

A NEW SPECIES OF THE GENUS "DROSOPHILA", WITH DISCUSSION ABOUT SPECIATION IN THE "MERCATORUM" SUB-GROUP¹

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(With 29 text-figures)

In the genus *Drosophila*, the *repleta* group is one of the most complexes, by the great morphological similarity of its species. As the new species described here was included in that group, we tried to study more carefully its quantitative characters, chitinized parts of the genitalia and also its genetic relationships with the resembling species, by means of interspecific crosses.

To Professor ANDRÉ DREYFUS, for his encouragement throughout the course of these investigations, and to him yet and other scientists that have kindly read the manuscript, for their valuable criticism, we express our gratitude. We are pleased to thank also to those that have cooperated with us, for collecting material, preparing slides, taking photographs and typewriting this paper.

MATERIAL AND METHODS

The analyzed specimens were descendants from a fertile female, that we collected near the Paraná river, at Capitão Heitor Port, in September 1944. Such descendants, 11 to 12 days old, were obtained at almost uniform conditions of temperature — $24.7 \pm 0.90^{\circ}\text{C}$ (σ), and nutrition (progeny from single pair matings moved day by day to fresh food tube).

Technique observed — Color of several characters determined by "A dictionary of color" of MAERZ & PAUL (1930). Measurements made on etherized flies, with exception of the wings and the legs. Size of the body: sum of the distance from the base of the antenna to limit of thorax-abdomen, plus the distance from the latter point to the tip of anal tubercle, at lateral sight. Measurements of the wings and the legs made on material distended on the glass slides, in 70% alcohol, and mounted in euparal. Length of the wing: distance

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from the insertion point to the tip, in a straight line. Counting of branches of anterior spiracle on material mounted directly in balsame. In the cases of pair organs (wings, eyes, etc.) the measurements were taken only from the right organ. The ratios between the several quantitative characters were computed on the means of the Table I, where are included also the standard deviation of each character, etc. It was applied the t test of significance in four cases, in which the coefficient of variation was larger than 10. For two values of t obtained (2nd oral bristle and greatest diameter of spermatheca), $P > 5\%$, and for two values of t (cheek and middle sternopleural bristle), $P > 1\%$. In that table, the characters significantly different (verified by the t test of significance of the difference between means — $P < 1\%$) in both sexes were separated from the similar characters ($P > 1\%$) in both sexes.

Chitinized parts of the genitalia obtained from flies diaphanized by the following method: I — Caustic potash (10%) at 57°C, 24 hours; II — Phenol at 57°C, 24 hours; III — Beech-wood creosote. Stain with acetic-orcein; differentiation with creosote. Internal genitalia analyzed in physiological solution. The terminology of the genitalia was taken from the work of HELENA SALLES (1947).

Larval ganglionic tissue stained by the acetic-orcein smear technique.

In the classification of specimens from other geographical strains, pair matings were also made.

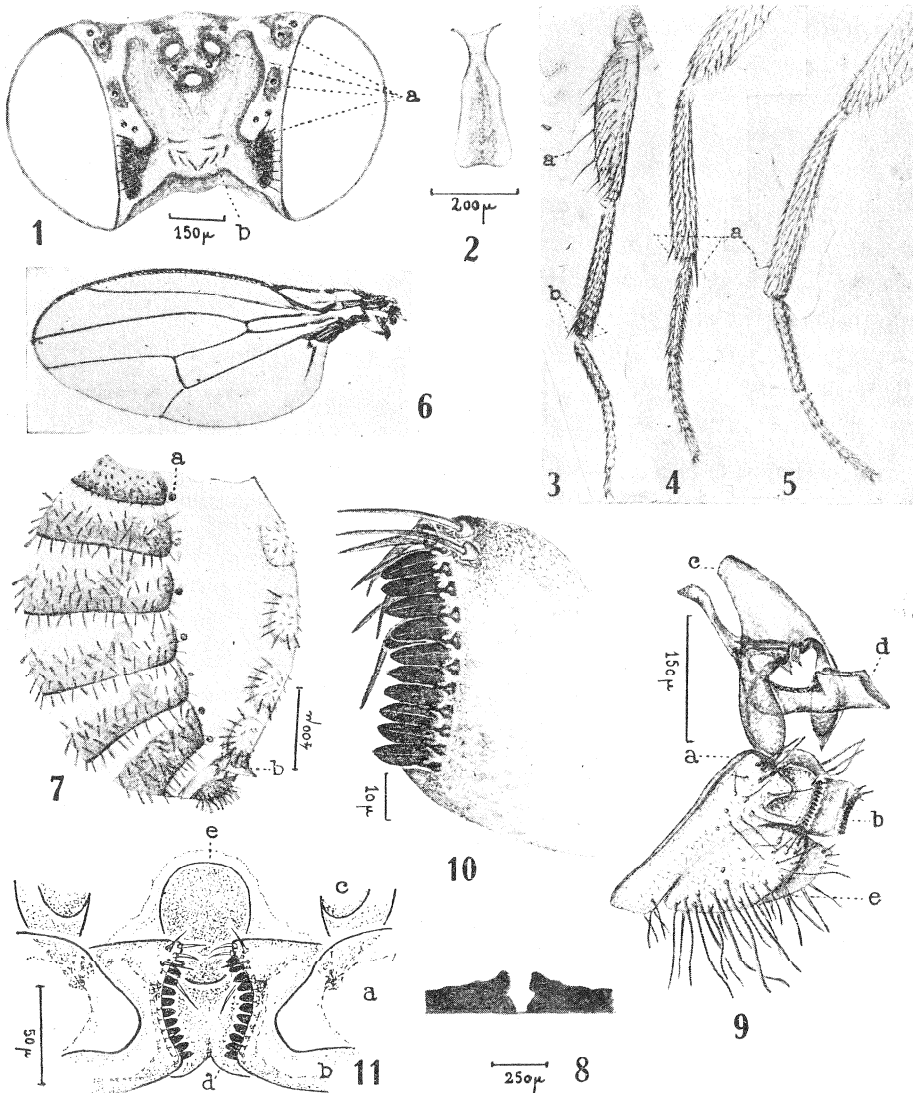
In the initial interspecific crosses, one pair of flies per tube was used at ordinary temperature. As by this method, the percentage of fertile females was very small, 3%, in certain types of cross, and 0% in others, experiments were made involving 10 aged pairs per bottle, in a total of 40 to 50 half-pint milk bottles, within each type of cross. The flies were transferred to fresh food bottles each three or four days, during the period of 15 to 20 days. First backcross, in pairs.

