SIBLING SPECIES OF THE *DROSOPHILA SERRATA* GROUP

FRANCISCO JOSE AYALA
Department of Zoology, Columbia University[1]

Accepted 24 May 1965

Drosophila serrata Malloch (1927) is a species belonging to the *melanogaster* section of the subgenus *Sophophora* and was originally described from a locality (Eidsvold) in Queensland, Australia. A redescription, in the form now accepted in *Drosophila* systematics, was made by Mather in 1955. Dobzhansky and Mather (1961) collected living specimens in eight localities of New South Wales and Queensland in Australia, and in New Guinea and New Britain. They distinguished two subspecies, the southern *D. serrata serrata* in New South Wales and most of Queensland, and the northern *D. serrata birchii* from Cairns, Queensland, to New Guinea and New Britain. The southern subspecies was described as having females with darker abdomens and males with two pairs of strong bristles in the genital arch. The northern group was characterized as having lighter females and three pairs of bristles in the male genitalia.

More recently, some 15 strains were collected by H. L. Carson and M. Wasserman. The present paper reports the results obtained from the study of all the available strains. It will be shown that the subspecies described by Dobzhansky and Mather are, in fact, two reproductively completely isolated species, *D. serrata* and *D. birchii*, which are sympatric at several localities. A strain collected in a locality of New Guinea (Madang) belongs to a third sibling species, fully isolated from the other two, which I have named *Drosophila dominicana*. A description of this new species has been published elsewhere (Ayala, 1965a).

GEOGRAPHIC DISTRIBUTION

The strains used in the present work, designated after the place they were collected, are listed below (see Fig. 1).

D. serrata: Sydney (from Bulahdelah, 130 miles north of Sydney), New South Wales, Australia, collected by Dobzhansky; Brisbane, Queensland, Australia, by Dobzhansky; Mundubbera, Queensland, by W. B. Mather; Heron Island, about 90 miles east of Rockhampton, Queensland, by H. L. Carson; Cooktown, Queensland, by M. Wasserman; Katherine Gorge, Northern Territory, Australia, by Wasserman; Roper River, Northern Territory, by Wasserman; Elsey Falls, Northern Territory, by Wasserman; Mataranka, Northern Territory, by Wasserman; Port Moresby, New Guinea, by Dobzhansky and Mather; Samarai, New Guinea, by Carson; Popondetta, New Guinea, by Wasserman; Wau, New Guinea, by Wasserman; Madang, New Guinea, by Carson; Rabaul, New Britain, by Wasserman.

D. birchii: Cairns, Queensland, collected by Dobzhansky; Port Moresby, New Guinea, by Wasserman; Popondetta, New Guinea, by Wasserman; Bulolo, New Guinea, by Dobzhansky and Mather; Goroka, New Guinea, by Carson; Rabaul, New Britain, by Dobzansky and Mather.

D. dominicana: Madang, New Guinea, collected by Carson.

D. serrata seems to be distributed continuously in Australia from just north of Sydney, through coastal New South Wales, coastal Queensland, and the Northern Territory. The species is also common in western New Guinea and New Britain. The distribution of *D. birchii* is less extensive. It is abundant in New Guinea and New Britain, but it has not been found south of Cairns in Australia. Professor Birch (personal communication) collected extensively during different seasons of the year in the area of Rockhampton, Queensland, without ever finding any specimens of *D. birchii*. Other attempts to find the species in places in Australia other than Cairns have been equally unsuccessful. *D. birchii* is found only in the deep forest while *D. serrata* lives around human habitation and in disturbed areas (Carson, personal com-

[1] Present address: Providence College, Providence, Rhode Island.

