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Enzyme variability in the *Drosophila willistoni* Group. V. Genic variation in natural populations of *Drosophila equinoxialis**

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SUMMARY

We have studied genetic variation at 27 loci in 42 samples from natural populations of a neotropical species, *Drosophila equinoxialis*, using standard techniques of starch-gel electrophoresis to detect allelic variation in genes coding for enzymes. There is considerable genetic variability in *D. equinoxialis*. We have found allelic variation in each of the 27 loci, although not in every population. On the average, 71 % of the loci are polymorphic – that is, the most common allele has a frequency no greater than 0·95 – in a given population. An individual is heterozygous on the average at 21·8 % of its loci.

The amount of genetic variation fluctuates widely from locus to locus. At the *Mdh-2* locus about 1 % of the individuals are heterozygotes; at the other extreme more than 56 % of the individuals are heterozygous at the *Est-3*. At any given locus the configuration of allelic frequencies is strikingly similar from locality to locality. At each and every locus the same allele is generally the most common throughout the distribution of the species. Yet differences in gene frequencies occur between localities. The pattern of genetic variation is incompatible with the hypothesis that the variation is adaptively neutral. Genetic variation in *D. equinoxialis* is maintained by balancing natural selection.

The amount and pattern of genetic variation is similar in *D. equinoxialis* and its sibling species, *D. willistoni*. Yet the two species are genetically very different. Different sets of alleles occur at nearly 40 % of the loci.

1. INTRODUCTION

Measuring the amount of genetic variation in natural populations is one of the outstanding problems of evolutionary genetics. The evolutionary potential at a given time of a population, or of a species, is a function of how much genetic variation the population, or species, has. As expressed by Fisher's (1930) fundamental theorem of natural selection, the rate of increase in fitness of a population at any time is equal to its genetic variance in fitness at that time.

Estimating the amount of genetic differentiation between closely related species is equally important. In outbreeding sexual organisms speciation occurs when

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reproductive barriers arise between populations which previously shared in a common gene pool through sexual reproduction. The question is whether closely related species are genetically very different or whether reproductive isolation usually develops between populations genetically little differentiated from each other.

Quantifying genetic variation within and between species proved to be an intractable problem for many years. More recently certain techniques, particularly the electrophoretic separation of enzymes and other proteins, have made possible the measurement at least approximately of the amount of genetic variation in populations. Since 1965 we have conducted a systematic survey of genetic variation in natural populations of a group of neotropical species related to *Drosophila willistoni*. There are six sibling species, four of which have widespread and largely overlapping distributions throughout the tropics of the New World. These species are therefore excellent materials for the study of the problems stated above. Variation among populations of a given species can be studied over large territories. Comparisons can be made between sympatric or allopatric populations of different species. We have published a summary of the genetic differentiation between species (Ayala *et al.* 1970), and have given detailed reports of the genetic variation in two sibling species, *D. willistoni* (Ayala, Powell & Dobzhansky, 1971; Ayala *et al.* 1972) and *D. paulistorum* (Richmond, 1972). We describe here genetic variation in a third, widely distributed sibling species, *D. equinoxialis*.

2. MATERIALS AND METHODS

Our samples of *Drosophila equinoxialis* cover most of the distribution range of the species in continental South America. They extend from Jaque, in eastern Panama, through Colombia, Venezuela, Trinidad, Guyana and northern Brazil. The localities are shown in Fig. 1. Triangles indicate the localities from which one, or only a few strains were available for our study. Circles indicate those localities from which many genomes were studied. A listing of the populations follows. The numbers in parentheses refer to the map (Fig. 1). Additional details concerning most of the collections can be found in Spassky *et al.* (1971).

Pamama: Jaque (1). *Columbia:* Piojo (2); Turbo (3); Teresita (4); Betoyes (5); Tame (6); Mesas (7); P. Lopez (8) – two collections about 3 km apart from each other; La Macarena (9), on the eastern slope of the mountain range of that name; Guayabero (10), on the south-west slope of La Macarena mountains near the left bank of the Guayabero river: Mitu (11) – three collections about 1 km apart from each other; Valparaiso (12) – two collections a few days apart; Leticia (13) – three collections: two 1 km apart from each other near the town of Leticia, the third in Marco, Brazil, about 3 km from the Leticia collections. *Venezuela:* Perija, Machiques (14); Rancho Grande (15); south-east of Caracas (16) – five collections a few km apart from each other: two in Marrero, two in Guatopo, and one in Burguillo; Puerto Ayacucho (17); Ocamo (18). *Trinidad* (19) – two collections, St Patrick and St Pablo, a few km apart from each other. *Guyana* (20) – two collections about

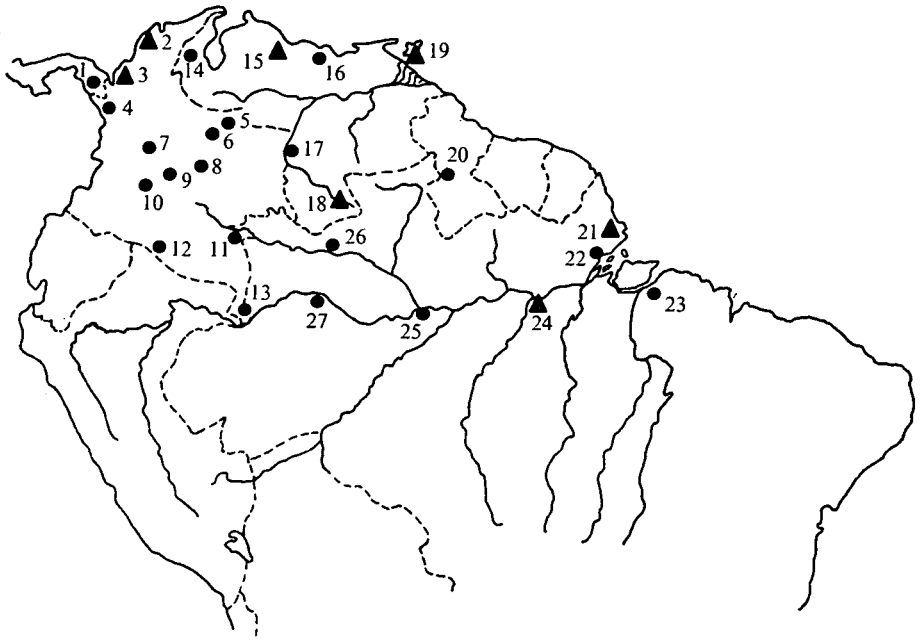

Fig. 1. Localities sampled in our study. Triangles: localities from which one or only a few strains were available; circles: localities from which many genomes were studied. 1, Jaque; 2, Piojo; 3, Turbo; 4, Teresita; 5, Betoyes; 6, Tame, 7, Mesas; 8, P. Lopez; 9, La Macarena; 10, Guayabero; 11, Mitu; 12, Valparaiso; 13, Leticia; 14, Perija; 15, Rancho Grande; 16, southeast of Caracas; 17, P. Ayacucho; 18, Ocamo; 19, Trinidad; 20, Guyana; 21, Tracajatuba; 22, Macapá; 23, Belem; 24, Santarem; 25, Manaus; 26, Tapuruquara; 27, Tefe.