✓ *Drosophila paranaensis* n. sp.

♂ and ♀ — Arista with 6-8 branches, 4-6 above and 2 below. Antennae beige (Pl 11=C 2); dark spots on three joints, the largest on third joint. Front (fig. 1) pollinose. Orbital lines and ocellar triangle beige. Dark spots at bases of a row of 6-10 short hairs in front of anterior orbitals, at bases of posterior orbitals, of anterior verticals and around the ocelli; posterior ocellar spots extending to bases of post-vertical bristles. Frontal lines brown (Pl 7 — C 12), forming a "v"; 7 to 12 short and convergent hairs on apex of "v". Middle orbital about 4/7 anterior and 1/2 posterior. Face light yellow with very diluted spots. Carina (fig. 2) trapezoid, slightly sulcated. Cheeks greyish yellow, their greatest width about 1/4 greatest diameter of eyes. One prominent oral bristle; second about 1/2 first. Palpi pale yellow with several hairs and two

thin bristles, apical the longest. Eyes brazil red (*Pl* 4 — *K* 12) with short black pile.

Acrostichal hairs in 8 rows, slightly irregular; 4 enlarged hairs in prescutellar position. Anterior scutellars convergent. Mesonotum pollinose,



Drosophila paranaensis n. sp. — Fig. 1: Front (a- dark spots in front of anterior orbital, at bases of posterior orbital, anterior vertical and around the ocelli; b- convergent hairs on apex of frontal "v"); fig. 2: carina; fig. 3: anterior leg (a- long bristles on the femur; b- apical and preapical bristles on the tibia); fig. 4: middle leg (a- apical and preapical bristles on the tibia); fig. 5: posterior leg (a- preapical bristles on the tibia); fig. 6: wing; fig. 7: abdomen of the male, diaphanized material (a- spiracle; b- external genitalia); fig. 8: interrupted black band on the tergite 5th; fig. 9: chitinized pieces of the genitalia of the male (a- genital arch; b- clasper; c- hypandrium; d- penis; e- anal plates); fig. 10: clasper; fig. 11: chitinized pieces of the genitalia of the male (a- genital arch; b- clasper; c- outer process of the hypandrium; d- bridge; e- platform of the penis arranged in the same plane of the bridge).

ivory (*Pl* 10 — *B* 2); bristles and hairs arising from dark spots, sometimes irregularly fused and tending to form more frequently in female, longitudinal stripes at each side of light midline. Scutellum pollinose, ivory; dark spots at bases of bristles and a semicircular spot on anterior part, sometimes fused to those of posterior bristles. Pleurae pollinose, beige; dark sinuous stripe from base of first coxae to halteres. Anterior sternopleural about 7/9 posterior, middle sternopleural about 3/10 posterior. Legs pale yellow with dark ringed spots at tips of femora and bases of tibiae. Anterior femora equally long in male and female ($t = 1.01$; $P > 5\%$), about 5/6 posterior femora, in male, and 4/5 posterior femora, in female; 6 to 9 long bristles. Anterior tibiae also equally long in male and female ($t = 0.30$; $P > 5\%$), about 8/9 anterior femora. Apical bristles on anterior and middle tibiae; preapical on all three (figs. 3-5). Recurved hairs on three tibiae and on three tarsi, more numerous on anterior tarsi than on others, and also on anterior tarsi of male, 26-43, than of female, 19-25 ($t = 2.70$; $P < 1\%$). Halteres pale yellow; first two segments with dark spots.

