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"DROSOPHILA PAGLIOLII" A NEW SPECIES SHOWING UNUSUAL CHROMATOGRAPHIC PATTERN OF FLUORESCENT SUBSTANCES [1]

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(With 5 text-figures)

During our collecting trips to secure material for ecologic and population-genetics studies we found an interesting new species that now is being described and named in honor of a great friend of our group of researchers, Prof. Dr. Elyseu Paglioli.

The description of this species contains also the chromosomal configurations of cerebral ganglions' metaphase plates. The two-dimensional paper chromatography pattern of fluorescent substances specially pteridines is presented due to its remarkable nature. The great amount of sepiapteridine and its deoxy derivative is the highest until now found by us after the study of about 70 different species of *Drosophila*. A complete report of these studies is being prepared

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Drosophila pagliolii sp. n.

Male and female – Arista with 8 to 9 branches and 4 to 6 smaller in a horizontal plane. Antennae dark brown with heavy pilosity. Front length/width 0.85/0.55, dark brown; ocellar triangle blackish brown. Anterior orbital 4/5 and middle 5/12 of the posterior. Two prominent oral bristle, the second about 2/3 of the first.

Face brown. Carina broader below and not sulcate. Palpi with yellow pilosity, three equally long bristles and two or three smaller ones. Cheeks brown, with the length of its greatest bristle and about 1/6 of the longest diameter of the eye. Eye dark red with yellow piles. Proboscis 0.5 mm.

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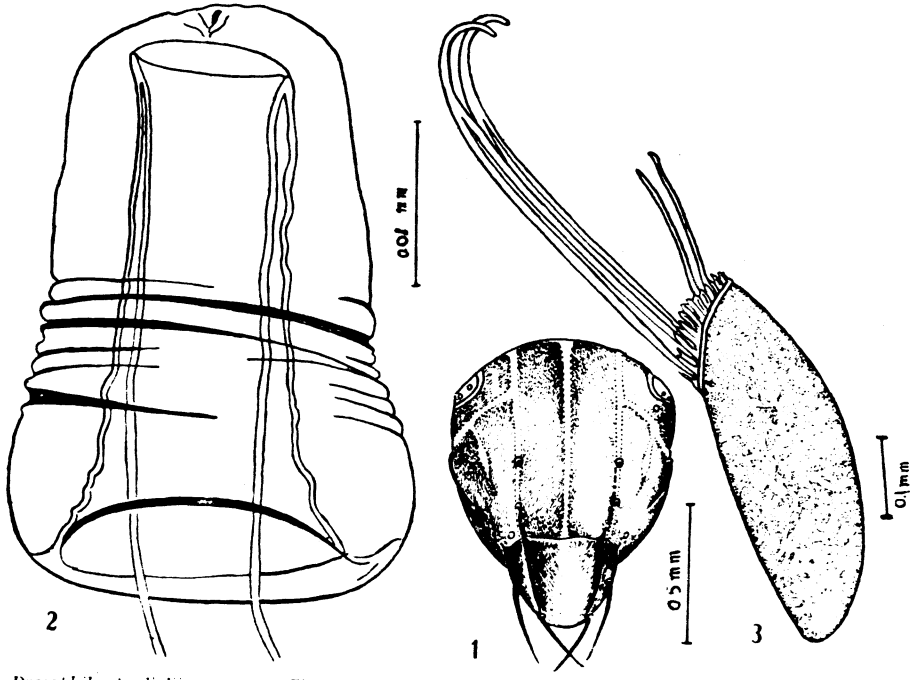
Acrostichal hairs in 8 rows. No prescutellars but all bristles between the four dorsocentral about twice as long as the other acrostichals. Anterior dorsocentral about 3/4 of the length of the posterior. Anterior scutellars convergent. Mesonotum dark brown, with three narrow yellowish longitudinal stripes (fig. 1), one median and the other two in the dorsocentrals' line. Pleurae and scutellum brown. Anterior sternopleural 5/6 and the middle 3/4 of the posterior. Haltere brownish. Legs yellowish; apical bristles on the first and second tibiae, preapical on all three. First leg with two parallel rows of short heavy bristles, in the internal side of the tarsal segments; second and third legs with two long and strong bristles on the basis of the first tarsal segment. Wings greyish brown clear, veins yellowish. Crossveins slight clouded. Two prominent bristles at the



Drosophila pagliolii sp. n. – Fig. 1: The mesonotum stripes, dorsocentral bristles excluded; fig. 2: spermatheca; fig. 3: egg, showing the four filaments and the surface marks

apex of the first costal section; third costal section with heavy bristles on its 3/5 basal portion, followed until the end of the fourth section by a pilosity slight heavier than the ones in the costal anal side. Costal index: 2.3-2.7; 4th vein index: 1.5-1.6; 5 x-index: 1.3-1.4.