2 months apart in the Kanuku mountains. *Brazil:* Tracajatuba, Amapa (21); Macapá, Amapa (22); Belem (23) – two collections a few km apart; Santarem (24); Manaus (25); Tapuruquara (26) – two collections a few km apart on the left bank of the Rio Negro; Tefe (27).

We have used standard techniques for starch-gel electrophoresis and assay of enzymes, with minor changes to suit our materials. The detailed procedures are described by Ayala *et al.* (1972). We have studied 27 loci coding for enzymes as follows: esterases, five loci (*Est-2*, *Est-3*, *Est-4*, *Est-5*, *Est-6*); acid phosphatases, two loci (*Acph-1* and *Acph-2*); octanol dehydrogenases, two loci (*Odh-1* and *Odh-2*); $NADP^+$-dependent malate dehydrogenases, two loci (*Me-1* and *Me-2*); adenylate kinases, two loci (*Adk-1* and *Adk-2*); hexokinases, three loci (*Hk-1*, *Hk-2*, and *Hk-3*); and one locus for each of the following enzymes: leucine aminopeptidase (*Lap-5*), alkaline phosphatase (*Aph-1*), aldolase (*Ald*), alcohol dehydrogenase (*Adh*), malate dehydrogenase (*Mdh-2*), α-glycerophosphate dehydrogenase (*αGpdh*), isocitrate dehydrogenase (*Idh*), glyceraldehyde-3-phosphate dehydrogenase (*G3pdh*), tetrazolium oxidase (*To*), Triose phosphate isomerase (*Tpi-2*), and phosphoglucomutase (*Pgm-1*). The abbreviations written in italics as given in parentheses are used to designate the gene loci. When several forms of a given

enzyme exist, each controlled by a different locus, a hyphenated numeral has been added to the gene symbol. Increasing numerals represent enzymes with increasing migration towards the anode in our gels. At each locus one allele is arbitrarily designed 1·00. Other alleles are named with reference to that standard. For example, an allele 0·95 migrates towards the anode 5 mm less, and an allele 1·04 migrates 4 mm more, than the standard. Following accepted procedures in genetic nomenclature we write alleles as superscripts to gene symbols $Adh^{0.98}$ symbolizes allele 0·98 at the *Adh* locus.

Our samples were studied as follows. When the flies collected in the field reach our laboratory the males are immediately used for electrophoresis. Females are placed in individual cultures. One F_1 progeny from each culture is then studied for each enzyme. Thus we sample two wild genes at each locus for each individual collected in nature, except for sex-linked loci which are carried by males in single dose.

3. RESULTS

We have studied 42 samples of natural populations of *Drosophila equinoxialis*. These samples come from 27 localities spread over most of the geographic distribution of the species in South America. We have found variation at every one of the 27 zones of enzymic activity studied in starch gels. The enzyme variants segregate as Mendelian entities.

Tables 1–15 (printed at the end of the paper) show the variation found at each of 23 loci. With one exception (*Est-3*) from several hundred to several thousand wild genomes have been sampled at each of these 23 loci. Relatively small samples were studied at four additional loci: *Acph-2*, *Ald*, *G3pdh* and *Odh-2*. The allelic frequencies observed at each locality are therefore not given for these four loci.

In Tables 1–15 we give for each locality the number of genes sampled, the allelic frequencies, and the proportion of individuals expected to be heterozygous on the assumption of Hardy–Weinberg equilibrium. In general there is good agreement between the expected and the observed proportion of heterozygotes. Where several samples were available from the same locality the actual allelic frequencies observed are given. The expected proportion of heterozygotes is the unweighted average of all samples consisting of 20 or more genes each. Data from localities from which we had only small samples have been pooled under the entry 'Other localities'. The last line of each table gives the allelic frequencies in the whole species. These frequencies are obtained by dividing the numbers of times each allele was found by the total number of genes sampled. When several alleles were found at very low frequencies their frequencies have been pooled (see Tables 4–6, 8, 12, 13); the number of different alleles found is given in parentheses.

Table 16 summarizes the amount of variation found at each of the 27 loci studied. For each locus the table shows the total number of genomes sampled, the proportion of polymorphic populations and the mean frequency of heterozygous individuals. Two criteria of polymorphism are used. By criterion 1 a population is considered polymorphic when the most common allele has a frequency

Table 16. *Proportion of polymorphic populations and average proportion of heterozygous individuals at each of 27 loci of* Drosophila equinoxialis

Gene	Genes sampled	Samples	Proportion of polymorphic populations* Criterion 1†	Criterion 2†	Mean frequency of heterozygous individuals*
Lap-5	2774	41	1·00	1·00	0·486 ± 0·023
Est-2	834	14	0·833	1·00	0·126 ± 0·019
Est-3	110	9	1·00	1·00	0·566 ± 0·029
Est-4	2682	32	1·00	1·00	0·373 ± 0·045
Est-5	2696	34	0·571	1·00	0·090 ± 0·011
Est-6	2756	32	1·00	1·00	0·256 ± 0·027
Aph-1	1913	29	0·813	1·00	0·133 ± 0·023
Acph-1	702	5	1·00	1·00	0·308 ± 0·010
Acph-2	56	2	1·00	1·00	0·296 ± 0·018
Ald	66	13	1·00	1·00	0·297 ± 0·007
Adh	1860	22	0·909	0·909	0·232 ± 0·032
Mdh-2	1900	25	0·000	0·250	0·010 ± 0·004
αGpdh	1888	24	0·000	0·583	0·027 ± 0·009
Idh	822	23	0·444	0·778	0·088 ± 0·023
G3pdh	28	1	1·00	1·00	0·196
Odh-1	508	4	1·00	1·00	0·293 ± 0·073
Odh-2	24	2	1·00	1·00	0·165
Me-1	734	21	0·00	0·500	0·025 ± 0·010
Me-2	292	15	1·00	1·00	0·308 ± 0·058
To	1347	14	0·571	0·714	0·131 ± 0·046
Tpi-2	550	22	0·00	0·571	0·037 ± 0·013
Pgm-1	524	12	1·00	1·00	0·444 ± 0·034
Adk-1	658	22	1·00	1·00	0·474 ± 0·034
Adk-2	576	22	0·250	0·625	0·096 ± 0·052
Hk-1	428	14	0·667	1·00	0·151 ± 0·028
Hk-2	662	15	0·571	0·857	0·146 ± 0·041
Hk-3	676	15	0·571	0·857	0·122 ± 0·038

Proportion of polymorphic loci per population: criterion 1, 0·711 ± 0·071; criterion 2, 0·876 ± 0·039. Proportion of heterozygous loci per individual 0·218 ± 0·030.

* Only samples with at least 20 genes have been used for these estimates except for *Acph-2* and *Ald* where all samples of ten or more genomes have been used.