♂ — Wings (fig. 6) clear, apex of first costal section black, with two prominent bristles. Heavy bristles to about 1/2 of third costal section. *Costal* index 2.60; *4th vein* index 1.68; *5x* index 1.25 and *4c* index 0.94.

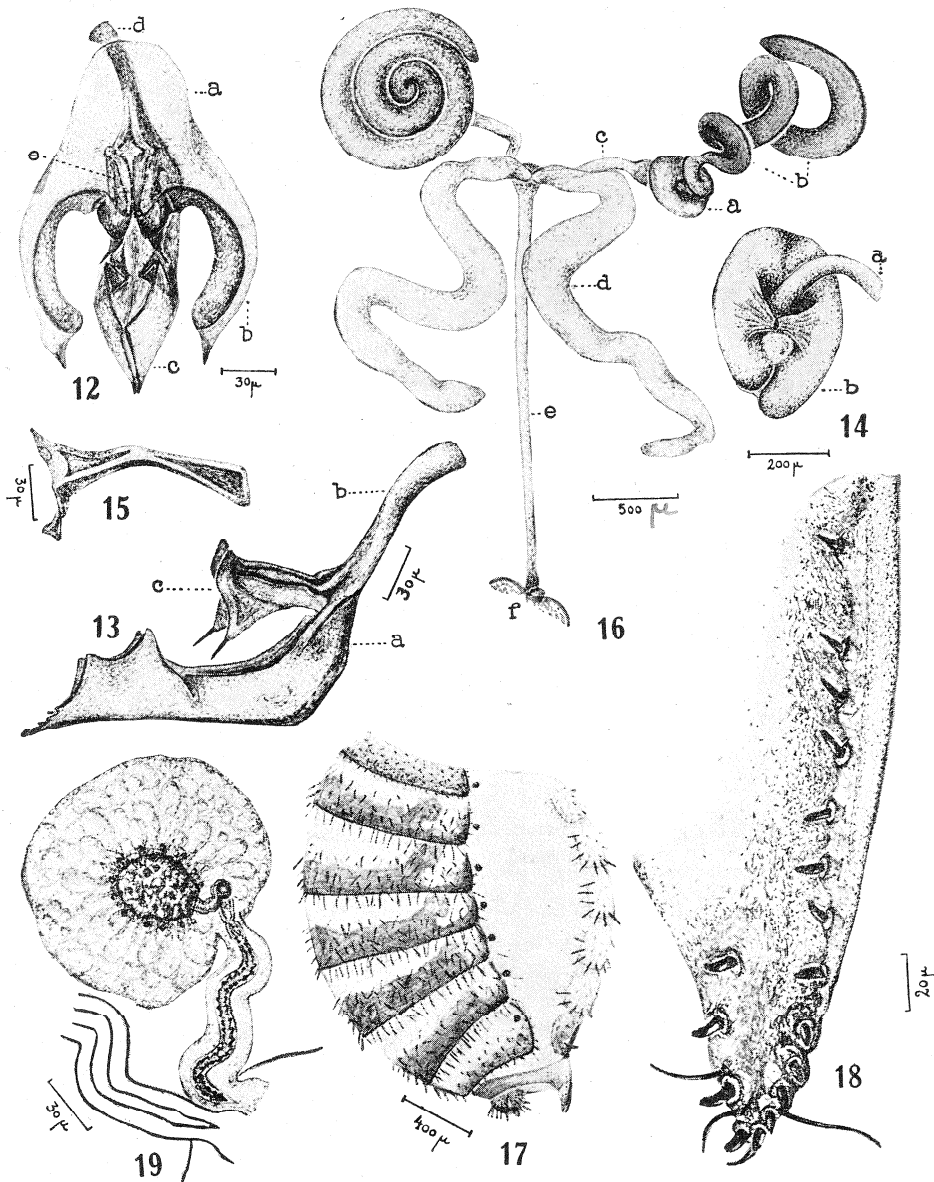
Abdomen (fig. 7) light yellow. Second to sixth tergites with black marginal bands, broader on sixth tergite, sharply delimited and interrupted in middle (fig. 8), leaving on second to fifth tergites an irregular trapezoid yellow area, laterally. First tergite with diffused and diluted black pigmentation, more condensed at lateral and posterior margins. Two spiracles on a level with sixth tergite, at each side. Chitinous pregenital plates, absent. Four sternites equally chitinized.