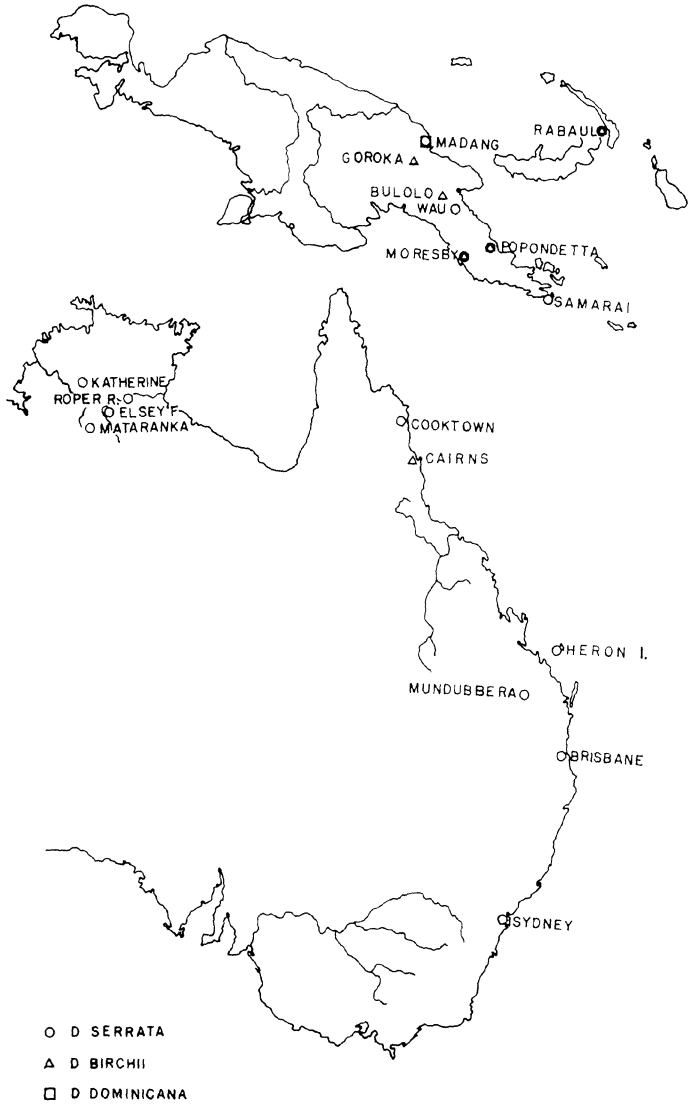

FIG. 1. Geographic origin of the strains of *Drosophila serrata*, *D. birchii*, and *D. dominicana* which served as material for the present investigation.

munication). *D. dominicana* has been collected so far only at Madang, New Guinea.

MORPHOLOGY

The most useful trait which permits distinguishing the strains of the three species is found in the male genitalia. The difference can be seen easily in both living and dried male specimens under moderately high magnification of a dissecting microscope. The males of *D. serrata* have two pairs of strong bristles on the genital arch (Fig. 2, left). The males of *D. birchii* have three pairs of strong bristles of about the same size on the genital arch (Fig. 2, center). The males of *D. dominicana* have

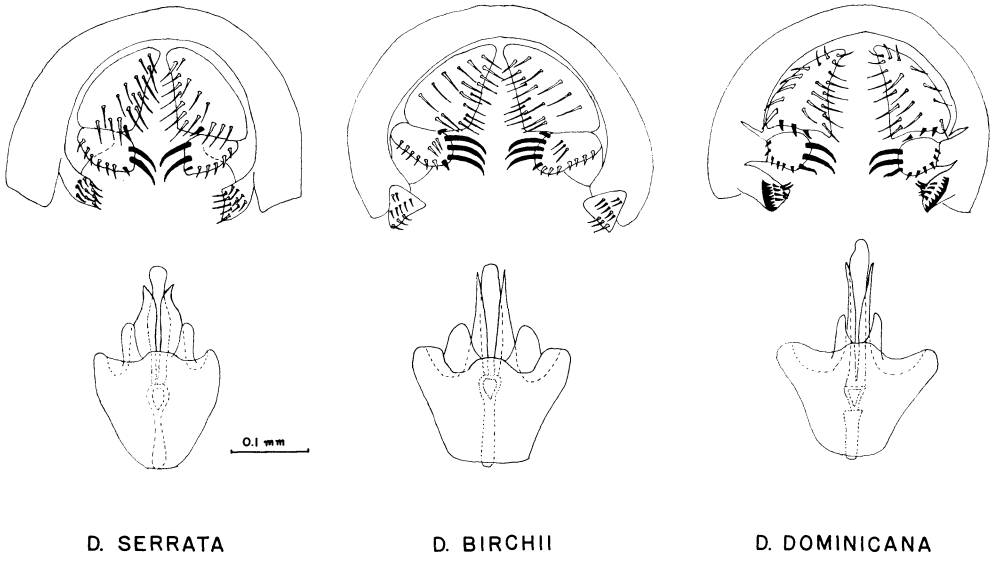

FIG. 2. Male genitalia of *Drosophila serrata* Malloch (left), *Drosophila birchii* Dobzhansky and Mather (center), and *Drosophila dominicana* Ayala (right).

two pairs of large bristles and another pair, anteriorly located with respect to the other two, somewhat smaller (Fig. 2, right). These differences are clear-cut, and for all practical purposes uniform in all the strains of the same species.

