

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

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Among the wild species of *Drosophila* found in neotropical America, the *willistoni* group has attracted the most attention. *Drosophila willistoni* itself has a natural distribution which extends from Florida to Argentina (Townsend, 1952; Pavan, 1952) and over much of this range it is the most abundant species of the genus in collections. The overwhelming biological success of this species appears to be correlated with the occurrence in its chromosomes of an extraordinary amount of naturally-occurring polymorphism due to the presence of inverted sections (da Cunha, Burla and Dobzhansky, 1950). Interest in this species is furthermore greatly enhanced by the fact that, within its general geographical range, there co-exist at least three "sibling species" which cannot be easily separated from *D. willistoni* on morphological grounds (Burla *et al.*, 1949). Isolation between these four entities is nonetheless absolute, both in nature and in the laboratory.

Perhaps because of preoccupation with the many problems presented by the existence of this tetrad of morphologically very close species of which *D. willistoni* is a part, the other neotropical species belonging to the *willistoni* group of the genus have not been intensively studied. These more distantly related species include *D. fumipennis*, *D. capricorni*, *D. nebulosa*, *D. sucinea* and *D. bocainensis*. Except for the work of Pavan (1946) and da Cunha, Brncic and Salzano (1953) on *D. nebulosa*, no quantitative studies of the population genetics of these forms have been made.

In order to begin the closing of this gap in the knowledge of this most significant group of flies and in order to

provide a basis for comparisons of evolutionary patterns within the *willistoni* group as a whole, the present study was undertaken. Quantitative data on populations of *Drosophila bocainensis* Pavan and da Cunha 1947 are presented. Within this older taxonomic designation, it will be shown that there exist three reproductively isolated Mendelian populations. Evidence for the specific distinctness of these three entities is given. Two of the three, although they are partially sympatric and essentially indistinguishable morphologically, show no crossing under natural conditions. This isolation, however, breaks down strikingly in the laboratory; under these conditions, the two species hybridize freely and produce abundant fertile offspring.

MATERIAL AND METHODS

Specimens of *Drosophila* were collected by the method of Pavan and da Cunha (1947) from a number of localities in the states of São Paulo and Rio Grande do Sul, Brazil during the period July to November, 1951. Each wild female which conformed to the morphological description of *Drosophila bocainensis* Pavan and da Cunha 1947 was placed in an individual culture tube containing banana-agar food inoculated with fresh Fleischmann's yeast. Cytological preparations of the salivary gland chromosomes of F_1 larvae from each wild female were made by the usual aceto-orcein technique.

DESCRIPTIONS OF SPECIES

In the body of this paper, quantitative cytogenetic evidence is adduced to show that three specific entities have previously

been included within the designation *Drosophila bocainensis* Pavan and da Cunha 1947. Only one of these three (*D. bocainoides* sp. nov.) is easily separable morphologically; the other two are very closely related sibling species. For one of the sibling species, the designation *Drosophila bocainensis* Pavan and da Cunha 1947 will be retained; the other will be described as *Drosophila parabocainensis* sp. nov. No single morphological character, or combination of characters, has been found whereby individual members of the two sibling species may be reliably separated. Statistical differences in certain structures, notably the male genitalia and in length of larval-pupal life are apparent and will be the object of further study. The species may be separated quickly and reliably by examination of the salivary gland chromosomes of F_1 larvae, as will be shown later. It may be convenient to refer to these three species as comprising the *bocainensis* subgroup of the *willistoni* group of *Drosophila*.

The type material of *Drosophila bocainensis* Pavan and da Cunha 1947 was collected in February, 1944 at a remote fazenda called Campos da Bocaina in the extreme northeastern corner of the State of São Paulo, Brazil. This fazenda lies in a tongue of the state which projects eastward into the State of Rio de Janeiro and is approximately equidistant (30 km.) from Quéluz, on the railroad Central do Brazil, and the town of Bananal.

As the laboratory strain from which this species was described is no longer in existence, it is not possible to assign the type material with certainty to one or the other of the species now recognized cytologically, nor has it been possible to make further collections from the type locality. Campos da Bocaina, however, lies in the coastal rainforest of the Serra do Mar, 270 km. (145 miles) north and east of Vila Atlântica, an area south of Santos from which extensive collections of *Drosophila* have been made (see Dobzhansky and Pavan, 1950). Both locations are closely

similar ecologically and lie in the same mountain range. At Vila Atlântica, only one of the two sibling species has been found; this same sibling has likewise been found abundantly at two other locations in eastern São Paulo from which *bocainensis*-like flies have been analyzed cytologically. These locations are the Horto Florestal in the Serra Cantareira, near the city of São Paulo, and Mogí das Cruzes.

For the above reasons, it seems logical to utilize the original name *Drosophila bocainensis* Pavan and da Cunha to designate the particular member of the sibling species pair found abundantly in coastal São Paulo. As the pinned type material prepared from flies collected at Campos da Bocaina has been lost, a neotype series (five males and five females) was prepared from a laboratory strain of flies derived from the offspring of a single wild female collected at Vila Atlântica in July 1951. These specimens, along with the syntypes of the two new species here described, have been deposited in the personal collection of H. D. Stalker, Washington University, St. Louis. This collection will ultimately be deposited at the American Museum of Natural History, New York. Duplicate series of all three species have been deposited in the Instituto de Zoologia, Museu Paulista, São Paulo, Brazil.

Drosophila bocainensis Pavan and da Cunha

Conforms to the original description of this species by Pavan and da Cunha 1947, pp. 18-20, except for geographical distribution, which is restated here as follows:

Distribution: Brazil: State of São Paulo: Campos da Bocaina (type material, February 1944; types lost; not verifiable cytologically); Vila Atlântica (paratype material, July 1951); Mogí das Cruzes; Horto Florestal, Serra Cantareira, near the city of São Paulo. State of Rio Grande do Sul: Feliz; Ponta Grossa. State of Minas Gerais: Montes Claros.

Drosophila parabocainensis sp. nov.

External characters of imagines

♂. Arista with about 11 branches. Antennae brownish yellow, third segment darker with dense light-colored pile; second joint with

slightly lighter lateral edges. Front uniformly brownish yellow; space between ocellae darker; small hairs curved towards the midline near the point where the frontal lines touch one another; a series of small hairs from the middle orbital to the base of the antenna following the edge of the eyes. Anterior orbitals slightly shorter than the posterior, middle $\frac{3}{7}$ the length of posterior. Two prominent oral bristles equally long. Face light brownish yellow. Carina rounded not sulcate. Cheeks approximately the same color as the face, their greatest width about $\frac{1}{7}$ the greatest diameter of the eyes. Palpus the same color as the face, slightly darker at apex. No prominent anteriorly directed bristle at apex. Anterior-posterior axis of labellum short, ventral edge not noticeably blackened. Eyes orange-red with short dark pile which is blond ventrally.

Acrostichal hairs in 8 irregular rows. Anterior scutellars divergent. No prescutellars. Mesonotum dull brownish yellow, pleurae somewhat lighter. Anterior sternopleural thin, $\frac{1}{2}$ the length of the posterior. Coxae and legs light brownish yellow; prominent apical bristle present on second tibia only, preapicals on all three.

Abdomen: First tergite grayish yellow; second, third and fourth with black (gray or light grayish brown in some specimens) posterior marginal bands, broadened at the midline and at the lateral edges. Anterior marginal band brownish yellow, forming a low crescent spot on each side of the tergite. The posterior tergites are entirely black (gray or light grayish brown in some specimens).

Wings slightly dusky; posterior crossvein usually clouded. Two prominent bristles at the apex of first costal section; third costal section with heavy bristles on its basal $\frac{2}{3}$ to $\frac{1}{2}$. Costal index 2.8-3.3; 4th vein index 1.6-1.8; 5x index about 1.7. Length of body (fresh specimens) 2.6-3.5 mm.; wing length 2.7-3.0 mm.