Genitalia: Genital arch (fig. 9) narrower in dorsal part, connected to anal plates by a chitinous bridge; partially it fuses to claspers, which are also covered by its posterior processes (figs. 9 and 11). Each clasper (figs. 9, 10 and 11) with 10 to 12 thick teeth at one row, 2 to 4 bristles, externally and 4 to 5 bristles, internally; two claspers linked by a weakly chitinized bridge, to the level of two internal long bristles. A racket-shaped plate (fig. 11), strongly chitinized, at right angle to bridge, forms a platform for the penis. Data are lacking about this plate, if it is a projection of the bridge or if it is fused to it. It seems that such a plate has not yet been observed in other species. Cerci, absent.

Anal plates (fig. 9) elliptic, grey, with long hairs.

Hypandrium (figs. 9 and 12) slightly convex; outer processes connected to genital arch, internally. Mantle of the penis, absent. Penis (figs. 9, 12 and 13) formed by two valves partially fused, with tip slightly indented. One pair of

gonapophysis bearing one bristle, each one. Penis, apodeme of the penis and gonapophysis fused in one piece (fig. 13). It seems that the hypandrium is fused also to them, by means of two internal folds of its posterior border (fig. 12).



Drosophila paranaensis n.sp. — Fig. 12: Chitinized pieces of the genitalia of the male (a- hypandrium; b- outer process of the hypandrium; c- penis; d- apodeme of the penis; e- gonapophysis); fig. 13: chitinized pieces of the genitalia of the male (a- penis; b- apodeme of the penis; c- gonapophysis); fig. 14: ejaculatory sac (a- anterior ejaculatory duct; b- left lobe); fig. 15: ejaculatory apodeme; fig. 16: internal genitalia of the male (a- inner coils of the testes; b- outer coils of the testes; c- seminal vesicles; d- paragonia; e- anterior ejaculatory duct; f- ejaculatory sac); fig. 17: abdomen of the female, diaphanized material; fig. 18: ovipositor valve; fig. 19: spermatheca.

Ejaculatory sac (fig. 14) with the right lobe shorter than left, fused at anterior part. There are not diverticula. Ejaculatory apodeme (fig. 15) *hammer-shaped*. Testes (fig. 16) with about $2\frac{1}{2}$ to $3\frac{1}{2}$ light yellow (*Pl 10 - C 1*) inner coils and $3\frac{1}{2}$ yellow (*Pl 10 - S 1*) outer coils. Seminal vesicle thin.

Length body: 3.04; length wing: 2.35 ^{mm}.

Two anterior Malpighian tubes free; posterior fused in a point, forming continuous lumen, or only apposing in it.

♀ — Wings: Heavy bristles to about $\frac{1}{2}$ of third costal section. *Costal* index 2.69; *4th vein* index 1.69; *5x* index 1.11 and *4c* index 0.92.

Abdomen (fig. 17) light yellow. Distribution of black bands on tergites, as at ♂. Seventh tergite with diluted pigmentation, on lateral and posterior margins; one pair of spiracles open in it. Six sternites; the last more chitinized, light brown and bifid posteriorly.

Genitalia: Ovipositor valves (fig. 18) internally linked by squamous membrane; posterior extremity in acute angle. Each valve with 16 to 19 teeth, thinner and further at the anterior part. Four long hairs, one at each interval between teeth, from last but one tooth forward.

Anal plates equal, grey, with long hairs.

Spermathecae (fig. 19) very small ($50\ \mu \times 32\ \mu$), not chitinized, oval with opening slightly invaginated. Ventral receptacle (fig. 20) ^{with} about 16 to 17 irregular coils, of which 5 free and 11-12 joined by a membrane.

Length body, 3.49, ^{mm} longer than male ($t = 5.81$; $P < 1\%$); length wing, 2.53, ^{mm} longer than male ($t = 3.64$; $P < 1\%$).

Egg (fig. 21) with 4 slightly sinuous filaments (sometimes 1 to 2 smooth); posterior a little longer than egg itself, anterior about $\frac{5}{6}$ posterior.

Puparia (fig. 22) brown-red (*Pl 12 - H 12*); *horn* index 0.20; anterior spiracle (fig. 23) with 16 to 19 branches.

Chromosomes — Metaphase plates (fig. 24): in female, one pair of *v's* (II), three pairs of rods (III, IV and X, the longer) and one pair of thin dots (V). In male, the Y chromosome is a rod about $\frac{1}{2}$ X.

Distribution — Brazil: Capitão Heitor Port at Paraná river (September, 1943); Cataratas and Foz do Iguassú (February, 1947); Pirassununga, State of S. Paulo (September, 1948); Belém, State of Pará (June, 1948); Livramento and Sto. Angelo, State of Rio Grande do Sul (May, 1949); Imperatriz, State of Maranhão (August, 1949). More frequent on moist places, near river at suburban zones, on decaying fruits.

