

THE *WILLISTONI* GROUP OF SIBLING SPECIES OF *DROSOPHILA*

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INTRODUCTION

Species in sexual cross-fertilizing organisms are reproductively isolated populations. Such populations may or may not be distinguishable in morphological characteristics. Mayr (1942) has proposed the designation "sibling species" for species that are morphologically similar or identical. Camp and Gilly (1943) have called such species phenons, and other authors have referred to them as physiological species (Lancefield, 1929), cryptic species, etc. The theoretical interest of sibling species lies in that their existence shows that reproductive isolation may arise without divergence in morphological traits, and that physiological differences are not necessarily accompanied by morphological ones.

As disclosed especially by the work of J. T. Patterson and his school, the genus *Drosophila* contains several groups of closely related species with small morphological differences between them. The present article reports the results of a study of a cluster of four sibling species native to tropical America, that previously have been confused under the name of *Drosophila willistoni* Sturtevant.

Drosophila willistoni Sturtevant and *Drosophila paulistorum* Dobzhansky and Pavan, *sp.n.*

In 1896, Williston described *Drosophila pallida*, a species from the isle of St. Vincent, in the West Indies. The name

being preoccupied, Sturtevant changed it in 1916 to *willistoni*. Sturtevant (1921) and Patterson (1943) recorded this species from a fair number of localities in a territory extending from the Bahamas, Florida, Cuba, and Mexico, through the West Indies and Central America to Brazil.

In 1943, Dobzhansky and Pavan examined a series of cultures of *willistoni*-like flies descended from progenitors captured in the state of São Paulo, Brazil. These cultures fell into two classes, the flies in some cultures being larger than those in others. A more detailed examination revealed other minor morphological differences between the large-bodied and the small-bodied flies, and an examination of the salivary gland chromosomes suggested that these were different as well. This led to the inference that two distinct although morphologically very similar species were involved, one of them being *willistoni* Sturtevant and the other a new one, to which the name *paulista* Dobzhansky and Pavan was given. Since neither living nor preserved material from outside Brazil was available to Dobzhansky and Pavan in 1943, they conjectured that the species found more frequently among their cultures, which happened to be the small-bodied species, corresponded to *willistoni*, while the less frequent species was a new one, to which the name *paulista* was assigned. We now believe that this conjecture was an unfortunate one.

Through the courtesy of Professor J. T. Patterson, we received a culture of *willistoni*-like flies from Quirigua, Guatemala, and Professor John A. Moore kindly collected several strains for us at Axtla, in the state of San Luis Potosí, Mexico. These strains proved to inter-

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cross freely with the larger-bodied flies from Brazil. If the species described by Williston from the Isle of St. Vincent is identical with that found in Mexico and Guatemala, a nomenclatorial confusion must be faced, because, in accordance with the Rules of Nomenclature, the name *willistoni* Sturtevant would necessarily apply to the large-bodied Brazilian species to which the name *paulista* was given by Dobzhansky and Pavan. The name *paulista* would thus become a synonym of *willistoni*. The smaller Brazilian species, designated as *willistoni* by Dobzhansky and Pavan, would then require a new name.

The situation can not, however, be definitively settled without examination of living material from St. Vincent and of Williston's types, which are preserved in the British Museum. We have been unable to examine either, and there is no immediate prospect of our doing so; furthermore, it may not be possible from examination of Williston's long-dried specimens to decide which of the four sibling species (see below) they represent. Under the circumstances, we are adopting the following course as temporary expedient that reduces to a minimum the confusion in the existing literature. The name *willistoni* Sturtevant is applied to the larger of the two species found in the state of São Paulo. It is identical with that known to live in Guatemala and in Mexico (the *willistoni* of Patterson, 1943; Patterson and Mainland, 1944; Dobzhansky and Mayr, 1944; and Dobzhansky, 1946; but not of Dobzhansky and Pavan, 1943). For the smaller of the two species found in the state of São Paulo, to which, apparently erroneously, the name *willistoni* was applied by Dobzhansky and Pavan (1943), the name *paulistorum* is hereby proposed.

Drosophila paulistorum Dobzhansky and Pavan, *species nova*

Body smaller than in *Drosophila willistoni* Sturtevant, wings shorter, costal index lower, 4th vein and 5 × indices

higher, branches in the arista fewer, width of the front between the eyes greater. The base of the middle orbital bristle only a little posterior and very close to the base of the first orbital.

Type locality: Mogi das Cruzes, state of São Paulo, Brazil.

Type in the Departamento de Zoologia of the Museum of São Paulo.

Ten strains of *paulistorum* from Mogi das Cruzes, in the state of São Paulo, and nine strains of *willistoni*, from the same locality, have been identified by their salivary gland chromosomes, as described below. Five females and five males were taken from each strain, and the length of the body, wing length, costal index, 4th vein index, and 5 × index were measured and the number of branches in the arista counted. The flies measured were raised under optimal feeding and temperature conditions. The body length was measured as the sum of the distances from the antenna to the base of a halter and thence to the end of the anal tubercle. The data are summarized in table 1. It can be

TABLE 1. Comparison of certain traits in *Drosophila paulistorum* sp. n. and *Drosophila willistoni* Sturtevant

Trait	Species	M ± m	Limits
Body-length (mm.)	<i>paulistorum</i> ♀	2.44 ± 0.02	2.00-2.70
Body-length (mm.)	<i>willistoni</i> ♀	2.65 ± 0.02	2.20-3.00
Body-length (mm.)	<i>paulistorum</i> ♂	2.10 ± 0.014	1.85-2.30
Body-length (mm.)	<i>willistoni</i> ♂	2.18 ± 0.015	2.00-2.40
Wing-length (mm.)	<i>paulistorum</i> ♀	2.27 ± 0.013	2.10-2.40
Wing-length (mm.)	<i>willistoni</i> ♀	2.47 ± 0.013	2.30-2.60
Wing-length (mm.)	<i>paulistorum</i> ♂	2.01 ± 0.013	1.80-2.20
Wing-length (mm.)	<i>willistoni</i> ♂	2.15 ± 0.012	2.00-2.35
Costal index	<i>paulistorum</i> ♀	2.01 ± 0.02	1.80-2.25
Costal index	<i>willistoni</i> ♀	2.12 ± 0.02	2.00-2.35
Costal index	<i>paulistorum</i> ♂	1.87 ± 0.02	1.70-2.10
Costal index	<i>willistoni</i> ♂	2.00 ± 0.02	1.90-2.15
4th vein index	<i>paulistorum</i> ♀	2.03 ± 0.02	1.80-2.30
4th vein index	<i>willistoni</i> ♀	1.92 ± 0.02	1.60-2.20
4th vein index	<i>paulistorum</i> ♂	1.93 ± 0.02	1.70-2.10
4th vein index	<i>willistoni</i> ♂	1.90 ± 0.02	1.65-2.40
5 × index	<i>paulistorum</i> ♀	2.01 ± 0.03	1.70-2.25
5 × index	<i>willistoni</i> ♀	1.78 ± 0.03	1.55-2.10
5 × index	<i>paulistorum</i> ♂	1.90 ± 0.03	1.65-2.30
5 × index	<i>willistoni</i> ♂	1.88 ± 0.03	1.65-2.35
Branches of arista	<i>paulistorum</i> ♀	10.66 ± 0.08	10-12
Branches of arista	<i>willistoni</i> ♀	11.51 ± 0.07	10-12