♀. A single, prominent, anteriorly-directed bristle at apex of palpus.

Internal characters of imagines

Anterior and posterior Malpighian tubules with free ends. Testes pale yellow with three outer and two inner coils. Spermatheca barely ellipsoidal, moderately chitinized, with a sharp apex. Ventral receptacle folded, resembling a skein of wool.

Other characteristics, relationship and distribution

Eggs: Two filaments with broadened tips, about $\frac{1}{2}$ the length of the egg.

Larvae: The larvae do not skip.

Puparia: Yellowish brown; horn very small with about 8 branches.

Chromosomes: Two pairs of V-shaped chro-

mosomes and one pair of rod-shaped chromosomes.

Distribution: Brazil: State of Rio Grande do Sul: Feliz (type material, July 1951); Ponta Grossa; Emboaba; Muitos Capões. State of São Paulo: Pirassununga; Horto Florestal, Serra Cantareira, near the city of São Paulo. State of Minas Gerais: Montes Claros.

Relationship: Belongs to the *willistoni* group of the subgenus *Sophophora*. This species cannot be reliably separated on morphological grounds from its sibling species *D. bocainensis*.

Drosophila bocainoides sp. nov.

External characters of imagines

♂. Arista with about 11 branches. Antennae brownish yellow, third joint darker and densely pilose. Front golden yellow; space between the ocellae darker; small hairs curved toward the midline near the point where the frontal lines touch one another; a series of small hairs from the middle orbital to the base of the antenna following the edge of the eyes. Anterior orbitals slightly shorter than the posterior; middle $\frac{1}{2}$ the length of posterior. Two prominent oral bristles equally long. Face grayish brown. Carina keel-like and prominent, not sulcate. Cheeks yellow, their greatest width $\frac{1}{3}$ greatest diameter of eyes. Palpus the same color as the face, a prominent anteriorly directed bristle at apex. Anterior-posterior axis of labellum forming a long keel, blackened along ventral edge. Eyes red with short, dark pile.

Acrostichal hairs in 8 irregular rows. Anterior scutellars divergent. No prescutellars. Mesonotum dull grayish yellow, pleurae contrastingly dark brownish black. Anterior sternopleural thin, $\frac{3}{5}$ the length of posterior. Coxae dark grayish brown; legs grayish yellow; prominent apical bristle present on second tibia only, preapicals on all three.

Abdomen: First tergite grayish brown, second, third and fourth with black posterior marginal bands irregularly narrowed at the midline and broadest at the lateral edges; anterior marginal band yellow. The posterior tergites are entirely black.

Wings slightly dusky, posterior crossveins with a very faint cloud. Two prominent bristles at the apex of the first costal section; third costal section with heavy bristles on its basal $\frac{2}{3}$ to $\frac{3}{5}$. Costal index 2.8-3.1; 4th vein index 1.4-1.6; 5x index about 1.5. Length of body (fresh specimens) 3.8-4.0 mm.; wing length 3.0-3.2 mm.

♀. Pleurae dull grayish yellow, only slightly darker than mesonotum. Coxae grayish-yellow, the same color as the legs.

Internal characters of imagines

Malpighian tubules not fused at apices; anterior with green or white bulbous swellings

TABLE 1. *Chromosomal evidence for non-interbreeding in nature between two sympatric entities, Drosophila bocainensis (GROUP 1) and Drosophila parabocainensis (GROUP 2). Data based on one F₁ test larva from each of 126 wild females collected at Feliz and Ponta Grossa, Rio Grande do Sul, Brazil, 1951.*

	Group 1 <i>D. bocainensis</i>	Group 2 <i>D. parabocainensis</i>
No. of wild females tested from:		
Feliz	60	42
Ponta Grossa	16	8
Total tested	76	50
<i>Chromosome X:</i>		
pattern at tip of left arm:*		
AA (female test larvae)	53	0
A (male test larvae)	23	0
aa (female test larvae)	0	36
a (male test larvae)	0	14
No. test larvae heterozygous for inversion XR-B	15	0
<i>Chromosome 2:</i>		
No. of test larvae heterozygous for inversion:		
2R-A	0	20
2R-B	6	0
<i>Chromosome 3:</i>		
No. of test larvae heterozygous for:		
0 inversions	16	50
1 inversion	0	0
2 inversions	11	0
3 inversions	7	0
4 inversions	11	0
5 inversions	18	0
6 inversions	13	0

* See figure 1.

distally. Testes very pale yellow with three outer and two inner coils. Spermatheca heavily chitinized; elongate, almost twice as long as its greatest width; obtuse at apex. Ventral receptacle folded, resembling a skein of wool.

Other characteristics, relationship and distribution

Eggs: Two filaments, $\frac{1}{2}$ the length of the egg, with very broad tips.

Larvae: The larvae do not skip.

Puparia: Yellowish brown; horn very small with about 10 branches.

Chromosomes: Two pairs of V-shaped chromosomes and one pair of rod-shaped chromosomes.

Distribution: Brazil: State of São Paulo: Vila Atlântica (type material, July 1951); Mogí das Cruzes; Horto Florestal, Serra Cantareira, near the city of São Paulo.

Relationship: Belongs to the *willistoni* group of the subgenus *Sophophora*. This species is

very closely related to *D. bocainensis* and *D. parabocainensis* but is easily separable morphologically from them in males by the black pleurae.

POPULATION SAMPLES FROM RIO GRANDE DO SUL, BRAZIL

Flies conforming morphologically to the description of *D. bocainensis* Pavan and da Cunha were collected at Feliz and Ponta Grossa in the state of Rio Grande do Sul during July and October, 1951. From each of the 126 wild females which produced offspring in the laboratory, one or more salivary gland chromosome smears of F₁ larvae were prepared. From each of the F₁'s, one of the larvae was designated as a "test larva"; this was

normally taken as the first female larva examined, as the use of a female permits the observation of two entire chromosomes of each pair transmitted by the wild-caught female. If no female larva was present among those examined, the first male larva was used; this is less desirable, as only a single X chromosome may be observed.

Inspection of the data from the test larvae reveals a sharp dichotomy (Groups 1 and 2, table 1). In the first place, the female test larvae showed either one of two alternate cytological appearances at the tip of the left arm of the X chromosome (A, B versus C, D, fig. 1). Inspection of the banding differences between these two alternate tips reveals them to be due to the homozygous presence (or absence) of a simple inversion of the segment 2-7, figure 1. Let us arbitrarily designate these two homozygous appearances as AA and aa. In no instance was the expected simple inversion loop of the heterozygote Aa found. This is a highly significant deviation from the Hardy-Weinberg equilibrium, as among the female test larvae, 53 AA, 36 aa and 0 Aa individuals were observed (table 1). The significance of the dichotomy in the data for the X chromosome is further strengthened by the fact that as well as the 126 test larvae reported in table 1, a total of 95 additional larvae were examined; thus a considerable number of the wild flies were tested by more than a single F_1 smear. In no case, in any given F_1 , was both types of X chromosome found; the X chromosomes of all the larvae examined from any individual wild female were either all AA or aa (A or a in males) in pattern at the tip of XL.

The separateness of the two groups in table 1 becomes even more clear when the evidence from the other chromosome variability is considered. For instance, 15 of the female test larvae were heterozygous for a short inversion, designated XR-B, near the base of the right arm of the X chromosome. All of these individuals had an AA, or "*bocainensis*" X chro-

mosome. In chromosome 2, 20 test larvae were heterozygous for a short inversion near the tip of the right arm (inversion 2R-A, table 1); all of these had the aa or "*parabocainensis*" X chromosome. Most striking of all, however, is the situation in chromosome 3; those F_1 's having "*bocainensis*" X chromosomes show an extraordinary amount of diversity for gene order in chromosome 3. About 80% of these test larvae (60 out of 76) were complex inversion heterozygotes in which the two homologues differed by from two to six inversions. The 50 F_1 's which showed the "*parabocainensis*" X chromosome, however, were exclusively homozygous for gene arrangement (table 1).

