ADAPTIVE CHROMOSOMAL POLYMORPHISM IN *DROSOPHILA WILLISTONI*[1]

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Introduction

Organic diversity is, in the main, a response of life to diversity in the environment. The process of evolution has brought forth many and varied species of organisms, because a variety of genotypes adapted to the multitude of environments which exist in the world can not be contained within a single interbreeding population. But species are rarely if ever genetically uniform, and much of the intraspecific polymorphism also has adaptive functions.

Populations of many species of *Drosophila* carry variant chromosomal structures due to the occurrence of inversions of blocks of genes in the chromosomes. This variability seemed adaptively fortuitous until seasonal changes in the incidence of chromosomal structures were found in some California populations of *D. pseudoobscura* (Dobzhansky, 1943) and in Russian populations of *D. funebris* (Dubinin and Tiniakov, 1945). Experiments on artificial populations kept in laboratories showed that individuals homozygous and heterozygous for chromosomal structures have different adaptive values (reviews in Dobzhansky, 1947a, b, 1949).

The biological function of the chromosomal polymorphism in *D. pseudoobscura* and *D. funebris* is, in part, adaptation to seasonal changes in the environment. Any species which inhabits a temperate or a cold climate finds itself exposed at different seasons to a succession of sharply different environments. *D. persimilis* (Dobzhansky, 1948; Spiess, 1950) and *D. robusta* (Carson and Stalker, 1947, 1949; Levitan, 1950) show, however, no pronounced seasonal changes in the incidence of chromosomal variants in the populations so far studied. Here the polymorphism is evidently a response to other than seasonal variations in the environment.

It appeared desirable to compare the chromosomal polymorphisms in temperate and in tropical species of *Drosophila*, and particularly in inhabitants of different tropical climates, some of which are seasonally more constant than others. Populations of *D. willistoni* from different bioclimatic zones of Brazil were chosen as test materials. Taken as a whole, this species shows a greater chromosomal polymorphism than any other organism so far studied. The climates of some parts of Brazil are among the seasonally most constant ones in the Western Hemisphere, while in other parts there is alternation of wet and dry seasons.

Material and Technique

The distribution area of *D. willistoni* extends from central Mexico and southern Florida at least to southern Brazil (Patterson and Wagner, 1943; Burla *et al.*, 1949). *D. willistoni* is, by and large, the commonest and most widespread species of *Drosophila* in Brazil. Its natural food consists of many kinds of fermenting fruit and other vegetable materials (Dobzhansky and Pavan, 1950). Samples of natural populations were collected in the regions of Brazil shown on the maps in figure 1 and figure 2. Smaller samples were obtained, through the courtesy of Professor J. A. Moore, from Costa Rica and from Mexico, and Miss Sophie Dobzhansky collected a sample in Trinidad.

[1] Contribution No. 7 of the cooperative research project of the University of São Paulo and Columbia University on genetics and ecology of tropical *Drosophila*.

These samples were brought or sent to the laboratories in São Paulo or in New York for study.

Single females were placed in culture bottles and allowed to produce offspring. When mature larvae appeared, the salivary glands of one or more larvae from each culture were prepared in temporary acetic orcein mounts. Although *D. willistoni* is not first-class material for studies on salivary gland chromosomes, good cells can be found, with some practice, in almost every slide. A slide was usually given a cursory examination under low magnification, in order to pick out the most favorable cell. Then the gene arrangements in the chromosomes were examined with the aid of an oil immersion (× 90) or a correction collar (× 40) objective. For statistical purposes, the gene arrangements were recorded in only a single larva from each culture.

The Chromosomal Complement

D. willistoni has three pairs of chromosomes: metacentric X or Y chromosomes, metacentric second, and acrocentric third chromosomes. In the cells of larval salivary glands, five chromosomal strands radiate from the heterochromatic chromocenter. Two of them correspond to the left and the right limbs of the X chromosome (designated XL and XR), two to the second (II L and II R), and one to the third chromosome (III). A strain from Belem, Pará, Brazil, has been chosen as standard. The disc patterns in the salivary gland chromosomes of the

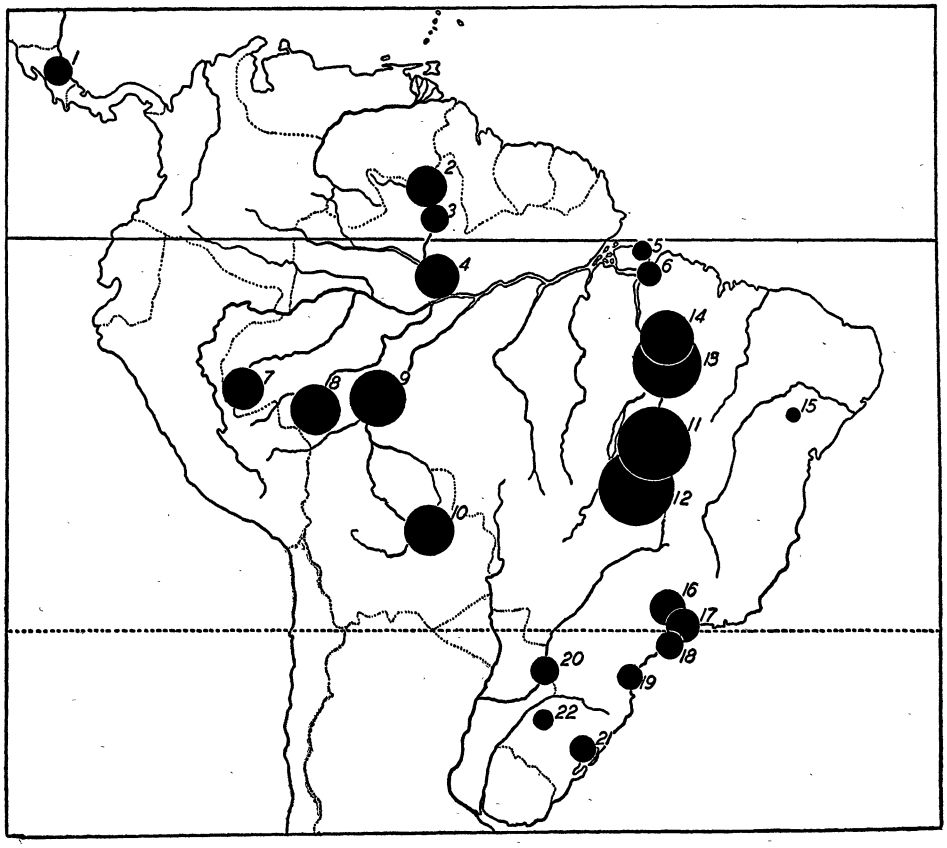

Fig. 1. Average numbers of heterozygous inversions per individual of *Drosophila willistoni* in different regions, symbolized by the diameters of the black circles. Numbers refer to the regions mentioned in tables 3 and 4.

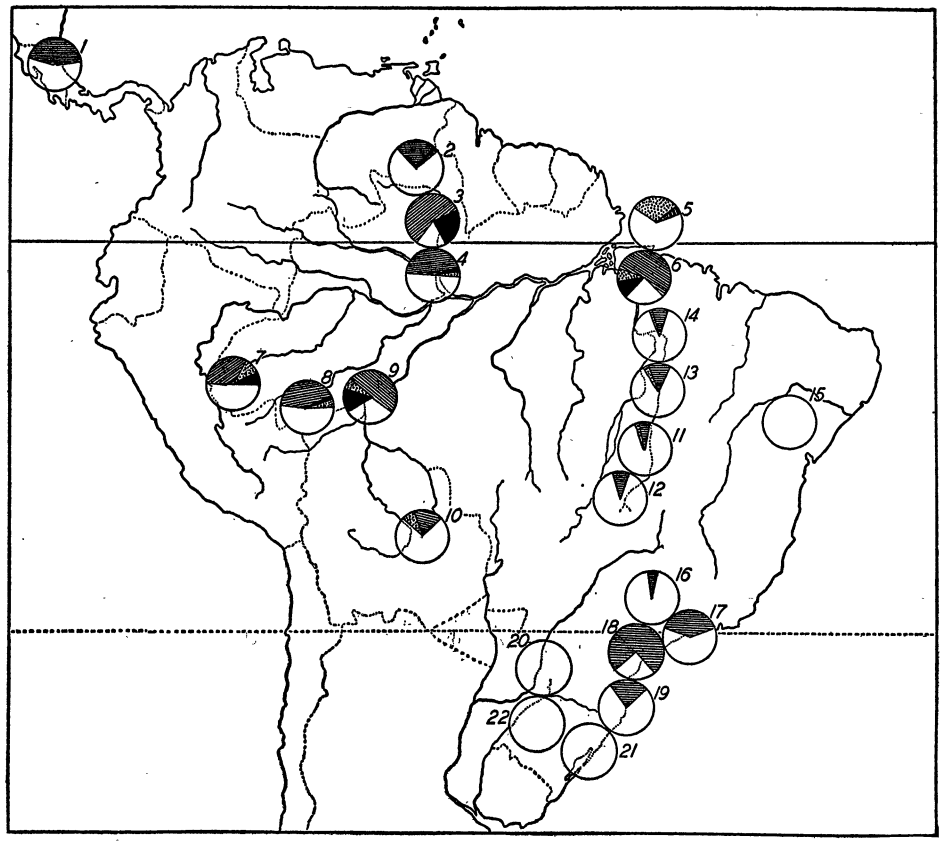

FIG. 2. Relative frequencies of *Drosophila willistoni* (white sectors), *D. paulistorum* (cross-hatched sectors), *D. equinoxialis* (black sectors) and *D. tropicalis* (stippled sectors) in different geographic regions.

standard strain have been drawn in the "maps" published elsewhere (Dobzhansky, 1950b). To facilitate description of the variant gene arrangements, the standard "maps" are divided into 100 arbitrary sections numbered 1–100. The XL strand contains sections 1–16; XR, 17–36; II L, 37–55; II R, 56–77; and III, 78–100.

The variations of gene arrangement known in natural populations of *D. willistoni* are all due to inversions of sections of chromosomes. In some species of *Drosophila,* notably *D. pseudoobscura, D. persimilis,* and *D. nebulosa,* the inversions are concentrated in one of the chromosomes, other chromosomes being largely or wholly free of inversions. As many as 40 inversions are known in *D. willistoni,* which is a much greater number than so far found in any other species. These inversions are distributed over all five chromosomal strands, no chromosome being free of inversions. The numbers of inversions known in different chromosomes are as follows:

XL—9	II R—5
XR—5	III—13
II L—8	χ^2—5.500

Although some of the chromosomes seem to be more variable than others, the differences are not significant statistically (probability about 0.25). The uniformity is especially obvious if one takes into account that chromosome III is longer than the rest, and hence should have more in-

versions in it than the shorter chromosomes.