seen that *paulistorum* has a smaller body, a shorter wing, lower costal index, higher 4th vein and $5 \times$ indices, and a lower number of branches in the arista than does *willistoni*. However, the variation in all these traits is sufficient to make the measurements of the two species overlap broadly. Additional, and more satisfactory, distinguishing characters are given below.

Drosophila willistoni strains, the specific identity of which has been ascertained either by hybridization with known strains of the same species or by cytological methods, have been found in the following localities: Mexico, San Luis Potosí: Axtla; Guatemala: Quiriguá; Brazil, Territory Rio Branco: Rio Uraricoera, north of Boa Vista, Rio Mucajaí south of Boa Vista; Amazonas: several localities along Rio Negro between the mouth of Rio Branco and Manaus; Territory of Acre: Rio Môa, Cruzeiro do Sul, Japiim, Palmares; Territory of Guaporé: Porto Velho; Isle of Marajó: Cape Maguari, Soure; Pará: Belem; Maranhão: Imperatriz, Carolina; Bahia: Salitre, Catuni; Goyaz: Palma, Monjolinho; Federal District: Rio de Janeiro; São Paulo: Pirassununga, Campinas, Mogi das Cruzes, São Paulo, Bertoga, Vila Atlantica, Itanhaem; Paraná: Paranaguá, Foz de Iguassu, Iguassu National Park; Rio Grande do Sul: Santo Angelo, Reuter; Bolivia: Santa Cruz de la Sierra; Argentina, Territory of Misiones, Iguassu.

Strains of *Drosophila paulistorum*, identified cytologically, have been collected in all the localities in Brazil and Bolivia in which *D. willistoni* has been found, except Catuni, and the places in the states of Paraná and Rio Grande do Sul; *D. paulistorum* has not been found in Mexico, Guatemala, or Argentina. The two species are, consequently, sympatric in a large territory including most of Brazil.

Drosophila equinoxialis Dobzhansky

A strain of flies from Tefé, Amazonas, which when collected was not distin-

guished from *willistoni*, proved to belong to a morphologically very similar but reproductively completely isolated species, described as *D. equinoxialis* Dobzhansky (1946). *D. equinoxialis* flies are similar to *D. willistoni*, although, as in the case of *D. paulistorum*, the variation curves overlap broadly. Professor H. T. Spieth discovered that the canal uniting the chitinous spermatheca with the vagina is cup-shaped in *willistoni* and vase-shaped in *equinoxialis* (Spieth, 1949).

Genetically or cytologically identified strains of *D. equinoxialis* have been collected in the following localities in Brazil. Territory of Rio Branco: Rio Uraricoera, north of Boa Vista, Rio Mucajaí; Amazonas: Tefé, several localities along the lower Rio Negro; Territory of Acre: Rio Môa, Japiim, Cruzeiro do Sul, Palmares; Territory of Guaporé: Porto Velho; Pará: Belem; Maranhão: Imperatriz, Carolina; Goyaz: Palma. The distribution area of *D. equinoxialis* is included in those of *D. willistoni* and *D. paulistorum*.

Drosophila tropicalis Burla and da Cunha, *species nova*

Male and female: Arista with 10–12 branches, 11 being the mean number. Antennae yellow, third segment darker, with a rather dense and long pilosity. Front dull yellow. Anterior orbitals slightly shorter than the posterior, middle one $\frac{2}{3}$ posterior. Two or three prominent orals. Face pale yellow. Carina short, gradually falling off below, not sulcate. Cheeks pale yellow, their greatest width about $\frac{1}{2}$ greatest diameter of eye. Eyes bright red with a short yellow pile.

Acrostichals in 6 rows, the lateral rows irregular. No prescutellars. Anterior scutellars parallel or slightly convergent. Thorax tannish-yellow, shining, pleurae lighter. Anterior sternopleural about $\frac{1}{2}$ posterior and much thinner, middle one slightly longer than the anterior. Legs pale yellow; apical bristles on first and second tibiae, preapicals on all three. Abdomen yellow with diffuse brown marginal bands not interrupted in the middle

and fading out laterally. Wings clear. Two prominent bristles at the apex of first costal section; third costal section with heavy bristles on its basal $\frac{1}{2}$. Costal index ♀ 1.9–2.2, ♂ 1.8–2; 4th vein index ♀ 1.9–2.1, ♂ 1.8–1.95; 5 × index ♀ 1.4–2.0, ♂ 1.3–1.7.

Length of body ♀ 2.4–2.7, ♂ 2.00–2.25 mm.; wings 2.2–2.8 mm. Two anterior and two posterior Malpighian tubes, ends free.

Testes yellow with 2 outer and 3 inner coils. Spermathecae spherical, weakly chitinized, with an indentation.

Ventral receptacle a long tube forming a flat spiral bent into a W-shaped plate resting on the vagina.

Eggs—two filaments greatly expanded and flattened distally, about $\frac{3}{5}$ as long as the egg itself.

Puparia—brownish yellow, horn very short, each anterior spiracle with 11–12 short branches.

Chromosomes—metaphase plate shows two pairs of V's, one of which is distinctly longer than the other, and a pair of rods.

Type in the Departamento de Zoologia of the Museum of São Paulo.