These data are summarized graphically in the two upper histograms in figure 2; detailed data on the inversions found in *D. bocainensis* are given in table 2. In view of the absolutely discontinuous distribution of the chromosomal variability in the two groups, there is no question that, although they are sympatric and are morphologically very similar, these two groups come from isolated Mendelian populations which do not interbreed. As will be shown later when the data from laboratory crosses between members of these two populations are presented, the above analysis reveals only to a small degree the actual amount of diversity of gene order between the two. Thus, it will be shown that F_1 hybrids between these two entities are heterozygous for from 13-26 inversions, most of which were not detected prior to the species crosses. This diversity is such that even a small amount of introgression between the two entities could be immediately recognized in examinations of test larvae. Although further evidence on this point will be adduced later, the two entities will be hereafter referred to as species.

POPULATION SAMPLES OF *D. bocainensis* FROM SÃO PAULO

Samples of flies were obtained from 1) Vila Atlântica, 2) Serra Cantareira, on

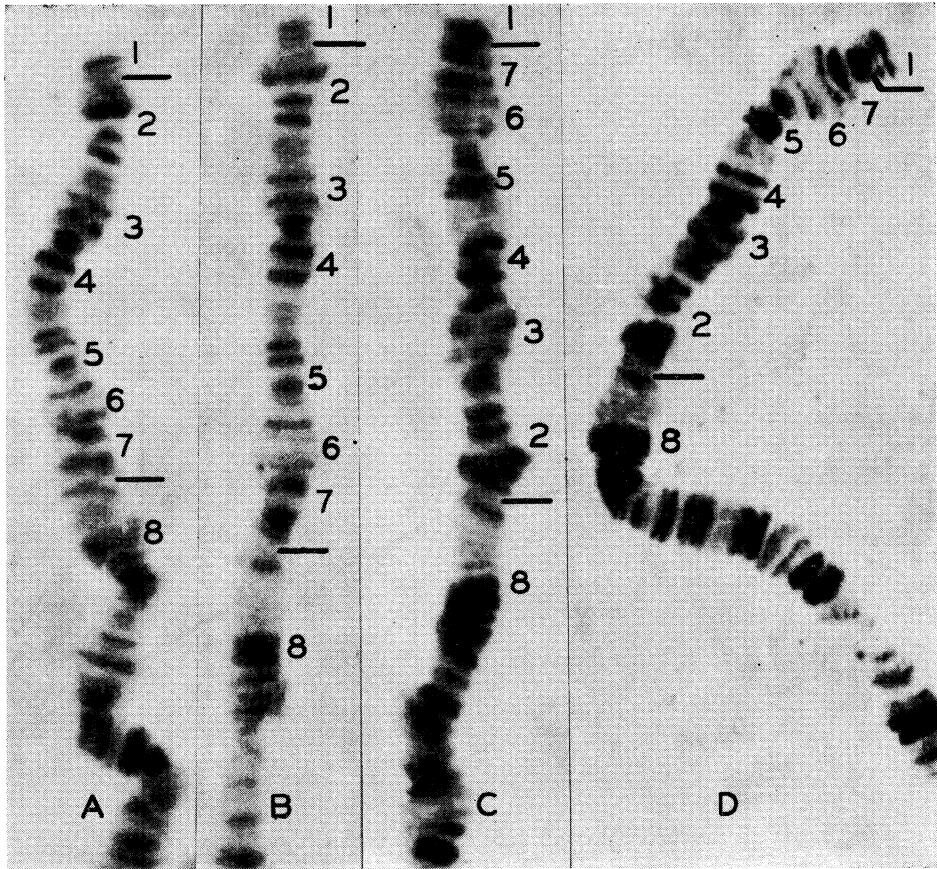


FIG. 1. The tip of XL in *D. bocainensis* (A, B) and *D. parabocainensis* (C, D). The species differ by a subterminal inversion in this region, the breakpoints of which are indicated by the heavy lines. Homologous regions are numbered 1-8.

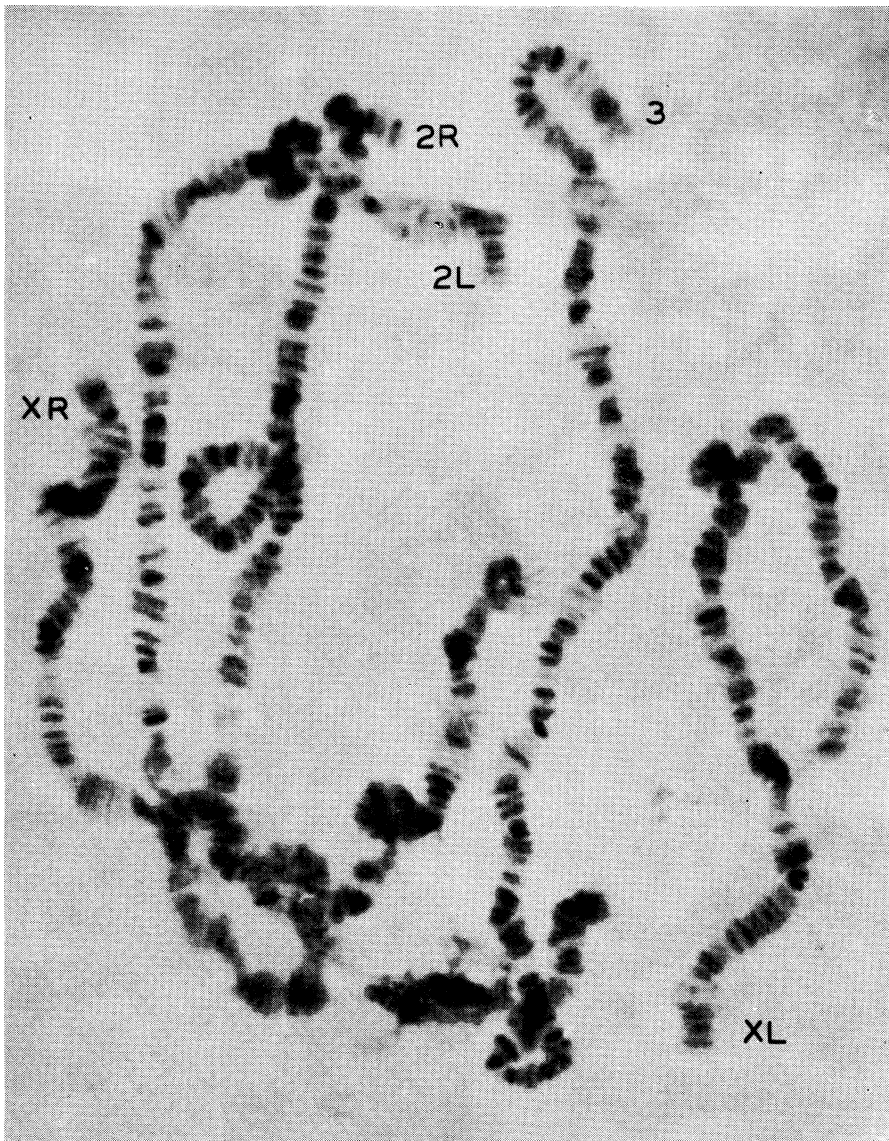


FIG. 4. Entire polytene chromosome complement in an individual of *D. parabocainensis* sp. nov. which is homozygous for gene arrangement. The distal end of each chromosome arm (XL, XR, 2L, 2R, 3) is labelled. XL has broken off from its base (center) and lies to the right of the picture.

the grounds of the Horto Florestal near the city of São Paulo and 3) Mogí das Cruzes. The chromosome variability in the test larvae from flies cytologically determined as *D. bocainensis* is recorded in the three right-hand columns of table 2, where they may be compared with the data from Rio Grande do Sul. A graphic summary of the data for the state of São Paulo is given in the lower histogram in figure 2.