It is further noteworthy that the majority of the inversions in *D. willistoni* are short ones, while in *D. pseudoobscura, D. ananassae, D. robusta, D. melanogaster, D. azteca,* but not in *D. nebulosa,* most inversions include relatively longer sections of the chromosomes. This fact is a source of technical difficulty since the small length of most inversions in *D. willistoni* has made it impractical to distinguish the homozygous standard and inverted gene arrangements. Our data consist, therefore, of records of frequencies of individuals in populations which were heterozygous for a given inversion. Furthermore, since the inversions are short, most of them are independent rather than overlapping or included. (The contrary is true in *D. pseudoobscura* and *D. persimilis,* Dobzhansky and Epling 1944.) Short independent inversions are combined and separated by crossing over, and for this reason the number of combinations of gene arrangements which exist in chromosomes of natural populations of *D. willistoni* is much greater than in any other species of *Drosophila* thus far known. In some samples from central Brazil no two of the hundred or so larvae examined had the same gene arrangement in all chromosomes. This made it impractical to score the frequencies of gene arrangements, and made it necessary to record most of the inversions separately, except some of the overlapping ones. No inversions with breaks in the chromocentral heterochromatin, and no pericentric inversions (including the centromeres), have been found. Their absence indicates that such inversions tend to be discriminated against in natural populations.

Some inversions are predominantly intra-populational, i.e., individuals heterozygous for them occur in many natural populations of the species. Other inversions distinguish geographical populations, i.e., flies of different geographic origin are homozygous for different gene arrangements, and heterozygotes for such inversions occur chiefly in some geographically intermediate populations. Since we recorded chiefly the inversions found in the progeny of wild females fertilized in nature by wild males, our data concern primarily the intra-populational inversions. We also made crosses of strains from different geographic regions to the standard Belem strain (and a similar strain from Marajó Island north of Belem). We do not feel, however, that the geographic distribution of the inversions is well known. A description of the inversions follows.

Inversions in the Left Limb of the X Chromosome

XL is the most "difficult" chromosome, owing chiefly to the prevalence of the geographically different gene arrangements. Crosses of the standard strain to strains from southern Brazil usually give the quintuple inversion shown in Plate 1 (B, D, F, G, H), while the triple inversion (B, C, D) is observed in crosses of the standard to Central American (Costa Rica, Guatemala, Mexico) strains. The quintuple and the triple inversions have two elements (B and D) in common, which means that with respect to these two inversions the Central American and the South Brazilian strains are actually more similar than either of them is to the standard strain which is of geographically intermediate origin. The inversions are shown in Plate 1.

Inversion A. Includes sections 3–5 of the standard map. These sections contain two segments which often form light bulbous swellings. Occurs rarely, chiefly in southern Brazil.

Inversion B. Includes the distal part of section 4, section 5, and the proximal part of section 6 of the standard map. Thus, only the distal part of the two "bulbs" is included in this inversion, which overlaps inversion A.

Inversion C. Includes sections from 6 up to the proximal part of 15, and is thus the longest inversion in the species. This inversion has not been seen alone, but it is an integral part of the triple inversion complex which distinguishes the strains from Central America from the standard strain.

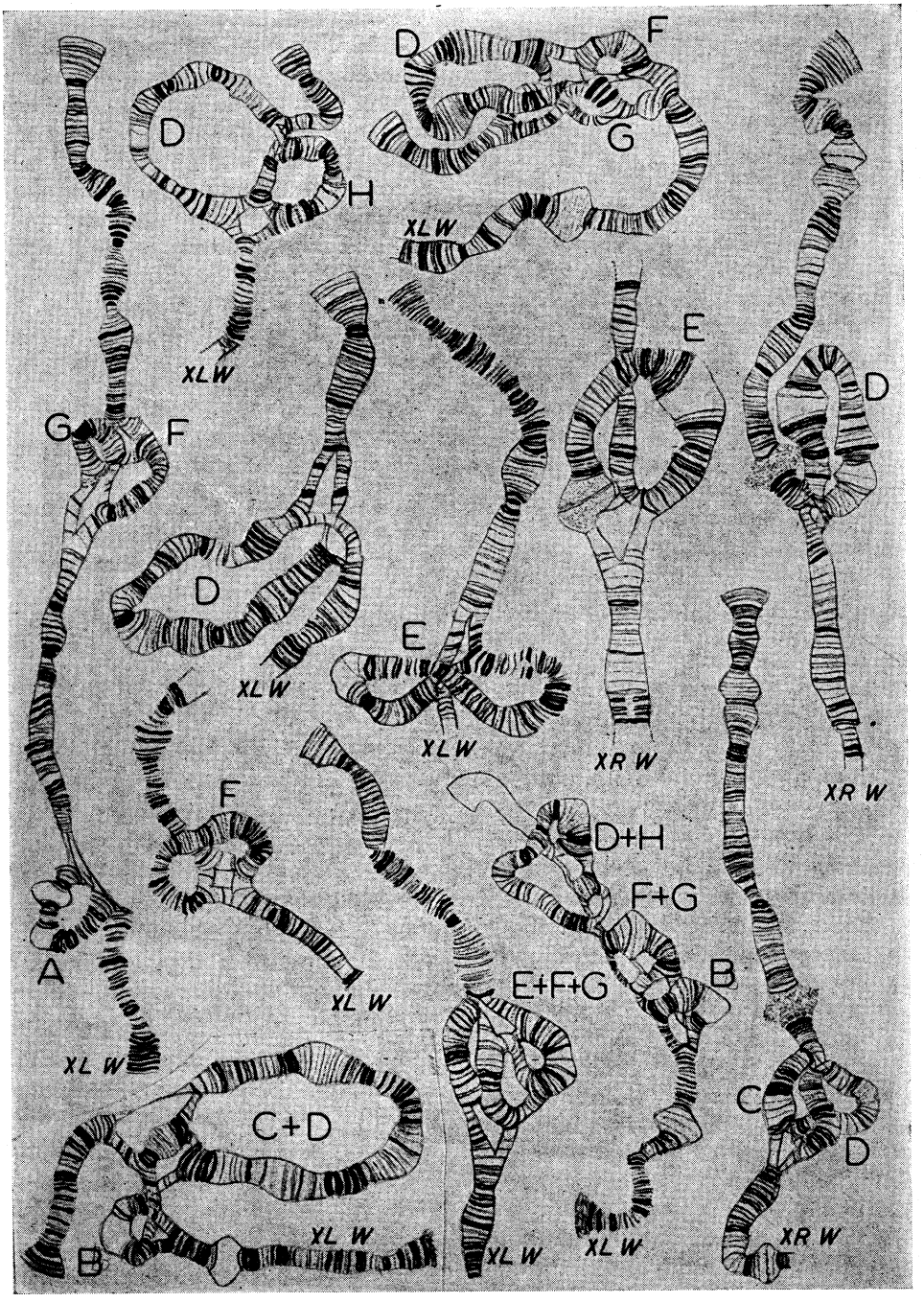
D
H
XL W
G
F
D
XL W
A
XL W
C+D
B
XL W
D
F
G
XL W
E
XL W
E
XR W
D
XR W
D+H
F+G
E+F+G
B
XL W
C
D
XR W

PLATE 1

Inversion D. Includes sections from the distal part of 7 till the proximal part of 15. The break in section 15 is close to, or identical with, that in inversion C. For this reason, simultaneous heterozygosis for C and D gives the configuration shown in the lower left corner of Plate I. When the short overlapping piece (sections 6 and a part of 7) fails to pair, the chromosome straightens out and shows merely two unpaired intercalations in the subterminal and the subbasal regions, respectively. Inversion D alone occurs in some flies from the Amazon Basin.

Inversion E. Includes sections from the distal portion of 9 to the proximal portion of 15. It has been seen only in a strain from Costa Rica, in which its position is median, since this strain was homozygous for C. Compounds including this inversion have, however, been seen in the Amazon Basin.

Inversion F. Includes sections 9 to 11 and occurs chiefly as an element of multiple inversions in central Brazil. The relations to Inversion E are not clear; the breaks in section 11 are close or even identical.

Inversion G. Includes sections 11 and a part of 7. This inversion is an integral part of the quintuple complex which distinguishes the standard from the south Brazilian strains.

Inversion H. Includes sections 7 to 9. Occurs as a part of the quintuple complex just mentioned, and also singly in south Brazilian strains. Since the latter appear to be homozygous for D, inversion H is located subterminally in the chromosome.

Inversion I. Some strains from southern Brazil contained a double inversion, in which H was certainly one of the elements. The other element was a larger inversion into which I was included, but apparently not quite as long as D. More data are needed to understand the relationships of these three inversions.

Inversions in the Right Limb of the X Chromosome

Some of the inversions known in XR are shown in Plate 1. Most of these inversions occur in the entire distribution area of the species, although the prevalent gene arrangements are different in different geographic regions.

Inversion A. Includes sections 18 to 21. This basal portion of the chromosome, separated from the chromocenter by only a short section No. 17, has two large bulbous swellings, and apparently some interstitial heterochromatin. The pairing in this region, with or without the inversion, is often disturbed, and care must be exercised in order not to mistake spontaneous lack of pairing for an inversion configuration.

Inversion B. Includes sections 18 and 19. The proximal break (in section 18) approximately coincides with that in inversion A, so that B seems to be included in A. Often found together with A.

Inversion C. Includes sections 23–27. Occurs sporadically in populations of central and northern Brazil. Independent of A and B, overlapping D.

Inversion D. Includes sections 23, 24, 25, 28, 29 and the proximal part of 30. Broadly overlaps C. A combination of C and D (see Plate) is characteristic of hybrids between standard and strains from southern Brazil. Inversion D alone is rather common in south Brazilian populations.

Inversion E. Includes sections 30, 29, 28, 23, 24, 25, the distal part of 30 and 31. Easily distinguishable from D because E does, and D does not, include the characteristic "repeat" area of section 31. Since inversion D overlaps C, and E overlaps D, the descent relationships of these inversions can be deduced as follows:

Gene arrangement:	Standard	↔	C	↔	D	↔	E
Distribution:	Amazon Valley		Central Brazil		Southern Brazil		Southern Brazil

Inversions in the Left Limb of the Second Chromosome

The inversions known in II L are shown in Plate 2. Most of them occur widely in the distribution area of the species.

Inversion A. A subbasal inversion including the block of genes from the proximal third of section 38 to the middle of 39.

Explanation of Plates 1–3

Camera lucida drawings of heterozygous inversion configurations in the salivary gland chromosomes of *Drosophila willistoni* (w) and *Drosophila paulistorum* (p). Plate 1—The right (XR) and the left (XL) limbs of the X-chromosome. Plate 2—The right (II R) and the left (II L) limbs of the second chromosome. Plate 3—The third chromosome. The large capital letters designate various inversions described in the text.