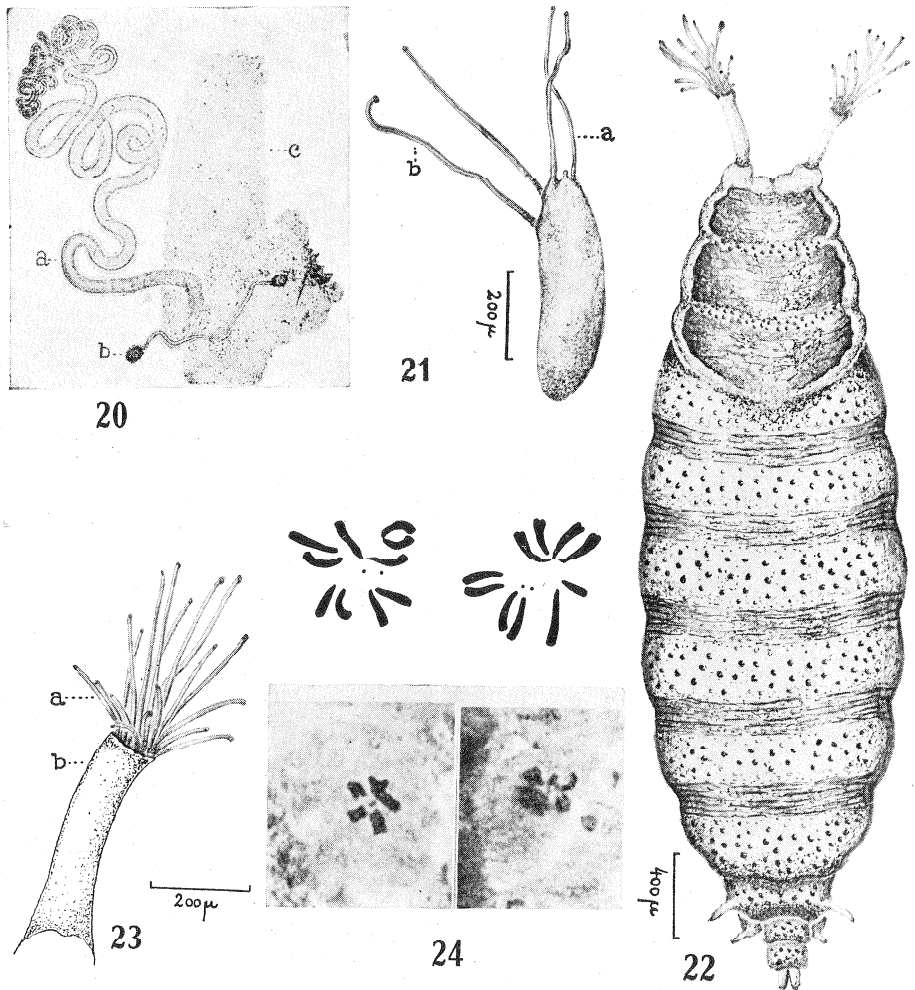
The *holotype* (♂), *allotype* and more eight paratypes (4 ♀♀ and 4 ♂♂) are preserved in the Instituto de Zoologia (Museu Paulista), Estado de São Paulo, and ten *paratypes* (5 ♀♀ and 5 ♂♂) are in the Museu Nacional, Rio de Janeiro, D. F., Brazil. All gonotypes from descendants of a fertilized female collected at Capitão Heitor Port.

TABLE I
Quantitative characters of *Drosophila paramensis* n. sp.