There are also differences among the species in the shape of the penis, gonapophyses, hypandrium, apodema, etc., as indicated in Fig. 2. How constant these characters are from strain to strain is uncertain. Some variability seems to exist within each species. The strains from which the drawings were made, are Brisbane (*D. serrata*), Cairns (*D. birchii*), and Madang (*D. dominicana*).

Dobzhansky and Mather observed differences between *D. serrata* and *D. birchii* in the coloration of the dorsal part of the abdomen of the females. This trait, which is observed in females several days after emerging from the pupa, does not seem sufficiently reliable because of great variability within each species, especially in *D. serrata*. Females of *D. birchii* have light abdomens. Dark brown bands are found only in the posterior margins of the second to fifth tergites. These bands narrow laterally, and are more or less sharply delineated, leaving most of the tergite brownish yellow. The females of *D. serrata* are more variable in color, sometimes even within the same strain. The dorsal part of the abdomen of some strains is similar to that of *D. birchii* females. These are all the strains from New Britain and New Guinea, except Popondetta, and the strains from Roper River, Katherine Gorge, and Elsey Falls in the Northern Territory. The strains from Sydney, Brisbane, and Mundubbera have much darker abdomens. The dark brown bands along the posterior borders of the abdominal tergites are expanded laterally towards the antero-lateral angles of the tergites; the outlines of these bands are not sharp, making the whole abdomen seem fuscous-brown in color. The Mataranka strain is clearly bimodal, with females of the two types indicated above. The Heron Island strain is also bimodal, but the darker females have the sixth, seventh, and eighth tergites very dark brown through the whole dorsal area, so that the tip of these females appears conspicuously dark. All the females of the Popondetta strain also have this dark tip of the abdo-

men. The females of *D. dominicana* have dark abdomens similar to the phenotype of the Sydney strain.

The dorsal part of the abdomen of the males is light in coloration, and all three species are very similar. Only the males of the Elsey Falls strain of *D. serrata* are darker and can be differentiated from the others in terms of this character alone.

Both males and females of *D. dominicana* have a characteristic dark brown pleural stripe in both sides of the thorax. A single individual of *D. dominicana* can be identified in terms of this trait alone. It remains to be known if this character will be present in other populations of this species.

Sexual Isolation

The technique used to study sexual isolation was the following. Groups of 10 virgin females from one strain were placed in ordinary half-pint culture bottles with about 10 males of the same or of a different strain, and kept in a constant temperature room at 25 ± 0.5° C. Five days later the females and males were transferred, without etherization, to fresh culture bottles. After five more days the surviving females were dissected, and their reproductive tracts examined under a microscope for the presence or absence of the sperm. This was the method used by Dobzhansky and Mather, and I followed it to make the data comparable.

In all, some 10,000 females of the three species were dissected; the data are summarized in Table 1. In each column the first figure represents the number of females tested, and the second figure the percentage of those females which were inseminated by the corresponding males. There is a clear-cut sexual isolation between the species.

Table 2 presents the percentages of females inseminated in intraspecific or interspecific crosses between the strains. The number of the females tested in each case is omitted to make the table less cumbersome. It is worth noting, however, that the data on the intraspecific crosses between strains of *D. birchii* are based on a larger number of females tested, usually about 100 for each cross.

TABLE 1. *Sexual isolation within and between the species* Drosophila serrata, D. birchii, *and* D. dominicana. *The first figure in each column represents the number of females tested; the second figure indicates the percentage of those females inseminated by the corresponding males.*

♀♀	♂♂		
	D. serrata	*D. birchii*	*D. dominicana*
D. serrata	3,841–90	1,246– 1	395– 1
D. birchii	699– 1	2,458–77	250– 0
D. dominicana	163– 0	537– 4	43–93

Intercrosses of the strains of *D. serrata* from different localities occur quite easily. The total average number of females inseminated is 90 per cent. The joint sexual isolation index (percentage of females inseminated by males of the same strain minus percentage of females inseminated by males of different strain, divided by the sum of both percentages) for all the intraspecific crosses of *D. serrata* has the value 0.039. This value is, however, significantly different from zero, because of the large number of females tested. The *t*-value for homogametic versus heterogametic matings between strains of *D. serrata*, using the binomial variance, is 5.54, with $P < 0.001$. There exists therefore some beginning of sexual isolation between geographic strains of *D. serrata*. From Table 2 it appears that Rabaul and Heron Island are the two strains relatively more sexually isolated from the rest. This seems interesting since these two strains are also geographically isolated from the rest of the species.