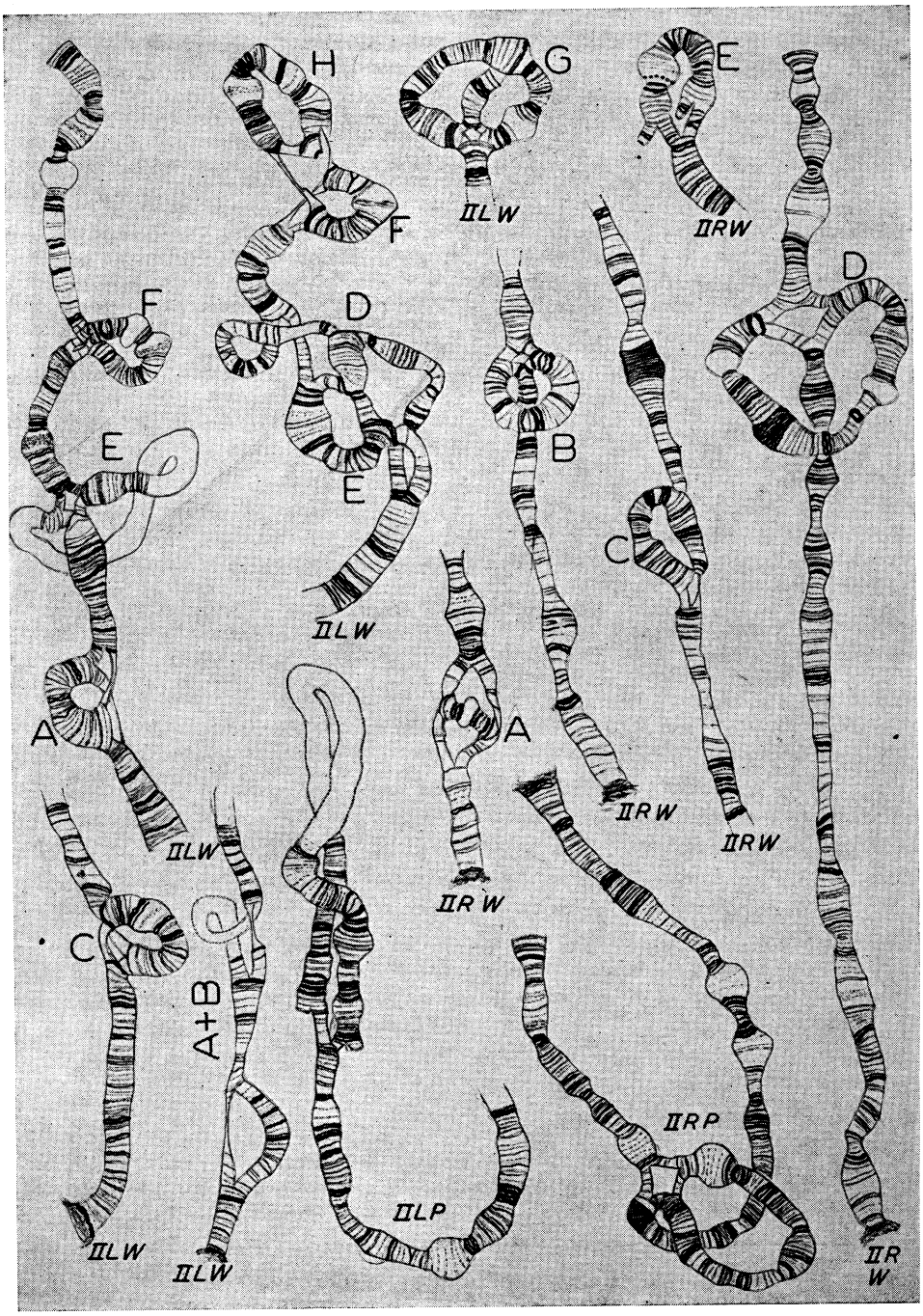
H
G
E
F
IILW
IIRW
F
D
D
E
B
E
C
IILW
A
A
IILW
IIRW
IIRW
IIRW
C
A+B
IIRP
IILP
IILW
IILW
IIR
W

PLATE 2

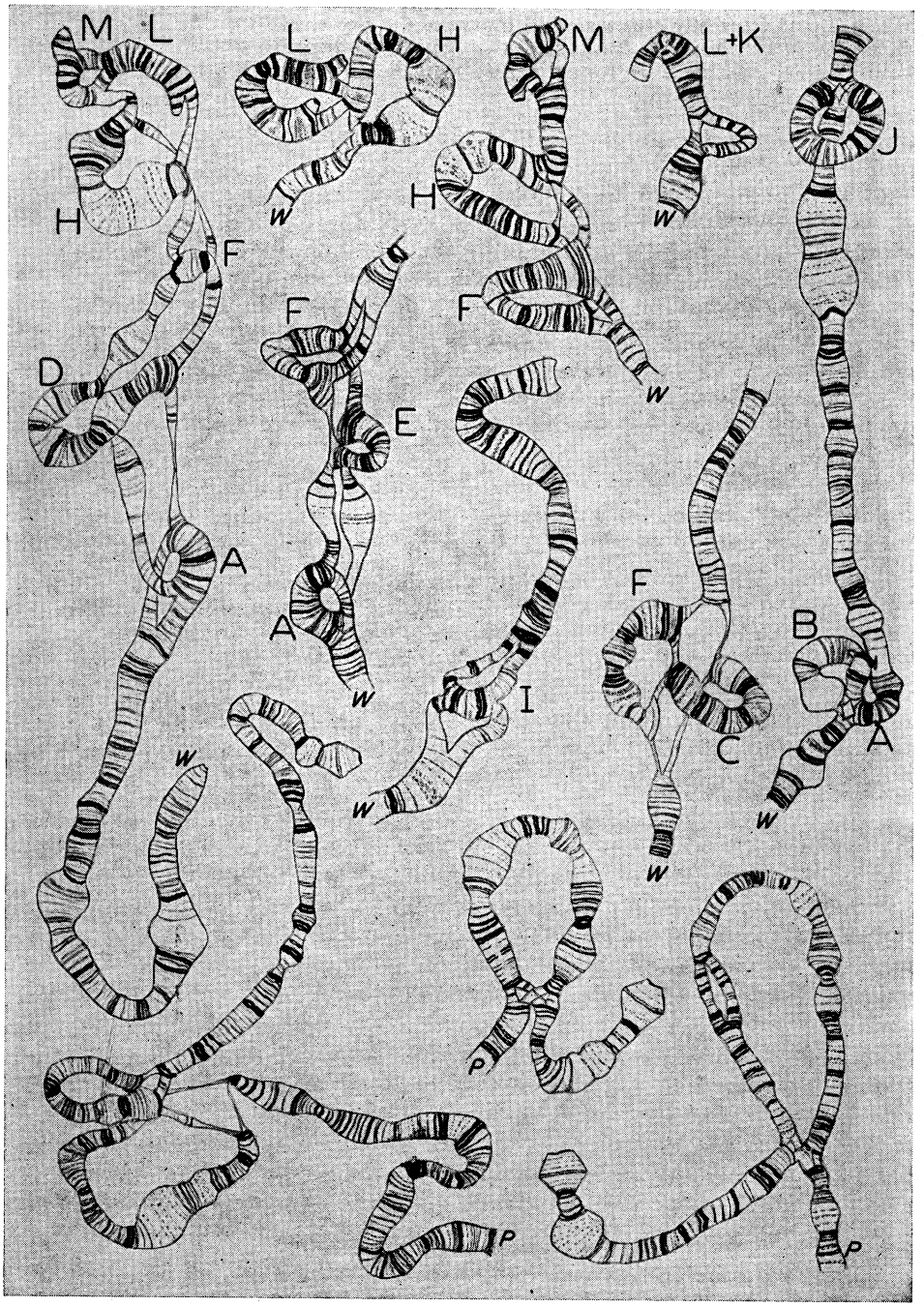

PLATE 3

Inversion B. Includes sections from the middle of 39 to the middle of 40. Broadly overlaps inversion A, and frequently occurs as A/B subbasal double inversion. The drawing of this double inversion in Plate 2 represents a case of incomplete pairing.

Inversion C. Includes sections 40 and 41, overlaps B but not A.

Inversion D. A relatively short median inversion including sections 44, 43, 42 and 48. The inverted gene arrangement differs from standard by two inversions (see Plate 2), the second being inversion E.

Inversion E. A relatively long median inversion which extends from section 42 to 47. Overlaps D, and is often difficult to distinguish from the latter if present alone in a preparation, especially because this part of the chromosome abounds in heterochromatic and repeat areas. The phylogenetic relationships are standard ↔ E ↔ D.

Inversion F. Includes sections 50 to the middle of 52.

Inversion G. A rare subterminal inversion which includes sections 50 to 54 and the proximal part of 55. This gene arrangement is apparently derived from H, thus: standard ↔ H ↔ G.

Inversion H. A relatively short but common subterminal inversion which extends from the middle of 52 up to almost the free end of the chromosome, but not including the few terminal discs which are sometimes paired as shown in Plate 2. The proximal break in H more or less coincides with the distal one in F. Broadly overlaps and almost includes G.

Inversions in the Right Limb of the Second Chromosome

II R is both geographically and individually the least variable chromosome. The five inversions known in it are represented in Plate 2.

Inversion A. A subbasal inversion which includes the distal part of section 57, section 58, and the proximal part of 59.

Inversion B. Includes sections from the distal part of 60 to 62. Independent of A and C.

Inversion C. A median inversion which extends from section 65 to 68. In both terminal sections some groups of discs seem to be partly heterochromatic, which causes irregular pairing in this part of the chromosome in some cells. These irregularly paired chromosomes should not be confused with true inversion heterozygotes.

Inversion D. A rare inversion which includes sections from 67 to 73. It must overlap C, but has never been seen together with the latter.

Inversion E. An apparently terminal inversion, extending from the middle of section 74 to the free end of the chromosome.

Inversions in the Third Chromosome

As many as 13 inversions have been recorded in chromosome III (Plate 3). All inversions are concentrated in the distal two-thirds of the length of the chromosome.

Inversion A. Includes section 87 and the proximal part of 88. This is one of the commonest inversions in southern and central Brazil. It can be seen in three drawings in Plate 3.

Inversion B. Includes sections 88 and 89, thus overlapping inversion A. Although B contains fewer dark discs than A, the two inversions have not been distinguished in the early part of our work. As a consequence, our records show the frequencies of A and B combined, except when both inversions were present simultaneously, in which case they form the configuration shown in Plate 3 (A + B). Inversion B is more common in southern than in northern Brazil.

Inversion C. Includes sections 90 and 91, probably does not overlap B but is included in D. This is a rather rare inversion found in central Brazil.

Inversion D. A relatively long inversion, which extends from section 90 to the basal part of 93, broadly overlaps or includes C and E, and overlaps F. Common in central Brazil but rare or absent in the south.