CHARACTERS	♂						♀						t'	P (%)		
	N	M	σ	t(1%)σ	fiducial limits	V (%)	N	M	σ	t(1%)σ	fiducial limits	V (%)				
					+	-					+	-				
Length of body.....	15	3041	185.54	552.91	3594	2488	6.10	15	3486	231.47	689.78	4176	2796	6.64	5.81	1
Length of posterior femur.....	15	801	22.90	68.24	869	733	2.86	15	830	18.89	56.29	886	774	2.27	3.78	1
Length of posterior tibia.....	15	797	2.81	53.10	850	744	3.11	15	831	18.96	40.57	872	790	2.33	4.22	1
Length of wing.....	15	2347	15.09	459.19	2806	1888	6.56	15	2530	119.01	354.65	2885	2175	4.70	3.64	1
2nd section of costal vein.....	15	1282	88.27	263.04	1545	1019	6.86	15	1416	83.67	249.34	1665	1167	5.91	4.27	1
3rd section of costal vein.....	15	493	21.90	65.53	559	427	4.46	15	526	23.35	69.58	596	456	4.44	3.98	1
4th section of fourth vein.....	15	525	33.99	101.30	626	424	6.47	15	571	26.79	79.83	651	491	4.69	4.12	1
Posterior cross-vein.....	15	881	50.03	149.09	1030	732	5.68	15	964	54.50	162.41	1126	802	5.66	4.35	1
Heavy bristles on 3rd costal section.....	15	218	14.27	42.52	261	175	6.56	15	245	13.17	39.25	224	206	5.38	3.39	1
	15	239	17.30	51.35	291	187	7.24	15	257	17.39	51.82	309	205	6.76	2.84	1
CHARACTERS	♂ AND ♀												t'	P (%)		
	N	M	σ	t(1%)σ	fiducial limits	V (%)	N	M	σ	t(1%)σ	fiducial limits	V (%)	t'	P (%)		
Anterior orbital bristle.....	30	212	17.33	47.65	260	164	47.65	30	212	17.33	47.65	260	164	8.17	0.95	5
Middle orbital bristle.....	29	121	9.48	26.16	147	95	26.16	29	121	9.48	26.16	147	95	7.83	0.80	5
Posterior orbital bristle.....	30	226	17.37	47.76	274	178	47.76	30	226	17.37	47.76	274	178	7.08	2.68	1
Eye — greatest diameter.....	32	602	24.74	68.03	670	534	68.03	32	602	24.74	68.03	670	534	4.11	1.48	5
Cheek — greatest diameter.....	30	150	15.39	44.50	193	107	44.50	30	150	15.39	44.50	193	107	10.39	1.91	5
1st prominent oral bristle.....	32	270	19.82	54.50	325	215	54.50	32	270	19.82	54.50	325	215	7.34	0.50	5
2nd oral bristle.....	32	136	22.76	62.59	199	133	62.59	32	136	22.76	62.59	199	133	16.73	0.52	5
Anterior sternopleural bristle.....	30	321	22.08	62.37	383	259	62.37	30	321	22.08	62.37	383	259	7.06	0.52	5
Middle sternopleural bristle.....	27	123	13.92	38.55	162	84	38.55	27	123	13.92	38.55	162	84	11.31	2.17	2
Posterior sternopleural bristle.....	30	409	30.28	83.27	492	326	83.27	30	409	30.28	83.27	492	326	7.40	0.99	5
Length of anterior femur.....	30	678	22.18	60.99	739	617	60.99	30	678	22.18	60.99	739	617	3.27	1.01	5
Length of anterior tibia.....	30	604	18.19	50.02	654	554	50.02	30	604	18.19	50.02	654	554	3.01	0.30	5
3rd section of fifth vein.....	30	272	15.12	41.58	314	230	41.58	30	272	15.12	41.58	314	230	5.55	1.71	5
Spermaheca — greatest diameter.....	15	50	5.97	7.79	68	32	7.79	15	50	5.97	7.79	68	32	11.89	0.95	5
Spermaheca — smallest diameter.....	15	32	1.03	3.07	35	20	3.07	15	32	1.03	3.07	35	20	3.18	0.80	5
Length of the puparium.....	11	4350	124.90	365.93	4746	3954	365.93	11	4350	124.90	365.93	4746	3954	2.87	0.80	5
Length of the anterior spiracle.....	11	877	30.35	96.21	973	781	96.21	11	877	30.35	96.21	973	781	3.46	0.80	5
Egg — greatest diameter.....	15	470	7.92	23.60	494	446	23.60	15	470	7.92	23.60	494	446	1.69	0.80	5
Anterior filament of egg.....	15	427	28.38	84.57	512	342	84.57	15	427	28.38	84.57	512	342	6.65	0.80	5
Posterior filament of egg.....	15	514	31.19	92.95	607	421	92.95	15	514	31.19	92.95	607	421	6.07	0.80	5

N = number of analyzed flies; M = mean, in micra; σ = standard deviation; V = coefficient of variation; t (1%) = maximum value of t at the 1% level; fiducial limits = calculated limits of variation, in micra, at the 1% level of t; t' = value of t obtained from the difference of the means between ♂ and ♀; P = probability.

Belongs to *repleta* group of the sub-genus *Drosophila*. It is closely related to members of *mercatorum* sub-group (Wharton, 1944): *Drosophila peninsularis* Patterson & Wheeler, 1942; *D. mercatorum mercatorum* Patterson & Wheeler, 1942; *D. mercatorum pararepleta*



Drosophila paranaensis n. sp. — Fig. 20: Internal genitalia of the female (a- ventral receptacle; b- spermatheca; c- oviduct); fig. 21: egg (a- anterior filament; b- posterior filament); fig. 22: puparium; fig. 23: anterior spiracle (a- branches; b- stalk); fig. 24: metaphase plates of female and male.

Dobzhansky & Pavan, 1943 and also to *D. meridiana rioensis* Patterson, 1943, a member of the *mulleri* sub-group (Wharton, 1944). Meanwhile, between them were found some morphological, physiological and genetic differences.

