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PATTERSON

Studies in the

GENETICS OF DROSOPHILA

IX. ARTICLES ON GENETICS, TAXONOMY, CYTOLOGY, AND RADIATION

Edited by

MARSHALL R. WHEELER

ASSOCIATE PROFESSOR OF ZOOLOGY

THE UNIVERSITY OF TEXAS

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The benefits of education and of useful knowledge, generally diffused through a community, are essential to the preservation of a free government.

SAM HOUSTON

Cultivated mind is the guardian genius of Democracy, and while guided and controlled by virtue, the noblest attribute of man. It is the only dictator that freemen acknowledge, and the only security which freemen desire.

MIRABEAU B. LAMAR

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Preface

This bulletin constitutes No. IX of the series of publications originated by Dr. J. T. Patterson in 1940. The 22 articles appearing here were contributed by the genetics staff, students, and visiting investigators.

Intensive investigations into the fields of taxonomy, species relationships, and evolution in Drosophila have been carried out since 1938, when Dr. Patterson began his studies of the populations on Capt. Aldrich's farm outside Austin. During the intervening years, collecting was extended from Texas to the remainder of the United States, and to Mexico. The work of our collaborators in Brazil indicated that Drosophila species in Central America and the islands of the Caribbean should be especially valuable in studies of evolution and relationship; accordingly, William B. Heed spent 1953–54 in San Salvador as a guest of the Instituto Tropical de Investigaciones Cientificas, Universidad Autonoma de El Salvador, where a tremendous wealth of Drosophila material was collected. We wish to thank Professor Adolph Meyer-Abich, Founder of the Institute, for helping arrange Mr. Heed's stay there. We also express our thanks to Dr. Aristides Palacios, Director General, and Dr. Helmut Meyer-Abich for their great help. Mr. O. C. Harper, Midland, Texas, kindly provided funds with which Mr. Heed extended his collections into Honduras and Nicaragua.

Our work has now expanded into the entire Caribbean area, and the expenses of the field collecting have been provided for by a grant from the National Science Foundation. The first 11 articles in this bulletin are based wholly or in part on material collected on expeditions financed by this grant. Our collectors have been Dr. W. B. Heed (principal collector), Dr. Marvin Wasserman, Dr. H. L. Carson. Mr. Hugo Hoenigsberg, and Mrs. Marta Breuer. Collections have been made in South America for us by Dr. Th. Dobzhansky, Dr. C. Pavan, Dr. Danko Brncic, and Mrs. Breuer.

The basic genetics laboratory is supported by the Rockefeller Foundation and The University of Texas; research in biochemical genetics is supported in part by the Welch Foundation. The Atomic Energy Commission, through grant AT-(40-1)-1323, has provided funds and support for the investigation of the Drosophila populations of the Marshall Islands.

In addition, a great many people have contributed their time and knowledge in helping our collectors. A simple listing of these people seems hardly adequate, but we want them all to know that the wonderful cooperation and friendliness which they have shown has been most sincerely appreciated. We are especially indebted to members of the Rockefeller Foundation in Colombia and Trinidad, and to the staff of the United Fruit Company in Colombia and Honduras, for their very great assistance.

In *Honduras*, we wish to thank Dr. Wilson Popenoe, Director, and Dr. Louis Williams, Escuela Agricola de Panamericana, Tegucigalpa; Dr. N. C. Thornton, Director, and Dr. Robert Roberts, of the Tropical Research Dept., United Fruit Co., La Lima; and Dr. Robert P. Armour, Director, Research Dept., Lancetilla.

In *Nicaragua*, we acknowledge the help of Mr. Raymond White, Director, Es-

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In *Costa Rica*, we wish to thank Dr. Jorge Leon, Chief Botanist, Instituto Interamericano de Ciencias Agricolas, Turrialba, and Dr. Bernal Fernandez, San Jose, a former student of The University of Texas, for his generous personal hospitality.

In *Panama*, Mr. James Zetek, Former Director, and Dr. Carl Koford, Resident Naturalist, Canal Zone Biological Area (Barro Colorado Island) were unusually helpful.

In *Colombia*, Mr. Hugo Hoenigsberg, Senior, of Barranquilla, helped our collectors in many ways, and the following members of the Rockefeller Foundation's Agricultural Program must also be acknowledged: Dr. Lewis M. Roberts, Director, Bogota; Dr. U. J. Grant, Local Director, Medellin; and Mr. Rafael Bravo, Facultad de Agronomia, Palmira.

On *Trinidad*, Dr. Wilbur Downs of the Trinidad Regional Virus Laboratory, Port of Spain, very graciously supplied laboratory facilities and other comforts.

In *Venezuela*, Mr. Walter Arp, Resident Director of the Estacione Biologia de Henry Pittier, Rancho Grande, Maracay, was of considerable help, as were Mr. William H. Phelps, and Mr. William H. Phelps, Jr., of the Coleccion Ornitologica Phelps, Caracas, and Dr. Pablo Cova Garcia, Jefe Sec. Estudios Especiales, Division de Malariologia, Maracay, has cooperated by sending a number of Venezuelan Drosophila to our laboratory.

On *Puerto Rico*, Dr. Irving Fox, School of Tropical Medicine, San Juan; Dr. George Wolcott, Agricultural Experiment Station, Rio Piedras; Dr. John B. Grant, Professor of Public Health and Medical Care, University of Puerto Rico, San Juan; and Dr. H. E. Warmke, U. S. Department of Agriculture, Federal Experiment Station, Mayaguez, have all been most helpful.

On *Jamaica*, we wish to thank Dr. Thomas H. Farr, Science Museum, Kingston for his help.

Finally, we wish to express our gratitude to our collaborators in South America, not only for the collections which they have made for us, but also for their great help in solving some of the problems in identification and relationship. Dr. Crodowaldo Pavan, Dr. A. B. da Cunha, Mrs. Marta Breuer and Mr. L. E. Magalhaes of the University of Sao Paulo, Brazil, have been especially helpful in this respect, as has Dr. Danko Brncic of the University of Chile, Santiago.

MARSHALL R. WHEELER,
for the Genetics Group

Austin, Texas
August 8, 1957.

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I. A Study of Interspecific Hybridization Between Members of the Tripunctata Group of Drosophila

J. T. PATTERSON

INTRODUCTION

At the time these tests were begun in June 1955, 31 species belonging to the tripunctata group had been described. All of these are included in the important monograph by Frota-Pessoa (1954), who established four subgroups, I, II, III, and IV. We had among our laboratory stocks eight of the 31 described forms, plus one newly described species *(D. paramediostriata)*. In addition two undescribed species were also in stock. These two have been given names as follows: *D. trapeza*, *D. trifiloides*, and they are described elsewhere in this volume. These made a total of 11 available species for this study. In Table 1 the 11 species are listed, together with the localities at which the original flies had been collected, and the names of the collectors.

TABLE 1

Subgroups and Species	Collecting Localities	Collectors
II. *medionotata* Frota-Pessoa 1954	La Palma, El Salvador	Heed
II. *mediopunctata* Dobzhansky and Pavan 1943	Itanhaen, Sao Paulo, Brazil	Nonata
II. *unipunctata* Patterson and Mainland 1943	La Palma, El Salvador	Heed
III. *crocina* Patterson and Mainland 1944	San Salvador, El Salvador	Heed
III. *mediostriata* Duda 1925	San Salvador, El Salvador	Heed
III. *paramediostriata* Townsend and Wheeler 1955	Rio Piedras, Puerto Rico	Townsend
III. *trapeza* Heed and Wheeler (this bulletin)	La Lima, Honduras	Heed
III. *trifiloides* Wheeler (this bulletin)	La Lima, Honduras	Heed
IV. *albicans* Frota-Pessoa 1954	San Salvador, El Salvador	Heed
IV. *metzii* Sturtevant 1921	San Salvador, El Salvador	Heed
IV. *tripunctata* Loew 1862	Gulfport, Mississippi	Wheeler

Past experience with hybridization tests among Drosophila species has shown that small mass matings are more effective in obtaining hybrids than are pair matings. Consequently, we used mass matings of about 20 pairs per each large food vial. Three such cultures were established for each cross, making a total of about 60 pairs per test, and since 11 species were employed, the total number of reciprocal crosses will be 110.

The males and females to be used were separated within 24 hours after the culture vials had been cleared of all flies. They were then aged for five days before making the crosses. After five days the flies were transferred to fresh food vials, and at the end of this period, 20 females from each cross were dissected and their reproductive tracts examined for the presence of sperm. All of the remaining flies were then changed to a fresh food vial and kept long enough to make it possible to determine whether the cross was fertile or incompatible. At first both the corn-meal and banana-agar media were used, but since the latter gave the better results, it was employed for all the tests.

HYBRIDIZATION TESTS

The results obtained for the 110 crosses are tabulated in Table 2, and reveal that they fall into three categories, as follows: first, crosses which produced no hybrids; second, crosses which also yielded no hybrids but the dissections revealed that some few females had been inseminated; and third, crosses which gave some form of hybridization. The first group included 97 of the 110 crosses, or slightly over 88% of the total. These 97 contained 11,644 flies, which is the equivalent of 5,822 pairs.

TABLE 2

Hybridization tests

Crosses (Females × Males)	Pairs tested	Hybrids	Remarks
1. *albicans* × *crocina*	57	none	incompatible cross
2. *crocina* × *albicans*	60	none	incompatible cross
3. *albicans* × *medionotata*	60	none	incompatible cross
4. *medionotata* × *albicans*	60	none	incompatible cross
5. *albicans* × *mediopunctata*	60	none	non-motile sperm in spermathecae of 1 female
6. *mediopunctata* × *albicans*	60	larvae 1♀ + 1♂	most larvae non-viable; female hybrid sterile
7. *albicans* × *mediostriata*	56	none	incompatible cross
8. *mediostriata* × *albicans*	60	none	incompatible cross
9. *albicans* × *metzii*	60	none	incompatible cross
10. *metzii* × *albicans*	60	none	inactivated sperm in V. R. of one female
11. *albicans* × *paramediostriata*	60	none	incompatible cross
12. *paramediostriata* × *albicans*	60	none	incompatible cross
13. *albicans* × *trapeza*	60	none	incompatible cross
14. *trapeza* × *albicans*	60	none	incompatible cross
15. *albicans* × *trifiloides*	60	none	incompatible cross
16. *trifiloides* × *albicans*	56	none	incompatible cross
17. *albicans* × *tripunctata*	60	none	non-motile sperm in spermathecae of one female
18. *tripunctata* × *albicans*	60	none	incompatible cross
19. *albicans* × *unipunctata*	60	none	incompatible cross
20. *unipunctata* × *albicans*	60	none	dead sperm in spermathecae of one female
21. *crocina* × *medionotata*	60	none	incompatible cross
22. *medionotata* × *crocina*	60	none	incompatible cross
23. *crocina* × *mediopunctata*	60	none	incompatible cross
24. *mediopunctata* × *crocina*	60	none	incompatible cross
25. *crocina* × *mediostriata*	60	none	incompatible cross
26. *mediostriata* × *crocina*	60	none	incompatible cross
27. *crocina* × *metzii*	60	none	incompatible cross
28. *metzii* × *crocina*	60	none	incompatible cross
29. *crocina* × *paramediostriata*	60	none	incompatible cross
30. *paramediostriata* × *crocina*	60	none	incompatible cross
31. *crocina* × *trapeza*	60	none	incompatible cross
32. *trapeza* × *crocina*	60	none	incompatible cross
33. *crocina* × *trifiloides*	63	none	1♀ with dead sperm; 1♀ with motile sperm in spth.
34. *trifiloides* × *crocina*	60	none	incompatible cross
35. *crocina* × *tripunctata*	60	none	incompatible cross
36. *tripunctata* × *crocina*	60	none	incompatible cross
37. *crocina* × *unipunctata*	60	none	incompatible cross
38. *unipunctata* × *crocina*	60	none	incompatible cross
39. *medionotata* × *mediopunctata*	60	none	incompatible cross
40. *mediopunctata* × *medionotata*	60	none	incompatible cross
41. *medionotata* × *mediostriata*	60	none	incompatible cross
42. *mediostriata* × *medionotata*	60	none	incompatible cross
43. *medionotata* × *metzii*	60	none	incompatible cross

TABLE 2—Continued

Hybridization tests

	Crosses Females	Males	Pairs tested	Hybrids	Remarks
44.	*metzii* × *medionotata*		60	none	incompatible cross
45.	*medionotata* × *paramediostriata*		60	none	incompatible cross
46.	*paramediostriata* × *medionotata*		60	none	incompatible cross
47.	*medionotata* × *trapeza*		60	none	incompatible cross
48.	*trapeza* × *medionotata*		60	larvae 1♀ + 1♂	most larvae non-viable both hybrids sterile
49.	*medionotata* × *trifiloides*		60	none	incompatible cross
50.	*trifiloides* × *medionotata*		60	none	incompatible cross
51.	*medionotata* × *tripunctata*		60	none	incompatible cross
52.	*tripunctata* × *medionotata*		60	none	incompatible cross
53.	*medionotata* × *unipunctata*		60	none	incompatible cross
54.	*unipunctata* × *medionotata*		60	none	incompatible cross
55.	*mediopunctata* × *mediostriata*		60	none	incompatible cross
56.	*mediostriata* × *mediopunctata*		60	none	incompatible cross
57.	*mediopunctata* × *metzii*		60	1♀	abnormal, sterile
58.	*metzii* × *mediopunctata*		60	none	incompatible cross
59.	*mediopunctata* × *paramediostriata*		60	none	incompatible cross
60.	*paramediostriata* × *mediopunctata*		60	none	incompatible cross
61.	*mediopunctata* × *trapeza*		62	none	non-motile sperm in spermathecae of one female
62.	*trapeza* × *mediopunctata*		56	none	incompatible cross
63.	*mediopunctata* × *trifiloides*		60	none	incompatible cross
64.	*trifiloides* × *mediopunctata*		60	none	incompatible cross
65.	*mediopunctata* × *tripunctata*		65	none	incompatible cross
66.	*tripunctata* × *mediopunctata*		64	none	incompatible cross
67.	*mediopunctata* × *unipunctata*		60	none	incompatible cross
68.	*unipunctata* × *mediopunctata*		60	5♀ + 5♂	$F_1 \times F_1$ fertile
69.	*mediostriata* × *metzii*		62	none	incompatible cross
70.	*metzii* × *mediostriata*		65	none	incompatible cross
71.	*mediostriata* × *paramediostriata*		70	larvae 26♀ + 22♂	$F_1 \times F_1$ fertile
72.	*paramediostriata* × *mediostriata*		70	larvae 5♀ + 4♂	$F_1 \times F_1$ fertile
73.	*mediostriata* × *trapeza*		60	none	incompatible cross
74.	*trapeza* × *mediostriata*		60	none	incompatible cross
75.	*mediostriata* × *trifiloides*		60	none	incompatible cross
76.	*trifiloides* × *mediostriata*		65	none	incompatible cross
77.	*mediostriata* × *tripunctata*		60	none	incompatible cross
78.	*tripunctata* × *mediostriata*		60	none	incompatible cross
79.	*mediostriata* × *unipunctata*		63	none	incompatible cross
80.	*unipunctata* × *mediostriata*		60	none	incompatible cross
81.	*metzii* × *paramediostriata*		60	none	incompatible cross
82.	*paramediostriata* × *metzii*		60	none	incompatible cross
83.	*metzii* × *trapeza*		60	none	incompatible cross
84.	*trapeza* × *metzii*		60	none	incompatible cross
85.	*metzii* × *trifiloides*		57	none	incompatible cross
86.	*trifiloides* × *metzii*		60	none	incompatible cross
87.	*metzii* × *tripunctata*		60	larvae pupae	no adults produced
88.	*tripunctata* × *metzii*		60	none	incompatible cross
89.	*metzii* × *unipunctata*		60	none	incompatible cross
90.	*unipunctata* × *metzii*		60	none	incompatible cross
91.	*paramediostriata* × *trapeza*		60	none	incompatible cross
92.	*trapeza* × *paramediostriata*		60	none	incompatible cross
93.	*paramediostriata* × *trifiloides*		60	none	incompatible cross
94.	*trifiloides* × *paramediostriata*		60	none	incompatible cross
95.	*paramediostriata* × *tripunctata*		58	none	incompatible cross
96.	*tripunctata* × *paramediostriata*		60	none	incompatible cross
97.	*paramediostriata* × *unipunctata*		60	none	incompatible cross
98.	*unipunctata* × *paramediostriata*		54	none	incompatible cross
99.	*trapeza* × *trifiloides*		60	none	incompatible cross
100.	*trifiloides* × *trapeza*		60	none	incompatible cross
101.	*trapeza* × *tripunctata*		60	none	incompatible cross

TABLE 2—Continued

Hybridization tests

Crosses Females	Males	Pairs tested	Hybrids	Remarks
102. *tripunctata* × *trapeza*		60	none	incompatible cross
103. *trapeza* × *unipunctata*		60	none	incompatible cross
104. *unipunctata* × *trapeza*		60	none	incompatible cross
105. *trifiloides* × *tripunctata*		60	none	incompatible cross
106. *tripunctata* × *trifiloides*		60	none	incompatible cross
107. *trifiloides* × *unipunctata*		66	none	incompatible cross
108. *unipunctata* × *trifiloides*		60	none	incompatible cross
109. *tripunctata* × *unipunctata*		60	none	incompatible cross
110. *unipunctata* × *tripunctata*		60	none	incompatible cross

The second group included but six crosses, which are indicated in the last column of Table 2. In Cross 5 (*albicans* ♀ × *mediopunctata* ♂) a single female contained non-motile sperm in the spermathecae. Cross 10 (*metzii* ♀ × *albicans* ♂) also yielded a single female with non-motile sperm, which were located in the ventral receptacle. In Cross 17 (*albicans* ♀ × *tripunctata* ♂) one female had non-motile sperm in the spermathecae. In Cross 20 (*unipunctata* ♀ × *albicans* ♂) one female contained dead sperm in the spermathecae. In Cross 33 (*crocina* ♀ × *trifiloides* ♂) two females had been inseminated. One had dead sperm and one had motile sperm, both in the spermathecae. In Cross 61 (*mediopunctata* ♀ × *trapeza* ♂) a single female had non-motile sperm in the spermathecae.

The third group included seven crosses which gave some form of hybridization (Table 2). Cross 6 (*mediopunctata* ♀ × *albicans* ♂) yielded many non-viable larvae, and one female and one male hybrid. In the test for the possible fertility of these two flies, the $F_1 \times F_1$ cross failed to produce offspring. The female was backcrossed simultaneously to both types of parental males, but failed to produce progeny. But the male hybrid backcrossed in the same manner to both types of parental females yielded 12 females and 17 males. The results from these tests show that the female was sterile, while the male was fertile.

Cross 48 (*trapeza* ♀ × *medionotata* ♂) yielded many non-viable larvae and one female and one male hybrid. These hybrids proved to be completely sterile in inbred and backcross tests. Cross 57 (*mediopunctata* ♀ × *metzii* ♂) produced a single hybrid female which was highly abnormal and incapable of breeding.

In the matings between *mediopunctata* and *unipunctata* the cross was incompatible when *mediopunctata* was used as the female parent (Cross 67), but slightly fertile when *unipunctata* represented the female parent, yielding 5 female and 5 male hybrids (Cross 68). The $F_1 \times F_1$ cross was fertile and gave nine females and 14 males. The following results were obtained in the backcross tests:

F_1 female mated to *unipunctata* males failed to produce offspring;
F_1 female mated to *mediopunctata* males gave nine females and 10 males;
F_1 male mated to *unipunctata* females failed to produce offspring;
F_1 male mated to *mediopunctata* females gave 19 females and 19 males.

Crosses 71 and 72 include the reciprocal matings between the two closely related species of *mediostriata* and *paramediostriata*. The first cross (*mediostriata* ♀ × *paramediostriata* ♂) produced a total of 26 females and 22 males. The

$F_1 \times F_1$ cross was fertile. The following results were obtained from the backcross tests. The F_1 females crossed to both types of parental males were fertile and produced a total of 22 ♀ ♀ and 13 ♂ ♂, plus several non-viable larvae. The F_1 males mated to both types of parental females produced 16 ♀ ♀ and 19 ♂ ♂ to *mediostriata* females, and none to *paramediostriata* females, not even non-viable larvae. The reciprocal Cross 72 (*paramediostriata* ♀ × *mediostriata* ♂) gave many non-viable larvae and only nine adults, five ♀ ♀ and four ♂ ♂. The F_1 flies were all used for inbred tests and produced 35 pupae, from which 13 females and 11 males emerged.

In Cross 87 (*metzii* ♀ × *tripunctata* ♂) many non-viable larvae and some pupae appeared in the culture, but no adults emerged from these pupae.

In Table 3 are summarized the results of the 110 reciprocal crosses. The table was constructed in order to facilitate the discussion and conclusions. The species included in Frota-Pessoa's subgroup I were not available for study. Only three species were available from his subgroup II, five from subgroup III, and three from subgroup IV. The symbols "Hy" and "In" represent the seven cases of hybridization and six of insemination, respectively. The numbers following these symbols refer to the numbers of the crosses as given in Table 2.

Among the reciprocal crosses within subgroup II, only one yielded hybrids (Hy-68), and none gave an insemination. The matings of the females of these three species to the males of the five of subgroup III gave a single case of insemination (In-61), which was found in the cross between *mediopunctata* females to *trapeza* males. The females of subgroup II mated to the males of subgroup IV gave two cases of hybrids (Hy-6, Hy-57) and one of insemination (In-20). It is interesting to note that *medionotata* females did not cross with any of the males of the other ten species, while the females of *mediopunctata* crossed to the males of three other species, and those of *unipunctata* mated to the males of two other species.

The females of subgroup III crossed to the males of subgroup II yielded a single case of hybridization. This was from *trapeza* females to the males of *medionotata* (Hy-48). The matings within subgroup III produced two cases of hybrids and one of insemination. The hybrids were from the reciprocal crosses between the two related species *mediostriata* and *paramediostriata* (Hy-71, Hy-72). The single case of insemination was from the cross of *crocina* females to *trifiloides* males (In-33).

In subgroup IV the females of *albicans* mated to the males of *mediopunctata* produced a single case of insemination (In-5). There were no successful crosses between members of subgroups IV and III. But in crosses within subgroup IV one case of hybridization (Hy-87) and two of insemination occurred (In-10, In-17). These results indicate that the species of these two subgroups are completely isolated from each other, for in a total of thirty crosses not a single case of either hybridization or insemination was obtained.

CONCLUSIONS

One may conclude from the results obtained in the cross tests that these 11 species of the tripunctata group are rather highly isolated from one another. There are, however, two cases in which the members of two species are rather

TABLE 3

FEMALES	MALES Subgroup II medio-notata	Subgroup II medio-punctata	Subgroup II uni-punctata	crocina	Subgroup III medio-striata	Subgroup III paramedio-striata	trapeza	tri-filoides	Subgroup IV albicans	Subgroup IV metzii	Subgroup IV tri-punctata
II. medionotata	...	0	0	0	0	0	0	0	0	0	0
II. mediopunctata	0	...	0	0	0	0	In–61	0	Hy–6	Hy–57	0
II. unipunctata	0	Hy–68	...	0	0	0	0	0	In–20	0	0
III. crocina	0	0	0	...	0	0	0	In–33	0	0	0
III. mediostriata	0	0	0	0	...	Hy–71	0	0	0	0	0
III. paramediostriata	0	0	0	0	Hy–72	...	0	0	0	0	0
III. trapeza	Hy–48	0	0	0	0	0	...	0	0	0	0
III. trifiloides	0	0	0	0	0	0	0	...	0	0	0
IV. albicans	0	In–5	0	0	0	0	0	0	...	0	In–17
IV. metzii	0	0	0	0	0	0	0	0	In–10	...	Hy–87
IV. tripunctata	0	0	0	0	0	0	0	0	0	0	...

similar morphologically, with the possibility that gene exchanges could occur. The first of these include *mediopunctata* and *unipunctata*, which show considerable resemblance to each other, but the cross was fertile only when *unipunctata* represented the female parent. The F_1 hybrids were fertile in the inbred test, and partially fertile in the backcrosses. There is one striking difference in the two species in the behavior of the larvae during pupation. The larvae of *mediopunctata* pupate at the surface of the food, while those of *unipunctata* pupate within masses of food which they had carried up on the inner surface of the vial. The second pair of species between which gene exchanges could occur includes *mediostriata* and *paramediostriata*, which are very similar morphologically. The reciprocal crosses were fertile, but went better when *mediostriata* was employed as the female parent. The F_1 flies of both crosses were fertile, as indicated above.

There is also a possibility of gene exchange in a third case. This is Cross 6 (*D. mediopunctata* ♀ × *D. albicans* ♂) in which the F_1 male was fertile in backcrosses.

The final point of interest is the degree of isolation that exists between the different species. The results obtained in the tests show that several isolating mechanisms operate to prevent the exchange of genes. The most important of these is sexual isolation, or the failure of the sexes to mate. Of the 110 reciprocal crosses, 97, or slightly more than 88%, were incompatible. The writer never observed a single copulating pair in all of these cultures. Occasionally a male would perform feeble courtship movements, but in every instance the female gave no response.

A second type of isolating mechanism is present in Crosses 5, 10, 17, 20, 33, and 61. In each cross the female had been inseminated, but in each case the sperm had become inactivated or even killed in the reproductive tract of the alien female. This type of isolation has been termed sperm or gametic mortality.

A third type of isolating mechanism is represented by crosses in which the larvae are non-viable and fail to pupate, or pupate without producing adults (Cross 87). Even when hybrids are produced the cultures may contain dead larvae (Crosses 71, 72). This form of isolation has been termed zygotic mortality.

Finally, a fourth isolating mechanism is revealed in matings producing only a few hybrids, such as seen in Crosses 6, 48, and 57, as well as in matings yielding larger numbers of progeny, in which some of the F_1 flies exhibited hybrid sterility.

Geographical isolation must be an important means of preventing gene exchanges between the different members of the group. At present we do not have enough accurate data on distribution to justify an extended discussion of the subject. However, we do know that *D. tripunctata* Loew is the only species of the group thus far collected in the United States, although Dr. W. B. Heed has collected a closely related form in Costa Rica, Panama, and Colombia.

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II. Intraspecific Relationships of *Drosophila crocina* Patterson and Mainland from Three Localities

WILLIAM B. HEED[1]

The distribution of *Drosophila crocina* Patterson and Mainland extends from central Mexico to northern South America. *Drosophila campestris* Burla is quite possibly a synonoym; if this is so, then the distribution is extended into Brazil. *D. crocina* has also been taken on all of the islands of the Greater Antilles. The species may be recognized by the presence of a row of short bristly spines along the inner surface of the fore femora and by the clouding of the anterior and posterior crossveins. It is a member of the tripunctata group of the subgenus Drosophila.

D. crocina is variable in abdominal pattern from one region to another. The most outstanding variation is the darkened body of the island forms. Strains from three widely separated localities, showing different degrees of variation, were tested for their ability to cross with one another. The strains used are as follows: No. H15.8, from El Salvador; No. H109.17, from Trinidad; and No. H131.2, from Puerto Rico. Other less obvious morphological differences became evident in the strains chosen for testing; these differences are difficult to measure, but may be summarized as follows:

	El Salvador	*Trinidad*	*Puerto Rico*
abdomen	brownish yellow	brownish yellow	brownish gray
mesonotum	yellowish brown	brown	grayish brown
crossveins	clouded	strongly clouded	clouded
wings	paler	grayish	paler
eye size	regular	larger	regular

On the basis of these characters, it may be seen that the Trinidad strain is unique in three respects, the Puerto Rico strain in two, and the El Salvador strain in one. In another sense, the Trinidad and Puerto Rico strains differ from each other in five features, Trinidad and El Salvador in four, and El Salvador and Puerto Rico in two. The morphological differences coincide with the results of the hybridization tests given below.

Table 1 shows the results from pair mating tests of the three strains, mated in all combinations. We have used the letter "S" for El Salvador, "T" for Trinidad

TABLE 1

Pair matings of *D. crocina* from three localities

♀ ♂	No. pairs tested	Percent fertile	Total F_1	♀♀	♂♂	Average No. F_1 per fertile ♀	F_2
S × S	22	50	691	366	325	62.8	yes
T × T	22	64	952	486	466	68.0	yes
P × P	23	26	403	207	196	67.2	yes
S × T	24	42	820	393	417	82.0	few
S × P	30	33	965	511	454	96.5	yes
T × S	21	0	...	...	...	...	...
T × P	21	14	239	136	103	79.7	no
P × S	27	0	...	...	...	...	...
P × T	20	5	79	36	43	79.0	no

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and "P" for Puerto Rico. The F_1 from these matings were mass mated to test for further fertility. Table 2 shows the results from mass matings of the three strains; the F_1 from these matings were further mass mated to derive an F_2. When no F_2 generation was produced, the first date of emergence of the F_1 is recorded to show the number of days they were under test.

TABLE 2

Mass matings of *D. crocina* from three localities

♀ ♂	Number mated	Date mated	Total F_1	♀♀	♂♂	Average no. F_1/♀	Earliest date F_1 emerged	F_2	Date Discarded
S × S	11♀, 13♂	5/8	285	158	127	25.9		yes	6/12
T × T	12♀, 13♂	5/8	150	79	71	12.5		yes	6/12
P × P	12♀, 14♂	5/8	202	121	81	16.8		yes	6/12
S × T	10♀, 10♂	4/27	246+	135+	110+	24.6+	5/6	no	6/12
	21♀, 8♂	5/8	many				5/26	no	6/13
	12♀, 12♂	5/8	many				5/26	no	6/13
S × P	10♀, 10♂	4/27	358	182	176	35.8		yes	6/12
	14♀, 14♂	5/8	many					yes	6/13
T × S	10♀, 10♂	4/27	75	41	34	7.5		yes	6/12
	15♀, 24♂	5/8	125+	74+	51+	8.3+		yes	6/13
T × P	10♀, 10♂	4/27	157	96	61	15.7		few	6/12
	14♀, 11♂	5/8	many				5/23	no	6/13
P × S	10♀, 10♂	4/27	61	29	32	6.1		yes	6/12
	13♀, 12♂	5/8	45	29	16	3.5		yes	6/13
P × T	10♀, 10♂	4/27	230	123	107	23.0	5/13	no	6/12
	14♀, 12♂	5/8	many				5/26	no	6/13

A general summary of the results for the production of a second generation is given below; in some crosses the F_1 males were dissected and the testes examined for active sperm before being discarded. Backcrosses were made of the hybrid females to both parental males in every case; the test was then discontinued when larvae were produced.

♀ ♂	F_2 from pair matings	Active sperm in F_1 males	F_2 from mass matings	Active sperm in F_1 males	Backcross	
S × T	few	25/79 (31.6%)	no	1/16 (6.2%)	(S × T) ♀ × T ♂	fertile
T × S	no		yes	3/8 (37.5%)	(S × T) ♀ × S ♂	fertile
S × P	yes		yes		(S × P) ♀ × P ♂	fertile
P × S	no		yes		(S × P) ♀ × S ♂	fertile
T × P	no	2/24 (8.3%)	few	2/21 (9.5%)	(T × P) ♀ × P ♂	fertile
P × T	no		no	0/24 (0 %)	(T × P) ♀ × T ♂	fertile

It may be seen that the Trinidad strain is highly differentiated from the Puerto Rico strain and less so from the El Salvador strain, while these two are quite interfertile. The presence or absence of active sperm in the testes of the male does not show good correlation with the hybridization tests.

The pair matings (Table 1) show that the El Salvador males are presumably reluctant to mate with females of the other two strains. All inter-strain pair matings that crossed gave more offspring per female than the controls; this shows a heterosis effect in the F_1 whether or not the F_1 individuals proved to be fertile. Further tests should be made to determine the genetic mechanisms underlying the incipient isolation illustrated here.

III. Thirteen New Species in the Genus Drosophila from the Neotropical Region

WILLIAM B. HEED[1] AND MARSHALL R. WHEELER

INTRODUCTION

The species described here were collected mainly by the senior author in four trips to the Neotropical Region from 1952 to 1956. He was assisted part of this time by his wife, Sarah, Drs. Hampton L. Carson and Marvin Wasserman and also by Mr. Hugo Hoenigsberg. The metaphase chromosome preparations were analyzed by Drs. Frances E. Clayton, Marvin Wasserman, and Lawrence E. Mettler.

Ten of the species belong to the tripunctata group of the subgenus Drosophila; they are listed below according to their respective subgroups as established by Frota-Pessoa (1954). Two species belong in the cardini group of the subgenus Drosophila and one species is unclassified. The new species of the tripunctata group are as follows:

Subgroup I. *D. setula*, n. sp.
Subgroup II. *D. facialba*, n. sp., *D. mediodelta*, n. sp.
Subgroup III. *D. converga*, n. sp., *D. mediopictoides*, n. sp., *D. trapeza*, n. sp.
Subgroup IV. *D. mediodiffusa*, n. sp., *D. medioparva*, n. sp., *D. spinatermina*, n. sp., *D. tristriata*, n. sp.

As noted previously (Heed, this bulletin) the tripunctata group replaces the repleta group as the dominant species group in the Neotropical Region south of the desert plateau of central Mexico. Frota-Pessoa *(op. cit.)* lists 31 members of the group, and points out that *D. metzii* and *D. albirostris* may also belong here rather than in the cardini group. We have examined a type specimen of *metzii* (described by Sturtevant from Cuba) and a type specimen of *albirostris* (described by Sturtevant from Panama) and believe them to be conspecific with two species which we have collected widely in Central America and northern South America. We believe, also, that *D. albicans* Frota-Pessoa, of the tripunctata group, may be synonymous with *albirostris* but further confirmation is necessary. Both *albirostris* and *metzii* clearly belong in the tripunctata group.

In our opinion *D. campestris* Burla is probably synonymous with *D. crocina* Patterson and Mainland, but this is still unconfirmed. This still leaves 31 species in the group, with the addition of *metzii* and *albirostris*, and the presumed synonymy of *albicans* and *campestris*.

Three other previously described forms belong in this group: *D. triangula* Wheeler, *D. fragilis* Wheeler, and *D. paramediostriata* Townsend and Wheeler. The genitalia of *fragilis* are very similar to that of *trifilum* Frota-Pessoa and the species may thus be placed in subgroup III. Finally, *D. trifiloides* Wheeler, described as new elsewhere in this bulletin, belongs in the tripunctata group. With the addition of this species, the ten species described in this report, the three species named above, and the 31 forms previously placed in the group, there are thus a total of 45 forms at present placed in the tripunctata group. There remain,

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however, many more species from Central and South America yet to be described. Our types are in The University of Texas collection.

DESCRIPTION OF NEW SPECIES

Drosophila setula Heed and Wheeler, new species.

External characters of imagines.

♂, ♀. Arista with 6–7 dorsal and 3 ventral branches in addition to the terminal fork. Front dull brown with few frontal hairs; face and cheeks paler. Carina prominent, broader below, not sulcate. One strong oral bristle. Proclinate orbital about 7/8 length posterior reclinate; anterior reclinate 1/4–1/3 proclinate. Greatest cheek width about 1/6 greatest diameter of eye; cheek very narrow directly below eye; eye large, dark red with a magenta cast dorsally and light colored pile. Palpi with 3–4 long bristles on antero-lateral margin and 14–18 shorter bristles on ventral surface.

Acrostichal hairs in 6 rows; no prescutellars. Anterior scutellars divergent. Mesonotum and scutellum semi-shining brown; pleura, halteres and legs lighter. One pair of low-lying bristles anterior to suture, in dorsocentral row, about 3/4 length of anterior dorsocentrals. Anterior sternopleural about 1/2 length posterior; mid-sternopleural about 1/2 anterior. Fore femur with 3–4 long bristles on lateral extensor surface and 4–5 long bristles on flexor surface; bases of fore and mid metatarsi with a single strong black bristle; base of hind metatarsus with three. Hind metatarsus not exceptionally wide; recurved hairs on fore tarsus and tibia. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings with a slight grayish cast; posterior crossvein clouded. Costal index about 4.0; 4th vein index about 1.4; 5x index about 1.0; 4c index about 0.6. Third costal section with heavy bristles on the basal 1/3–1/2. One prominent bristle at apex of first costal section.

Abdomen shining brownish yellow with posterior black bands on all tergites which fade at the angle of the tergite and sometimes continue to the lateral margin as a thin line. The bands are narrowly or incompletely interrupted mid-dorsally on tergites 2–5. Band of 6th with a mid-dorsal extension that reaches 1/2 the distance to the preceding tergite. Abdominal pattern of both sexes similar.

Body length (etherized) about 3.0 mm. in ♂, 3.5 mm. in ♀; wing length about 3.0 mm. (♂), 3.0 mm. (♀).

Internal characters of imagines and genitalia.

Anterior Malpighian tubes branched basally near gut; posterior tubes apposed without a continuous lumen. Testes with about 6–7 yellowish white outer coils and 3–4 white, thicker inner coils. Sperm pump diverticula about four times length of pump. Lower tip of anal plate with a finger-like projection that bears a row of 4–6 bristles at its base. Forceps narrow with 7–9 primary teeth in an irregular S-shaped row, and with 3–6 marginal bristles that come on to upper surface. Toe very closely associated with forceps, with 3–4 bristles. Hypandrium broad with one long bristle and 1–2 thinner bristles only 1/3–1/2 length of former. Head of penis with no special processes, the extreme tip with a pair of slightly toothed plates.

Spermathecae chitinized, elongate (about 0.1 mm. wide and 0.16 mm. long);

inner duct funnel-shaped with a basal constriction followed by a bulb-like expansion just before leaving spermatheca. Ventral receptacle with about 40 coils. Ovipositor plate long and slender, with 23–27 teeth.

Other characteristics, relationship, and distribution.

Eggs. With 2 pairs of thin filaments, longer than the egg.

Larvae. Do not skip; posterior spiracles yellow.

Puparia. Red-brown; anterior spiracles with 19–20 pale branches; horns, including spiracles, about ⅓ length puparium. Posterior spiracles yellow, parallel and rather long, nearly ⅕ length of puparium.

Chromosomes. Determination of the metaphase chromosome complement from larval brain cells was unusually difficult; there appear to be either 3 or 4 pairs of rods, one pair of V-s, and a pair of dots. The X and Y chromosomes could not be identified. The analysis was made by Dr. Wasserman using stocks No. H81.15 from Barro Colorado, Canal Zone, and No. H187.9 from El Recuerdo, Colombia.

Relationship. Belongs in subgroup I of the tripunctata group of the subgenus Drosophila; it is most similar to *platitarsus* Frota-Pessoa from which it is distinguished by the normal width of the hind metatarsus, the pair of bristles anterior to the transverse suture, and the finger-like projection on lower tip of anal plate.

Distribution. Collected by the senior author in 1955 in Costa Rica and Panama (Oct.), Colombia (Nov., Dec.) and Trinidad (Dec.); this trip is designated as (a). Further collections were made in company with Drs. Wasserman and Carson in 1956 in Costa Rica (July, Aug.) and Panama and Colombia (Aug.); this trip is designated as (b). Localities are: *Costa Rica:* San José 3000′ (a, b); Turrialba, 2000′ (a, b); La Lola, 128′ (b); San Isidro de General, 2000′ (b); *Panama:* Barro Colorado Island (a, b) and Cerro La Campana, 2700′ (b); *Colombia:* Fusagasuga, 5700′ (a) and El Recuerdo in the Sierra Nevada Mts., 3000′ (a, b); *Trinidad:* many localities including Arima Valley and Sangre Grande (a).

Types. Holotype male and 2 male, 4 female paratypes from stock No. H95.14 from Fusagasuga, Colombia.

Drosophila facialba Heed and Wheeler, new species.

External characters of imagines.

♂, ♀. Arista with 6–7 dorsal and 3 ventral branches in addition to the terminal fork. Carina prominent, broader below where it slopes to the oral margin; face and oral margin white. Front tan with few frontal hairs. Third antennal joint whitish tan. Proclinate orbital ⅝ length posterior reclinate; anterior reclinate about ⅓ length proclinate and closer to eye margin. Two long thin orals of equal length. Greatest cheek width about ⅙ greatest diameter of eye; cheek width directly below eye about 1/12. Eyes bright red with silvery pile. Palpi with 4–5 long bristles on lateral margin and 20–30 shorter bristles on posterior surface and lateral margin.

Acrostichal hairs in 6 rows; no prescutellars. Anterior scutellars divergent. Mesonotum and scutellum subshining brown; pleura and legs whitish tan. An-

terior sternopleural about ½ posterior; mid-sternopleural ½–⅝ anterior. Flexor surface of fore femur with 2 long bristles; lateral extensor surface with 4 long bristles. A few recurved hairs on fore tarsus. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings fuscous; anterior and posterior crossveins clouded. Costal index about 4.7; 4th vein index about 1.5; 5x index about 1.1; 4c index about 0.5. One strong and one weak bristle at apex of 1st costal section. Third costal section with heavy bristles on the basal ½.

Abdomen of male shining yellow with posterior black bands extending slightly beyond the angle of the tergite on all segments. The bands are interrupted mid-dorsally on 2nd tergite, expanded to posterior half of dorsal region on 3rd, expanded to a heavy mid-dorsal triangle on 4th, and forming heavy mid-dorsal rectangles on 5th and 6th tergites. Abdomen of female similar to that of male but 5th and 6th tergites with narrow light mid dorsal stripes with little or no banding.

Body length (etherized) about 2.4 mm. (♂), 3.0 mm. (♀); wing length about 2.4 mm. (♂), 3.0 mm. (♀).

Internal characters of imagines and genitalia.

Anterior Malpighian tubes branched at basal ⅕; posterior tube with a continuous lumen. Testes with about 5 thin outer and 3 thicker inner coils, all white. Sperm pump diverticula about ¾ greatest diameter of pump. Anal plate with a tuft of about 6 hairs on lower tip. Toe with 3–4 short apical bristles. Forceps with 7–9 primary teeth in a row which curve inward on last two posterior teeth; about 13 marginal bristles which come around to upper surface. Spermathecae lightly chitinized, oval; inner duct broadly funnel-shaped in upper half. Ventral receptable with over 25 coils. Ovipositor plate with 19–20 teeth.

Other characteristics, relationship, and distribution.

Eggs. With two pairs of thin filaments.

Larvae. Posterior spiracles pale; do not skip.

Puparia. Red-brown, posterior spiracles pale. Each anterior spiracle with about 16 yellow branches, the horn, including spiracles, about ⅕ length of puparium.

Chromosomes. Determined by Dr. Mettler from stock H67.5 from El Salvador: 3 pairs of rods, 1 pair of V's (but see below), and a small pair of dots. The X chromosome is about twice the length of the autosomes, and the latter are about equal to the length of one arm of the V. There are satellites on the centromere end of the X and on one pair of small rods. The Y is the same length as the X and also has a satellite; it is uncertain whether it is a rod or is J-shaped.

Dr. Clayton examined another stock from El Salvador (H26.2a) and determined the V as being two rods. She reports that the salivary chromosomes show 5 arms and the dot, with a large mass of heterochromatin associated with the X.

Relationship. Belongs to subgroup II of the tripunctata group of the subgenus Drosophila. This is the first member of this subgroup with a distinctly white face.

Distribution. Restricted to the highlands of southern Mexico and Central America. Collected by the senior author as follows: *Mexico:* 19 miles east of San

Andres Tuxtla, Veracruz, 1700′, July 1952 (with M. Wasserman); *El Salvador:* Volcan El Boqueron and Volcan Santa Ana, 5000–6000′, throughout the year, 1953–54, and Oct. 1955; *Nicaragua:* Hacienda Santa Maria de Ostuma, 7 miles north of Matagalpa, 4000′, June 1954; *Costa Rica:* San José, 3000′, Oct. 1955.

Types. Holotype male and 1 male, 3 female paratypes, all descendants from stock No. H67.5 from Volcan El Boqueron, El Salvador.

Notes. This species has been found feeding on fermenting coffee berries and on the sweet white pulp of pepete pods (*Inga spuria* H. and B.) in El Salvador. It is difficult to maintain stocks on banana medium.

Drosophila mediodelta Heed and Wheeler, new species.

External characters of imagines.

♀, ♂. Arista with 7–8 dorsal and 3–4 ventral branches in addition to the terminal fork. Face and antennae brown; front brown with few frontal hairs. Carina extending nearly to level of 1st oral. Proclinate orbital 5/8 length posterior reclinate; anterior reclinate 1/3 length proclinate. Three strong orals (usually); cheek and area below eye narrow; eye large, red with pale pile. Male palpi with one long subapical bristle and 4–6 progressively shorter bristles on outer margin. One bristle at apex about equal to length of first few on outer margin. Female palpi usually with 2 long bristles on antero-lateral margin and 6–8 shorters ones.

Acrostichal hairs in 8–10 irregular rows; no prescutellars. Anterior scutellars widely divergent. Mesonotum and scutellum dull dark brown except on a small central area directly behind head which appears paler on pinned specimens. Halteres, pleura and legs lighter brown. Anterior and middle sternopleurals equal in length, about 3/7 length posterior one. Numerous recurved hairs on fore tarsi, a few on hind tarsi. Fore femora with 2 prominent bristles on posterior flexor surface and usually with 3 on lateral extensor surface. Several long yellow bristles at base of hind metatarsus. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings with brownish cast; posterior crossvein clouded. Costal index about 4.3; 4th vein index about 1.3; 5x index about 1.0; 4c index about 0.5. Two strong bristles at apex of 1st costal section; 3rd section with heavy bristles on the basal 1/2.

Abdomen brownish yellow with black apical bands on tergites 2–4, interrupted dorsally, the interruptions becoming progressively wider posteriorly. Band of 4th tergite sometimes with paramedian extensions leading anteriorly (usually on females). Tergite 5 usually with a thin interrupted apical band with paramedian extensions that fuse anteriorly into a central delta-shaped spot, but this spot is sometimes quite faint and isolated, with no apical band and sometimes the fusion is incomplete so that no spot is formed. Tergite 6 with a median rectangular to triangular black spot. Females are usually more heavily marked on all tergites.

Body length (pinned) about 3.0–3.5 mm. (♂), 4.0–4.5 mm. (♀); wing length about 3.0 mm. (♂), 3.8 mm. (♀).

Internal characters of imagines and genitalia.

Posterior Malpighian tubes apposed but apparently without continuous lumen. Testes yellowish white with about 4 outer and 4 inner thicker coils. Sperm pump

diverticula short, about ½ diameter of pump. Forceps with 7–9 primary teeth in a slightly curved row and 8–12 marginal bristles. Toe without bristles; 1–2 long bristles on genital arch near point of attachment of forceps (*lingueta* of Salles, 1947). Lower tip of anal plate with about 10 loosely grouped small bristles. Head of penis divided, with a small "tooth," bearing 3–4 notches, extending from the tip of each division; there are also a pair of pointed plate-like processes extending back toward the apodeme and which are notched along both inner and outer borders. Spermathecae about 0.13 mm. long and 0.09 mm. wide; inner duct wide with a slight constriction at about the middle. Ovipositor plate with 29–30 teeth.

Other characteristics, relationship, and distribution.

Eggs. Two pairs of filaments, the anterior pair thin and about equal to egg length, posterior pair thicker and longer.

Puparia, larvae, and chromosomes. Unknown.

Relationship. Belongs to subgroup II of the tripunctata group of the subgenus Drosophila; it appears to be close to *mediopunctata* and to *unipunctata*.

Distribution. Collected during 1954–56 by the senior author in the following localities: *El Salvador:* Volcan El Boqueron, 5000′, Oct., Feb., May, July; Volcan Santa Ana, 5000′, July; San Salvador, 2000′, Aug.; *Nicaragua:* Santa Maria de Ostuma, about 7 miles north of Matagalpa, 4000′, June; *Costa Rica:* San Isidro de General, 2000′, Aug.; *Colombia:* El Recuerdo, about 15 miles southeast of Santa Marta, 4000′, Aug.

Types. Holotype male and 1 paratype male, El Recuerdo, Colombia (No. H186.19); 1 ♂ and 2 ♀ paratypes from El Salvador.

Notes. In general, very few individuals were collected at any one time except on Volcan Santa Ana where *D. mediodelta* was by far the dominant species (18% of 2,056 flies); here it was found feeding on fruits of *Citharexylum donnell-smithii* Greenman (Verbenaceae). The species does not breed well on laboratory food. Diagnostic characters are: the large eyes, 3 strong orals, dull mesonotum, delta-shaped spot connecting with the apical band on tergite 5, large size, high altitude preference, and characteristic processes of the male genitalia.