Remarks—The differences from *D. willistoni*, *D. paulistorum*, and *D. equinoxialis* are minor and overlapping, although doubtless significant statistically.

Geographic distribution — Brazil, Goyaz: Palma (type locality), Monjolinho; Territory of Rio Branco: Rio Uraricoera, north of Boa Vista, Rio Mucajá; Amazonas: several localities along the lower Rio Negro; Territory of Acre: Rio Mõa, Cruzeiro do Sul, Japiim, Palmares; Guaporé: Porto Velho; Island of Marajó: Cape Maguari; Pará: Belem; Maranhão: Imperatriz, Carolina; Bolivia: Santa Cruz de la Sierra. *D. tropicalis* is, thus, sympatric with the other three sibling species.

CHROMOSOMAL DIFFERENCES

The four sibling species have the same metaphasic chromosome groups: a pair of V-shaped X or Y-chromosomes, a pair of V-shaped second, and of rod-shaped third

chromosomes. In the nuclei of the larval salivary gland cells, there are five chromosome strands that can be recognized by the patterns formed by the stainable discs which they contain (Plate 1); the strands designated as XR and XL correspond to the X-chromosome and carry the sex-linked genes; IIR and IIL are the two branches of the second chromosome and carry the second linkage group; and III is the chromosome which is rod-shaped at metaphase and carries the third linkage group. (The correspondence between cytologically visible chromosomes and linkage groups has been established by examination of certain chromosomal aberrations in *D. willistoni*, unpublished data).

The disc patterns in the free terminal portions of the XR, XL, IIR and IIL chromosomes are sufficiently similar in the four sibling species, so that these chromosomes can easily be recognized as such in all four species. The fifth chromosome strand, which must represent the third chromosome, is however strikingly and characteristically different (Plate 1). In *D. willistoni* the free end of the third chromosome contains three fairly dark discs, followed by a short light area and by a group of four very dark discs; at about the middle of the length of the portion drawn in Plate 1, there is a long section that contains only faint discs composed of dots, and which in most cells appears as a somewhat swollen "bulbous" segment. In *D. paulistorum* and *D. equinoxialis*, the free end of the third chromosome forms a spindle-shaped "head" with a fairly dark disc in the tip and two dark discs in the "neck"; this is followed proximally by a long segment with only light discs which is, however, not swollen; proximally to that is a segment in which some dark discs appear, and another light and often swollen section (Plate 1). In *D. tropicalis* the free end is light and usually expanded fan-like, followed by a group of four very dark discs, and by a light and often much swollen area (Plate 1). The free end of the third chromosome conclusively serves to identify *D. wil-*

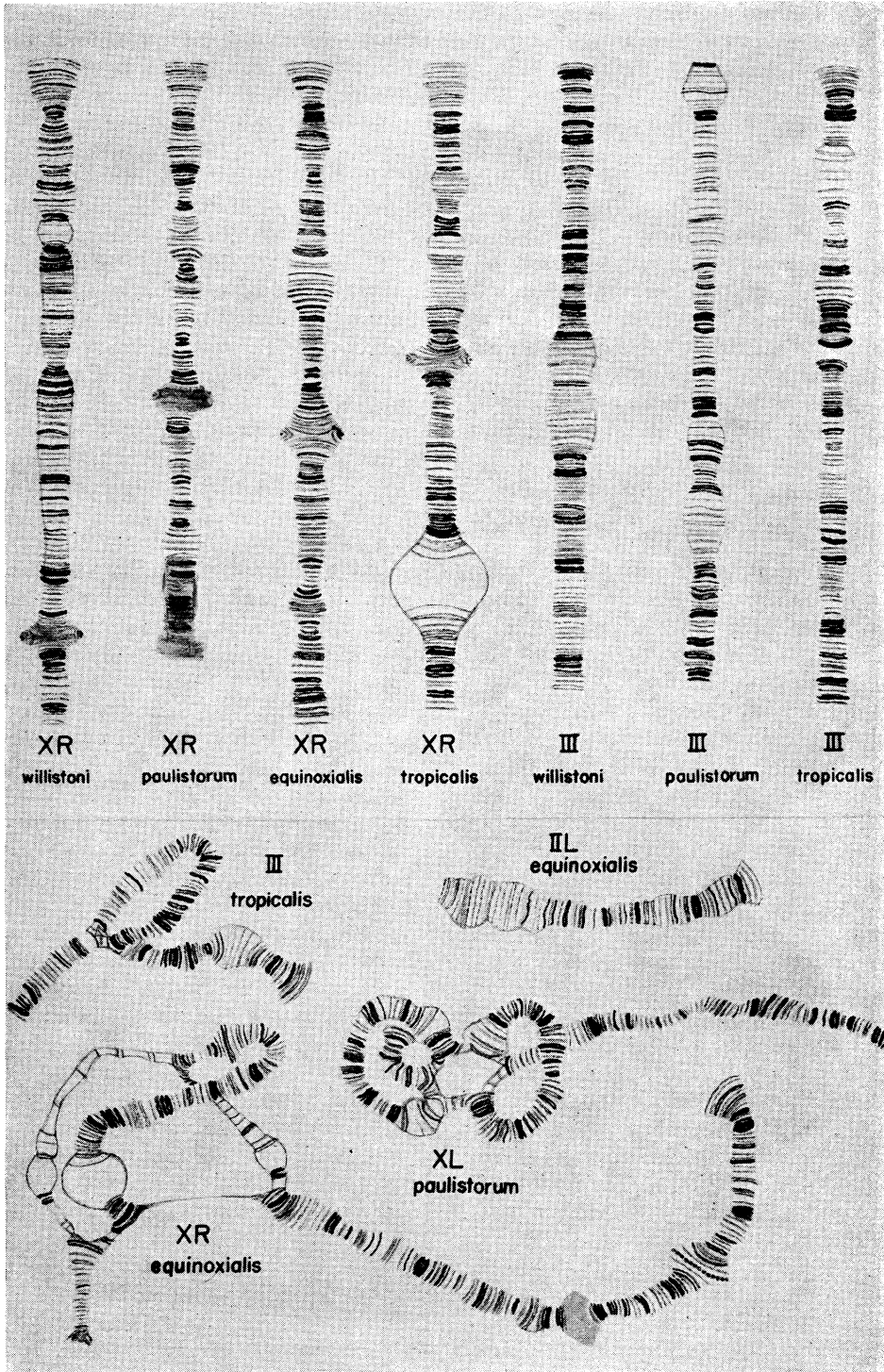


PLATE 1. The distal portions of the right limb of the X-chromosome, of the third chromosome, and some of the inversions found in natural populations of *Drosophila willistoni* and its relatives.

listoni and *D. tropicalis*, and to distinguish both of them from *D. paulistorum* and *D. equinoxialis*.