MORPHOLOGICAL DIFFERENCES

a) on etherized material

Species	Color of front	Position of 3rd orbital spot	Spot on 7th tergite, laterally, in ♀
<i>D. paranaensis</i>	beige-brown	bases of anterior vertical bristles	absent
<i>D. peninsularis</i>	tannish-brown	bases of anterior vertical bristles	present
<i>D. m. mercatorum</i>	light-brown	bases of anterior and posterior vertical bristles	absent
<i>D. m. pararepleta</i>	yellowish-grey	bases of anterior and posterior vertical bristles	absent

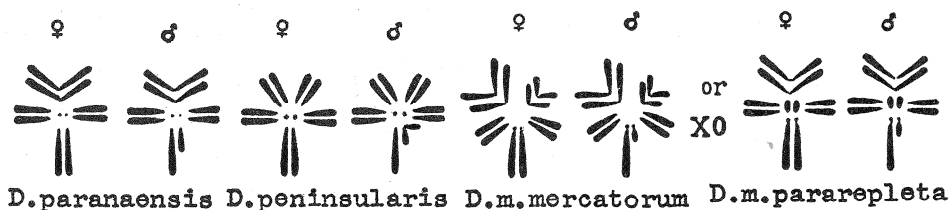
b) on dissected material

Species	Shape of spermatheca	Number of coils of receptacle
<i>D. paranaensis</i>	oval	16—17 (only 5 free)
<i>D. peninsularis</i>	hemispheric	24 (free)
<i>D. m. mercatorum</i>	spheric	6 (linked)
<i>D. m. pararepleta</i>	oblong pumpkin	9 (linked)

The numbers were taken from original description.

Comparative studies on the external genitalia and on quantitative data will be made later.

c) on metaphase configurations



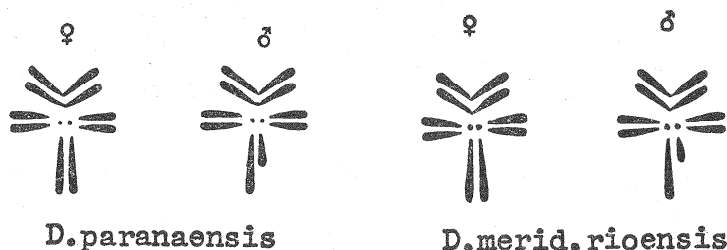
DREYFUS & BARROS observed that the salivary chromosomes of *paranaensis* — *m. pararepleta* hybrids (1947 and 1949) and also of *paranaensis* — *m. mercatorum* hybrids fail to synapse at the tips and at the bases.

In accordance to the description of *D. meridiana rioensis*, of which we have no specimens, *D. paranaensis* differs from it:

a) on etherized and dissected materials

Species	Color of eyes	Spermatheca
<i>D. paranaensis</i>	red	not chitinized
<i>D. merid. rioensis</i>	sepia	chitinized

b) on metaphase configurations



PHYSIOLOGICAL DIFFERENCES

We have verified that in *D. paranaensis*:

- a) the time of maturation is longer than in *D. m. mercatorum* and in *D. m. pararepleta*.
- b) the time of development of the larvae and imago is also longer than in *D. m. mercatorum* and in *D. m. pararepleta*.
- c) the percentage of females with sperm in the paraovaria, intraspecifically, is lower than in *D. m. mercatorum* and in *D. m. pararepleta*. Sperm were never found in paraovaria of *D. peninsularis*.
- d) there is no formation of cysts (degenerating mass formed either by sperm, or sperm plus egg) in genital organs, intraspecifically, as also in *D. peninsularis*, while we have observed them in *D. m. mercatorum* and in *D. m. pararepleta*.

GENETIC DIFFERENCES

Analyzed by means of the following interspecific crosses (10 pairs of flies per vial):

- a) *D. paranaensis* (Capitão Heitor) female x *D. peninsularis* (Florida — U. S. A.) male — is cross-sterile. Descendants did not appear in 50 vials of culture. This experiment was repeated at the temperature of $24.7 \pm 0.90^{\circ}\text{C}$.

The females were dissected after 17 days of exposure. 24.5 per cent of 50 females had sperm only in the vagina, or only in the spermatheca, or in those two organs together. It was also scarcely found in ventral receptacle. The remaining females were not inseminated.

b) *D. peninsularis* (Fl.) female x *D. paranaensis* (C. H.) male — is also cross-sterile. In 50 vials of culture, no offspring were produced. This experiment was repeated in just the same conditions of the cross *a*. Forty per cent of 50 dissected females were inseminated; receptacle with few or, more frequently, none sperm.

c) *D. paranaensis* (C. H.) female x *D. m. mercatorum* (Sta. Barbara — U.S.A.) male — gives only females. In media, 24 females per vial appeared in 62.50 per cent of 40 vials of culture. All were sterile. Larvae, that did not reach the pupal stage, were produced.

d) *D. m. mercatorum* (Sta. B.) female x *D. paranaensis* (C. H.) male — gives some females and males. In media, 3 females and 1 male per vial were produced in 10 per cent of 40 vials of culture. All were sterile. Males very weak in appearance, with rudimentary testes and abnormal abdomen; they died 2 to 3 days after birth.

e) *D. paranaensis* (C. H.) female x *D. m. pararepleta* (Jacarepaguá) male — gives only females. Also in this cross, were produced descendants that died in the larval stage. In media, 96 descendants per vial, mostly sterile, appeared in 60 per cent of 50 vials of culture. So, from the first backcross using *paranaensis* males, involving 210 pair matings, only 15.71 per cent produced offspring, in media, 8 descendants per tube. It was verified by the dissection, that the remaining 177 female hybrids had rudimentary ovaries (figs. 25 and 26).

f) *D. m. pararepleta* (J.) female x *D. paranaensis* (C. H.) male — gives few females and fewer males. In media, 14 females and 3 males per vial were produced in 30 per cent of 50 vials of culture. Females, in majority sterile; males as those of cross *d*, and also with rudimentary testes (figs. 27-29).