Drosophila converga Heed and Wheeler, new species.

External characters of imagines.

♂, ♀. Arista with 5, rarely 6, dorsal branches and 3 ventral branches in addition to the fork. Face, antennae and cheeks brown; front lighter brown. Carina short, not sulcate. Proclinate orbital about ⅝ length posterior reclinate; anterior reclinate minute; proclinate and large reclinate set rather close together. Two orals, the second usually shorter. Greatest width of cheek about ⅙ greatest diameter of eye. Eye orange red with a light magenta cast dorsally and with light pile. Palpi with one bristle at apex and 4–5 marginal bristles that decrease in size posteriorly; ventral surface with about 9 shorter bristles.

Acrostichal hairs in 6 rows; no prescutellars. Anterior scutellars convergent. Mesonotum and scutellum brown, very shiny; pleura and legs light brown. Anterior sternopleural usually ½ posterior; mid-sternopleural usually ½ anterior, but the anterior and middle bristles are sometimes weaker and of equal length, in

which case they are about ⅓ length posterior one. Numerous recurved hairs on fore tarsus, a few on hind tarsus. A row of 11–13 peg-like bristly spines on inside edge of fore femur. First and 2nd metatarsi each with a single black bristle at base, hind metatarsus with three such bristles. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings with darkened posterior crossvein and a very slight cloud. Costal index about 3.0; 4th vein index about 1.7; 5x index about 1.2; 4c index about 0.9. One prominent bristle at apex of 1st costal section; 3rd section with heavy bristles on the basal ½.

Abdomen shining brownish yellow with black apical bands on most tergites. On males the bands fade at the angle of the tergite except on 4th (and sometimes on 5th) where the band nearly reaches lateral margin. The bands are sometimes faintly and incompletely interrupted mid-dorsally on tergites 2–4. Tergites 5–6 with very narrow bands, usually with slight midline extensions. Female usually with no markings on last two tergites; tergites 2–4 frequently with incompletely interrupted bands.

Body length (etherized) about 2.0 mm. (♂), 2.5 mm. (♀); wing length about 2.0 mm. (♂), 2.3 mm. (♀).

Internal characters of imagines and genitalia.

Anterior and posterior Malpighian tubes branched basally near gut; posterior tube with apposed ends but without continuous lumen. Testes with 4–5 yellow outer coils and 3 whitish inner coils. Sperm pump diverticula long, about 10 times length of pump. Forceps with 6–7 primary teeth, 5–8 long stout secondary teeth and 7–10 long marginal bristles that come around on upper surface. Toe rounded with 1–3 bristles; genital arch, just behind toe, with 2–4 bristles that are sometimes continuous with those of the toe. Gonopophysis with 2 long bristles immediately adjacent to each other. Head of the penis rounded, the neck region with a pair of narrow, lateral, saw-toothed, fringed plates. Basal to the plates and extending distally and dorsally, nearly reaching the head of the penis, is a pair of narrow, curved, sword-like projections.

Spermatheca pear-shaped, brown; inner duct funnel-shaped. Ventral receptacle with 40 or more coils. Ovipositor plate with 22–25 teeth.

Other characteristics, relationship, and distribution.

Eggs. With 2 pairs of thin filaments.

Larvae. Posterior spiracles tan; do not skip.

Puparia. Yellow-brown; anterior spiracle with 20–24 yellow filaments; horns, including spiracles, about ⅓ length of puparium.

Chromosomes. Metaphase preparations show 5 pairs of rods and a pair of dots. One pair of rods is short, and one long pair possesses satellites on the centromere end. The preparations were made by Dr. Mettler using stock No. H180.2 from San José, Costa Rica.

Relationship. Belongs in subgroup III of the tripunctata group of the subgenus Drosophila. It is closely related to *crocina*, *mediostriata*, and *mesostigma* which also possess peg-like bristles on the fore femur.

Distribution. A widely distributed, but uncommon, species of the Neotropical region. Collectors from the Texas laboratory (1954–56) have taken it at various

localities in *El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Colombia* and *Trinidad* at elevations from sea level to 4000 feet.

Types. Holotype male, 2 ♂ and 2 ♀ paratypes, stock No. H180.2, from San José, Costa Rica.

Notes. The only great concentration of *converga* was found at Lancetilla, Honduras in April where it made up 53% of a micropopulation of 266 individuals collected over mangosteena fruits *(Garcinia)*, an Asiatic tree. Stocks are difficult to establish but can be maintained on banana medium.

D. converga can be separated from the three closely related species by the following combination of characters: small, very shiny, anterior scutellars convergent, posterior crossveins only slightly clouded, and the characteristic genitalia.

Drosophila mediopictoides Heed and Wheeler, new species.

External characters of imagines.

♂, ♀. Arista with 5 dorsal and 3 ventral branches in addition to the fork. Front tan with a few minute frontal hairs arranged in a V; face and cheeks paler. Oral margin of male sometimes whitish. Carina rounded, short; two strong orals, the 2nd slightly shorter. Proclinate orbital 7/8 length posterior reclinate; anterior reclinate minute. Greatest cheek width 1/5–1/6 greatest diameter of eye. Eye red with light pile. Palpi with one strong subapical bristle, one slightly shorter bristle and two bristles about half length of first two on outer margin; ventral side with about 10 shorter bristles.

Acrostichal hairs in about 6 rows; no prescutellars. Anterior scutellars divergent. Mesonotum semi-shining light brown; pleura, legs and halteres somewhat lighter. Anterior sternopleural 3/7 posterior; middle bristle about 1/2 anterior. Fore femur with 2–3 long bristles on flexor surface, 2 distal and 3–4 thinner, more basal bristles on lateral extensor surface. Fore tarsus, especially of male, with numerous recurved hairs. Base of fore metatarsus with 1–2 strong bristles; base of hind metatarsus with 3 strong yellow bristles. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings clear, the posterior crossvein with very slight clouding. Costal index about 4.2; 4th vein index about 1.4; 5x index about 1.2; 4c index about 0.6. Two prominent bristles at apex of 1st costal section; 3rd section with heavy bristles on the basal 2/5.

Abdomen shining tannish-yellow with thin, indistinct, brownish to grayish posterior bands on tergites 2–6, interrupted mid-dorsally except on 2nd and 3rd where the bands are sometimes merely narrowed. On some individuals the bands may nearly reach the lateral margin as a thin line except on 6th tergite, this one sometimes without markings.

Body length (etherized) about 2.5 mm. (♂), 2.9 mm. (♀); wing length about 2.4 mm. (♂), 2.8 mm. (♀).

Internal characters of imagines and genitalia.

Anterior Malpighian tubes branched at the basal 1/4; posterior tubes apposed without a continuous lumen. Testes with about 5 yellowish outer coils and 3 whitish thicker inner coils. Sperm pump diverticula about as long as the pump. Forceps with 6–8 primary teeth in a straight row, 8–15 secondary teeth and 11–

13 marginal bristles. Most of the secondary teeth are arranged into two rows when they are numerous, but when few then about 5 teeth are arranged in a posterior row. Toe rounded with 4 long subapical bristles; lower tip of anal plate with a dense tuft of bristles. Hypandrium with a long and a short bristle; head of penis large and complex, wtih 2 chitinized processes, sawtoothed along their outer margin, extending posteriorly from the apodeme.

Spermatheca chitinized, distinctly rounded, about 0.15 mm. in both width and length; upper ⅔ of inner duct funnel-shaped, narrowing abruptly to a constriction followed by a slight bulblike expansion before the point of exit. Ventral receptacle with about 50 coils; ovipositor plate with 20–24 teeth, not broad apically.

Other characteristics, relationship, and distribution.

Eggs. With 2 pairs of thin filaments, as long as the egg.

Larvae. Posterior spiracles develop a burnt brown color in older larvae; do not skip.

Puparia. Red-brown; anterior spiracles with 17–18 branches; horns, including spiracles, about ¼ length of puparium.

Chromosomes. Metaphase preparations show 5 pairs of rods and a pair of large V's; 4 rod pairs are about equal in length and about ¾ as long as one arm of the V; the 5th rod pair is half the length of the others and in one cell this pair occupied the "dot position" in the center. No differences were seen in male and female. Salivary chromosomes showed 5 long arms and a dot; the X is a single arm and has much heterochromatin; one heterozygous inversion was seen in an autosome. The analysis was made by Dr. Mettler using stock No. H73.12 from Turrialba, Costa Rica.

Relationship. Belongs in subgroup III of the tripunctata group of the subgenus Drosophila; it is very similar to *mediopicta* Frota-Pessoa from which it is distinguished by the lack of any central markings on tergites 5–6, and by the smaller, rounded spermathecae; male genitalia of the two species are quite similar.

Distribution. Collected by the senior author in 1955 at Turrialba, *Costa Rica*, 2000′, Oct., and Facultad de Agronomia, Medellin, *Colombia*, 5000′, Nov. Collectors in 1956 included Wasserman, Carson and Hoenigsberg; localities are: *Costa Rica:* Turrialba, Aug.; La Lola, 128′, Aug.; San Isidro de General, 2000′, Aug.; San José, 3000′, Aug.; *Panama:* Cerro La Campana, 2700′; *Colombia:* El Recuerdo, Sierra Nevada Mts., 3000′.

Types. Holotype male, 4 ♂ and 4 ♀ paratypes, from stock No. H73.12 from Turrialba, Costa Rica.

Drosophila trapeza Heed and Wheeler, new species.

External characters of imagines.

♂, ♀. Arista with 6 dorsal and 3 ventral branches in addition to the fork. Front tan, face and cheeks somewhat paler. Carina not sulcate, reaching nearly to level of vibrissae. Proclinate orbital about ⅝ posterior reclinate; anterior reclinate about ¼ proclinate. Two strong orals, the second shorter than the first. Greatest cheek width 1/5–1/7 greatest diameter of eye. Eyes bright red with

light pile. Palpi usually with 2 long bristles on antero-lateral margin followed by 2 thinner ones; ventral surface with 11–17 shorter bristles.

Acrostichal hairs in 6 rows; no prescutellars. Anterior scutellars divergent. Mesonotum brown, semi-shining; pleura and legs lighter. Pleura with a faint brown notopleural stripe and with faint brown spots on pteropleura and upper posterior corner of sternopleura. Anterior sternopleural about ⅓ length posterior; middle bristle about ¾ length of anterior one. Front tarsus with numerous recurved hairs; base of fore and mid-metatarsus with a strong bristle, base of hind tarsus with 3 strong bristles. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings with posterior crossvein clouded; costal index about 3.5; 4th vein index about 1.7; 5x index about 1.7; 4c index about 0.75. One prominent bristle at apex of 1st costal section; 3rd section with heavy bristles on the basal ¼. Vein 2L bends to the costa at its end.

Abdomen of male shining yellow; tergites 2–5 with narrow posterior black bands that fade at the angle. The bands are incompletely interrupted on tergites 2–3, and completely interrupted on 4–5. Sixth tergite with a large central trapezoidal black spot that almost reaches the base of the tergite. Strains from Palmira and Medellín, Colombia are more strongly marked, all bands are interrupted in the midline, the 5th and sometimes the 4th bands are expanded into paramedian spots or triangles, and the trapezoidal spot on the 6th usually reaches the anterior margin.

Female abdomen as in the male except that the thin band of 5th tergite is more often broken into apical spots; 6th with a pair of small delicate, paramedian triangles. The more heavily marked form from Colombia has the bands on the 5th and sometimes the 4th expanded into paramedian spots or triangles; 6th with a pair of large paramedian triangles or spots which are sometimes partly fused and reach posterior margin.

Body length (etherized) about 2.3 mm. (♂), 2.7 mm. (♀); wing length about 2.0 mm. (♂), 2.3 mm. (♀).

Internal characters of imagines and genitalia.

Anterior Malpighian tubes branched basally near gut; posterior tubes apposed without continuous lumen. Testes with about 8 very pale yellow outer coils and 4–5 white inner coils. Sperm pump diverticula short, about half greatest diameter of pump. Forceps with 6–8 primary teeth, 5–6 strong secondary teeth on upper surface and 6–8 marginal bristles. Toe usually with 3 long bristles; 2–6 bristles on genital arch in back of toe, sometimes continuous with toe bristles. Head of penis arrow-head shaped with 2 horn-like processes pointing back toward the apodeme. Shells of hypandrium very narrow.

Spermathecae pear-shaped, about 0.13 mm. long, yellow brown in color; inner duct funnel-shaped. Ventral receptacle with about 60 coils. Ovipositor plate with about 19–21 teeth, apex rounded.

Other characteristics, relationship, and distribution.

Eggs. With 2 pairs of thin filaments.

Larvae. Posterior spiracles not black; do not skip.

Puparia. Red-brown; anterior spiracle with about 12 gray branches, the horn, including spiracles, about ¼ length of puparium.

Chromosomes. Five pairs of rods and one pair of large dots. The longest rod pair represents the X chromosomes; the Y is a rod about ⅔ the length of the X. Salivary preparations show 5 long arms and the dot chromosome. Determinations are by Dr. Clayton, using stock No. H29.16 from San Salvador, El Salvador, and by Dr. Mettler, using stock No. H167.1 from La Lola, Costa Rica.

Relationship. Belongs in subgroup III of the tripunctata group of the subgenus Drosophila.

Distribution. A widely distributed but relatively uncommon species of the Neotropical region. Collected by the senior author and other Texas collectors (1952–56) in Vera Cruz, *Mexico*, *El Salvador*, *Honduras*, *Costa Rica*, *Nicaragua*, *Colombia*, *Venezuela* and *Trinidad* at elevations from sea-level to 5000 feet; also collected at the Agricultural Experiment Station, Río Piedras, *Puerto Rico*, and at Kingston, *Jamaica*, B.W.I., in Feb., 1956.

Types. Holotype male, 2 ♂ and 3 ♀ paratypes, from stock No. H167.1 from La Lola, Costa Rica.

Notes. *D. trapeza* is attracted to banana bait but it is never found in great quantities in any collection. The largest number taken on any day was 22 individuals (4.8% of the total collection) collected over zapote fruits *(Calocarpum)* at San Salvador in May. It has been reared in El Salvador from the following plants: *Helosis* (Balanophoraceae), *Inga preussii* Harms (Leguminosae), *Castilla gummifera* Pittier (Moraceae), and *Brosimum terrabanum* Pittier (Moraceae).

Distinguishing characters are: the typical trapezoidal mark on 6th tergite of males, the small delicate paramedian triangles on 6th tegite of females, and the slight markings of the pleura. In general view, the head seems somewhat too small for the size of the body.

Drosophila mediodiffusa Heed and Wheeler, new species.

External characters of imagines.

♂. Arista with 5–6 dorsal and 3 ventral branches in addition to the fork. Antennae and front brown; few frontal hairs. Proclinate orbital about ⅝ length posterior reclinate; anterior reclinate ¼ proclinate. Only one strong oral, the 2nd about ⅓ as long. Carina prominent, a bit longer than 3rd antennal segment. Face whitish but not strikingly so. Greatest width of cheek about ⅛ greatest diameter of eye, extremely narrow directly below the eye. Eyes scarlet with silvery pile. Palpi with 8–10 strong bristles on lateral margin and 30–40 shorter bristles ventrally.

Acrostichal hairs in 6 rows; no prescutellars. Anterior scutellars straight or slightly divergent. Mesonotum shining brown, scutellum darker. Pleura very light tan to nearly whitish. Anterior sternopleural about 0.3 length posterior, the middle bristle usually shorter than anterior. Legs tan; numerous recurved hairs on fore tarsi; flexor and lateral extensor surfaces of fore femur with several long bristles; several strong bristles near base of hind metatarsus. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings grayish, posterior crossvein clouded, anterior crossvein dark or slightly

clouded. Costal index about 3.3; 4th vein index about 1.7; 5x index about 1.2; 4c index about 0.8. Two prominent bristles at apex of 1st costal section; 3rd section with heavy bristles on the basal ⅓. Vein 2L bends to costa near its tip.

Abdomen shining tan with grayish shading dorsally which is darker on tergites 2 and 3. Body length (etherized) about 2.1 mm.; wing, 2.0 mm.

Female. Palpi with 4–5 strong bristles on lateral margin and about 12 shorter bristles on ventral surface. A few recurved hairs on fore tarsi. Abdomen shining tan with a diffuse gray band as broad as the tergite on 2nd and 3rd segments which terminate at the angle; the remainder of the abdomen varies from a grayish dorsum with a slight clear midstripe, to shining tan. Body length (etherized) about 2.5 mm.; wing, 2.3 mm.

Internal characters of imagines and genitalia.

Anterior Malpighian tubes branched at about the basal 1/10; posterior tubules with a continuous lumen. Testes with 3 pale yellow outer coils and 2 white inner coils. Sperm pump diverticula slightly longer than greatest diameter of the pump. Forceps with 6–8 primary teeth in a straight row, 4–6 long bristles on upper surface and 4–9 short marginal bristles. No bristles on toe; head of penis with 2 prominent horn-like processes extending back toward the apodeme. Spermatheca chitinized, brown, the width about equalling the length; inner duct usually with uniform diameter but sometimes the distal portion is widened into a cup. Ventral receptacle with only about 6 irregular coils. Ovipositor plate with 23–28 teeth.

Other characteristics, relationship, and distribution.

Eggs. With 2 pairs of thin filaments.

Puparia. Red-brown; anterior spiracle with 15–16 white branches, the horns, including spiracles, about ¼ length of puparium.

Larvae. Posterior spiracles yellow; do not skip.

Chromosomes. Five pairs of rods and one pair of dots; the X is about twice as long as the autosomes and as the Y; one autosomal pair has satellites on the centromere end. The Y is a rod and the dot pair is very small. The analysis was made by Dr. Mettler using stock No. H130.6 from Puerto Rico.

Relationship. Belongs in subgroup IV of the tripunctata group of the subgenus Drosophila; it is most similar to *albescens* and *tristriata.*

Distribution. Collected by the senior author in January and February, 1956, in *Haiti, Jamaica,* and *Puerto Rico.* Mrs. Marta Breuer also collected the species in *Cuba* in January, 1956.

Types. Holotype male and 6 paratypes of both sexes, descendants of stock No. H129.16 from El Yunque Resort Area, Caribbean National Forest, Puerto Rico at about 2000 feet elevation.

Notes. D. mediodiffusa comes readily to fruit baits and is easily cultured on banana medium. The species is named for the diffuse, indistinct abdominal pattern.

Drosophila medioparva Heed and Wheeler, new species.

External characters of imagines.

♂, ♀. Arista with 7 dorsal and 3 ventral branches in addition to the fork. Face white; carina reaching nearly to level of vibrissae, rounded at tip, not sulcate.

Antennae, cheeks and front brown. Proclinate orbital about ⅝ length posterior reclinate; anterior reclinate about ¼ length proclinate. One strong oral; greatest cheek width about ⅛ greatest diameter of eye, extremely narrow directly below eye. Eyes red with pale pile. Palpi with about 4 long bristles along antero-lateral margin, gradually decreasing in size posteriorly.

Acrostichal hairs in 6 rows; no prescutellars. Anterior scutellars divergent. Mesonotum and scutellum semi-shining brown; pleura and legs paler. Anterior and mid-sternopleurals about ½ length posterior one. Fore femur usually with 5 prominent bristles on lateral extensor surface and 3 on flexor surface. Several recurved hairs on fore tarsus. No heavy black bristles at base of hind metatarsus. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings with grayish cast; posterior crossvein clouded. Costal index about 4.0; 4th vein index about 1.5; 5x index about 1.4; 4c index about 0.6. One prominent bristle at apex of 1st costal section; 3rd section with heavy bristles on the basal ½.

Abdomen brownish yellow with black apical bands on tergites 2–4 which are incompletely interrupted on 2–3 and usually completely interrupted on 4th. The widest band is on the 3rd tergite where it is about half the tergite width. All bands fade at the angle of the tergites. Tergite 5 usually without markings, but rarely with a pair of faint, small, paramedian spots. Tergite 6 with a large round median spot that extends almost the width of the tergite. Sexes similar.

Body length (pinned specimens) about 2.2 mm.; wing length about 2.3 mm. (all measurements made on males).

Internal characters of imagines and genitalia.

Posterior Malpighian tubes apposed but apparently without a continuous lumen. Testes with 8–9 outer yellowish coils and 3–4 inner coils. Sperm pump diverticula short, about ¾ length of pump. Forceps with 8 primary teeth in a curved row, and 12 marginal bristles that come around onto upper surface. Toe large with about 8 bristles. Gonapophysis without conspicuous bristle. Basal part of anal plate with a straight row of about 6 small, well-spaced bristles.

Spermathecae elongate, about 0.18 mm. long and 0.1 mm. wide; inner duct tubular with a median constriction. Ventral receptacle with about 40 coils. Ovipositor plate with 21 teeth, the apex rounded.

Other characteristics, relationship, and distribution.

Eggs. With 2 pairs of thin filaments.

Larvae. Posterior spiracles black.

Puparia and chromosomes. Unknown.

Relationship. Belongs in subgroup IV of the tripunctata group of the subgenus Drosophila. It is quite similar to *D. albicans* in the male genitalia, but the two species are easily distinguished on other features.

Distribution. Collections were made by the senior author and Drs. Carson and Wasserman in July and August, 1956, as follows: *Costa Rica:* Turrialba, 2000′; La Lola, 128′; *Panama:* Cerro La Campana, 60 klm. southwest of Panama City, 2700′; *Colombia:* El Recuerdo, 24 klm. southeast of Santa Marta, 4000′. Mr. Hoenigsberg assisted in the last locality.

Types. Holotype male and 3 paratype males, No. H154.10, from Turrialba, Costa Rica.

Notes. Collected over hule fruits *(Castilla)* in Costa Rica and on rotting celery cabbage in Panama. It was not common in any collection. Two males were reared by Dr. Wasserman from fruit of *Anthurium* (Araceae). We have been unable to raise this species on banana medium as yet.

The species may be recognized by its small size, white face, large round spot on 6th tergite, rather large eye, and semi-shining mesonotum. The only striking difference in male genitalia from *albicans* is the arrangement of small bristles along the base of the anal plate, since in *albicans* these are formed into a closely-set row of 10 or more bristles.

Drosophila spinatermina Heed and Wheeler, new species.

External characters of imagines.

♂, ♀. Arista with 5–6 dorsal and 2–3 ventral branches in addition to the terminal fork. Face and 3rd antennal joint tan; front dull brown with paler areas at bases of orbitals and in ocellar triangle, the latter extending as a thin line to anterior margin. Proclinate orbital about 4/5 length posterior reclinate; anterior reclinate about 1/4 proclinate. Two strong orals of equal length. Carina prominent with rounded edges, not sulcate. Greatest cheek width about 1/4 greatest diameter of eye, about 1/6 directly below eye. Eyes bright red with light pile. Palpi with 2–3 long bristles on antero-lateral margin and about 20 shorter ones on ventral surface and margin.

Acrostichal hairs in 6 rows; no prescutellars. Anterior scutellars divergent. Mesonotum and scutellum subshining brown; pleura brown. Anterior sternopleural about 1/2 length posterior; middle bristle about half length anterior one. Legs light brown; a few recurved hairs on fore tarsus. Lateral extensor surface of fore femur with 4 long bristles; flexor surface with 3 long bristles. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings clear; posterior crossvein clouded. Tip of 3L usually with slight shading. Costal index about 2.7; 4th vein index about 1.7; 5x index about 1.5; 4c index about 0.9. Two prominent bristles at apex of 1st costal section; 3rd section with heavy bristles on the basal 1/2. Vein 2L bends toward costa at its tip, and 3L usually bends toward 4th at its tip, the apex of the wing appearing rounded.

Abdomen shining yellow with very narrow dark brown posterior bands on 2nd and 3rd tergites that are interrupted in the midline and which extend to the angle of the tergite. Remainder of abdomen without marks.

Body length (etherized) about 2.0 mm. (♂), 2.4 mm. (♀); wing length about 1.9 mm. (♂), 2.1 mm. (♀).

Internal characters of imagines and genitalia.

Anterior Malpighian tubes branched at about the basal 1/10; posterior tubes with continuous lumen. Testes with about 7 thin outer coils and 4 thicker inner coils; white. Sperm pump diverticula about half length of pump itself. Anal plate with a single prominent spine on lower tip; toe with 4 bristles; forceps with a row of 8–9 primary teeth, 5–8 secondary teeth and 8–9 marginal bristles which continue around to upper surface. Head of penis rather large and complex, in a filiform envelope. Spermathecae dark brown, oval, the greatest width 3/4 the length; inner duct broadly funnel-shaped, narrowing gradually to a constriction

near base with a slight bulblike expansion just before the point of exit. Ventral receptacle with over 50 coils. Ovipositor plate with 15–17 teeth, the tip somewhat blunt.

Other characteristics, relationship, and distribution.

Eggs. With 4 thin filaments, the apical pair thinner and shorter than the basal pair.

Larvae. Posterior spiracles yellow; do not skip.

Puparia. Red-brown; anterior spiracle with 24–26 thin yellow branches; horns, including spiracles, about ⅓ length of puparium.

Chromosomes. Three pairs of rods, one pair of V's and one pair of dots. One rod pair is longer than the other two, and longer than the arms of the V. Determination made by Dr. Mettler on stock No. H106.1 from Port of Spain, Trinidad.

Relationship. A rather atypical member of subgroup IV the tripunctata group of the subgenus *Drosophila.* It resembles *tripunctata* in some respects, including the low costal index, darkening at apex of 3L, small size, and shape of ovipositor plate. It resembles members of the cardini group in having a spine on the lower tip of the male anal plate and large secondary teeth on the forceps.

Distribution. Known from two individuals (and their descendants) collected by the senior author while sweeping terrestrial aroids near the Queen's Savannah, Port of Spain, *Trinidad* in December, 1955.

Types. Holotype male, 3 ♂ and 4 ♀ paratypes, descendants from stock No. H106.1 from Port of Spain, Trinidad.

Notes. Some individuals from the stock show a doubling of ocellar bristles on one or both sides, and a few females have the proclinate and posterior reclinate bristles doubled on one side or the other. Such abnormalities were found in about 30% of the 52 individuals examined.

Drosophila tristriata Heed and Wheeler, new species.

External characters of imagines.

♂, ♀. Arista with 5–6 dorsal and 3 ventral branches in addition to the fork. Antennae and front brown; few frontal hairs. Proclinate orbital ¾ posterior reclinate; anterior reclinate ¼ proclinate. One strong oral, 2nd about half length 1st. Carina widened below, not sulcate. Face whitish in females, tan to whitish in males; on pinned specimens the face turns rather brownish. Greatest cheek width about 1/7 greatest diameter of eye; cheek exceedingly narrow directly below eye. Eyes red with silvery pile. Male palpi with about 8–10 strong bristles on lateral margin and 15 shorter bristles on ventral surface. Female palpi with 4–6 strong bristles on lateral margin and about 9 shorter bristles on ventral surface.

Acrostichal hairs in 6 rows; no prescutellars. Anterior scutellars slightly divergent. Mesonotum and scutellum shining brown; pleura whitish. Anterior sternopleural ⅓–½ posterior; middle one ¼–½ anterior. Legs whitish tan; recurved hairs on fore tarsi. Flexor surface and lateral extensor surface of fore femur with 3–5 long bristles each. Several long yellow bristles present on base of hind metatarsus. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings grayish; posterior crossvein clouded, anterior crossvein dark to slightly clouded. Costal index about 3.2; 4th vein index about 1.6; 5x index about 1.3; 4c index about 0.7. One prominent bristle at apex of 1st costal section; 3rd section with heavy bristles on the basal ½ or slightly less. Vein 2L bends to costa at its tip and is slightly darkened apically.

Abdomen shining yellow with posterior black bands on 2nd and 3rd tergites that fade at the angle and are usually diffusely interrupted medianly, but sometimes with diffuse mid-dorsal extensions. Tergites 4–6 in male with median longitudinal stripes that usually extend the length of the tergite; last 3 segments sometimes with narrow posterior bands in male, quite variable in females, sometimes with median stripes as in males, or with only median dots, or without markings. A narrow posterior band usually present on 4th when median markings are present.

Body length (etherized) about 2.0 mm. (♂), 2.5 mm. (♀); wing length about 2.0 mm. (♂), 2.2 mm. (♀).

Internal characters of imagines and genitalia.

Anterior Malpighian tubes branched basally near gut; posterior tubes with continuous lumen. Testes with 3 outer yellow coils and 2 inner white coils. Sperm pump diverticula about ¾ greatest diameter of the pump. Genital arch with well developed anterior process; forceps with 8–9 primary teeth in a straight row, usually 6 long pale bristles on upper surface and 6 long marginal bristles. Toe with 1–2 bristles subapically; lower tip of anal plate with a loose tuft of about 10 hairs. Head of penis rounded, with a lateral pair of short rounded chitinized processes (ventral view). The median hornlike projection connecting the arms of the hypandrium, between the forceps, unusually narrow.

Spermatheca round, brown; inner duct with uniform diameter. Ventral receptacle with about 15 irregular coils. Ovipositor plate with 22–27 teeth, the apex fairly broad.

Other characteristics, relationship, and distribution.

Eggs. With 2 pairs of thin filaments.

Larvae. Posterior spiracles yellow; do not skip.

Puparia. Yellowish brown; anterior spiracle with about 16 whitish branches; horns, including spiracles, about ¼ length of puparium.

Chromosomes. Five pairs of rods and one pair of dots; one rod pair is nearly twice the length of the others and bears satellites; two pairs of shorter rods also bear satellites. The X and Y were not recognized. The analysis was made by Dr. Wasserman using stock No. H109.22 from Port of Spain, Trinidad.

Relationship. Belongs in subgroup IV of the tripunctata group of the subgenus Drosophila and is most similar to *albescens* and *mediodiffusa*. It may be distinguished from *mediodiffusa* by the presence of a rather strong 2nd oral, abdominal stripes (when present) and characteristic genitalia. *D. albescens* differs from the other two by the nearly transparent mesonotum and absence of sperm pump diverticula, and from *mediodiffusa* by the absence of prominent hornlike processes on the head of the penis; it differs from *tristriata* also in the absence of bristles on the toe, and the lack of an unusually narrowed connection between the forceps.

Distribution.—Collected by the senior author in *El Salvador*: San Salvador, throughout the year, 1953–54; *Honduras:* La Lima, April 1954; *Trinidad:* Port of Spain, Dec. 1955; *Puerto Rico:* Río Piedras Experiment Station, Jan. 1956; and *Jamaica:* Bath and Kingston, Feb. 1956.

Types.—Holotype male, 3 ♂ and 7 ♀ paratypes, from collection No. H109.22 from the vicinity of Port of Spain, Trinidad.

Notes.—Near Port of Spain, Trinidad the species was collected in a cacao finca on the fallen red flowers of *Erythrina micropteryx* Poepp. (Leguminosae) where, on Dec. 24, 1955, the species made up 7.5% of the collection of 374 flies, and on Dec. 26, 1955, this species accounted for 6.5% of the 369 flies collected.

Drosophila neomorpha Heed and Wheeler, new species.

This new member of the cardini group is most similar to *polymorpha* and *parthenogenetica;* it occurs in both a dark and a light form.

External characters of imagines.

♂, ♀. Arista with 5 dorsal and 2 ventral branches in addition to the fork. Face, cheeks and antennae light tan; two orals of equal length; carina fairly broad, reaching below 3rd antennal joint, not sulcate. Proclinate orbital equal to length of posterior reclinate; anterior reclinate about 1/5 the other two. Front tan; base of orbitals and ocellar triangle shining, the latter extending forward to level of orbital bases. Few frontal hairs present. Greatest width of cheek about 1/5 greatest diameter of eye; eye red with light colored pile. Palpi of male with 2–3 long bristles on antero-lateral margin and 25–30 only slightly shorter ones (3/4 length long ones) on anterior and ventral surfaces. Palpi of female with one strong bristle subapically and about 16 shorter ones (1/3 length of long bristle) on anterior and ventral surface.

Acrostichal hairs in 6 rows; no prescutellars. Anterior scutellars convergent. Mesonotum, scutellum and pleura shining yellowish-brown; legs and halteres lighter. Anterior sternopleural about 3/8 posterior; mid-sternopleural about 1/3 anterior.

Fore femur with one prominent bristle on basal lateral extensor surface; flexor surface with 1–2 prominent bristles about equal to greatest width of femur in length and 1–2 shorter bristles about 1/2–3/4 length of longer ones in addition to several still shorter ones. Posterior surface of fore femur fairly densely haired, especially in male. Numerous recurved hairs on fore tibia and tarsus; fore metatarsus with one, sometimes two, prominent bristles at base. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings clear, crossveins not clouded. Costal index about 3.2; 4th vein index about 1.7; 5x index about 1.5; 4c index about 0.8. One strong and one weak bristle at apex of 1st costal section, this area slightly darkened; 3rd costal section with heavy bristles on the basal 1/2.

Abdomen shining brownish yellow with polymorphic pattern. The light and dark forms of both sexes will be described, but several grades of intermediates exist.

Light form, male: narrow posterior black bands on tergites 2–4, interrupted medianly, fading at the angle; 5–6 sometimes without markings or with 5th

segment only with a suggestion of a posterior band; 6 with a pair of small paramedian spots at posterior border. Genital arch darkened.

Dark form, male: tergites 2–4 with shining black posterior bands, interrupted medianly and extended basally to preceding tergite at the angle but failing to reach lateral margin; 5 shining black except for a narrow dorsal interruption, a central narrow area along edge of preceding segment and a narrow area along lateral margin; 6 shining black except along lateral margin.

Light form, female: tergites 2–4 with medianly interrupted black bands about ⅓ width of tergite; the bands stop rather abruptly at the angle and continue toward but fail to reach lateral margin as a much narrower line or band. Interrupted band of tergite 5 very narrow, almost reaching lateral margin; 6 with only a suggestion of a band. Segments 3–6 with an extra narrow mark on anterior border slightly beyond the angle. Genital arch darkened.

Dark form, female: bands become progressively wider posteriorly until segments 5–6 where they are slightly more than half the width of the tergite; at the angle the bands of tergites 3–6 extend forward to basal border; 6 with non-interrupted band with paramedian extensions that reach base; area around anal plates darkened.

Body length (etherized) about 2.5 mm. (♂), 2.8 mm. (♀); wing length about 2.1 mm. (♂), 2.4 mm. (♀).

Internal characters of imagines and genitalia.

Anterior Malpighian tubes branched at their distal ⅙; posterior tubes apposed, without a continuous lumen. Testes with about 6 outer yellow coils and 3 thicker inner white coils. Sperm pump diverticula about twice the greatest diameter of the pump. Forceps with 7–8 primary teeth, 8–10 strong secondary teeth and about 8 marginal bristles that come around onto upper surface. Lower tip of anal plate with 3 strong spine-like bristles that are thicker and shorter than the usual anal plate bristles. Hypandrium with one long bristle; base of genital arch with 1–2 long bristles. Spermatheca vase-shaped, the inner duct broadly funnel-shaped in its upper ¾. Ventral receptacle with about 70 coils. Ovipositor plate with 18–22 teeth.

Other characteristics, relationship, and distribution.

Eggs.—With 2 pairs of thin filaments.

Larvae.—The larvae skip.

Puparia.—Reddish brown; anterior spiracle with about 18 grayish branches; horns, including spiracles, about ⅓ length of puparium.

Chromosomes.—Two pairs of V's, one pair of rods, and a pair of dots. One V-pair has slightly unequal arms; the X is a rod with a satellite on the centromere end and is slightly longer than an arm of the metacentric V. The Y is slightly shorter than the X and lacks a satellite. Salivary chromosomes show 5 long arms and the dot; the X has much heterochromatin at the centromere end. No inversions were seen in 5 preparations. Analysis made by Dr. Mettler using stock No. H80.8 from Barro Colorado Island, Canal Zone.

Relationship.—Belongs to the cardini group of the subgenus Drosophila and is most similar to *D. parthenogenetica* and *D. polymorpha.*

Distribution.—Collected at Chapulhuacan, Hidalgo, *Mexico* (Wasserman and

Heed, June 1952); Lancetilla, *Honduras* (Heed, April 1954); Barro Colorado Island, *Canal Zone* (Heed, Oct. 1955) (Heed, Wasserman, Carson, Aug. 1956); Cerro La Campana, *Panama* (Heed, Wasserman, Carson, Aug. 1956); Arima Valley, *Trinidad* (Heed, Dec. 1955).

Types.—Holotype male, 2 ♂, 2 ♀ paratypes, descendants from stock No. H80.8 from Barro Colorado Island, Canal Zone; 1 ♂, 1 ♀ paratype from stock No. H107.21 from Arima Valley, Trinidad.

Notes.—D. neomorpha may be distinguished from *polymorpha* and *parthenogenetica* by the following combination of characters: long bristles on flexor surface of fore femur, strong spine-like bristles on lower tip of male anal plate, and long secondary bristles on palpi.

Dr. Harrison D. Stalker, Washington University, St. Louis, Missouri, tested this species for parthenogenetic tendencies; a total of 14,278 eggs was examined from 40 virgin females. Seven eggs began development but died before hatching; a single larva hatched from the egg but died within 24 hours. No adults were produced by parthenogenesis.

Drosophila nigrodunni Heed and Wheeler, new species.

External characters of imagines.

♂. Arista with 5 dorsal and 2–3 ventral branches in addition to the fork. Front dull brown; bases of orbitals and ocellar triangle shining brown; few frontal hairs. Proclinate orbital equal to length of posterior reclinate; anterior reclinate ⅓ other two. Two moderately strong orals of equal length. Carina prominent, ⅔ as broad as 3rd antennal joint, fused to mouth margin. Face brown; greatest cheek width about ⅕ greatest diameter of eye, about 1/7 directly below eye. Eyes garnet with short, pale pile. Palpi with 6–8 strong bristles on anterior distal margin and about 20–25 shorter bristles on ventral surface.

Acrostichal hairs in 6 rows; no prescutellars. Anterior scutellars convergent. Mesonotum subshining grayish brown, darker on scutellum and pleura. Anterior sternopleural about ⅖ posterior; middle bristle ½ length anterior one. Legs tan; numerous recurved hairs on fore tarsus. Flexor surface of fore femur with 2–4 long bristles; lateral extensor surface with 1–3 long bristles. Apical bristles on 1st and 2nd tibiae, preapicals on all three.

Wings clear; posterior crossvein dark but not clouded. Costal index about 3.2; 4th vein index about 1.8; 5x index about 1.6; 4c index about 0.8. Two prominent bristles at apex of 1st costal section; 3rd section with heavy bristles on the basal half. Vein 2L bends to costa at apex.

Abdomen generally shining brownish black with a tan pattern dorsally. Tergite 2 with a faint posterior gray band vaguely interrupted, and a tan spot anteriorly at the angle; 3–4 with darker wide posterior bands, usually interrupted, and with narrow paramedian extensions reaching basal border. Entire lateral area from angle of tergite brownish black. The pattern thus obtained is a pair of dorsal rectangular tan areas on 3–4; 5–6 shining black. Body length (etherized) about 2.2 mm.; wing, 2.0 mm.

♀. Palpi with 4–6 strong bristles on anterior distal margin and about 10–16 shorter bristles on ventral surface; few recurved hairs on fore tarsus. Abdomen with shining black bands with definite brownish cast on 2–3. Paired rectangular

dorsal tan areas sometimes on all tergites. Posterior half of 5th and all of 6th usually entirely black. Body length about 2.5 mm.; wing, 2.1 mm.

Internal characters of imagines and genitalia.

Anterior Malpighian tube branched at its distal 1/6; posterior tubes apposed without a continuous lumen. Testes white, with 5 outer and 3 inner coils. Sperm pump diverticula slightly longer than greatest diameter of pump. Forceps with 6–7 primary teeth in a straight row, 5–8 secondary teeth and 8–10 marginal bristles. Spermathecae chitinized dark brown with a slight indentation at distal tip; inner duct funnel-shaped. Ovipositor plate with 18–21 teeth.

Other characteristics, relationship, and distribution.

Eggs.—With 2 pairs of thin filaments.

Larvae.—The larvae skip

Puparium.—Brown, redder with age; anterior spiracle with 9–10 branches; horns including spiracles, about 1/5 length of puparium.

Chromosomes.—Two pairs of V's, one pair of rods and one pair of dots. The rods are slightly longer than an arm of the V's. Analysis made by Dr. Mettler using stock No. H119.4 from Barbados.

Relationship.—Belongs to the cardini group of the subgenus Drosophila and is most similar to *D. dunni.*

Distribution.—*Barbados*, British West Indies. Collected by the senior author over fruit bait in Turner's Hall Woods, and in several orchards.

Types.—Holotype male, 4 ♂, 3 ♀ paratypes, descendants from stock No. H119.4 collected Jan. 1956, at Grant's Castle, Barbados.

Drosophila aracea Heed and Wheeler, new species.

This interesting new species cannot, as yet, be assigned to any of the established subgenera of Drosophila.

External characters of imagines.

♂, ♀. Arista with 3 dorsal and 2 ventral branches of moderate length in addition to the terminal fork. Face, cheeks and antennae brown; carina of moderate length, rounded. One strong oral, 2nd much weaker, lateral to 1st rather than ventral to it. Front brown, about as broad as long; frontal hairs present. Base of orbitals and ocellar triangle shining brown. Proclinate and posterior reclinate orbitals usually nearly of equal length but somewhat variable; anterior reclinate about 1/4 proclinate and laterad of the other two. Postverticals about 4/5 (sometimes equal) length of two long orbitals. Eye castillian red with light colored pile; eyes round. Cheeks wide, the greatest width about 3/8 diameter of eye. Palpi with 3 long bristles on antero-lateral margin; numerous shorter bristles on ventral surface.

Acrostichal hairs in about 6 irregular rows; no prescutellars. Anterior scutellars convergent; posterior scutellars usually directed upwards at a 45° angle. Usual anterior dorsocentrals about 5/8 length posterior ones and placed rather far forward (1/4–1/5 distance from transverse suture to scutellum), the distance between anterior dorsocentrals equalling that from anterior to posterior pair so that the two pairs form a square. Anteriorly in each dorsocentral row, immediately behind transverse suture, there is a shorter bristle, a little larger than acrostichal

hairs and about half length of anterior dorsocentrals. Other hairs in the dorsocentral row may sometimes be enlarged and bristle-like. Mesonotum, scutellum and pleura shining light brown; halteres and legs paler brown. No middle sternopleural; anterior one about ⅓ posterior. Fore femur with 5 prominent bristles on flexor surface and 4 prominent bristles on lateral extensor surface. Recurved hairs on all tibiae and tarsi. First 4 segments of all tarsi with 2–3 stouter and longer apical bristles; distal tarsal joint of all legs somewhat enlarged, with large empodium and pulvilli. Hairs and bristles on all legs somewhat elongated. Apical bristle developed on 2nd tibia only, preapicals on all three.

Wings clear, veins yellow. Costal index about 3.0; 4th vein index about 1.5; 4c index about 0.8; 5x index about 1.1. One prominent bristle at apex of 1st costal section; 3rd section with heavy bristles on the basal ¼ or less. The double row of recurved hairs along costa enlarged more than usual in the genus, giving the wing a pectinate appearance.

Abdomen considerably wider than thorax, brownish yellow with black apical bands that are widest at the angle of the tergite and fade away just before reaching lateral margins. Bands of tergites 2–5 usually with slight mid-dorsal extensions, also sometimes incompletely interrupted here. Sixth tergite with a faint, incomplete band. Posterior borders of tergites 5–6 lined with long thin bristles. Sexes similar.

Body length (etherized) about 3.1–3.5 mm.; wings, 2.8 mm.

Internal characters of imagines and genitalia.

Anterior and posterior Malpighian tubes branched basally near gut, the latter with continuous lumen. Testes with 10–15 bright yellow outer coils and 1–2 thicker white inner coils. Sperm pump diverticula long, about 8–10 times the length of the pump. Anal plates fused ventrally by a strong chitinized bow; forceps with 8–10 primary teeth, 5 marginal bristles and 3–6 bristles on upper surface. Toe short and wide, the surface thickly covered with hairs. Base of genital arch with an extended thin pitted shell of about the same size and shape as the hypandrium shell, and which overlies the toe and forceps. Arch of hypandrium short, not extending beyond shells, these with one median bristle. Head of penis acuminate, without special processes.

Spermathecae lightly chitinized, almost round. Ventral receptacle with about 120 tight coils. Ovipositor plate with one row of 23–24 teeth, broad until the very tip where a small extended apex bears the first 5 teeth, a long bristle between teeth 6–7.

Other characteristics, relationship, and distribution.

Eggs.—With 2 pairs of flattened filaments which taper to a point and which are equal in length and equal to egg length.

Larvae.—Posterior spiracles yellow; do not skip.

Puparium.—Red-brown; horns, including spiracles, about ⅓ length of puparium; each anterior spiracle with 22–24 yellow branches.

Chromosomes.—There are 3 pairs of chromosomes: one pair of rods with a subterminal constriction, one pair of large J's, and one pair of V's. Salivary chromosomes show 5 long arms and a dot chromosome; several inversions were seen. The chromocenter is small and very little heterochromatin is present.

Determined by Dr. Clayton using stocks No. H46.28 (El Salvador) and H57.69 (Nicaragua).

Relationship.—Of uncertain relationship in the genus Drosophila.

Distribution.—Collected by the senior author as follows: *El Salvador*: near San Salvador, 2000′, Oct. 1953, Feb., May, June and Aug. 1954; San Marcos Lempa, 150′, May 1954; *Honduras:* La Lima, 100′, March 1954; *Nicaragua:* Santa Maria de Ostuma, 11 klm. north of Matagalpa, 4500′, July 1954; *Costa Rica:* San José, 3000′, Oct. 1955.

Types.—Holotype male, 5 ♂, 4 ♀ paratypes, descendants from stock No. H57.69 from Santa Maria de Ostuma, Nicaragua.

Notes.—*D. aracea* has been collected only from inside the white spathes of aroid flowers (Araceae), where they were feeding and laying eggs on the sticky yellow gynecium at the base of the spadix. A total of 49 individuals has been reared from flowers of the genus *Xanthosoma* and 10 came from flowers of *Syngonium* in El Salvador. The species seems to prefer fresh water swamps where "elephant ears" (*Xanthosoma*) are abundant, but it has also been taken in a wet cloud forest in Nicaragua from aroid flowers.

In both flowers and laboratory culture vials, individuals have a habit of "flocking" together in one place; the species is reared fairly readily on banana medium. Distinguishing characters are: forward position of anterior dorsocentrals; small pair of additional dorsocentral bristles; elongated recurved hairs on costa; enlarged terminal tarsal segment, empodium and pulvilli; flower feeder.

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IV. *Drosophila insularis*, a New Sibling Species of the Willistoni Group

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INTRODUCTION

For about two centuries, insect species names were given to single specimens, or to series of specimens, of dried remains of insect bodies, preserved on pins in museum drawers. The chief criterion of validity of such "species" was that the specimens be distinguishable without undue trouble, preferably with the aid of no more than a simple hand lens, from other pinned specimens. The necessity of such "alpha taxonomy" for a preliminary orientation in the otherwise baffling and bewildering diversity of insect forms can hardly be questioned. "Alpha taxonomy" continues to be necessary in little known groups and in little known faunae.

However, after the taxonomic foundation has been laid, the methods of the so-called "new systematics" are made use of. New systematics studies species not as series of specimens but as biological phenomena. The "alpha taxonomists" have already had the intuition of the existence in nature, among sexually reproducing and cross-fertilizing organisms, of very significant biological units. These are Mendelian populations, or groups of such populations, within which the gene exchange normally occurs, but between which it is impeded or prevented by reproductive isolating mechanisms. The remarkable fact is that these reproductively isolated groups of populations coincide, in most cases, with the species of taxonomists. They coincide in most and yet not in all cases; the reproductive isolation, which is the touchstone of biological species in sexual organisms, is strongly but not absolutely correlated with easy morphological recognizability, which is the touchstone of "good" species of "alpha taxonomy."

The chief interest of systematics of Drosophila lies in that most of the insects belonging to this genus are singularly amenable to methods of investigation which shed light on species as biological phenomena. Most Drosophilae can be easily and cheaply maintained in laboratory cultures. A variety of tests disclosing the presence or absence of certain forms of reproductive isolation can readily be imposed. As material for ecological studies in nature Drosophila proved rather intractable; nevertheless, some ecological data can also be secured. In Drosophila one is, then, able in most instances to evaluate the degree of correspondence between the species of "alpha taxonomy" and biological species.