Two inversions not found in the Rio Grande do Sul populations are present in

São Paulo; these are the rare inversion XR-A and inversion 2L-A. In general, the frequencies of inversions in chromosomes X and 2 are greater in the São Paulo populations than in Rio Grande do Sul, although the situation with respect to inversion 2R-B is not clear, because of the very small sample obtained at Mogí das Cruzes. In all the populations studied, inversion variability in chromosome 3 is very great and the extent of each individual inversion has not been mapped for this study. There appears, however,

TABLE 2. *Chromosomal variability in D. bocainensis Pavan and da Cunha in Brazil, 1951*

	Locality					
	<i>Rio Grande do Sul</i>			<i>São Paulo</i>		
	Feliz		Ponta Grossa Oct.	Vila Atlântica July-Sept.	Serra Cantareira Aug.-Sept.	Mogí das Cruzes Nov.
July	Oct.					
No. of wild females tested	22	38	16	48	106	7
No. tested by single F ₁ female larva	12	31	9	34	71	4
No. tested by single F ₁ male larva	10	7	7	14	35	3
<i>Chromosome X:</i>						
% of female test larva heterozygous for:						
inversion XL-B	0.0	0.0	22.2	20.6	28.2	50.0
inversion XR-A	0.0	0.0	0.0	2.9	1.4	0.0
inversion XR-B	8.3	35.5	33.3	32.4	45.1	75.0
<i>Chromosome 2:</i>						
% of test larvae heterozygous for:						
inversion 2R-B	9.1	10.5	6.3	2.1	0.0	28.7
inversion 2L-A	0.0	0.0	0.0	20.8	45.7	0.0
inversion 2L-B	0.0	0.0	6.3	33.3	35.2	0.0
inversion 2L-C	0.0	2.6	0.0	33.3	40.0	0.0
<i>Chromosome 3:</i>						
% of test larvae heterozygous for:						
0 inversions	27.3	18.4	25.0	10.4	11.4	0.0
1 inversion	0.0	0.0	0.0	20.8	9.5	14.2
2 inversions	22.7	5.3	18.7	10.4	19.0	14.2
3 inversions	4.5	15.8	0.0	25.0	18.1	0.0
4 inversions	18.2	15.8	6.3	16.7	24.8	28.7
5 inversions	18.2	26.3	25.0	12.5	9.5	14.2
6 inversions	9.1	18.4	25.0	4.2	6.7	28.7
9 inversions	0.0	0.0	0.0	0.0	1.0	0.0

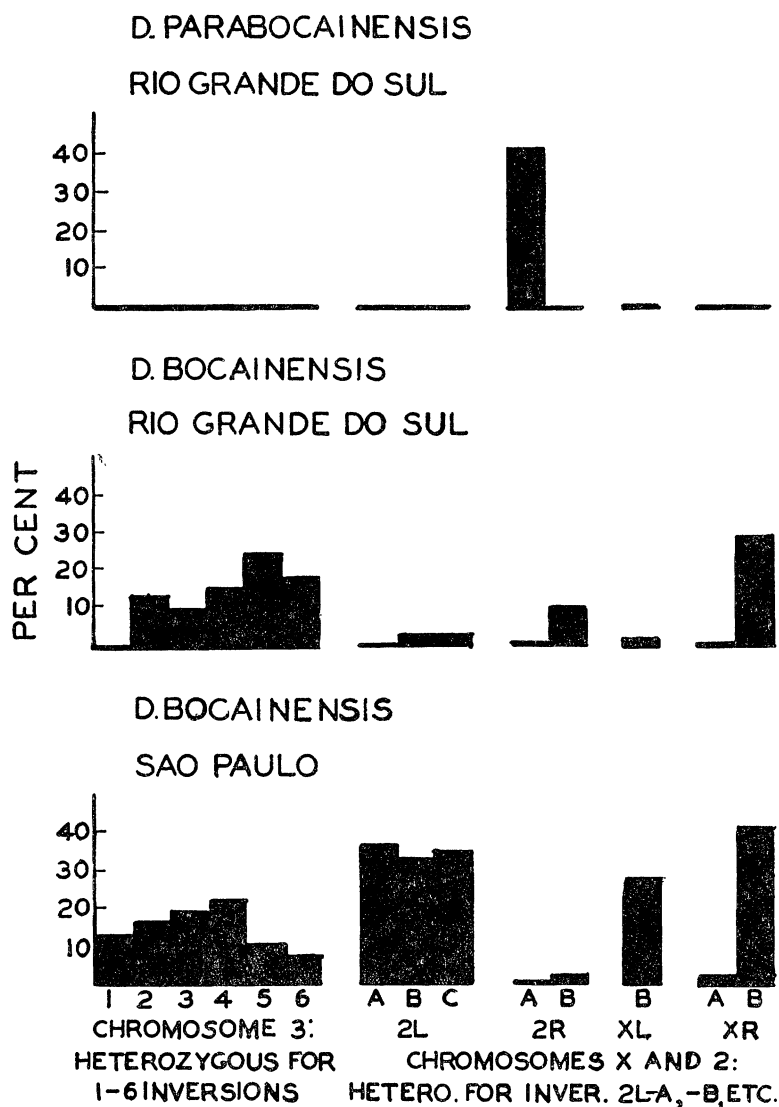


FIG. 2. Chromosome variability within sympatric populations of *D. parabocainensis* (upper histogram) and *D. bocainensis* (middle histogram). Although the differences in variability pattern are apparent, this diagram does not attempt to show all of the differences in chromosomal arrangement between the species. For comparison, São Paulo populations of *D. bocainensis* are summarized (lower histogram). Detailed data are given in tables 1 and 2 and in the text.

to be a maximum of about 10 inversions. The variability in this chromosome has been expressed in terms of the number of inversion differences between the two homologues observed in the test larvae (table 2). This method, however, expresses somewhat less than the total varia-

bility encountered, because it is clear that there is a considerable variety of configurations within, for instance, the 1-inversion and 5-inversion difference classes. Despite the inadequacies of the data for chromosome 3, certain differences between the Rio Grande do Sul populations and those

from São Paulo are apparent (table 2; fig. 2). For example, no instance of a single-inversion difference was encountered among the Rio Grande do Sul flies, whereas in the São Paulo populations this class runs as high as 20%. Correlated with this difference appears to be the fact that in São Paulo, a relatively smaller percentage of complex figures (differing by 4-6 inversions) are found, although the individually most complex configuration, a 9-inversion difference, came from the Serra Cantareira.

POPULATION SAMPLES OF *D. bocainoides*
FROM SÃO PAULO

At the three locations discussed above, no specimens of *D. parabocainensis* were obtained. Among the *bocainensis*-like flies that were analyzed, however, 35 of the F₁ progenies (23 from Vila Atlântica; 12 from Cantareira) showed banding patterns in the chromosomes which differed markedly from both *D. bocainensis* and *D. parabocainensis*. Close inspection revealed that the general similarities make it possible to homologize the chromosome arms with those of the sibling species, but in most cases direct band homologies could not be drawn.

Separation and examination of the progenies of these females revealed that the emerging flies showed, especially in males, clear morphological differences from the sibling species, the degree of divergence in the chromosomes paralleling, more or less, the morphological divergence. This species is described in this paper as *Drosophila bocainoides* sp. nov. Twelve of the test larvae were heterozygous for a short inversion slightly distal to the center of 2R and three were heterozygous for a short inversion close to the tip of XR. This latter inversion was found only in flies collected at Vila Atlântica. Males of *D. bocainoides* were identified in collections from Mogí, but no females were present among the small sample available from this location.