† Criterion 1: the frequency of the most common allele is $\leqslant$ 0·95; criterion 2: the frequency of the second most common allele is $\geqslant$ 0·01.

no greater than 0·95. Criterion 2 is less stringent; a population is classified as polymorphic when the second most common allele has a frequency not smaller than 0·01. The mean frequency of heterozygous individuals is the unweighted mean, with its standard error, of the frequency of heterozygotes in all samples containing at least 20 genomes. A summary of genetic variation in the whole species is given at the bottom of Table 16. The average proportion of polymorphic loci per population and the proportion of heterozygous loci per individual are obtained by averaging over all loci the figures given in the last three columns of the table; the standard errors for these averages are also given.

The amount of variation found in each locality is summarized in Table 17. We have listed the 19 localities in which an average of ten or more genes per locus have been studied. For each locality the table shows the number of loci studied, the average number of genes sampled per locus, the proportion of polymorphic loci and the proportion of loci at which an individual is heterozygous. This last statistic is obtained by averaging over all loci the proportion of heterozygous individuals at each locus.

Populations of *D. equinoxialis* contain enormous amounts of genetic variation. On the average a population is polymorphic at 71·1 % of the loci if the 95 % criterion of polymorphism is used. In 87·6 % loci a given population has at least two alleles with frequencies higher than 0·01. An individual is heterozygous, on the average, at $21{\cdot}8 \pm 3{\cdot}0$ % of its loci (Table 16). No two individuals are likely to be genetically identical.

The amount of genetic polymorphism varies considerably from locus to locus (Table 16). More than 50 % of the individuals are heterozygous at the *Est-3* locus but only about 1 % at the *Mdh-2* locus. We may classify the 27 loci according to their levels of polymorphism into four categories. The actual boundaries between these categories are, of course, arbitrary since variation in the degree of polymorphism is nearly continuous. The four categories, going from more to less polymorphism, are as follows:

(1) Loci with more than 40 % heterozygous individuals. Four loci reach very high levels of polymorphism: *Lap-5*, *Est-3*, *Pgm-1* and *Adk-1*.

(2) Loci with 10–40 % heterozygous individuals. This category of fairly highly polymorphic loci comprises 16 loci, nearly two-thirds of the total: *Est-2*, *Est-4*, *Est-6*, *Aph-1*, *Acph-1*, *Acph-2*, *Ald*, *Adh*, *G3pdh*, *Odh-1*, *Odh-2*, *Me-2*, *To*, *Hk-1*, *Hk-2*, and *Hk-3*.

(3) Loci with 5–10 % heterozygous individuals. Three loci are only moderately polymorphic: *Est-5*, *Idh* and *Adk-2*.

(4) Loci with less than 5 % heterozygous individuals. Four loci have little polymorphism: *Mdh-2*, *αGpdh*, *Me-1*, *Tpi-2*.

That some loci are more polymorphic than others is to be expected. What is perhaps surprising is that the amount of genic polymorphism varies little from population to population. The average heterozygosity per individual is given for each of 19 populations in the last column of Table 17. Since we have not studied the same loci in every population, the frequency of heterozygous loci depends on which loci have been studied in a given population. For purposes of comparisons between populations, those localities should be used in which a sufficiently large number of loci have been studied. We have studied 16 or more loci in nine localities. The average heterozygosity per individual ranges in these nine localities from 15·4 % loci in Guayabero (10 in Fig. 1) to 22·2 % in Tefe (27). Likewise, little variation from locality to locality in the amount of polymorphism has been found in two sibling species of *D. equinoxialis*, namely *D. paulistorum* (Richmond, 1972) and in *D. willistoni* (Ayala, Powell & Dobzhansky, 1971; Ayala *et al.* 1972). A similar situation obtains for other *Drosophila* species. In sharp contrast, the

Table 17. *Number of loci studied for each of 19 populations of* Drosophila equinoxialis; *proportion of loci which are polymorphic in each population; and average proportion of loci at which an individual is heterozygous*

Locality	No. of loci studied	Average sample size per locus	Proportion of polymorphic loci*		Proportion of heterozygous loci per individual
			Criterion 1	Criterion 2	
1. Jaque	20	42	0·650	0·850	0·203
4. Teresita	13	347	0·615	0·692	0·175
5. Betoyes	22	237	0·636	0·909	0·175
6. Tame	19	16	0·632	0·684	0·171
7. Mesas	9	11	0·556	0·556	0·173
8. P. Lopez	21	287	0·667	0·857	0·183
9. La Macarena	5	120	0·800	1·00	0·333
10. Guayabero	21	62	0·524	0·857	0·154
11. Mitu	5	35	1·00	1·00	0·261
12. Valparaiso	4	33	0·750	1·00	0·464
13. Leticia	5	105	0·800	1·00	0·297
14. Perija	5	28	1·00	1·00	0·355
16. S.E. of Caracas	4	54	0·500	1·00	0·226
20. Guyana	18	18	0·444	0·667	0·212
22. Macapa	13	18	0·615	0·615	0·182
23. Belem	18	30	0·500	0·667	0·177
25. Manaus	16	16	0·625	0·625	0·180
26. Tapuruquara	22	53	0·682	0·909	0·201
27. Tefe	21	200	0·762	0·857	0·222

* Criterion 1: the frequency of the most common allele is $\leqslant 0{\cdot}95$; criterion 2: the frequency of the second most common allele is $\geqslant 0{\cdot}01$.

amount of chromosomal polymorphism generally fluctuates widely from locality to locality (see below).

Similarity between localities occurs not only in the amount of genetic variation but also, and most interestingly, in the *pattern of the variation*. We have surveyed a large number of populations of *D. equinoxialis*. The territory embraced by our samples extends from eastern Panama to the estuary of the Amazon and from Caracas to central Amazonia – more than 4 million km^2. Yet the configuration of allelic frequencies remains fairly constant throughout this enormous territory. The same alleles occur at high and intermediate frequencies in almost every locality. Even rare alleles reappear again and again over large distances. Nevertheless the allelic frequencies are not identical everywhere; sometimes they fluctuate widely. The same striking phenomenon was discovered in *D. willistoni*. Some 70 samples were studied, obtained from localities extending from Mexico through Central America, the Caribbean and much of South America, to southern Brazil (Ayala *et al.* 1971; Ayala *et al.* 1972). The configuration of the allelic frequencies remains fairly constant throughout the species, although the allelic frequencies are by no means identical everywhere. Two generalizations were formulated to summarize the pattern of the variation in *D. willistoni*. The same two generalizations apply to *D. equinoxialis*: (1) *At any given locus the same allele is* (with few

and minor exceptions) *most common throughout the whole distribution of the species.* (2) Nevertheless, *the allelic frequencies are not uniform everywhere.*

Examples of significant differences in gene frequencies between localities or between regions can be found at nearly every locus. A few examples will be pointed out for purposes of illustration.

The frequency of $Lap\text{-}5^{1.07}$ is substantially lower, and that of $Lap\text{-}5^{1.09}$ substantially higher, in La Macarena (9) than in most other localities (Table 1). The frequency of $Lap\text{-}5^{1.07}$ is about twice as large in Tefe (27) ($0{\cdot}83 \pm 0{\cdot}02$) as in La Macarena (9) ($0{\cdot}39 \pm 0{\cdot}05$). The standard deviations are calculated from the binomial distribution.

