DA CUNHA *et al.* (1959) based on a series of studies of chromosomal polymorphism in *D. willistoni*. These authors found that populations along the western coasts of Colombia and Ecuador

tended to have low degrees of chromosomal heterogeneity when compared to populations of northern and central South America. AYALA *et al.* (1971b) in their study of allozyme loci in *D. willistoni* also found a relatively low degree of individual heterozygosity in the Teresita population but their sample from Malaga Bay shows an intermediate level of genic heterogeneity. The low amount of individual heterozygosity in the Mora Forest sample from Trinidad does not match the figure for the second Trinidad sample, Arima Valley, which has a level of heterozygosity similar to that found in the mainland samples from Venezuela. This may be explained by the rather small sample of 24 genomes from Mora Forest. DA CUNHA *et al.* (1959) also found low levels of chromosomal heterogeneity at the southern terminus of the range of *D. willistoni* in Brazil and Argentina. The sample of *D. paulistorum* from this region (Mirassol, Brazil) has an average of 26.5% individual heterozygosity, one of the highest figures for *D. paulistorum*. However the data of AYALA *et al.* (1971b) for *D. willistoni* from Mirassol do indeed show that this population has a relatively low degree of genic heterogeneity. PRAKASH *et al.* (1969) conclude that geographic marginality in populations of *D. pseudoobscura* does not result in genic homozygosity. As they point out this finding is not in accordance with the predictions of the "homoselection" theory of CARSON (1959) which is based upon studies of chromosomal polymorphism in *D. robusta*. The data derived from *D. paulistorum* and *willistoni* unfortunately yield conflicting results in this case. Some populations of both species which were considered to be in distributionally marginal areas by DA CUNHA *et al.* (1959) have intermediate or even high levels of genic heterogeneity. AYALA, POWELL and DOBZHANSKY (1971a) have recently compared the degrees of allozyme and chromosomal heterogeneity in continental and oceanic island (West Indies) populations of *D. willistoni*. They find that the amount of genic heterozygosity found in island *vs.* continental populations is 16.2% and 18.4% respectively. In contrast however, the mean numbers of heterozygous, autosomal, inversions in island *vs.* continental populations is 1.06 ± 0.26 and $4.03 \pm .47$ respectively. There appears to be little or no correlation between inversion and genic polymorphisms in this case. As the authors point out and PRAKASH *et al.* (1969) predicted, this data gives valuable information about the nature of the differences between chromosomal and genic polymorphisms. CARSON (1959) has hypothesized that a small newly formed, marginal population of a species is likely to be homozygous for inversion systems as well as for loci which show genic polymorphism in central populations. A process which Carson termed "homoselection" acts to specialize the gene pool of the population to marginal conditions resulting in even further reductions in chromosomal and genic polymorphisms. As the population increases in size its gene pool will be restricted to those inversions carried by the founder individuals since inversions are probably of monophyletic origin, but mutation at individual loci will create a store of genic polymorphism. CARSON's theory may well hold true for both chromosomal and genic polymorphisms in the earliest stages of the development of a new, marginal population but with time the only evidence of the "homoselection" process

will be low chromosomal heterogeneity. In every case the populations of both *D. paulistorum* and *willistoni* which have been studied are large and probably number in the millions. This data leads us to the conclusion that studies of genic polymorphism in well established marginal populations of a species will not yield information about the nature of the "homoselection" process.

KASTRITSIS (1967, 1969) has made an extensive series of investigations of the chromosomal inversions found in *D. paulistorum*. He finds that the T semispecies is the most polymorphic of the semispecies. This result is confirmed by the data of Table 19 which shows that the T semispecies has the highest proportion of polymorphic loci per population. KASTRITSIS (1967, 1969) has also found that some inversions are fixed in all strains of a semispecies studied. For example, the AB strains studied by KASTRITSIS (1967) were fixed for two inversions in the left arm of the *X* chromosome. This observation may explain the presence of an essentially unique allele, $To^{0.59}$, in the AB strains studied in this investigation. RICHMOND and POWELL (1970), however, were unable to demonstrate a correlation between *X*-chromosome inversions and this allele in the Mirassol, Brazil population. Further attempts to correlate specific inversions with allozyme alleles have not been made in *D. paulistorum*.