POPULATION SAMPLES OF *D. parabocainensis*

Other than the specimens obtained from Ponta Grossa and Feliz, Rio Grande do Sul, only 9 wild females of this species have been analyzed, 7 from Pirassununga, São Paulo and 2 from Montes Claros, Minas Gerais. In Rio Grande do Sul, the species is homozygous for gene arrangement, except for a short inversion near the tip of 2R (2R-A, table 1) which is present in about 40% of the test larvae. This inversion is also present at Pirassununga and Montes Claros. At these two latter locations, despite the small number of flies analyzed, three additional inversions, all in chromosome 2, have been found. The two inversions found in the left arm (Pirassununga only) resemble 2L-B and 2L-C of *D. bocainensis* but their identity with these inversions has not been definitely established. In the right arm, a large basal inversion has been found in specimens from both Montes Claros and Pirassununga. These data make it probable that when satisfactory samples of the São Paulo populations of *D. parabocainensis* are eventually obtained, the apparent tendency toward inversion homozygosity, relative to *D. bocainensis*, will be shown to be spurious.

Although among the recently-collected *D. parabocainensis* all have been homozygous for gene order in both chromosomes X and 3, examination of an old laboratory stock of this species from Pirassununga revealed that it was carrying a chromosome 3 which differed from its homolog by five inversions. The possibility of contamination of this stock with *D. bocainensis* cannot be excluded.

HYBRIDIZATION EXPERIMENTS BETWEEN
D. parabocainensis AND
D. bocainensis

1. P₁ hybridizations

Reciprocal crosses between *D. parabocainensis* and *D. bocainensis* were made, using F₁ flies from wild females (table 3).

TABLE 3. Results of P_1 reciprocal crosses between *D. parabocainensis* and *D. bocainensis*. The fractions given indicate the number of tubes out of the total in each category which produced abundant offspring. Each tube contained 5 males and 5 females.

A. *parabocainensis* females \times *bocainensis* males

		<i>Parabocainensis</i> females	
		Feliz	Pirassununga
<i>Bocainensis</i> males	Feliz	5/5	0/1
	Vila Atlântica	5/5	1/1

B. *bocainensis* females \times *parabocainensis* males

		<i>Bocainensis</i> females	
		Feliz	Vila Atlântica
<i>Parabocainensis</i> males	Feliz	8/8	6/6
	Pirassununga	2/3	2/2

For each test, 5 virgin females and 5 males of approximately the same age were placed together in a large shell vial and each tube was examined daily for the appearance of eggs and larvae. A total of 18 strains were employed in the crosses: 7 *parabocainensis* from Feliz, 1 *parabocainensis* from Pirassununga, 5 *bocainensis* from Feliz, and 5 *bocainensis* from Vila Atlântica. A series of 17 control (intraspecific) crosses were made at the same time and were prepared and followed in the same way.

Of 31 interspecific crosses made, 29 produced abundant larvae at the same time and in the same quantity as the controls (table 3). The fact that two of the crosses failed is not considered significant in view of the fact that of the 17 intraspecific control crosses, three also failed. All of the latter failures were intrastrain matings of *D. bocainensis*. No difference was noted in the success of sympatric and allopatric crosses. The interspecific crosses did not differ from the controls in abundance of larvae produced, time of pupation and emergence of adult flies. No dying larvae or pupae were observed and the F_1 hybrid flies were fully as vigorous as the controls, if not more so.

Study of the sex ratio in the F_1 generation from the crosses described above was carried out on 7 of the crosses, 2 Feliz \times Feliz, 3 Feliz \times Vila Atlântica and 2 Vila Atlântica \times Pirassununga. The data were obtained by isolating the five individual females used in each cross after they had already produced larvae in several successive tubes. Each female was placed without males in a separate tube and changed regularly to a fresh one until her supply of sperm was exhausted. The F_1 hybrid individuals were thus raised under conditions where no crowding occurred and all individuals emerging were counted. The results of these counts are given in table 4. It will be noted that whereas the sex ratio among the allopatric crosses did not significantly deviate from a 1:1 ratio, there is a highly significant shortage of

TABLE 4. Sex ratios in the offspring of reciprocal crosses between *D. parabocainensis* and *D. bocainensis*

P_1 cross female \times male	Sex ratio in F_1 hybrids					
	Sympatric (Feliz \times Feliz)			Allopatric (Feliz \times Vila Atlântica) (Vila Atlântica \times Pirassununga)		
	No. females	No. males	Sex ratio	No. females	No. males	Sex ratio
<i>para.</i> \times <i>boca.</i>	125	93	74.4	220	199	90.5
<i>boca.</i> \times <i>para.</i>	295	166	56.3	249	269	108.0
Total	420	259	61.7	469	468	99.8

TABLE 5. Fertility of interspecific hybrids between *D. parabocainensis* and *D. bocainensis*. The fractions given indicate the numbers of tubes out of the total in each category which produced F₂ offspring. ++ indicates abundant offspring produced; + indicates few offspring.

A. tests of F₁ hybrids from *D. parabocainensis* female × *D. bocainensis* male

Test No.	female × male	Feliz × Feliz sympatric	allopatric
1	hybrid × hybrid (mass)	++ 4/4	++ 5/5
2	hybrid × hybrid (tubes)	++ 2/2	++ 2/2
3	hybrid × <i>para.</i>	++ 2/2	++ 2/2
4	hybrid × <i>boca.</i>	++ 1/2	++ 2/2
5	<i>para.</i> × hybrid	++ 1/2	++ 2/2
6	<i>boca.</i> × hybrid	++ 2/2	++ 2/2

B. Tests of F₁ hybrids from *D. bocainensis* female × *D. parabocainensis* male

	female × male	Feliz × Feliz sympatric	allopatric
7	hybrid × hybrid (mass)	++ 7/7*	+ 3/9†
8	hybrid × hybrid (tubes)	– 0/2	– 0/2
9	hybrid × <i>para.</i>	++ 2/2	+ 2/2
10	hybrid <i>boca.</i>	+ 1/2	+ 2/2
11	<i>para.</i> × hybrid	– 0/2	– 0/2
12	<i>boca.</i> × hybrid	– 0/2	– 0/2

* Delay 1–5 weeks; 4 of the 7 with abundant offspring.

† Delay 2–5 weeks; none of the 3 with abundant offspring.

males from the sympatric cross, regardless of the way in which the original cross is made. This effect is noticeable in the progenies of each of the 9 individual females which produced offspring; in no individual progeny does the number of males equal or exceed that of the females.

2. Fertility of F₁ hybrids

The fertility of the F₁ hybrid individuals was tested by mass matings of F₁ individuals from 25 of the successful crosses. Four of the original crosses (reciprocals; sympatric and reciprocals; allopatric) were selected for more intensive tests by F₁ matings and backcrosses. These data are summarized in table 5.

Of 9 original crosses in which *D. parabocainensis* was the female parent, all produced vigorous F₂ generations in mass cultures containing approximately 25 individuals of each sex, as shown in test No. 1, hybrid × hybrid (mass), table 5. The sympatric crosses were equally as successful as the allopatric ones (Feliz × Vila Atlântica and Pirassununga × Vila Atlântica). The abundance of offspring

equalled that of the controls and F₂ larvae were produced without delay (6–8 days after emergence). Two of the original crosses (Feliz × Feliz No. 2 and Feliz × Vila Atlântica No. 2) were selected for more intensive study. Tubes containing 5 females and 5 males each were prepared of F₁ hybrids (test No. 2, table 5) and backcrosses (tests No. 3–6, table 5). As might be expected from the results obtained in mass cultures, all combinations showed a high degree of fertility. The fact that one of the tubes failed in each of tests 4 and 5 is not considered significant in view of the fact that abundant offspring were produced in each case in the duplicate tube.