At the *Est-6* locus (Table 5) allele 1·00 is very rare in some localities like Teresita (4) (0·003; sample size, 974) and Tefe (27) (0·01; sample size, 496), but reaches $0{\cdot}06 \pm 0{\cdot}02$ in La Macarena (9), and occurs at intermediate frequencies in other localities.

$Odh\text{-}1^{0.96}$ was found 20 times in a sample of 44 genes from Tapuruquara (26) (Table 10), while in Betoyes (5) its frequency is only $0{\cdot}07 \pm 0{\cdot}01$ (sample size, 412). Allele 1·00 has a frequency $0{\cdot}55 \pm 0{\cdot}07$ in Tapuruquara (26) but is 50% more frequent, $0{\cdot}85 \pm 0{\cdot}02$, in Betoyes (5).

Evidence of regional differentiation exists at the *Est-4* locus (Table 4). Allele 0·98 is very rare in the Llanos of Colombia near the eastern slope of the Andes. Its average frequency in this region is less than 0·02 [total sample size from Betoyes (5), P. Lopez (8), La Macarena (9) and Guayabero (10) is 806]. In the central Amazonian region [Belem (23), Tapuruquara (26), Tefe (27)] the frequency of this allele is greater than 0·20. Another instance of regional differentiation between the Llanos of Colombia and central Amazonia occurs at the *To* locus (Table 12). Allele 0·98 has been found only three times among the 891 genomes sampled from Betoyes (5), P. Lopez (8) and Guayabero (10), while it occurs at frequencies of about 0·10 in Belem (23), Tapuruquara (26) and Tefe (27).

Differences in allelic frequencies sometimes occur between localities not very distant. Betoyes (5) is about 350 km by air from P. Lopez (8). $Pgm\text{-}1^{1.04}$ (Table 6) has a frequency $0{\cdot}72 \pm 0{\cdot}03$ in Betoyes (5) but only $0{\cdot}49 \pm 0{\cdot}03$ in P. Lopez (8). The frequency of $Adh^{1.00}$ (Table 7) is $0{\cdot}91 \pm 0{\cdot}01$ in Betoyes (5), $0{\cdot}82 \pm 0{\cdot}02$ in P. Lopez (8). At the *Adk-1* locus the frequencies of alleles 1·00 and 1·06 are approximately reversed between these two localities (Table 13). The frequencies of $Adk\text{-}1^{1.00}$ are $0{\cdot}29 \pm 0{\cdot}03$ and $0{\cdot}53 \pm 0{\cdot}03$; those of $Adk\text{-}1^{1.06}$ are $0{\cdot}65 \pm 0{\cdot}03$ and $0{\cdot}38 \pm 0{\cdot}03$ in Betoyes (5) and P. Lopez (8), respectively. The *Adk-1* locus provides one exception to our first generalization. Allele 1·06 is the most common in every locality except P. Lopez (8), where the most common allele is 1·00, which is the second most common elsewhere. Significant differences in allelic frequencies between Betoyes (5) and P. Lopez (8) exist also at the *Hk-2* locus (Table 14). The frequency of allele 1·00 is $0{\cdot}97 \pm 0{\cdot}01$ in Betoyes (5); $0{\cdot}88 \pm 0{\cdot}02$ in P. Lopez (8). The differences at these four loci between Betoyes (5) and P. Lopez (8) are not likely to be due to sampling accidents. The samples are too large for that; more than 180 genomes were sampled from each locality at each locus.

Differentiation at some loci occurs between localities which have nearly identical frequencies at some other loci. No differences in allelic frequencies occur between Betoyes (5) and P. Lopez (8) at several loci, like *Est-4*, *Est-5*, *Acph-1*, *Mdh-2*, *αGpdh*, *Idh*, *Me-1* and *Hk-3*. Very small, if any, differences occur at the *Lap-5*, *Est-2*, *To*, and other loci.

4. DISCUSSION

Using techniques of starch-gel electrophoresis we have assayed 16 different enzymes in *D. equinoxialis* and found 27 separate zones of activity. The banding patterns in these zones of activity are clearly defined and reproducible. We have found variation in enzyme mobility within each of the 27 zones of activity. Standard genetic tests show that the variation within each zone of enzymatic activity is governed by alleles of single gene loci.

Natural populations of *D. equinoxialis* have large amounts of genetic variation. If a locus is considered polymorphic when the most common allele has a frequency no greater than 0·95, then 71·1 % of the 27 loci are, on the average, polymorphic in a given population. If a locus is considered polymorphic when the second most frequent allele has a frequency not smaller than 0·01, then 87·6 % loci are, on the average, polymorphic per population. An individual fly is heterozygous on the average at 21·8 % of the loci studied.

Very large amounts of genetic variation have also been found in other sibling species of the *D. willistoni* group. In *D. willistoni*, Ayala *et al.* (1972) studied 28 loci coding for enzymes. The proportion of polymorphic loci is 58·1 % by the '0·95 criterion' and 86·1 % by the '0·01 criterion'; a *D. willistoni* fly is heterozygous on the average at 18·4 % of the loci. Of the 17 enzyme loci studied in *D. paulistorum* 55 and 67 % are polymorphic per population, by the 0·95 and 0·01 criteria, respectively; an average fly is heterozygous at 21 % of the loci (Richmond, 1972). Similar amounts of genetic variation are found in *D. tropicalis* (Ayala, 1972). Much allelic variation has been found also in other *Drosophila* species. The proportion of heterozygous loci per individual ranges from about 8 to 28 % (Gillespie & Kojima, 1968; Kojima, Gillespie & Tobari, 1970; O'Brien & MacIntyre, 1969; Prakash, 1969; Prakash, Lewontin & Hubby, 1969).

The enzyme loci studied in *D. equinoxialis*, and in other *Drosophila* species, represent hopefully a random sample with respect to variation of all such loci. They were chosen independently of their having much or little variation, but rather because techniques were available for favourable assay of the enzymes coded. However, the amount of variation discovered in our samples cannot readily be generalized for the whole genome. Several difficulties arise. First, the techniques used probably underestimate the amount of variation (Lewontin & Hubby, 1966; Ayala *et al.* 1970). The *Drosophila* studies deal with only one class of genes – those coding for soluble proteins – and little or nothing is known about allelic variation in genes regulating the function of other genes or in those coding for structural proteins (see, however, Mross & Doolittle, 1967, for variation in fibrinopeptides among artiodactyls). To estimate the amount of variation in absolute, rather than

Table 18. *Percentage of loci which are diagnostic between any two sibling species of the* Drosophila willistoni *group* (*from Ayala & Powell*, 1972)

	D. tropicalis	*D. equinoxialis*	*D. paulistorum*	*D. pavlovskiana*	*D. insularis*	Average
D. willistoni	17·9	21·4	25·0	25·0	32·1	24·3
D. tropicalis	—	21·4	35·7	28·6	28·6	26·4
D. equinoxialis	—	—	14·3	25·0	28·6	22·1
D. paulistorum	—	—	—	14·3	32·1	24·3
D. pavlovskiana	—	—	—	—	32·1	25·0
D. insularis	—	—	—	—	—	30·7

proportional, values we would also need to know how many genes exist in the genome of the species. Estimates of the haploid number of genes in insects like *Drosophila* run from about 10000 to several million (Ayala, 1972). Moreover, we do not know what proportion of them are genes coding for soluble enzymes, although they are likely to be a substantial proportion, and perhaps a majority, of the total. In spite of these uncertainties, it is likely that the genome of *Drosophila* contains at least several thousand loci coding for soluble proteins. Based on our observation that 71–88 % loci are polymorphic, we conclude that at least several thousand loci are polymorphic in a given population of *D. equinoxialis*, and that an average individual is heterozygous probably at more than 1000 loci. That is, indeed, a very large amount of genetic variation.