Abdomen brown with blackish brown distal bands in all tergites except the first that is completely dark brown.

The dark bands in the second to fifth tergites of male or female expanded laterally reaching the anterior edge in the margins, and near the middle of each tergite there is an expansion followed by a depression almost reaching the

posterior margin. Sixth tergite in the male darker than in the female, the dark band reaching the anterior margin in the middle and laterally of the tergites.

Internal characters of the imago — Malpighian tubes pale yellowish, anterior and posterior ends free. Testes yellowish brown to brownish in old specimens with four outer and 3 inner coils. Spermatic pump large. Seminal vesicle coiled. Ventral receptacle with about 89-90 coils. Spermatheca bell-shaped (fig. 2) with wrinkled exterior sheet.

Adult size: male, body 2.25-2.50; wing 3-3.2 mm; female, body 3.50-4.0; wing 3-3.5.

Egg with four filaments, the posterior stronger and three times the length of the anterior (fig. 3).

Puparia brown: horn index 5.1, anterior spiracle with 20-22 free branches.

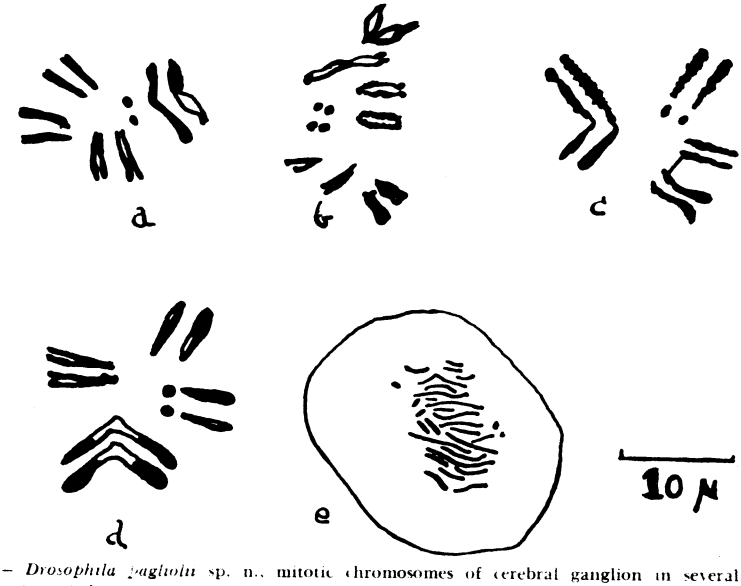


Fig. 4 — *Drosophila pagliolii* sp. n., mitotic chromosomes of cerebral ganglion in several stages. Note in *b* the division of dots, in *c* the proximity of the dots with one pair and the apparent connection of two rods (?); in *d* the V shaped chromosomes shows a lack of coloration (by acetic orcein and, in *e* a polyploid cell is pictured with part of its chromosomes, many of them appeared

Chromosomes — One pair of V shaped, three pairs of rod shaped and one pair of dot-like chromosomes associated in many cells to one of the rods (fig. 4).

Geographic distribution — Since 1954 this species has been found at low frequencies but consistently specially during Autumn, Winter and Spring in several localities of Rio Grande do Sul: Bexiga (Sta. Maria County), Eldorado (Guaiba County) and Garruchos, São Pedro (Torres County) and in 1962 it was found at a "cerrado" near Brasilia, D.F.

Several specimens have been preserved in Barber liquid and others in the dry pinned "Reference Collection" maintained in our Department.

Relationships – This new species is related to *Drosophila fumosa* Pavan & Da Cunha, 1947 from which it differs in the chromosome complements, the spermathecae, and some other characteristics.

THE TWO-DIMENSIONAL PAPER CHROMATOGRAPHIC PATTERN

The chromatographic technique used by HADORN & MITCHELL (1951), BUZZATI-TRAVERSO (1953) and FOX (1956) was followed to develop the two--dimensional paper descending chromatograms.