It is perhaps gratifying that by and large this correspondence amounts to identity, as shown most convincingly particularly by Patterson and Stone (1952) in their admirable work on the evolution of Drosophila. And yet some noteworthy exceptions have been found (see Patterson and Stone, page 180). The exceptions concern mainly sibling species. Sibling species have been defined by Mayr (1942) as species "which are morphologically very similar and indistinguishable, but which possess specific biological characteristics and are reproductively isolated." It would be premature at present to decide whether sibling

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species are more frequent among Drosophila than among other insects, although taking the data at their face value this would appear to be so. The work of Sonneborn and his school has shown that sibling species (there dubbed "varieties") are much more numerous among certain infusorians than they are among Drosophila. However that may be, and despite some disingenuous suggestions that have been made that Drosophila systematics should be kept within the bounds of "alpha taxonomy," the study of sibling species is certainly important because of the light which it sheds on the nature of species in general. The group of sibling species related to *D. willistoni* is perhaps one of the most interesting among those known in the genus.

THE *WILLISTONI* SPECIES GROUP

The *willistoni* species group is a subdivision of the sub-genus *Sophophora* of the genus Drosophila. Patterson and Stone (1952, pages 51 and 52) list nine species, and three further species have since been added to the group. They are all native in the Neotropical faunal region, although *D. willistoni* reaches southern Florida, and *D. nebulosa* occurs in southern Texas and has been found as far north as Nebraska, doubtless an accidental introduction by man. According to Burla *et al.* (1949), the sibling species related to *D. willistoni* are four in number, namely *D. willistoni* Sturtevant, *D. paulistorum* Dobzhansky and Pavan, *D. equinoxialis* Dobzhansky, and *D. tropicalis* Burla and da Cunha. A fifth sibling, *Drosophila insularis* Dobzhansky may now be added.

The siblings are morphologically very close. Although Burla *et al.* (1949) have listed several minor differences, both external and internal, identification of single specimens, even living ones, used to be practically out of the question. The situation has changed thanks to Spassky's discovery of slight but constant differences in the external male genitalia (Spassky, an article in this volume). Male specimens may now be identified as to the species. As correctly pointed out by Spassky himself, this does not alter the status of the five species as siblings. The morphological differences between these species are still so small that no one would recognize them as distinct species if it were not for other relevant evidence. This evidence shows that the species are completely isolated reproductively. Burla *et al.* (1949) found that the four sibling species known to them fail to cross. Insemination is rarely observed even when females of one species are confined with males of another in the absence of conspecific males (except that *D. paulistorum* females of the strain used accepted males of the other species relatively easily, though much less so than their own males). However, regardless of the frequency of insemination by foreign males, no hybrid larvae or adults have ever appeared. It remains unknown whether fertilization of eggs by foreign spermatozoa occurs at all, and if so at what stage the embryos die out.

At least equally decisive as the above evidence is the evidence that no gene exchange between the sibling species takes place in nature. Some of the chromosomes of the sibling species (especially the third) are distinguishable in the preparations of salivary gland cells, and the populations of each species contain species-characteristic inversions. If effective introgression were taking place, these cytological markers would occasionally be exchanged between the species. Rather extensive studies of natural populations failed to disclose a single indi-

cation of anything of this sort (da Cunha and Dobzhansky 1954, and much unpublished evidence).

The geographic distribution of the sibling species is interesting. *D. willistoni* is distributed most widely from the Argentine Pampa to the West Indies, southern Florida, and north-central Mexico (San Luis Potosi), and from the eastern slope of the Andes in Peru and northern Argentina to the Atlantic. The distribution areas of the other siblings seemed to be included into each other, in the order *willistoni-paulistorum-tropicalis-equinoxialis* (Burla *et al.* 1949), but the study of the abundant material collected by the expeditions of the genetics group of the University of Texas in Central America and the West Indies discloses a somewhat different situation (Dobzhansky 1957). Namely, *D. paulistorum* is more at home in the south, while its nearest relative, *D. equinoxialis*, has a more northern distribution. The former species occurs from southern Brazil (Rio Grande do Sul) to Colombia, Panama, Costa Rica and Salvador; it is often the dominant species in superhumid rainforest regions of Brazil, but it seems to be entirely absent in the West Indies, except in Trinidad. *D. equinoxialis* is, on the contrary, rare in Brazil, but it is frequent or even dominant in Central America and on the islands of the Greater Antilles. The Lesser Antilles have only *D. willistoni* and *D. insularis* (see below). *D. tropicalis* extends from central Brazil (Goyaz) where it is rare, to Central America and the Greater Antilles, where it is common and locally even dominant. It is tempting to speculate that *D. paulistorum* and *D. equinoxialis*, and *D. willistoni* and *D. tropicalis* were at some time in the past respectively the southern and northern subspecies of their ancestral species. After having evolved complete reproductive isolation, the now full-fledged sibling species all became sympatric in a large territory, embracing the Amazon Valley, the northern part of the continent of South America, a part of Central America, and the Island of Trinidad. The newly discovered, the fifth, sibling species, *D. insularis*, may now be described.

DROSOPHILA INSULARIS DOBZHANSKY, *SPECIES NOVA*

Male and female: Arista with 10-11 branches, both numbers being about equally frequent. Front dusky yellow. Anterior orbital bristles shorter than the posterior, middle orbital one-third posterior. Two prominent oral bristles. Face yellow. Carina short and broad, not sulcate. Cheeks yellow, their greatest width about one-tenth greatest diameter of eye. Eyes bright red with a short brownish pile.

Acrostichals in 6 to 8 rows, often quite irregular. Thorax dusky yellow, darker on the average and in flies of equal age than in *Drosophila willistoni* Sturtevant; pleurae lighter than the thorax. Anterior and middle sternopleurals at most half as long as the posterior and much thinner. Legs greyish yellow. Abdomen yellow with diffuse dark brown bands expanded in the middle and fading out laterally. Wings clear, proportions like in *Drosophila willistoni* but slightly darker in color, especially in old individuals.

Length of body in laboratory-bred specimens: ♀ 2.9–3.2 (mean 3.03), ♂ 2.5–2.8 (mean 2.65) mm.; wings ♀ 2.1–2.3 (mean 2.16), ♂ 2.0–2.1 (mean 2.04) mm.

Reproductive organs of the adults, eggs, larvae, and pupae like those in *Dro-*

sophila willistoni, except for minute but constant differences in the external male genitalia which will be described in a separate publication by Mr. B. Spassky.

Remarks. Closely related to *Drosophila willistoni*, from which it differs by a slightly larger body size and a darker pigmentation. These differences, though fairly clear in flies grown in similar environments, are not reliable for identification of single specimens grown in diverse environments. However, the reproductive isolation between *Drosophila insularis* and *Drosophila willistoni* is, as shown below, complete.

Geographic Distribution. In the sample of about 80 females of *willistoni*-like species collected on the Island of St. Kitts by Dr. W. B. Heed in January of 1956 and sent to the laboratory in Columbia University, 77 females which yielded progenies belonged to *D. willistoni* and 3 to *D. insularis*. The sample from the Island of St. Lucia collected by Dr. Heed in the same month contained 105 fertile females of *D. willistoni* and 1 of *D. insularis*. The samples from Trinidad, Barbados, or the Greater Antilles, contained no *D. insularis*. This species is probably confined to the Lesser Antilles, but how widespread it is on the islands of this chain cannot be decided from the data now available.

Chromosomes. The metaphase group consists of two pairs of metacentric and one pair of acrocentric chromosomes. The "dots" are absent. This metaphasic configuration is invariant in all known species of the *willistoni* group, and, with one unconfirmed exception, in all examined species of the second characteristically Neotropical group of the subgenus Sophophora, namely the *saltans* species group. The giant chromosomes in the salivary gland cells show slight but characteristic differences in the different sibling species of the *willistoni* group (Burla *et al.* 1949). Most characteristic are the disc patterns in the distal portions of the third chromosomes (cf. Plate 1, page 304, in Burla *et al.*, *l.c.*). The disc pattern of this chromosome in *D. insularis* is most nearly akin to that in *D. tropicalis*, except for the absence of the distinct light "bulb" located proximally from the group of heavy doublets characteristic of the latter species. A further resemblance of *D. insularis* to *D. tropicalis* lies in the disc patterns of the right limbs of the X-chromosomes (XR), which have the characteristic "repeat" area in a more nearly subterminal position, while in *D. willistoni* it is in a submedian position.

SEXUAL ISOLATION

As mentioned above, Burla *et al.* (1949) found that the four sibling species of the *willistoni* group known to them showed strong sexual isolation from each other. We have, consequently, studied the situation in this respect of the fifth sibling, *D. insularis*. The usual "multiple choice" method was used in the experiments, a mixture of females of two species being confined with males of one of them.

Newly hatched females and males were divided in groups of three. Three females of one species, three females of another, and three males conspecific with one of the kinds of females were placed togethed in small containers ("creamers") and left undisturbed for 8-10 days at room temperature (about 22° C. with fluctuations of several degrees). To make the identification of the females of the sibling species easy, the tips of the wings were clipped either on the right or on

the left side. The dissection of the females and the examination of the contents of their sperm receptacles under a microscope yielded the data summarized in Table 1. The isolation index was completed according to Stalker (1942).

TABLE 1

Numbers of females tested and per cent found inseminated in various crosses of *D. insularis* with other sibling species

Males	Females	Homogamic n	Homogamic %	Heterogamic n	Heterogamic %	Isolation Index
insularis	insularis + paulistorum	95	96.8	94	1.06	0.976
insularis	insularis + equinoxialis	166	95.1	169	6.47	0.873
insularis	insularis + tropicalis	86	96.5	82	4.57	0.903
insularis	insularis + willistoni (St. Kitts)	180	92.3	180	3.8	0.879
insularis	insularis + willistoni (Recife)	182	94.5	182	10.3	0.791
paulistorum	paulistorum + insularis	159	96.85	155	5.03	0.901
equinoxialis	equinoxialis + insularis	72	100	72	8.33	0.846
tropicalis	tropicalis + insularis	171	98.2	171	8.76	0.836
willistoni	willistoni (St. Kitts) + insularis	180	100	180	1.59	0.957
willistoni	willistoni (Recife) + insularis	180	97.2	180	13.3	0.759

It can be seen that the sexual isolation is quite strongly pronounced in all the crosses studied. And yet, cross-inseminations have been observed in every one of the ten combinations of heterospecific parents which have been tried. In the experiments of Burla *et al.* (1949) several of the crosses between the sibling species of the *willistoni* group showed no insemination at all, and only the crosses to *paulistorum* males have a considerable frequency of inseminations. There seems to be, therefore, a real difference between the behavior of *D. insularis* with respect to the other sibling species and that of these sibling species with respect to one another. There is one further fact that should be noted. The crosses *insularis* ♀ × *willistoni* ♂ and *willistoni* ♀ × *insularis* ♂ have been made using two different strains of the *willistoni* parent, namely a strain from the Island of St. Kitts and from Recife, Brazil. Now, *D. insularis* shares the islands of St. Kitts and St. Lucia only with *D. willistoni* and with no other species. The crosses *D. insularis* x *D. willistoni* from St. Kitts are, therefore, crosses between strains which are sympatric in origin. The Recife strain of *D. willistoni* comes, on the contrary, from a locality quite remote from the islands in which *D. insularis* is known to live. The isolation coefficients in the crosses which involve the Recife strain are ostensibly, though not quite significantly, lower than those in the corresponding crosses with *D. willistoni* from St. Kitts. The chi-square test gives $0.05 < p < 0.10$ in both crosses.

INTERSPECIFIC HYBRIDIZATION

Systematic experiments have been made crossing *D. insularis* to the other four sibling species. All the hybrids so far obtained came from the crosses in which *D. insularis* was used as the female parent. It would, nevertheless, be premature to conclude that the reciprocal crosses are wholly incapable of producing viable hybrids, since perhaps an insufficient number of attempts have been made, and since insemination of females of other species by *D. insularis* males does occur (see above). The technique of the experiments was to place

several (up to about a dozen) virgin females with approximately equal numbers of males of a foreign species in a culture bottle. After a lapse of about a week, inspection disclosed the presence or absence of hybrid larvae. If hybrids were absent, the parents were transfered to fresh cultures, to give them another chance to produce hybrids. Flies of *D. insularis* from every one of the four St. Kitts strains were used in these experiments indiscriminately.

The cross *insularis* ♀ × *tropicalis* ♂ (the strain from Palma, Goyaz, Brazil) yields hybrids most easily. Several hundred such hybrids have been examined from a number of different cultures. Hybrids of both sexes appear, the sex ratio being about 1:1. The external morphology of the hybrids is apparently normal; study of the copulatory organs of hybrid males by Mr. Spassky (in press) showed these to be approximately intermediate between those of the parental species. The internal reproductive organs are also normal, except for the gonads themselves which are grossly abnormal. The testes of the males are reduced to tiny rudiments, smaller in size than the testes of fully grown larvae. No histological examination has been made, except for observation under an intermediate magnification (about × 600) of a microscope of freshly dissected gonads flattened under a cover-slip. This examination shows nothing resembling normal spermatogenesis. The remains of the testes consist of fairly large undifferentiated cells. The ovaries of the hybrid females are composed of normal-looking egg strings and ovarioles, in which the oocytes and the nurse cells are clearly differentiated. In the ovaries of hybrid females which are about a week old one finds abnormal eggs, with a formed chorion and with two filaments which are shorter than those in the eggs of either parental species. Such females were kept with males of both parental species, but, as expected, no progenies resulted. The hybrid females, like the hybrid males, are wholly sterile.

The crosses *insularis* ♀ × *willistoni* ♂ (using strains of *D. willistoni* derived from flies collected on the Island of St. Kitts) succeed not quite as easily as those to *tropicalis* males. Nevertheless, hybrids of both sexes have been obtained and studied. Their characteristics are quite similar to those of the *insularis* ♀ × *tropicalis* ♂ hybrids described above. They are completely sterile. The crosses *insularis* ♀ × *equinoxialis* ♂ (using the strain from Teffe, Amazonas, Brazil) produce mostly no hybrids at all. However, one cross yielded several pupae which failed to hatch, and in another cross five hybrid females, and no males, were obtained. These hybrid females were kept for some time in a culture with males of both parental species, but proved to be sterile. The cross *insularis* ♀ × *paulistorum* ♂ (a brown-eyed mutant strain) is most refractory. Some larvae have, however, been noted in one of the cultures, but they failed to produce adult hybrids.

A most interesting peculiarity of the *insularis* ♀ × *tropicalis* ♂ and the *insularis* ♀ × *willistoni* ♂ hybrids, which as far as we are aware has no analogue in any other interspecific hybrids, concerns the behavior of the chromosomes in the salivary gland cells of the hybrid larvae. This matter will be described in more detail elsewhere (Dobzhansky, in press in "Chromosoma"). The essence of the story is as follows. The heterochromatic parts adjacent to the centromeres in all the chromosomes come together in the hybrid cells to form a compact chromocenter which resembles the chromocenter of normal cells. However, the

euchromatic parts of the chromosomes of the two species evince no attraction toward each other, and remain completely unpaired. The hybrid males, thus, have eight chromosomal strands radiating from the chromocenter. Two of these eight strands are the two arms of the X-chromosome. Female larvae must have 10 chromosomal strands, four of them being X-chromosomes, but no cell has been found in which all strands were identifiable. The lack of pairing of the euchromatic chromosome strands certainly cannot be due to lack of linear homology, since the chromosomes remain easily recognizable as homologues by their disc patterns. Another interesting fact is that the X-chromosomes in the male larvae were clearly greater in average diameter than are the autosomes in the same cells, or than the X-chromosomes or the autosomes in female larvae. Anybody with any experience in studying the chromosomes in the salivary gland cells of Drosophila larvae knows that the X-chromosome in male larvae (which is single) differs in a paler color, but not in average diameter, from the X-chromosome of female larvae (which is double). The observations on the *insularis* ♀ × *tropicalis* ♂ hybrid larvae prove that this behavior of the X-chromosome depends upon the sex of the cell and not upon the chromosome being paired or unpaired.

VARIATIONS IN THE GENE ARRANGEMENT IN *D. INSULARIS*

The sibling species of the *willistoni* group tend to be highly polymorphic with respect to the gene arrangements in their chromosomes. In fact, natural populations of *D. willistoni* show a diversity of chromosomal structures which is, as far as we know, greater than that recorded in any other species. It is interesting that chromosomal polymorphism exists even in *D. insularis*, the populations of which are isolated on islands.

The four strains of *D. insularis* from St. Kitts were out-crossed to each other and to the strain from St. Lucia. As expected, the crosses went easily and the hybrids were fertile. Three different inversions exist in the St. Kitts population. One of them includes the sub-terminal portion of the right limb of the second chromosome (II R). The homologue of the heavily-banded "repeat" located in this chromosome, corresponding to the section 70 in the II R of *D. willistoni* (see the map in Dobzhansky, 1950), is included in this inversion. The position of this landmark in the chromosome is more nearly subterminal in *D. insularis* than in *D. willistoni*. Another inversion has been found in the subbasal portion of the left limb of the second chromosome (II L). This inversion, like that in II R, is short, but it includes no conspicuous landmark, and its position in terms of the standard map in *D. willistoni* has not been determined. The third inversion has been found in the subterminal portion of the third chromsome (III). All three inversions exist within the St. Kitts population of *D. insularis*, and the only available strain from St. Lucia happens to be free from inversions.

The precise limits of the inversions in *D. insularis* in terms of the maps of the salivary gland chromosomes have as yet not been determined. A comparison of the inversions in the species with those recorded in the other four siblings has nevertheless been made, using the published descriptions and the unpublished notes. The inversions in *D. insularis* are not identical with any found in other

species. Thus far, no inversion has been encountered in more than one of the five siblings.

CONCLUSIONS AND SUMMARY

Drosophila insularis resembles morphologically the other four sibling species of the *willistoni* group. It shows some slight differences in the external appearance, a difference in the structure of the male copulatory organs (discovered by Mr. B. Spassky), and differences in the disc patterns of the chromosomes in the salivary gland cells. Nevertheless, the reproductive isolation between any two of the five siblings is complete. Of the isolating mechanisms so far discovered, sexual isolation is probably most important in natural populations. Nevertheless, this isolation is not absolute, and cross-insemination has been observed under experimental conditions, especially when *D. insularis* females were exposed to males of the other species. Viable hybrids are produced particularly in the cross *D. insularis* ♀ × *D. tropicalis* ♂. These hybrids are, however, completely sterile in both sexes.

D. insularis is the only one of the five sibling species which is capable of producing some hybrids with every one of the remaining four siblings. These latter have never been observed to produce any progenies in the intercrosses with each other. The hybrids are, however, completely sterile, thus making *D. insularis* a reproductively absolutely isolated species. Sexual isolation has been tested between *D. insularis* and the other species with the aid of the multiple choice method. A pronounced isolation has been observed in every cross, though perhaps less strong on the average than the sexual isolation between the four sibling species other than *D. insularis*, as studied by Burla *et al.* (1949). It is tempting to correlate the fact that the isolating mechanisms between these latter species are more nearly perfect than those between them and *D. insularis* with the geographic distribution of these species. The four siblings other than *D. insularis* have their geographic distributions widely overlapping, so that they are sympatric in extensive territories in South and Central America. *D. insularis* is, on the other hand, endemic on some of the islands of the Lesser Antilles, and the only other species which occurs there is *D. willistoni*. Another interesting fact in this connection is that this sexual isolation between *D. willistoni* and *D. insularis* is at least ostensibly stronger if the strains tested are sympatric in origin than when they are allopatric in origin. All these facts are consistent with the hypothesis that reproductive isolating mechanisms between populations of sympatric species may be perfected owing to pressure of natural selection.

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V. Morphological Differences Between Sibling Species of Drosophila

B. SPASSKY[1]

INTRODUCTION

Mayr (1942) has defined sibling species as "pairs or even larger groups of related species which are so similar that they are considered as belonging to one species until a more satisfactory analysis clears up this mistake." The genus Drosophila is known to contain several groups of sibling species, which are supposedly indistinguishable in external morphology, and yet can be shown by genetic and other techniques to represent independent and separate biological species. Clearly, morphological similarity is a relative matter, dependent upon the methods of investigation used. What Mayr has called a "satisfactory analysis" needed to "clear up this mistake" of supposedly complete morphological identity of sibling species takes more effort in some than in other cases. The purpose of the present work is to show that some of the longest known and best studied groups of sibling species of Drosophila can be differentiated by inspection of the external genitalia of males, and that, using a sufficient magnification of a microscope, these species can be recognized in living individuals as well as in cleared preparations.

Morphology of External Male Genitalia

The external genitalia of Drosophila males have been described and figured in detail by Salles (1947), Malogolowkin (1948, 1951), and Hsu (1949). The nomenclature of these authors will be followed in the present investigation.

The genital arch (GA in Figure 1, see also Figures 2 and 30) is a more or less crescent-shaped sclerite which, according to Dobzhansky (1928), corresponds to the fused eighth and ninth abdominal tergites. To it are attached the genital claspers (CL in Figures 1 and 11, see this part also in Figures 2–4, 11–18, and 30–32), which are a pair of plates armed with various kinds of teeth, spines, and bristles. Neither the genital arch nor the claspers enter the vagina of the female at copulation. It appears, however, the claspers function to hold the genitalia of the female during copulation. In etherized copulating pairs of species of *saltans* group observed with the aid of high magnification of dissecting microscope it can be seen that the relaxation of the claspers causes the genitalia of the pair to separate at the termination of the copulation. The inside margin of the genital arch delimits a depression in which are located the anal tubercle (AT, Figures 1 and 2) and the penis with its appendages. This depression may be called the genital chamber. The shape of the genital chamber is often characteristically different in closely related species, particularly in the sibling species of *willistoni* group (Figures 11–18). In copulating pairs it can be observed that the female genitalia fit accurately in the opening of the genital chamber of the male.

The penis (P in Figures 3, 5, 6, and 18, see also Figures 4, 8, 10, 12–18, 25–38) is an unpaired organ, often of species-characteristic shape and adorned with

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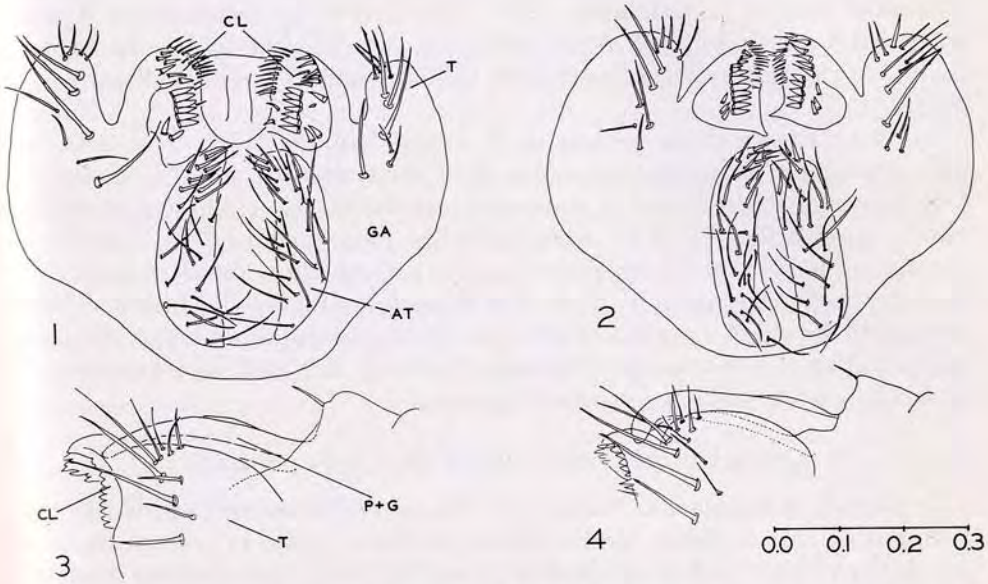
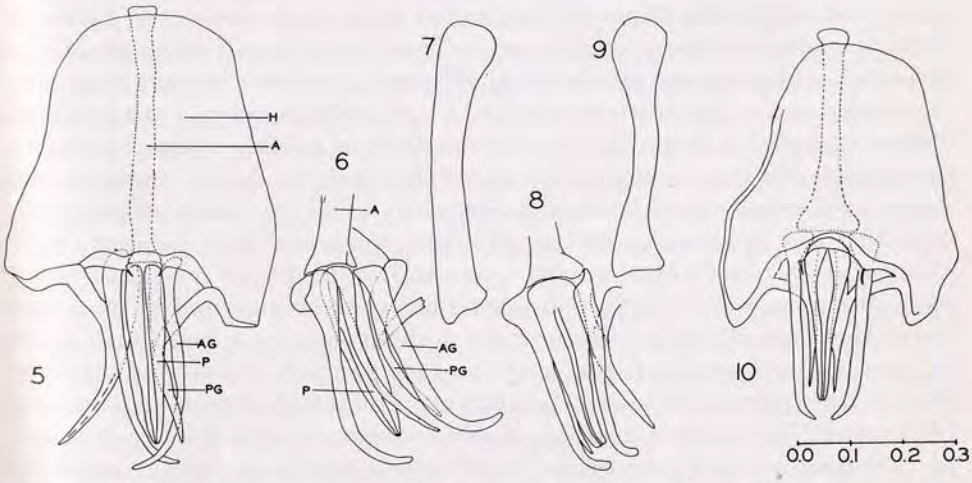
species-characteristic bristles or spines (see particularly Figures 33–38). On either side of the penis lie two pairs of gonapophyses (G), which can be distinguished as anterior (AG) and posterior (PG) gonapophyses (Figures 3, 5, 6, see also Figures 4, 10, 25–29, 33–35). During the copulation, the gonapophyses enter the vagina together with the penis, and their rapid twitchings inside the vagina can be seen clearly in etherized pairs.



FIGS. 1 and 3—*Drosophila pseudoobscura*. FIGS. 2 and 4—*D. persimilis*. FIGS. 1 and 2—genital arches (GA), claspers (CL), anal tubercles (AT), and "toes" of the genital arch (T). FIGS. 3 and 4—side views of the penis—gonapophyses complex; CL—Claspers, PG—penis and gonapophyses, T—"toes". The scale in this and the following figures represents 0.3 mm.



FIGS. 5–7—*Drosophila pseudoobscura*. FIGS. 8–10—*D. persimilis*. A—apodeme: AG—anterior gonapophyses; H—hypandrium; P—penis; PG—posterior gonapophyses.

The penis together with the gonapophyses is articulated to the apodeme (A in Figures 5 and 6, also Figures 7–10, 25–29, 33–38). The apodeme is a heavily sclerotized endoskeletal rod, to which are attached the muscles which move the penis complex and the gonapophyses. The muscles which move the penis complex together with the apodeme are attached to the hypandrium, to which is articulated also the genital arch. The hypandrium (H in Figure 5, see also Figures 10–24, 39–41) is a roughly triangular plate which may correspond to the eighth abdominal sternite (Dobzhansky, 1928). The form of the hypandrium is very important for distinguishing closely related species. In some species the hypandrium has characteristic bristles or teeth (HT in Figure 11, see also Figures 12–24, 39–41).

For the purposes of the present study, it was found desirable to observe the male genitalia in living flies, as well as after maceration in hot 10% solution of KOH in water. Examination in macerated preparations alone can lead to serious errors, since in flies killed by etherization the penis-gonapophyses complex is usually pushed out, and a prolonged maceration sometimes dissolves some delicate parts and makes them invisible. The drawings in Figures 1–41 have all been made with the aid of a camera lucida in macerated preparations and at the same magnification of a microscope. The magnification is indicated for every group of figures by a scale, which represents 0.3 of a mm.

Drosophila pseudoobscura and Drosophila persimilis

Drosophila pseudoobscura Frolova and *Drosophila persimilis* Dobzhansky and Epling are a pair of sibling species which have been studied in perhaps the most detail. They were first distinguished by Lancefield (1929) because they produced sterile male hybrids when crossed; Lancefield regarded them as "two races or physiological species," although he found them to be morphologically indistinguishable. Dobzhansky and his co-workers (a review in Dobzhansky and Epling, 1944) found that the two species have overlapping geographic distributions and show partial ecological and sexual reproductive isolation, sterility of F_1 hybrid males, and hybrid breakdown in the progeny of the backcrosses of F_1 hybrid females to males of either parental species. These facts, as well as the presence of several chromosomal and physiological differences, led these authors to conclude that the process of speciation has reached a complete separation of the gene pools of the two species, and accordingly they proposed the suitable nomenclatorial recognition of this fact. Subsequent studies confirmed the specific status of these forms, by showing that no gene exchange occurs where the species are sympatric.

Mather and Dobzhansky (1939) and Reed, Williams, and Chadwick (1942) found that *D. pseudoobscura* and *D. persimilis* differ in the mean numbers of teeth in the sex combs, in certain dimensions of the wings, and in some other traits. However, all these differences are so slight that single individuals cannot be classified as to species. Rizki (1951) was the first to find a morphological difference which permitted such a classification. This difference concerns the shape of the penis. This organ is relatively longer and more parallel-sided in *D. psuedoobscura* than it is in *D. persimilis*. Cooper and Lewontin (personal communications) noted in 1954 a difference in the shape of the hypandrium and a possible difference in the number of teeth on the claspers in the two species.

The work of the present writer has confirmed the existence of a difference in the shape of the penis, which is relatively longer and gradually tapering towards the distal end in *D. pseudoobscura* (Figure 5) and is shorter but inflated at base in *D. persimilis* (Figure 10). The hypandrium of *D. pseudoobscura* is somewhat larger and its sides converge frontwards less rapidly than in *D. persimilis* (Figures 5 and 10). The apodeme of *D. pseudoobscura* (Figure 7) is longer and more nearly cylindrical, while in *D. persimilis* it is shorter, inflated at both ends and thinner in the middle.

However, for practical identification of living specimens the above characteristics are not suitable, since they are clearly visible only in macerated and mounted preparations. A more convenient and more easily visible characteristic is the shape of the penis-gonapophyses complex, especially as seen in a lateral aspect (Figures 3, 4, 6, 8). A male fly to be classified as to species should be etherized and placed on his side on a plate under a binocular dissecting microscope; a needle is then placed lightly across his abdomen, and with the aid of a gentle pressure the penis complex is extruded. Care should be taken not to make the gonapophyses flare apart from the penis. In *D. pseudoobscura* (Figures 3, 6) the gonapophyses are bent sabre-like, while in *D. persimilis* they are straight except at the free ends which are bent ski-like (Figures 4, 8). With some practice, this method permits mistake-free identification; some hundreds of cultures of unknown species were classified by the present writer, and the classification was subsequently verified by Prof. Th. Dobzhansky by means of examination of the salivary gland chromosomes.

The willistoni group of five sibling species of Drosophila

Drosophila willistoni Sturtevant is a new name for the species described in 1896 by Williston from the island of St. Vincent in the West Indies under the preoccupied name of *Drosophila pallida* Williston. In 1943, Dobzhansky and Pavan noticed the existence of two externally very similar but non-interbreeding species resembling *D. willistoni* in southern Brazil. One of these is on the average larger in body size than the other. A wrong guess led to the larger species being described as a new species, *Drosophila paulista* Dobzhansky and Pavan, but when it was found that this is a synonym of *D. willistoni*, the smaller species was given the name of *D. paulistorum* Dobzhansky and Pavan. A third species, morphologically very similar to the preceding two but reproductively completely isolated from them, was described from the Upper Amazon as *Drosophila equinoxialis* Dobzhansky (1946). Burla *et al.* (1949) added a fourth species, *Drosophila tropicalis* Burla and da Cunha from Central Brazil, and reviewed the morphology, cytology, and genetics of the four sibling species. Several minute morphological differences have been recorded between the sibling species, involving the structure of the maxillary palpi, position of the orbital bristles, coloration of the ocelli, the shape of the vaginal plates, of the hypandrium, and of the chitinous spermatheca of the females. Nevertheless, the authors concluded that: "The variability is great enough to make identification of species in single individuals hazardous." This contrasts, however, with complete reproductive isolation of these species and the relative facility of their recognition by the structure of their chromosomes in the salivary glands cells. Malogolowkin (1952) made a more

detailed study of the genitalia of males of the *willistoni* group, and discovered several additional characteristics which differentiate the species in cleared and macerated preparations.

Further work brought to light the existence of morphologically cryptic but genetically explicit differentiation within the sibling species. Townsend (1954) described *Drosophila tropicalis* subsp. *cubana* Townsend from Cuba, which can be crossed to *D. tropicalis tropicalis* from Brazil, but which produces sterile male and fertile female hybrids. He also noticed some minute morphological differences between the two subspecies, which however do not permit recognition of single individuals. Cordeiro (1952) found a difference in the shape of the hypandrium between certain strains of *D. paulistorum* from northern and southern Brazil. These strains cross freely, and analysis of the F_2 generation hybrids shows that the differences in the hypandrium shape observed between these strains of *D. paulistorum* have a rather simple genetic basis. The present writer (unpublished) found in 1955 that strains of *D. paulistorum* collected by Dr. W. B. Heed in Honduras differ from the Brazilian strains of this species. The crosses Honduras ♀ × Brazil ♂ and the reciprocal produce F_1 hybrids which are sterile as males and fertile as females when backcrossed to the parental forms. Finally a fifth sibling species, *Drosophila insularis* Dobzhansky (this bulletin) has been discovered among the *willistoni*-like flies collected by Dr. W. B. Heed on the islands of St. Kitts and St. Lucia in the Lesser Antilles. This species is reproductively completely isolated from the other four siblings, although it did produce a few sterile male and female hybrids with *D. willistoni* and *D. tropicalis*.

Dr. O. Frota Pessoa, of the University of Rio de Janeiro, Brazil, and the present writer have, independently, observed that the five sibling species of *willistoni* group can be recognized by inspection of the external genitalia in single living

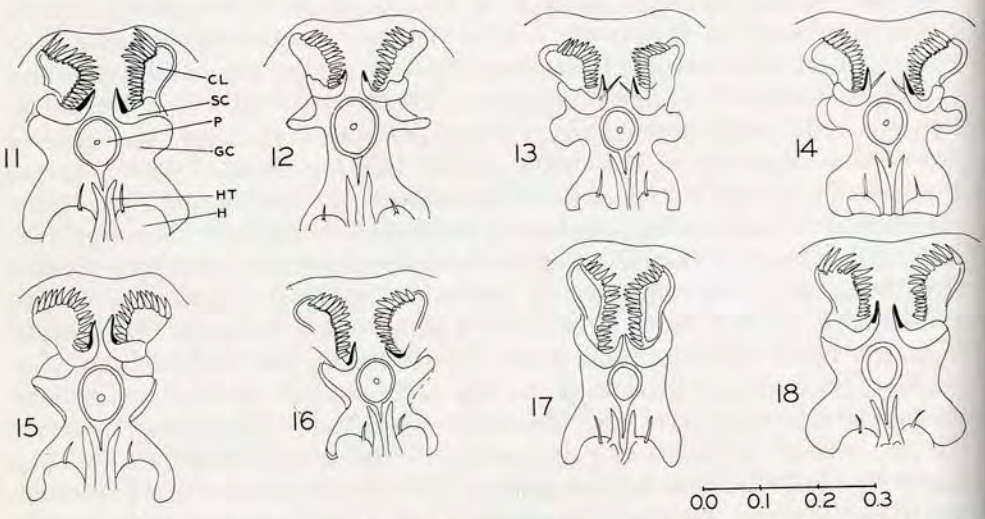
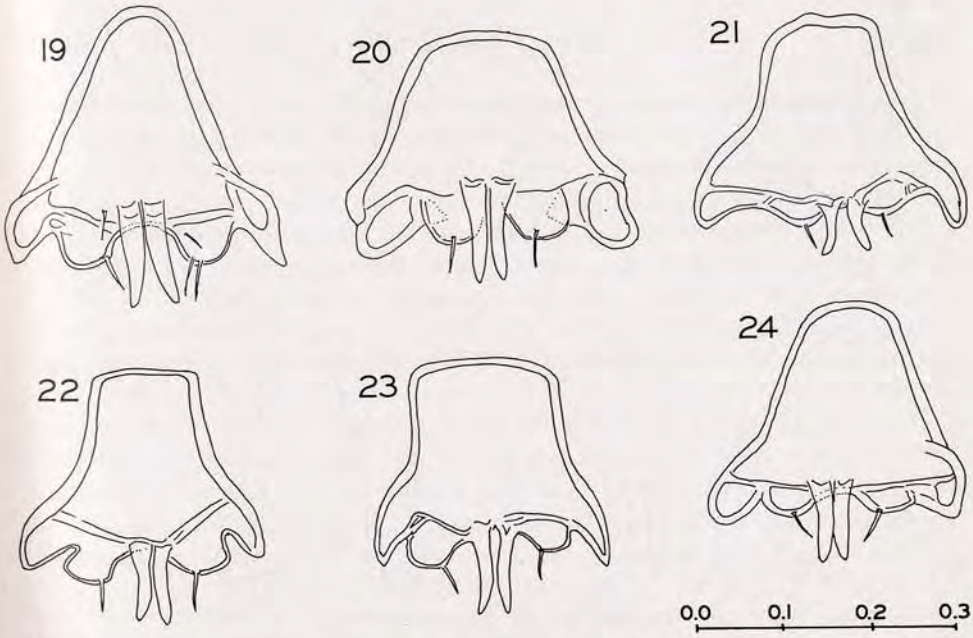


Fig. 11—*Drosophila willistoni;* Fig. 12—*D. equinoxialis;* Fig. 13—*D. paulistorum* from Brazil; Fig. 14—*D. paulistorum* from Honduras; Fig. 15—*D. tropicalis* from Goyaz, Brazil; Fig. 16—*D. tropicalis* from Cuba; Fig. 17—*D. insularis;* Fig. 18—*D. insularis* ♀ × *D. willistoni* ♂ hybrid. CL—posterior clasper; GC—genital chamber; H—hypandrium; HT—hypandrium tooth; SC—anterior claspers.

males, under a binocular dissecting microscope with a magnification 60–80 times. The most convenient trait is the outline and the margin of the genital chamber (GC), together with the shape of the genital claspers (CL), and the size of the hypandrium teeth (HT) relative to that of the hypandrium lobes (H, Figures 11–18). The genital chamber is widest in *D. willistoni* (Figure 11) and its lateral margins flanking the penis (P) are heart-shaped. In *D. equinoxialis* (Figure 12) the margins are more sharply sinuate, forming two rather narrow slits, while in *D. paulistorum* (Figures 13 and 14) they are rounded, and *D. tropicalis* (Figures 15 and 16) wedge-shaped. *D. insularis* has the narrowest genital chamber (Figure 17) and its sides are more nearly rectilinear. The hybrids of *D. insularis* with *D. willistoni* (Figure 18) have a genital chamber intermediate between those of the parental species.

Malogolowkin (1952) studied the number of the teeth on the genital claspers and found them different on the average in the different species. The observations of the present writer confirm those of Dr. Malogolowkin, but for the purpose of identification of the species the outlines of the claspers as a whole seem to be more useful, at least in living material. In *D. equinoxialis* (Figure 12) the claspers with their teeth form two curved lines, while in the other species they are more or less sharply angular (Figures 11, 13–18). The characteristic shapes of these organs can best be seen in the figures rather than described in words; the only pair of species which are hard to distinguish on the basis of this trait alone are *D. paulistorum* and *D. tropicalis* (Figures 13–16).

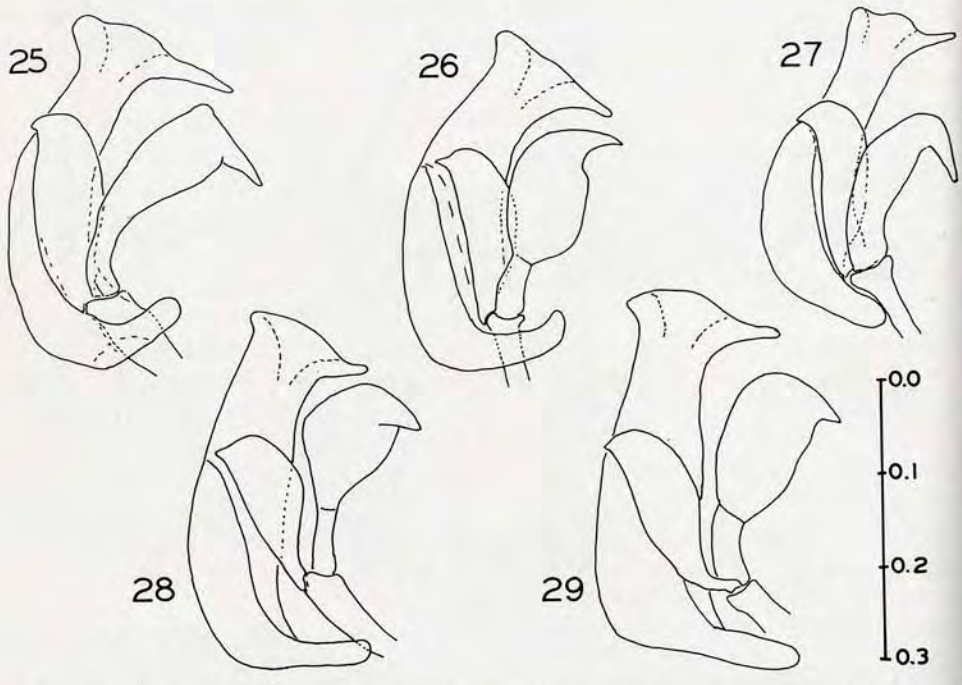
The hypandria of the sibling species are shown in Figures 19–24, drawn from



FIGS. 19–24—Hypandria of: FIG. 19—*D. tropicalis;* FIG. 20—*D. willistoni;* FIG. 21—*D. insularis;* FIG. 22—*D. paulistorum* from Brazil; FIG. 23—*D. paulistorum* from Honduras; FIG. 24—*D. equinoxialis.*

macerated preparations. However, the lobes and the teeth of the hypandrium are well visible also in living males. The hypandrium is relatively broadest in *D. willistoni* (Figure 20); its teeth are long, and the lobes are about ⅔ as long as the teeth. *D. tropicalis* (Figure 19) has, on the contrary, a long triangular hypandrium. The teeth are about as long as in *D. willistoni*, and the lobes about ½ as long as the teeth. *D. insularis* has by far the shortest teeth and hypandrial lobes (Figure 21). *D. paulistorum* (Figures 22 and 23) has the anterior (proximal) margin of the hypandrium more nearly straight than in the other species, making the hypandrial plate trapezoidal rather than triangular. The teeth are moderately long, and the lobes about ⅓ as long as the teeth. Finally, *D. equinoxialis* has a subtriangular hypandrium with moderately short teeth and lobes.

The shapes of the penis and of its gonapophyses also differ in the sibling species, but these differences are visible chiefly in macerated and cleared preparations, and therefore are relatively less convenient for purposes of identification (figures 25–29). The gonapophyses, especially the posterior ones, are so extremely thin and delicate in the *willistoni* group of species that they become virtually invisible in strongly macerated preparations. As shown in the figures, the most noticeable specific differences reside in the terminal (distal) portion of the penis. In *D. tropicalis* this portion is extended to form a long finger-like protusion (Figure 25), which is shorter in *D. willistoni* (Figure 26), and shortest in *D. insularis* (Figure 27). In the three species just named the penis is rather strongly curved, while in *D. paulistorum* and *D. equinoxialis* (Figures 28 and 29) it is relatively straight.



FIGS. 25–29—Penis and gonapophyses of: FIG. 25—*Drosophila tropicalis;* FIG. 26—*D. willistoni;* FIG. 27—*D. insularis;* FIG. 28—*D. paulistorum;* FIG. 29—*D. equinoxialis.*

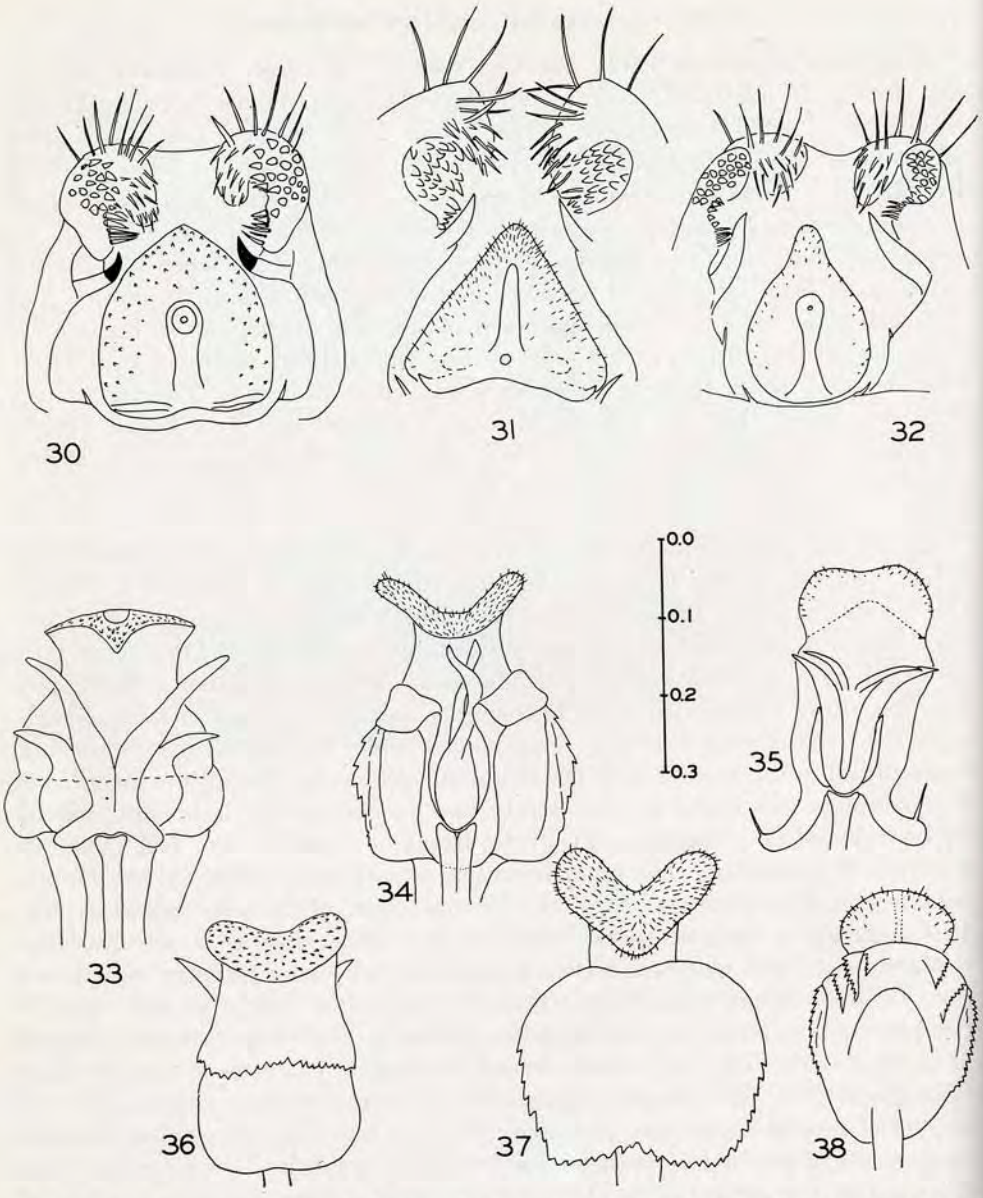
Drosophila prosaltans and Its Close Relatives

Drosophila prosaltans Duda was described in 1925 from Paraguay, and redescribed as *D. sellata* Sturtevant from Guatemala and Mexico. Dobzhansky and Streisinger (1944) intercrossed three strains from Mexico, one strain from Guatemala, one strain from equatorial Brazil, and two strains from southern Brazil. When a mixture of females of northern and southern origins are exposed to males from any locality, a certain proportion of females of both kinds are inseminated. But, curiously enough, greater proportions of northern females are inseminated both by males of northern and of southern origins. It appears that northern males are either more aggressive in pursuing the females, or the females regardless of their origin accept more readily the northern males, or both. However this may be, F_1 hybrids are easily produced in all crosses. This writer has, however, found that the crosses Mexico (Chilpancingo) ♀ × Brazil (Pirassununga) ♂ produce hybrid sons which are completely sterile. The F_1 hybrid females are fertile in backcrosses with both parental races. The reciprocal cross, Brazil ♀ × Mexico ♂ gives fertile hybrids of both sexes.