A substantial amount of evidence has been accumulated that points to the conclusion that genetic variation at enzyme loci in natural populations is maintained by the action of selective factors (DOBZHANSKY 1970). PRAKASH *et al.* (1969) have reviewed the evidence for *D. pseudoobscura* and the present investigations corroborate their findings as well as those of AYALA *et al.* (1971b) on *D. willistoni* The presence of a single predominant allele at each locus in the vast majority of populations of a single semispecies and in some cases all populations of the species is strong evidence for the action of selective factors. There is also evidence for clinal variation at several loci in two semispecies. At both the *Est-5* and *Est-7* loci (Tables 4, 6) of the I semispecies, the regressions of the frequency of allele, $Est\text{-}5^{1.00}$, on latitude and the frequency of allele $Est\text{-}7^{1.00}$, on longitude border on statistical significance, $b = 0.01$ and 0.02; $0.05 < P < 0.10$ for each case respectively. Similarly at the *Est-6* and *Lap-5* loci (Tables 5, 8) of the A semispecies, the regressions of the frequencies of alleles, $Est\text{-}6^{1.02}$ and $Lap\text{-}5^{1.00}$, on longitude and latitude respectively border on statistical significance, $b = 0.02$ and 0.01; $0.05 < P < 0.10$ for both cases. The regression of $Lap\text{-}5^{1.00}$ frequencies on latitude does not include the Teresita sample which has an uncharacteristically high frequency of this allele.

The *To* locus (Table 13) presents an example of a locus which is widely polymorphic in only one semispecies. The AB semispecies has the widest distribution of the six semispecies, and polymorphism at this locus may represent an adaptation to a distribution which extends much farther south than any of the remaining semispecies (SPASSKY *et al.* 1971). RICHMOND and POWELL (1970) have presented evidence that indicates that this locus may be maintained in at least one population (Mirassol, Brazil) by heterotic, balancing selection.

In summary these investigations have shown a very similar pattern of genic

variability to that found in *D. pseudoobscura* and *willistoni* and reinforce the conclusions of PRAKASH *et al.* (1960), AYALA *et al.* (1971b) and others that such variation is maintained by balancing selection.

KOJIMA, GILLESPIE and TOBARI (1970; see also GILLESPIE and KOJIMA 1968) have presented evidence that variation at enzyme loci is dependent upon the metabolic system in which the particular enzyme under study functions. Their comparison revealed that glucose-metabolizing enzymes (Group I) were significantly less genetically variable than non-glucose-metabolizing enzymes (Group II). The loci studied in *D. paulistorum* have been similarly compared and the results support the general hypothesis of KOJIMA *et al.* (1970). Five loci (*Mdh, αGpdh, Tpi, Pgm, Me*) in Group I have an average of 3.6 alleles per locus, and $3 \pm 1\%$ individual heterozygosity. None of these loci is polymorphic in any semispecies by the 5% criterion. The remaining twelve loci which fall in Group II have an average of 4.7 alleles per locus and an individual heterozygosity of $21 \pm 4\%$. On the average 65.8% of these loci are polymorphic in *D. paulistorum*. It is noteworthy that the evidence for differentiation of gene frequencies in *D. paulistorum* comes from Group II enzyme loci. This further supports the contention of KOJIMA and his colleagues that Group II loci are subject to "ecologic" environmental heterogeneity.

Several of the polymorphic loci which show evidence of differentiation of gene frequencies both within and between the semispecies can be used to give an indication of the genetic relationships among the semispecies. At the *Est-2* locus (Table 2) the frequencies of alleles, $Est\text{-}2^{0.85}$ and $Est\text{-}2^{1.00}$ indicate that the C, T, and AB semispecies are more similar than the O, I and A semispecies which have rather similar allele frequencies. A similar situation is found for alleles, $Est\text{-}4^{0.85}$ and $Est\text{-}4^{1.00}$ (Table 3). At the *Est-6* locus (Table 5), populations of the A semispecies have a high frequency of allele $Est\text{-}6^{1.00}$ while the remaining five semispecies have low frequencies of this allele but high frequencies of allele $Est\text{-}6^{1.02}$. These observations lead to the conclusion that the O, I and A semispecies are more closely related to each other than the C, T and AB semispecies and *vice versa*. However, the A semispecies is clearly the most distinct of the six. These conclusions are in agreement with those of PEREZ-SALAS *et al.* (1970) and SPASSKY *et al.* (1971) who made ethological, genetic, and cytological tests in addition to examining a few allozyme loci in *D. paulistorum*. Indeed a close genetic relationship between the T and AB semispecies was indicated by the work of MALOGOLOWKIN (1963).

A last point which is impressively apparent from Tables 2–17 is that none of the enzyme loci studied can serve as a diagnostic tool for separating the semispecies. Such a result is in agreement with the results of PRAKASH (1969) who compared allozyme variation *D. pseudoobscura* and *persimilis*. These findings have again raised the problem of the degree of genetic differentiation among semispecies and species; originally posed by DOBZHANSKY (1959) and MAYR (1963) and investigated by HUBBY and THROCKMORTON (1968) using molecular techniques. This point will be explored later in a separate publication using data on the sibling species of the *D. willistoni* group.

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