The terminal portions of the XR chromosome are similar in the four species but the middle and the basal parts, which contain characteristic mirror-image "repeat" areas (drawn in all four species in the upper portion of Plate 1) and large, light, usually strongly swollen "bulbs" (shown in XR of *tropicalis* in the upper, and XR of *equinoxialis* in the lower part of Plate 1), are different. The light bulbs are located at about the basal third of the length of XR in *D. tropicalis*, and within the basal fourth in the three other species. The "repeat" lies more proximally in *D. willistoni* than in *D. paulistorum* and in *D. equinoxialis*, and in the latter two slightly more proximally than in *D. tropicalis*. The XR chromosome can be used as well as the third chromosome for the identification of the species, except that *D. paulistorum* and *D. equinoxialis* are similar.

The basal portions of the chromosome strands (attached to the chromocenter) are as characteristic for chromosomes and for species as are the terminal portions. The IIR and IIL strands have similar combinations of basal and terminal portions in *D. willistoni* and *D. tropicalis*. The same basal and terminal parts are recognizable also in *D. paulistorum* and *D. equinoxialis*, but in these two species the base of what in *D. willistoni* is designated as the IIR strand goes with the terminal portion of what in the latter species is the IIL strand, and vice versa. This situation shows that a pericentric inversion (including the centromere) has taken place in the phylogeny of the sibling species under consideration, so that the second chromosomes of *D. willistoni* and *D. tropicalis* differ from those of *D. paulistorum* and *D. equinoxialis* in the distribution of the genetic materials among the right and the left limbs.

The IIL strand of *D. equinoxialis* (Plate 1) differs from its homologues in the other three species in having a

light and slightly swollen portion, somewhat resembling that in the third chromosome of *D. willistoni*, in a subterminal position.

The arrangement of the discs in the salivary gland chromosomes is known to reflect the linear arrangement of the genes. The phylogeny of the four species here described has, consequently, entailed a considerable amount of gene rearrangement, yet an amount not great enough to make the chromosomes unrecognizable by their disc patterns. The third chromosome has suffered more reconstructions in the phylogeny than did other chromosomes, as attested by the fact that the third chromosomes, except in *D. paulistorum* and *D. equinoxialis*, are identifiable only by a process of exclusion, since their disc patterns have no obvious similarities. Concentration of rearrangements in one of the chromosomes of a set is known to occur in natural populations of several species, such as *D. pseudoobscura* and *D. persimilis* (Dobzhansky, 1944) and *D. nebulosa* (Pavan, 1946), although the causes of this concentration are still obscure.

By the criterion of similarity of the disc patterns, *D. paulistorum* and *D. equinoxialis* are much closer to each other than they are to the other two species, or than these latter are among themselves. A reservation that must be made is that comparison of the disc patterns in chromosomes of species that can not be hybridized discloses only the major rearrangements that involve long sections of chromosomes, while changes in position of small groups of discs may be overlooked.

MORPHOLOGICAL DIFFERENCES BETWEEN THE SPECIES

As far as the traits used in the formal description now standard for *Drosophila* species are concerned, *D. willistoni*, *D. paulistorum*, *D. equinoxialis*, and *D. tropicalis* show only slight, although statistically significant, differences. With the exception of the position of the anterior

scutellar bristles, which distinguishes *tropicalis* from the other three species, these differences are subject to so much variation and overlapping that determination of single specimens is often not reliable. We have therefore made a search

for other differentiating traits, using flies from a series of cultures identified as to species by investigation of their chromosomes. Small differences indeed exist in almost every part of the body of the flies, and they give a characteristic "habi-