Among the *prosaltans*-like flies collected at Pirassununga, state of Sao Paulo, Brazil, and sent to this laboratory through the courtesy of Professor C. Pavan, this writer discovered the existence of two distinct species. Although they appear very similar in most traits used in the conventional systematics of Drosophila, they proved to be completely isolated reproductively. Females of either of these were kept in culture bottles with males of the other species for a week or more, whereupon the females were dissected. Examination under the microscope disclosed no sperm at all in the ventral or in the chitinous receptacles. One of the forms from Pirassununga was found to cross easily and to produce fertile female progeny with strains of *D. prosaltans* either from Brazil or from Mexico. This form evidently is *D. prosaltans*. The other form has refused to cross not only to *D. prosaltans* from Pirassununga but to the Mexican strains of the same species as well. It is evidently a separate species which is described below as *D. austrosaltans*.

Male genitalia of species of *saltans* group seem to be more complex in structure than those in *obscura* and *willistoni* groups. The penis is very large relative to the two pairs of gonapophyses, and the latter appear as mere outgrowths on the penis (Figures 33–35). The penis itself carries denticulate processes of various kinds (Figures 34–38). The claspers (Figures 30–32) carry relatively large numbers of bristles of several kinds—peg-like, tooth-like, and hair-like, the numbers and arrangements of which are characteristic for different species. The large horn-like processes on the genital arch (the anterior claspers) have the same function in copulation in *prosaltans* as they have in the *willistoni* group. The hypandria (Figures 39–41) are very large and of characteristic shapes.

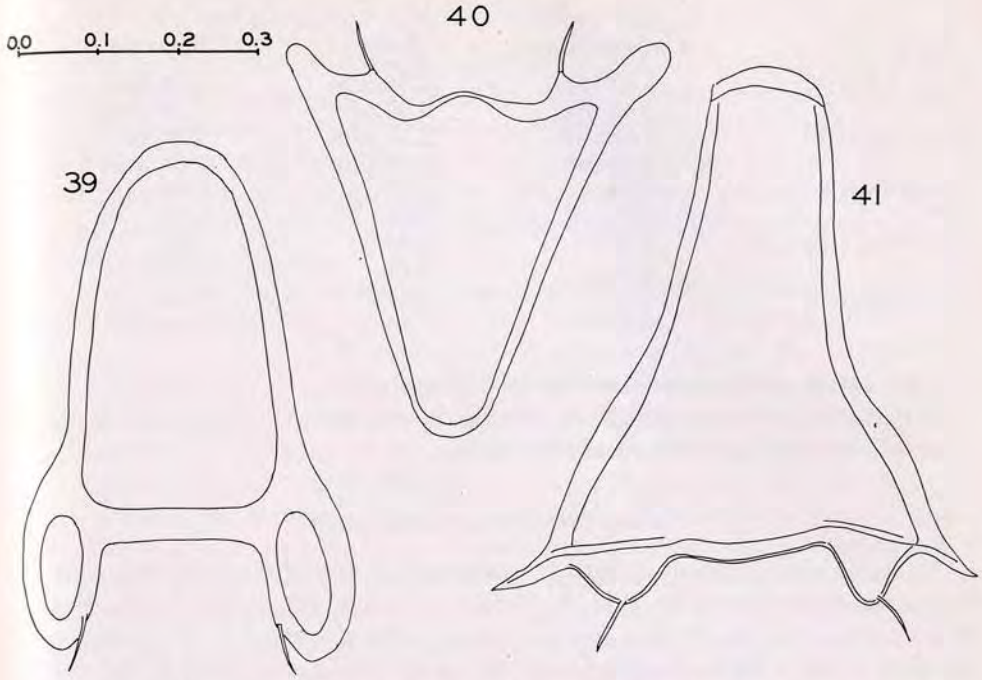
In six of the strains of *D. prosaltans* kept in this laboratory the penis and the adjacent parts of the genital arch have, observed from the ventral side, the appearance shown in Figure 30. All these strains are derived from flies collected in various localities in Brazil (Bertioga in the state of Sao Paulo; Monjolinho, Goyaz; Barreiras, Bahia; Fordlandia and Marajo Island, Para; and Içana, Amazonas). In the two northern strains (Guatemala and Chilpancingo, Mexico) these parts appear as shown in Figure 31, the head of the penis being triangular rather than persimmon-shaped. Finally, in *D. austrosaltans* (Figure 32) the head



FIGS. 30–32—genital arch and the tips of the penis of—FIG. 30—*Drosophila prosaltans* from Brazil; FIG. 31—*D. prosaltans* from Mexico; FIG. 32—*D. austrosaltans*. FIGS. 33–38—distal parts of the penis of—FIGS. 33 and 36—*D. prosaltans* from Brazil; FIGS. 34 and 37—*D. prosaltans* from Mexico, and FIGS. 35 and 38—*D. austrosaltans*.

of the penis is pear-shaped. There exists also a characteristic difference in the color of the gonapophyses in living young males—they are amber yellow in Brazilian *D. prosaltans*, dark brown in *D. prosaltans* from Mexico and Guatemala, and black in *D. austrosaltans*.

In macerated and cleared preparations one can observe also extensive differences in the shape and ornamentation of the penis and of the gonapophyses



FIGS. 39–41—Hypandria of, FIG. 39—*Drosophila austrosaltans*. FIG. 40—*D. prosaltans* from Brazil, and FIG. 41—*D. prosaltans* from Mexico.

(Figures 33–38). In Brazilian *D. prosaltans* the tip of the penis carries minute teeth-like bristles, in those from Mexico and Guatemala it has numerous minute hair-like bristles, while in *D. austrosaltans* it is sparsely covered with hairs. In *D. austrosaltans* the body of the penis is covered with denticulate ridges resembling the edge of a saw (Figure 38). The hypandria of *D. prosaltans* are triangular, with two pairs of processes, one of which carries a bristle (Figures 40 and 41). In *D. austrosaltans* the hypandrium is rounded off, and the processes are fused to form ear-like lobes which carry a bristle on their inner margins (Figure 39). A description of *D. austrosaltans* follows.

Drosophila austrosaltans, Species Nova

Female and Male.—Arista with 9–10 branches, 9 being the mode. Antennae, head, carina, cheeks, eyes, and palpi like in *Drosophila prosaltans* Duda (see a re-description of the latter species in Dobzhansky and Pavan, 1943 (pp. 17–19), except that the middle orbital bristle is a little longer relative to the anterior and posterior orbital, being about half as long as the latter.

Thorax with its bristles, legs, and wings like in *D. prosaltans*, except that the pleurae on the average a little darker than in the latter species. Abdomen and the opaque areas on the posterior part of the sixth abdominal tergite in female like in *D. prosaltans*, the opaque areas a little broader.

Measurements of 10 ♀♀ and 10 ♂♂ each of *D. austrosaltans* and of the Belem and Barreiras strains of *D. prosaltans* gave the following results:

	D. austrosaltans Pirassununga	*D. prosaltans* Belem	Barreiras
Body Length, ♀	3.12 mm.	2.98 mm.	2.96 mm.
" " ♂	2.43 mm.	2.34 mm.	2.58 mm.
Wing Length, ♀	2.38 mm.	2.15 mm.	3.26 mm.
" " ♂	2.02 mm.	1.83 mm.	2.01 mm.
Costal Index, ♀	2.31	2.09	2.14
" " ♂	2.12	2.06	1.99
4th Vein Index, ♀	2.06	2.07	2.05
" " " ♂	2.16	2.09	2.24
5 × Index, ♀	1.82	1.57	1.74
" " ♂	2.08	2.11	2.14

Eggs, pupae, and chromosomes like in *D. prosaltans*.

Distribution.—Pirassununga, Sao Paulo, Brazil, Drs. C. Pavan and A. B. da Cunha Collectors, April 1951 and May 1954.

Tests of Reproductive Isolation

As stated above, strains of both *D. austrosaltans* and of *D. prosaltans* were simultaneously collected by Prof. C. Pavan at Pirassununga, Sao Paulo, Brazil. The existence of two distinct species was not even suspected on examination of the flies under a binocular dissecting microscope. Only after some of the strains refused to cross to laboratory strains of *D. prosaltans* containing certain mutant genes (derived mostly from ancestors collected at Belem, Brazil) it was finally realized that *D. austrosaltans* is a reproductively isolated species, and this was further borne out by the examination of the genitalia. The Pirassununga strain of *D. austrosaltans* was crossed, both as female and as male, to the following strains of *D. prosaltans*, no larvae appearing in any of the crosses after repeated transfer of the potential parents to fresh food:

Barreiras, Bahia, Brazil	Marajo, Para, Brazil
Palina, Goyas, Brazil	Içana, Amazonas, Brazil
Fordlandia, Para, Brazil	Guatemala City, Guatemala
Belem, Para, Brazil	Chilpancingo, Guerrero, Mexico

Pirassununga, Sao Paulo, Brazil

The intercrosses of the southern and northern races of *D. prosaltans* yield invariably ample progenies of F_1 hybrids. The hybrid females are fertile, but the hybrid males are sometimes sterile, as can be seen from the following tabulation of the crosses made and of their outcomes:

Içana ♀ × (Guatemala ♀ × Içana ♂) F_1 ♂ = Sterile
Guatemala ♀ × (Pirassununga ♀ × Guatemala ♂) F_1 ♂ = Few Larvae
(Guatemala ♀ × Içana ♂) F_1 ♀♀ × ♂♂ = Sterile
(Pirassununga ♀ × Guatemala ♂) F_1 ♀♀ × ♂♂ = Some Larvae
(Içana ♀ × Pirassununga ♂) F_1 ♀ × (Guatemala ♀ × Içana ♂) F_1 ♂ = Sterile
(Pirassununga ♀ × Guatemala ♂) F_1 ♀ × (Içana ♀ × Pirassununga ♂) F_1 ♂ = Fertile
(Içana ♀ × Pirassununga ♂) F_1 ♀ × (Pirassununga ♀ × Guatemala ♂) F_1 ♂ = Some Larvae

DISCUSSION

A detailed examination of the external male genitalia in two pairs and in a group of five sibling species has disclosed small, perhaps recondite, but quite constant and diagnostically valid species differences. The usability of these differences for determination of the species of individual males has been tested repeatedly in living specimens. Genetic and cytological examination disclosed very few errors while the writer was learning the distinctions, and none after he acquired a certain practice.

This need not be interpreted to mean that the species concerned should no longer be regarded sibling species. It is clear from Mayr's definition (1942) that sibling species are not a special and discrete category of species. The morphological distinguishability or indistinguishability are evidently relative matters. It remains as true now as it was before the present study, that it is much harder to discriminate between males of *Drosophila pseudoobscura* and *D. persimilis* by simple inspection of their morphology than it is to discriminate between, say, *D. pseudoobscura* and *D. affinis*, which are still fairly closely related species. Moreover, the females of the sibling species remain, as far as known, morphologically indistinguishable. Perhaps a very detailed comparison of their structures could yield discriminating characters. However that may be, it is a fact that some pairs of species are much easier to distinguish by inspection than other pairs. To say the very least, it takes considerably more time to separate males of *D. persimilis* and *D. pseudoobscura* from a mixed lot than it takes to distinguish them from *D. affinis* males, and the females of the former two species can be distinguished only by breeding experiments. As far as old fashioned dried museum specimens are concerned, the males can now be classified, although in most cases this would necessitate preparation of cleared mounts of the genitalia.

The five sibling species of the *willistoni* group are now easily recognizable in living male material, and with some labor also in dried specimens, although the writer has insufficient experience with the latter. The situation in the *saltans* group is very instructive and deserves some comment. *D. austrosaltans* and *D. prosaltans* are sympatric in at least a part of their distribution, and they are completely isolated reproductively. The morphological difference observed between the male genitalia of these species is, however, not strikingly greater than that between the genitalia of *D. prosaltans* from Brazil and from Central America. Known morphological differences in traits other than genitalia are scarcely significant in either case. The question may, then, be asked whether it is justified to distinguish *D. prosaltans* and *D. austrosaltans* as full species and to regard the varieties of the former as races of the same species. The study of the reproductive isolation supplies the answer. The Central and the South American *D. prosaltans* are allopatric, and no secure reproductive isolation between them has been built by natural selection. Hybrids are produced easily, true sexual isolation is completely absent (Dobzhansky and Streisinger, 1944), female hybrids are quite fertile, and only male hybrids are sterile in some crosses. The evidence is, then, reasonably clear that unobstructed gene exchange is possible even between populations as remote in space as those of Mexico and of southern Brazil, although this gene exchange would have to occur via a long chain of con-

necting populations extending clear across the intertropical zone of the Western Hemisphere.

SUMMARY

A study of the external genitalia of the males permits distinguishing the species of even single living specimens, and perhaps also of dried specimens, of the following members of groups of sibling species of Drosophila:

D. pseudoobscura — D. persimilis
D. prosaltans — D. austrosaltans
D. willistoni—D. paulistorum—D. equinoxialis—D. tropicalis—D. insularis

ACKNOWLEDGMENTS

The writer is deeply indebted to Professor Th. Dobzhansky for many constructive suggestions and criticisms, and for assistance in the preparation of the manuscript. It is a pleasure to express our appreciation of the kindness of Drs. W. Heed, W. S. Stone, and M. R. Wheeler of The University of Texas who have sent us a rich material of *willistoni*-like species collected by them in the West Indies and in Central America, among which the new species *Drosophila insularis* was encountered; to Dr. C. Pavan and A. B. da Cunha, of the University of Sao Paulo, Brazil, for sending us a collection of *prosaltans*-like flies among which *Drosophila austrosaltans* was encountered; and to Drs. Chana Malagolowkin and O. Frota Pessoa of the National University of Brazil for useful advice concerning the nomenclature of some parts of the fly's morphology and the technique for their investigation.

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VI. Ecological and Distributional Notes on the Drosophilidae (Diptera) of El Salvador

WILLIAM B. HEED[1]

INTRODUCTION

The study of the evolution in any group of animals or plants implies a knowledge of the number and distribution of the species involved and the population structure and habits of the species in relation to their environment. The family of Acalyptrate Diptera, Drosophilidae, is world wide in distribution. The largest genus in the family, *Drosophila*, is also cosmopolitan. Our knowledge of this group in the Neotropical region is incomplete.

The author collected material in El Salvador from October, 1953, until August, 1954, while he was a guest at the Instituto Tropical de Investigaciones Cientificas, San Salvador. During this time he also collected in Honduras (March 19 to April 5) and Nicaragua (June 13 to July 3). This work is not reported on here. Dobzhansky and Pavlovsky (1955) report on a heterosis study of *D. tropicalis* from Honduras.

A total of 18 genera and approximately 222 species were collected in El Salvador. The genera are listed in Table 1. Also the number of species in each genus

TABLE 1

Genera and number of species of the family Drosophilidae from El Salvador

	Described	Undescribed	Total
1. Sinophthalmus	1	1	2
2. Amiota	1		1
3. Gitona	1		1
4. Stegana	7	14	21
5. Rhinoleucophenga	1	1	2
6. Leucophenga	8	5	13
7. Neotanygastrella	1		1
8. Chymomyza	1	1	2
9. Scaptomyza	3	3	6
10. Paraliodrosophila	2		2
11. Dettopsomyia	1		1
12. Mycodrosophila	1		1
13. Paramycodrosophila	2	2	4
14. Zygothrica	6	11	17
15. Microdrosophila	1	1	2
16. Diathoneura	7	18	25
17. Cladochaeta	1		1
18. Drosophila	53	67	120
Total	98	124	222

is listed according to whether they are described or as yet undescribed forms. It can be seen that approximately 124 (56%) of the species are new. It also may be seen that the genus *Drosophila* makes up about 54% (120 species) of the family Drosophilidae in El Salvador. Table 2 shows the classification to subgenera and species groups of the genus *Drosophila*. This information is again listed according to described and undescribed species.

[1] Zoological Laboratory, Univ. Pennsylvania, Philadelphia.

TABLE 2
Classification of the species of Drosophila from El Salvador

	Described	Undescribed	Total
Subgenus			
Hirtodrosophila	6	8	14
Pholadoris	1		1
Phloridosa	1		1
Siphlodora	1		1
Sophophora			
Species Group			
saltans	3		3
willistoni	7		7
melanogaster	3		3
obscura	2	1	3
alagitans	1	1	2
bromeliae	1		1
Drosophila			
Species Group			
tripunctata	6	23	29
repleta	6	7	13
cardini	2	1	3
immigrans	1		1
macroptera	2		2
rubrifrons	1	1	2
annulimana	1	2	3
guarani	1		1
pallidipennis	1		1
dreyfusi	1		1
calloptera	1		1
canalinea	1	3	4
Unclassified	3	20	23
Total	53	67	120

The large number of new forms shows that our knowledge of Neotropical drosophilids is incomplete and that this group is rich in genetic variability. Patterson and Stone (1952) list 613 species in the genus *Drosophila* from the six zoogeographic realms of the world. Of these, 200 are from the Neotropic region. An addition to this of 67 species from El Salvador alone indicates that much is yet to be learned about the group. In Appendix A are listed the 98 species already described in the family Drosophilidae from El Salvador. Also listed is the author and country from which the species was first described.

In the following text and tables are mentioned types of flies which have not yet been described. The names given to them are convenient manuscript names only. There is absolutely no attempt of a taxonomic description of any kind. Such designations as "tripunctata K," "medium brown," "medionotata-L." and "unknown yellow" are not to be construed as valid species names.

In this respect there are several new names used in the text and tables that are described as new species by the author elsewhere in this bulletin. They are *D. trapeza, D. mediodelta, D. converga, D. facialba* and *D. aracea.*

DISTRIBUTION

Drosophila species are not found evenly distributed throughout El Salvador due in part to the uneven terrain of the country. Instead there are areas of concentration. The areas have at least one element in common and that is shade from the direct rays of the sun. As a result they are also cooler than the surround-

ing territory. Five general habitats were collected in El Salvador which fulfill these conditions. Table 3 lists the habitats, the number of collecting stations for each and their location. The gallery forest, upland forest and cloud forest are described for El Salvador by Lauer (1954). The barranco (ravine) habitat is described by Lotschert (1953). El Salvador has several upland fresh water swamps. One distinguishing characteristic of the swamps is the scattered stands of a big leafed aroid plant, *Xanthosoma* (Araceae). The overlapping leaves give continuous shade throughout the day and support a varied population of drosophilids beneath them. The aroid swamps may be considered a Drosophila habitat. The remaining general habitat includes coffee fincas interlaced with patches of secondary growth generally near domestic habitation. The fincas make excellent collecting areas because of the fermenting fruits available during much of the

TABLE 3

Location of Collecting Stations

Habitat	Altitude (feet)	No. of Stations and Location	No. Different Months Collected
Gallery Forests	0–300	1. Puente Cuscatlan 2. San Marcos Lempa 3. Puerto Triunfo	4
Barrancos	2000	1. Instituto Tropical 2. 17 klm. W. San Salvador	8
Aroid Swamps	2000	1. 12 klm. NW. Santa Tecla 2. Laguna de Zapotitan	6
Coffee Fincas, Upland Forests and Domestic Habitats	2000	1. Instituto Tropical 2. Lago de Coatepeque	7
Coffee Fincas, Upland Forests and Domestic Habitats	3000	1. La Palma 2. Finca San Nicolas	2
Coffee Fincas, Upland Forests and Domestic Habitats	5000	1. Volcan Boqueron 2. Volcan Santa Ana	5
Cloud Forest	7000	1. Cerro de Monte Cristo	1

year upon which many of the species in the genus *Drosophila* feed. In October and November the adults and larvae utilize the rotting coffee berries and in May, June, July and August they utilize the fallen fruits from the shade trees such as mango, zapote, pepete, aguacate and the like.

Table 4 lists the number of species in each genus in the family Drosophilidae collected in the five general habitats. Some habitat preferences may be seen. For instance members of the genus *Stegana* are definitely partial to barrancos and more *Scaptomyza* species were collected in the cloud forest clearings than anywhere else. In general, though, there is much overlapping; that is, many of the same species may be found in a barranco as well as an aroid swamp or a coffee finca at one time of year or another so long as these habitats are at approximately the same altitude above sea level. Only a study on a microhabitat basis would indicate species preferences. Table 4 shows that there are fewer species in the cloud forest and gallery forest. This is so because these habitats constitute more stable environments, uninterrupted to a large extent by domestic activities.

Table 5 shows the vertical distribution of drosophilids in El Salvador. The altitudes at 2000 feet and 5000 feet are taken as reference points since most of the species were collected here, and also since there is no difference (except for one

TABLE 4

Number of species in each genus collected in five general habitats

Altitude (feet)	Gallery Forests 0–300	Barrancos 2000	Aroid Swamps 2000	Coffee Fincas, Upland Forests and Domestic Habitats 2000	3000	5000	Cloud Forest 7000
1. Sinophthalmus						2	
2. Amiota		1		1		1	
3. Gitona	1			1			
4. Stegana	1	18	2	9	3	5	
5. Rhinoleucophenga	1	1	1	1			
6. Leucophenga	6	9	3	7	4	6	
7. Neotanygastrella	1	1	1	1	1		
8. Chymomyza		1		1		1	
9. Scaptomyza		1	1	1		2	5
10. Paraliodrosophila					1	2	1
11. Dettopsomyia			1				
12. Mycodrosophila	1	1	1	1	1	1	
13. Paramycodrosophila	2	3		2		2	
14. Zygothrica		4		3	9	13	2
15. Microdrosophila	1	1	1	1	1	1	
16. Diathoneura	5	14	6	8	8	11	9
17. Cladochaeta		1	1	1	1	1	
18. Drosophila	25	44	36	58	48	56	10
Total	44	100	54	96	77	104	27

TABLE 5

Vertical distribution of the Drosophilidae of El Salvador

Elevation (feet)	0–300	2000	3000	5000	7000	Total
Drosophila						
No. of species	25	76	48	56	10	
No. common to next higher altitude	25	24	7	8		
No. peculiar to that altitude	0	58	14	36	2	110
No. common to (or too few at) 2000 and 5000 feet						10
						120
Other Genera						
No. of species	19	66	29	48	17	
No. common to next higher altitude	18	11	8	10		
No. peculiar to that altitude	1	49	2	29	7	88
No. common to (or too few at) 2000 and 5000 feet						14
						102

species) in the fauna from sea level to 2000 feet and little difference (9 species) from 5000 to 7000 feet. A total of 108 species was collected only in the lowlands (sea level to 2000 feet) and 74 species were collected only in the highlands (5000 to 7000 feet). This represents 81.9% of the 222 forms from El Salvador that are restricted to either one or the other general altitude.

The La Palma collecting station at 3000 feet is considered an intermediate altitude having affinities with the highlands and lowlands. From Table 5 it can be

seen that 16 species (14 *Drosophila* and two of other genera) have been taken only at La Palma. Most of these species would probably be taken also either in the highlands or lowlands with further collecting. La Palma has more species in common with the lowlands (35) than with the highlands (15) probably because it has more physical continuity with the lowlands, especially through the river bottoms. Of the total of 77 species from La Palma, only 12 were taken at altitudes both above and below 3000 feet. This fact alone illustrates a rather sharp division between highland and lowland faunas.

Table 5 shows that 10 species of *Drosophila* and 14 species belonging to other genera were collected at both the 2000 and 5000 foot level. Of the 10 forms of *Drosophila*, seven were taken in sufficient numbers at both altitudes to be considered members of both the highland and lowland faunas. Of the 14 species belonging to genera other than *Drosophila*, seven are considered members of both highland and lowland faunas. In effect then the 14 species of drosophilids common to both altitudes represents only 6.4% of the total number of species from El Salvador.

SEASONAL VARIATION

Noticeable seasonal changes in population size were found in three of the habitats studied. They are the barrancos, aroid swamps and coffee fincas, all at an altitude of 2000 feet. Table 6 shows the seasonal distribution of the number of species and number of individuals collected in each habitat. Also the rainfall in inches for each month is listed. It will be noticed that the peak collecting periods are not synchronous from one habitat to another.

The best collecting season in the barrancos and aroid swamps is in the dry season. The coffee fincas are very poor in drosophilid numbers at this time. The fincas have two population peaks. One is in November during the coffee season and the other is in May when many of the shade trees are in fruit. The aroid swamps are poorest in the middle of the wet season probably because of the higher water level at this time. Many species concentrate in the barrancos during the dry season in part because this is the coolest habitat at this time of year. Also the barrancos are probably used as flyways. Also many species seem well adapted to the bare lower walls of the barranco, especially forms of the genus *Stegana*. It can be seen from Table 6 that there are definite cycles of abundance in each habi-

TABLE 6

Seasonal variation in drosophilid numbers in three general habitats at 2000 feet

	Inches Rain	Barranco		Aroid Swamps		Coffee Fincas	
		Species	Individ.	Species	Individ.	Species	Individ.
November	..	..	..	..	..	33‡	1430§
December	0.2	63‡	603	30	421§	11	131
January	0.04	59†	393	..	..	1	2
February	0.6			22	206§	..	..
March	0.0	38†	822§	..	..	..	..
April	20.4	4	7	6	251§	..	..
May	7.4	4	5	18	266	20*	1470
August	11.8	30	112	12	24§	29*	514

* 2 collections, † 4 collections, ‡ 6 collections, § baited.

tat, but they are at different times of the year for each one. Rainfall is the only major variable in El Salvador from a macroclimatic point of view. This divides the year into a wet season (mid-May to mid-October) and a dry season. Nearly all of the annual 71 inches is accumulated in the wet season. Temperature and humidity are fairly constant throughout the year.

LARVAL FEEDING SITES

Throughout the year at El Salvador fermenting fruits and other succulent material were collected to obtain data on larval feeding sites. The method was to place one or several fruits of the same type in a jar enclosed with a cotton stopper and to collect the adults daily as they emerged. A total of 3,142 individuals was reared from fruits, or other succulent material, of 13 families of flowering plants and one group of fungi (Basidiomycetes). In Appendix B are listed the species of plants according to family and the habitat in which they were collected. In all cases except four, the fruits of the plants were utilized. The whole plant was utilized by larvae in the case of the fungi and also the root parasite, *Helosis*. In the case of the papaya, *Carica*, the larvae were found in a broken stem.

Table 7 lists the species and numbers of individuals reared from the 22 plant types. In the case of the willistoni and cardini groups there are sibling species which were not distinguished in this count. The willistoni group sibling species identified by Mr. B. Spassky for El Salvador are *D. willistoni*, *D. tropicalis*, *D. equinoxialis* and a subspecies of *D. paulistorum*. In the cardini group, *D. cardini*, *D. cardinoides* and an undescribed species have been taken in El Salvador.

A total of at least 31 species were reared from the 22 plant types. Table 7 illustrates that most of the species are general fruit feeders in the larval stage. Strict plant host dependency does not seem to be the general rule. The fruit *per se* is

TABLE 7

Species reared from different plant types

	Xanthosoma		Syngonium 1		Syngonium 2		Persea		Enterolobium	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. paranaensis			59	72.8			5	71.4	4	100
2. paranaensis-L.			1	1.2						
3. repleta			6	7.4						
4. cardini gp.	4	6.7	5	6.2	4	100	2	28.6		
5. aracea	49	81.7	10	12.3						
6. unkn. yellow	7	11.7								
Total	60		81		4		7		4	

	Mangifera		Spondias		Gilibertia		Psidium		Citharexylum	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. Diath. dubia					1	3.3				
2. willistoni gp.	18	9.4	108	98.2			23	8.0		
3. sucinea									1	100
4. nebulosa	1	0.5								
5. mel. and sim.	172	90.1	1	0.9			232	81.1		
6. ananassae							21	7.3		
7. metzii-L.			1	0.9						
8. crocina					29	96.7	1	0.3		
9. cardini gp.							9	3.1		
Total	191		110		30		286		1	

TABLE 7—Continued

Species reared from different plant types

	Inga preussii No.	%	Inga spuria No.	%	Brosimum No.	%
1. G. bivisualis	1	0.5				
2. pictiventris					7	9.0
3. sucinea			4	20.0		
4. mel. and sim.	3	1.5				
5. ananassae	177	91.2				
6. crocina					38	48.7
7. mediostriata			1	5.0		
8. metzii-L.					2	2.6
9. trapeza	4	2.1			7	9.0
10. fulvimacula			15	75.0		
11. cardini gp.	9	4.6			24	31.0
Total	194		20		78	

	Helosis No.	%	Bracket Fungus No.	%	Leather Fungus No.	%	Carica No.	%	Coffea No.	%
1. L. pulcherrima			58	87.9						
2. prognatha-L.					1	100				
3. thoracis			1	1.5						
4. willistoni gp.									6	54.5
5. nebulosa									5	45.5
6. medionotata-L.	65	91.5					29	70.7		
7. trapeza	3	4.2								
8. onca-L.							4	9.8		
9. cardini gp.	3	4.2	7	10.6			8	10.5		
Total	71		66		1		41		11	

	Ficus No.	%	Castilla No.	%	Citrus No.	%	Calocarpum No.	%
1. G. bivisualis			1	0.1				
2. Diath. opaca	6	3.0						
3. sturtevanti					2	3.1		
4. willistoni gp.	23	11.4	145	12.5			71	15.4
5. sucinea	50	25.0						
6. fumipennis			1	0.1				
7. nebulosa			4	0.3			3	0.7
8. mel. and sim.	45	22.4	893	77.0	54	83.1	217	47.2
9. ananassae	9	4.5						
10. crocina	21	10.4	33	2.8				
11. albicans	5	2.5	3	0.3				
12. metzii-L.	41	20.4						
13. trapeza			1	0.1				
14. fulvimacula					9	13.8	28	6.1
15. paranaensis							89	19.3
16. cardini gp.			79	6.8			2	0.4
17. gibberosa							50	1.6
18. st. castanea-L.	1	0.5						
Total	201		1160		65		460	

probably not too important a factor if it acts as a suitable substrate to support the microorganisms such as yeast and bacteria on which the larvae obtain their nutrition. Dobzhansky and da Cunha (1955) showed that two species of yeast mixed with sterile banana bait differentially attracted sibling species of the willistoni group and *D. nebulosa*, *D. fumipennis*, *D. cardinoides* and several other

forms in Brazil. It is necessary to realize the importance of Drosophila species in the general ecology of a forest floor or coffee finca. In the adult stage the flies distribute microorganisms from one fruit to another. In the larval stage they work through the fruits. All this contributes to the more rapid physical and chemical breakdown of the ground litter clearing the forest and finca floors.

Although most of the species proved to be general feeders, certain ones were found to be restricted to only one type of plant. The most outstanding example. *D. aracea*, was collected only in the inflorescence of aroids such as *Xanthosoma* and *Syngonium* of the family Araceae, and was reared only from their fruits. It results, then, that the success of this form depends upon the success of its host plant. The aroids are one of the commonest and most abundant plant types throughout Central America. *Drosophila aracea* has been taken in El Salvador, Honduras and Nicaragua at elevations of a few hundred feet up to 5000 feet.

It is now pertinent to discuss the limitations one fruit or fruit type exhibits in supporting a varied population of larvae. Table 7 shows that as many as 10 species of flies were reared from fruits of the genera *Ficus* and *Castilla* (Moraceae) and eight from *Calocarpum* (Sapotaceae). In some instances the species emerging from one fruit only were recorded. The best example in this respect was with the zapote fruits, *Calocarpum*. This is a large reddish fleshy edible berry the size of a small grapefruit with a hard coating and large oval seed. Table 8 shows the variety of species and number of individuals reared from each of the five individual fruits collected in a coffee finca (2000′) in May. In one case six

TABLE 8

Species reared from five individual fruits of zapote.
Calocarpum mammosum (L.) Pierre

	Fruit 1		Fruit 2		Fruit 3		Fruit 4		Fruit 5	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. willistoni gp.			5	6.1	29	36.3	34	22.1	3	3.9
2. nebulosa	3	4.4								
3. melanog. and sim.	65	95.6	65	79.3	21	26.3	2	1.3	64	84.2
4. paranaensis					14	17.5	75	48.7		
5. fulvimacula					5	6.3	23	14.9		
6. cardini					2	2.5				
7. gibberosa			12	14.6	9	11.3	20	13.0	9	11.8
Total	68		82		80		154		76	

species and 80 individuals emerged from one fruit. It is difficult to estimate the amount of interspecific and intraspecific competition of the larvae for food and pupation space since the fruits are large and the food seemingly plentiful. It is interesting to note however that in fruits 1, 2 and 5 where the *D. melanogaster* and *simulans* population is 80% or above that the number of species is few.

ADULT FEEDING SITES

Sweeping over natural feeding sites provides ecological data not to be expected with the banana trapping technique. It also gives some index as to actual population size and complexity on a micro basis. A micropopulation is defined here as a group of flies that have been collected over a definite feeding site which usually

consists only of one type of fruit, flower or fungus. The size of such a feeding site may vary from several square inches, as in the case of a small bracket fungus, to as large as an acre, as in the coffee berries or pepete (bean) pods scattered on the ground in a finca. However, the usual size is that found under one particular fruiting tree such as a mango, jocote, ujushte, etc. A large number of micropopulations were analyzed throughout the year in El Salvador but only one group of observations will be reported.

On Volcan Santa Ana at an elevation of 5000 feet from July 25–27 seven different micropopulations were encountered in an area of about one-half of a square mile. Identification of the feeding sites is as follows: small red drupes, *Citharexylum donnell-smithii* Greenman (Verbenaceae); acorns, *Quercus skinneri* Bertham (Fagaceae); figs, *Ficus sp.* (Moraceae); pepete pods, *Inga spuria* H. and B. (Leguminosae); small flowers from unidentified tree; and bracket fungus No. 1 and No. 2 ((Basidiomycetes).

A total of 30 of the 47 species and 1103 of the 1471 individuals collected in this region were found over at least one of the above feeding sites. This means the choice of feeding sites for 75% of the total collected population is known. The three dominant species of all seven micropopulations together are *D. mediodelta*, 236 individuals (23%); *D. alagitans-L.*, 164 individuals (16%) and *D. capnoptera*, 136 individuals (13%). Table 9 lists the three dominant species of each micropopulation.

TABLE 9

Three dominant micropopulation species, Volcan Santa Ana

Total indiv.	Acorns 283 %	Flowers 100 %	Figs 189 %	Beans 129 %	Fungus 1 39 %	Fungus 2 72 %	Drupes 219 %
Paralio. dudai					8	63	
Paralio. bipart.					8		
sucinea			21	21			
alagitans-L.	30	3	30				20
capnoptera	28						55
mediodelta	24		15		8		
mediostriata		50		18			
fragilis		42					5
tripunctata K						6	
uninubes				16	56	18	
medium brown							

These data show that no two micropopulations have a similar structure and rather distinct feeding preferences are thus indicated. The most abundant species in all micropopulations together, *D. mediodelta*, is the only form taken on all seven feeding sites. However, it is the most abundant at only one feeding site. This also illustrates a choice of food.

Choice of egg laying sites is more difficult to determine. It is very doubtful if the acorns were utilized for egg deposition since they were too durable for the larvae to work. Four of the six food types were collected to test later fly emergences. The results are as follows: *Leucophenga pulcherrima* from fungus (58); *D. thoracis* from fungus (1); *D. sucinea* from figs (50); beans (2) and drupes (1); and *D. fulvimacula* from beans (6). Of the four species that emerged, only

D. sucinea was collected by net on Volcan Santa Ana in July. This illustrates the possible incompleteness of any micropopulation record and the difficulty in correlating feeding sites with egg laying sites.

FAUNAL AFFINITIES

Recent work on the Drosophilidae of the Neotropic region has centered mostly in Mexico and Brazil. It is pertinent then to discuss the faunal relations of El Salvador with that of Mexico and South America. The conclusions reached in these comparisons are only tentative since much collecting is yet to be done. Very little net sweeping as compared to banana trapping has been done in Mexico and consequently the knowledge of the fauna other than the genus *Drosophila* is limited because most of the members of the related genera do not frequent fermenting fruit. Even considering *Drosophila* alone much is yet to be learned, for the sweeping technique brings to light forms which do not ordinarly come to traps. Also Mexico has not yet been collected south of southern Veracruz and Oaxaca. Patterson and Mainland (1944) have reported on the Drosophilidae of Mexico.

Nearly all the recent work in South America has centered in Brazil where Dobzhansky, Pavan, da Cunha and their co-workers have collected rather extensively. They have reported only on the genus *Drosophila* (Dobzhansky and Pavan, 1943; Pavan and da Cunha, 1947; Pavan, 1950). Other genera such as *Rhinoleucophenga, Neotanygastrella,* and *Diathoneura (Clastopteromyia)* have also been reviewed (Malogolowkin, 1946; Frota-Pessoa and Wheeler, 1951; Frota-Pessoa, 1947). In addition Duda (1927) gives descriptions of species in the entire family mostly from Bolivia, Peru and Paraguay.

Several other taxonomic works on the family Drosophilidae should be mentioned for the Neotropic region. They include for Costa Rica, Duda (1925) and Malloch (1924); for Panama, Malloch (1936); and for Panama and the Antilles, Sturtevant (1921). Wheeler (1952) has a recent key to the Nearctic genera and to the species of each genus other than *Drosophila.* This is especially valuable for nearly all of the genera listed are also found in the Neotropical region.

Stalker (1953) and Forta-Pessoa (1954) have recently reviewed the cardini and tripunctata groups respectively.

Table 10 illustrates the faunal affinities as well as can be determined of El

TABLE 10

Faunal affinities of El Salvador with Mexico and South America

	Mexico No.	Mexico %	South America No.	South America %
Drosophila common with 120 species from El Salvador	60	50.0	26	21.6
Species of other genera common with 102 species from El Salvador	23	22.5	20	19.6
Total	83	37.3	46	20.7

	Drosophila	Other Genera	Total
Species common to Mexico, El Salvador and S. America	19	6	25

Salvador with Mexico and South America. Of the 120 *Drosophila* from El Salvador. six are considered cosmopolitan species and are usually found in domestic habitats. These forms are: *D. melanogaster, D. simulans, D. ananassae, D. repleta, D. hydei* and *D. immigrans*. They are included in the analyses in Table 10. Aside from these forms, 12 *Drosophila* and six forms belonging to other genera are also common to all three areas under discussion and are listed below.

Amiota steganoptera	D. prosaltans
Leucophenga frontalis	D. willistoni
Rhinoleucophenga sp.*	D. nebulosa
Scaptomyza adusta	D. crocina (campestris)
Zygothrica dispar	D. sp. (tripunctata gp.)*
Zygothrica sp.*	D. cardinoides
D. thoracis	D. pallidipennis
D. latifasciaeformis (baeomyia)	D. calloptera
D. sturtevanti	D. albicans

* Comparison of pinned material

The list will undoubtedly be extended with future collecting and with a clarification of literature names.

Table 10 shows a total of 83 species in the family Drosophilidae common to El Salvador and Mexico. This represents 37.3% of all the forms collected in El Salvador. Of these 83 species, 60 belong in the genus *Drosophila*. This represents 50% of all *Drosophila* collected in El Salvador. The remaining 23 species belong in other genera and they represent 22.5% of the forms common to El Salvador and Mexico. In one respect these data show that our knowledge of Mexican drosophilids is incomplete, as explained previously, since it is believed that there will be a higher correlation between the two countries when southern Mexico has been properly collected. The Pacific slope of Middle America is generally the same climatically and vegetationally from the Isthmus of Tehuantepec to Panama. The very humid Puerto Golfito region of Costa Rica is one big exception.

Patterson and Stone *(op. cit.)* list 89 species of *Drosophila* from the Nearctic and Neotropical transition zone in Mexico (19 to 22 degress N. latitude). Since then other localities within this zone have been collected, especially San Andres Tuxtla in Veracruz, and at least 20 additional forms have been added to this list which brings the total to 109. This list will be extended no doubt with further collecting, especially in other seasons than in summer. In any event it is interesting to compare this total with the 120 forms from El Salvador. It is indeed surprising to find so rich (or as rich) a fauna in so small a country that obviously does not have the physiographic and climatic variety that is found in Central Mexico. It is interesting to note that the species group containing the most forms in Central Mexico is the repleta group, while the dominant group in El Salvador is the tripunctata group. This is shown below:

	Central Mexico	*El Salvador*
repleta group	25	13
tripunctata group	7	29

The large number of species of the repleta group in Mexico is due to the desert conditions found on the high central plateau to which these forms have become

adapted. The large number of species of the tripunctata group found in El Salvador cannot so easily be accounted for except to say that they are forest forms, many of which apparently have become adjusted to coffee fincas. This group is also well represented in South America.

The faunal affinities of El Salvador with South America are not as great as with Mexico. This may be seen in Table 10. A total of 46 forms is common to the two areas. This represents 20.7% of all the species in El Salvador. The lack of a higher correlation is partly due to incomplete collecting, but also these two areas, because of their greater separation, if nothing else, should not be expected to have as many species in common as those of El Salvador with Mexico. The geologic history also no doubt accounts for some differences.

The events of the late Mesozoic to recent times in the Americas are described by Dunn (1931) and Simpson (1943) in working with fossil and recent turtles; and Simpson (1950), in working with fossil and recent mammals. Present evidence does not allow speculation as to the historical relationship of the Middle and South American drosophilid fauna except to say that for the genus *Drosophila* there are apparently no species groups (and this is the level on which comparisons can be made most conveniently) in South America different from those found in Middle America. The distribution of some Drosophila species is apparently continuous from Central Mexico to Brazil (such as *crocina*, *albicans*, *calloptera*, *willistoni* and *cardinoides*). Other distributions are apparently continuous only from Costa Rica to Brazil (such as *bifilum*, *mesostigma* and *angusti bucca*.)* The continuity implies that the dispersal of these and other species between the two continents is a comparatively recent event. In this connection it is interesting to add that Cockerell (1923) reports a record of a *Drosophila* in amber from South America. His description, however, resembles more closely a *Diathoneura*. He believes the specimen may be Tertiary, but more likely Pleistocene, in age. Comparative breeding tests and chromosomal analyses on a large scale should give some evidence of the sequence of events through time. The recent and very interesting work of Dobzhansky (in press) on the distribution of inversions in *D. willistoni* throughout the Neotropical region is of much significance in this respect.

SUMMARY

Species of the acalyptrate family, Drosophilidae, were collected in El Salvador, Central America, from October 7, 1953 until August 20, 1954, in five general habitats. The habitats are: gallery forests at 300 feet; barrancos (ravines) at 2000 feet; aroid swamps at 2000 feet; coffee fincas, upland forests and domestic habitats (considered together) at 2000, 3000 and 5000 feet; and cloud forests at 7000 feet.

A total of 222 species representing 18 genera in the family *Drosophilidae* were collected. A total of at least 96 of the forms are known in the literature and they are listed. The number of species in each subgenus and species group in the genus *Drosophila* are also listed.

* The author has recently collected more extensively in Central America and Colombia. In general there is much similarity of the lowland Colombian drosophilid fauna to Central America (especially Costa Rica and Panama) even as high as 4,000 feet in the Santa Marta Mountains. However at elevations of 8–11000 feet in the Andes (above Medellin and Bogota) one encounters many new forms mostly in the tripunctata group and at least one new genus has been taken there.

The number of species in each genus collected in the five general habitats is listed. There is a direct correlation in the degree of domestic disturbance and the variety of species collected. A few genera are seen to have an affinity for one habitat in particular. The majority of forms of most genera however inhabit many ecological types (at least on a macro basis) and it is only on an altitudinal basis that they can be separated. An analysis of the vertical distributions shows at least 82% of the species belong to either a highland or lowland fauna and about 6% may be considered members of both faunas. The 3000 foot level is intermediate.

Seasonal variation in population size is shown for the habitats more intensively studied. Two population peaks of *Drosophila* in the coffee fincas at 2000 feet are correlated with the appearance of coffee berries in November and the shade tree fruits in May. The barrancos were most productive in the dry season (November–March) and their numbers declined sharply at the beginning of the wet season (April). The aroid swamps had a population peak in December and a low in the middle of the wet season (August) apparently due to the higher water level.

Fermenting fruits and other succulent material representing 13 families of flowering plants and one group of fungi were collected from sea level to 6000 feet. A total of 3142 individuals representing four genera and 31 species were bred from them. It is shown that many of the forms are nonspecific feeders in the larval stage in regard to the fruit itself. Several species are specific to a certain plant type and one of them is discussed. The limitations one fruit or fruit type exhibits in supporting a varied population of larvae is also discussed.

A micropopulation for adult flies is defined and seven of them are analyzed. Definite preferential feeding is indicated in that the dominant species are usually not the same even when the different types of feeding sites are within close proximity.

An analysis of the faunal affinities of El Salvador with Mexico and South America is given. A total of at least 83 species is found to be common to Mexico and El Salvador, and at least 46 species are common to El Salvador and South America.

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Appendix A

Described species of the family Drosophilidae from El Salvador

1. Sinophthalmus pictus Coquillett. U.S.
2. Amiota steganoptera Malloch. Costa Rica.
3. Gitona bivisualis Patterson. U.S.
4. Stegana uniformis Malloch. Costa Rica.
5. Stegana interrupta Malloch. Costa Rica.
6. Stegana schildi Malloch. Costa Rica.
7. Stegana nigrita Malloch. Costa Rica.
8. Stegana nigrimana Malloch. Panama.
9. Stegana flavifrons Malloch. Costa Rica.
10. Stegana tempifera Malloch. Costa Rica.
11. Rhinoleucophenga bezzii Duda. Costa Rica.
12. Leucophenga obscuripennis (Loew). Cuba.
13. Leucophenga frontalis (Williston). St. Vincent.
14. Leucophenga undulata Hendel. Peru.
15. Leucophenga varia (Walker). U.S.
16. Leucophenga bimaculata (Loew). Cuba.
17. Leucophenga pulcherrima Patterson and Mainland. Mexico.
18. Leucophenga elegans Duda. Peru.
19. Leucophenga maculosa var. fenestrata Duda. Peru.
20. Neotanygastrella chymomyzoides Duda. Peru.
21. Chymomyza laevilimbata Duda. Peru.
22. Scaptomyza vittata (Coquillett). U.S.
23. Scaptomyza paravittata Wheeler. U.S.
24. Scaptomyza adusta (Loew). U.S.
25. Paraliodrosophila bipartita Duda. Costa Rica.
26. Paraliodrosophila dudai Wheeler. Mexico.
27. Dettopsomyia formosa Lamb. Seychelles.
28. Mycodrosophila projectans (Sturtevant). Santa Domingo.
29. Paramycodrosophila costaricana Duda. Costa Rica.
30. Paramycodrosophila centralis Wheeler. U.S.
31. Zygothrica dispar (Wiedemann). Brazil.
32. Zygothrica poeyi (Sturtevant). Cuba.
33. Zygothrica vittatifrons (Williston). St. Vincent.
34. Zygothrica microstoma Duda. Costa Rica.
35. Zygothrica aldrichi Sturtevant. Panama.
36. Zygothrica scutellaris Wheeler. Mexico.
37. Microdrosophila quadrata (Sturtevant). U.S.
38. Diathoneura dubia (Sturtevant). Cuba.
39. Diathoneura minuta Duda. Costa Rica.
40. Diathoneura opaca (Williston). St. Vincent.
41. Diathoneura superba (Sturtevant). Guatemala.
42. Diathoneura metallica (Sturtevant). Cuba.
43. Diathoneura splendida (Williston). St. Vincent.
44. Diathoneura cruciata Duda. Costa Rica.
45. Cladochaeta nebulosa Coquillett. Puerto Rico.
46. Drosophila pictiventris Duda. Costa Rica.
47. Drosophila thoracis Williston. St. Vincent.
48. Drosophila nigrohalterata Duda. Costa Rica.
49. Drosophila glabrifrons Duda. Costa Rica.
50. Drosophila mexicoa (Wheeler). Mexico.
51. Mycodrosophila pleuralis Williston. St. Vincent.*
52. Drosophila floricola Sturtevant. U.S.
53. Drosophila latifasciaeformis Duda. Africa.
54. Drosophila subsigmoides Patterson and Mainland. Mexico.
55. Drosophila sturtevanti Duda. Brazil.
56. Drosophila emarginata Sturtevant. Guatemala.
57. Drosophila prosaltans Duda. South America.
58. Drosophila willistoni Sturtevant. St. Vincent.
59. Drosophila tropicalis Burla and da Cunha. Brazil.
60. Drosophila equinoxialis Dobzhansky. Brazil.
61. Drosophila paulistorum Dobzhansky and Pavan. Brazil.
62. Drosophila nebulosa Sturtevant. Cuba.
63. Drosophila fumipennis Duda. Costa Rica.
64. Drosophila sucinea Patterson and Mainland. Mexico.
65. Drosophila melanogaster Meigen. Austria.