Tests of F₁ hybrid individuals from the 16 crosses which were the reciprocal of the above, that is, in which *D. bocainensis* was the female parent, were tested in a similar manner in mass cultures (test No. 7, table 5) and in tubes (tests 8–12, table 5). It was soon apparent that, compared to the hybrids from the reciprocal cross and the controls, some degree of sterility existed. For this reason all of the tests

7-12 were followed intensively for a period of two months, during which time the flies were periodically transferred to fresh food and any dead flies in the crosses being followed in tubes were replaced with fresh ones.

Using this procedure, all of the sympatric crosses of this type were observed to produce offspring, although there was a delay of from 1-5 weeks beyond the control time for the production of offspring. Four of these crosses eventually produced abundant offspring, whereas the other three produced only a small F_2 . F_1 hybrids from the original allopatric crosses in mass culture were noticeably less successful. Of 9 such crosses followed, only 3 produced F_2 larvae. Although egg production by the females was enormous over the two-month period, very few offspring were obtained; in all cultures the appearance of larvae was delayed from 2-5 weeks beyond the normal time. Of 5 crosses of Feliz by Vila Atlântica, only two produced offspring; one of these resulted in the hatch of only 1 female, the other produced 8 females and 1 male. Two crosses of Vila Atlântica \times Pirassununga were negative and of two crosses of Feliz \times Pirassununga, one was sterile and the other produced 16 females and 13 males.

Two of the reciprocal crosses in which *D. bocainensis* was the female parent were followed in detail, as explained in a previous section. These were Feliz \times Feliz No. 3 and Vila Atlântica \times Feliz No. 5. The results are given in tests 8-12, table 5. It will be noted that all tests involving F_1 hybrid males failed (tests 8, 11, 12, table 5) despite the fact that they were carried for two months. F_1 hybrid females of this type proved to be highly fertile when backcrossed to *D. parabocainensis* males (test 9). The backcrosses to *D. bocainensis* males were somewhat less successful, but 3 out of 4 such tests produced moderate numbers of offspring.

The discrepancy between the results of mass cultures and tube experiments

and backcrosses (tests 7-12, table 5) may be best explained as indicating that the F_1 hybrid males from the cross *D. bocainensis* female \times *D. parabocainensis* male are only occasionally fertile and become so only after considerable delay. Thus, the success of the mass cultures may have been due to the fertility of a very few males or conceivably a single male. All hybrid females, in fact most of the species females as well, produced enormous quantities of eggs when in tubes with sterile males; in a number of cases, the hybrid females were still active and vigorous and were producing large quantities of eggs at the time they were discarded, two months from the time of emergence. A number of putative sterile hybrid males were dissected; in all cases the testis resembled that of the species controls and of fertile hybrid males from the reciprocal cross; motile spermatozoa were observed in the ejaculatory duct. These males are furthermore able to inseminate females; dissections of their mates revealed that the seminal receptacles were filled with actively motile sperm. Examination of eggs laid by these females, however, showed them to be uncleaved.

3. Crosses involving *D. bocainoides*

As has been stated previously, this species is, on morphological and cytological grounds, somewhat removed phylogenetically from the sibling species discussed above. *D. bocainoides*, moreover, is difficult to rear in the laboratory. Six hybridization attempts, two with *D. bocainensis* and four with *D. parabocainensis* were made, using 5 flies of each sex as in the experiments described above. No offspring were obtained and further attempts were abandoned.

SALIVARY GLAND CHROMOSOME STUDIES OF INTERSPECIFIC HYBRIDS, F_2 'S AND BACKCROSSES

Studies on F_1 larvae from wild females, described in an earlier section, have shown

TABLE 6. *Chromosomal differences between D. bocainensis and D. parabocainensis as revealed in species crosses and in studies of intraspecific variability*

Chromosome arm	Number of inversions observed	
	Minimum	Maximum
XL	4	5
XR	5	7
2L	0	5
2R	0	2
3	4	7
Total	13	range 26

that there is considerable divergence of gene order within one of the species (*D. bocainensis*) and at least one consistent difference (an inversion near the tip of XL) between the species. This method of analysis, involving the recognition of inversion heterozygotes and chromosome ends, reveals only a small measure of the differences of gene order between these species. This fact is apparent from the study of the chromosomes of F_1 hybrids. The salivary gland chromosomes of a total of 47 F_1 hybrid larvae were examined and proved to be heterozygous for from 13 to 26 inversions, depending on the strain of *bocainensis* used in the cross (table 6). In the crosses from Feliz, strains of *D. bocainensis* homozygous for gene arrangement in the X chromosome were used. As all strains of *D. parabocainensis* are entirely homozygous for gene arrangement in this chromosome, such crosses should reveal the basic differences in gene order between the species. In addition to the nearly terminal inversion in XL (the configuration Aa, predicted from the two homozygous appearances in the two species), all larvae were heterozygous for 8 additional inversions in the X chromosome. Thus, in the interspecific hybrids, XL shows four inversions, a simple sub-terminal one (Aa), an overlapping inversion figure centrally and another single inversion proximally. In XR there are 5 inversions, bearing a complex relationship to one another.

In most of the crosses examined, the 3rd chromosome forms excessively complicated figures which are difficult to analyze. In one case the difference amounted to 8 inversions. A great variety of these complex figures was found in the larvae because of the widely diverging gene orders found within *D. bocainensis*. Chromosome 2 represents an interesting case in that unlike the X (and apparently the 3rd) chromosome, the two species share a common gene arrangement. Thus, in the right arm, the interspecific hybrids show either two inversions (2R-A and 2R-B), 2R-A alone, 2R-B alone, or no inversions. The latter condition was recorded in a Feliz by Feliz cross. In the left arm of chromosome 2, the most common condition in the F_1 hybrids is a very complex configuration which has not been satisfactorily analyzed, although it appears to be due to a 5-inversion difference. In two instances, however, the difference between the two was simpler; in crosses between Vila Atlântica and Pirassununga some crosses showed only a two-inversion difference and in one instance the gene arrangement in the two species proved to be the same, that is, no inversions were found. These observations show that the two species share a common gene arrangement in both 2L and 2R. A more detailed analysis is necessary before the derived arrangements found within the species may be clarified relative to a "standard" arrangement.

An examination of the salivary gland chromosomes of 28 larvae of the F_2 generation and backcrosses was made. Although this examination was cursory, the following facts are clear. Segregation in the F_2 is random and involves principally whole chromosomes in both males and females. A number of recombinations between arms were also detected. Thus, in several cases it was observed in chromosomes X and 2 that a given hybrid female transmitted a right arm derived from one species along with a left arm derived from the other.

GEOGRAPHICAL DISTRIBUTION AND
ECOLOGY

Most species of *Drosophila* which have been subjected to detailed microevolutionary studies are relatively abundant in banana-bait collections, at least seasonally or locally. Wild-caught adults of these species, unless in some state of reproductive arrest, usually breed readily when brought to the laboratory and may be maintained on standard laboratory media with little difficulty. The members of the *bocainensis* subgroup unfortunately do not display a full measure of these favorable attributes. Like perhaps the majority of species of the genus, they show ecological specialization; concomitant technical difficulties occur if they are handled by routine laboratory methods.