Crosses involving 1♀ x 2♂♂ of *D. paranaensis* were made as control. Each one of 46 fertile females gave, in media, 210 descendants.

Our results show that, in spite of the great morphological similarity between *D. paranaensis* and the other species studied here, which are hardly distinguished by their external morphology, there is already a high grade of evolutionary divergence between them. By this divergence are responsible, at least, several types of reproductive isolation: a) sterility; b) little viability of zygotes; c) abnormal sex-ratio, of which was made cytological analysis by DREYFUS & BARROS (1947 and 1949); d) abnormal reaction of insemination, that will be studied better in a further work; e) partial sexual isolation, as show the experiments of N. PEREIRA & DREYFUS (1947).

Very interesting are these facts verified by DREYFUS (1948 and 1949):
I) the rate of sexual isolation (one of ^{the} most important mechanisms of

speciation) between *D. paranaensis* female x *D. m. pararepleta* male is the same in presence or absence of conspecific females in the end of a certain period of time; II) *D. paranaensis* females are inseminated by *D. m. pararepleta* males in presence of conspecific males and females.

D. paranaensis and *D. m. pararepleta* seem to be partially sympatric. We have obtained strains of *D. m. pararepleta* from thirty five localities of Brazil. *D. paranaensis* has been found only in seven of those localities. It seems that *D. paranaensis* is allopatric with the others species.

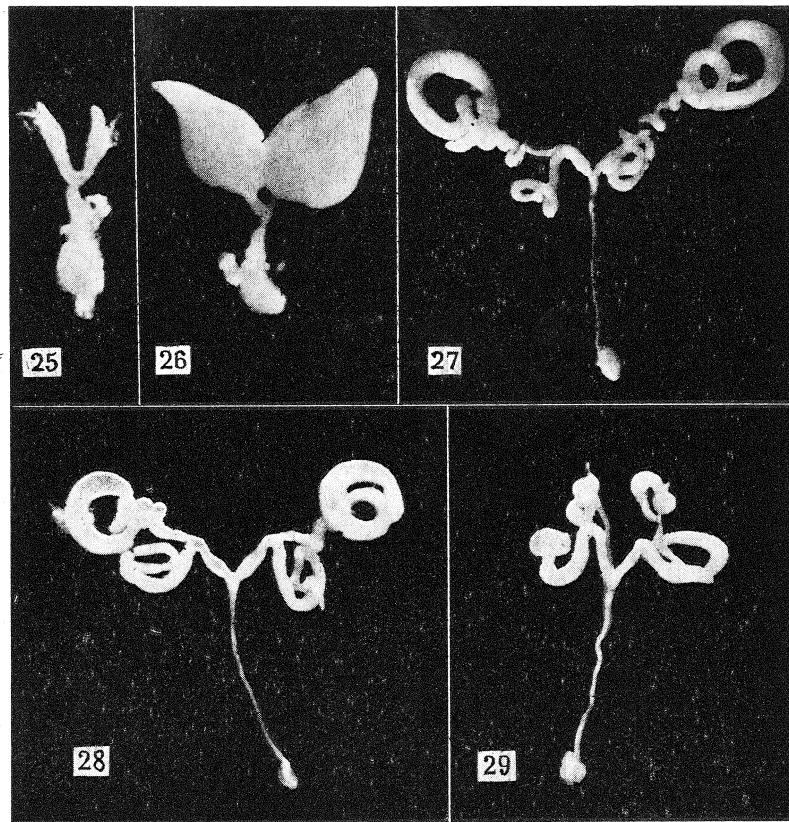


Fig. 25 - Rudimentary ovaries of a female hybrid from *D. paranaensis* female x *D. m. pararepleta* male. Fig. 26 - Normal ovaries of *D. paranaensis* female. Fig. 27 - Testes of *D. paranaensis* male. Fig. 28 - Testes of *D. m. pararepleta* male. Fig. 29 - Testes of a male hybrid from *D. m. pararepleta* female x *D. paranaensis* male.

So, by the data we know, besides others we have obtained, several stages of speciation have been observed in the *mercatorum* sub-group:

1) *D. peninsularis* of the Lake McKethan - *D. peninsularis* of Tarpon Springs (Florida - U. S. A.), which attained already a certain grade of reproductive isolation, but did not reach yet the taxonomic level of subspecies (PATTERSON & WHEELER, 1947).

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