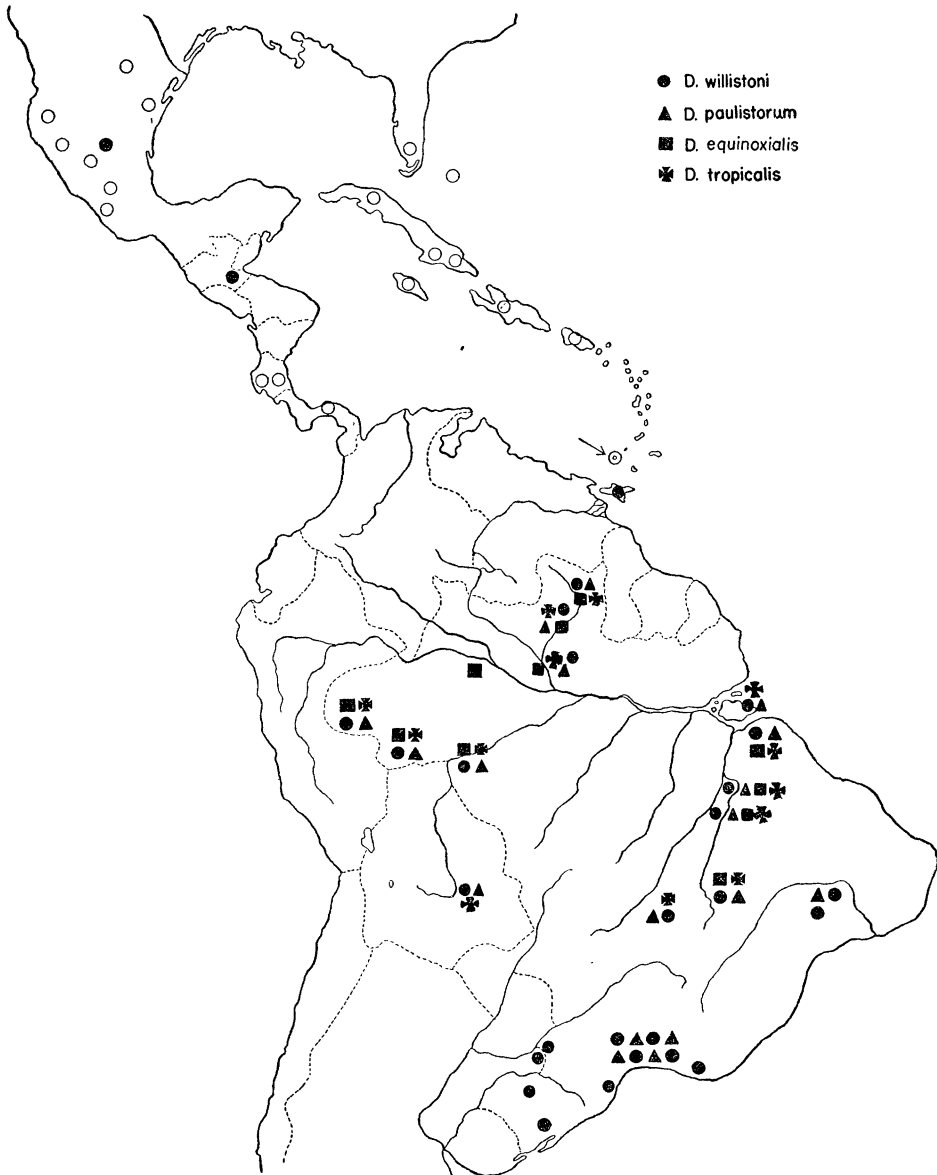


FIG. 1. Geographic distribution of *Drosophila willistoni* and its relatives. Localities in which the presence of *D. willistoni* has been ascertained by genetic or cytological tests are indicated by black circles, and the literature records of this species by open circles. The arrow points to the Isle of St. Vincent, the type locality of *D. willistoni*.

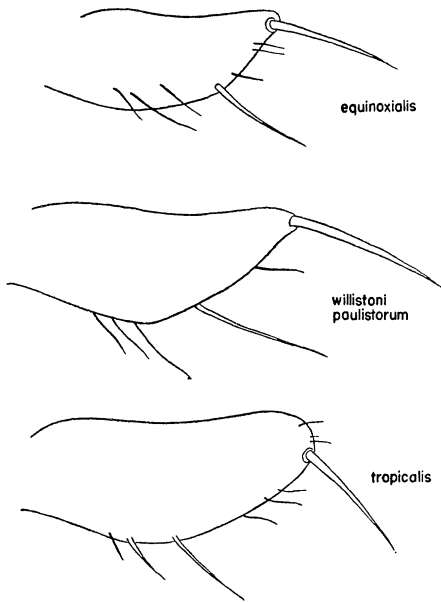


FIG. 2. Maxillar palpi of *Drosophila willistoni* and its relatives.

tus" to every species. Some of these differences prove clear enough to be used as traits for distinguishing the four species. Five traits are external and visible at magnifications of 20–50 times; these include the shape of the maxillar palpi, relative positions of the orbital bristles, relative width of the front, coloration of the ocelli, and shape of the apex of the vaginal plates. Three further traits demand dissection of the flies and preparation of microscopic slides.

D. tropicalis has the maxillar palpi inflated and broadly rounded at the apex, while the other three species have the palpi more slender and acuminate at the apex (fig. 2). The relative positions of the orbital bristles, shown in figure 3, permit one to distinguish *D. willistoni* from *D. paulistorum*. In *paulistorum* the base of the middle orbital lies only a little behind, and very close to the base of the first one. In *D. willistoni* the base of the middle orbital lies definitely between the anterior and the posterior ones. The distances between the bases of the orbital bristles have been measured in camera

lucida drawings made from microscopic preparations. Taking the distance between the first and the third orbitals as 100, the distance between the first and the second varies between 8 and 22 (mean 15.7) in *paulistorum* and between 22 and 41 (mean 29.8) in *willistoni*. Figure 3 shows that the width of the front between the eyes is distinctly greater in *D. paulistorum* than in *D. willistoni*. The other two species, with respect to this and the preceding traits, are intermediate between *paulistorum* and *willistoni*, these traits being useless for their identification. *D. equinoxialis* differs from the other species in having a lighter coloration of the ocelli, this difference not being subject to age changes.

The vaginal plates differ in shape in every species (fig. 4). In *D. tropicalis* they are broadly rounded at the apex; this

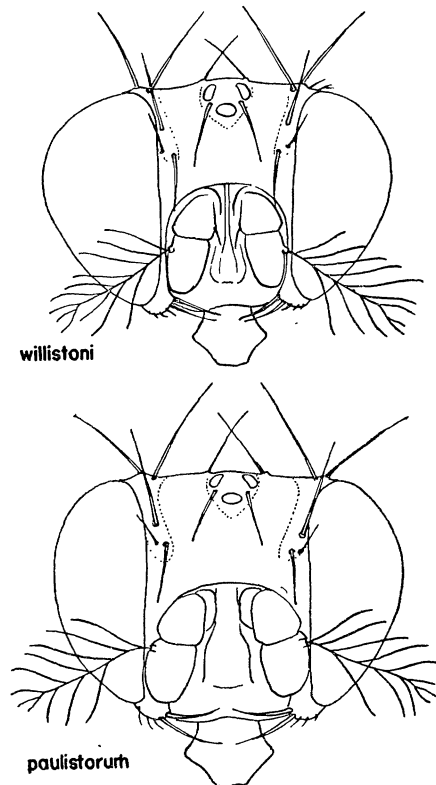


FIG. 3. Frontal view of the heads of *Drosophila willistoni* and *D. paulistorum*.

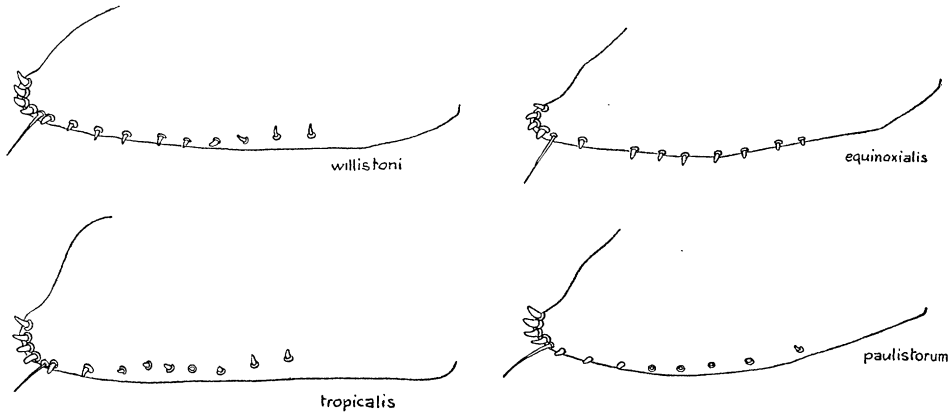


FIG. 4. Vaginal plates of *Drosophila willistoni* and its relatives.

trait, together with the shape of the palpi and the position of the anterior scutellar bristles, identifies this species conclusively. In *D. equinoxialis* the vaginal plates are acuminate at the apex. *D. willistoni* and *D. paulistorum* have vaginal plates intermediate in shape, making it rather difficult to distinguish these species from each other and from the preceding two by this trait alone.