Another goal of our studies is to compare the genetic constitution of closely related species. Is the amount and pattern of genetic variation similar in different sibling species? How much genetic diversification occurs among the species? To answer the first question we compare the data in Table 16 with similar data for the same loci in *D. willistoni* (from table 19 in Ayala *et al.* 1972). Using the proportion of heterozygous individuals as a measure of genetic variation at each of the 27 loci studied in both species, the correlation between the two species is 0·625, which is significantly positive ($P < 0{\cdot}001$). The correlation in the frequencies of the most common allele is 0·388, also significantly greater than zero ($P < 0{\cdot}01$). It appears, then, that at any given locus similar amounts of genetic variation tend to occur in these two sibling species.

There is, nevertheless, considerable genetic differentiation between these sibling species. Ayala *et al.* (1970) compared *D. willistoni*, *D. equinoxialis*, *D. tropicalis* and *D. paulistorum* at 15 enzyme loci. On the average, individuals of two different species are genetically different from each other at 60 % of their loci. The sibling species of *D. willistoni* are morphologically so similar that the species can hardly or not at all be identified by their external morphology. Yet the species of single individuals can be easily identified by their protein variants; that is, allozyme variants can be used as diagnostic characters (Ayala & Powell, 1972). Table 18 gives the proportion of enzyme loci which are diagnostic between any two species. A locus is considered diagnostic in a pair of species when the probability of correctly identifying the species of a single individual is 0·99 or higher. On the average, about 25 % of the loci are species diagnostic (Ayala & Powell, 1972).

What is the evolutionary significance of the large amount of genetic variation found in *D. equinoxialis*? Are the allelic variants in these enzyme loci adaptively equivalent? It has recently been suggested (Kimura, 1968; Kimura & Ohta, 1971; King & Jukes, 1969) that most of the genetic variation found in loci coding for soluble proteins may be selectively neutral. Allelic frequencies would then fluctuate exclusively due to errors of sampling through the generations. Differences between populations as well as between species would not have any adaptive significance since they would be the result of random processes.

The effective number of neutral alleles, n, per locus maintained in a population is, at equilibrium, given by the expression:

$$n = 4Nu + 1, \tag{1}$$

where N is the effective size of the population and u is the mutation rate to neutral alleles (Kimura & Crow, 1964). If the effective population size is one hundredth of the reciprocal of the mutation rate, the effective number of alleles is 1·04, and about 4 % of the individuals will be heterozygous per locus. If population size is one-tenth of the reciprocal of the mutation rate, the effective number of alleles is 1·4 and the average percentage heterozygosity per locus is 29. If population size is one-fourth of the reciprocal of the mutation rate, the effective number of alleles is 2 and 50 % individuals will be heterozygous per locus. In somewhat more than half of the *D. equinoxialis* loci the heterozygosity per locus falls between 4 and 29 %. If the genetic variation is selectively neutral, this is the amount of heterozygosity expected if the effective population size is between 0·01 and 0·10 of the reciprocal of the mutation rate. The amount of heterozygosity observed at the most polymorphic loci could be accounted for if the effective population size is between 0·10 and 0·25 of the reciprocal of the mutation rate. Unfortunately there is no precise information about mutation rates in enzyme loci nor about the effective population size of local natural populations of *Drosophila equinoxialis*.

Equation (1) is derived assuming that an enormously large, effectively infinite, number of selectively neutral allelic states can exist at a given locus. If the genetic variation is adaptively neutral *different sets of alleles should occur in different populations*. This prediction stands in sharp contrast with the facts. As Tables 1–15 show, the same alleles, and at highly correlated frequencies, occur throughout the species.

Kimura & Ohta (1971) have proposed an escape from this difficulty. If a certain amount of migration occurs between neighbouring populations, the species may effectively approximate a single panmictic population. Different local populations would effectively represent samples from one single interbreeding population. The same alleles and at similar frequencies would occur in different local populations. If this hypothesis holds true the number of neutral alleles per locus would still be given by (1), but N would represent in such case the effective population size of the *species* rather than of the local population. As stated above, little is known about the effective size of local populations. We do know, however, something about the lower limit of the size of the breeding population of the species. The distribution

of *D. equinoxialis* embraces a territory of several million square kilometres. Throughout its distribution *D. equinoxialis* is one of the most abundant drosophilids. A collector can obtain several hundred individuals in 2 or 3 h within a few hundred square metres. Within such an area the number of *D. equinoxialis* is at least several thousand. The total size of the species at a given time must reach at least many billions. If we take for the breeding population of the species a low estimate of 10^9 individuals, and if the mutation rate is of the order of 10^{-7}, the effective number of neutral alleles segregating at a given locus would be in the hundreds. The proportions of heterozygous individuals at a given locus should be greater than 99%. Clearly, this is not so. Even if we assume a very low mutation rate to neutral alleles of 10^{-8}, the effective number of alleles would be 41, and the proportion of heterozygotes per locus should be greater than 97%. At the levels of polymorphism observed in *D. equinoxialis*, the similarity of allelic frequencies throughout the species cannot be explained by migration between populations of selectively neutral alleles.

Migration cannot account for the genetic similarity between populations, since geographically isolated populations exhibit the same configurations of allelic frequencies as those centrally located. The Macapá and P. Lopez populations, for instance, live in small gallery forests surrounded by thousands of square kilometres of dry savannah. In *D. willistoni*, Ayala *et al.* (1971) studied genetic variation in six small oceanic islands of the Windward group of the Lesser Antilles. The same alleles were found, and in similar frequencies, as in continental populations. The isolation of the island populations was confirmed by great differentiation among the islands, and between the islands and continental populations, in the chromosomal polymorphisms.

The hypothesis that most of the enzyme variation in natural populations is selectively neutral is inconsistent with the facts. Natural selection is responsible for the large amount of genetic variation observed in natural populations. Various forms of balancing selection are likely to be involved. Heterosis has been demonstrated in two different loci of *Drosophila* (Richmond & Powell, 1970; Wills & Nichols, 1971). Kojima and his collaborators have shown that frequency-dependent selection is involved in the maintenance of certain polymorphisms (Huang, Singh & Kojima, 1971, and references therein). Recently, Powell (1971) has shown that the amount of polymorphism is directly related to the degree of environmental heterogeneity.