In the first place, flies of this subgroup are exceedingly rare in banana trap collections. This might be taken to mean that populations are generally small were it not for the fact that the evidence indicates rather strongly that under most circumstances fermenting banana does not attract the flies well. The most striking evidence for this fact comes from the collecting activities of Pavan (1952) at Vila Atlântica during September, 1951. Approximately one week of collecting from banana baits resulted in a catch of only 18 *bocainensis* subgroup out of 6920 flies captured, or 0.3%. Fermenting orange, hitherto suspected of being a good attractant for these species, yielded only 0.4% out of 1325 flies caught. That neither of these baits was adequately sampling the population in the area, however, is shown by the fact that of 1472 *Drosophila* swept from rotting *Myrciaria*¹ fruits in one restricted spot on the forest floor near the collecting area, 462, or 31.4%, were *bocainensis* subgroup.

From these data, it is clear that *bocainensis* subgroup (specifically, about 75% *D. bocainensis* and 25% *D. bocainoides*)

¹ *Myrciaria delicatula* (B. C.) Berg, family Myrtaceae. Kindly determined by Dr. M. G. Ferri.

were in this case selectively attracted to the wild fruits, and if the collecting at Vila Atlântica at this time had been made exclusively from banana baits, an erroneous impression of the size of the *bocainensis* populations would have been obtained. As most of the routine sampling of South American populations of *Drosophila* has been done with banana bait (e.g., Dobshansky and Pavan, 1950; Pavan, 1952) it is probable that published figures on the incidence of *D. bocainensis* do not accurately reflect the state of its natural populations.

The ecological relationships of the flies of the *bocainensis* subgroup to fruits of trees of the family Myrtaceae, especially of the genus *Myrciaria*, are much in need of investigation. Good collections of these species have been made from wild fruits of members of this genus on a number of occasions. Preliminary tests of commercial relatives of these wild species of trees indicate that certain of their fruits are promising as specific attractants. The fruits tested were: jaboticaba, *Myrciaria* (*Eugenia*) *jaboticaba* or *M. cauliflora* and uvaia, *M. uvalha* or *M. pyriiformis*.

Pavan (1952) has recorded small numbers of flies of this subgroup from various of the northern states of Brazil (Amazonas, Maranhão, Goyaz, Acre) as well as from the south (São Paulo, Paraná). Table 7 gives the geographical origin and relative frequency in collections made with banana or orange bait of the three species recognized in the present paper. All determinations, except as noted, are based on cytological examination.

Despite the doubtful attractant qualities of banana and orange bait, as discussed above, several facts are apparent. In the first place, the data from Feliz, Rio Grande do Sul, suggest the existence of a reciprocal seasonal cycle of abundance of the sibling species *D. bocainensis* and *D. parabocainensis*, with the latter the more frequent species in winter (July). As the data stand, the change from July to October in the frequency of these two

TABLE 7. *Geographical distribution and relative frequency of Drosophila bocainensis, D. parabocainensis and D. bocainoides in Brazil.* All records verified cytologically, except as indicated.

Locality	Total	Per cent		
		<i>bocainensis</i>	<i>parabocainensis</i>	<i>bocainoides</i>
Rio Grande do Sul, 1951	129	61.2	38.8	0.0
Feliz, July	51	43.1	56.9	0.0
Feliz, October	54	75.9	24.1	0.0
Ponta Grossa, October	24	66.7	33.3	0.0
São Paulo, 1951-52	206	83.0	0.0*	17.0
Vila Atlântica	72	68.1	0.0	31.9
Cantareira	120	90.0	0.0*	10.0
Mogi das Cruzes	7†	95.0	0.0	5.0
Pirassununga	7	100.0	0.0	0.0
Minas Gerais, 1953				
Montes Claros	3	33.3	66.7	0.0

* Mr. F. M. Salzano reports the presence of this species in a collection from Cantareira in 1952.

† All 7 flies tested cytologically were *D. bocainensis*. The percentages are based on the relative number of *bocainoides* males in the collection.

species is highly significant statistically but the possible effect of many intangible and uncontrolled factors make statistical comparisons of relative frequency open to question. For example, both orange and banana were used in the October collections and the collection period extended over a number of days. A single collection, using banana only was made in July. The species are not spacially separated in the forest; both may be captured over the same bait pile with a single sweep of the net.

The high frequency of *D. parabocainensis* at the cooler, drier season in Rio Grande do Sul correlates directly with its occurrence, so far to the exclusion of *D. bocainensis*, at Pirassununga, São Paulo. This station is located on the dry interior plateau. Conversely, *D. bocainensis*, with large populations at Vila Atlântica and in the Cantareira is apparently a fly of the coastal rain and cloud forest. *D. bocainoides* is definitely less frequent at the higher altitudes near the city of São Paulo than it is on the coast, suggesting that its distribution may be more characteristic of areas of high temperature, rainfall and humidity than the other two species.

In most cases, the number of specimens

originally captured was much greater than the number which could be successfully analyzed cytologically. This is due primarily to the fact that many wild females of this subgroup lay only a few or no eggs after being brought to the laboratory. Wild-caught flies of both sexes feed well in the laboratory and live for long periods if changed periodically to fresh food. Disinclination to oviposit in the laboratory appears to be about equally pronounced in the three species. As far as general laboratory culture is concerned, all three species may be raised on banana food; *D. parabocainensis* is the most vigorous and is the easiest to raise. *D. bocainoides* requires the most attention; careful aging and repeated changes of the flies are necessary to assure well-populated culture bottles. Hybrid stocks are highly vigorous and have been maintained without difficulty for several years (Carson, 1953).

CYTOLOGICAL SYNOPSIS

The X chromosome

General. The left and right arms of the X chromosome are joined by a small heterochromatic mass at the chromocenter region. As in the *D. willistoni* subgroup and in *D. nebulosa*, XL is shorter than XR (fig. 3). The banding pattern at the tip of XR appears to be identical

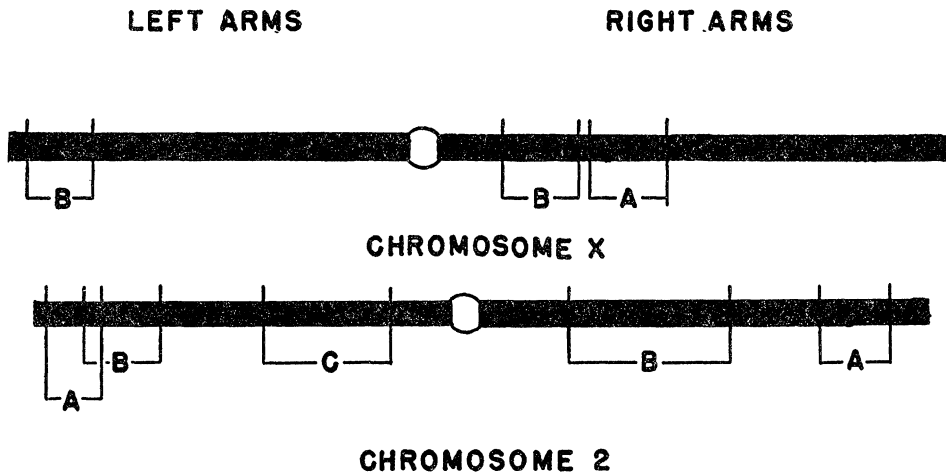


FIG. 3. Diagrams of the left and right arms of chromosomes X and 2 of *D. bocainensis* and *D. parabocainensis*. The light areas in the middle represent the position of the heterochromatin and the centromeres; the bracketed lettered sections refer to inversions XL-B, XR-B, etc. All of the inversions shown, with the exception of 2R-A, are characteristic of *D. bocainensis* only.

in all three species of this subgroup; this pattern, furthermore, appears to be very similar to the regions of like position in *D. willistoni* (region 36, Dobzhansky, 1950) and *D. nebulosa* (region 35, Pavan, 1946). The banding pattern at the tip of the left arm serves as the easiest cytological means to distinguish *D. bocainensis* from its sibling species, *D. parabocainensis* (fig. 1; see also XL, fig. 4). The difference is due to an interspecific inversion, designated as XL-A.