Inversion E. Includes sections 91 and 92, hence broadly overlaps C and is included in D. Occurs rarely, and forms a double inversion configuration when combined with D which resembles the A + B configuration but can be distinguished from the latter by its more distal position.

Inversion F. Includes section 93 and the distal portion of 92 and the proximal portion of 94. Overlaps slightly inversions E and D. Is shown in four figures in Plate 3. Common in Amazonia and in central Brazil, but is rare in southern Brazil.

Inversion G. Extends from section 94 to 97 inclusive, hence includes H and probably also I, but overlaps F only slightly if at all. G has been seen only in two chromosomes, one from the savanna portion, and the other from the forested portion of the territory of Rio Branco. Unfortunately no good drawing is available.

Inversion H. Includes the distal portion of section 94, 95, 96 and the proximal part of 97. Appears in three figures in Plate 3. Does not overlap F but probably does overlap L slightly.

Very common in most of Brazil, except in the south.

Inversion I. Includes sections 96 and the proximal part of 97, hence is included in H, but probably does not overlap J or L. Rare, but has been found in São Paulo, central Goyaz, and Maranhão. No satisfactory drawing available.

Inversion J. This is probably the commonest inversion in the species, distributed from Costa Rica to Rio Grande do Sul. Includes the distal portion of section 97, 98, 99 and the heavy doublet in the proximal portion of 100. Is included in L and overlaps M.

Inversion K. Found only in two chromosomes from Goyaz, both times in combination with inversion L (Plate 3). Includes parts of sections 98 and 99, and is not derived from the standard gene arrangement but from that modified by L.

Inversion L. Includes sections 97 to 100, except for a few terminal discs in the latter. Slightly overlapping H and I, and probably including J, K and M. Common in most of Brazil, except in the south. Shown in three figures in Plate 3.

Inversion M. Includes the distal portion of the chromosome involved in inversion L, and is apparently derived from the latter. Most often seen as a double subterminal inversion (M + L, Plate 3), and less often by itself (M, Plate 3). The distal break coincides very closely with that in inversion L, making this an almost, but not quite, terminal inversion. Widespread but less common than L.

Temporal Changes in the Chromosomal Constitution of Populations

As stated in the Introduction, cyclic seasonal changes in relative frequencies of inversions occur in certain populations of *D. pseudoobscura* and *D. funebris.* The inversions contain gene complexes which make their carriers better adapted to the environments prevailing at some than at other seasons. During each season, natural selection augments the frequency of the better adapted variants, and reduces that of the less well adapted ones. No seasonal changes occur, however, in *D. persimilis, D. robusta,* and in some populations of *D. pseudoobscura.* This certainly does not mean that the seasonally constant inversions are adaptively neutral. Their adaptive significance is presumably related to other than seasonal variations in the habitats, such as different food substances, etc. The adaptive importance of these inversions is indirectly confirmed by the fact that they show not only geographical but also elevational gradients, and in some cases undergo temporal changes not connected with the alternation of seasons (Carson and Stalker, 1947, 1949; Dobzhansky, 1947b, 1948).

Since populations of *D. willistoni* show an unprecedented diversity of gene arrangements, the problem of whether temporal changes occur in this species is especially intriguing. We were able to make periodic collections, at about two month intervals, in three localities in the state of São Paulo. The first locality, Vila Atlantica, is in the rainforest at the foot of the Serra do Mar, some 8 kilometers from the Ocean. Despite its location slightly to the south of the Tropic of Capricorn (lat. 23°50′ south), this locality has a superhumid tropical climate. Monthly temperature means range from 18.6° C. (July) to 25.2° C. (February), and rainfall from 103 mm. (July) to 298 mm. (March) (data for Santos, some 30 km. away). Large populations of *Drosophila* are active throughout the year. The second locality, Mogí das Cruzes, is at an elevation of about 800 m., on the plateau about 77 km. from Vila Atlantica and 36 km. from the coast. Here the winters are too cool and dry (14.7° C. and 30 mm. precipitation in July) for much activity on the part of the flies, but summers are warm and humid (21.5° C. and 208 mm. in January, data for São Paulo, some 50 km. away). The third locality, Pirassununga, is some 213 km. north of Mogí, in the continental interior, with warm but dry winters (18.4° C. and only 14 mm. precipitation in July), and hot and humid summers (23.5° C. and 188 mm. in January).

The observed frequencies of chromosomes with different inversions are summarized in table 1. (Since many chromosomes in natural populations carry more than a single inversion, the sums of

TABLE 1. *Frequencies (in per cent) of inversions, and numbers of chromosomes studied, in the state of São Paulo*

Inversion \ Date	Vila Atlantica						Mogí						Pirassununga						
	Sept. 26	Nov. 20	Jan. 10	March 16	May 28	Total	Sept. 4–19	Oct. 23	Dec. 17	Febr. 20	April 19	Total	Sept. 7	Oct. 13–19	Dec. 8–9	Febr. 10	April 8	June 14	Total
XL Chromosome																			
None	83.3	84.0	75.5	64.5	70.4	74.4	65.2	70.0	41.9	64.5	65.1	61.9	72.7	64.1	69.1	53.5	70.8	76.1	67.0
A *or* B	8.3	—	1.9	1.6	7.4	3.7	4.3	—	7.0	3.2	—	2.9	3.0	2.6	5.9	2.8	—	1.5	2.6
F	—	—	—	—	—	—	—	—	7.0	—	—	1.2	6.1	—	—	—	3.1	—	1.0
H	16.7	16.0	20.8	29.0	25.9	22.3	28.3	30.0	55.8	29.0	32.6	34.4	15.2	28.2	26.5	33.8	21.5	20.9	25.4
H + I	—	—	1.9	3.2	—	1.4	2.2	—	—	3.2	2.3	1.2	6.1	6.4	1.5	8.5	4.6	1.5	4.7
D + F + G	—	—	—	1.6	—	0.5	—	—	—	—	1.6	0.4	—	—	—	—	—	—	—
Chromosomes studied	48	25	53	62	27	215	46	50	43	62	43	244	33	78	68	71	65	67	382
XR Chromosome																			
None	85.4	76.0	75.5	69.4	70.4	75.3	63.0	90.0	51.2	58.1	46.5	62.3	81.8	76.9	80.9	66.2	72.3	80.6	75.9
A	—	—	—	1.6	3.7	0.9	—	2.0	9.3	9.7	—	4.5	—	—	1.5	1.4	—	1.5	0.8
A + B	—	—	—	—	—	—	—	—	7.0	—	—	1.2	—	—	—	—	1.5	—	0.3
D	14.6	20.0	24.5	24.2	25.9	21.9	23.9	8.0	37.2	22.6	44.2	26.2	12.1	17.9	16.2	28.2	18.5	16.4	18.8
E	—	4.0	—	4.8	—	1.9	13.0	2.0	9.3	9.7	9.3	8.2	6.1	6.4	1.5	4.2	7.7	1.5	4.4
Chromosomes studied	48	25	53	62	27	215	46	50	43	62	43	244	33	78	68	71	65	67	382
II L Chromosome																			
None	22.7	29.7	28.8	25.0	25.6	26.1	13.8	16.4	20.6	12.0	14.7	15.2	20.9	8.2	17.1	13.0	11.0	11.0	12.7
A	9.1	2.7	5.0	9.0	9.3	7.4	8.6	—	11.1	18.0	17.3	11.8	20.9	24.5	23.8	20.0	23.1	20.0	22.2
C	15.2	13.5	8.8	13.0	18.6	13.2	32.8	13.4	14.3	18.0	16.0	18.5	18.6	16.4	19.0	11.0	7.7	23.0	15.8
D	40.9	35.1	33.8	27.0	23.3	31.9	51.7	40.3	36.5	33.0	42.7	39.9	53.5	58.2	33.3	42.0	38.5	40.0	43.5
E	33.3	13.5	15.0	35.0	27.9	26.4	27.6	32.8	23.8	24.0	22.7	25.9	37.2	52.7	38.1	27.0	25.3	26.0	34.6
F	40.9	45.9	47.5	39.0	41.9	42.6	43.1	32.8	44.4	47.0	56.0	45.2	41.9	47.3	46.7	45.0	49.5	59.0	48.8
H	7.6	8.1	3.8	12.0	7.0	8.0	6.9	6.0	9.5	10.0	5.3	7.7	2.3	13.6	8.6	10.0	16.5	15.0	11.8
Chromosomes studied	66	37	80	100	43	326	58	67	63	100	75	363	43	110	105	100	91	100	549

TABLE 1.—*Continued*

Inversion \ Date	Vila Atlantica						Mogí						Pirassununga						
	Sept. 26	Nov. 20	Jan. 10	March 16	May 28	Total	Sept. 4–19	Oct. 23	Dec. 17	Febr. 20	April 19	Total	Sept. 7	Oct. 13–19	Dec. 8–9	Febr. 10	April 8	June 14	Total
II R Chromosome																			
None	87.9	100.0	95.0	87.0	93.0	91.4	89.7	94.0	85.7	78.0	90.7	86.5	93.0	87.3	81.9	87.0	84.6	90.0	86.5
A	1.5	—	—	—	—	0.3	—	1.5	1.6	2.0	—	0.6	—	1.8	1.0	1.0	—	1.0	0.5
B	—	—	—	—	—	—	—	—	1.6	2.0	—	0.8	—	2.7	1.9	1.0	—	1.0	0.9
C	1.5	—	—	2.0	—	0.9	—	—	4.8	3.0	—	1.7	—	—	—	—	—	—	—
D	—	—	—	—	—	—	—	—	—	—	1.3	0.3	—	—	—	—	—	—	—
E	9.1	—	5.0	11.0	7.0	7.4	10.3	4.5	6.3	15.0	8.0	9.4	7.0	8.2	16.2	11.0	15.4	8.0	11.3
Chromosomes studied	66	37	80	100	43	326	58	67	63	100	75	363	43	110	105	110	91	100	549
Third Chromosome																			
None	22.7	29.7	23.8	17.0	39.5	24.2	25.9	26.9	31.7	18.0	25.3	24.8	30.2	11.8	15.2	14.0	12.1	16.0	15.1
A *or* B	51.5	59.5	60.0	54.0	41.9	54.0	50.0	37.3	54.0	53.0	46.7	48.5	32.6	47.3	63.8	60.0	60.4	51.0	54.5
A + B	9.1	—	1.2	8.0	2.3	4.9	1.7	3.0	1.6	14.0	13.3	7.7	16.3	14.5	7.6	12.0	12.1	12.0	12.0
D	—	—	—	—	—	—	—	—	—	3.0	1.3	1.1	—	0.9	—	3.0	8.8	1.0	2.4
E + F	—	—	—	—	—	—	—	—	—	—	—	—	—	3.6	—	—	—	3.0	1.2
F	—	—	—	2.0	2.3	0.9	3.4	—	3.2	8.0	4.0	4.1	2.3	6.4	3.8	6.0	5.5	4.0	4.9
H	—	2.7	—	7.0	2.3	2.8	1.7	1.5	3.2	9.0	6.7	5.0	7.0	1.8	11.4	13.0	16.5	6.0	9.3
J	43.9	32.4	38.7	41.0	41.9	40.2	46.6	52.2	44.4	50.0	48.0	48.5	39.6	43.6	46.7	43.0	41.8	41.0	43.0
L	—	—	—	1.0	—	0.3	—	—	—	—	1.3	0.3	—	—	1.9	2.0	—	—	0.7
M	—	—	—	—	—	—	—	—	1.6	—	—	0.3	—	—	—	—	—	—	—
L + M	—	2.7	—	4.0	2.3	1.8	1.7	1.5	3.2	8.0	5.3	4.4	7.0	14.6	3.8	11.0	14.3	5.0	9.5
Chromosomes studied	66	37	80	100	43	326	58	67	63	100	75	363	43	110	105	100	91	100	549