APPENDIX A—Continued

Described species of the family Drosophilidae from El Salvador

66. Drosophila simulans Sturtevant. U.S.
67. Drosophila ananassae Doleschall. Amboina.
68. Drosophila tolteca Patterson and Mainland. Mexico.
69. Drosophila azteca Sturtevant and Dobzhansky. U.S.
70. Drosophila capnoptera Patterson and Mainland. Mexico.
71. Drosophila bromeliae Sturtevant. Cuba.
72. Drosophila crocina Patterson and Mainland. Mexico.
73. Drosophila mediostriata Duda. Costa Rica.
74. Drosophila fragilis Wheeler. Mexico.
75. Drosophila albicans Frota-Pessoa. Brazil.
76. Drosophila unipunctata Patterson and Mainland. Mexico.
77. Drosophila bipunctata Patterson and Mainland. Mexico.
78. Drosophila paranaensis de Barros. Brazil.
79. Drosophila repleta Wollaston. Madeira.
80. Drosophila fulvimacula Patterson and Mainland. Mexico.
81. Drosophila nigricruria Patterson and Mainland. Mexico.
82. Drosophila hydei Sturtevant. U.S.
83. Drosophila subviridis Patterson and Mainland. Mexico.
84. Drosophila cardini Sturtevant. Cuba.
85. Drosophila cardinoides Dobzhansky and Pavan. Brazil.
86. Drosophila immigrans Sturtevant. U.S.
87. Drosophila (?) submacroptera Patterson and Mainland. Mexico.
88. Drosophila (?) aurea Patterson and Mainland. Mexico.
89. Drosophila uninubes Patterson and Mainland. Mexico.
90. Drosophila gibberosa Patterson and Mainland. Mexico.
91. Drosophila limbinervis Duda. Costa Rica.
92. Drosophila pallidipennis centralis Patterson and Mainland. Mexico.
93. Drosophila briegeri Pavan and Breuer. Brazil.
94. Drosophila calloptera Schiner. South America.
95. Drosophila canalinea Patterson and Mainland. Mexico.
96. Drosophila castanea Patterson and Mainland. Mexico.
97. Drosophila argenteifrons Wheeler. Mexico.
98. Drosophila crassa Patterson and Mainland. Mexico.

* Belongs in the subgenus *Hirtodrosophila*.

APPENDIX B

List of plants utilized by drosophilid larvae and the habitat from which they were collected

Family	Species and Local Name	Habitat	Altitude (ft.)
Anacardiaceae			
	1. Mangifera indica L. (mango)	Coffee finca	2000
	2. Spondias mombin L. (jocote)	Gallery forest	300
Araceae			
	3. Xanthosoma sp.	Aroid swamp	2000
	4. Syngonium sp. 1.	Gallery forest	100
	5. Syngonium sp. 2.	Upland forest	4000
Araliaceae			
	6. Gilibertia arborea (L.) March. (mano de leon)	Coffee finca	2000
Balanophoraceae			
	7. Helosis sp. (planta de tierra)	Upland forest	2000
Basidiomycetes			
	8. Bracket fungus	Coffee finca	5000
	9. Leather fungus	Gallery forest	300
Caricaceae			
	10. Carica papaya L. (papaya)	Upland forest	2000
Lauraceae			
	11. Persea americana Mill. (aguacate)	Coffee finca	2000
Leguminosae			
	12. Inga preussi Harms. (cujun)	Coffee finca	3000
	13. Inga spuria H. & B. (pepete)	Coffee finca	5000

APPENDIX B—Continued

List of plants utilized by drosophilid larvae and the habitat from which they were collected

Family	Species and Local Name	Habitat	Altitude (ft.)
	14. Enterolobium cyclocarpum Griseb. (guanacaste)	Domestic	2000
Mirtaceae			
	15. Psidium guajava L. (guava)	Domestic	2000
Moraceae			
	16. Ficus spp. (capulamate, amate)	Gallery forest	100
		Domestic	2000
		Coffee finca	5000
	17. Castilla gummifera Pittier (hule)	Gallery forest	300
		Coffee finca	3000
	18. Brosimum terrabanum Pittier (ojushte)	Gallery forest	300
Rubiaceae			
	19. Coffea arabica L. (cafe)	Coffee finca	2000
Rutaceae			
	20. Citrus sinensis (L.) Obseck (naranja)	Domestic	2000
Sapotaceae			
	21. Calocarpum mammosum (L.) Pierre (zapote)	Coffee finca	2000
Verbenaceae			
	22. Citharexylum donnell-smithii Greenman	Upland forest	6500

VII. Taxonomic and Distributional Studies of Nearctic and Neotropical Drosophilidae

MARSHALL R. WHEELER

During the past several years we have been engaged in a large scale study of the Drosophilidae of the Caribbean region. Our field collectors, financed largely by the National Science Foundation, have been Dr. William B. Heed (principal collector), Dr. Marvin Wasserman, Dr. H. L. Carson, and Mr. Hugo Hoenigsberg. Additional material has been collected for us by Dr. Th. Dobzhansky, Dr. C. Pavan, Dr. Danko Brncic, and Mrs. Marta Breuer, to whom we are greatly indebted. We have also had the use of some 6000 pinned specimens belonging to the United States National Museum, and we are grateful to Dr. C. H. Curran who graciously permitted us to borrow a number of specimens, including types, from the collection of the American Museum of Natural History.

These various collections have resulted in the finding of a great many undescribed species of Drosophila, of other genera of Drosophilidae, and other acalyptrate Diptera. In addition, information concerning the geographical distribution of many species has been tremendously increased. Some of the new species are being described in this paper, with occasional remarks on the taxonomy and distribution of other species.

A few undescribed species of Drosophila are still being found in North America, and I am taking this occasion to describe a few of them.

In view of the difficulties in taking care of laboratory cultures of many of our more exotic species, I want to express my sincerest gratitude to Mrs. Mary Budd for her loyal assistance. Mr. Peter Thompson kindly prepared the description of one of the new species and the figures for it, and the wing photographs were made by Dr. Frances Clayton.

The type specimens have been desposited in the Drosophila Type and Reference Collection of The University of Texas unless it is stated that they are the property of another museum.

Genus Drosophila

Subgenus *Siphlodora*

Drosophila flexa Loew

Drosophila flexa Loew, 1865. Berl. Ent. Zeit. 9:182 (Cent. VI, No. 89).
Drosophila subsigmoides Patterson and Mainland, 1944. Univ. Texas Publ. 4445:26. New Syn.

D. flexa was described from Cuba, *subsigmoides* from Mexico; our collections indicate that both names refer to the same, widespread species. We have been unable to detect any differences in the male genitalia of island and mainland specimens, and since the species cannot as yet be raised in culture in the laboratory we are forced to assume that only one species is concerned. The following summary of distribution includes, in addition to our own records, the reports of some of our collaborators in South America. *D. flexa* is now known from: Mex-

ico, Guatemala, El Salvador, Nicaragua, Costa Rica, Panama, Colombia, Venezuela, Ecuador, Peru, Brazil, and Cuba, Haiti, and Puerto Rico.

Subgenus *Hirtodrosophila*

Drosophila alabamensis Sturtevant

Drosophila alabamensis Sturtevant, 1918. Jour. N. Y. Ent. Soc. 26:38.

The holotype ♀ from Kushla, Alabama (AMNH) has been re-examined, and we now feel confident that we have collected this species from a number of localities: Austin, Texas (Oct., Nov.), Carlinville, Ill. (Aug., Sept.), Equality, Ill. (Sept.), Lithium, Mo. (June), Mongomery Co., Va. (Max Levitan, coll., April), Algonquin Park, Ont. (D. D. Miller, coll.). I have also seen a specimen from E. Lansing, Mich. (April). Williams and Miller (1952) report a specimen, as *alabamensis*-like, from Lincoln, Nebr. (May); their identification was probably correct.

Subgenus *Pholadoris* Sturtevant

Okada (1956) uses for this subgenus the name *Paradrosophila* Duda, 1924, in which Duda placed a number of species with prescutellar bristles. A type species was designated by Sturtevant in 1927, who chose one of the species included by Duda, *Drosophila pictipennis* Kertesz 1901. Although many of the species placed in *Paradrosophila* by Duda are co-subgeneric with the species now included in *Pholadoris*, it seems fairly certain that *pictipennis* is not co-subgeneric, and hence the only species which can logically be included in *Paradrosophila* are those which are, in turn, co-subgeneric with *pictipennis*.

We have not seen *pictipennis* Kertesz; however, Duda (1923. Ann. Mus. Nat. Hung. 20:43) reports briefly on the type ♀ in the Budapest museum, bearing the label "N.-Guinea, Biró, 1898", and later (1924. Arch. f. Naturg. 90A3: 206) gives some descriptive notes in his key and a photograph of the wing (Pl. IV, Fig. 59), characterizing the species as follows: wing very narrow, intensely black along the costa and especially broadly so on the basal and apical thirds, and with a large black cloud over the posterior crossvein; thorax yellow to brownish; proclinate orbital at most as long as posterior reclinate; anterior reclinate midway between the other two or closer to the proclinate. The photograph of the wing shows that it is not unusually narrow but the intense darkening is as described. The costal index appears to be near 3.0.

Drosophila (Pholadoris) latifasciaeformis Duda

Drosophila (Paradrosophila) latifasciaeformis Duda, 1940.

Synonyms are: *D. finitima* var. *gracilipes* Duda, *D. mirim* Dobzhansky and Pavan, *D. baeomyia* Wheeler. (See discussion in Burla, H. 1954. Rev. Suisse de Zool. 61:115 ff.).

There is evidence that this species is expanding its territory rather rapidly. Duda described it from specimens collected in 1913 in Uganda, Africa, and Burla *(op. cit.)* found it common in the region of the Ivory Coast. *D. mirim* was found in the State of Sao Paulo, Brazil in 1943, and the first records from Mexico were made by us in 1947. In 1948 we found it for the first time in the United States

where we have found it from Florida to South Carolina. Our more recent collecting shows that it is widely distributed throughout the Caribbean region: Cuba, Jamaica, Puerto Rico, St. Lucia, St. Kitts, Barbados, El Salvador, Nicaragua, Honduras, Costa Rica, Panama, Colombia, Trinidad, and Venezuela. It is also known from Peru and, as stated above, from Brazil.

Subgenus *Sophophora*
saltans group

Drosophila pulchella Sturtevant

Drosophila bellula Williston, 1896, not *bellula* Bergroth, 1894.
Drosophila pulchella Sturtevant, 1916. Ann. Ent. Soc. Amer. 9:327.

The type (AMNH collection) is a male in fair condition and clearly belongs to the saltans group. It is most similar to both *subsaltans* Magalhaes and *parasaltans* Magalhaes, having a light body color, but shows small differences from both of them as shown in the following comparisons:

pulchella—arista with 8 branches; middle orbital ¼ posterior; subcarinal hairs long and numerous; pleura pale.

parasaltans—arista with 10 branches; middle orbital ½ posterior; subcarinal hairs moderately long and numerous; sternopleura distinctly darkened above.

subsaltans—arista with about 12 branches; middle orbital 4/10 posterior; subcarinal hairs scanty, short, often absent; pleura more uniformly discolored.

The type was from the island of St. Vincent, B. W. I.; we have not yet taken this species in our own collecting.

Subgenus *Drosophila*
tripunctata group

Drosophila trifiloides Wheeler, new species.

External characters of imagines.

♂, ♀. Arista with 6 dorsal and 3 ventral branches in addition to the fork. Front tan, orbits and ocellar triangle paler; face and cheeks tan, clypeus a little darker. Proclinate orbital about 5/6 length posterior reclinate; anterior reclinate thin, about ¼ length proclinate. Carina broad, rounded, not sulcate; one prominent oral, the 2nd ⅓–½ as long; palpi with a strong subapical bristle, directed outward, and 3 others spaced along outer side. Face sometimes with slight whitish pollinosity.

Mesonotum tan, scutellum darker. Acrostichal hairs in 6 rows; no prescutellars; basal scutellars divergent. Pleura paler than mesonotum; halteres pale. Posterior sternopleural stout and black, anterior one only 0.6 as long. Legs pale; 1st femur with 2 strong bristles on inner side, and about 4 on outer side. Base of hind tarsus with only pale bristles below.

Abdomen light tan with apical bands and median spots as shown in Figure 1. In the ♀, tergites 2–4 have narrow apical bands, medianly interrupted, 5 has a still narrower band plus a poorly outlined median spot, and 6 has only a large black median spot, variable in shape but more or less rounded. In the ♂ tergite 5

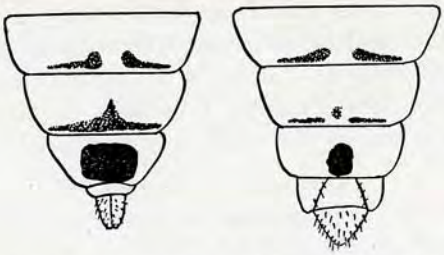


FIG. 1. *Drosophila trifiloides*, abdominal pattern of posterior tergites. Male, left; female, right.

has the band extended forward in the middle, and 6 has a large black median spot, usually somewhat rectagular but sometimes with the anterior margins more rounded.

Wings hyaline; posterior crossvein with a narrow light brown cloud, the anterior crossvein dark but without cloud. Costal index 3.4–4.1; 4th vein index 1.3–1.4; 5x index about 1.0; 3rd costal section with the small black bristles on the basal half.

Body length (live specimen), ♂, 2.6 mm., wing, 2.0 mm.; ♀, 3.2 mm., wing, 2.8 mm.

Other characteristics, relationship, and distribution.

Internal features.—Spermatheca elongate, pear-shaped (Fig. 9). Ventral receptacle with 60 or more coils. Testes pale whitish.

Eggs.—With 3 filaments, the anterior pair thin, pale, about ⅘ the egg length; the single large posterior filament is longer than the egg, this filament and the operculum both sclerotized, tan.

Puparia.—Tan; posterior spiracles pale, parallel and rather long; anterior spiracular horns short, about ⅙ length of the body, with about 12–14 pale branches.

Chromosomes.—These are described in detail by Clayton and Wasserman (this bulletin).

Distribution.—This species has been collected by us only twice: H50.10b, La Lima, Honduras, March 1954, W. B. Heed; H192.39A, Rionegro, near Bucaramanga, Colombia, September 1956, H. L. Carson, M. Wasserman, and H. Hoenigsberg.

Types.—Holotype male and 9 paratypes of both sexes, Rionegro, Colombia.

Relationship.—*D. trifiloides* apparently belongs in subgroup III of the tripunctata group of the subgenus Drosophila. The egg filaments suggest a relationship with *trifilum* from which it differs in many respects (one prominent cral, unclouded anterior crossvein, abdominal banding pattern, etc.). Using the key of Frota-Pessoa (1954) the species keys to the vicinity of *albicans* from which it is distinct in many characters including the egg filaments.

Notes.—The male genitalia were examined by Dr. Frota-Pessoa who reported as follows (comparing with *trifilum*): conical gonopophyses with bristles are absent; the cut in the midline of the posterior border of the hypandrium is narrower and deeper, and the bristles of the hypandrium are closer together. The clasper has 5–6 primary teeth, usually with the 6th separated from the others by a gap, the row followed by a cluster of long pale bristles.

repleta group

Drosophila aureata Wheeler, new species.

External characters of imagines.

♂, ♀. Arista usually with 3 dorsal and 2 ventral branches in addition to the fork. Front golden brown, rather velvety, turning more reddish on dead specimens, the orbits and ocellar triangle grayish brown pollinose. Antennae light brown; carina exceptionally broad below, shallowly sulcate, light brown; antennal foveae and palpi pale tan, clypeus dark brown. Cheeks pollinose tan, rather broad; 2 strong orals. Eyes medium red with short pale pile. Proclinate orbital nearly as long as posterior reclinate, the anterior reclinate about ¼ as long.

Acrostichals 4–6 rowed between the dorsocentrals, irregularly 8 rowed just anterior to them; no prescutellars. Anterior scutellars convergent. Mesonotum with dense pollinosity, dark golden to golden brown, with a few irregularly shaped darker brown spots: a pair anteriorly, one at the inner edge of each transverse suture, a median posterior stripe between middle acrostichal rows, and a few other less obvious marks. Scutellum golden brown pollinose, much darker on the basal lateral sides. Pleura brown but with an intense grayish, rather metallic, sheen when viewed from most angles. Halteres pale. Anterior sternopleural 0.6 length posterior, the middle one scarcely evident. Legs tan to light brown, femora sometimes a little darker. Inner surface of 1st femur of both sexes with a row of 8–10 widely spaced stiff bristles which are distinctly shorter and thicker than usual. Male 1st tarsus with very numerous short recurved hairs, their length generally less than the diameter of the tarsus.

Abdominal tergites with large dull black bands occupying most of the area, interrupted medianly with yellow, the two colors strongly contrasting; the bands are a little narrowed before reaching the angle, then expand into solid black lateral areas. Male genital arch dark; sternites pale.

Wings hyaline, the 1st vein darkened. First costal section with one strong bristle at apex; 3rd section with the small black bristles on the basal ½. Costal index about 3.1; 4th vein index about 1.8; 5x index about 0.9.

Body length (live flies), ♂, 3.0 mm.; wing, 2.5 mm.; ♀, 3.7 mm.; wing, 3.0 mm.

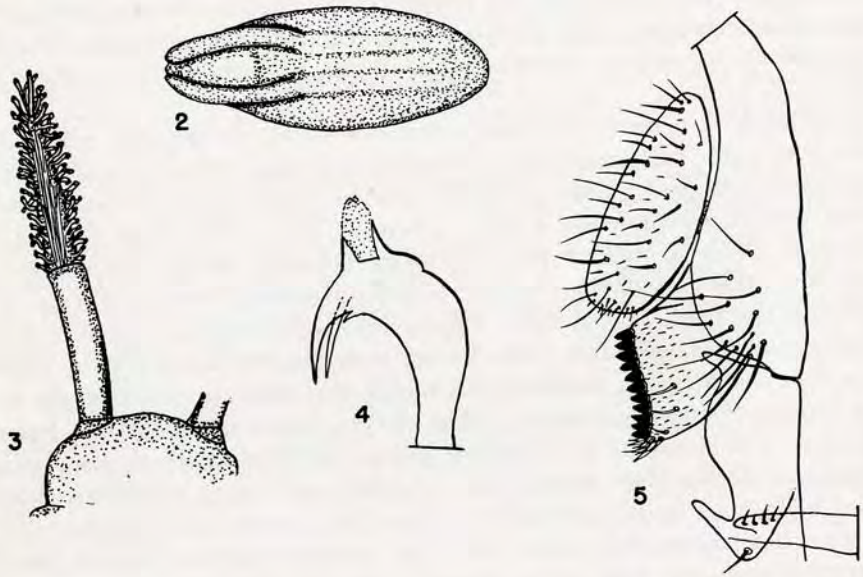
Internal characters of imagines.

Spermatheca with very small, slightly sclerotized center; ventral receptacle of medium length, with about 13–15 tight coils basally, more irregularly tangled distally. Ovipositor with about 18 coarse, pale teeth, a long apical bristle, and the usual ventral one (opposite tooth No. 7), plus two secondary bristles. Anterior Malpighian tubes with the common stalk about ¼ their total length; common stalk of posterior pair shorter, the ends apposed but without continuous lumen. Testes pale lemon yellow, irregularly formed into about 3½ coils; sperm pump slightly bulbous below but without true diverticula. Genital arch of male, with some of the attached pieces as shown in Figure 5; penis (Fig. 4) with two stout diverging spines, and an apical non-sclerotized sac-like process arising from sclerotized protuberances. Anal plate with only a slight connection to the genital arch.

Other characteristics, relationship, and distribution.

Eggs.—With only two filaments (Fig. 2), the usual posterior ones, which are always closely appressed to the opercular region.

Puparia.—Pale tan; anterior spiracles (Fig. 3) appearing rather feathery, with many short branches on a central stalk; horn plus spiracle equals half length body; posterior spiracles short, surrounded by two rows of fleshy processes whose surfaces bear numerous minute spines.



FIGS. 2–5. *Drosophila aureata.* FIG. 2, egg; FIG. 3, anterior spiracle of puparium; FIG. 4, head of penis, profile view; FIG. 5, genital arch, clasper, and attached pieces.

Chromosomes.—These are described by Clayton and Wasserman (this bulletin).

Relationship.—*D. aureata* appears to be an aberrant member of the repleta group of the subgenus Drosophila.

Distribution.—We have collected this species in Mexico (San Andres Tuxtla, Vera Cruz), El Salvador (San Salvador, and Laguna de Zapotitan), Costa Rica (San Jose), Panama (Cerro La Campana), and Trinidad. Collectors were W. B. Heed, H. L. Carson, and M. Wasserman.

Types.—Holotype male and 8 paratypes of both sexes, from stock No. H180.42, which originated from a collection of 13 individuals taken from a slime flux of a large willow tree, San Jose, Costa Rica, Aug. 1956. The other specimens were taken at banana-baited traps.

pallidipennis group

The species *pallidipennis* consists of two subspecies, *D. pallidipennis pallidipennis* Dobzhansky and Pavan from South America, and *D. p. centralis* Patterson and Mainland, known only from Mexico. Patterson and Dobzhansky (1945) reported that crosses between the two subspecies produced fertile F_1 females and

sterile males. Our more recent collecting has shown that the species is generally distributed throughout Central America. We have made a number of crosses between the various strains in the laboratory, including one from Tingo Maria, Peru, the results of which are shown in Table 1. The stock from Peru appears to be the only one representing the subspecies *pallidipennis*, all other strains being quite interfertile and representing *centralis*. Although all possible crosses have not been completed, it is apparent from the table that only four crosses between the subspecies gave the same results as those reported by Patterson and Dobzhansky, while two crosses produced no F_1 adults and two others produced sterile females as well as males. Finally, in the three cases where the cross was made reciprocally, different results were obtained when the Peruvian parent was the female and when it was the male.

TABLE 1

Crosses between strains of *D. pallidipennis*

♂ \ ♀	62.58 Salv.	91.25 Colo.	191.48 Colo.	1804.3 Mexico	2263.21 Mexico	29.27 Salv.	183.17 Panama	2373.5 Peru
62.58	X	F	F	F			F	F♀ S♂
91.25	F	X	F		F	F	F	Few F_2 larvae, died
191.48	F	F	X	F	F		F	F_1 sterile ♂, ♀
1804.3	F		F	X	F	F		
2263.21		F	F	F	X	F	F	
29.27		F		F	F	X		
183.17	F	F	F		F		X	F_2 larvae, 1 pupa, no adults
2373.5	F_1 sterile ♂, ♀	F♀ S♂	F♀ S♂		F♀ S♂			X

F = fertile, producing an F_2
S = sterile

calloptera group

In the excellent summary of this group by Burla and Pavan (1953) five, possibly six, species were recognized; we have seen additional material of all of them, and have records of two new species. With some exceptions, the known distributions of the species are shown in Fig. 6. *D. quadrum* is not shown, since most of the localities are farther south in Brazil; *calloptera*, *schildi*, and *atrata* are also well known from southern Brazil. *D. calloptera* has the most extensive distribution: from Tamazunchale, S. L. P., Mexico, to Sao Paulo, Brazil; it occurs on Trinidad but on the islands of the West Indies it is replaced by *ornatipennis*, which we have taken in Cuba, Jamaica, Puerto Rico, Haiti, St. Kitts, and St. Lucia. We are still unable to say whether *schildi* and *poecila* are one or two species but our collections indicate that there is only one widely distributed species, known from Costa Rica, Panama, Colombia, Venezuela, Trinidad, and Brazil.

D. atrata has been known only from Brazil; Dr. Wasserman collected 15 individuals at Caripe, Venezuela, where both *calloptera* and *schildi* were taken

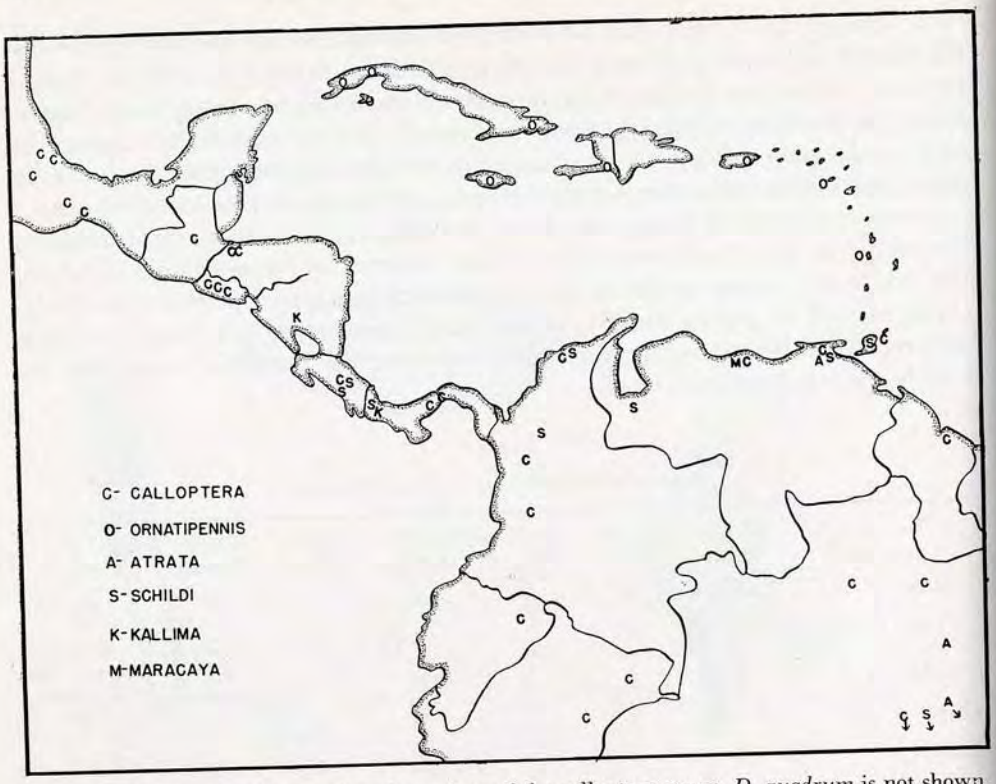


FIG. 6. Geographic distribution of members of the calloptera group. *D. quadrum* is not shown, nor are most Brazilian records of *calloptera*, *atrata*, and *schildi*.

simultaneously. Previous records of *D. quadrum* are from the State of Sao Paulo, Brazil, to Rio Grande do Sul; we can report three additional specimens, from the USNM collection: 2, Perene, Peru (R. C. Shannon); 1, Surukum, Venezuela (P. Anduze.)

We have been able to maintain *schildi* in culture for only about three generations, but *calloptera*, *ornatipennis*, and *atrata* have been maintained with comparative ease. Stocks of these three species have been tested for interfertility, with the results shown in Table 2. We obtained no hybrids between species, but all crosses between strains of *calloptera* were successful.

TABLE 2

Crosses between strains of the calloptera group

	calloptera				*atrata*	*ornatipennis*
♂ \ ♀	74.3 Costa Rica	181.16 Panama	192.11 Colombia	203.21 Venezuela	203.23 Venezuela	2378.2 Cuba
74.3	X	F	F	F	S	S
181.16	F	X	F	F	S	S
192.11	F	F	X	F	S	S
203.21	F	F	F	X	S	S
203.23	S	S	S	S	X	S
2378.2	S	S	S	S	S	X

F = fertile, producing an F_2
S = sterile; no F_1

Drosophila kallima Wheeler, new species.

External characters of imagines.

♂, ♀. Most similar to *schildi*, differing most obviously in the wing pattern (Fig. 7). Front whitish yellow, brown around ocelli and bases of verticals; proclinate orbital ⅔ length posterior reclinate; anterior reclinate thin, hairlike, about ⅙ posterior. Face, antennae, clypeus except base, and palpi except base, all pale yellowish; bases of clypeus and palpi brown; proboscis brown with pale labellum. Arista with 7 dorsal and 4 ventral branches in addition to the fork. One strong oral; cheeks narrow, brown below eye becoming grayish yellow posteriorly.

Mesonotum dull reddish brown with a pattern of pale tan to whitish pollinose spots, streaks and blotches. Generally there is a poorly defined pale area on anterior ⅔ between dorsocentral rows, within which there are 3 medium large brown blotches; from this a thin irregular median line continues to scutellum. About 6 pale marks laterad of dorsocentral rows. Acrostichal hairs in 4 rows between anterior dorsocentrals, irregularly 6-rowed in front; one or more bristles in dorsocentral rows enlarged, an especially prominent one just anterior to edge of transverse suture.

Scutellum dark brown with 5 gray pollinose areas: one at base on each side and 3 apical ones which may be partly fused. Basal scutellars divergent. Pleura mottled with dark brown, tan and gray pollinose areas, not organized into a describable pattern. Anterior sternopleural 0.7 length posterior; middle bristle thinner but of about same length as anterior one. Halteres with brownish discoloration on front surfaces.

Legs brown, especially coxae and femora; tibiae paler with a tendency toward basal and subapical darker bands; tarsi pale. Hind metatarsus with 2 large, 1 smaller, stout black bristles among the pale ones basally below.

Abdomen with pattern of dark dull brown apical bands, expanded in the middle and at lateral margins, the large paramedian basal areas thus formed being grayish pollinose, sometimes almost metallic. Anal plates and ovipositor of female pale.

Wings as in Figure 7. Vein 2L with spur veins as in *schildi* and *quadrum*. Costal index 3.1–3.3; 4th vein index 1.3–1.4; 5x index about 0.7.

Body length (pinned female) 3.2 mm., wing, 4.0 mm.; males smaller.

Other characteristics, relationship, and distribution.

Male clasper with 7–9 primary teeth followed by a cluster of pale hairs going around onto lower side, and with 4–5 long bristles in secondary position; genital arch with a single strong bristle just above attachment of clasper, and 2 bristles on heel area; lower tip of anal plate with a cluster of small bristles. Ovipositor slender, pointed, with about 17 primary teeth, 6 secondary teeth, and 4–5 pale bristles near tip. Spermatheca, as observed in cleared preparation of pinned specimen, dark, shaped about as in Figure 17.

Distribution and types.—Holotype male, 9 paratypes of both sexes, plus 7 additional specimens, from Hacienda Santa Maria de Ostuma, about 11 klm. north of Matagalpa, Nicaragua, June 1954, W. B. Heed collector. Two specimens (USNM) labelled Panama, Volcan Chiriqui Prov., 9-XII-1952, F. S. Blanton.

Relationship.—Belongs to the calloptera group of the subgenus Drosophila.

Drosophila maracaya Wheeler, new species.

External characters of imagines.

♂, ♀. Similar to *calloptera,* but with an entirely different wing pattern (Fig. 8), the 5th vein with a spur vein. Arista with 6 dorsal and 4 ventral branches in addition to the fork. Front, antennae, face and clypeus whitish yellow, ocellar area, orbits and bases of verticals a little darker; clypeus brownish at base. Palpi dark tan; cheeks narrow, grayish brown, darker behind.

Mesonotum mottled, dark brown with golden brown pollinose blotches and spots, the appearance varying with the viewing angle; in general the anterior

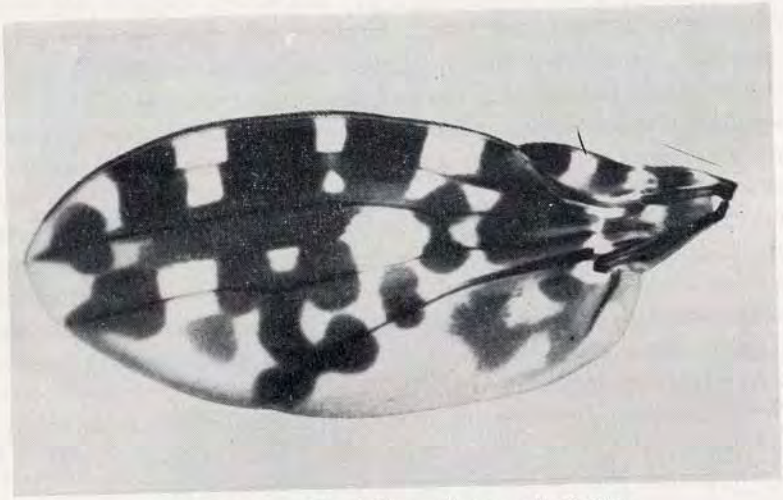


FIG. 7. *Drosophila kallima,* photograph of wing.

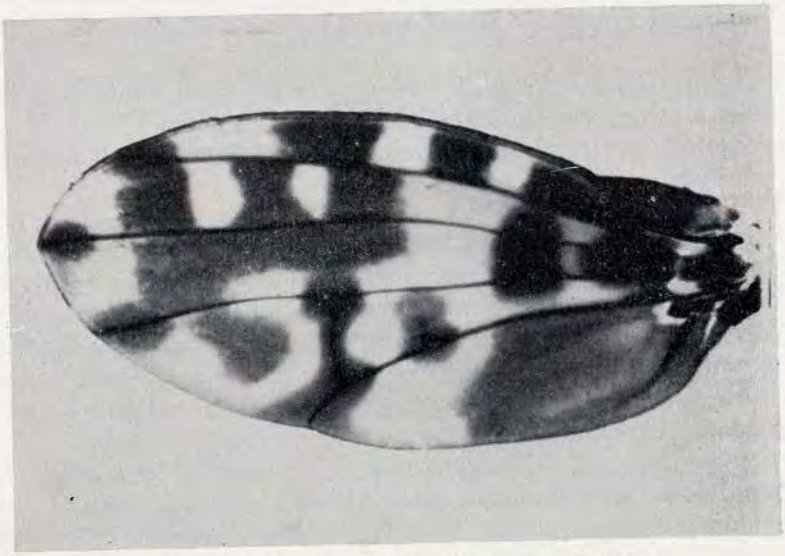


FIG. 8. *Drosophila maracaya,* photograph of wing.

⅓ is golden with a partial median brown streak, but which appears dark brown to black when seen from behind; posteriorly and laterally there are a number of golden brown spots and blotches. Scutellum broadly brown except on sides where it is more tan. Acrostichal hairs rather sparse, irregularly 6-rowed; no prescutellars; an enlarged bristle near inner edge of transverse suture. Basal scutellars divergent. Pleura dark brown to blackish brown, pollinose. Posterior sternopleural stout and long, the other two equal in size, about 0.6 length posterior. Halteres tan.

Legs mostly brown, including coxae; tibiae paler, tarsi grayish yellow. Base of hind metatarsus below with 2 bristles darker than the others. Abdomen with dark brown apical bands with median extensions, leaving paired tan basal markings. Male tergite 2 mostly brown, 3, apical band with large median expansion, 4–5, with narrower expansions, the band of 5 thicker; 6 all dark brown; all bands form solid lateral areas. Female as in male, but tergite 6 with small paired basal tan areas, 7 all dark; anal plates and ovipositor tan.

Wings as in Figure 8, vein 2L lacking spur veins but 5L with a spur in the large dark spot before posterior crossvein. Third costal section with the small black bristles on the basal 0.4; costal index about 3.4; 4th vein index about 1.2; 5x index about 0.6.

Body length (pinned female) up to 3.5 mm., wing, 3.2 mm.; ♂ smaller.

Relationship.—Belongs to the calloptera group of the subgenus Drosophila.

Distribution and types.—Holotype ♂, 9 paratypes of both sexes, Rancho Grande, near Maracay, Venezuela, Nov. 1956, M. Wasserman collector.

melanderi group
Drosophila ordinaria Coquillett

Drosophila ordinaria Coquillett, 1904. Proc. Ent. Soc. Wash. 6:190.

An examination of the holotype female showed that this rare species belongs to the melanderi species group, and is quite similar in appearance to both *melanderi* and *magnafumosa*. The type, in the U.S. National Museum collection, is from the White Mountains, N. H., and I have examined a second specimen, in the Museum of Comparative Zoology, from St. John's County, Quebec. Sturtevant (1921:86) lists a specimen from Chester, Mass.

The melanderi group now consists of the following species: *melanderi* Sturtevant, *magnafumosa* Stalker and Spencer, *ordinaria* Coquillett, all from North America, *cameraria* Haliday (=*pallida* Zetterstedt) from Europe, and *makinoi* Okada from Japan. As far as known, all the species are mushroom feeders and are poorly attracted to fruit baits; they also show many morphological similarities to the subgenus Hirtodrosophila.

polychaeta group
Drosophila polychaeta Patterson and Wheeler

Drosophila polychaeta Patterson and Wheeler, 1942. Univ. Texas Publ. 4213:102.

Our original specimens came from the wharfs at Galveston, Texas. The species has since been reported from various widely scattered localities, including the Netherlands, Liverpool (on ships from west Africa and Malaya), Hawaii, and Guam, and I have seen specimens from Saipan and Guam (Marianas) and

Koror (Palau). New American records are: 1, Cativa, Colon Prov., Panama; 6, Cocos Island, Wafer Bay. Cocos is a small island belonging to Costa Rica, nearly midway between the latter and the Galapagos Islands. These 7 specimens are in the U. S. National Museum collection.

canalinea group

Recent collections have shown that there are perhaps 6–8 species in Central and South America that are related to *canalinea* Patterson and Mainland. We have not succeeded in culturing most of these, but hybridization tests between the seven stocks which we did have available showed that three species were present in the laboratory. The results of these tests are given in Table 3. The geographic origins of the stocks used are as follows:

canalinea: H50.31 La Lima, Honduras
H101.7 Palmira, Colombia
H188.21 Santa Marta, Colombia
canalinioides: H38.6 San Salvador, El Salvador
H66.7 San Salvador, El Salvador
H181.55 Barro Colorado Island, Canal Zone
paracanalinea: H129.19 El Yunque, Puerto Rico

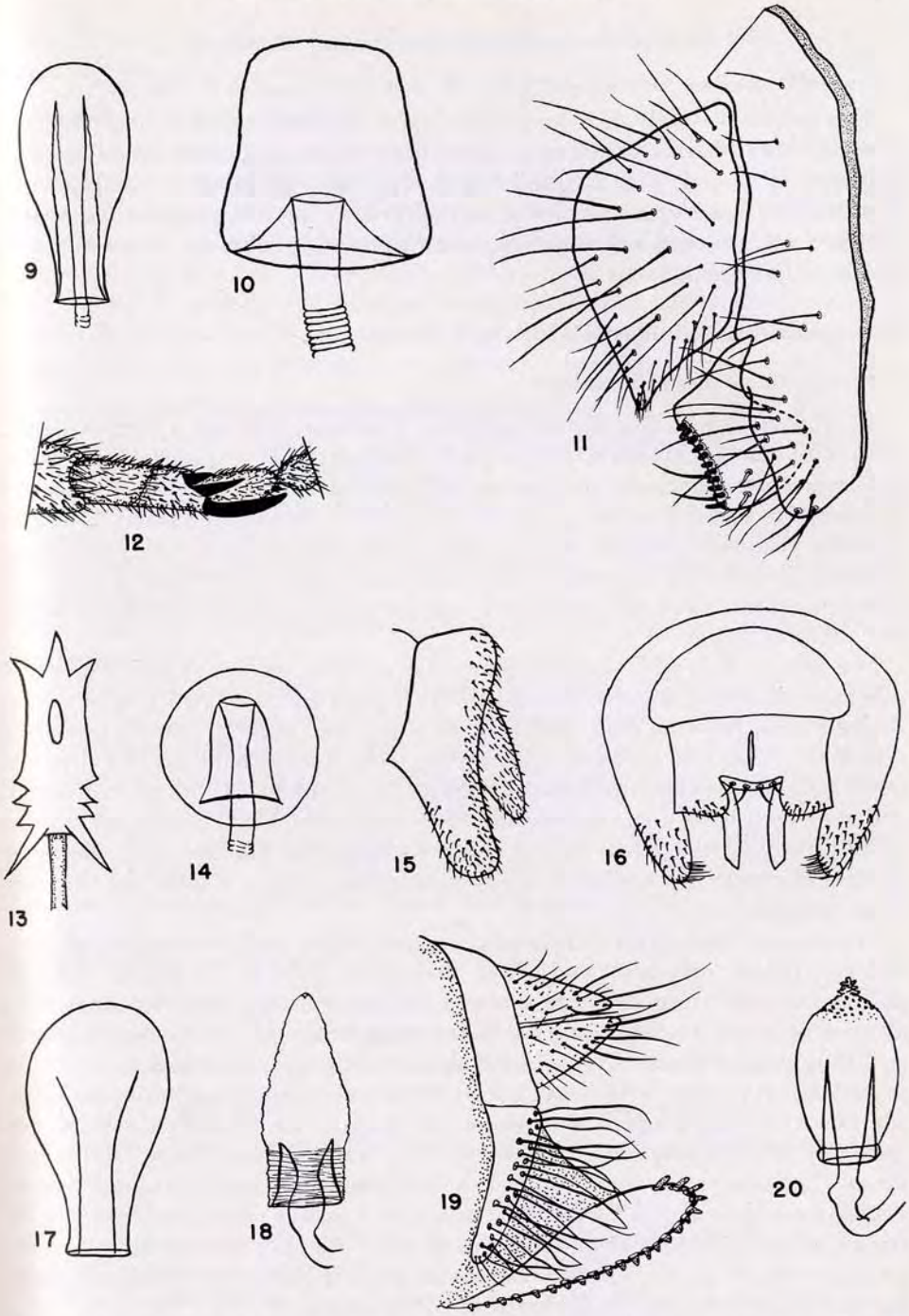
As the table shows, intraspecific crosses are fully fertile, and interspecific crosses are sterile with the single exception of *canalinea* ♀ (50.31) × *paracanalinea* ♂ (129.19). The cross was made by mass matings of about 10 ♂, 10 ♀; the first attempt yielded three F_1 males which were backcrossed to parental females and proved to be fertile. The cross was made again and watched more closely. The cross produced an estimated two dozen larvae from which only seven pupae developed; from these only three F_1 males emerged, the remaining pupae undergoing an internal degeneration. These males were similarly backcrossed to parental females and proved to be fertile.

TABLE 3

Results of crosses between members of the *canalinea* complex

	Intraspecific Cross ♀ ♂		Interspecific Cross ♀ ♂		Interspecific Cross ♀ ♂	
canalinioides	38.6 × 66.7	F	38.6 × 50.31	S	50.31 × 38.6	S
	38.6 × 181.55	F	38.6 × 101.7	S	50.31 × 66.7	S
	66.7 × 38.6	F	38.6 × 129.19	S	50.31 × 181.55	S
	66.7 × 181.55	F	66.7 × 50.31	S	50.31 × 129.19	few
	181.55 × 38.6	F	66.7 × 101.7	S	101.7 × 38.6	S
	181.55 × 66.7	F	66.7 × 129.19	S	101.7 × 66.7	S
			181.55 × 50.31	S	101.7 × 181.55	S
canalinea	50.31 × 101.7	F	181.55 × 101.7	S	101.7 × 129.19	S
	101.7 × 50.31	F	181.55 × 129.19	S	129.19 × 38.6	S
	188.21 × 101.7	F	129.19 × 181.55	S	129.19 × 66.7	S
			129.19 × 50.31	S	129.19 × 101.7	S

F = fertile F_1 produced
S = sterile; no F_1



FIGS. 9–20. FIG. 9, *D. trifiloides*, spermatheca; FIG. 10, *D. paraguttata*, spermatheca; FIG. 11, *D. paraguttata*, genital arch and clasper; FIG. 12, *Laccodrosophila heedi*, part of front tarsus, ventral view, diagrammatic; FIG. 13, *D. sticta*, head of penis, ventral view; FIG. 14, *D. sticta*, spermatheca; FIG. 15–16, *Pseudiastata* sp. from Cuernavaca, Mexico, genital arch and genitalia from below; FIG. 17, *D. kallima*, spermatheca; FIG. 18, *D. canalinioides*, spermatheca; FIG. 19, *D. canalinioides*, female genitalia showing paragenital fringe, semi-diagrammatic; FIG. 20, *D. paracanalinea* and *canalinea*, spermatheca.

Drosophila canalinea Patterson and Mainland

Drosophila canalinea Patterson and Mainland, 1944. Univ. Texas Publ. 4445:50.

This species is widely distributed throughout the Neotropical region. We have material from Mexico, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Trinidad, Ecuador, and Brazil. The only collection of canalinea-like flies from the Caribbean islands was on Puerto Rico, but this stock showed nearly complete reproductive isolation from *canalinea* and is being described as a new species, *D. paracanalinea*.

Drosophila canalinioides Wheeler, new species.

External characters of imagines.

♂, ♀. Arista with 5–7 dorsal branches, 3 ventral ones and a terminal fork. Front dull blackish brown with strong pollinose gray to brown areas along orbits, two large spots anteriorly, and on the large ocellar triangle, the latter extended anteriorly to ptilinal suture, gradually narrowing. Proclinate orbital ¾ length posterior reclinate; anterior reclinate tiny. Antennae blackish with pale yellow apices on 2nd and 3rd segments. Carina broad, rounded; face yellowish gray, clypeus and cheeks black, the latter with grayish pollen; palpi and proboscis black. One prominent oral.

Mesonotum dull, with complex, somewhat variable, pattern of dark brown and pale tannish brown areas with many of the bristles and hairs which arise within the pale areas showing dark spots at their bases; the resulting pattern resembles that of *canalinea* plus scattered *repleta*-type spots. Acrostichal hairs in 8 rows; no prescutellars. Anterior scutellars convergent. Scutellum black with grayish brown pollen on each basal angle and broadly over apex. Pleura dull black with mottled grayish pruinescence when viewed from certain angles. Halteres pale, darkened at base. Anterior sternopleural about ¾ posterior one, a middle one scarcely differentiated.

Legs mostly black, with annulated tibiae and yellow tarsi. Femoral-tibial junction pale; tibiae with basal and apical black areas, paler in the middle, the pale portion faint on 1st leg, larger on 2nd and still larger on 3rd. Male 1st tarsus with a series of about 14 long recurved hairs along inner side, their length nearly equalling tibial diameter. Base of hind tarsus with pale bristles below.

Abdominal tergites with broad apical bands, interrupted medianly, the interruptions becoming progressively narrower on posterior tergites; the bands are wide next the interruption, then narrow, then widen again to form solid lateral areas. The basal yellow area on each side with whitish to silvery pruinosity when viewed from most angles. Circumanal tergite of ♀ with a prominent "paragenital fringe" of about 24 long slender bristles on each side (Fig. 19); as far as known, a similar sort of fringe occurs on the females of all species of the *canalinea* group, but has not yet been seen on species of any other group.

Wings light brown, paler posteriorly; apex of 1st costal section a bit enlarged, black, with 2 bristles; 3rd costal section with the small black bristles on the basal ⅘. Posterior crossvein with a moderately narrow black cloud, the anterior one with a small cloud. Costal index about 2.2; 4th vein index about 1.6; 5x index about 0.9.

Body length, live ♂, about 3.2 mm., wing, 2.6 mm.; female, about 3.8 mm., wing, 2.6 mm.

Internal characters of imagines.

Ventral receptacle long with many tight coils; spermatheca (Fig. 18) pale tan, elongate but narrowed apically, most of the surface with fine wrinkles except basally with numerous fine sulci. Ovipositor with about 23 primary teeth, longer than usual, and 4 slender secondary bristles. Posterior Malpighian tubes apposed at the tips, without a continuous lumen. Testes orange (young ♂♂) to rusty red (older ♂♂); sperm pump with two long thick, somewhat tangled, posterior diverticula. Clasper with a slightly curved row of about 10 teeth, the lower 2–3 distinctly more pointed; penis shaped nearly as in *canalinea*.

Other characteristics, relationship, and distribution.

Eggs.—With 4 filaments, the anterior pair short and thin, the posterior pair thicker and longer, nearly ⅔ the egg length.

Larvae.—The larvae skip, but infrequently.

Puparium.—Dark tan; anterior spiracular horns short, about equalling the distance between their bases, bearing 9–10 branches. Posterior spiracles rather long, pale, parallel.

Chromosomes.—The chromosomes are described by Clayton and Wasserman (this bulletin).

Distribution.—Our collectors have taken this species rather commonly in El Salvador (San Salvador, Santa Tecla), and on Barro Colorado Island, Canal Zone. The U.S. National Museum collection has specimens from: Costa Rica (San Mateo), Panama (Patino Point, San Carlos, Barro Colorado Island, Loma Borracha, Tocumen), and Venezuela (Carife).