A rather higher sexual isolation between some geographic strains occurs in *D. birchii*. The joint sexual isolation index for all the strains of *D. birchii* is 0.121 as compared to 0.039 for *D. serrata*. Table 3 presents above the diagonal the joint sexual isolation indexes between strains, and below the diagonal the corresponding *P* values, using the binomial variance for the estimation of *t*. All the sexual isolation in-

TABLE 2. *Sexual preferences among strains of* Drosophila serrata, D. birchii, *and* D. dominicana. *The figures given represent the percentage of females inseminated by the corresponding males.*

♀ \ ♂		*D. serrata*																*D. birchii*							*D. dominicana*
		Sydney	Brisbane	Mundubbera	Heron I.	Cooktown	Elsey F.	Mataranka	Roper R.	Katherine G.	Moresby	Samarai	Popondetta	Wau	Madang	Rabaul	TOTAL	Cairns	Moresby	Popondetta	Bulolo	Goroka	Rabaul	TOTAL	
D. serrata	Sydney	97	100	100		48	95	100	100	100	88	100	95	38	82	21	82	0		0	0	0	0	0	0
	Brisbane	95	94	100	73	72	100	100	100	100	96	85	100	76	100	95	91	2	0	0	5		0	1	0
	Mundubbera				82	57	100	100	95	94							80				0		0	0	
	Heron I.	87	100		100	79	75		90		69	67	87	59	100	18	76	0	0	0			0	0	0
	Cooktown	100	100		95	100	100	96	55	96	96	97	100	100	79	60	93	0	0	0	0		0	0	0
	Elsey F.				100	100					92		97	97	100	100	98	0		0	0		3	1	0
	Mataranka				97	98					63	96			100	82	89	6					0	3	2
	Roper R.				95	90		100	96		100						96	0		0		0	0	0	
	Katherine G.				100	100					90						96	0	0				0	0	
	Moresby				55	86	100	100	95	68	97	97	95	69	100	100	88	0	0	0		0		0	0
	Samarai	92	100		99	100	100	100			100	100	100	100	97	88	98	0		0			0	0	0
	Popondetta	90				100		95			83	100		100	97	70	91	0		0			0	0	0
	Wau	100				100	100				100	100	100	100	100	75	95	0	0	29		0		5	14
	Madang	89	92		98	100		100			100	97	100	98	94	93	96	0		0		0		0	0
	Rabaul		75			86	80				81	78		88	90	96	85	0		0			0	0	0
	TOTAL	93	94	100	93	83	96	99	93	88	89	94	98	85	96	78	90	1	0	2	1	0	0	1	1
D. birchii	Cairns	0	3	5	0	8	0		6			2	0	0	0	0	2	92	56	72	54	44	57	63	0
	Moresby	0					0				0						0	93	94	99	83	70	89	89	0
	Popondetta										0	0	0				0	83	93	90	57	89	88	83	2
	Bulolo	0	0	0	0	0								0			0	100	82	73	96	80	96	85	0
	Goroka																	94	50	93	100	100	53	82	0
	Rabaul	4	0		0	0	0		0						0	0	1	86	67	51	55	47	95	69	0
	TOTAL	1	1	2	0	2	0		4		0	1	0	0	0	0	1	89	77	76	74	68	76	77	0
D. dominicana			0	0		0	0	0			0	0	0	0	0		0	3	4	0	0	0	10	4	93

Number of females tested: *D. serrata*, 5,482; *D. birchii*, 3,407; *D. dominicana*, 743; total, 9,632.