TABLE 2. *Degree of homogeneity of the frequencies of certain inversions at different seasons*

Chromosome and inversion	Vila Atlantica		Mogí		Pirassununga	
	χ^2	P	χ^2	P	χ^2	P
XL, Inv. H	2.60	0.6	7.08	0.15	4.53	0.5
XR, Inv. D	1.73	0.8	14.02	0.008	4.58	0.5
II L, Inv. C	2.35	0.7	10.07	0.04	9.43	0.09
II L, Inv. F	0.92	0.9	4.34	0.35	3.01	0.7
II L, Inv. H	3.89	0.4	1.80	0.75	7.32	0.2
II R, Inv. E	5.39	0.25	5.92	0.2	6.20	0.3
III, A or B	1.98	0.75	2.61	0.6	10.26	0.07
III, A + B	9.62	0.05	15.93	0.003	2.92	0.7
III, J	0.87	0.9	0.50	0.95	0.60	0.95
III, H	—	—	—	—	15.10	0.01
III, L + M	—	—	—	—	11.40	0.04
Degrees of freedom	4		4		5	

the percentages of all inversions in a sample are often greater than 100 per cent.) In all, 32 different inversions (out of the 40 known in the species) occur in the three localities under consideration. Some of them are common, and others rare. The observed frequencies of the inversions fluctuate in the different samples taken in the same locality in different months. The statistical significance of these fluctuations can be evaluated by computing chi-squares for homogeneity. Such computations have been made for all inversions which are frequent enough in the samples to make the application of the chi-square method free of difficulties. (No tests were made for inversions D and E in chromosome II L because these inversions are not easily distinguishable and their recorded frequencies may be inaccurate.) The results are summarized in table 2.

For most of the inversions, the frequencies observed in samples taken at different times in a given locality are alike within the limits of sampling errors. Inversion D in the XR chromosome at Mogí, A + B in the third chromosome at Mogí, and H and perhaps L + M in the third chromosome at Pirassununga are exceptions because the frequencies of these inversions varied significantly during the period of observation.

Since our observations extended only from September, 1948, to June, 1949, we do not know whether the observed changes in the frequencies of the inversions are a part of a seasonal cycle, or whether these changes reflect other than seasonal alterations in the environment. Both seasonal and non-seasonal changes have been observed in *D. pseudoobscura* and *D. robusta*. As an attempt to get additional information bearing on this point, the data in Table 1 have been re-calculated by summing up, for each locality and inversion, the records for the hot months (December–March), and for the cooler months (September–November and April–June). Chi-squares, having each one degree of freedom, were then computed for these hot season-cool season comparisons. If the frequencies of some inversions varied seasonally one could expect that they would be different during climatically most distinct seasons. In reality, not one of the 31 chi-squares came up to the 5 per cent probability level, not even for those inversions which gave significant heterogeneities in comparisons of individual samples. This suggests, although it does not prove, that the observed changes are not seasonal.

It is commonplace that seasonal changes in the purely physical parameters of the environment (temperature, length of day,

etc.) are smaller in the tropics than in the temperate zones, and some tropical climates, such as that of Vila Atlantica, are more constant than others, such as that of Pirassununga. The indicated absence of seasonal changes in the frequencies of inversions may, then, seem trivial. In reality, the environment is far from seasonally constant even at Vila Atlantica because of changes in biotic parameters, such as the availability of different species of fruits on which the flies feed. Dobzhansky and Pavan (1950) have shown that the relative frequencies of the 30 to 35 species of Drosophila which occur at Vila Atlantica, Mogí, and Pirassununga, undergo changes from month to month, which are at least as great as the changes recorded by Patterson (1943) for species of *Drosophila* in Texas. It is, however, an open question whether the changes in the relative abundance observed in the Brazilian localities are cyclic and seasonal. Some of the data of Dobzhansky and Pavan indicated that they are not regularly seasonal, and Dr. Pavan kindly informs us that his most recent observations tend in the same direction. When as many as 30 ecologically fairly similar species compete for a group of kindred habitats in the same locality, the biotic system is highly complex. Now, the environment varies not only from season to season but also from year to year, and, in fact, a given constellation of physical and biotic variables is never repeated. This makes regular cycles either in the relative abundance of species or in relative frequencies of inversions improbable in regions where many species share the same habitat.

The three localities in which periodic sampling has been done are in the state of São Paulo, at the southern margin of the tropical zone. It is, therefore, interesting that temporal variations in the relative abundance of *Drosophila* species occur also in the equatorial climate of Belem do Pará, where samples in the same neighborhood were taken in June, 1948, and in May and July–September, 1949 (Dobzhansky and Pavan, 1950). Analysis of the chromosomes of *D. willistoni* was made in the samples of May and of July–September, 1949. Despite the small number of individuals analyzed (77 and 78, respectively), one of the inversions, namely F in the third chromosome, proved to be significantly more frequent in the May than in the July–September samples ($\chi^2 = 7.08$, with 1 degree of freedom, $P = 0.008$). The reverse situation obtained for the subbasal inversions (A and B) in II L chromosome, which were less frequent in May than in July–September ($\chi^2 = 8.91$).

Indication of Adaptive Superiority of Inversion Heterozygotes

Apart from the seasonal changes in the frequency of inversions, the evidence that the inversions found in natural populations are adaptively important comes from two sources. First, experiments on artificial populations kept in population cages in the laboratory show that, under certain conditions, natural selection modifies the frequencies of the inversions (Wright and Dobzhansky, 1946; Dobzhansky, 1947 for *D. pseudoobscura;* Dubinin and Tiniakov, 1947 for *D. funebris;* Spiess, 1950 for *D. persimilis;* Levitan, 1950 for *D. robusta*). Secondly, in *D. pseudoobscura,* inversion heterozygotes have been shown to be more frequent in nature, compared to homozygotes, than they would be if their viabilities were alike (Dobzhansky and Levene, 1948). Neither type of evidence is yet available for *D. willistoni,* so that our assumption of adaptive importance of the inversions in this species rests almost entirely on analogy with the species which have been studied in this respect. In no species have naturally occurring inversions proven to be adaptively neutral. The only more direct evidence for *D. willistoni* is as follows:

If homozygotes and heterozygotes for an inversion are equally viable between the egg and the adult stage, the highest frequency which the heterozygotes can reach in a panmictic population is 50 per

TABLE 3. *Frequencies (in per cent) of inversions and numbers of chromosomes in different regions*

Inversion \ Region	1. Costa Rica	2. Rio Branco Savanna	3. Rio Branco Forest	4. Rio Negro Amazonas	5. Marajo Island	6. Belem, Para	7. Western Acre	8. Eastern Acre	9. Guapore	10. Santa Cruz Bolivia	11. Palma Goyaz	12. Monjolinho Goyaz	13. Carolina Maranhão	14. Imperatriz Maranhão	15. Catuni, Bahia	16. Pirassununga São Paulo	17. Mogi São Paulo	18. Vila Atlantica São Paulo	19. Paranagua Parana	20. Iguassu Parana	21. Reuter, Rio Grande Sul	22. Santo Angelo Rio Grande Sul
XL Chromosome																						
None	62.5	76.7	72.0	42.4	100.0	96.1	74.7	56.4	61.5	37.5	34.1	25.3	17.6	53.4	72.6	67.0	61.9	74.4	88.8	93.0	100.0	94.7
A *or* B	12.5	2.3	—	9.1	—	3.0	—	—	—	50.0	20.5	42.1	5.9	3.4	1.8	2.6	2.9	3.7	—	2.3	—	—
D	—	—	4.0	9.1	—	1.0	—	—	—	12.5	36.4	30.5	17.6	—	25.7	—	—	—	—	—	—	—
E	37.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F	—	—	—	—	—	1.0	2.7	17.9	23.1	—	—	—	5.9	3.4	—	1.0	1.2	—	—	—	—	—
E + F	—	2.3	—	6.1	—	—	22.7	25.6	15.4	—	—	—	—	—	—	—	—	—	—	—	—	—
D + E	—	9.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
G	—	—	—	—	—	—	—	—	—	—	6.8	8.4	—	—	—	—	—	—	—	—	—	—
F + G	—	—	—	—	—	—	—	—	—	—	6.8	3.6	35.3	3.4	—	—	—	—	—	—	—	—
D + F + G	—	4.6	—	9.1	—	1.0	—	—	—	—	11.4	20.5	29.4	39.6	—	—	0.4	0.5	—	1.2	—	—
H	—	—	—	—	—	—	—	—	—	—	29.5	42.1	5.9	—	—	25.4	34.4	22.3	11.1	3.5	—	5.3
D + H	—	7.0	24.0	36.4	—	—	—	—	—	—	4.6	—	—	—	—	—	—	—	—	—	—	—
H + I	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.7	1.2	1.4	—	—	—	—
XR Chromosome																						
None	87.5	65.1	92.0	69.7	73.7	92.2	93.3	87.2	69.3	100.0	29.5	36.8	47.1	65.5	100.0	75.9	62.3	75.3	88.9	96.5	50.0	100.0
A *or* B	—	2.3	4.0	21.2	—	6.9	2.7	5.2	7.7	—	59.1	36.8	—	31.0	—	1.1	5.7	0.9	5.5	1.2	20.0	—
C	—	—	—	—	—	1.0	—	—	—	—	—	6.2	5.9	—	—	—	—	—	—	—	—	—
D	—	2.3	4.0	9.1	—	—	4.0	7.7	23.1	—	27.3	36.8	—	—	—	18.8	26.2	21.9	5.5	—	20.0	—
C + D	12.5	30.2	—	—	26.3	—	—	—	—	—	—	50.0	47.1	3.4	—	—	—	—	—	—	—	—
E	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.4	8.2	1.9	—	3.5	0.0	—
X-chromosomes studied	8	43	25	33	19	102	75	39	13	8	44	83	17	58	113	382	244	215	18	86	10	19
II L Chromosome																						
None	40.0	38.3	72.2	25.8	67.6	33.5	28.3	16.4	5.9	15.4	8.3	7.0	9.4	6.0	82.0	12.7	15.2	26.1	33.3	23.9	40.0	77.8
A *or* B	10.0	17.3	11.1	29.0	17.7	18.7	33.3	35.8	41.2	30.7	40.3	39.1	37.5	22.0	—	22.2	11.8	7.4	20.8	20.9	—	—
A + B	—	22.2	16.7	27.4	11.8	27.1	19.2	11.9	17.6	—	26.4	8.6	21.9	16.0	1.3	—	—	—	—	—	—	—
C	10.0	9.9	—	—	2.9	0.6	—	—	17.6	7.7	6.9	7.8	6.2	2.0	—	15.8	18.5	13.2	16.7	7.5	33.3	3.7
D	—	11.1	—	11.3	—	—	5.1	6.0	29.4	15.4	8.3	47.7	40.6	2.0	—	43.5	39.9	31.9	20.8	37.3	33.3	3.7
E	20.0	1.2	2.8	—	2.9	0.6	11.1	6.0	29.4	15.4	26.4	28.1	12.5	—	15.3	34.6	25.9	26.4	16.7	29.1	13.3	—
F	40.0	17.3	8.3	33.9	17.6	25.8	43.4	50.7	41.2	84.6	54.2	50.0	62.5	51.0	2.7	48.8	45.2	42.6	20.8	22.4	26.6	11.1
G	—	—	—	—	—	—	—	—	—	—	2.8	—	—	—	—	—	—	—	—	—	—	—
H	—	2.5	—	9.7	—	—	3.0	13.4	29.4	7.7	4.2	7.8	3.1	1.0	—	11.8	7.7	8.0	20.8	15.7	—	7.4