The sibling species of the *D. willistoni* group are chromosomally also very polymorphic. At least 50 distinct inversions have been recorded in *D. willistoni* alone; inversion polymorphisms exist in every chromosome. Nevertheless, the chromosomal and the enzyme polymorphisms are strikingly different with respect to their geographic distributions. Differences occur at two levels. First, in *D. willistoni* as in *D. equinoxialis* the same allozyme variants occur in similar frequencies in every population. In contrast, only three inversions are species-wide in *D. willistoni* while many others are endemic to certain regions or localities. Moreover the frequency of a given inversion varies greatly among the localities

where it occurs (Da Cunha, Burla & Dobzhansky, 1950; Da Cunha & Dobzhansky, 1954; Dobzhansky, 1957; Da Cunha *et al.* 1959).

Secondly, the chromosomal and enzyme polymorphisms differ in the degree and extent of interlocality variation. The amount of chromosomal polymorphism in *D. willistoni* as related to the geographical and ecological characteristics of the population according to some simple rules. Populations from the central distribution area and from ecologically rich and diversified habitats are more polymorphic than peripheral or island populations; populations occupying ecologically marginal environments show little variation (Da Cunha *et al.* 1959). On the contrary, there is little interlocality variation in the amount of enzyme polymorphism. Ayala *et al.* (1971) compared the allozyme and the chromosomal polymorphisms in ten populations of *D. willistoni*. Four continental populations were sampled in the Llanos of Colombia; the other six populations are from six islands in the Windward group of the Lesser Antilles. In Colombia the mean number of heterozygous autosomal inversions per individual is greater than 5; in the islands it ranges from 0·48 to 1·40 with a mean of $0{\cdot}79 \pm 0{\cdot}05$. In spite of this substantial difference in the amount of chromosomal polymorphism little difference exists between the continental and the island populations in the amount of enzyme polymorphism. Twenty-four enzyme loci were studied in each population. The average proportions of heterozygous loci per individual are $0{\cdot}184 \pm 0{\cdot}009$ and $0{\cdot}162 \pm 0{\cdot}006$ in the continental and island populations, respectively. It is clear, then, that the extensive enzyme polymorphisms occurring in these species cannot be explained for the most part by the association of specific alleles with specific inversions. The processes which maintain the enzyme polymorphisms act independently, at least in part, of the processes responsible for the chromosomal polymorphisms.

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Table 1. *Frequencies of alleles at the* Lap-5 *locus of* Drosophila equinoxialis

Locality	Genes sampled	Alleles 1·03	1·05	1·07	1·09	1·12	Proportion of heterozygous individuals
1. Jaque	108	0·02	0·19	0·74	0·06	—	0·414
4. Teresita	822	0·002	0·22	0·73	0·05	—	0·419
5. Betoyes	246	0·02	0·24	0·70	0·04	—	0·450
8. P. Lopez	358	0·003	0·22	0·71	0·07	—	0·442
9. La Macarena	108	—	0·21	0·39	0·38	0·02	0·659
10. Guayabero	82	—	0·34	0·57	0·07	0·01	0·549
11. Mitu	38	—	0·13	0·63	0·24	—	0·528
12. Valparaiso	28	—	0·18	0·57	0·25	—	0·579
13. Leticia	74	—	0·19	0·55	0·24	0·01	0·559
14. Perija	24	—	0·25	0·54	0·21	—	0·601
16. S.E. of Caracas	80	—	0·35	0·59	0·06	—	0·513
20. Guyana	30	—	0·33	0·57	0·10	—	0·558
22. Macapa	26	—	0·12	0·85	0·04	—	0·288
23. Belem	44	—	0·30	0·66	0·05	—	0·477
26. Tapuruquara	76	—	0·05	0·84	0·11	—	0·320
27. Tefe	504	0·004	0·11	0·83	0·04	0·01	0·291
Other localities	126	—	0·27	0·70	0·02	0·01	
Total	2774	0·004	0·205	0·712	0·075	0·004	

Table 2. *Frequency of alleles at the* Est-2 *locus of* Drosophila equinoxialis

Locality	Genes sampled	Alleles 0·98	1·00	1·02	1·04	1·10	Proportion of heterozygous individuals
5. Betoyes	204	0·005	0·02	0·95	0·03	—	0·104
8. P. Lopez	372	0·01	0·01	0·96	0·01	0·003	0·081
10. Guayabero	74	—	0·04	0·96	—	—	0·078
23. Belem	38	0·26	0·10	0·87	—	—	0·234
26. Tapuruquara	74	0·03	0·05	0·92	—	—	0·151
27. Tefe	28	—	0·07	0·93	—	—	0·133
Other localities	44	0·07	0·11	0·82	—	—	
Total	834	0·014	0·032	0·939	0·013	0·001	

Table 3. *Frequency of alleles at the* Est-3 *locus of* Drosophila equinoxialis

Locality	Genes sampled	Alleles 0·96	0·98	1·00	1·02	Proportion of heterozygous individuals
26. Tapuruquara	40	—	0·50	0·38	0·12	0·594
27. Tefe	26	—	0·42	0·38	0·19	0·636
Other localities	44	0·02	0·48	0·43	0·07	
Total	110	0·009	0·473	0·400	0·118	

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Table 4. *Frequencies of alleles at the* Est-4 *and* Est-5 *loci of* Drosophila equinoxialis

	Est-4					*Est-5*					
Locality	Genes sampled	0·98	1·00	1·02	Proportion of heterozygous individuals	Genes sampled	0·95	1·00	1·05	Other (3)	Proportion of heterozygous individuals
1. Jaque	74	0·27	0·58	0·15	0·567	66	0·05	0·94	0·02	—	0·115
4. Teresita	948	0·18	0·72	0·10	0·441	958	0·05	0·94	0·01	—	0·107
5. Betoyes	230	0·01	0·95	0·04	0·100	228	0·04	0·92	0·04	—	0·149
8. P. Lopez	362	0·02	0·94	0·05	0·118	346	0·03	0·93	0·03	0·01	0·129
9. La Macarena	132	0·03	0·89	0·08	0·202	132	0·01	0·97	0·02	—	0·059
10. Guayabero	82	0·01	0·94	0·05	0·116	82	0·02	0·95	0·01	0·01	0·094
11. Mitu	34	0·15	0·79	0·06	0·344	34	—	0·94	0·06	—	0·111
13. Leticia	114	0·11	0·80	0·10	0·353	114	0·01	0·97	0·02	—	0·043
14. Perija	28	0·14	0·64	0·21	0·520	28	0·04	0·93	0·04	—	0·135
20. Guyana	16	0·31	0·69	—	0·430	28	—	0·96	0·04	—	0·069
23. Belem	46	0·48	0·46	0·07	0·555	40	—	0·97	0·03	—	0·049
26. Tapuruquara	78	0·37	0·56	0·06	0·495	80	0·03	0·94	0·03	0·01	0·058
27. Tefe	450	0·22	0·70	0·08	0·456	452	0·02	0·93	0·05	0·002	0·138
Other localities	88	0·23	0·68	0·09		108	0·01	0·94	0·06	—	
Total	2682	0·150	0·769	0·081		2696	0·033	0·940	0·024	0·002	