The chitinous spermathecae are more or

less spherical in all species except in *D. tropicalis*, in which the width of the spermatheca is distinctly greater than its height (fig. 5). In *D. paulistorum* the spermatheca has folds that make the outlines of the organ uneven; in *D. willistoni* no external folds are present but the posterior part of the spermatheca is furrowed on the inside surface, and in *D. equinoxialis* neither folds nor furrows are noticeable. In *D. equinoxialis*, *D. paulistorum*

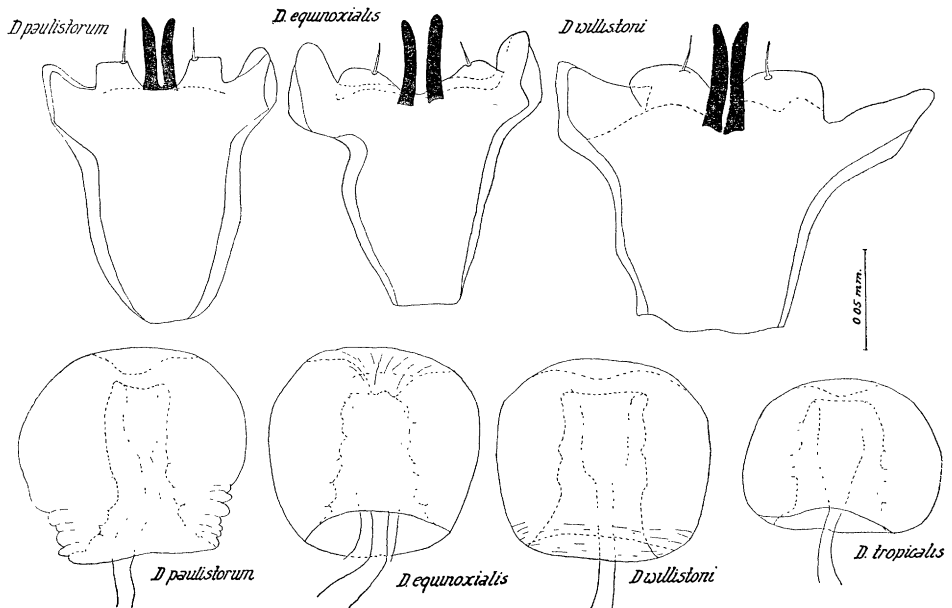


FIG. 5. Hypandria (above) and spermathecae (below) of *Drosophila willistoni* and its relatives.

and *D. tropicalis*, the surface of the sheath enclosing the spermathecal duct has tiny spines not noticeable in *D. willistoni* (fig. 5).

The hypandrium in male genitalia shows differences in the four species, especially in the shape of its posterior edge (directed upward in fig. 5) as may be noted in the figures. The hypandria of *D. paulistorum* and *D. tropicalis* seem to be alike in shape although the size is greater in the latter species. *D. willistoni* and *D. equinoxialis* also differ in the shape of the interior margin of the genital arch near the forceps (see Sales, 1947), which has a fairly large hook-shaped fold in the latter, and only a small tubercle in the former species. *D. paulistorum* is intermediate between the two species, and *D. tropicalis* is close to *D. willistoni* in this trait.

Reviewing the morphological differences between the four species under consideration, we find that species most different in some traits may be similar in other traits. *D. tropicalis* is on the whole the most distinctive species, and yet the shape of the internal process of the hypandrium in *D. tropicalis* and *D. paulistorum* is similar. *D. paulistorum* and *D. equinoxialis* are most similar in external morphology as well as in the disc patterns of the salivary gland chromosomes, and are distinguishable from each other principally by the color of the ocelli, by a slightly different shape of the vaginal plates and by the shapes of the spermatheca and of the hypandrium. *D. willistoni* is in many respects intermediate between *D. paulistorum* and *D. tropicalis*, and yet *D. willistoni* has the most distinctive hypandrium.

[Note added in September 1949. The above descriptions are based on examination of strains of the four sibling species coming from southern Brazil, from Goyaz, and from Acre. When, however, further strains, identified by inspection of their chromosomes, were examined from the equatorial part of Brazil (Rio Branco, Rio Negro, Pará), some of the traits, es-

pecially in *D. willistoni*, were found to be geographically variable. Thus, examination of a single female from each of 13 strains from Belem (Pará), 22 strains from Rio Branco, and 14 strains from Vila Atlantica (southern Brazil), all strains known to be *D. willistoni* gave the following results:

Scutellar Bristles	Belem	Rio Branco	Vila Atlantica
Divergent	23%	23%	86%
Parallel	62%	68%	14%
Convergent	15%	9%	0%

It is evident that this trait can not be relied upon to distinguish some strains of *D. willistoni* from *D. tropicalis*. These two species can still be discriminated by the shape of their palpi, but, unfortunately, this trait is not workable in dried material. *D. willistoni* from Vila Atlantica has a narrow front in 100% of the flies examined, but among the Belem flies only 69% have this condition. As many as 57% Vila Atlantica flies, and only 15% of the examined Belem flies, have 12 branches in at least one of their arista. Comparison of cytologically identified *D. paulistorum* flies from Vila Atlantica and from Belem disclosed that divergent anterior scutellars are found in 70% of Vila Atlantica and 53% of Belem flies. The first and third orbitals are close to each other in 100% of Vila Atlantica and in only 40% of Belem flies.]