TABLE 3.—*Continued*

Inversion \ Region	1. Costa Rica	2. Rio Branco Savanna	3. Rio Branco Forest	4. Rio Negro Amazonas	5. Marajo Island	6. Belem, Para	7. Western Acre	8. Eastern Acre	9. Guapore	10. Santa Cruz Bolivia	11. Palma Goyaz	12. Monjolinho Goyaz	13. Carolina Maranhão	14. Imperatriz Maranhão	15. Catuni, Bahia	16. Pirassununga São Paulo	17. Mogi São Paulo	18. Vila Atlantica São Paulo	19. Paranagua Parana	20. Iguassu Parana	21. Reuter, Rio Grande Sul	22. Santo Angelo Rio Grande Sul
II R Chromosome																						
None	50.0	70.3	94.4	85.5	94.1	93.5	44.4	43.3	64.7	69.2	27.8	31.3	78.1	91.0	89.3	86.5	86.5	91.4	100.0	91.0	93.3	96.3
A	30.0	1.2	—	—	—	—	—	7.5	5.9	—	6.9	4.7	6.3	—	—	0.5	0.6	0.3	—	—	—	—
B	—	4.9	—	—	—	—	23.2	19.4	23.5	—	31.9	19.5	—	1.0	—	0.9	0.8	—	—	0.7	—	—
C	—	6.2	—	—	—	1.3	2.0	6.0	11.8	—	9.7	10.9	9.4	3.0	—	—	1.7	0.9	—	0.7	—	—
D	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.3	—	—	—	—	—
E	30.0	19.8	5.6	14.5	5.9	5.2	43.4	35.8	5.9	30.8	47.2	53.9	9.4	5.0	10.7	11.3	9.4	7.4	—	7.4	6.7	3.7
III Chromosome																						
None	40.0	17.3	27.7	11.3	64.7	32.3	11.1	6.0	5.9	23.1	6.9	3.1	3.1	14.0	80.0	15.1	24.8	24.2	20.8	13.4	26.7	29.6
A *or* B	—	34.6	11.1	25.8	8.8	15.5	58.6	62.7	23.5	53.8	40.3	61.7	59.4	37.0	1.3	54.5	48.5	54.0	37.5	54.5	33.3	37.0
A + B	—	7.4	5.5	1.6	—	1.3	—	1.5	11.8	—	6.9	8.6	—	—	—	12.0	7.7	4.9	20.8	24.6	13.3	14.8
C	—	1.2	—	—	—	12.9	—	—	—	—	2.8	1.6	—	5.0	—	—	—	—	—	—	—	—
D	20.0	27.2	27.7	38.7	17.6	13.5	17.2	17.9	41.2	23.1	16.7	28.9	18.8	45.0	0.7	2.4	1.1	—	—	—	—	—
E	—	3.7	—	—	—	3.2	—	—	—	—	2.8	0.8	—	—	—	1.2	—	—	—	—	—	—
F	20.0	13.3	27.7	33.9	23.5	40.0	41.4	35.8	64.7	15.4	51.4	54.7	37.5	26.0	0.7	6.1	4.1	0.9	—	1.5	—	3.7
G	—	1.2	2.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
H	10.0	29.6	27.7	46.8	—	7.7	57.5	65.7	82.4	61.5	55.6	61.7	65.6	39.0	6.0	9.3	5.0	2.8	—	3.7	—	3.7
I	—	—	—	—	—	—	—	—	—	—	1.4	0.8	9.4	—	—	—	—	—	—	—	—	—
J	20.0	35.8	16.7	25.8	14.7	38.7	5.0	4.5	—	15.4	4.2	5.5	21.9	50.0	13.3	43.0	48.5	40.2	41.7	44.8	40.0	59.3
K	—	—	—	—	—	—	—	—	—	—	1.4	—	—	—	—	—	—	—	—	—	—	—
L	30.0	12.3	30.6	29.0	—	0.6	—	—	—	—	15.3	12.5	21.9	—	—	0.7	0.3	0.3	—	—	—	—
M	—	—	—	1.6	—	—	—	—	—	—	15.3	9.4	—	—	—	—	0.3	—	—	—	—	—
L + M	—	9.9	—	1.6	—	0.6	51.5	55.2	41.2	23.1	29.2	44.5	21.9	17.0	0.7	9.5	4.4	1.8	—	1.5	—	—
Autosomes studied	10	81	36	62	34	155	99	67	17	13	72	128	32	100	150	549	363	326	24	134	15	27

cent. This occurs if the two alternative gene arrangements differing in the inversion are equally common, i.e., have gametic frequencies equal to 0.5. Yet, in the population samples studied by ourselves frequencies above 50 per cent are recorded for some inversions (tables 1 and 3). In most cases the excess over 50 per cent is not statistically assured. However, inversion H in the third chromosome showed frequencies higher than 50 per cent in all samples from a large territory which extends from Santa Cruz de la Sierra, in Bolivia, to Acre, Guaporé, Goyaz, and Carolina, in Maranhão (table 3). The deviations from 50 per cent are more than twice as large as their standard errors for Eastern Acre, for Guaporé, and for Monjolinho, in Goyaz.

Geographic Distribution of Inversions

Most of the inversions recorded in the chromosomes of *D. willistoni* are distributed very widely. Although the sample studied from Costa Rica consisted of only 10 flies, and was studied two generations after capture, it contained 11 autosomal inversions, 7 of which occur also throughout Brazil, including the southernmost state of Rio Grande do Sul. The remaining 4 inversions do not seem to reach Rio Grande do Sul, but go at least as far as the state of São Paulo, on the Tropic of Capricorn (cf. table 3 and fig. 1). Vice versa, among the 42 flies studied from Rio Grande do Sul, 11 autosomal inversions were found, every one of which occurs throughout Brazil, including the equatorial Amazonian regions.

A few of the inversions seem to be endemic (XL–E in Costa Rica, XL–I in São Paulo, II L–G in Goyaz, II R–D in São Paulo, III–G in the territory of Rio Branco, III–K in Goyaz) but most of these endemics are rare even where they have been found. The great amplitude of the geographic distribution of the inversions in *D. willistoni* exceeds what is known in such "natural" species as its relative *D. paulistorum,* or as *D. pseudoobscura, D. robusta,* and *D. persimilis,* and compares with the situation in "domestic" species like *D. ananassae* and *D. melanogaster.* However, in the latter the wide distribution of inversions is due to transport by man.

Nevertheless, populations of *D. willistoni* are differentiated into geographic races which differ in relative frequencies of certain inversions (table 3). Thus, the samples from Acre contain many inversions, F and E + F in XL, B in II R, and H and L + M in III. Flies from northwestern Amazonia (Rio Branco, Rio Negro) frequently have the combination of the inversions D + H, but not E + F, in XL chromosome, little B in II R, and much L but little L + M in III. The savannas and forests of central and north-central Brazil (Goyaz, Maranhão) have high concentrations of many inversions, among which A, B, D, and D + F + G in XL, B, C, and E in II R, and H in III are conspicuous. São Paulo has much inversion H in XL, D in XR, no A + B in II L, little D, F, H, L, and M, but much J in III. The extreme south of Brazil (Paraná, Rio Grande do Sul) resembles São Paulo, but has less H in XL and much A + B in III. The small samples from Bolivia and from the territory of Guaporé (Porto Velho) seem to resemble Acre, while Marajó is related to Pará, but fails to include some inversions common in the latter territory.

Since the data in table 3 indicate the frequencies of inversion *heterozygotes* they do not reflect the full extent of the racial differentiation of the species. Absence in geographically remote regions of heterozygotes for a certain inversion may be due to the populations of these regions being uniformly homozygous for different gene arrangements. To detect such "racial" gene arrangements, some strains from different regions were crossed to the standard strains from Belem do Pará and from Marajó Island. If the strains crossed differ in gene arrangement, the hybrids will show heterozygous inversions.