Table 5. *Frequency of alleles at the* Est-6 *and* Aph-1 *loci of* Drosophila equinoxialis

	Est-6							*Aph-1*					
Locality	Genes sampled	1·00	1·04	1·06	1·08	other (3)	Proportion of hetero-zygous individuals	Genes sampled	1·00	1·02	1·04	Other (4)	Proportion of hetero-zygous individuals
1. Jaque	74	0·01	0·88	0·01	0·09	—	0·219	66	0·02	0·92	0·06	—	0·142
4. Teresita	974	0·003	0·78	0·04	0·17	0·002	0·356	520	0·01	0·93	0·05	0·01	0·127
5. Betoyes	226	0·02	0·91	—	0·05	0·02	0·166	186	0·03	0·90	0·06	0·01	0·189
8. P. Lopez	324	0·02	0·87	0·01	0·09	0·01	0·239	291	0·03	0·93	0·03	—	0·117
9. La Macarena	132	0·06	0·86	0·02	0·06	—	0·247	98	0·01	0·87	0·12	—	0·233
10. Guayabero	82	0·01	0·90	—	0·07	0·01	0·180	70	—	0·94	0·06	—	0·108
11. Mitu	34	0·03	0·88	—	0·09	—	0·213	34	—	0·94	0·06	—	0·111
12. Valparaiso	32	—	0·78	—	0·22	—	0·342	40	0·03	0·88	0·07	0·03	0·228
13. Leticia	114	0·04	0·85	0·02	0·05	0·04	0·253	110	0·04	0·86	0·09	0·01	0·276
14. Perija	28	—	0·89	—	0·11	—	0·191	26	0·08	0·81	0·12	—	0·329
20. Guyana	26	0·04	0·77	0·08	0·12	—	0·388	24	—	0·96	0·04	—	0·080
22. Macapa	22	—	0·86	0·05	0·05	0·05	0·248	24	0·04	0·88	0·08	—	0·233
23. Belem	44	—	0·93	0·02	0·05	—	0·122	44	0·02	0·95	0·02	—	0·088
26. Tapuruquara	82	—	0·79	0·06	0·10	0·05	0·364	80	0·03	0·97	—	—	0·057
27. Tefe	496	0·01	0·89	0·01	0·07	0·01	0·197	242	0·02	0·91	0·07	—	0·169
Other localities	66	—	0·83	0·06	0·11	—		58	0·02	0·93	0·05	—	
Total	2756	0·014	0·843	0·023	0·111	0·009		1913	0·020	0·919	0·057	0·003	

Table 6. *Frequencies of alleles at the* Acph-1 *and* Pgm-1 *loci of* Drosophila equinoxialis

	Acph-1						*Pgm-1*					
Locality	Genes sampled	0·94	1·00	1·04	1·08	Proportion of heterozygous individuals	Genes sampled	0·96	1·00	1·04	Other (2)	Proportion of heterozygous individuals
1. Jaque	—	—	—	—	—	—	26	—	0·19	0·81	—	0·311
5. Betoyes	248	0·02	0·18	0·80	0·004	0·329	182	0·01	0·26	0·72	0·01	0·415
6. Tame	12	—	0·25	0·67	0·08	0·486	24	—	0·38	0·62	—	0·469
8. P. Lopez	360	0·01	0·16	0·82	0·005	0·292	206	0·01	0·48	0·49	0·01	0·527
10. Guayabero	82	0·01	0·18	0·80	—	0·319	58	0·02	0·24	0·72	0·02	0·417
Other localities	—	—	—	—	—		28	—	0·39	0·57	0·04	
Total	702	0·013	0·172	0·811	0·004		524	0·011	0·353	0·622	0·013	

Table 7. *Frequency of alleles at the* Adh *locus of* Drosophila equinoxialis

Locality	Genes sampled	Alleles					Proportion of heterozygous individuals
		0·90	0·98	1·00	1·06	1·12	
1. Jaque	42	—	—	1·00	—	—	0·00
4. Teresita	56	0·02	0·04	0·95	—	—	0·103
5. Betoyes	588	—	0·09	0·91	0·002	—	0·167
8. P. Lopez	354	0·003	0·18	0·82	—	—	0·289
10. Guayabero	60	—	0·08	0·92	—	—	0·153
16. S.E. of Caracas	46	—	0·15	0·85	—	—	0·258
20. Guyana	24	0·04	0·17	0·79	—	—	0·344
22. Macapa	24	—	0·08	0·92	—	—	0·153
23. Belem	42	—	0·19	0·81	—	—	0·219
24. Manaus	22	—	0·23	0·77	—	—	0·351
26. Tapuruquara	86	—	0·24	0·76	—	—	0·263
27. Tefe	488	0·002	0·16	0·83	0·002	0·002	0·284
Other localities	28	—	0·04	0·96	—	—	
Total	1860	0·002	0·135	0·861	0·001	0·001	

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Table 8. *Frequency of alleles at the* Mdh-2 *and* αGpdh *loci of* Drosophila equinoxialis

	Mdh-2					*αGpdh*					
Locality	Genes sampled	0·86	0·94	1·00	Proportion of heterozygous individuals	Genes sampled	0·94	1·00	1·06	Other (3)	Proportion of heterozygous individuals
1. Jaque	40	—	1·00	—	0·00	42	0·02	0·95	0·02	—	0·092
4. Teresita	24	—	1·00	—	0·00	56	—	1·00	—	—	0·00
5. Betoyes	598	—	0·997	0·003	0·007	598	0·01	0·98	0·005	0·002	0·033
8. P. Lopez	362	0·006	0·99	0·003	0·018	362	0·01	0·99	0·003	—	0·023
10. Guayabero	82	—	1·00	—	0·00	42	—	1·00	—	—	0·00
16. S.E. of Caracas	44	0·02	0·98	—	0·044	44	0·02	0·95	—	0·02	0·088
20. Guyana	24	—	0·96	0·04	0·080	24	—	1·00	—	—	0·00
22. Macapa	24	—	1·00	—	0·00	22	—	1·00	—	—	0·00
23. Belem	46	—	1·00	—	0·00	46	0·02	0·98	—	—	0·043
25. Manaus	22	—	1·00	—	0·00	22	—	1·00	—	—	0·00
26. Tapuruquara	94	0·01	0·99	—	0·014	86	—	0·99	0·01	—	0·014
27. Tefe	494	0·002	0·99	0·006	0·016	502	0·01	0·99	0·002	0·002	0·028
Other localities	46	—	1·00	—		42	0·10	0·90	—	—	
Total	1900	0·003	0·994	0·004		1888	0·12	0·983	0·004	0·002	

Table 9. *Frequency of alleles at the* Idh *and* Tpi-2 *loci of* Drosophila equinoxialis