TABLE 3. *Sexual isolation among strains of* Drosophila birchii. *The joint sexual isolation indexes are given above the diagonal. Below the diagonal: values of* P *for these isolation indexes.*

	Cairns	Moresby	Popondetta	Bulolo	Goroka	Rabaul	Average
Cairns		0.11	0.08	0.08	0.16	0.13	0.11
Moresby	<0.001		0.02	0.07	0.24	0.10	0.11
Popondetta	<0.001	>0.1		0.18	0.02	0.14	0.09
Bulolo	<0.001	<0.001	<0.001		0.04	0.12	0.10
Goroka	<0.001	<0.001	>0.3	<0.01		0.32	0.16
Rabaul	<0.001	<0.001	<0.001	<0.001	<0.001		0.16

dexes are highly significant except two, corresponding to the matings Popondetta × Moresby and Popondetta × Goroka. Goroka and Rabaul have the highest average sexual isolation indexes. The Rabaul strain is also geographically isolated from the rest. In the Goroka strain the high sexual isolation index seems to be due to the low sexual activity of the males of this strain (see Table 2).

The sexual isolation indexes given here are not directly comparable with the indexes used in other papers. In most studies on *Drosophila* the "male choice" method is used to test for sexual isolation. Virgin females from two strains to be tested are confined with an equal number of males of one of the strains. The duration of the exposure is adjusted to secure the insemination of approximately one-half of all the females exposed, and usually varies from a few to 48 hours. In the present investigation, only one kind of male and one kind of female are confined together; there is no possible alternative mating. The exposure is extended for 10 days so that every chance is given to the flies to mate.

HYBRID STERILITY

Crosses between strains of *D. serrata* produce abundant F_1 hybrid progenies which are fertile in all cases tested. For *D. birchii* the story is different. All hybrids having Rabaul as one of the parents or Cairns as the male parent produce no F_2 progenies. Hybrids having Cairns as the female parent produce, however, F_2 progenies. F_2 progenies are easily produced by hybrids between any two strains from New Guinea. The sterility of the hybrid progenies having Rabaul as one of the parents or Cairns as the male parent is due to sterility of the hybrid males. The hybrid females are fertile, and produce backcross progenies with males of either parental strain.

Considering the geographic relationships and the reproductive isolation due to hybrid sterility, one might distinguish three subspecies among the strains of *D. birchii*. The strains from Cairns and from Rabaul could be considered as the only representatives of the Australia and New Britain subspecies respectively, while all the strains from New Guinea could be grouped into a third subspecies.

The following interspecific crosses produced hybrid progeny: *D. serrata* Mataranka × *D. dominicana* Madang; *D. serrata* Wau × *D. birchii* Popondetta; *D. serrata* Wau × *D. dominicana* Mandang; *D. dominicana* Madang × *D. birchii* Moresby. Only a few individuals were obtained from the first cross, and they failed to produce F_2 progeny. In all three other crosses, F_2 progenies were obtained.

DISCUSSION

The genus *Drosophila* abounds in pairs or groups of morphologically very similar species whose genetic architecture is, in some cases, widely different. A case worth noting is the pair *D. willistoni* and *D. paulistorum* extensively studied by Dobzhansky and co-workers. These species are hardly distinguishable morphologically, although Spassky (1957) has discovered slight but diagnostically reliable differences in the male genitalia. The two species, however, represent very different, if not

antithetic, genetic architectures. *D. willistoni* inhabits an enormous territory from southern Florida and the middle of Mexico down to La Plata in Argentina. Nevertheless the species is genetically almost monolithic; there is ample chromosomal polymorphism but strains of any geographic origin cross easily (Dobzhansky, 1963). On the other hand, *D. paulistorum* is a complex of at least six races or incipient species some of which have, in fact, reached a degree of reproductive isolation enabling them to coexist in some territories, apparently without gene exchange (Dobzhansky and Spassky, 1959; Dobzhansky *et al.*, 1964; Malogolowkin-Cohen *et al.*, 1965).

More information is needed to understand the genetics of the sibling species *D. serrata, D. birchii,* and *D. dominicana,* but some facts are clear enough. *D. serrata* is the most successful species; it is apparently the most abundant in the localities where it is sympatric with any of the other two species, and surely has a considerably greater distribution area from just north of Sydney, through Queensland, and the Northern Territory in Australia, up to New Guinea and New Britain. *D. birchii* has never been found south of Cairns. In spite of its wide geographic range, strains of *D. serrata* cross easily giving very low sexual isolation indexes. *D. birchii* appears to be genetically more heterogeneous. Sexual isolation indexes between strains are considerably higher than in *D. serrata,* and hybrid sterility has developed so that three subspecies may be distinguished. Gene-flow between its subspecies is severely handicapped by the geographic isolation and by hybrid sterility. Even if a close relation between the amount of genetic differentiation and behavior is not always expected, we can see that the Rabaul strain is also the most sexually isolated. The Cairns strain is also considerably isolated sexually; the joint sexual isolation index of this strain is moderate due to the great sexual activity of its males.