Such crosses have indeed shown that populations of *D. willistoni* in different parts of the distribution area have become homozygous for different gene arrangements in some chromosomes. Thus, all hybrids between the standard strain and strains from southern Brazil (São Paulo, Paraná, Rio Grande do Sul) are heterozygous for the inversions C and D, but usually not for B, in XR chromosome. If the standard gene arrangement is denoted bcd, the south Brazilian populations are bCD (although some bCd individuals also occur). In central and northern Brazil all the alternatives of B-b, C-c, and D-d are found. Hybrids of standard with Guatemala (1 strain) and central Mexican strains (4 strains from Axtla, San Luis Potosí, studied) have inversion B but not C or D. Central American strains are, therefore, mostly Bcd. In the XL chromosome, hybrids between standard and south Brazilian strains have the quintuple inversion BDFGH (Plate 1). This indicates that the south Brazilian race is predominantly BDFGH in XL chromosome, while the Pará race, in equatorial Brazil is bdfgh. In central Brazil (Goyaz, Maranhão) the population is mixed. The hybrids of standard and Central American strains are heterozygous for inversions BCD, showing that the Central American race is mostly BCD, while the Brazilian races are all c. In the autosomes, there is relatively little differentiation, although the standard (Pará race) seems to be rather exceptional in predominance of the e gene arrangement

TABLE 4. *Mean numbers of heterozygous inversions per individual* Drosophila willistoni *in different regions of Brazil and in Costa Rica*

Region	Mean	Limits	Region	Mean	Limits
1. Costa Rica	♀3.25±0.85	2–4	12. Monjolinho, Goyaz	♀9.36±0.26	2–14
	♂2.00±0.71	1–3		♂6.56±0.31	2–10
2. Rio Branco, Savanna	♀4.77±0.43	0–11	13. Carolina, Maranhão	♀8.65±0.58	4–13
	♂3.00±0.23	0–7		♂4.33±0.42	1–7
3. Rio Branco, Mucajaí	♀2.67±0.46	0–8	14. Imperatriz, Maranhão	♀5.47±0.37	1–12
	♂2.50±0.49	0–5		♂3.24±0.21	1–6
4. Rio Negro, Amazonas	♀5.03±0.49	1–9	15. Catuní, Bahía	♀0.81±0.03	0–5
	♂4.03±0.36	0–7		♂0.42±0.05	0–2
5. Marajó Island	♀2.05±0.48	0–7	16. Pirassununga São Paulo	♀4.13±0.10	0–11
	♂0.93±0.43	0–5		♂3.52±0.13	0–8
6. Belem, Pará	♀2.85±0.18	0–7	17. Mogi, São Paulo	♀3.97±0.13	0–13
	♂2.53±0.23	0–7		♂2.92±0.34	0–9
7. Western Acre	♀4.68±0.26	0–9	18. Vila Atlantica São Paulo	♀3.06±0.12	0–9
	♂4.58±0.41	0–8		♂2.34±0.12	0–7
8. Eastern Acre	♀5.49±0.31	1–9	19. Paranaguá, Paraná	♀2.61±0.39	0–5
	♂4.86±0.35	1–9		♂2.50±0.68	0–4
9. Guaporé	♀6.39±0.66	1–10	20. Iguassú, Paraná	♀3.20±0.19	0–8
	♂5.25±0.99	3–7		♂2.81±0.22	0–7
10. Santa Cruz, Bolivia	♀5.50±0.73	3–8	21. Reuter, Rio Grande do Sul	♀2.90±0.75	0–7
	♂3.00±1.22	0–7		♂2.40±0.53	1–4
11. Palma, Goyaz	♀9.11±0.44	3–16	22. Santo Angelo, Rio Grande do Sul	♀1.90±0.24	0–4
	♂5.57±0.36	2–9		♂1.25±0.42	0–3

in the II R chromosome, while other regions have chiefly E. Further studies are needed to map the distribution of the chromosomal races in *D. willistoni.*

Geographic Variation in the Frequency of Inversion Heterozygosis

Most interesting and significant proved to be the geographic variations in the average number of heterozygous inversions per individual. The data are summarized in table 4. The observed numbers of heterozygous inversions are higher in females than in males for the simple reason that, having only one X chromosome, a male cannot be heterozygous for any inversion in that chromosome.

Examination of table 4 and the maps in figure 1 and figure 2 shows that the frequencies of heterozygous inversions vary greatly from region to region. In the desert (caatinga) of the state of Bahia a female is heterozygous, on the average, for 0.8 inversions, while in the savannas of central Brazil, in Goyaz, this number is more than ten times as high; a Bahia male carries only some 0.4 heterozygous inversions, compared to about 6 in a Goyaz male. All intermediate values also occur.

The highest frequencies of inversion heterozygosis occur in the central portion of the geographic distribution of the species, in central and northern Brazil—from Goyaz to Acre, and from Maranhão to the territory of Rio Branco, north of the Equator. The frequencies of heterozygosis decline away from this central region, and reach minimal values in the deserts of northeastern Brazil (Bahia), in southern Brazil (Rio Grande do Sul), and in Costa Rica (nothing is known about the situation in Central America, Mexico, and the West Indies). The centripetal gradients are, however, by no means regular: the lowest frequency of inversions is observed in Bahia, which is relatively close to the maximal frequency in Goyaz. A low frequency is found also in Belem, Pará, and Marajó Island; the forested part of the territory of Rio Branco (Mucajaí) has significantly fewer inversions than regions north and south of there (savanna of Rio Branco and Rio Negro). These "irregularities" in the gradients are most significant and, as we hope to show, revealing.

Discussion

Our fundamental assumption is that the chromosomal inversions found in natural populations of *D. willistoni,* either because of position effects or, more likely, because of the genes they carry, influence the adaptive properties of their carriers. This assumption is justified by analogy with the situation in other species of *Drosophila* for which experimental data are available, by the observed temporal changes in some Brazilian populations, and, finally, by some inversion heterozygotes reaching frequencies in natural populations in excess of 50 per cent which would be impossible if the homozygotes and heterozygotes were equally viable. If this assumption is granted, the degree of variability of chromosome structure becomes one of the measures of the adaptive versatility of populations. Now, the data disclose great differences in the amount of chromosomal variability in different bioclimatic regions of Brazil. These differences require explanation.

Superficially considered, the only regularity which appears from the data is that the degree of variability is greatest in populations which reside in the central part of the distribution area of the species, and falls off away from this center. According to Vavilov's (1926) theory of centers of origins of cultivated plant species, the region of the highest diversity of genetic types should be the territory in which the species arose and from which it spread elsewhere. The basin of the Amazon, and particularly that of the Tocantins, would, according to this view, represent the center of origin of *D. willistoni.*

It must be noted, however, that the genetic variability on which Vavilov based

his generalization was, or was supposed to be, adaptively neutral. Vavilov's conception was essentially that the degree of accumulation of neutral genetic variants is more or less proportional to the time during which a numerically large population occupies a territory. This view is not directly applicable to adaptively effective variants, especially to variants acted upon by selective forces as powerful as those affecting inversions in natural populations of *Drosophila*. The distribution of such inversions in space will be governed by natural selection, and not by mutation pressure or by genetic drift. Vavilov's idea must be supplemented.

We submit the working hypothesis that the amount of adaptive polymorphism carried in a population tends to be proportional to the variety of habitats (ecological niches) which this population exploits in a territory it occupies. Now, the evolutionary process which produces adaptive polymorphism, and thereby permits the population to penetrate and to control more and more habitats, requires time. Other things being equal, the longer a territory is occupied by a species the greater will be the supply of adaptive variants, and the greater the variety of ecological niches conquered by the species. As the species expands its geographic distribution, it invades territories in which it is able to occupy progressively fewer habitats. At the margins of the distribution area, unless the species is stopped by an impenetrable geographic barrier, the species has a toehold in only few ecological niches, because it has not had time to evolve adaptive polymorphism that would permit it both to expand its control of the environment locally and to spread further geographically. It is very important in this connection that the gene contents of the same inversion in *D. pseudoobscura* are known to be adaptively different in different parts of the distribution area. This means that an inversion modifies its gene contents in accordance with the adaptive requirements of each geographic region. The fact that many of the inversions of *D. willistoni* occur at least from Costa Rica to southern Brazil certainly does not mean that these inversions are similar in gene content throughout this enormous territory.

The limitation of Vavilov's theory is clearly that the polymorphism, in so far as it is adaptive, is more directly related to the number of ecological niches occupied than to the antiquity of the population itself. It is conceivable that a species arises in an ecologically rather uniform territory, spreads to one containing a large variety of ecological niches, and after some time develops a greater adaptive polymorphism in the latter than in its former home. It is a suggestive fact that the greatest polymorphism in *D. willistoni* is found in the savannas and forests of the basin of the Amazon, a region of one of the richest and most diversified fruit producing floras in the world; *D. willistoni* develops in nature on a great variety of fermenting fruits (Dobzhansky and Pavan, 1950). Geologically, the valley of the Amazon is a new territory, of late Tertiary and Quaternary origin, while central and southern Brazil (Goyaz, São Paulo, Paraná, Bahia and the part of Maranhão along the Tocantins) are more or less ancient. The degree of polymorphism is, thus, not correlated with the geological age of the terrain where a population now lives, but rather with the variety of habitats available there.[2] The information at our disposal does suggest that

[2] It may be interesting to consider from this point of view another generalization, similar in principle to that of Vavilov, set forth even earlier by Matthew (1915). Matthew believed that phyletically advanced types of land animals arise chiefly in the central portion of the Holarctic land mass, while the more primitive forms are pushed out and preserved at continental peripheries and on islands. This may mean that continental centers are biotically more complex than peripheries, and consequently offer more varied challenges to which organisms may respond by adaptive evolutionary changes. The more limited diversity of organic types in peripheral and island biota permits lower rates of evolutionary advance.

the "center of origin" of *D. willistoni* is somewhere in the tropical part of South America, but it does not permit of more precise localization.

Consideration of the variety of ecological niches available to *D. willistoni* in different parts of its distribution area helps to understand the observed variations in the extent of chromosomal polymorphism in the regions studied. Although the southern boundary of the species area is not known, it is unlikely that it extends far southward into Argentina and Uruguay; in the northern Hemisphere *D. willistoni* does not live much outside the tropical zone. The populations of Rio Grande do Sul and Paraná are, thus, geographically marginal, and the low frequency of inversion heterozygotes in them is expected on this basis. Furthermore, *D. willistoni* was a relatively rare species in the two collections made in Rio Grande do Sul, which again suggests that it occupies only a limited variety of ecological niches there. More collecting would, however, be needed to establish this point securely.

Another, and very striking, fact is the low frequency of inversions observed in the interior of Bahia, in northeastern Brazil. This is a region which is climatically close to the limit of toleration for most species of *Drosophila.* Little precipitation falls during most of the year in the "caatinga" deserts, and the short but intense rainy season is quite variable from year to year (Anonymous 1941). The peculiar trees and bushes of this region mostly shed their foliage and become dormant during the severe and prolonged dry season. We have collected mainly in a range of low mountains outside the true caatinga, and yet found there fewer species of *Drosophila* than in any other of the 15 regions of Brazil in which collection was made on a comparable scale. Furthermore, by far the commonest species on the caatinga is *D. nebulosa,* and *D. willistoni* is relatively rare (Dobzhansky and Pavan, 1950). It is clear that the caatinga is ecologically inhospitable to *D. willistoni* which controls only a small array of habitats there, and carries little chromosomal variability. A contrasting situation is found a few hundred kilometers westward, in Goyaz and Maranhão. This is a region of exuberant savanna and gallery forests, in which, despite a pronounced dry season, there is no general dormancy of the vegetation as on the caatinga. A considerable variety of fruits mature throughout the year, and our collecting on them revealed a dense and diversified fauna of species of *Drosophila,* among which *D. willistoni* is the dominant species for at least a part of the year (*D. nebulosa* being the chief competitor). The chromosomal polymorphism reaches its maximum in Goyaz; a female larva heterozygous for 16 inversions, which is the highest number found in any species, was found in the progeny of Goyaz flies (table 4).