PHYSIOLOGICAL DIFFERENCES

No systematic study of physiological properties of the four species has been made, but some differences between them were noted in the course of the work. If many single female cultures of *D. willistoni* and *D. paulistorum* are started simultaneously, the first cultures that produce grown-up larvae fit for dissection and study of salivary gland chromosomes are invariably *willistoni*, while among the last maturing cultures a large proportion are *paulistorum*. Thus, *willistoni* larvae,

despite their larger size, mature more rapidly than the smaller *paulistorum*. Cultures of *D. tropicalis* give uniformly excellent slides, the chromosomes being large, with clear disc patterns, and well spread. Conversely, *D. paulistorum*, and especially *D. equinoxialis*, give very inferior slides, with small, thin chromosomes, that seldom spread well. The nature of the physiological differences that lead to the different appearances of the chromosomes in preparations would be interesting to study from the standpoint of cyto- and chromosome chemistry.

SEXUAL ISOLATION AND LACK OF HYBRIDS

Dobzhansky (1946) found that when males of *D. willistoni* are placed together with females of *D. equinoxialis*, or vice versa, very few of the females are inseminated even after several weeks of exposure to males of the foreign species. Furthermore, the inseminated females fail to produce viable hybrid offspring. Spieth (1947) discovered that the courtship methods in the two species are different, *D. equinoxialis* males being more "lethargic" than *D. willistoni*. Thus the two species show a strong, though not absolute, sexual isolation, and the hybrid zygotes, if formed, are inviable. We have made similar experiments with all four species, using flies from cultures identified as to the species by cytological criteria.

Females and males to be used in the experiments were aged separately in vials with food for 4 to 6 days after their hatching from the pupae. Thereupon, 10 females of one and 10 males of another species were placed together in vials 25 × 95 mm. or 20 females and 20 males in vials 35 × 95 mm., left undisturbed for from 8 to 12 (mostly for 10) days, after which the females were dissected and their ventral receptacles as well as chitinous spermathecae examined for spermatozoa under a microscope. All experiments were made at room temperature (close to 25° C.). Since a single species of fe-

TABLE 2. Numbers of females dissected and percentages of them found inseminated in experiments in which no choice of mates was available

Males	Females	Number dissected	Per cent inseminated
<i>willistoni</i>	<i>paulistorum</i>	69	5.7
<i>willistoni</i>	<i>equinoxialis</i>	545	1.5
<i>willistoni</i>	<i>tropicalis</i>	105	3.8
<i>paulistorum</i>	<i>willistoni</i>	230	51.3
<i>paulistorum</i>	<i>equinoxialis</i>	165	12.7
<i>paulistorum</i>	<i>tropicalis</i>	104	65.0
<i>equinoxialis</i>	<i>willistoni</i>	729	3.8
<i>equinoxialis</i>	<i>paulistorum</i>	94	0.0
<i>equinoxialis</i>	<i>tropicalis</i>	105	0.0
<i>tropicalis</i>	<i>willistoni</i>	112	0.0
<i>tropicalis</i>	<i>paulistorum</i>	115	27.8
<i>tropicalis</i>	<i>equinoxialis</i>	143	0.0

males and a single species of males were present in the vials, no "choice" of mates was available in these experiments. The results are summarized in table 2. The data for *willistoni* × *equinoxialis* crosses in this table are copied from Dobzhansky 1946.

It can be seen that the four sibling species show varying degrees of sexual isolation from each other. Males of *equinoxialis* failed entirely to inseminate females of *paulistorum* and *tropicalis*, and inseminated only a few *willistoni* females. Similarly, *tropicalis* males failed to inseminate *willistoni* and *equinoxialis* females. *Willistoni* males inseminated small numbers of females of all three sibling species. In all these crosses sexual isolation is thus very strong or even complete. Quite different are the results in experiments in which *paulistorum* males are involved, for they inseminated from 12.7% (*equinoxialis*) to as many as 65% (*tropicalis*) females of other species. Females of *paulistorum* were not inseminated by *equinoxialis* males, rarely by *willistoni*, but frequently by *tropicalis* males. On the assumption that sexual isolation depends upon females repelling males of foreign species, made probable

by the observations of Streisinger (1948) and Bateman (1948), our results indicate that *paulistorum* males are either sexually more active than males of the other species, or that they are accepted without difficulty by females of all four species. This does not, however, account for the fact that females of *paulistorum* are inseminated rather readily by *tropicalis*, but not by *equinoxialis* or *willistoni* males. It seems necessary to assume that sexual isolation is less strong between *paulistorum* and *tropicalis* than between these and the other two species. It is possible that courtship or copulation techniques of *paulistorum* and *tropicalis* males are similar in some essential respect that satisfies females of either species, but that these techniques differ in some respect that causes *paulistorum* males to be accepted and *tropicalis* males to be rejected by *willistoni* and *equinoxialis* females.

Small scale experiments were made in which males of one of the species were kept for 9–12 (mostly 10) days with females of their own and of a foreign species at room temperatures. The flies were 4–6 days old when placed together. A summary is presented in table 3, which also includes the data for *willistoni* × *equinoxialis* crosses taken from Dobzhansky (1946). The results obtained agree well enough with those found in the experiments in which no choice of mates was available. Homogamic matings (i.e., those between individuals of the same species) occur easily, and a majority of the conspecific females is found inseminated. Heterogamic matings (between individuals of different species) occur with difficulty, except that *tropicalis* males are accepted by *paulistorum* females.

Occurrence of heterogamic copulation and insemination does not guarantee that interspecific hybrids will be produced, since the sex cells of different species may have a lowered affinity or the hybrid zygotes may die at any stage of the life cycle. Some of the early experiments in which females and males of different species

were together have produced progenies, but careful scrutiny showed that experimental errors were involved in these cases (these experiments are not included in tables 2 and 3). Otherwise, the interspecific matings, where they occur, produce no hybrids. Whether this is due to lack of fertilization or to inviability of the hybrids is unknown.