Due to the size of these flies, only *two* decapitated bodies, of males or females, aged 14 days, have been used for each chromatogram. They are quickly rinsed in 90% ethanol boiled in distiled water for one minute and smashed directly on the paper. The first solvent was 1 part of *n*-propanol to 2 parts of 7% ammonia; the second: the upper phase of *n*-Butanol satured with acetic-acid--water (4 : 1 : 5).

After two hours of saturation in the chromatocabs the solvent was poured and let to migrate 9 hours in the first solvent and 4 hours in the second at 22°C approximately.

After drying in an air forced owen the chromatograms were observed with the aid of a 3650Å UV light and the fluorescent spots were delineated with a pencil. The intensity of fluorescence was scored visualy in four categories: very strong, medium, and weak.

The dubious, "very weak" spots were not included.

Due to the quickly loss of fluorescence exhibited by many substances we did not attempt in this work to study quantitative differences with the aid of a fluorometer. All the process was executed in darkroom conditions.

Two months after this examination the chromatograms have been observed at the UV (3650Å) light again. New spots with a faint green or "ice blue" fluorescence were observed. *The results* are summarized in Table I and fig. 5.

The most stricking characteristic is the great amount of sepiapterin and its derivate *isosepiapterin* in the *males* a large spot of an unknow blue fluorescent substance (N.º 5) that appears some time after the first examination. The females shows all the known pteridines that occur in their males, except the Drosopterins. This fact is expected because most of the body pterins are at the testicles.

The six unknown spots that appeared after the first examination under UV (1 to 6) are another unusual feature of this species.

The females have the yellow 1, and the yellow 2, spots not detected in males. The uric acid has been detected in both sexes as a small rounded spot a little over the isoxanthopterin (fig. 5 U).

The xanthina oxidase with a cofactor, the diphosphopyridine nucleotide, are necessary to convert hypoxanthine through xanthine to *uric acid*. The same

TABLE I

Occurrence and relative intensity of the fluorescent spots in two-dimensional paper chromatograms of Drosophila pagliolii

SUBSTANCES AND SPOTS	MALES	FEMALES	Rf VALUES	
			1st SOLV	2nd SOLV
Isoxanthopterin	+++	+++	0.10 ± 0.02	0.20 ± 0.02
Sepiapterin	++++	++	0.31 ± 0.03	0.32 ± 0.02
Isosepiapterin	+++	—	0.46 ± 0.03	0.50 ± 0.03
Biopterin	++	++	0.30 ± 0.03	0.34 ± 0.03
2-amino —4 hydroxypteridin e (AHP)	++	++	0.27 ± 0.02	0.27 ± 0.02
Flavine mononucleotide	+++	+++	0.07 ± 0.01	0.04 ± 0.02
Riboflavin like	+++	+++	0.27 ± 0.01	0.22 ± 0.02
Drosopterins	+++	—	0.02	0.05
Unknown spots				
Yellow Y	++	—		
Yellow$_1$ Y_1	—	++	0.17	0.19
Yellow$_2$ Y_2	—	++	0.07	0.09
Yellow$_3$ Y_3	++	+++	0.23	0.11
Ice blue 1	+	—	0.31	0.09
Blue 2	++	—	0.37	—
Yellow 3	++	++	0.31	0.09
Ice blue 4	++	—	0.43	0.13
Blue 5	++++	++	0.32	0.19
Green 6	++	—	0.34	0.32
			0.17	0.19

enzyme catalyse the conversion of *2-amino-4 hydroxypteridine* to *isoxanthopterin* (FORREST, 1962).

The *uric acid* appears in median amounts in males and more weak in females of *D. pagliolii*.

The amount of Drosopterins and yellow pterins in males place this species among the "primitive *Drosophila*" stage of evolution suggested by THROCKMORTON (1962). These characteristics are exhibited by the *repleta*, *cannalinea* and *mesophragmatica* "Groups" of species for example. The role of pteridines as precursors of the red eye pigments and their relations with the ommochromes (brown) pigments have been discussed recently by FORREST (1959, 1962) and ZIEGLER (1961). The adaptive importance of these pigments can be illustrated by the fact that no mutant lacking such pigments (white, sepia, etc.) can reach high frequencies in natural or experimental populations. On other hand, the racial, sub-specific and specific difference of the pteridine pattern was demonstrated by the work of BUZZATI-TRAVERSO & RECHNITZER (1953), RASMUSSEN (1955), CORDEIRO, LEWGOY & TONDO (1960), CORDEIRO (1959), HUBBY & THROCKMORTON (1960), and by the extensive analyse of THROCKMORTON (1962). The compound nature of the red eye pigment was first demonstrated by TONDO, LEWGOY & CORDEIRO (1960) by electrophoresis. This fact is of importance in connection with the comparative biochemistry studies of evolution in *Drosophila* genus.