Seasonal diversity, vs. relative uniformity of the climate, might be expected to enhance the variety of habitats available for *Drosophila,* and consequently the extent of the chromosomal polymorphism. For a time we believed that our accumulating data on *D. willistoni* supported this hypothesis, only to discover later that the facts fit another view even better, namely, that the amount of polymorphism is greater in abundant and widespread species, and less in ecologically subordinate forms. Indeed, the greatest polymorphism is observed in Goyaz (table 4) which is also characterized by alternation of very arid and rainy seasons. The polymorphism decreases westward (Goyaz—Guaporé—eastern Acre—western Acre), as well as northward (Goyaz—Maranhão—Pará) and southward (Goyaz—Pirassununga—Mogí—Vila Atlantica, table 4), and in every case the seasonal climate variations become less and less pronounced (anonymous, 1941). Western Acre, Pará and Vila Atlantica have relatively equable climates.

The correlation between seasonal climatic changes and chromosomal polymorphism breaks down in Bahia, and es-

pecially on Rio Negro and Rio Branco. Table 4 and figure 1 show that the frequency of inversions is high on Rio Negro, which is the region of one of the most exuberant rainforests in the world and one of the most equable climates in the Western Hemisphere. An only slightly more limited polymorphism is found in the equatorial savanna of Rio Branco (along Rio Uraricoera), where the months from May to July have more than 300 mm. rainfall each, while the months from December to March have less than 50 mm. each. The forested zone of the territory of Rio Branco (along Rio Mucajaí) is intermediate both geographically and climatically between Rio Negro and Uraricoera, and yet it has significantly less chromosomal polymorphism than either of the others. The situation becomes comprehensible if one takes into consideration that *D. willistoni* is a less common species on Mucajaí than its near relative, *D. paulistorum,* while on Rio Negro and in the savannas of Rio Branco *D. willistoni* is the predominant species. The map in figure 2 shows graphically the proportions of *D. willistoni, D. paulistorum, D. tropicalis* and *D. equinoxialis* found in the various collecting regions. These four sibling species are very close morphologically, at least sometimes live together on the same fruits, and may be regarded most immediate ecological competitors (Burla *et al.,* 1949; Dobzhansky and Pavan, 1950).

On Rio Negro about 49 per cent of the *willistoni*-like flies were *D. willistoni* and 43 per cent *D. paulistorum;* in the savanna the percentages are, respectively, 74 and 25; while on Rio Mucajaí *willistoni* is relatively rare, 15 per cent of the total, and *paulistorum* and *equinoxialis* dominant with, respectively, 60 and 25 per cent of the total population. It is clear that, if several related species subdivide among themselves the habitats available in a given region, then each of them will dominate fewer habitats than it might if it were the sole possessor of the environment. And although the population density of a species is not necessarily proportional to the number of ecological niches it controls, by and large the more numerous species may be presumed to be ecologically more versatile than its less abundant competitors. The less abundant species should have, in general, less genetic polymorphism to master the environment than a more abundant one. The relatively low variability of the Mucajaí population is, hence, expected.

A similar situation is observed in the state of São Paulo. At Pirassununga *D. willistoni* is predominant at all seasons (throughout the year, *willistoni* forms 95 per cent, and *paulistorum* 5 per cent of the total), and the population contains relatively many inversions. At Mogí, *willistoni* is dominant in winter but *paulistorum* is dominant in summer, and the inversions are fewer (the difference happens not to be statistically significant for females, but is significant for males, Table 4). At Vila Atlantica, *willistoni* is somewhat less common than *paulistorum* in winter and much less common in summer, and the concentration of inversions is significantly lower than in Mogí.

Most remarkable is the low concentrations of inversions at Belem and on Marajó Island, in Pará. The luxuriant equatorial rainforest near Belem assuredly contains a great variety of adaptive niches for *Drosophila,* and *willistoni*-like species have very dense populations there. However, in these populations, *D. paulistorum* is decidedly more abundant than *D. willistoni,* apparently throughout the year. At Belem, *D. willistoni* dominates only those ecological niches not controlled by the apparently stronger competitor, *D. paulistorum.* The Marajó Island sample came from two ecologically quite different regions: forests near Soure on the eastern coast, and patches of forest near Cape Maguarí. The latter region is a grass-covered plain submerged for several months each year under water, with tree vegetation confined to scattered sand hummocks. The species composition of the *Drosophila* fauna is here unlike any

other place visited, with *willistoni*-like species being a decided minority, and *D. tropicalis* being more common than *D. willistoni*. A small population sample collected in the Maguarí region showed the lowest frequency of inversion heterozygotes (less than 0.5 per female) ever recorded in *D. willistoni*.

The amount of adaptive polymorphism present in the population of a species in a given region is, in general, proportional to the variety of habitats which the species has mastered. The variety of habitats mastered is, however, a function of at least three variables: (1) The number of ecological niches potentially available to a given form of life in a given region. The ecological niches can hardly be enumerated at the present level of knowledge, but it is obvious that a region with a varied fruit-bearing flora can offer more ecological niches for a fruit-feeding insect like *Drosophila* than a region with a limited flora and few fruits. Similarly, predators and parasites may find numerous ecological niches in a region having many species of potential prey or hosts, and water dwelling forms in a region with diversified bodies of water. (2) A species is likely to control more ecological niches in a territory which it inhabits for a long time than in a recently colonized one (Vavilov's "center of origin" factor). (3) Presence of competing species with similar ecological requirements. Related species, if they occur in the same region, subdivide among themselves the available habitats so that each of them is able to exploit fewer habitats than it could have in the absence of competitors. Seasonal constancy or changeability of the climate in a physical sense (temperature, precipitation) appears to be a less important variable in the tropics than it is in temperate countries, although it doubtless influences the number of potentially available habitats. As shown by Dobzhansky and Pavan (1950), the biotic environment of tropical *Drosophila* is seasonally quite variable even in regions with rather uniform climate.

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Summary

Natural populations of *Drosophila willistoni* contain many individuals heterozygous for inverted sections in their chromosomes. At least 40 different inversions have thus far been recorded; inversions occur in every chromosome and chromosome limb; one individual has been found heterozygous for 16 inversions.

Most of the inversions are very widespread geographically. Nevertheless, the incidence of certain gene arrangements is quite different in different parts of the geographic range of the species. Every population can be described in terms of the relative frequencies of the gene arrangements it carries, and different populations are racially distinct.

The relative frequencies of gene ar-

rangements in populations of some localities vary from month to month. Whether these variations are cyclic and seasonal cannot be decided from the available data.

The mean numbers of heterozygous inversions per individual vary in different bioclimatic regions from 0.8 (deserts of Bahia) to 9 (central Goyaz). These variations can be accounted for on the supposition that the amount of chromosomal polymorphism, and in general the amount of adaptive genetic variability, is proportional to the variety of habitats which the species has mastered in the environment of a given region.

Literature Cited

Anonymous. 1941. Normais climatológicas. Serviço de Meteorologia, Ministério da Agricultura. Rio de Janeiro.

Burla, H., da Cunha, A. B., Cordeiro, A. R., Dobzhansky, Th., Malogolowkin, C., and Pavan, C. 1949. The willistoni group of sibling species of Drosophila. Evolution, **3**: 300–314.

Carson, H. L., and Stalker, H. D. 1947. Gene arrangements in natural populations of *Drosophila robusta* Sturtevant. Evolution, **1**: 113–133.

——. 1949. Seasonal variations in gene arrangement frequencies over a three-year period in *Drosophila robusta* Sturtevant. Evolution, **3**: 322–329.

Dobzhansky, Th. 1943. Genetics of natural populations. IX. Genetics, **28**: 162–186.

——. 1947a. Adaptive changes induced by natural selection in wild populations of Drosophila. Evolution, **1**: 1–16.

——. 1947b. A directional change in the genetic constitution of a natural population of *Drosophila pseudoobscura*. Heredity, **1**: 53–64.

——. 1948. Genetics of natural populations. XVI. Genetics, **33**: 158–176.

——. 1949. Observations and experiments on natural selection in Drosophila. Proc. 8th Internat. Congr. Genetics, Hereditas Suppl., 210–224.

——. 1950a. Genetics of natural populations. XIX. Genetics, **35**: in press.

——. 1950b. The chromosomes of *Drosophila willistoni*. J. Heredity, **41**: in press.

——, Burla, H., and da Cunha, A. B. 1950. A comparative study of chromosomal polymorphism in sibling species of the willistoni group of Drosophila. Amer. Natur., **84**: in press.

——, and Epling. 1944. Contributions to the genetics, taxonomy, and ecology of *Drosophila pseudoobscura* and its relatives. Carnegie Inst. Washington Publ., **554**: 1–183.

——, and Levene, H. 1948. Genetics of natural populations. XVII. Genetics, **33**: 537–547.

——, and Pavan, C. 1950. Local and seasonal variations in relative frequencies of species of Drosophila in Brazil. Journ. Animal Ecology, in press.

Dubinin, N. P., and Tiniakov, G. G. 1945. Seasonal cycles and the concentration of inversions in populations of *Drosophila funebris*. Amer. Natur., **79**: 570–572.

——. 1946a. Natural selection and chromosomal variability in population of *Drosophila funebris*. Jour. Heredity, **37**: 39–44.

——. 1946b. Inversion gradients and natural selection in ecological races of *Drosophila funebris*. Genetics, **31**: 537–545.

Levitan, M. Experiments on chromosomal variability in *Drosophila robusta*. (In press.)

Matthew, W. D. 1915. Climate and evolution. Ann. New York Acad. Sci., **24**: 171–318.

Pavan, C. 1946. Chromosomal variation in *Drosophila nebulosa*. Genetics, **31**: 546–557.

Patterson, J. T. 1943. The Drosophilidae of the Southwest. Univ. Texas Publ. **4313**: 7–216.

——, and Wagner, R. P. 1943. Geographical distribution of species of the genus Drosophila in the United States and Mexico. Univ. Texas Publ., **4313**: 217–281.

Spiess, E. 1950. Experimental populations of *Drosophila persimilis* from an altitudinal transect of the Sierra Nevada. Evolution, **4**: 14–33.

Stalker, H. D., and Carson, H. L. 1949. Seasonal variation in the morphology of *Drosophila robusta* Sturtevant. Evolution, **3**: 330–343.

Vavilov, N. I. 1926. Studies on the origin of cultivated plants. Leningrad.

Wright, S., and Dobzhansky, Th. 1946. Genetics of natural populations. XII. Genetics, **31**: 125–156.