The difference in genetic architecture between *D. serrata* and *D. birchii* is also indicated by the experimental studies of Ayala (1965b). The relative fitnesses of the widely geographically separated strains of *D. serrata* from Sydney, Cooktown, and Popondetta were very similar, and hybrid populations between strains of different geographic origins failed to show any breakdown after many generations of equilibrium with the environment. On the other hand, there was a large difference in performance between the Cairns and the Popondetta strains of *D. birchii.* Hybrid populations between these two strains were intermediate in fitness, but after eight or nine generations one of them increased to the level of the better parental strain. These results seem to indicate that there exist considerable genetic differences between the strains of *D. birchii.*

A comment should be made in reference to the results obtained by Dobzhansky and Mather (1961). Their two "subspecies" appeared as completely reproductively isolated from each other, except for the Moresby strain, which morphologically belonged to the *birchii* group while being intermediate between *serrata* and *birchii* in sexual behavior. The strain was established with descendants of several impregnated females collected in nature. The results observed by Dobzhansky and Mather could be explained if the mass cultures of this strain were a mixture of both species, and this situation was not detected when the genitalia of the males were examined.

In conclusion, *D. serrata, D. birchii,* and *D. dominicana* appear as another evolutionarily interesting case of sibling species in *Drosophila.* A more thorough study of the available strains and more collecting in nature is necessary in order to understand adequately the evolutionary status of these species.

Summary

Three reproductively isolated species, *Drosophila serrata, D. birchii,* and *D. dominicana,* can be distinguished among 22 strains of the *D. serrata* group. The genitalia of the males present the most reliable morphological traits for distinguishing

these species. *D. serrata* has a wider geographic distribution, extending from New South Wales, through Queensland and the Northern Territory, up to New Guinea and New Britain; nevertheless, intercrosses of the strains from different localities occur quite easily. *D. birchii* is abundant in New Guinea, and has been found in one locality (Cairns) in Australia and in New Britain (Rabaul). Sexual isolation between strains of *D. birchii* is higher than in *D. serrata*. Hybrid *D. birchii* males are sterile when either parent is from the Rabaul strain, or when the male parent is from Cairns. *D. dominicana* is recorded from only a single locality, Madang, in New Guinea.

ACKNOWLEDGMENTS

I am greatly indebted to Professor H. Levene for advice and many valuable criticisms; to Professor Th. Dobzhansky for advice, encouragement, and many suggestions; and to Dr. Ch. Malogolowkin-Cohen who taught me the techniques of dissection of the male genitalia and helped me continuously with her advice. It is a pleasure to express my appreciation to Miss Marian Quirk who typed the manuscript. This work was done while the author was a recipient of a Columbia University President's Fellowship.

LITERATURE CITED

AYALA, F. J. 1965a. *Drosophila dominicana,* a new sibling species of the serrata group. Pacific Insects, **7**: 619–621.

——. 1965b. Relative fitness of populations of *Drosophila serrata* and *Drosophila birchii.* Genetics, **51**: 527–544.

DOBZHANSKY, T. 1963. Species in Drosophila. Proc. Linnean Soc. London, **174**: 1–12.

DOBZHANSKY, T., L. EHRMAN, O. PAVLOVSKY, AND B. SPASSKY. 1964. The superspecies *Drosophila paulistorum.* Proc. Nat. Acad. Sci., **54**: 3–9.

DOBZHANSKY, T., AND W. B. MATHER. 1961. The evolutionary status of *Drosophila serrata.* Evolution, **15**: 461–467.

DOBZHANSKY, T., AND B. SPASSKY. 1959. *Drosophila paulistorum,* a cluster of species in *statu nascendi.* Proc. Nat. Acad. Sci., **45**: 419–428.

MALLOCH, J. R. 1927. Notes on Australian Diptera. X. Proc. Linnean Soc. New South Wales, **52** (2): 1–16.

MALOGOLOWKIN-COHEN, C., A. S. SIMMONS, AND H. LEVENE. 1965. A study of sexual isolation between certain strains of *Drosophila paulistorum.* Evolution, **19**: 95–103.

SPASSKY, B. 1957. Morphological differences between sibling species of Drosophila. Univ. Texas Publ., **5721**: 48–61.