	Idh					*Tpi-2*				
Locality	Genes sampled	0·96	1·00	1·04	Proportion of heterozygous individuals	Genes sampled	1·00	1·06	1·10	Proportion of heterozygous individuals
1. Jaque	52	—	0·98	0·02	0·019	28	0·04	0·96	—	0·069
4. Teresita	40	—	0·90	0·10	0·180	18	—	1·00	—	0·00
5. Betoyes	194	—	0·96	0·04	0·070	104	0·01	0·98	0·01	0·038
8. P. Lopez	284	0·004	0·97	0·03	0·060	230	0·03	0·97	—	0·079
10. Guayabero	60	0·02	0·97	0·02	0·065	56	0·02	0·98	—	0·035
20. Gayano	20	—	1·00	—	0·00	20	—	1·00	—	0·00
26. Tapuruquara	58	—	0·91	0·09	0·158	24	—	1·00	—	0·00
27. Tefe	40	—	0·90	0·10	0·180	20	—	1·00	—	0·00
Other localities	74	—	0·99	0·01		50	—	1·00	—	
Total	822	0·002	0·960	0·04		550	0·020	0·978	0·002	

Table 10. *Frequency of alleles at the* Odh-1 *locus of* Drosophila equinoxialis

Locality	Genes sampled	Alleles 0·92	0·96	1·00	1·04	1·08	Proportion of heterozygous individuals
5. Betoyes	412	0·01	0·07	0·85	0·07	0·005	0·148
6. Tame	14	—	0·07	0·86	0·07	—	0·255
10. Guayabero	38	—	0·05	0·92	0·03	—	0·148
26. Tapuruquara	44	—	0·45	0·55	—	—	0·496
Total	508	0·008	0·104	0·827	0·057	0·004	

Table 11. *Frequencies of alleles at the* Me-1 *and* Me-2 *loci of* Drosophila equinoxialis

	Me-1				*Me-2*				
Locality	Genes sampled	1·00	1·04	1·06	Proportion of heterozygous individuals	Genes sampled	0·96	1·00	1·04	Proportion of heterozygous individuals
1. Jaque	26	—	1·00	—	0·00	16	0·31	0·69	—	0·430
4. Teresita	40	0·03	0·97	—	0·049	—	—	—	—	—
5. Betoyes	190	0·005	0·99	0·005	0·021	72	0·07	0·89	0·04	0·203
8. P. Lopez	286	0·003	0·997	—	0·009	160	0·13	0·82	0·05	0·315
10. Guayabero	56	0·04	0·96	—	0·069	—	—	—	—	—
26. Tapuruquara	28	—	0·96	0·04	0·069	—	—	—	—	—
27. Tefe	20	—	1·00	—	0·00	20	0·15	0·75	0·10	0·405
Other localities	88	—	1·00	—		24	0·25	0·54	0·21	
Total	734	0·007	0·990	0·003		292	0·137	0·801	0·062	

Table 12. *Frequency of alleles at the* To *locus of* Drosophila equinoxialis

Locality	Genes sampled	Alleles				Proportion of heterozygous individuals
		0·98	1·00	0·02	Other (3)	
5. Betoyes	528	0·004	0·99	—	0·002	0·011
8. P. Lopez	293	—	0·99	—	0·01	0·014
10. Guayabero	70	0·01	0·99	—	—	0·028
22. Macapa	22	0·05	0·91	—	0·05	0·169
23. Belem	38	0·18	0·79	0·02	—	0·342
26. Tapuruquara	72	0·10	0·86	0·05	—	0·265
27. Tefe	274	0·09	0·89	0·01	0·004	0·204
Other localities	50	0·04	0·96	—	—	
Total	1347	0·034	0·956	0·006	0·004	

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Table 13. *Frequency of alleles at the* Adk-1 *and* Adk-2 *loci of* Drosophila equinoxialis

	Adk-1						*Adk-2*				
Locality	Genes sampled	1·00	1·06	1·12	Other (2)	Proportion of heterozygous individuals	Genes sampled	1·00	1·04	Other (5)	Proportion of heterozygous individuals
1. Jaque	24	0·17	0·83	—	—	0·278	24	0·04	0·96	—	0·080
4. Teresita	36	0·36	0·61	0·03	—	0·495	40	—	1·00	—	0·00
5. Betoyes	186	0·29	0·65	0·06	—	0·495	98	0·02	0·90	0·08	0·189
8. P. Lopez	264	0·53	0·38	0·07	0·02	0·541	244	0·07	0·92	0·01	0·209
10. Guayabero	28	0·39	0·57	0·04	—	0·518	26	—	1·0	—	0·00
23. Belem	28	0·25	0·68	0·07	—	0·405	18	—	1·0	—	0·00
26. Tapuruquara	24	0·29	0·63	0·08	—	0·517	24	—	1·0	—	0·00
27. Tefe	22	0·18	0·73	0·09	—	0·430	46	0·02	0·96	0·02	0·084
Other localities	46	0·30	0·70	—	—		56	0·02	0·96	0·02	
Total	658	0·386	0·547	0·059	0·008		576	0·038	0·941	0·021	

Table 14. *Frequency of alleles at the* Hk-1 *and* Hk-2 *loci of* Drosophila equinoxialis

	Hk-1					*Hk-2*					
Locality	Genes sampled	0·97	1·00	1·04	Proportion of heterozygous individuals	Genes sampled	0·96	1·00	1·04	1·12	Proportion of heterozygous individuals
1. Jaque	26	0·15	0·85	—	0·260	30	—	0·87	0·07	0·07	0·240
5. Betoyes	106	0·09	0·91	—	0·171	190	—	0·97	0·03	—	0·061
8. P. Lopez	142	0·06	0·94	—	0·090	278	0·03	0·88	0·05	0·04	0·185
10. Guayabero	58	0·07	0·90	0·03	0·190	58	0·02	0·97	0·02	—	0·067
26. Tapuruquara	28	0·14	0·86	—	0·080	34	—	0·91	0·09	—	0·161
27. Tefe	20	0·13	0·87	—	0·255	20	—	0·85	0·05	0·10	0·265
Other localities	48	0·04	0·96	—		52	—	0·96	0·04	—	
Total	428	0·082	0·914	0·005		662	0·014	0·920	0·044	0·023	

Table 15. *Frequencies of alles at the* Hk-3 *locus of* Drosophila equinoxialis

Locality	Genes sampled	Alleles				Proportion of heterozygous individuals
		0·98	1·00	1·04	1·12	
1. Jaque	26	0·08	0·81	0·12	—	0·328
5. Betoyes	194	—	0·97	0·03	—	0·060
6. Tame	24	—	1·00	—	—	0·00
8. P. Lopez	388	0·003	0·96	0·03	0·003	0·068
10. Guayabero	58	0·03	0·95	0·02	—	0·101
26. Tapuruquara	32	—	0·91	0·09	—	0·170
27. Tefe	20	—	0·90	0·05	0·05	0·185
Other localities	34	—	1·00	—	—	
Total	676	0·007	0·954	0·036	0·003	

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