ABSENCE OF GENE EXCHANGE BETWEEN SPECIES IN NATURE

Laboratory experiments have disclosed the existence of sexual isolation between the four sibling species; this isolation is incomplete in some instances, especially in the case of *D. paulistorum*, which copulates fairly readily with the other three. Females inseminated by males of foreign species fail, however, to produce hybrid progenies. Therefore, the reproductive isolation between the four species is complete, and they can coexist in the same territory without exchanging genes. Indeed, although the geographic distributions are far from perfectly known, it is certain that all four species are sympatric in an extensive territory (fig. 1). Within that territory they actually occur side by side, although the proportions of the four species in the population vary from one microenvironment to another, indicating that they have somewhat different ecological preferences. For example, in the five localities in the territories of Acre and Guaporé, population samples contained the following percentages of the four species (identified by cytological examination):

Locality	Strains examined	<i>willistoni</i>	<i>paulistorum</i>	<i>equinoxialis</i>	<i>tropicalis</i>
Cruzeiro do Sul	213	53	36	8	3
Rio Mõa	60	33	18	3	45
Japiim	46	54	20	9	17
Porto Velho	53	32	49	13	6

D. tropicalis has been found most frequently in the interior of the "varzea" jungle along Rio Mõa, and least fre-

TABLE 3. Numbers of females dissected (*n*) and percentages of them found inseminated (%) in experiments in which a choice of mates was available

Males	Females	Homogamic		Heterogamic	
		n	%	n	%
<i>willistoni</i>	<i>willistoni</i> + <i>paulistorum</i>	86	100	85	0
<i>willistoni</i>	<i>willistoni</i> + <i>equinoxialis</i>	71	65	73	0
<i>paulistorum</i>	<i>paulistorum</i> + <i>willistoni</i>	37	89	35	0
<i>paulistorum</i>	<i>paulistorum</i> + <i>equinoxialis</i>	10	100	9	11
<i>equinoxialis</i>	<i>equinoxialis</i> + <i>willistoni</i>	266	77	256	0.5
<i>equinoxialis</i>	<i>equinoxialis</i> + <i>paulistorum</i>	10	100	10	0
<i>tropicalis</i>	<i>tropicalis</i> + <i>willistoni</i>	105	100	112	0
<i>tropicalis</i>	<i>tropicalis</i> + <i>paulistorum</i>	45	100	42	50
<i>tropicalis</i>	<i>tropicalis</i> + <i>equinoxialis</i>	50	100	51	0

quently in "terra firme" forests of Palmares and Porto Velho. In the state of São Paulo, *D. paulistorum* is much commoner, relative to *D. willistoni*, on the very humid coast, and less common in the drier interior of the state, and much commoner during the warm season than during the cold.

Evidence, quite independent of laboratory experiments, is available to show that no gene exchange takes place in natural populations of the four species, despite their being sympatric to a large extent. Many of the individuals found in nature are heterozygous for inverted sections in their chromosomes. This phenomenon of structural heterozygosis will be dealt with in detail in another publication. What is important for us is that up to the present 42 different inversions have been discovered in populations of *D. willistoni*, that individuals that are not heterozygous for inversions are in the minority at most localities, and that inversions are found in all five chromosome limbs. In *D. paulistorum*, the frequency of inversions is considerably lower than in *D. willistoni*, and yet at least 20 different inversions have been recorded in all five chromosome limbs. Plate 1 shows the left limb of the X chromosome (XL) of *D. paulistorum* with two, apparently independent or tandem, inversions in heterozygous state. Most individuals of *D. equinoxialis* are inversion homozygotes, but one individual from Belem do Pará

was heterozygous for an inversion in the basal portion of the right limb of the X chromosome (XR), shown in Plate 1. Another individual, from Cruzeiro do Sul, contained an inversion in the median part of the third chromosome. In *D. tropicalis* several individuals have been found in the Territory of Acre that had a subterminal inversion in the third chromosome shown in Plate 1. One individual from Porto Velho, Guaporé, was heterozygous for two inversions in the third chromosome, one identical with that pictured in Plate 1 and another in a more proximal position, apparently slightly overlapping the first. Finally, two individuals from Palmares, Acre, were heterozygous for a double inversion in the median part of the left limb of the second chromosome (IIL). Thus, 4 different inversions have been found in *D. tropicalis*.

The fundamental fact disclosed by the cytological studies on the four species under consideration is that each of the numerous inversions found in them is completely restricted to a single species. In other words, none of the inversions found in *D. willistoni*, for example, ever occurs in *D. paulistorum*, or vice versa. Now, if these species would intercross in nature so that a channel for effective gene exchange would be maintained between them, chromosomes or chromosome sections of one species would be found in populations of the other. This would be the case even if effective hybridization

were rare, for, unless foreign chromosome sections are rapidly eliminated by natural selection, they would accumulate in the populations. Let it be noted that while the absence of similar inversions in populations of sympatric species proves that effective hybridization between these species does not occur, their presence would not necessarily prove the opposite, because similar gene arrangements in chromosomes may be inherited by two or more species from their common ancestor. This is exactly what has been observed in the sibling species *D. pseudoobscura* and *D. persimilis* (Dobzhansky, 1944) and in *D. guaru* and *D. subbadia* (King, 1947).

SUMMARY

The *willistoni* species group in tropical America contains four sibling species, *Drosophila willistoni* Sturtevant, *D. paulistorum* Dobzhansky and Pavan, *D. equinoxialis* Dobzhansky, and *D. tropicalis* Burla and da Cunha. In at least part of their area of distribution the four species occur together, sympatrically.

The four species are so much alike in their external morphological characters that they can be distinguished only with difficulty. Small differences are present in several traits that are generally used in *Drosophila* systematics, but the variability is great enough to make identification of species in single individuals hazardous. Several differences have been detected in traits not usually considered in describing *Drosophila* species which, after some practice, permit identification of individual flies, at least in fresh material.

The four species can always be recognized by the disk patterns in the chromosomes of larval salivary glands. The third chromosomes are very differently built in *willistoni*, *tropicalis*, and *paulistorum*, but similar in *paulistorum* and *equinoxialis*. The latter two species are in general most similar in chromosome structure, but can be differentiated by the structure of the

left limb of the second chromosome. Speciation in the *willistoni* group has, consequently, involved major changes in the gene arrangement, without, however, altering beyond recognition the disk patterns in the chromosomes, except in the third.

The four species show varying degrees of sexual isolation from each other. *D. equinoxialis* and *D. willistoni* males seldom inseminate females of species other than their own. *D. tropicalis* males rather readily inseminate *D. paulistorum* females, and *D. paulistorum* males copulate fairly easily with females of the other three species. The degree of sexual isolation between any two species is proportional neither to morphological nor to chromosomal differences between them. Interspecific insemination does not result in production of hybrid progenies.

Numerous inversions have been found in natural populations of all species, especially *D. willistoni* and *D. paulistorum*. However, no inversion has ever been found in more than a single species. This proves that the reproductive isolation between these species is effective in nature.

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