This species would be classified biochemically as a *sepia* or *sepiaoid* mutant. The eye color of this species are *darker* than the ones of *tripunctata* group that have very low sepiapterin according THROCKMORTON (1962) and our

unpublished observations. Nevertheless, the *guarani* group that shows dark eyes have quite low sepiapterin accumulation (THROCKMORTON, 1962).

A proper balance of production and use in the metabolic pathways of pteridines was attained by some groups of species only.

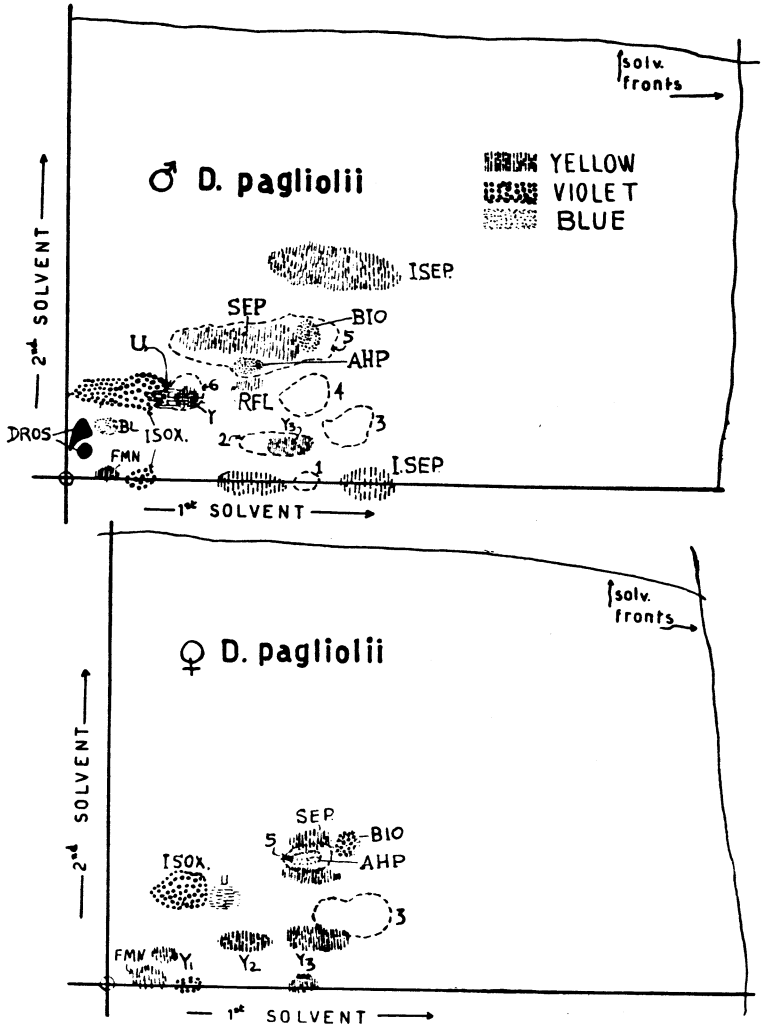


Fig. 3 – *D. pagliolii* sp. n. Outlines of actual two-dimensional paper chromatography of *male* and *female*. *DROS* = *drosopterins*, red and orange; *FMN* = flavine mononucleotide, *ISOX* = isoxanthopterin, *SEP* = sepiapterin, *I.SEP* = isosepiapterin, *BIO* = *biopterin*, *AHP* = 2 amino–4 hydroxypterin, *RFL* = = riboflavin like substances, Y Y_3 = Yellow fluorescent unknown substances and the spots *1* to *6* are unknown substances that appeared *after* the first examination.

SUMMARY

Drosophila pagliolii sp. n. is described and the outstanding accumulation of *sepiapterin, isosepiapterin* and an unknown substance in the male bodies, are presented, and discussed in relation with other species groups of this genus.

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