D. bocainensis. Left arm pattern commonly as in figures 1A and 1B. Only a single inversion is known within the species (XL-B, fig. 3). The distal break of this inversion is just proximal to the three large bands designated "2" in figures 1A and 1B. Although the appropriate mapping has not been done, it is probable that this inversion overlaps XL-A at its proximal end. Two inversions are known in the right arm; XR-B is a very short inversion close to the base and XR-A is immediately distal to it and is about the same length (fig. 3).

D. parabocainensis. The tip of the left arm banding is exclusively as in figures 1C and 1D; no inversions in either arm are known within the species. In crosses to *D. bocainensis* which lacks XL-B, XR-A and XR-B, the hybrid shows regularly four inversions in the left arm; a nearly terminal single inversion (XL-A), two inversions which overlap centrally in the arm and a single large inversion near the base. Interspecific hybrids show a complex figure in the right arm which is apparently due to a five-inversion difference.

D. bocainoides. The terminal portion of XL cannot be homologized with either of the sib-

lings; XL is best identified by elimination, as the pattern at the tip of XR makes this arm easily identified. A short inversion (XR-C), with its distal break seven bands from the tip, occurs within the species.

Chromosome 2

General. The left and right arms of the second chromosome are usually joined by a large, conspicuous heterochromatic mass, as in *D. willistoni* and *D. nebulosa*. As in the latter two species, 2L may be recognized by the fact that it is shorter than 2R and contains much interstitial heterochromatin, causing it to be kinky and ordinarily unfavorable cytologically.

D. bocainensis. Three inversions are known in the left arm, 2L-A, 2L-B, 2L-C (fig. 3). These may be most easily recognized by their positions in the arm. In the right arm, this species has a single long inversion (2R-B) located centrally in the arm, somewhat more towards the base than the tip.

D. parabocainensis. The left arm is not completely analyzed (see section on hybrids). Inversions are present in both the left and right arms. They are not given letter designations here, however, because their relationship to *bocainensis* inversions of similar position have not been determined. 2R-A is a very common inversion near the tip of the arm; it is the only inversion known from all populations of this species. It does not occur within *D. bocainensis*.

D. bocainoides. The banding patterns at the tips of the second chromosome arms are not recognizably homologous to the sibling species. 2L is easily recognized, however, by its interstitial heterochromatin. There is a short in-

version (2R-C) near the center of the right arm.

Chromosome 3

This element, which is the rod-shaped chromosome of the metaphase group, is easily recognized in both the *bocainensis* and *willistoni* subgroups and in *D. nebulosa* by the fact that it has very little or no heterochromatin at its base. In salivary gland preparations, it appears as a single chromosome arm which tends to separate from the rest of the chromosome group (fig. 4). As has been shown previously, there is a large number of inversions in this chromosome in *D. bocainensis*; natural populations of *D. parabocainensis* and *D. bocainoides*, however, are apparently homozygous for gene arrangement in this chromosome.

DISCUSSION

The implications and uses of the term "sibling species" have been fully discussed by Mayr, Lindsley and Usinger (1953). The term is commonly used to designate two or more non-interbreeding Mendelian populations or species, the individuals of which are so similar morphologically that often separation of the species by one or more qualitative characters is not possible. The most significant recent development in this field is not the abandonment of morphological criteria alone for the recognition of species, a step which was taken long ago, but the development of highly refined cytogenetic and experimental methods for the recognition of sibling species. In cases where these methods can be readily applied, the list of valid cases continues to lengthen.

The present case of sibling species was uncovered by cytological means. The situation is especially clear because the differences in gene sequence between the species are extensive and well-distributed over the chromosome set. Thus even a small amount of interbreeding in nature could be quickly and easily detected in the chromosomes of larvae from wild females. None was detected and like the majority of other cases of sympatric sibling species known in *Drosophila*, isolation in nature appears to be complete.

The completeness of the isolation be-

tween the two species in nature is all the more remarkable because there is little or no easily recognized isolation between them in the laboratory. This is in sharp contrast to the situation in the other case of sibling species in the same group of flies, the *willistoni-paulistorum-tropicalis-equinoxialis* cluster (Burla et al., 1949). In these cases no interspecific offspring are produced either in nature or in the laboratory. In *bocainensis-parabocainensis*, furthermore, the species hybrids are not only produced in abundance but, as is perhaps the most unique feature of the present case, all types of hybrids are fertile, with only one type of male having fertility reduced relative to the controls.

The case in *Drosophila* which most closely parallels that of *bocainensis-parabocainensis* is that of *pseudoobscura-persimilis* (Dobzhansky and Epling, 1944). In this case, the entities concerned are also sympatric; crossing in nature does not occur and reciprocal laboratory crosses between the species result in abundant offspring. The main point of difference is that in *pseudoobscura-persimilis*, the F_1 hybrid males are sterile, no matter which way the original cross is made.

The germ plasms of *D. bocainensis* and *D. parabocainensis*, a pair of species apparently formed by divergent speciation, are thus compatible and are still capable of being easily fused. This has been done experimentally in the laboratory; two of four laboratory strains kept for 30 generations still retained some chromosomes derived from both species (Carson, 1953). Under natural conditions, however, the species have remained separate. It is highly unlikely that the small amount of hybrid sterility found could play more than a minor role, if any, in keeping the two species apart. This sterility is better interpreted as a by-product of the speciation process and it appears more likely that some subtle but efficient ecological separation exists in natural populations. This need not be an actual spacial separation; indeed, both species may be col-

lected over baits with a single sweep of the net and such separation appears to be excluded, at least where artificial baits are used. The potentiality for crossing in nature between these species is certainly very great and might conceivably lead to introgression under conditions of disturbance of the delicate ecological balance between them (cf. Anderson, 1948).

That the number of known cases of sibling species should continue to increase for the genus *Drosophila* is not surprising. As a genus, these flies may be looked upon as small flying insects of conservative form which have become adapted, through extensive speciation, to exist on some micro-organism or constellation of microorganisms. In other words, the major factors of organic diversity to which these flies are adapting in their evolution is itself morphologically cryptic but at the same time highly diverse biochemically. Just as complex morphological flower form and complex morphology of pollinating insects are correlated in their evolution, so one might expect "sibling species" among yeasts, for example, to encourage the formation of correlated sibling species among the flies adapted to them.

SUMMARY

1. The *bocainensis* subgroup of the *willistoni* group of *Drosophila* consists of three species from southern Brazil. Two are described in this paper.

2. *D. bocainensis* and *D. parabocainensis* are sibling species which are indistinguishable morphologically. They occur together in the same collections but chromosome analyses prove that crossing in nature does not occur. The species differ profoundly in gene arrangement (13-26 inversions).

3. Despite the lack of natural hybridization, laboratory crosses between the species are highly successful and resemble intraspecific ones in abundance and vigor of F_1 progeny. All hybrids from both reciprocal crosses appear to be as fertile as the controls except for F_1 males which

have *bocainensis* mothers. In these, fertility appears to be strongly reduced.

4. It is suggested that the sibling species are kept apart in nature by unknown subtle differences in ecological factors to which the species are differentially adapted.

5. *D. bocainoides* is morphologically distinguishable from and will not hybridize with the sibling species. Data on chromosome variability within each of the three species is given.

6. *D. parabocainensis* appears to be a fly of the dry, seasonally cool interior plateau of southern Brazil, whereas *D. bocainoides* is characteristic of the super-humid coastal rainforests of São Paulo. *D. bocainensis* appears to thrive in intermediate habitats. All appear to be relatively specialized and to have some particular relationship to fruits of trees of the genus *Myrciaria* (Myrtaceae).

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