Types.—Holotype ♂ and 9 paratypes (♂, ♀) from stock No. H66.7 from San Salvador, El Salvador, Sept. 1956, W. B. Heed, collector. This stock originated from 6 individuals collected by Dr. Heed from bracket fungus.

Drosophila paracanalinea Wheeler, new species.

This new species is quite similar in appearance to *canalinea*, but a side-by-side comparison of living individuals of a stock from Puerto Rico with individuals of *canalinea* (Honduras stock) shows some fairly reliable differences:

canalinea	*paracanalinea*
1. mesonotum dull; ground color dark brown	1. slightly shining; brownish black
2. eyes very dark red	2. brighter red
3. abdominal bands dark brown, the yellow areas pronounced	3. blacker bands, yellow areas not pronounced
4. sternites pale, grayish	4. sternites darker
5. ♀ circumanal tergite pale below, with *ca.* 7 hairs in paragenital fringe	5. this tergite dark below, with *ca.* 9 hairs in paragenital fringe

Dissections of male and female genitalia show small, possibly not significant differences: clasper of *canalinea* with usually 9 primary teeth *vs. paracanalinea*

with usually 10; spermatheca (Fig. 20) of *paracanalinea* generally more irregularly warty over the apical ½, and the inner duct usually a little shorter.

The chromosomes also differ, and are described by Clayton and Wasserman (this bulletin).

Distribution.—Collected by Dr. W. B. Heed in El Yunque, Caribbean National Forest, Puerto Rico, January 1956, and at the Agricultural Experiment Station, Rio Piedras, Puerto Rico, February 1956.

Types.—Holotype ♂, and 9 paratypes (♂, ♀), from El Yunque, Puerto Rico.

Drosophila annularis Sturtevant

Drosophila annulata Williston, not Fallén.
Drosophila annularis Sturtevant, 1916. Ann. Ent. Soc. Amer. 9:327.

A female labelled Type, from the collection of the American Museum of Natural History, is headless and a portion of the abdomen is missing; it clearly shows, however, the *canalinea*-like pattern on the mesonotum and legs, and has an especially well-developed paragenital fringe, the hairs being longer and more numerous than on any other specimen we have seen. The apex of the 1st costal section is enlarged and strongly blackened, but the crossveins show no clouding at the present time. On the remaining part of the abdomen one can see that the midline stripe (interruptions) are strongly grayish to silvery pruinose. We conclude that this species is distinct from any we have collected, and that the various published records of this species from Central and South American localities are to be viewed with suspicion.

Drosophila panamensis Malloch

Drosophila panamensis Malloch, 1926. Proc. U.S.N.M. 68 (21):28.

This species is probably best placed as an aberrant member of the canalinea group. We have examined the type in the USNM, and a single ♀, AMNH collection, also from Barro Colorado Island, Canal Zone. The general appearance is not that of *canalinea*, having a golden yellow face and front (the latter with a V-shaped brown mark on the type), and a different sort of mesonotal pattern. The female has, however, a paragenital fringe, a rather typical abdominal pattern and leg markings. The wing is quite dark, with still darker clouds over the crossveins; the 3rd costal section has the small black bristles reaching all the way to the 3rd vein, and the 4th vein clearly bends toward the 3rd at its apex.

fenestrarum group (Subgenus uncertain)

Drosophila basdeni Wheeler, new species.

=*Scaptomyza sp. B*, Wheeler, 1952. Univ. Texas Publ. 5204:197, 208.

External characters of imagines.

♂. Front tannish yellow, the orbits paler, less than half width of head. Anterior reclinate orbital about ⅓ proclinate, ¼ posterior reclinate. Antennae pale yellow, 2nd segment with 4 stout bristles, 3rd small; arista with 5 dorsal and 2 ventral branches in addition to the fork. Face whitish yellow, low, a carina scarcely indicated; cheeks whitish, very narrow. Vibrissa thin, 2nd oral about ¾ as long and placed rather far behind 1st. Palpi pale with a strong apical bristle.

Mesonotum and scutellum pale tan, rather shiny; acrostichal hairs in four rows; no prescutellars. Apical scutellars cruciate and bent somewhat upright. Two nearly equal humerals; posterior sternopleural large, the other two thin, middle one longer than 1st. Pleura tan, a trifle darker on mesopleura. Legs wholly yellowish.

Abdominal tergites blackish, distinctly shining; tergites 1–2 paler. Halteres pale. Sternites pale. Wings clear, rather narrow and somewhat pointed. Two prominent bristles at distal costal break; 3rd costal section with the small black bristles on the basal ⅝. Costal index about 3.2; 4th vein index about 2.0; 5x index about 1.9. Anal vein greatly reduced.

Body length (pinned specimen) about 2.1 mm.; wing, 1.9 mm.

Distribution and types.—Holotype ♂ (USNM), E. Lansing, Mich., May 4, 1949, Ryoji Namba collector. Paratype ♂, Mohawk Park, Ohio, May 10, 1937, H. D. Stalker collector.

Relationship.—This new species belongs to the fenestrarum species group and is the first member of the group to be reported from North America. It is quite distinct from any of the Palaearctic species: *fenestrarum* Fallén, *forcipata* Collin, and *acuminata* Collin.

Collin (1952) gives the following features of the fenestrarum group: acrostichals irregularly 4–6 rowed; facial carina small; middle sternopleural longer than anterior one; arista with 2 ventral branches basal to the fork; male fore metatarsus ventrally with an apical tuft of long pale hairs, and with a less obvious tuft on the 2nd joint; male hypopygia generally rather large. Collin has pointed out that the group occupies a somewhat isolated position in the genus. Hackman (1954) comments that many features, including those of the genitalia, suggest a relationship with some of the species of *Scaptomyza*, and is of the opinion that a new subgenus should be erected for the group. Little is known of the food habits of the species of this group, but since Basden (1954) reports taking both *fenestrarum* and *forcipata* by sweeping over watercress *(Nasturtium microphyllum)* in Scotland, a position intermediate between *Drosophila* and *Scaptomyza* is again indicated.

The holotype specimen of *basdeni* was compared with the Palaearctic species by Mr. E. B. Basden of Edinburgh who has graciously permitted the use of his notes. He states that *basdeni* is most similar to *acuminata*, especially in the form of the genital arch and the lack of large claspers. *D. acuminata* also shows, according to Basden, the following obvious differences: tergites with more obvious apical bands; cheeks pale brownish; palpi blackened; frons broader; mesonotum tannish brown but darker posteriorly and on scutellum; much of the pleura darkened ; wings broader; 3rd costal section with stronger bristles on the basal ¼; etc. In both *fenestrarum* and *forcipata* the thoracic and abdominal color is subject to considerable variability.

Subgenus **Drosophila** (unclassified species)

Drosophila carsoni Wheeler, new species.

External characters of imagines.

♂. Front dull reddish brown, the orbits and enlarged ocellar triangle darker brown, well-marked, posterior orbits around verticals much paler. Middle orbital

small, ¼ posterior, proclinate strong, about ⅔ length of posterior reclinate. Antennae brown, 3rd segment darker. Arista with 4 dorsal and 2 ventral thin branches in addition to the terminal fork; there are up to 7 thin lateral branches, more prominent than is usual. Face tan; carina of moderate size, a bit flattened on top. Cheeks, palpi and proboscis pale tan, clypeus darker, especially medianly. Palpi of ♂ quite bristly, with numerous thin long hairs and bristles, thickest around apex. 1st oral strong, 2nd thinner, about ½ length 1st. Cheeks rather broad, especially broad and bristly behind. Inner and outer verticals strong; a 3rd vertical, just behind outer one and bent inwardly, small.

Mesonotum subshining brown to dark brown, lighter far anteriorly and on humeri; pleurae diffusely browned, paler over sutural area between sterno- and mesopleura. Acrostichal hairs 6-rowed, a bit irregular; usually a few enlarged hairs anteriorly in dorsocentral row. No prescutellars; anterior scutellars divergent. Three humerals, upper one small, middle one large, lower one strong, about ⅔ length middle one. Two stout sternopleurals, anterior one about ⅚ length posterior.

Legs pale tan, rather long and slender. Bristles of 1st femur prominent: about 4 on lower side, 5 on outer side of which the apical one is strongest and placed further dorsad. Preapicals on all tibiae; a strong apical on 2nd, no apicals evident on 1st or 3rd tibiae. Halteres pale.

Abdominal tergites subshining brown to blackish brown, sometimes paler; two basal segments paler, remaining ones often somewhat paler in midline.

Wings clear; two strong bristles at apex of 1st costal section; 3rd section with the small black bristles on the basal ⅔. Costal index about 3.5; 4th vein index about 1.4; 5x index about 0.9–1.0.

Body length to 3.5 mm., wing about 3.5 mm.

Female: Usually paler than male, abdominal tergites often more tan with diffuse brownish apical bands with median interruptions. Palpi less bristly. Body length to 4.0 mm., wing about 4.0 mm.

Other characteristics, distribution, and types.

Eggs.—With 4 very thin filaments about equal to egg length.

Chromosomes.—The metaphase and salivary gland chromosomes are described by Clayton and Wasserman (this bulletin).

Distribution.—About 25 individuals have been captured, from 10 states as follows: Maine, Vermont, New York, Ohio, Tennessee, Missouri, Wisconsin, South Dakota, Colorado, and New Mexico. Male genitalia of specimens from Wisconsin and Colorado have been compared; they agree in all respects.

Types.—Holotype male, Mellen, Wisconsin, July 1952, H. L. Carson collector; 9 paratypes from: Mellen, Wisconsin, Steelville, Missouri, Espanola, New Mexico, and Pagosa Springs, Colorado.

Drosophila sticta Wheeler, new species.

External characters of imagines.

♂, ♀. A pale tan to yellowish tan species with unmarked wings and a spotted abdominal pattern. Head yellowish tan, including front, face, cheeks, palpi, antennae, and proboscis. Proclinate orbital about ⅝ posterior reclinate, middle or-

bital minute. Arista with 4–5 dorsal and 2 ventral branches in addition to the fork. Carina broad, rather bulbous, not sulcate. One strong oral, the 2nd about ⅜ its length, thin. Orbits and ocellar triangle only slightly differentiated.

Mesonotum tan, thinly pollinose, not shiny. Acrostichal hairs in about 6 rows, sometimes more irregular; no prescutellars. Basal scutellars widely divergent. Two humerals, nearly equal. Anterior sternopleural thin, about ⅔ length posterior one; a middle one present, small. Pleura yellowish tan; halteres pale. Legs pale without unusual bristling.

Abdomen tan, subshining, with small dark brown markings apically on tergites, rather irregularly formed into a series of paired spots (Fig. 21); there is variation in the shape of the larger paramedian spots and considerable variation

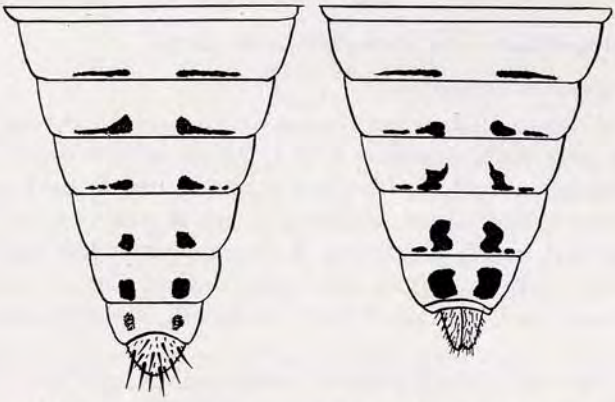


FIG. 21. *Drosophila sticta*, abdominal pattern. Male, left; female, right.

in the extent to which the various lateral markings are broken up into spots. Female with sub-anal spines—a pair of small sclerotized spines just below lower base of anal plates and above base of ovipositor.

Body length (live flies), ♀, 2.5 mm., wing, 2.2 mm.; males smaller.

Other characteristics, relationship, and distribution.

Eggs.—With 4 slender filaments, tending to be irregularly bent and twisted at tips; posterior pair about ⅗ egg length, anterior pair a little shorter.

Puparia.—Anterior spiracles with 25–30 long white branches; stalk of anterior spiracle about ⅕ length of puparium; posterior spiracles pale, rather long, divergent or parallel.

Internal features.—Posterior Malpighian tubes apposed at their tips but without a continuous lumen. Ventral receptacle spirally coiled, of medium length; spermatheca (Fig. 14) dark, round, with a strong ridge at base; ovipositor with blunt tip. Testis pale yellow; sperm pump with 2 short blunt diverticula. Clasper (forceps) with 8–9 teeth, plus strong marginal bristles; head of penis (Fig. 13) with 3 strong spines distally, 2 basally, and 3 smaller ones on each side.

Distribution.—*D. sticta* has been collected in Nicaragua (El Recreo), El Salvador (Santa Tecla, Laguna Alegria), Honduras (Lancetilla), and Colombia (Palmira, Rionegro). Collectors at Rionegro, Colombia were H. L. Carson, M. Wasserman, and H. Hoenigsberg; all other localities were collected by Dr. W. B. Heed.

Types.—Holotype male, 9 paratypes of both sexes, from stock No. H51.15 from Lancetilla, Honduras, collected in April 1954.

Relationship.—Belongs to the subgenus Drosophila, but cannot be placed satisfactorily in any of the established species groups. Dr. Frota-Pessoa has examined the male genitalia and reports that the ring of the hypandrium is present but it is of a different shape than in any of the species groups possessing such a ring (Malogolowkin, 1953, lists these groups: quinaria, tripunctata, cardini, guarani, calloptera, macroptera, rubrifrons, testacea, and pallidipennis). In other features this new species does not agree too well with any of these groups. Its position in the subgenus thus remains undecided.

Chromosomes.—The chromosomes are discussed in detail by Clayton and Wasserman (this bulletin).

Drosophila paraguttata Peter Thompson, new species.

External characters of imagines.

♂, ♀. A medium-sized, dark tan species with a pair of light spots on the mesonotum, known only from Jamaica, B.W.I. Arista with 4 dorsal and 2 ventral branches in addition to the fork; front and orbits uniformly dark tan, ocellar area darker. Proclinate orbital about equalling length of posterior reclinate; anterior reclinate about half length proclinate. Antennae somewhat darker than front; face, cheeks, and clypeus grayish tan, palpi darker. Carina broadened below, cuneate; one strong oral, the 2nd about ⅓ its length. Pseudotracheae of labellum 8/8 (♂), 9/9 (♀).

Mesonotum dark tan, faintly striped, with a pair of light spots just anterior to and medial to transverse suture, and with less conspicuous light spots at bases of posterior supra-alars and medial to humerus. Scutellum uniformly dark tan. Acrostichal hairs in 8 rows; no prescutellars; anterior scutellars convergent. Anterior sternopleural thin, about 0.8 length posterior. Legs pale gray.

Wings faintly brownish, most obviously so in marginal and submarginal cells; crossveins unclouded. Third costal section with the small black bristles on the basal ⅜; 2 bristles of equal size at apex of 1st section. Costal index about 2.0; 4th vein index about 1.8; 5x index about 1.3.

Abdominal tergites pale grayish tan at base and diffusely banded at apex, the apical bands of the basal tergites interrupted in midline.

Body length, ♂, 2.9 mm., wing, 2.8 mm.; ♀ 3.8 mm., wing, 3.0 mm. The foregoing description was prepared from living material.

Internal characters of imagines.

Ventral receptacle with 12–15 tight coils; spermatheca (Fig. 10) small. Testes pale yellow with about 5 coils; ejaculatory bulb without diverticula. Genital arch and clasper as in Figure 11; clasper large, bearing a primary row of 12 teeth, 2 large marginal bristles, and a line of 6 pale bristles from upper side to below margin.

Other characteristics, relationship, and distribution.

Eggs.—With 4 filaments about 1.5 times the egg length.

Puparia.—Amber; posterior spiracles pale, slightly divergent; anterior spiracle with 10 branches, the horn plus branches about 1/7 length of body.

Chromosomes.—The metaphase chromosomes, described by Clayton and Wasserman (this bulletin), consist of 5 pairs of rods and one pair of dots.

Distribution and types.—Holotype male and 9 paratypes of both sexes, from stock No. H136.34 which originated from flies collected by Dr. W. B. Heed near Bath, Jamaica, B.W.I., February 1956.

Relationship.—Belongs to the subgenus Drosophila, possibly close to the dreyfusi group.

Drosophila castanea Patterson and Mainland

Drosophila castanea Patterson and Mainland, 1944. Univ. Texas Pub. 4445:51.

This species has now been collected over a wide area, from Mexico to Colombia and Venezuela. Intraspecific crosses have been made between 13 geographic strains: Mexico (2), El Salvador (3), Costa Rica (5), Colombia (2), and Venezuela (1). Of the 24 crosses attempted, including reciprocals, only one failed to produce fertile F_1 resulting in an F_2. Figure 22 illustrates many of these crosses; solid lines represent fertile crosses, and the dotted line represents the cross: Medellin, Colombia ♀ × Huatusco, Mexico ♂. Two *castanea* females were received in the laboratory from Medellin and when they failed to establish stocks each was mated to males from the Huatusco culture. One female died shortly thereafter, but the other produced many F_1 flies. These failed to produce an F_2 on inbreeding and were then backcrossed to the Huatusco parent stock. The backcross

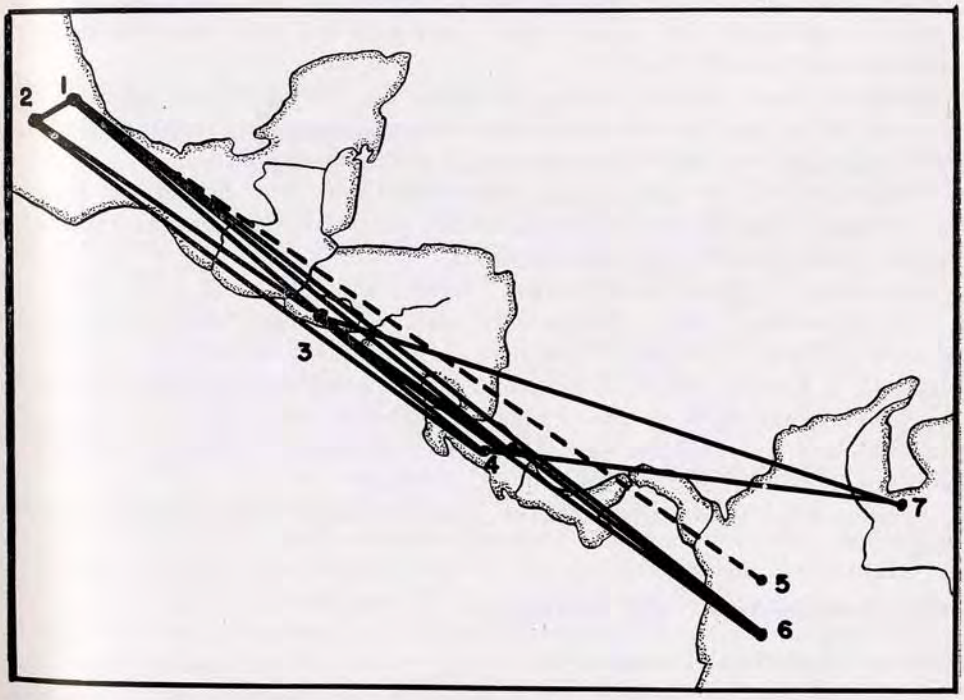


FIG. 22. Crosses between geographic strains of *Drosophila castanea*. Localities are: 1, Huatusco, Mexico; 2, Atlixco, Mexico; 3, El Salvador (3 stocks); 4, Costa Rica (5 stocks); 5, Medellin, Colombia; 6, Palmira, Colombia; 7, Merida, Venezuela. Solid lines show crosses producing fertile F_1; the dotted line shows the cross which produced sterile F_1.

was also sterile, indicating that both F_1 males and females were sterile. As is shown in the figure, however, *castanea* from Palmira, Colombia, crossed readily to several other stocks, producing fertile offspring.

Drosophila suturalis Wheeler, new species.

External characters of imagines.

♂, ♀. This new species simulates *Zapriothrica dispar* (Schiner) but it appears to be a case of convergent evolution rather than phylogenetic kinship. Front broad, dull tan to orange-brown, the ocellar area and elongated orbits darker brown, the latter a bit shiny. Head bristles rather short and stout, ocellars arising from outside the ocellar area on a level with the anterior ocellus. Proclinate orbital far forward, 0.9 length posterior reclinate, the middle orbital hairlike, midway between the other two. Antennae, face, cheeks, clypeus, palpi and proboscis pale tan; arista with short branches, about 8 dorsal and 5 ventral, progressively shorter toward apex. Carina broad, rather low, rounded, the foveae deep. One prominent oral; cheeks broad, nearly half eye-height; eyes rather small, dark red with long thick pile.

Mesonotum long and narrow, dark blackish brown; acrostichal hairs in 4 rows; no prescutellars. Posterior dorsocentrals in usual position, anterior pair far forward, at transverse suture, about ⅔ length posterior ones. Scutellum colored as mesonotum, basal bristles convergent to parallel. Pleura pale tan, contrasting with the dark mesonotum; sternopleurals hairlike, short, the anterior one 0.8 length posterior. Halteres pale. Legs pale tan; tarsi long and slender; empodium and pulvilli small.

Abdomen dark blackish brown, becoming tan laterally; male genital arch and anal plates tan, the latter noticeably elongated ventrally; female anal plates pale, ovipositor dark tan with exceptionally strong teeth apically.

Wings hyaline; 1st costal section with several stout short bristles, the longest one at apex; costa beyond the break weakly pectinate. Costal index about 2.6; 4th vein index about 1.5; 5x index about 1.2.

Body length (pinned female) about 3.0 mm., wing, 2.4 mm.; ♂ smaller.

Distribution and types.—Known only from 45 specimens taken from a single *Monstera* flower, Turrialba, Costa Rica, July 29, 1956; collectors were W. B. Heed, H. L. Carson, and M. Wasserman who reported that the flies moved very fast for Drosophilids. We were not able to establish a culture, but a few eggs were observed, and possessed one pair of very short filaments. Holotype male, and 9 paratypes of both sexes, from Turrialba, Costa Rica.

Relationship.—Of uncertain affinity, possibly related to the subgenus Phloridosa.

Drosophila tibialis Wheeler, new species.

External characters of imagines.

♂, ♀. This species simulates *Zapriothrica dispar* (Schiner) and *D. suturalis*, described above, but is probably not closely related to either. Front orange-tan, ocellar triangle and elongated orbits gray pollinose; head bristles strong; proclinate orbital slightly shorter than posterior reclinate, middle orbital ⅔ length

proclinate and only slightly nearer it than posterior one. Antennae and face tan; arista with 3 dorsal and 1 ventral branches in addition to the often irregular terminal fork. Carina high, narrow and long, rounded; one strong oral. Cheek narrow, a bit brownish; clypeus pale brown, darker on sides; palpi tan; proboscis pale with darkened labellum. Eyes dark red with thin pile.

Mesonotum long and narrow, dark brown pollinose, a little lighter above humeri; dorsocentrals in the usual position. Acrostichal hairs 6-rowed; no prescutellars. Scutellum darker than mesonotum, basal bristles divergent. Pleura nearly as dark as mesonotum, the sutures all paler; anterior sternopleural half length posterior, a middle one not developed. Halteres brownish. Legs, including coxae, pale tan except for hind tibiae which are dark brown to black, but variable in intensity. Front femur thick, the inner side with a row of stubby bristles, the distal 4–5 of which arise from small tubercles.

Abdomen uniformly dark blackish brown, pollinose, though some individuals show narrow basal tan areas on the tergites. Wings weakly smoky throughout; 3rd costal section with the small black bristles on the basal ⅓. Costal index about 3.7; 4th vein index about 1.2; 5x index 0.9.

Body length (pinned female) about 3.2 mm., wing, 2.5 mm.; ♂ a trifle smaller. The eggs have two pairs of thin filaments.

Distribution and types.—Holotype male, 9 paratypes and 16 other specimens, Turrialba, Costa Rica, Oct. 1955, W. B. Heed collector, from flowers of *Dimerocostus* (Zingiberaceae); an additional 18 specimens, Cerro La Campana, Panama, Aug. 1956, from flowers of *Costus* (same family).

Drosophila reticulata Wheeler, new species.

External characters of imagines.

♂, ♀. The wing of this very beautiful fly is shown in Figure 23, and is sufficiently unusual that a new genus is suggested. Many other features, however, are not so bizarre, and we doubt if this species is more than subgenerically distinct.

Arista with 6 dorsal and 2 ventral branches in addition to the fork; front broad, dull yellowish white, strongly sloping so that it and the face are continuous; head bristles normal; proclinate orbital ⅔ length posterior reclinate, the middle orbital minute. Antennae tan, 3rd joint elongate; face whitish yellow, carina evenly rounded. One prominent oral; cheek of medium width, brown below eye, creamy yellow posteriorly; clypeus pale, rather large. Palpi elongate and pointed, black at base, white at apex, the separation between the two colors nearly longitudinal. Eyes elongate oval with long white pile.

Mesonotum rather tan to brown with shining bluish reflection; acrostichal hairs 4-rowed, sparse and thin, difficult to see; no prescutellars. Basal scutellars divergent; dorsocentrals normal. Pleura whitish with 5 brown spots, variable in intensity: just below and behind humerus, on lower middle of mesopleura, on upper sternopleura, below wing base, and over posterior spiracle. Halteres pale. Legs, including coxae, pale yellow but the "knees" darkened, and front femur with a small brown mark on inner side just beyond middle.

Abdomen of female with broad apical bands expanded in the middle to base of previous tergite where they form narrow basal bands; remaining areas pale

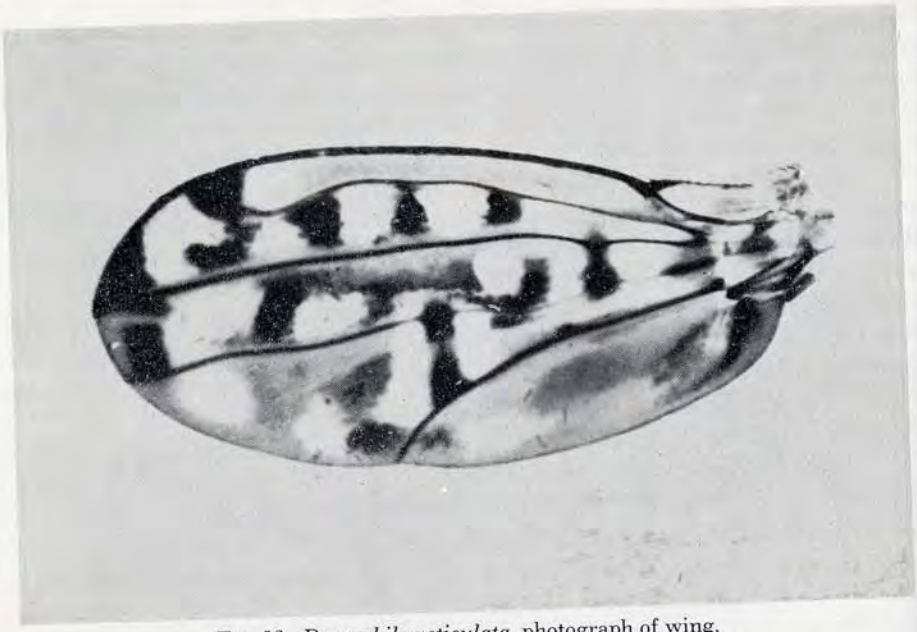


FIG. 23. *Drosophila reticulata*, photograph of wing.

yellowish; laterally the apical bands gradually disappear but the basal ones become a little larger. In the male the apical bands of the basal tergites continue to the sides, the basal bands are smaller, and tergite 6 has no basal band.

Wings glassy and wrinkled, with the pattern shown in Figure 23; the darker spots in submarginal and posterior cells produced as bullae. Apex of 1st costal section with two bristles; 3rd section with the small black bristles on the basal $\frac{2}{5}$–$\frac{1}{2}$. Costal index about 2.8; 4th vein index about 1.7; 5x index about 1.2.

Body length (pinned female) about 3.0 mm., wing, 3.3 mm.; ♂ smaller.

Internal characters of imagines.

Anterior Malpighian tubes with the common stalk $\frac{1}{3}$ their total length; posterior tubes with their tips apposed, but without a continuous lumen. Ventral receptacle short, with 2–3 tight coils basally, remainder uncoiled. Spermathecae with very small chitinized centers, the surrounding glandular portion unusually large and frothy in appearance. Parovaria long and elliptical. Eggs with 2 pairs of filaments, the anterior pair $\frac{2}{3}$ egg length, posterior pair $\frac{3}{4}$ egg length; in the ovaries examined there was but a single developed egg in each ovary.

Distribution and types.—Holotype male, and 3 paratypes, Medellin (30 klm. northwest), Colombia, 8000 ft., Nov. 1955, W. B. Heed collector. Other paratypes as follows: 3, Bucaramanga, Colombia; 2, Rionegro, Colombia; 4, Rancho Grande, near Maracay, Venezuela. Eight specimens from Ecuador differ slightly in wing pattern are not being considered paratypes.

GENUS CHYMOMYZA

Chymomyza bicoloripes (Malloch)

Drosophila bicoloripes Malloch, 1926. P. U. S. N. M. 68 (21):31.

The generic combination used here has never been made officially although it has been used in an article on the moldy rot of rubber trees in Costa Rica (J. B.

Carpenter. 1954. Plant Disease Reporter 38(5): 334–337), the determination of the flies being credited informally to Dr. W. W. Wirth. Carpenter reports that the species has been found constantly associated with active moldy rot lesions and saprophytic colonies of the mold *Ceratostomella fimbriata*, and it was suspected that the flies were primarily responsible for the spread of the mold.

There has been some confusion concerning the type locality. Dr. Paul H. Arnaud, U.S.D.A., has been very helpful in settling this question. The type (♂) bears the labels: "Las Cascadas, Canal Zone, Panama"; "A. H. Jennings Collector"; and "Type No. 28467, USNM." Dr. Arnaud states that "I am inclined to believe that somehow Malloch incorrectly stated the type locality as Higuito, San Mateo, Costa Rica (P. Schild) both with the original description and in the type book."

Dr. Arnaud reports that the USNM collection possesses, in addition to the type (which we have seen), 30 specimens from La Francia, Costa Rica, 11 from Turrialba, Costa Rica, and 4 from Panama. Our own field collectors have taken this species on Barro Colorado Island, Canal Zone, "off fresh cut log," and at Villavicenico, Colombia, in August and September.

Malloch's species is clearly a *Chymomyza* although it is unusual in having a strong black cloud over the posterior crossvein and in lacking a row of femoral spines. *Chymomyza maculipennis* Hendel (1936. Ann. Naturhist. Mus. Wien. 47:97) from the Amazon region of Brazil is, judging from the description, most likely the same species. However, he describes the pleura as lighter than the mesonotum, and we are unable to interpret satisfactorily his description of the 2nd vein as "*winkelig gebrochen.*"

Chymomyza mexicana Wheeler

Chymomyza mexicana Wheeler, 1949. Univ. Texas Publ. 4920:162.

The description was based upon a male from Puebla, Mexico. We have now seen five additional specimens as follows: 4, Santa Maria de Ostuma, Nicaragua, June 1954 (W. B. Heed); 1, Volcan Boqueron, El Salvador, 4500 feet, July 1954 (W. B. Heed). The present specimens do not agree exactly with the description, especially the pigmentation pattern of the front, but we believe that they are conspecific.

Genus Cladochaeta

Cladochaeta nebulosa Coquillett

Cladochaeta nebulosa Coquillett, 1900. P. U. S. N. M. 22:264.

This species is apparently quite widespread on both the mainland and the islands of the Caribbean region. We have seen 18 specimens from the USNM collection, including the type from Puerto Rico, from Mexico (Vera Cruz, Tampico), Panama (Ft. Clayton, Darien Pr.), and Puerto Rico (many localities). Our collectors have saved more than 50 specimens, from Mexico (San Luis Potosi); El Salvador (San Salvador, Santa Tecla, Laguna de Zapotitan); Nicaragua (El Recreo); Colombia (Medellin, Bucaramanga); Trinidad (Sangre Grande); Venezuela (Merida); St. Lucia; Jamaica; Haiti (Kenscoff); and Cuba (Santiago de Cuba). It has also been recorded from Florida and from the Virgin Islands (St. Croix).

We have never been successful in culturing this species in the laboratory.

Genus Neotanygastrella

Neotanygastrella nigricosta (Malloch), new comb.

Drosophila nigricosta Malloch, 1926. Proc. U. S. Nat. Mus. 68 (21):30.

I have examined the type female from Costa Rica (U.S.N.M.) and a second female from El Recreo, Nicaragua (June 1954, W. B. Heed). There can be no doubt about the generic reference, although the anterior reclinate orbital is a little larger than usual, the face less bulbous below, and the legs all yellow. The large postverticals and absence of femoral spines serve to separate it from *Chymomyza* which it superficially resembles. The very dark costal and marginal cells are quite distinctive.

Neotanygastrella antillea Wheeler, new species.

External characters of imagines.

♂, ♀. Similar in general appearance to *leucopoda* and *boliviensis* (see Frota-Pessoa and Wheeler, 1951), differing most obviously as follows: 2nd antennal joint black, 3rd whitish yellow; palpi yellow; face with a shiny brownish black longitudinal stripe bordered by white on each side, the broad whitish areas beginning beneath the antennae and continuing down around the cheeks which gradually become black posteriorly.

Front velvety black, strongly whitish pruinose posteriorly when viewed from certain angles; ocelli on a raised prominence, placed rather far forward, nearly equidistant between postverticals and ptilinal suture. Anterior reclinate orbital small, about ⅓ length proclinate and placed slightly to the side of and anterior to the latter. Arista with 4 dorsal and 2 ventral branches in addition to the fork. Clypeus dark, small; proboscis dark apically.

Mesonotum dark brown to blackish, rather shiny but with thin gray pollen when seen from the side. Scutellum black, velvety, with yellowish apex. Pleura and prosternum dark brown. First legs with white coxae, trochanters, and 4 apical tarsal segments, and with black femora, tibiae and metatarsi. Coxae of 2nd legs also black, remainder of legs yellow.

Abdomen uniformly dark brown to black; 1st tergite and part of 2nd yellowed. Anal plates of female yellow, those of male rather small, yellow. Halteres whitish. Wings clear. Costal index about 1.4; 4th vein index about 3.8; 5x index about 1.8.

Body length (pinned specimen), ♀, about 2.5 mm.; wing, 2.0 mm.; male slightly smaller.

Distribution and types.—Holotype male and 6 paratypes of both sexes from Jamaica, British West Indies, collected February 1956, by Dr. W. B. Heed. Approximately 50 additional specimens were taken by Dr. Heed in the Montego Bay area of Jamaica in July 1957.

Neotanygastrella ornata Wheeler, new species.

External characters of imagines.

♂, ♀. Similar to *tricoloripes* Duda but with mesopleura velvety black and strongly contrasting with the pale tan sternopleura, and with more extensive

darkening on the mesonotum. Arista with 4 dorsal and 2 ventral branches in addition to the fork. Front velvety black, whitish pruinose behind when seen from most angles. Antennae pale tan. Face bulbous below, with a broad black median stripe, narrowly yellow along sides; clypeus black, palpi yellow, cheeks yellow becoming black behind. One strong oral. Postverticals strong; anterior reclinate orbital ½ length proclinate and placed lateral of and slightly anterior to the latter.

Mesonotum basically tan with a broad blackish central stripe between the dorsocentral lines, widening anteriorly to reach humeri; it is also black above and in front of the wing base thus leaving an irregular, winding yellow area from humerus (and fore coxa) upwards and then back to dorsocentrals. Acrostichals irregularly 8-rowed; no prescutellars. Basal scutellars parallel. Scutellum velvety black with white tip. Postscutellum dark brown, this color continued laterally to around haltere base; haltere pale. Legs yellow except: fore coxae whitish, fore femur, tibia and metatarsus black, the 4 apical joints white.

Abdomen black, a bit velvety, the basal two tergites paler. Wings clear; 3rd costal section with the small black bristles on the basal ⅔. Costal index about 1.3; 4th vein index about 3.6; 5x index about 2.4.

Body length (pinned specimen), ♀, 2.0 mm.; wing, 2.0 mm.; males a little smaller.

Distribution and types.—Holotype male and one paratype female, from Merida, Venezuela, collected in October 1956, by Dr. Marvin Wasserman.

Neotanygastrella tricoloripes Duda

Neotanygastrella tricoloripes Duda, 1925. Ann. Mus. Nat. Hung. 22:224.

Duda's specimens came from Costa Rica; our own collections have shown that this species is widely distributed: Honduras, Costa Rica, Panama, Colombia, Trinidad, Peru, Brazil, and Puerto Rico (El Yunque). Judging from the 70+ specimens in our collection, we are unable to find any consistent differences from *N. brasiliensis* (Frota-Pessoa), but a careful comparison of male genitalia has not yet been made.

Neotanygastrella chymomyzoides Duda

Neotanygastrella chymomyzoides Duda, 1927. Arch. Naturg. 91A 11–12 (1925):71.

The species was described from specimens from Peru and Bolivia; I have examined one specimen (American Museum of Natural History) labelled "Cotypus" from "Bolivia-Mapiri." We have preserved about 180 specimens of this species and have noticed that while most of the specimens show the typical color pattern of the front legs (metatarsus black, apical joints pale), about 30 of them have all of the fore tarsi dark. The significance of this is not yet known. In the following distributional list, starred countries are those from which dark-legged specimens were taken: El Salvador, Nicaragua, Honduras, Costa Rica,* Panama,* Colombia,* Venezuela, Trinidad, Brazil,* Ecuador,* Peru,* and St. Lucia (B. W. I.).

Genus Dettopsomyia

Dettopsomyia formosa Lamb

Dettopsomyia formosa Lamb, 1914. Trans. Linn. Soc. London 16:350.

This very small, pretty species is apparently quite widespread. Lamb's material came from the Seychelles, and it is also known from Hawaii, the Palau and Solomon Islands, Yap, and the Philippines. We can now report it from Central America where Dr. Heed collected about 70 specimens as follows: El Salvador (Santa Tecla, Laguna de Zapotitan); Honduras (La Lima). Collections were made in March and May, 1954. Live specimens from El Salvador were kept in the laboratory for a short while; the eggs had 4 short filaments, each about ⅓ the egg length.

Genus Clastopteromyia

Clastopteromyia superba (Sturtevant)

Drosophila superba Sturtevant, 1916. Ann. Ent. Soc. Am. 9:342.

I have examined the type from Guatemala (USNM collection); it agrees quite well with the 11 specimens taken by our collectors. Localities are: El Salvador (San Salvador, Santa Tecla, Laguna de Zapotitan); Honduras (Lancetilla); Costa Rica (Turrialba, La Lola); Colombia (El Recuerdo). We have also examined 3 specimens (USNM) from the Panama Canal Zone.

The description states that only a single pair of dorsocentrals is present; on our specimens when the anterior dorsocentrals are missing, one can still detect the empty socket, indicating that two pairs are normally present.

Clastopteromyia longipennis Malloch

Clastopteromyia longipennis Malloch, 1926. Proc. U. S. Nat. Mus. 68:34.

The type, from Costa Rica, shows that the very dark brown prosternum, which contrasts strongly with the pale legs, is a very distinctive feature. We have 10 specimens from: El Salvador (San Salvador), Honduras (Lancetilla), Costa Rica (San Isidro de General), and Canal Zone (Barro Colorado Is.). One individual, from Trinidad, appears to be an undescribed species very closely related to *longipennis*.

Clastopteromyia guttipennis (Duda)

Diathoneura guttipennis Duda, 1925. Ann. Mus. Nat. Hung. 22:171; 1927. Arch. Naturg. 91A 11 (1925):91.

Clastopteromyia guttipennis, Frota-Pessoa, 1947. Sum. Brasil. Biol. 1:36.

Duda described the species from 2 ♂♂, 1 ♀ from Costa Rica and later recorded 1 ♂, 1 ♀ from Peru. We have seen a single specimen from Almirante, Bocas del Toro, Panama (USNM) which we identify as *guttipennis* although it shows these small differences: marginal cell of wing with an additional white spot, rather small, nearly in line with the posterior crossvein (thus between the two prominent white stripes of the wing); halteres somewhat darkened and

palpi definitely brownish; face and orbits without noticeably whitish areas along edge.

Clastopteromyia bomplandi (Malloch)

Diathoneura bomplandi Malloch, 1934. Dipt. Patag. Chile 6:438.

Clastopteromyia bomplandi, Frota-Pessoa, 1947. Sum. Brasil Biol. 1:18.

We have a single specimen, from San Salvador, El Salvador, which extends the known distribution considerably. Malloch's type and paratype came from Bompland, Argentina, and Frota-Pessoa reports it from Brazil. The species is quite similar to *taeniatipennis* Duda from Costa Rica, but the latter has considerable darkening along the leading edge of the marginal cell, and the other dark areas are much more prominent than in *bomplandi*.

Clastopteromyia aberrans Wheeler, new species.

The species described here differs from the typical members of the genus in (1) the absence of posterior scutellar bristles and (2) the presence of only two rows of acrostichal hairs. In other respects it appears to be fairly closely related to other members of this genus. The highly marked wings are rather similar to those of *guttipennis* and *borgmeieri*.

♂, ♀. Head a little broader than thorax, front longer than broad, light brown but darker in front and more tannish behind; orbits rather long, subshining, rest of front pollinose. Middle orbital small, at most ¼ length proclinate and placed nearly to the side of the latter; proclinate about ⅚ length posterior reclinate. Ocellars long; post-verticals long, about ⅔ length ocellars; inner verticals unusually long, the outer ones only about half their length. Antennae light brown; 2nd segment with 2 stronger bristles; 3rd segment small, with thick pale hairs. Arista with 4–5 dorsal branches, 2 ventral branches, in addition to the fork.

Face whitish yellow with a diffuse dark brown mark centrally just above clypeal margin; face not wholly flat, but with a low median ridge which disappears at the dark central mark. Vibrissa single, the following oral hairs inconspicuous. Cheek very narrow, pale along eye, narrowly darkened along lower margin. Clypeus tan to brown; palpi elongate, blackish, with a stout apical bristle.

Mesonotum subshining brown with a pair of semi-distinct tannish longitudinal stripes which disappear posteriorly, the stripes located just within the dorsocentral rows, leaving a median darker stripe. Acrostichal hairs in two sparse rows (about 8 hairs per row) placed on the median dark stripe; no prescutellars. Two pairs of dorsocentrals, both well-developed, the anterior ones rather far forward, just behind transverse suture; some hairs of dorsocentral row more developed, especially one just anterior to transverse suture. Scutellum brown; basal bristles quite long, in the usual position; no apical scutellars. Pleura all brown; 1 humeral; 1 long presutural; 2 notopleural; no propleurals or mesopleurals; 2 sternopleurals, the anterior one about 0.6 length posterior. Knob of haltere white, the basal joints darkened.

Legs long and slender; 2nd and 3rd femora dark brown to black, and 3rd tibiae often somewhat darkened, the rest of the legs tan to light brown. An api-

cal bristle visible on 2nd tibia, a preapical present on all three. Abdomen slender, uniformly shining black.

Wings with striking color pattern of dark brown and white areas (Fig. 24). Costa reaches 4th vein; 2nd vein somewhat undulating; apices of 3L and 4L bend backwards to reach costa. Anal angle wholly absent. Costal index about 1.7; 4th vein index about 1.4; 5x index about 0.8 (unusually low due to the long posterior crossvein). Dr. Heed, the collector, records in his field notes that the flies wave their wings like members of the genus *Chymomyza*.

Distribution and types.—Holotype male and 3 paratypes, from Kenscoff, Haiti (4000 feet), collected February 1956, by Dr. W. B. Heed.

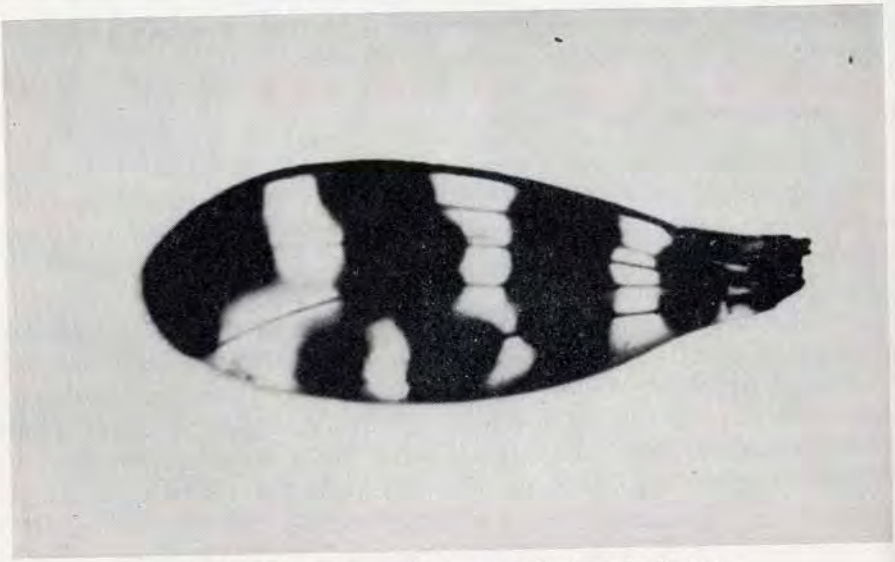


FIG. 24. *Clastopteromyia aberrans*, photograph of wing.

Clastopteromyia albinota Wheeler, new species.

External characters of imagines.

♂, ♀. A very distinctive species, with pale yellow to milky white mesonotum, scutellum, front and face, but dark brown abdomen and pleura, the latter with a narrow longitudinal yellow stripe. Arista with 4–5 dorsal and 3 ventral branches in addition to the fork. Mesonotum, scutellum and front pale yellowish with a dull whitish surface, the whitish appearance less extreme on some individuals. Postverticals minute; proclinate and posterior reclinate orbitals equal in size, a middle one not evident. Second antennal joint yellow, 3rd darker. Face yellow to whitish, darker at vibrissal base and sometimes darkened more extensively. Palpi black; cheeks pale behind the vibrissal area, narrow.

Acrostichal hairs in 6 irregular rows; no prescutellars; 2 pairs of dorsocentrals; basal scutellars divergent. Scutellum rather rounded above, wholly pale yellow to whitish, including sides and underside. Pleura dark brown with a narrow pale stripe from base of 1st coxa, along meso-sternopleural suture to haltere base; halteres brown. Anterior sternopleural thin, about 0.6 length posterior one, a middle one not developed. Legs tan, 1st coxae usually, and all femora sometimes

darker. Base of hind metatarsus with two black bristles below. Abdomen dark brown, without pattern.

Wings uniformly brown. Costal index about 2.0; 4th vein index about 1.7; 5x index about 2.0.

Body length (pinned specimen), ♂ and ♀, about 2.0 mm.; wing, 2.0 mm.

Distribution and types.—Holotype male, Cerro La Campana, Panama (about 60 klm. SW Panama City), collected August 1956, by W. B. Heed, H. L. Carson, and M. Wasserman. Two paratypes, same data; 8 paratypes, Barro Colorado Is., Canal Zone. We have, in addition, about 35 specimens from: Nicaragua, Honduras, Costa Rica, Colombia, Venezuela, Trinidad, and Brazil.

Genus Paraliodrosophila

Paraliodrosophila antennata Wheeler, new species.

External characters of imagines.

♂, ♀. Arista with 4 dorsal, 1 ventral branch in addition to the fork. Front dark brown to black, the large shiny frontal triangle gradually tapering to a point at the ptilinal suture; orbits also shiny, rest of front velvety brown. Second antennal joint whitish yellow, strongly contrasting with the dark front, and with the brown 3rd joint. Postverticals rather small; proclinate and posterior reclinate orbitals equal in size, the middle one about ⅓ as long. Face dark tan, the carina of moderate size, rather short; oral margin dark brown as is the area around the vibrissae; cheeks white posteriorly. One strong oral. Clypeus black, dull; palpi small, light brown.

Mesonotum dark brownish black, shining; acrostichal hairs in 6 rows, absent posteriorly from the level of posterior dorsocentrals. No prescutellars; basal scutellars convergent. Scutellum rather rounded, more pollinose than mesonotum. Anterior dorsocentral short, about ⅓ posterior. Pleura pale whitish yellow except for a narrow area above, which is velvety black beginning near base of 1st coxa and going to wing base. Basal joints of halteres brownish, the knob tan. Legs wholly yellow.

Abdomen with large dark bands, semi-shining, with creamy yellow areas; on both male and female tergites 1–3 are paler medianly, 4–5 are dark in the middle, leaving basal yellow areas on each side, that of 5 extended all the way laterally; remaining tergites dark, but all dark bands cease before reaching lateral edge, the edges thus all yellow. Anal plate of ♀ yellow; ovipositor pale, truncate, with dark teeth.

Wings hyaline, a little darkened below distal costal break. Small black bristles of 3rd costal section poorly differentiated, but occur on about the basal 0.6. Costal index 1.4; 4th vein index about 2.5; 5x index about 3.0.

Body length (pinned specimen), ♀, up to 2.2 mm.; wing, about 2.4 mm.; males smaller.

Distribution and types.—Holotype male and 9 paratypes, from near Bath, Jamaica, B. W. I., collected February 1956, by Dr. W. B. Heed. There are six additional specimens from the same locality.

Genus Amiota

The type species is *leucostoma* Loew, designated by Coquillett (1910. Proc. U. S. Nat. Mus. 37 (No. 1719): 505) and not *humeralis* as I have stated earlier (Wheeler, 1952. Univ. Texas Publ. 5204:166).

Amiota humeralis Loew

Amiota humeralis Loew, 1862. Berlin Ent. Zeit. 6:229 (Cent. II, No. 93).

An examination of the holotype female, in the collection of the Museum of Comparative Zoology at Harvard, shows that my earlier concept of this species was in error (Wheeler, *op. cit.*, p. 168). The specimen is in fair condition, but, aside from size, shows only one outstanding feature that may serve to recognize it: the front is noticeably golden pruinose when viewed from most angles. The only other species known to me with this character is *setigera* Malloch, of which we have 1 ♂ from Rutledge, Tenn. and 1 ♂ from Lithium, Mo. Of the 9 females from Lithium which we consider are the females of *setigera*, some show the pruinosity and others have it less developed. There is a possibility, then, that the male of *humeralis* is *setigera*, but for the present there seems to be no way to verify this.

Amiota steganoptera Malloch

Amiota steganoptera Malloch, 1926. Proc. U. S. Nat. Mus. 68:31.

This species seems not to have been recorded since its description, but we find that it has a very extensive distribution. It is easily recognized by the presence of thorn-like "warts" on the 3rd costal section, quite like those characteristic of *Leucophenga* and *Stegana*.

The type, from Higuito, San Mateo, Costa Rica (USNM) has been examined. We have seen 30 additional specimens as follows: 1, Saucier, Miss.; 1, Kushla, Ala.; 1, Blacksburg, Va.; Mexico; El Salvador; Nicaragua; Honduras; Costa Rica; Panama; Brazil; and Puerto Rico (Rio Grande). It should be mentioned that there are at least three undescibed species closely related to *steganoptera* occurring in Brazil.

Genus Pseudiastata

Sabrosky (1951) summarizes all that is known about this rare genus. All of the species so far recorded are quite similar to the type species, *nebulosa*, but there are obvious differences in male genitalia. Among the specimens loaned to us from the National Museum collection were four such individuals, and we borrowed another from the American Museum of Natural History. The latter, from Guatemala, is a female, and cannot be determined further. Two are males, and have been dissected and examined. One, from Cano Saddle, Gatun Lake, Panama, is *pseudococcivora* Sabrosky; the other male, from Cuernavaca, Mor. ,Mexico, has a genitalial arrangement not figured by Sabrosky. This genitalia is shown in Figures 15–16, but the species is not being named at the present time.

The remaining two specimens are females, and have an entirely different wing pattern; further, the 3rd vein is bristled, a character not previously known in the Drosophilidae. These are described below as a new species, *P. armata*.

Pseudiastata armata Wheeler, new species.

With the general appearance of *nebulosa*, but with clearly different wing pattern (Fig. 25). Many stout prescutellar bristles, the middle pair quite long and reaching to scutellar apex, the rest (about 6) only half this long. Fringe of calyp-

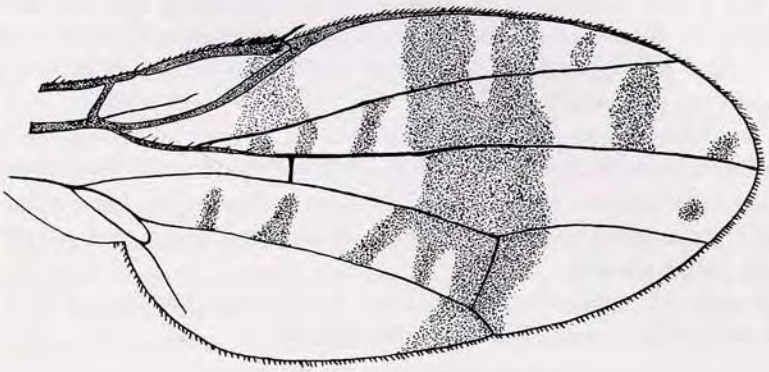


FIG. 25. *Pseudiastata armata*, wing.

ter especially long. Ovipositor with numerous pale hairs. All femora rather thick, the 3rd ones especially so, nearly globose. Third vein bristled, both above and below, from the bifurcation of 2L and 3L to just beyond the anterior crossvein, with about 6 bristles visible dorsally on the best-preserved wing, and 3 ventrally: 1 at the bifurcation, 1 just before anterior crossvein, and 1 about midway between the other two. Second wing of this specimen missing. On female 2, the wings are bent downward in such a way that the ventral surfaces cannot be studied, and the upper sides are considerably rubbed. However, on one wing there are 6 empty sockets dorsally plus 4 small bristles, the distal one just a little beyond the level of the posterior crossvein. It is apparent, therefore, that the actual distribution of these bristles is variable.

Distribution and types.—Holotype ♀, USNM collection, labelled "Mojinga Swamp; Ft. Sherman, C.Z." (Canal Zone, Panama); "6–17–52"; "F. S. Blanton Collector." Paratype ♀, same locality, collected 5–13–52.

GENUS LACCODROSOPHILA

Duda erected the genus for two species from Bolivia, and it seems not to have been recorded since. Since he did not designate a type species I am selecting his first species, *L. flavipes* Duda, as the type species.

Laccodrosophila flavipes Duda

Laccodrosophila flavipes Duda, 1927. Arch. Naturg. 91A 11 (1925):38.

Duda described the male only; we have a female (AMNH collection) with the following labels: "Minza Chica; V. Tungurahua; 3750 M.; Ecuad.; 2–14 IV, 1939; F. M. Brown."

The legs are all yellow; front and mesonotum black; pleura brownish black, subshining; antennae, face and palpi yellow. Postverticals large. Ocellars ap-

parently divergent, placed outside the ocellar triangle, in line with anterior ocellus; 3 orbitals extra thin, nearly equidistant from one another. Halteres yellowish white. Empodium and pulvilli unusually large. Front tarsi appearing to consist of but 4 joints (see Duda's figures 6 and 8) but as seen under higher magnification (120X) it can be observed that the apparent "metatarsus" consists of the fused 1st and 2nd joints, each of which bears a pair of stout black spines apically.

Two pairs of dorsocentrals, thin. Scutellum with marginal hairs, arising from the lower edge and upturned; both basal and apical scutellars arise from short black tubercles. Prescutellars present.

Duda described the male as having the 3 basal tergites black, 4th black at basal angles, otherwise reddish yellow, as is the 5th and 6th. On this female the tergites are yellow with narrow apical bands, broader at the angle. Anal plates yellow, long and flat; ovipositor long and hairy, of unusual shape and partly encased within a tubular sheath.

Wings pale, the posterior fringe long. First vein, dorsally, with 1–2 black bristly hairs just before its union with the costa.

Laccodrosophila atra Duda

Laccodrosophila atra Duda. 1927. Arch. Naturg. 91A 11 (1925):40.

Duda saw 4 ♂ ♂, 3 ♀ ♀ from Bolivia; we have a female which we tentatively identify with this spcies, from the vicinty of Bogota, Colombia (Nov. 1955, W. B. Heed), "off pink *Digitalis* flower." In this species the face is black as are the palpi and proboscis; abdomen shining black; arista with only a single dorsal branch plus the large fork. The scutellar margin is haired as in the former species, and the scutellars also arise from small tubercles. The legs are thicker, mostly black with yellowish tarsi. First tarsal joint of leg 1 with a very stout spine plus 7–10 smaller ones, the 2nd joint with a single smaller spine.

Laccodrosophila flavescens Wheeler, new species.

External characters of imagines.

♂, ♀. Quite similar to *flavipes*, differing primarily in the shape and color of the face and cheeks, and the much more yellowed abdomen. Face narrow with deep foveae separated by a strong ridge, the latter blackened, the foveae pale; the dark ridge is continuous with a dark structure on anterior surface of proboscis. Cheeks broad, the bristled area dark, the remainder tan (♀), shiny next the eye, becoming striated next to the bristled area; lower posterior corner dark reddish brown (♀), strongly rugulose, the creases parallel to lower cheek margin. In males, dark areas are more black, the non-striated portion of the cheek much darker than in females. Postverticals ⅔ length ocellars, latter long. Orbitals on the best preserved specimen flattened against head, proclinates mildly convergent, middle reclinates somewhat diverging, posterior reclinates inclined outward over the eye. One pair of strong orals.

Abdomen mostly yellow, wholly yellow on males, only the genital arch brownish; in females tergites 4–5 a little darker laterally, the apex shiny brown, including sides of sheath of ovipositor. Legs yellow, both sexes with 1st tarsal spines as in *flavipes*.

Wings broad, hyaline with yellow veins; apex of 1st vein a bit thickened and discolored at its union with costa, without hairs; costa somewhat pectinate, with small sparse black spines. Costal index about 1.3; 4th vein index about 2.0; 5x index about 0.9.

Body length (pinned female with extended ovipositor) about 4.5 mm.; wing, 4.0 mm.; ♂, 3.5 mm., wing, 3.7 mm.

Distribution and types.—Holotype ♂, 2 ♂, 1 ♀ paratypes, USNM collection, labelled: Pedregal, near Pobre Pena, Mexico, 14 Sept. 1942, W. F. Foshag; 1 ♀, USNM, L. Patzcuaro, Mich., Mexico, 29 Aug. 1945, W.F. Foshag.

Laccodrosophila heedi Wheeler, new species.

External characters of imagines.

♂, ♀. A shiny black species with broad cheek, unusually low costal index, and with the apex of 1st vein strongly blackened. Front black, rather shiny, with numerous small hairs; posterior reclinate orbital about 5/6 length proclinate, middle orbital 1/2 length posterior. Antennae and palpi dark tan; arista short, with one basal dorsal branch and one more distal ventral branch in addition to the fork. Face deeply sunken, without interfoveal ridge; one strong oral; oral margin, clypeus and cheeks black, the latter very broad, mostly shining, but with a shagreened quality on under side.

Mesonotum and scutellum black; acrostichals typical for this genus; prescutellars strong; scutellars tuberculate; scutellar margin typically haired. Pleura shining black; mesopleura with scattered small hairs on upper part (absent on *flavipes*, *flavescens*, and *atra*); legs mostly shining black including coxae. Tarsi of fore legs bearing three stout black spines (Fig. 12), fore tarsi black, other tarsi yellow except for blackened apical joint. Empodia and pulvilli large, pale.

Abdomen shining black; ♀ anal plate long, flat, yellow; ♂ anal plate not well observed but apparently pale. Wings hyaline, veins pale. Apex of 1st costal section, and apex of 1st vein enlarged and blackened.

Body length (pinned female with retracted ovipositor) 3.0 mm., wing, 2.5 mm. Male smaller. Costal index about 0.55, the 2nd costal section being only about half length 3rd; 4th vein index about 2.6; 5x index 2.0.

Distribution and types.—Holotype ♀, paratype ♂, from orchid flowers, orchid garden, Medellin, Colombia, Nov. 16, 1955, W. B. Heed collector.

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VIII. A Parthenogenetic Strain of *Drosophila mangabeirai* Malogolowkin

H. L. CARSON[1], M. R. WHEELER, AND W. B. HEED[2]

INTRODUCTION

Reproduction in the genus Drosophila has long been considered to be exclusively a biparental process. No exceptions to this rule have been recorded in the extensive treatises on the taxonomy, genetics and evolution of the genus (*e.g.*, Sturtevant, 1921; Patterson and Stone, 1952). Recently, however, Stalker (1954) has found that a number of normally bisexual species show a low rate of development of unfertilized eggs. So far only two species, *D. parthenogenetica* and *D. polymorpha* have been successfully maintained unisexually for an extended series of generations (25 or more) in the laboratory. Parthenogenesis is probably not important in the natural populations of either of these species, however, because both are normally bisexual and the rate of development of unfertilized eggs is low. Thus, in *D. parthenogenetica*, only about 1.5% of the eggs laid by virgin females develop into adult offspring.

The present paper records the existence of a wholly parthenogenetic strain of the little-known Central and South American species, *Drosophila mangabeirai* Malogolowkin (1951). The single strain of this species now being maintained in the laboratory displays about 60% hatchability of unfertilized eggs; of these about 80% survive to the adult stage. The strain is thelytokous and over a period of more than a year and a half has not been observed to produce any males.

TAXONOMY AND DISTRIBUTION

Drosophila (Sophophora) mangabeirai Malogolowkin (1951:432) was described from a female collected July 19, 1951 near the city of Salvador, State of Bahia, Brazil. Eighteen additional specimens, including one male, have been collected by one of us (WBH) but our only culture (stock H64.20) originated from a single female from El Salvador. We are especially indebted to Dr. O. Frota-Pessoa of Rio de Janeiro who compared the holotype with some examples of our flies and confirmed that they are identical, to Miss Chana Malogolowkin who studied the genitalia of the male specimen, and to Dr. Frances Clayton who prepared the photographs shown in Figure 1.

The following descriptive notes summarize the available information about this species.

External characteristics. A dull tan species with reduced abdominal pigmentation. Front dull tan, nearly devoid of hairs; orbits with 5–6 hairs anterior to the orbital bristles, and front with a small cluster of 6–8 postlunular hairs. Arista with 6 dorsal and 3 ventral branches in addition to the terminal fork; 3rd antennal joint with the hairs rather longer than usual. Carina exceptionally narrow and high, the upper surface flattened but not sulcate. Two nearly equal orals; cheeks very narrow; palpi with a strong apical bristle, turned outward,

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and with about 2 smaller bristles along outer side. Pseudotracheae of proboscis 5/5.

Acrostichal hairs in about 8 irregular rows; no prescutellars. Basal scutellars weakly convergent, sometimes straight. A strong posterior sternopleural, the middle one smaller (about 2/5 posterior), the anterior one still smaller (1/3 middle one and 1/6 posterior one). Third costal section with the small black bristles on its basal 2/3; costal index about 2.1; 4th vein index about 1.8; 5x index about 1.3. Legs pale tan; first femur (Figure 1) with a row of minute

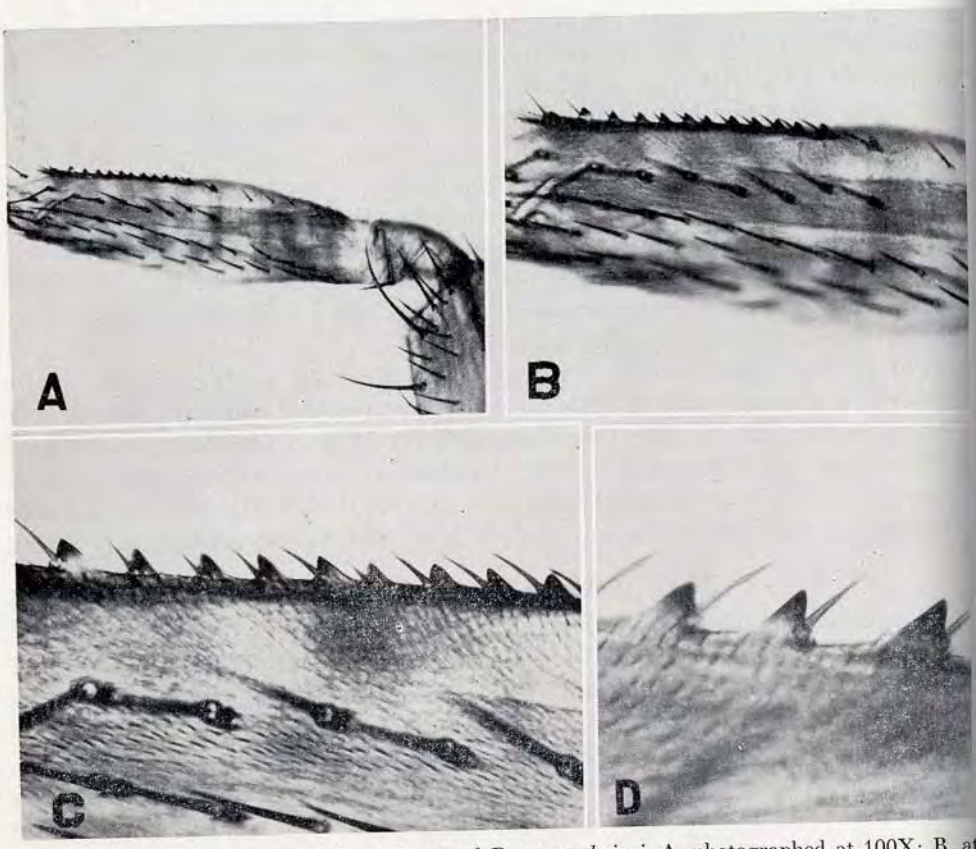

FIG. 1. Photomicrographs of front femur of *D. mangabeirai*. A, photographed at 100X; B, at 200X; C, at 475X; D, at 900X. Photographs by Dr. Frances Clayton.

spines, 10–12 in number, along the inner apical side, each spine subtended by a hair (seen only with high magnification). These femoral spines, shaped somewhat like shark's teeth, appear to be derived from a portion of the rim of the socket belonging to the hair.

Other characteristics. *Malpighian tubes* with free ends, the posterior pair with their tips not apposed. *Ventral receptacle* wound back and forth like a skein of wool, thick at the base, thinner beyond. *Spermathecae* (figured by Malogolowkin, *op. cit.*) with brownish centers, a bit narrowed basally and with several basal sulci. *Eggs* with two short, blunt, rather thick filaments, not expanded at the tips, the filament length about 1/4 the egg length. *Puparia* with the anterior spiracles

sessile, each with about 14 branches arranged radially, dark in the center and pale whitish peripherally; posterior spiracles pale, divergent.

Relationship. Belongs to the willistoni group of the subgenus Sophophora. On the basis of the original female, Malogolowkin placed the species in the melanogaster group and compared it with *ananassae* which it resembles in some respects. The genitalia of our only male specimen have been dissected and studied by Miss Malogolowkin; the detailed results of this study will be published by her elsewhere, but she has graciously supplied us with the following preliminary information. The hypandrium and the gonopophyses are similar to *bocainensis*, but with several differences. The genital arch is close to that of *bocainensis* and *sucinea*, while the penis is different from all other members of the group, being most like *bocainensis* and *nebulosa*.

Judging from the description, *Drosophila kerteszina* Duda (1925. Ann. Mus. Nat. Hung. 22:222) from Costa Rica may also be closely related to *D. bocainensis*.

Distribution and ecology. *D. mangabeirai* is so rare in collections that its habitat and food preferences are poorly known. In all, just 19 individuals (18 ♀♀, 1 ♂) have been collected; their collection records are given in Table 1. A majority of the specimens was taken by sweeping vegetation, but some were taken on fruit and over fallen flowers as shown in the table.

The ravine *(barranco)* at San Salvador, from which 10 of the specimens were taken, deserves special comment. It originates on the nearby Volcan Boqueron,

TABLE 1

Record of collections of *D. mangabeirai*

Locality	Date	Number	Habitat	Remarks
El Salvador:				
San Salvador, 2000 ft.	Dec. 17, 1953	2 ♀♀	ravine	
	Dec. 19, 1953	5 ♀♀	ravine	
	Jan. 20, 1954	1 ♀	ravine	
	Mar. 2, 1954	1 ♀	ravine	
	Sept. 27, 1955	1 ♀	ravine	origin of parthenogenetic stock
Honduras:				
Lancetilla, 100 ft.	Apr. 4, 1954	1 ♀	orchard	on mangosteenas fruit (*Garcinia* sp.)
Nicaragua:				
Santa Maria de Ostuma, 4000 ft.	June 30, 1954	1 ♀	coffee finca	banana-baited trap
	July 1, 1954	1 ♀	coffee finca	banana-baited trap
Costa Rica:				
Turrialba, 2000 ft.	Oct., 1955	1 ♀		
Panama Canal Zone:				
Barro Colorado Island, 200 ft.	Nov. 1, 1955	1 ♂	rain forest	only known male to date
Trinidad:				
	Dec. 21, 1955	1 ♀	Queen's Savannah	on fallen flowers of *Erythrina* sp.
Port of Spain, 100 ft.	Dec. 26, 1955	1 ♀	Cacao finca	
Sangre Grande	Dec. 28, 1955	1 ♀	Cacao finca	sweeping over breadfruit
Brazil:				
Salvador, Bahia	July 19, 1951	1 ♀		Holotype female

and at the Instituto Tropical de Investigaciones Cientificas, where the collections were made, it has a depth of from 20 to 60 feet and an average width of about 10 feet. The walls are nearly perpendicular and at intervals are quite densely covered with such vegetation as ferns, begonias, Selaginella, bromeliads *(Pitcairnea)* and bloodworts *(Xiphidium)*. Sweeping over this vegetation with a long-handled net yielded more than 100 species of drosophilids in an eleven month period.

CYTOLOGY AND REPRODUCTION

The single strain of *D. mangabeirai* now extant in the laboratory was established from a single wild female individual captured at San Salvador, El Salvador on September 27, 1955. Since this time the strain has been reared on standard culture media by routine methods in both the Texas and St. Louis laboratories. When the strain was first subjected to close scrutiny approximately one year after it was established, no male individuals could be found. In a systematic check of the sub-strain in the St. Louis laboratory, 2,365 hatching individuals were screened; all were normal females except for three females with slightly rotated genitalia. A simultaneous check of the strain at The University of Texas laboratory also showed only females and all specimens which had been previously pinned from this strain were likewise found to be females. There is thus no evidence that males have ever occurred in this strain.

Cytological studies on the brain and salivary glands of larvae were made using the conventional aceto-orcein technique. Brain metaphases were clearly observed in smears from 14 different larvae; each showed the same configuration, namely, the diploid condition of two pairs of V-shaped chromosomes and one pair of rods (similar to the willistoni group). Furthermore, Dr. H. D. Stalker kindly examined the wing cells of 671 individuals without finding any which showed a cell size suggestive of triploidy or tetraploidy.

Salivary gland chromosome preparations were examined and chromosome conditions recorded in a total of 50 individual larvae chosen at random from the stock. Each larva showed five salivary gland chromosome arms. Two of these arms tend to come out of the chromocenter attached to the same heterochromatic mass (Fig. 2, bottom, center). Without exception, in every individual examined, these two arms show the identical heterozygous configurations shown in Figure 2. There is a nearly terminal overlapping inversion configuration in one of the two arms (Fig. 2, left) and a simple, just barely subterminal inversion loop in the other (Fig. 2, right). The other three chromosome arms were homozygous for gene arrangement in every case. The X chromosome cannot be identified.

To summarize, this strain apparently consists exclusively of parthenogenetically-produced diploid females, each of which is heterozygous for the same structural karyotype.

In order to determine the hatchability of the unfertilized eggs of this species, newly-emerged flies were collected and aged for four days. Ten females were selected at random and each female placed without etherization into an individual culture vial. Each female was changed to a fresh vial every day for the succeeding 21 days. All females were still vigorous and healthy when they were discarded at the end of the three-week period of observation. Eggs were counted

FIG. 2. Heterozygous configurations of salivary gland chromosomes in *D. mangabeirai*.

daily. Larvae were counted daily as they hatched and were removed from the vial with a needle. They were either destroyed or transferred to a fresh yeasted vial. Table 2 shows the hatchability of eggs of each of the ten females.

The number of transferred larvae which survived to adults is given in Table 3 for each of the 26 vials so prepared. Survival between larva and adult was greater when 12 or fewer larvae were transferred (average survival: 86.6%) than when 13 or more were transferred (average survival: 72.8%). Some of the larval mortality observed is thus apparently due to larval crowding under the conditions of the experiment.

Even though *D. mangabeirai* is a small species (approximately the size of *D. melanogaster*), the generation time from egg to adult at 25.5° C. is long, that is, approximately 18 days under optimal conditions. Maturation of females takes about four days so that the minimum generation time is approximately 22 days. As in many other species, however, slight crowding or underfeeding of larvae results in very great extension of the length of larval life.

TABLE 2

Hatchability of unfertilized eggs of *Drosophila mangabeirai* Malogolowkin. Each female was changed to a new vial for 21 successive days. Eggs were counted daily.

Female number	Total number of eggs laid	Number of larvae hatching	Per cent hatchability
12	59	29	49.2
13	30	8	26.7
14	135	79	59.5
15	123	84	68.3
16	119	79	66.4
17	149	101	67.8
18	99	63	63.6
19	63	33	52.4
20	96	49	51.0
21	20	5	25.0
	893	530	59.4

TABLE 3

Survival of *Drosophila mangabeirai* from larva to adult. Newly hatched larvae were counted and transferred to fresh vials. The number of adults emerging from each vial were recorded.

Vial No.	No. of larvae transferred	No. of adults emerging	Per cent survival
1	4	3	75.0
2	6	6	100.00
3	9	7	77.8
4	12	12	100.00
5	8	6	75.0
6	18	15	83.3
7	13	8	61.5
8	17	11	64.7
9	19	14	73.7
10	15	11	73.3
11	17	10	58.8
12	15	14	93.3
13	10	7	70.0
14	9	9	100.0
15	12	10	83.8
16	8	7	87.5
17	19	17	89.5
18	15	10	66.7
19	17	13	76.5
20	19	11	57.9
21	12	11	91.7
22	11	9	81.8
23	12	10	83.8
24	12	11	91.7
25	12	10	83.8
26	12	11	91.7
Total	333	263	79.0

CONCLUSION

Whether thelytokous parthenogenesis as found in the El Salvador strain of *D. mangabeirai* is characteristic of wild populations of this species or not must remain a matter of conjecture at the present time. Although rare, the very existence of a male in the collections suggests that the species has at least a residual bisexuality. Nevertheless, the rate of parthenogenesis in this strain is so high

that culture of it in large numbers by routine laboratory methods is easily accomplished. Such an efficient method of parthenogenetic reproduction could hardly be a matter of chance occurrence in a single strain. Rather, it is probable that parthenogenesis has somehow been brought to its present level of efficiency by natural selection.

The type of parthenogenesis, that is, the manner in which diploidy is maintained in the absence of syngamy, is not yet known. From the genetic point of view it is important to know if the method involves meiotic reduction and fusion of two haploid nuclei *(automixis)* or whether the definitive nucleus of the egg is derived directly from a diploid cell of the mother without intervention of meiosis *(apomixis)*.

Further cytological or genetic evidence will be necessary before either of the above alternatives can be demonstrated. At present, however, the indications are as follows. That automixis may occur is suggested by the fact that Stalker (1954) has conclusively demonstrated this process in *Drosophila parthenogenetica;* it probably also forms the basis for parthenogenesis in the related species *D. polymorpha.* Automixis also apparently occurs in the parthenogenetic fly, *Lonchoptera dubia* (Stalker, 1956). This latter case is of special interest in that the cytologically-observed automictic mechanism apparently involves the fusion of the central two of the four pronuclei produced at meiosis. Such a process would serve to reconstitute exactly the original heterozygosity of the mother, thus maintaining intact the fixed heterozygous karyotype characteristic of each of the choromosomal races in *L. dubia.* Such a mechanism could also be operative in *D. mangabeirai* and reproduce the observed fixed heterokaryotype.

On the other hand, apomixis cannot be excluded; indeed, certain facts suggest it as a possibility. In the first place, if meiosis is essentially normal, then nondisjunction of X chromosomes should produce an occasional male. No males have been observed. Secondly, if automixis, involving fusion of haploid products of meiosis is occurring, then occasional triploids due to fusion of three nuclei would be expected. No triploids have been found. Finally, the maintenance of the fixed heterokaryotype appears to be complete. This suggests apomixis because under automixis, regularity could be assured only if fusion of central nuclei in the egg is likewise highly regular.

If automixis of the type which occurs in *D. parthenogenetica* has served as the starting place for the evolution of parthenogenesis in *D. mangabeirai*, then natural selection must have eliminated a number of the inefficient features of this system. Further study of *mangabeirai* may thus not only provide clues to the evolutionary origin of parthenogenesis in the Diptera but might also provide fundamental information on the manner in which nuclear and cellular dynamics themselves may respond to natural selection.

SUMMARY

Pertinent facts on the taxonomy, relationships, ecology and distribution of the sophophoran species *Drosophila mangabeirai* Malogolowkin are presented. The only known strain of this species is unique in the genus Drosophila in that it reproduces wholly by diploid parthenogenesis. About 60% of the eggs laid by virgin females hatch. No males have been produced; all individuals examined

have been diploid females and all are heterozygous for the same two inversion configurations. Parthenogenetic reproduction, as it now exists in this strain is efficient and it is considered likely that the system has evolved in natural populations and is not a chance occurrence in a single strain. Existing data do not permit a definitive statement as to whether the type of parthenogenesis in this strain is automictic or apomictic.

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IX. A Preliminary Note on the Cardini Group of Drosophila in the Lesser Antilles

WILLIAM B. HEED[1]

Townsend and Wheeler (1955) described a new species, *Drosophila dunni*, in the cardini group from Puerto Rico. More recently the author has found a northwest, south-east gradation in abdominal pattern of *dunni*-like forms on three islands of the Lesser Antilles. Collections were made in January 1956 on Barbados, St. Lucia and St. Kitts, British West Indies.

The abdominal markings of *dunni* consist mostly of light paramedian markings on the 3rd and 4th tergites; these markings become slightly heavier and more fused in the St. Kitts form, and they become still darker and broader in the form from St. Lucia. The abdomen of the Barbados form is most distinct of all, being mostly brownish black with a dorsal tan pattern. Superficial study of other characters, such as size, color of mesonotum, number of primary and secondary teeth on the forceps of the male genitalia, and extent of heavy bristles on the 3rd costal section of the wing show a disjunct relation in that the Puerto Rico and St. Lucia forms are more similar while the St. Kitts and Barbados forms are, in turn, more similar to each other.

Hybridization tests were made with *dunni* from Puerto Rico and the dark form from Barbados. When females from Puerto Rico were crossed to males from Barbados, hybrids intermediate in color were produced (Table 1). The hybrids failed to produce progeny after being tested for two weeks. The males were then dissected and examined. The testes were smaller than is typical for either parental form and neither active nor inactive sperm were seen. The hybrid females were not tested for fertility. The reciprocal cross (Barbados ♀ × Puerto Rico ♂) did not produce hybrids.

On the basis of the crosses and the clearly distinct abdominal pattern, the Barbados form is being described as a new species, *D. nigrodunni*, by Heed and

TABLE 1

Hybridization tests of *D. dunni* from Puerto Rico (P) and *D. nigrodunni* from Barbados (B).

Cross ♂ ♀	No. mated ♀♀	♂♂	Date mated	Date lumped	Date discarded	Total F_1	♀♀	♂♂
P × B	8	8	5/11					
	8	9	5/11	5/21	6/13	47	32	15
	12	9	5/11					
B × P	11	8	5/11					
	9	11	5/11	5/21	6/1*	0	0	0
	12	15	5/11					
	9	20	5/20		6/13*	0	0	0
P × P	6	8	5/11		6/13	267+	140	127
	5	7	5/14		6/13	150+	74	76
	12	12	5/14		6/13	91+	46	45
B × B	10	8	5/14		6/13	46+	22	24
	8	6	5/14		6/13	2	2	0
	22	17	5/17		6/13	19+	8	11

* Females dead at this time.

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Wheeler (this bulletin). The results of the crosses are given in Table 1. It may be seen from the control test that *nigrodunni* is difficult to maintain on banana medium. This is also true of the forms from St. Lucia and St. Kitts and further testing is impractical at this time.

The islands adjacent to the Lesser Antilles were also collected. Trinidad was collected in December, and Puerto Rico in February, 1956. No *dunni*-like forms were taken in Trinidad. This island shows true continental affinities in that five members of the cardini group, all represented on the mainland, have been taken here. They are *cardini*, *polymorpha*, *cardinoides*, *neomorpha*, and a form very similar to *parthenogenetica* (differs in having a shiny black abdominal pattern typical of most of the other cardini group species, and a slightly higher secondary bristle count on the palpi. *Drosophila neomorpha* is being described as new in this bulletin (article by Heed and Wheeler).

In Puerto Rico the only species of the cardini group other than *dunni* was *D. cardini*, which was also collected on St. Lucia and has been reported from Dominica (Sturtevant, 1921). One other species of the Lesser Antilles should be mentioned: *Drosophila similis*, described by Williston from the island of St. Vincent. The author has examined a type specimen from the American Museum of Natural History and has come to the conclusion that this specimen belongs to the *dunni*-like forms.

The cardini group shows an extremely interesting distributional pattern especially in the West Indies and the adjacent mainland. Part of this pattern is briefly outlined above. Future collecting and subsequent analysis following up Stalker's important contribution (1953) to the genetic affinities of the species then known in the cardini group will produce valuable information about the evolution of the group.

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X. Chromosomal Studies of Several Species of Drosophila

FRANCES E. CLAYTON[1] AND MARVIN WASSERMAN

INTRODUCTION

This report includes cytological analyses of a number of new species of Drosophila, as well as of additional strains of species which have been reported upon previously by the authors and other investigators.

The preparation of the salivary gland chromosomes and larval brain ganglion slides has been by our usual techniques. The larval ganglion is stained for approximately 15 minutes with aceto-orcein after which it is squashed in 50 per cent acetic acid, ringed with a paraffin-vaseline mixture, and studied as a temporary preparation. The salivary glands are pretreated in 1 N hydrochloric acid for about one minute, stained in aceto-orcein and mounted in 50 per cent acetic acid. Identifications of the species were made by Drs. M. R. Wheeler, W. B. Heed, and the junior author.

RESULTS AND DISCUSSION

The data resulting from this investigation are given in Table 1, and the chromosomal configurations of some of the strains are illustrated in Plate 1. In Table 1 the stock number and the geographic origin of each culture are given; starred species are those being described by various authors elsewhere in this bulletin. Many of the strains examined show no differences from previous accounts and will not be discussed further. Several species, however, have unusual cytological interest and are described below.

D. paranaensis. Fig. 5–7. We have found three types of metaphases among our material. Type (a) was first reported by Dreyfus and de Barros (1949); type (c) was described by Clayton and Ward (1954). Type (b), reported here, is intermediate between the other two, consisting of three pairs of rods, one pair of large V's, one pair of small V's with the Y a short rod. Crosses between the three types give interesting results (see Wasserman and Wilson, this bulletin); for example, F_1 males from type (a) crossed to type (b) are sterile; type (a) crossed to type (b) and type (b) crossed with type (c) give variable results.

D. fragilis. The stock from La Palma, El Salvador shows three pairs of rods, one pair of J's, and one pair of dots; the stock was about to be lost and only a single larva was dissected. Additional dots may have been present, as reported earlier (Clayton and Ward, 1954), but no other larvae were available to check on this point.

D. setula. Fig. 10–11. Metaphase plates of this species have been unusually difficult to interpret and the two stocks we have used seem to disagree with each other. Stock No. H187.9 appears to have four pairs of rods, one pair of V's, and one pair of dots, while stock No. H81.15 has the same V and dot chromosomes but shows only three pairs of rods. However, due to the poor quality of the preparations, these interpretations are still tentative.

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TABLE 1

Species	Stock No.	Locality	Metaphase	X	Y	Salivary
Hirtodrosophila						
pictiventris	H 15.10	San Vincente, El Salvador	1V, 1J, 1R, 1D	R	O	5A, 1D
longala (?)	2268.27	Cuernavaca, Morelos, Mexico	5R, 1D	R	O	5A, 1D
grisea	2359.6	Rustler Park, Arizona	5R, 1D	R	O (?)	5A, ID
Dorsilopha						
busckii	H 57.27	Santa Maria de Ostuma, Nicaragua	2V, 1R	R	J (?)	5A, 1D
Sophophora						
saltans group						
elliptica (?)	H 62.51	La Palma, El Salvador	2V, 1R			
willistoni group						
bocainensis (?)	H 50.4	La Lima, Honduras	2V, 1R	V	J	5A
	H 50.4B	La Lima, Honduras	1V, 1J, 1R			5A
fumipennis	H 57.29	Santa Maria de Ostuma, Nicaragua	2V, 1R			5A, 1D (?)
sucinea	H 57.23	Santa Maria de Ostuma, Nicaragua	2V, 1R	V	V	5A, 1D
bromeliae group						
bromeliae	H 51.24	Lancetilla, Honduras	2V, 1R, 1D			
bromelioides (?)	H 57.54	Santa Maria de Ostuma, Nicaragua	4V	V	J	
Drosophila						
tripunctata group						
albirostris	H 25.2	San Salvador, El Salvador	5R, 1D	R	R	5A, 1D
	H 29.10	San Salvador, El Salvador	5R, 1D	R	R	5A, 1D
bipunctata	H 41.25	Volcan Boqueron, El Salvador	5R, 1D	R	R	
crocina	H 25.30	San Salvador, El Salvador	5R, 1D	R	R	5A, 1D
	H 29.1b	San Salvador, El Salvador	5R, 1D	R	R	5A, 1D
facialba*	H 26.2a	Volcan Santa Ana, El Salvador	5R, 1D	R	R	5A, 1D
fragilis	H 62.1	La Palma, El Salvador	3R, 1J, 1D			
medionotata (?)	H 21.5	Lago de Coatepeque, El Salvador	5R, 1D	R	R	5A, 1D
	H 27.1	Lago Pichichuela, El Salvador	5R, 1D	R	R	5A, 1D
	H 29.18	San Salvador, El Salvador	5R, 1D	R	R	5A, 1D
	H 50.10	La Lima, Honduras	5R, 1D	R	R	5A, 1D
	H 62.10	La Palma, El Salvador	1V, 1J, 2R, 1D	R	R	5A, 1D
mediopunctata	H 29.1a	San Salvador, El Salvador	5R, 1D	R	R	5A, 1D
mediostriata	H 35.15	San Salvador, El Salvador	5R, 1D	R	R	5A, 1D
	H 50.41	La Lima, Honduras	5R, 1D	R	R	5A, 1D
	H 51.6	Lancetilla, Honduras	5R, 1D	R	R	5A, 1D
	H 56.95	El Recreo, Nicaragua	5R, 1D	R	R	5A, 1D
metzii	H187.9	Santa Marta, Colombia	4R, 1V, 1D			
setula*	H 81.15	Barro Colorado Is., Canal Zone	3R, 1V, 1D (?)			
trapeza*	H 29.16	San Salvador, El Salvador	5R, 1D	R	R	5A, 1D

TABLE 1—Continued

Species	Stock No.	Locality	Metaphase	X	Y	Salivary
trifiloides*	H 50.10b	La Lima, Honduras	5R, 1D	R	R	
tristriata*	H109.22	Trinidad, B. W. I.	5R, 1D			5A, 1D
repleta group						
aldrichi (?)	H 52.12	Puerto Triunfo, El Salvador	5R, 1D	R	R	5A, 1D
aureata*	H180.42	San Jose, Costa Rica	5R, 1D	R	J	5A, 1D
fulvimaculoides*	H163.31	Turrialba, Costa Rica	5R, 1D	R	J	5A, 1D
martensis*	H188.12	Santa Marta, Colombia	5R, 1D	R	V	5A, 1D
mercatorum	1781.8	San Pedro, Coah., Mexico	3R, 2V	R	R	
paranaensis type a	H113.5	Trinidad, B. W. I.	3R, 1V, 1D	R	R	5A, 1D
	H194.44	Villavicencio, Colombia	3R, 1V, 1D	R	R	5A, 1D
type b	H186.33	Santa Marta, Colombia	3R, 2V	R	R	5A, 1D
	H 73.6	Turrialba, Costa Rica	3R, 2V	R	R	5A, 1D
	H 75.10	Heredia, Costa Rica	3R, 2V	R	R	5A, 1D
type c	H 56.94	El Recreo, Nicaragua	3R, 2V	R	R	5A, 1D
	H 34.20/32	San Salvador, El Salvador	3R, 2V	R	R	5A, 1D
	H101.12	Palmira, Colombia	3R, 2V	R	R	5A, 1D
peninsularis	2327.3	Rio Piedras, Puerto Rico	5R, 1D	R	V	5A, 1D
repleta	H 17.8	Santa Tecla, El Salvador	5R, 1D	R	R	5A, 1D
	H 25.32E	San Salvador, El Salvador	5R, 1D	R	R	5A, 1D
	H 34.30	San Salvador, El Salvador	5R, 1D	R	R	5A, 1D
immigrans group						
immigrans	H 57.21	Santa Maria de Ostuma, Nicaragua	3R, 1V	R	V	4A, 1D
macroptera group						
submacroptera (?)	2257.1b	Chapulhuacan, Hid., Mexico	2V, 1J, 1D			5A
	2257.20	Chapulhuacan, Hid., Mexico	2V, 1J, 1D			5A
	2261.16b	Tehuacan, Puebla, Mexico	2V, 1J, 1D			5A
	2262.4b	Huatusco, Vera Cruz, Mexico	2V, 1J, 1D			5A
	2263.3b	Tezuitlan, Puebla, Mexico	2V, 1J, 1D			5A
guarani group						
guaramunu (?)	2263.17	Tezuitlan, Puebla, Mexico	4R, 1V			5A (?)
limbinervis	H 26.29	Volcan Santa Ana, El Salvador	5R, 1D	R	R	5A, 1D
	H 42.26	Volcan Boqueron, El Salvador	5R, 1D	R	R	5A, 1D
pallidipennis group						
pallidipennis centralis	H 29.27 ♂ × H 34.24 ♀	San Salvador, El Salvador	4R, 1V, 1D			5A, 1D
	H 62.58	La Palma, El Salvador	4R, 1V, 1D			5A, 1D
calloptera group						
atrata	H203.23	Caripe, Venezuela	5R, 1D	R	R	

TABLE 1—Continued

Species	Stock No.	Locality	Metaphase	X	Y	Salivary
ornatipennis	2378.2	St. Vicente, Cuba	3R, 1V, 3D			5A, 1D
dreyfusi group						
briegeri	H182.2	Cerro La Campana, Panama	3V, 1D	V	V	5A, 1D
	H 80.5	Barro Colorado Is., Canal Zone	3V, 1D	V	V	5A, 1D
camargoi	H 51.12	Lancetilla, Honduras	3V, 1J	V	J	5A, 1D
cardini group						
cardini	H 15.20	San Vincente, El Salvador	6R	R	R	5A, 1D
	H 26.6	Volcan Santa Ana, El Salvador	6R	R	R	5A, 1D
	H 29.20	San Salvador, El Salvador	6R	R	R	5A, 1D
	H 57.26	Santa Maria de Ostuma, Nicaragua	6R	R	R	5A, 1D (?)
cardinoides	H 27.9	Lago Pichichuela, El Salvador	2V, 1R, 1D			4A, 1D (?)
canalinea group						
canalinea	H 50.31	La Lima, Honduras	2V, 1R, 1D			5A, 1D
canalinioides*	H 25.24	San Salvador, El Salvador	6R	R	R	5A, 1D
	H 66.7	San Salvador, El Salvador	6R	R	R	5A, 1D
paracanalinea*	H129.19	El Yunque, Puerto Rico	1R, 1V, 1J, 1D			5A, 1D
Unassigned species						
castanea	H 29.7	San Salvador, El Salvador	1R, 2V, 1D	V	J	5A, 1D
	H 25.13	San Salvador, El Salvador	1R, 2V, 1D	V	J	5A, 1D
	H 64.13	San Salvador, El Salvador	1R, 2V, 1D			5A, 1D
	H 73.8	Turrialba, Costa Rica	1R, 2V, 1D			5A, 1D
	H180.21	San Jose, Costa Rica	1R, 2V, 1D			5A, 1D
	H209.9	Merida, Venezuela	1R, 2V, 1D			5A, 1D
	H209.39	Merida, Venezuela	1R, 2V, 1D			5A, 1D
	1802.8	Atlixco, Puebla, Mexico	1R, 2V, 1D	V	J	5A, 1D
fulvalineata	2075.9	Cliff, New Mexico	5R, 1V	R	V	5A, 1D
fulvalineata (?)	2158.13	Patagonia, Arizona	3R, 2V	V	J	6A, 1D
aracea*	H 17.1	Santa Tecla, El Salvador	1V, 1J, 1R			5A, 1D
	H 46.28	Santa Tecla, El Salvador	1V, 1J, 1R			5A, 1D
	H 57.69	Santa Maria de Ostuma, Nicaragua	1V, 1J, 1R			5A, 1D
carsoni*	2351.9	Espanola, New Mexico	2R, 2J, 1V, 1D			8A, 1D
sticta*	H 51.15	Lancetilla, Honduras	5R, 1D	R	R	5A, 1D
	H 57.61	Santa Maria de Ostuma, Nicaragua	5R, 1D	R	R	5A, 1D
	H192.32	Rio Negro, Colombia	varies			
paraguttata*	H136.34	Jamaica, B. W. I.	5R, 1D	R	R	5A, 1D

* New species described elsewhere in this bulletin.

PLATE I.

1. STICTA – a

2. STICTA – b

3. STICTA – c

4. STICTA – d

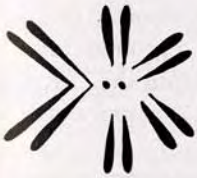
5. PARANAENSIS – a

6. PARANAENSIS – b

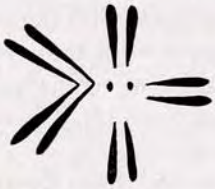
7. PARANAENSIS – c

8. ORNATIPENNIS

9. BRIEGERI



10. SETULA – a



11. SETULA – b

12. CARSONI

13. FULVALINEATA – a

14. FULVALINEATA – b

15. PARACANALINEA

D. submacroptera. The metaphase reported for this species was one pair of large V's, two pairs of small V's, one pair of rods and one pair of dots. All of the strains we have examined differ from this configuration, possessing one pair of large V's, one pair of smaller V's, one pair of J's, and one pair of dots.

D. guaramunu. The stock studied (Tezuitlan, Mexico) is probably a new species related to *guaramunu;* its chromosomes differ from the original report in several respects.

D. atrata. As was pointed out by Clayton and Ward (1954) the metaphase of this species was reported previously by Dobzhansky and Pavan (1943) under the name of *calloptera*, where it was described as five pairs of rods and one pair of dots. Our preparations agree, with one pair of long rods, four pairs of slightly shorter rods (one pair with satellites), and a pair of dots.

D. ornatipennis. Fig. 8. Our determination, based upon a stock from Cuba, differs from the earlier reports. Metz (see discussion in Clayton and Ward, 1954) reported one pair of large V's, three pairs of rods, and one pair of microchromosomes. We find one pair of large V's, three pairs of rods (one long pair and two shorter pairs), and three pairs of dots. This is a haploid complement of seven, previously reported for two other species, *trispina* and *fragilis*.

D. briegeri. Fig. 9. Pavan and Breuer (1954) described the metaphase in the species description as follows: three pairs of V's, one pair of dots. They state that the large V's (sex chromosomes) are heteropycnotic, with one arm and half of the other arm positively heteropycnotic in prophase. Our preparations showed approximately the same thing. Salivary chromosomes show some banding patterns recognizable on the basic *repleta* map. We consider that one pair of smaller V's represents a 2–5 fusion, and the other pair of smaller V's represents a 3–4 fusion, using the *repleta* configuration as the standard.

D. camargoi. The original description of this species gives the following metaphase configuration: one pair of long J's, one pair of rods, and two pairs of small V's. Our determination, using a stock from Lancetilla, Honduras, differs slightly, since we find three pairs of V's, rather than two, and no rods.

D. canalinea. Our preparations agree with the metaphase reported in the original species description, but disagree with that recorded in Patterson and Stone (1952:159) where it is evident than an error was made, listing the chromosome complement as 1R, 1V, 1D rather than 1R, 2V, 1D. Of the two pairs of V's, one pair is much larger than the other.

D. paracanalinea. Fig. 15. The stock from Puerto Rico shows one pair of large V's, one pair of large J's with satellites, one pair of rods and one pair of dots. Salivary preparations show five arms and a dot, and certain portions are recognizable in terms of the *repleta* chromosomes. An X-4 fusion forms one of the metacentrics, and the other is uncertain, using the *repleta* configuration as the standard.

D. castanea. Wasserman (1954) reported that many portions of the salivary chromosomes showed recognizable *repleta* patterns. The new strains agree with those examined previously in having a 2–4 fusion and an X-3 fusion, using the *repleta* chromosomes as a standard.

D. fulvalineata. Fig. 13–14. The stock from New Mexico shows the same metaphase as that reported previously but we see only five arms and the dot in salivary

preparations rather than six arms. The stock from Arizona, however, has an entirely different metaphase, and is possibly an undescribed species. The metaphase of this strain consists of one pair of large V's, one pair of long rods, one pair of shorter rods, one pair of chromosomes which are probably rods, and one pair of small V's. The X is a large V while the Y is a large J-chromosome. Salivary preparations show six arms and a dot, with two arms rather short.

D. carsoni. Fig. 12. Metaphase preparations show one pair of long rods with satellites, one pair of shorter rods, two pairs of J's, one pair of V's (with a satellite seen on one V of the pair in several individuals), and one pair of large dots. The X and Y could not be determined. Salivary preparations showed eight arms and the dot; two arms were about half the length of the others, and one chromosome had a mass of heterochromatin in the middle, this chromosome usually being separated from the chromocenter.

D. sticta. Fig. 1–4. The metaphase configurations found in stock No. H192.32 from Colombia show an unusual type of variability. The basic chromosomes are apparently five pairs of long rods and one pair of dots. All preparations consistently showed the five long rods, but the remaining chromosomes were either (1) one pair of dots, or (2) one pair of dots and a short rod, or (3) one dot and one short rod, or (4) two short rods and no dots. Nine larvae, chosen at random from the culture, gave the following distribution:

1 ♀, 2?	:	one pair dots, no rods
1 ♀	:	no dots, one pair rods
1 ♂	:	One dot, one rod
1 ♂, 3?	:	one pair dots, one rod

Thus the "supernumerary" rod may be absent, present once or twice, and the dot may be absent, present once or twice. There is no apparent relation between rods or dots and sex. The X and Y chromosomes could not be identified with certainty.

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XI. Further Studies on the Repleta Group

MARVIN WASSERMAN AND FLORENCE D. WILSON

This is a continuation of the work started by the senior author in 1954 on the repleta group of the genus Drosophila. At that time an examination was made of twenty species, mainly from the United States and Mexico. During the past two years the Genetics Foundation of the University of Texas has been carrying out a series of collecting trips to Central and South America and the Caribbean islands, aided by a grant from the National Science Foundation. This investigation is based primarily on cultures derived from these collections. Drs. Marshall R. Wheeler and William B. Heed have made most of the species determinations; the field collecting has been carried out mainly under the leadership of Dr. Heed assisted by Dr. H. L. Carson and the senior author. We are also indebted to Dr. W. S. Stone for suggestions and encouragement, to Mrs. W. B. Mather for the use of some of her unpublished data, and to Miss Gail Kaufman for her aid in the preparation of the manuscript.

The repleta group is one of the largest known species groups in the genus Drosophila. Most of the species are either cytologically homozygous or have but few gene sequences. The differences from species to species are also usually slight, and cytological changes from form to form can be readily determined (Wharton, 1942, 1943; Warters, 1944; Dreyfus and de Barros, 1948, 1949; de Barros, 1950; Ward and Stone, 1952; Wasserman, 1954; Stone, 1955).

In addition there is a wealth of information on isolating mechanisms among the species in the group. Patterson and Stone (1952: 412) summarized the crosses attempted between members.

The group has been subdivided into three or four subgroups; Wharton (1944), Wheeler (1949), and Patterson and Stone (1952) recognize the melanopalpa, hydei, and mulleri subgroups. In addition, Wharton (1944) proposed the mercatorum subgroup for *D. mercatorum* and *D. peninsularis*. Patterson and Stone (1952) also recognize the mercatorum subgroup and have included in it *D. paranaensis;* they agree with Wheeler, however, in placing *peninsularis* in the mulleri subgroup. There is also a considerable number of species which have not as yet been assigned to any subgroup, in some cases because not enough information is available, and at other times because they seem to be intermediate in many of their characteristics.

The present investigation deals primarily with the cytological analyses of several members of the repleta group, namely, *D. repleta*, the *fulvimacula* complex, the *mercatorum* subgroup, and the *mulleri* subgroup. Some data on the hybridization of certain species are presented and two new species are described.

MATERIALS AND METHODS

The 59 geographic strains of the eight species studied are listed in Table 1. Mass matings (20 ♂, 20 ♀) were made to obtain F_1 larvae for cytological examination. Several of the strains were also identified by crosses to known laboratory

stocks. If no progeny had been produced from the matings after four weeks, the cross was considered sterile. Cytological examinations were made on the salivary gland chromosomes of the pure strains and on those hybrids which were obtained from the mass matings. In most cases eight slides were made of each stock to test for homozygosity. The observed gene sequence or sequences found in each strain were compared to that found in *Drosophila repleta*, using the *repleta* map of Wharton (1942).

The symbols used to represent the different inversions have been modified somewhat from the pattern established in the earlier report on the repleta group (Wasserman, 1954). The use of a single alphabet letter (a, b, c, etc.) remains as before, but we are substituting the symbols a^2, b^2, c^2, etc. for the inversions previously recorded as aa, bb, cc, etc., and, due to the great number of inversions in chromosome 2, we are also employing the symbols a^3, b^3, c^3, rather than the cumbersome forms aaa, etc.

I. MELANOPALPA SUBGROUP

Wharton (1942) and Ward and Stone (1952) have made cytological studies on the melanopalpa subgroup. They have found that *D. repleta*, *D. limensis*, *D. canapalpa*, *D. melanopalpa* and *D. neorepleta* form a close-knit group. Among the strains tested they found that only 6 inversions have taken place in the cytological divergence of these 5 species. Ward and Stone (1952) have also demonstrated that *melanopalpa*, *canapalpa* and *neorepleta* share some of these inversions. Thus a Mexican *melanopalpa* strain was homozygous for a gene sequence in the fifth chromosome which was lacking in an Arizona strain of *melanopalpa*. The strain of *canapalpa* used was homozygous for this sequence. Similarly, *neorepleta* and the Arizona strain of *melanopalpa* were homozygous for a gene sequence in the second chromosome. The Mexican strain of *melanopalpa* was heterozygous for this sequence.

Wasserman (1954) examined the salivary gland chromosomes of *D. nigrospiracula*. This species had been placed in the melanopalpa subgroup on morphological grounds. Its cytology, however, places it closer to the mulleri and hydei subgroups.

Drosophila repleta Wollaston, 1858

The species *D. repleta* has been examined a number of times (Wharton, 1942, 1943; Ward and Stone, 1952; and Wasserman, 1954). It has never been known to vary in its gene sequence. The strains examined in this study (Table 1) were all also homozygous for the standard *repleta* sequence.

The *fulvimacula* complex

Drosophila fulvimacula was described by Patterson and Mainland from several Mexican localities but the species did not reproduce in the laboratory. Their description was, therefore, based entirely on the morphology of the imago and the egg. In 1947, M. R. Wheeler and F. A. Cowan collected a single female from Oaxaca, Mexico; a laboratory strain (1808.37) was established from this female, which was identified as *D. fulvimacula*. In 1949, Th. Dobzhansky sent a Brazilian strain (1975.5) to the Texas laboratory. Crosses were carried out between

TABLE 1

Chromosomal Configuration of Stocks Studied

Subgroup	Species	Stock	Locality	X	2	3	4	5
	repleta	2257.23	Jacala, Mexico	+	+	+	+	+
		H63.7	Merida, Mexico	+	+	+	+	+
		H75.9	Heredia, Costa Rica	+	+	+	+	+
		H138.1	Jamaica, B.W.I.	+	+	+	+	+
		H118.1	Barbados, B.W.I.	+	+	+	+	+
		H34.30	El Salvador	+	+	+	+	+
		H25.32	El Salvador	+	+	+	+	+
melanopalpa		H27.14	El Salvador	+	+	+	+	+
		H17.8	El Salvador	+	+	+	+	+
		H155.1	Turrialba, Costa Rica	+	+	+	+	+
		H87.2	Canal Zone, Panama	+	+	+	+	+
	fulvimacula	1808.37	Oaxaca, Mexico	+	i^2, j^2, k^2, z^2, h^2	b	+	+
		H66.24	El Salvador	+	i^2, j^2, k^2, z^2, h^2	b	+	+
		H51.13	Lancetilla, Honduras	+	i^2, j^2, k^2, z^2, h^2	b	+	+
		H80.4	Barro Colorado Is., Canal Zone	+	i^2, j^2, k^2, z^2, h^2	b	+	+
		H181.8	Barro Colorado Is., Canal Zone	+	i^2, j^2, k^2, z^2, h^2	b	+	+
		H186.37	Santa Marta Mts., Colombia	+	i^2, j^2, k^2, z^2, h^2	b	+	+
		1975.5	Belem, Brazil	+	i^2, j^2, k^2, z^2, h^2	b	+	+
		H194.28	Villavicencio, Colombia	+	$i^2, j^2, k^2, z^2, h^2, l^2/+$	b	+	+
	fulvimaculoides	H29.15	El Salvador	+	$i^2, j^2, k^2, z^2, l^2, a^3, b^3, c^3$	b	+	+
		H57.56	Santa Maria de Ostuma, Nicaragua	+	$i^2, j^2, k^2, z^2, l^2, a^3, b^3, c^3$	b	+	+
		H73.23	Turrialba, Costa Rica	+	$i^2, j^2, k^2, z^2, l^2, a^3, b^3, c^3$	b	+	+
		H158.4	Turrialba, Costa Rica	+	$i^2, j^2, k^2, z^2, l^2, a^3, b^3, c^3$	b	+	+
		H161.1	Turrialba, Costa Rica	+	$i^2, j^2, k^2, z^2, l^2, a^3, b^3, c^3$	b	+	+
		H163.11	Turrialba, Costa Rica	+	$i^2, j^2, k^2, z^2, l^2, a^3, b^3, c^3$	b	+	+
		H163.31	Turrialba, Costa Rica	+	$i^2, j^2, k^2, z^2, l^2, a^3, b^3, c^3$	b	+	+
	mercatorum	1412.9b	Sao Paulo, Brazil	+	d, t, e, u, v	b, f, h, n/+	+	+
		H191.30	Bucaramanga, Colombia	i	d, t, e, u, v	b, f, h, "g"	+	+
		H191.46	Bucaramanga, Colombia	i	d, t, e, u, v	b, f, h, "g"	+	+

		H62.60	La Palma, El Salvador	+	d, t, e, u, v	b, f, h	+	+
		2370.5	Oahu, Hawaii	+	d, t, e, u, v	b, f, h	+	+
		2393.1	Cocha Bamba, Bolivia	+	d, t, e, u, v	b, f, h	+	+
mercatorum		2395.12	Cuzco, Peru	+	d, t, e, u, v	b, f, h	+	+
	paranaensis	H62.13	La Palma, El Salvador	+	d, t, e	b, f	+	+
		H194.44	Villavicencio, Colombia	+	d, t, e	b, f	+	+
		H34.20/32	El Salvador	+	d, t, e	b, f	+	+
		H73.6	Turrialba, Costa Rica	+	d, t, e	b, f, i	+	+
		H56.94	El Recreo, Nicaragua	+	d, t, e	b, f, i	+	+
		H163.16	Turrialba, Costa Rica	+	d, t, e	b, f, i	+	+
		H101.12	Palmira, Colombia	+	d, t, e	b, f, i, g/+	+	+
		H75.10	Heredia, Costa Rica	+	d, t, e	b, f, i, g/+	+	+
		H186.33	Sierra Nevada de Santa Marta, Colombia	+	d, t, e	b, f, i	b/+	+
		H113.5	Trinidad	+	d, t, e, m^2	b, f, i/+, g/+	b/+	+
	nigricruria	2268.7	Cuernavaca, Morelos, Mexico	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2:s^2/y^2	b	c, d	f
		1796.2	Morelia, Michoacan, Mexico	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2:s^2/y^2	b	c, d	f
		1797.14	Mexico City, Mexico	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2:s^2/y^2/+	b	c, d	f
mulleri		2264.14	San Andres Tuxtla, Veracruz, Mexico	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2:s^2	b	+	f
		2263.15	Tezuitlan, Mexico	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2	b	c, d	f
		2266.15	Sierra Madre del Sur, Mexico	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2:s^2/s^2y^2	b	c, d	f
		2261.7	Tehuacan, Puebla, Mexico	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2	b	+	f
		H62.29	La Palma, El Salvador	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2	b	+	f
		H75.11	Heredia, Costa Rica	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2	b	+	f
		H163.29	Turrialba, Costa Rica	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2	b	+	f
		.....	Costa Rica A	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2	b	+	f
		H206.19	Caripe, Venezuela	a, b, c, k	a, b, t^2, u^2, v^2, w^2, x^2	b	+	f
	martensis	H188.12	Santa Marta, Colombia	a, b, c, j	a, b, c, d^2, e^2, f^2, g^2/+	a, b, k	+	+
		H208.1	Barquesimeto, Venezuela	a, b, c, j	a, b, c, d^2, e^2, f^2, g^2/+	a, b, k	+	+
	"*aldrichi*"	H52.12	Puerto Triunfo, El Salvador	a, b, c	a, b, c, f, g	a, b, c	+	+
		H103.2	Sevilla, Colombia	a, b, c	a, b, c, f, g	a, b, c	+	+

it and the strain from Mexico, and Patterson (1952) described the Brazilian form as a new subspecies, *D. fulvimacula flavorepleta*.

Recently a number of strains were collected in Central and South America by W. B. Heed, H. L. Carson and the senior author. These strains have now been tested, and the results show that two species are present. The pertinent data are presented below. The two forms are very similar morphologically, and, without breeding tests, they might quite easily be considered one species. The diagnostic characters are unfortunately either cytological or those which living specimens exhibit best.

All of the original specimens of *fulvimacula* except the holotype have been lost. Therefore the specimens available for study consist of the holotype and those flies which have been obtained since 1947. However, the authors believe that they have probably identified the holotype of *fulvimacula* with one of the two species present in the laboratory. We give below a complete redescription of *D. fulvimacula*, as we interpret the species, and a description of *Drosophila fulvimaculoides*, a new species.

Drosophila fulvimacula Patterson and Mainland, 1944.

External characters of imagines.

♂, ♀. Arista with about 8 branches; antennae brownish yellow, 3rd joint darker; 2 prominent bristles on the 2nd joint. Front brownish yellow; orbits lighter with brown areas ventrally; ocellar triangle slightly darker. There may be very faint brownish yellow spots at the bases of the posterior orbitals and inner verticals. Middle orbital about .55 to .625 the other two. Carina flat, slightly broadened ventrally. Face light yellow, tending toward white. Cheeks yellowish white, their greatest width about 1/6 greatest diameter of eye. Eyes scarlet-vermilion (Pl. 2-J12), with dense, short black pile.

Acrostical hairs in 8 rows; no prescutellars, but hairs of all the acrostical rows immediately anterior to the scutellar suture enlarged. Anterior scutellars convergent. Sterno-index about 0.76. Mesonotum pollinose brownish yellow, hairs and bristles arising from light yellowish brown spots which tend to fuse giving two indistinct stripes between the dorsocentral rows in the darker forms. Sternopleurae yellowish; mesopleurae and pteropleurae slightly darker. Legs yellowish, with a slight indication of darker basal bands on the tibiae. Apical bristles on the first and second tibiae, preapicals on all three.

Abdomen yellowish with slight greenish cast, each segment with a dark apical band, interrupted medially, which fades at the angle of the tergite, and with anterior projections at the interruption and sometimes at the angle of the tergite. In dead pinned specimens the lateral margin of the tergite also darkens.

Wings clear with a yellowish cast, veins brown. Costal index about 3.0; 4th vein index about 1.6; 5x index about 0.9; 4c index about 0.5. Apex of first costal section with two prominent bristles. Third costal section with heavy bristles on its basal 3/8 to 1/2.

Length of body about 4 mm; wing about 3.75 mm.

Internal characters of imagines.

Testes pale yellow (Pl. 9-H1), with about 5½ outer coils and 5 thin, lighter

colored, inner coils. Ventral receptacle with about 100 coils, irregularly coiled. Spermathecae not chitinized, bell-shaped.

Other characteristics, relationship, and distribution.

Eggs.—With 4 filaments, the anterior pair about 4/9 length posterior pair.

Puparia.—Yellowish brown (Pl. 9-L7), each anterior spiracle with about 16 branches. Horn index about 2.0 (including the spiracle branches).

Chromosomes.—Metaphase plate shows 5 pairs of rods and one pair of dots (Clayton and Ward, 1954).

Relationship.—Belongs to the melanopalpa subgroup of the repleta group of the subgenus Drosophila.

Distribution.—The holotype is from Sedeño Cañon, near Jalapa, Mexico. Other known localities, listed in Table 1, are El Salvador, Honduras, Panama Canal Zone, Colombia, and Brazil.

Drosophila fulvimaculoides Wasserman and Wilson, new species.

External characters of imagines.

♂, ♀. Arista with about 8 branches; antennae yellowish brown, the 3rd joint darker; two prominent bristles on the second joint. Front brown; orbits pollinose brownish yellow with brown areas ventrally; ocellar triangle brown. The posterior orbitals and the inner verticals arise from brown spots. Middle orbital about ⅔ length of anterior and ½ posterior orbital. Carina slightly keeled dorsally, flattened and slightly widened ventrally. Face yellowish gray. Cheeks yellowish gray, their greatest width about ⅙ greatest diameter of the eye. Eyes red (Pl. 2-K11), with dense, short black pile.

Acrostical hairs in 8 rows; no prescutellars, but hairs of all the acrostical rows immediately anterior to the scutellar suture enlarged. Anterior scutellars convergent. Sterno-index about 0.84. Mesonotum pollinose brownish yellow, hairs and bristles arising from brown spots which tend to fuse into two stripes between the dorsocentral rows. Pleurae yellowish, with indistinct brown stripes from the base of the wing to the humerus, from the haltere to the base of the fore coxa, and along the margin between the sternopleura and the meso- and pteropleurae. Legs yellowish, with a dark band at the apices of the femora and the bases of the tibiae. Apical bristles on the first and second tibiae, preapicals on all three.

Abdomen yellowish with a slight greenish cast, each segment with a black apical band, interrupted medially, with anterior extensions at either side of the interruption, at the angle of the tergite, and at the lateral margin.

Wings clear with a slight yellowish cast, veins brown. Costal index about 3.0; 4th vein index about 1.4; 5x index about 1.05; 4c index about 0.5. Apex of the first costal section with two prominent bristles. Third costal section with heavy bristles on the basal ½.

Length of body, about 4 mm; wing, about 3.75 mm.

Internal characters of imagines.

Testes pale yellow (Pl. 9-H1), with 3 inner and 5 outer coils. Ventral receptacle with about 90 coils, irregularly coiled. Spermathecae not chitinized, bell-shaped.

Other characteristics, relationship, and distribution.

Eggs.—With 4 filaments, the anterior pair 4/5 length posterior pair.

Puparia.—Yellowish brown (Pl. 11-K12), each anterior spiracle with about 16 branches. Horn index about 1.67 (including spiracle branches).

Chromosomes.—Metaphase plate shows 5 pairs of rods and one pair of dots. The Y chromosome is J-shaped (Clayton and Wasserman, this bulletin).

Relationship.—Belongs to the melanopalpa subgroup of the repleta group of the subgenus Drosophila. It is quite similar to *D. fulvimacula;* among the strains present at the Texas laboratory, *fulvimaculoides* can be distinguished from *fulvimacula* by its darker, more distinct pattern on the abdomen and mesonotum, the banding on the pleurae, and the coloring on the front and orbits.

Distribution.—The type locality is Turrialba, Costa Rica. The holotype and 9 paratypes are in the collection of the University of Texas. It has also been collected at San Salvador, El Salvador, and at Santa Maria de Ostuma, Nicaragua (see Table 1).

Matings

Mass matings were carried out among the various strains of *fulvimacula* and *fulvimaculoides*. The results are shown in Table 2. In all interspecific crosses where *fulvimaculoides* was the female, no F_1 progeny were obtained. In the reciprocal crosses there was a certain number of F_1 males and females. No F_2 was obtained. The F_1 males proved to be sterile; in four dissected males the internal genitalia appeared normal but the testes were not as highly pigmented as those of either parental male, nor were any motile sperm observed. Some F_1 females from the cross Brazilian *fulvimacula* (1975.5) to Costa Rican *fulvimaculoides* (H163.31) were backcrossed to H163.31 males; these females proved to be fertile.

TABLE 2

Crosses between strains of the *fulvimacula* complex.

	Intraspecific Crosses ♀ ♂	Results	Interspecific crosses ♀ ♂	Results
fulvimacula	H181.8 × H186.37	F_2	H163.31 × 1808.37	no progeny
	reciprocal	F_2	H163.31 × H181.8	no progeny
	H181.8 × H194.28	F_2	H163.31 × H186.37	no progeny
	reciprocal	F_2	H163.31 × H194.28	no progeny
	H181.8 × 1975.5	F_2	H163.31 × 1975.5	no progeny
	reciprocal	F_2	1808.37 × H163.31	no progeny
	H186.37 × H194.28	F_2	H181.8 × H163.31	no F_2
	reciprocal	F_2	H186.37 × H163.31	no F_2
	186.37 × 1975.5	F_2	H194.28 × H163.31	no F_2
	reciprocal	F_2	1975.5 × H163.31	sterile ♂ ♂
	H194.28 × 1975.5	F_2		fertile ♀ ♀
	reciprocal	F_2		
	H186.37 × 1808.37	F_2		
fulvimaculoides	H163.31 × H161.1	F_2		
	reciprocal	F_2		
	H163.31 × H163.11	F_2		
	reciprocal	F_2		
	H163.31 × H158.4	F_2		
	reciprocal	F_2		

Cytology

The cytology of the salivary gland chromosomes of the two species are as follows:

X Chromosome: The X chromosome is identical to that of *repleta*.

2 Chromosome: The changes which have taken place in the 2 chromosome, using the *repleta* 2 as the standard, are shown in Figure 1.

3 Chromosome: This chromosome differs from repleta in that inversion 3b is present.

4 and 5 Chromosomes: These chromosomes, like the X, are identical to those of *repleta*.

6 Chromosome: This is the dot chromosome; no comparisons were made among the various dot chromosomes of the repleta group.

Discussion

The species *fulvimacula* had been placed in the melanopalpa subgroup by Wheeler (1949) on morphological grounds. Cytological evidence indicates that both *fulvimacula* and *fulvimaculoides* have diverged from the other members of the repleta group from the 3b ancestor and not directly from *repleta*. This, however, does not invalidate its placement in the melanopalpa subgroup. The original 3b sequence appears to be the focal point from which the melanopalpa subgroup, the mercatorum subgroup, the species *peninsularis*, and the Xa, Xb, Xc, 2a, 2b, 3b ancestor of the hydei and mulleri subgroups diverge from each other. Cytologically, the *fulvimacula* complex is quite different from the melanopalpa complex (*melanopalpa*, *repleta*, *canapalpa*, *limensis*, and *neorepleta*). However, it is cytologically equally or more distinct from any other subgroup.

Both *fulvimacula* and *fulvimaculoides* are quite homozygous as to their gene sequences; however, one exception, the *fulvimacula* strain from Villavicencio, Colombia (H194.28), is quite interesting, being heterozygous for the gene sequence 21^2. Aside from this arrangement, which no other *fulvimacula* strain has, it is like the other strains. All of the *fulvimaculoides* strains are homozygous for this arrangement. In addition, they are homozygous for $2a^3$ which overlaps 21^2 and therefore must have occurred after 21^2. This is similar to what had been found by Ward and Stone (1952) among *melanopalpa*, *canapalpa* and *neorepleta*, and within the mulleri subgroup (Wasserman, 1954). The authors believe that the precursor of the two closely related species was heterozygous for this inversion in some areas. Most of the populations which gave rise to *fulvimacula* did not receive or lost this arrangement. As far as is known, all of the populations which have evolved into *fulvimaculoides* have maintained this arrangement in the homozygous condition and have added $2a^3$.

Cytologically these forms have also proven interesting. The inversion $2z^2$ has breakage points which are very similar to those of 2a. Inversions $2h^2$ of *fulvimacula* and $2c^3$ of *fulvimaculoides* have arisen independently. However, they both apparently have D3a as one of the breakage points. The other breakage points are quite distinct although fairly close to each other, one being C5b, the other C7–.

Our data do not give us information as to the sequence of origin of $2i^2$, $2j^2$, and $2k^2$. We postulate these three inversions with only four breakage points as a

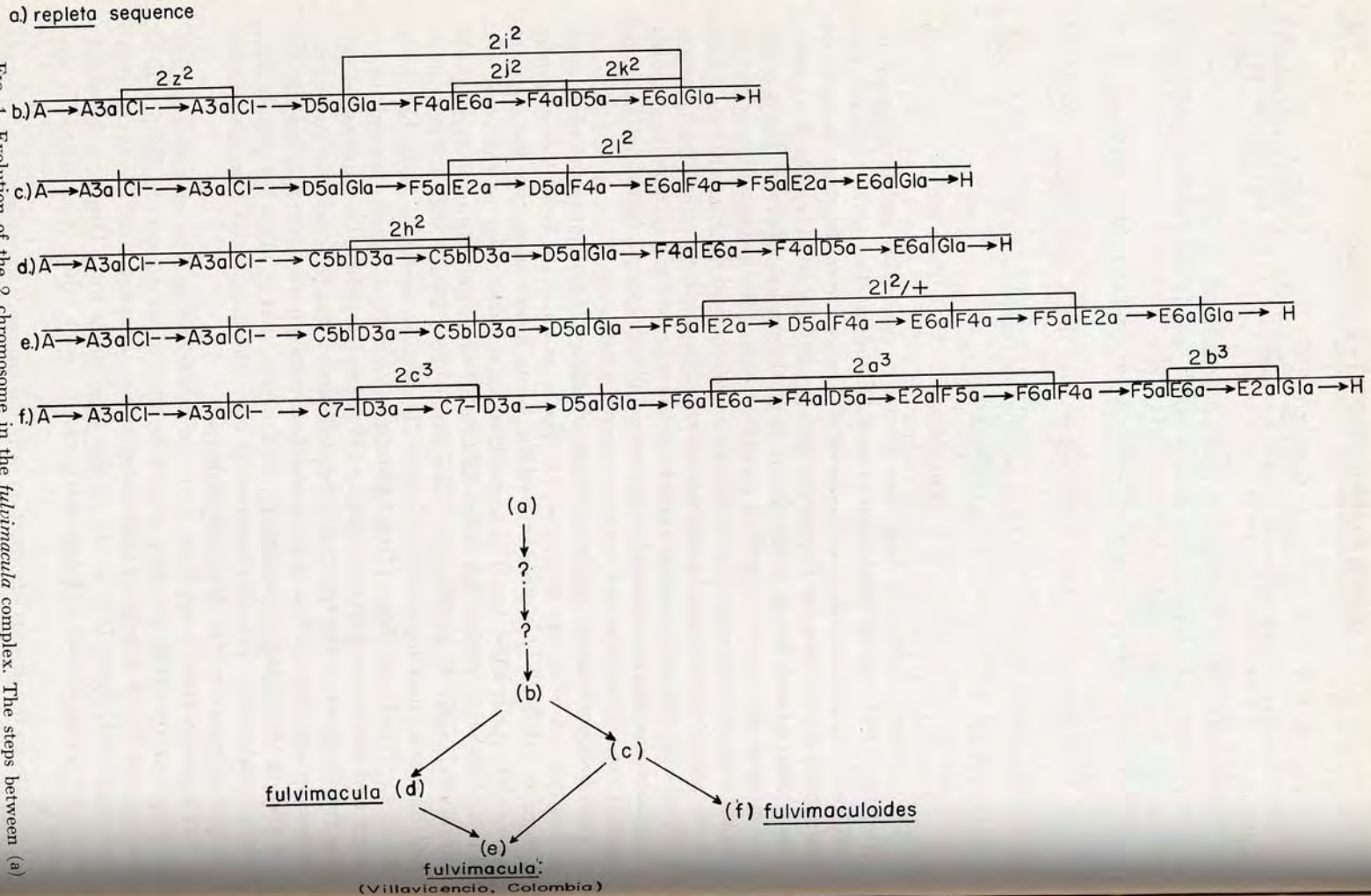

Fig. 1. Evolution of the 2 chromosome in the *fulvimacula* complex. The steps between (a) and (b) are unknown at present.

working model to explain the chromosomes as we interpret them. Our breakage points are, of course, too inaccurate to definitely assert that the chromosome has broken at the same place more than once. However, there is definitely a tendency to have certain areas involved in rearrangements.

II. MERCATORUM SUBGROUP

The mercatorum subgroup is composed of three described forms: the species *D. paranaensis*, and the two subspecies *D. mercatorum mercatorum* and *D. m. pararepleta*. *D. paranaensis* is known from Mexico to Brazil; the subspecies *mercatorum* has a distribution from Hawaii and the United States, south through Mexico and Central America to Peru and Bolivia. *D. m. pararepleta* is known only from Brazil.

Crosses between *paranaensis* and *mercatorum* have yielded a variety of results. In all cases, *paranaensis* females crossed to *mercatorum* males yield only female F_1. When *pararepleta* males are used, these F_1 females are fertile (Dreyfus and de Barros, 1948 and 1949). When *mercatorum* males are used, the F_1 females are sterile (de Barros, according to Patterson and Stone, 1952). The reciprocal crosses produce both male and female F_1; *mercatorum* female parents produce sterile males and females, while *pararepleta* female parents produce fertile F_1 females and sterile males.

Cytologically these two species present an interesting picture. Dreyfus and de Barros (1948, 1949) have found that the F_1 hybrids of Brazilian *paranaensis* × *pararepleta* are always heterozygous for 2 inversions in the II-E chromosome. There are also two other inversions which are heterozygous within *paranaensis* in this same arm.

Wasserman (1954), studying 4 Mexican strains and 1 Honduras strain of *paranaensis* and 14 stocks of *mercatorum* from various localities, found 5 inversions among the 2 species. All of the *mercatorum* stocks differed from the *paranaensis* strains by two inversions (2u and 2v). These are probably the same inversions found by Dreyfus and de Barros in Brazil, the II–E chromosome being equivalent to the 2 chromosome of *repleta*. One inversion, 3h, occurs only in *mercatorum*. Another, 3g, apparently is found heterozygous in both species. The third inversion, 3i, is found in *paranaensis*. Wasserman (1954) reported that 3i and 3g were both present in a Brazilian strain of *pararepleta*. A reexamination of this strain (1412.9B) has led us to believe that this is incorrect. The two inversions reported by Dreyfus and de Barros as being heterozygous within the *paranaensis* strains of Brazil were not found in the strains from Mexico and Honduras.

Matings

Crosses were carried out primarily to obtain F_1 hybrids for cytological analyses. The F_1 were checked for fertility to ensure correct identification. Mrs. W. B. Mather has made a considerable number of matings among the various strains of *paranaensis* and has kindly made available her unpublished data.

D. mercatorum × *D. mercatorum:* All attempted mass matings between strains of *mercatorum* yielded fertile F_1. Our new data, not previously reported, are shown in Table 3, bringing the total intraspecific crosses to 40. These include the extremes of the known range. For example, Hawaiian strains have been crossed

TABLE 3

Crosses in the mercatorum subgroup. One vial of about 20 pairs in mass was made of each cross except the *mercatorum* St. × *paranaensis* (H113.5) where two attempts were made.

	Crosses ♀ ♂	F_1	Crosses ♀ ♂	F_1
mercatorum ♀ × mercatorum ♂	St. × 2370.5	Fertile	St. × 2395.12	Fertile
	2370.5 × St.	Fertile	2395.12 × St.	Fertile
	St. × H191.46	Fertile	St. × H62.60	Fertile
	H191.46 × St.	Fertile	H62.60 × St.	Fertile
	St. × 2393.1	Fertile	St. × H191.30	Fertile
	2393.1 × St.	Fertile	H191.30 × St.	Fertile
mercatorum ♀ × paranaensis ♂	St. × H73.6	None	St. × H113.5	2 ♂ ♂
	St. × H186.33	None	St. × H34.20/32	None
	St. × H75.10	None	2370.5 × St.	None
	St. × H194.44	None	H191.30 × St.	None
	St. × H56.94	None	H191.46 × St.	None
	St. × H62.31	9 ♂ *, 22 ♀ *	H62.60 × St.	None
	St. × H163.16	None	2393.1 × St.	None
	St. × H101.12	None	2395.12 × St.	None
paranaensis ♀ × mercatorum ♂	St. × H191.30	None	H62.31 × H62.60	212 S ♀
	St. × H191.46	None	H73.6 × St.	14 ♀
	St. × 2370.5	None	H186.33 × St.	20 ♀
	St. × 2395.12	None	H75.10 × St.	82+ ♀
	St. × 2393.1	20 S ♀	H194.44 × St.	None
	St. × H62.60	45 ♀	H62.31 × St.	None
	H56.94 × St.	1 pupa	H163.16 × St.	41 S ♀
	H101.12 × St.	7 S ♀	H113.5 × St.	None
	H34.20/32 × St.	None		

St. = standard strain.
S = sterile in backcross.
* = no F_2.

to strains from the United States, Mexico, Costa Rica and Brazil. United States strains have been crossed to those from Hawaii, Mexico, Costa Rica, Colombia and Peru.

D. mercatorum females × *D. paranaensis* males: Table 3 gives the results of the interspecific crosses involving new strains. Only two crosses yielded progeny. A Mexican strain of *mercatorum* crossed to a Salvador *paranaensis* yielded 9 males and 22 females. No F_2 was obtained from these flies. The other cross was a Salvador *mercatorum* to a Trinidad *paranaensis*. Two males were obtained.

D. paranaensis females × *D. mercatorum* males: Table 3 shows the results of these crosses. They were either incompatible or only females were obtained. All of the F_1 females which were tested by backcrossing to one of the parental stocks proved to be sterile.

D. paranaensis × *D. paranaensis:* Unlike *mercatorum*, there is genetic isolation between geographical localities in this species. Table 4 shows the results obtained. The Mexican standard (2263.13) crossed with all available strains from Mexico (Wasserman, 1954) south to Palmira, Colombia. Occasionally a vial of 10 or 20 pairs did not produce progeny, but repeating the crosses gave fertile F_1. However, the Mexican standard, crossed to the Villavicencio, Colombia (H194.44) and the Trinidad (H113.5) strains, although yielding hundreds of F_1 flies, never produced an F_2.

The Trinidad strain was also used as a standard to the other strains. In most

TABLE 4

Crosses between strains of *D. paranaensis*. Mather's data was derived from mass matings of 10 pairs per vial; the number in parenthesis indicates the number of such vials. All other crosses were made as mass matings with about 20 pairs per vial; a second attempt was usually made when the first attempt gave no F_1.

Cross ♀ ♂	Mather's data (F_1)	F_1 (1st)	F_1 (2nd)	Cross ♀ ♂	Mather's data	F_1
M × H34.20/32		Fertile		T × H34.20/32		Many*
reciprocal		Fertile		reciprocal		Many*
M × H62.31	(6) Fertile	Fertile		T × H62.31	(6) +	Many*
reciprocal	(5) Fertile	None	Fertile	reciprocal	(4) *	Many*
M × H56.94	(6) Fertile	None		T × H56.94	(8) *	Many*
reciprocal	(5) Fertile	Fertile		reciprocal	(1) None	*
M × H73.6	(3) Fertile	Fertile		T × H73.6	(6) +	Many*
reciprocal	(2) Fertile	Fertile		reciprocal	(2) *	Many*
M × H75.10		None	Fertile	T × H75.10		Many*
reciprocal		Fertile		reciprocal		Many*
M × H163.16		Fertile		T × H163.16		Many*
reciprocal		None	Fertile	reciprocal		None
M × H101.12		Fertile		T × H101.12		Many*
reciprocal		Fertile		reciprocal		Many*
M × H186.33		None		T × H186.33		Fertile
reciprocal		Fertile		reciprocal		Fertile
M × H194.44		None		T × H194.44		Fertile
reciprocal		*	Many*	reciprocal		Fertile
M × T	(11) Pupae	None	†	T × M	(3) +	Many*
H73.6 × H62.31	(4) Fertile			H73.6 × H56.94	(3) Larvae	
reciprocal	(4) Fertile			reciprocal	(3) None	
H56.94 × H62.31	(8) Fertile			H62.31 × H56.94	(4) Fertile	

M = Mexico strain (2263.13).
T = Trinidad strain (H113.5).
+ = F_1 not tested further.
* = F_1 ♂ ♂ and ♀ ♀, no F_2.
† = F_1 ♂ ♂ sterile, ♀ ♀ fertile.

crosses, many F_1 flies were obtained. However, only in the crosses to the Villavicencio and the Santa Marta, Colombia (H186.33) strains were F_2 flies recovered.

Some of the F_1 females obtained from the cross between the two standards, Mexican females to Trinidad males, were backcrossed to Mexican males. These females proved fertile. Apparently the F_1 males of this cross, and probably the other crosses as well, were sterile.

Mather has unsuccessfully attempted to cross a Turrialba, Costa Rica strain (H73.60) with a Nicaraguan strain (H56.94). Both of these yield fully fertile F_1 when crossed to the Mexican standard.

Cytology

Clayton and Wasserman (this bulletin) report on the metaphase chromosomes of a number of strains of *paranaensis*. There are three karyotypes. Type (a) shows a large pair of metacentric chromosomes, three pairs of rods and one pair of dots. Type (b) has one pair of large metacentrics, three pairs of rods and a pair of small metacentrics. Type (c) has two pairs of large metacentrics and three pairs of rods. Type (a) was found in Villavicencio, Colombia; Trinidad; and was reported by Dreyfus and de Barros (1949) in Brazil. Type (b) was found in the Santa Marta Mountains of Colombia and from Heredia and Turrialba, Costa Rica. All of the other strains examined had type (c), including the 5 strains from Mexico and Honduras reported by Clayton and Ward (1954).

This karyotype information is related to our crosses. Crosses between strains of type (a) and type (c) produce the sterile males. The crosses involving type (b) produce variable results (see Table 4). We are not certain as to the exact mechanisms involved in the sterility encountered. But it seems evident that the amount of heterochromatin present on the dot chromosome affects the fertility of the hybrid males.

The salivary gland chromosomes of the new strains have been examined; and may be described as follows:

X Chromosome: The X chromosome of the mercatorum group seems to be identical to the *repleta* X. All stocks, except the two strains from Bucaramanga, Colombia have the *repleta* sequence. These two, H191.30 and H191.46, are homozygous for a new gene sequence, Xi. Using the *repleta* map, region C2c to E3c has inverted to form Xi.

2 Chromosome: One new sequence was found among the new strains. The Trinidad strain of *paranaensis* is homozygous for $2m^2$, whose breakage points are approximately C5d to G1–. It includes gene sequence 2e, whose breakage points are nearer to C6– and F3a than C6– and F1c, as reported in 1954. The resulting 2 chromosome of this strain is shown in Figure 2.

3 Chromosome: In 1954 Wasserman reported that a strain of *pararepleta* from Brazil (1412.9B) was heterozygous for 3i and 3g. We have recently examined

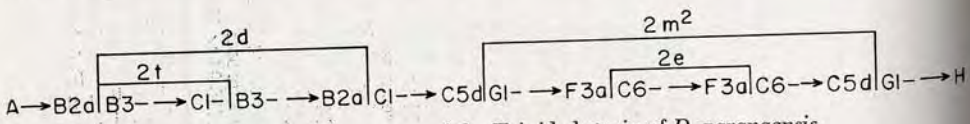

FIG. 2. Second chromosome of the Trinidad strain of *D. paranaensis*.

about 40 slides of this strain and have failed to find these inversions. We did, however, find at least one new inversion, 3n. Its breakage points are approximately D3d to F2a. In addition, one occasionally finds individuals which have the region around D1a clumped together into a small compact mass. We are not certain whether or not this is a very small heterozygous inversion. We believe that 3n and this compact mass was probably misidentified as 3i and 3g by Wasserman (1954).

4 Chromosome: The 4 chromosome, like the X, is also probably identical to the *repleta* 4. Two strains, H113.5 from Trinidad and H186.33 from the Santa Marta Mountains of Colombia, are heterozygous for a new gene arrangement, 4b. Regions B1– to C2d of *repleta* have inverted to form 4b.

5 Chromosome: All of the strains in this subgroup have the *repleta* 5.

Discussion

Table 1 lists the stocks and the chromosomal complement of the strains in the mercatorum subgroup. Figure 3 shows the phylogenetic relationships based on

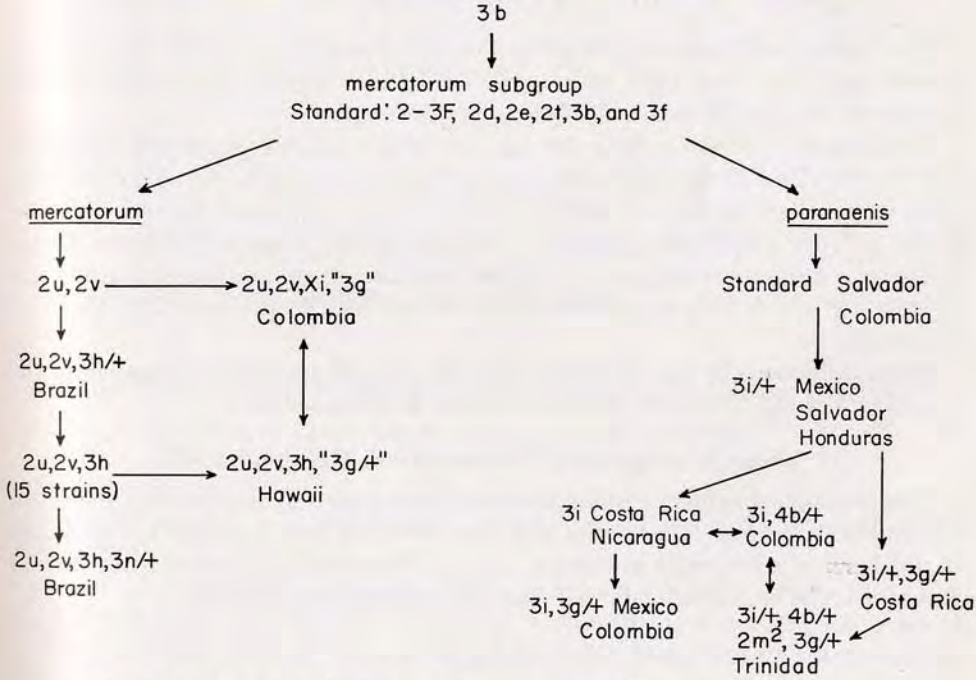

FIG. 3. Phylogenetic relationships between strains in the mercatorum subgroup.

the gene sequences found. Evidence had been presented (Wasserman, 1954) to show that the *repleta* sequence, or one differing from the *repleta* sequence by 3b, is the primitive type of the group. All of the strains of the mercatorum subgroup differ from the *repleta* sequence by having a 2–3 fusion, 2d, 2e, 2t, 3b and 3f. This is the standard arrangement of the mercatorum subgroup.

The data can be summarized as follows:

1. Several of the strains of *paranaensis* still retain the standard sequence of the subgroup.

2. The gene sequences 3i and 3g are widespread throughout the *paranaensis* distribution range, from Mexico to Colombia to Trinidad. The 3i gene sequence includes the 3g sequence. All *paranaensis* strains which have 3g also have 3i.

3. The Trinidad strain is unique for $2m^2$, and it also is heterozygous for 4b, an inversion which was found also in the Santa Marta strain.

4. All of the strains of *mercatorum* are homozygous for 2u and 2v, which have never been found in *paranaensis*.

5. All *mercatorum* strains except the Bucaramanga strains, have 3h. This has never been seen in *paranaensis*.

6. Two localities of *mercatorum* (Hawaii and Bucaramanga, Colombia) have a gene sequence which we have not been able to separate from 3g. It seems unlikely that this is 3g, because these strains do not have 3i, and, at least in *paranaensis*, 3i seems to have preceded 3g. The 3g sequence is included in 3i and the migration of the two from chromosome to chromosome in a double heterozygote, although possible, seems very unlikely.

III. MULLERI SUBGROUP

The mulleri subgroup as defined by Wheeler (1949) contains 15 species. Two species described since 1949, *Drosophila wheeleri* Patterson and Alexander and *Drosophila stalkeri* Wheeler, also belong to this subgroup.

Wasserman (1954) studied the salivary gland chromosomes of 12 of these species. Only one, *Drosophila peninsularis* Patterson and Wheeler, has a chromosome morphology which is at variance with the others. Its genic sequence, as seen in the salivary gland chromosomes, is very primitive, differing from the 3b ancestor by a unique inversion, 2o. All other members examined have evolved from an ancestor which had, in addition to 3b, the mulleri X (probably Xa, Xb, Xc), 2a and 2b.

From collections in the Caribbean area we have obtained several species of the mulleri subgroup. Strains of three species will be discussed here.

Drosophila nigricruria Patterson and Mainland, 1943

Our cytological evidence to be presented here causes us to place this species in the mulleri subgroup. The species had been recorded from Encinillas, Chihuahua in northern Mexico, south to Oaxaca, Mexico. We now have strains from Central America and Venezuela, and it has also been collected in Colombia.

Matings

We have carried out a number of matings among the various strains to determine whether these forms are compatible. Not all attempts were successful. However, there is no clear-cut break in fertility. In one case, repeating the cross which was sterile produced fertile F_1. The data are shown in Table 5.

Cytology

X Chromosome: In addition to the Xa, Xb, Xc gene sequence previously described by Wasserman (1954), the species *nigricruria* is homozygous for a unique sequence, Xk, whose breakage points are approximately C1a to D2e. This in-

TABLE 5

Crosses between strains of *D. nigricruria*. One vial with a mass mating of about 20 pairs was made of each cross except 1796.2 × 2266.15 where two attempts were made.

	Crosses ♀ ♂	Results	Crosses ♀ ♂	Results
4c,d × 4c,d	1796.2 × 2263.15	Fertile F_2	2263.15 × 2266.15	Fertile F_2
	1796.2 × 2266.15	Ster.; fertile F_1	2263.15 × 2268.7	Fertile F_2
	2263.15 × 1796.2	Fertile F_2	2266.15 × 2263.15	Fertile F_2
4c,d × St. 4	1796.2 × H62.29	Fertile F_2	2266.15 × 2261.7	F_1*
	1796.2 × C. Rica A	Fertile F_1	2266.15 × H62.29	Fertile F_2
	1796.2 × H75.11	Sterile	2266.15 × C. Rica A	Fertile F_2
	1796.2 × H206.19	Sterile	2266.15 × H75.11	Fertile F_2
	2263.15 × H62.29	Fertile F_2	2266.15 × H163.29	F_1*
	2263.15 × C. Rica A	Fertile F_1	2266.15 × H206.19	Fertile F_1
	2263.15 × H75.11	Fertile F_2	2268.7 × 2261.7	F_1*
	2263.15 × H163.29	Sterile	2268.7 × H62.29	Fertile F_2
	2263.15 × H206.19	Fertile F_2	1797.14 × 2261.7	F_1*
St. 4 × 4c,d	2261.7 × 2263.15	F_1*	H75.11 × 2263.15	Fertile F_2
	2261.7 × 2266.15	F_1*	H75.11 × 2266.15	Sterile
	H62.29 × 1796.2	Fertile F_2	H163.29 × 1796.2	Fertile F_2
	H62.29 × 2263.15	Fertile F_2	H163.29 × 2263.15	Fertile F_3
	C. Rica A × 1796.2	Sterile	H163.29 × 2266.15	Sterile
	C. Rica A × 2263.15	Fertile F_2	H163.20 × 2268.7	Sterile
	C. Rica A × 2266.15	Fertile F_1	H206.19 × 1796.2	1 pupa
	H75.11 × 1796.2	Fertile F_1	H206.19 × 2263.15	Sterile
St. 4 × St. 4	H62.29 × C. Rica A	Fertile F_2	H163.29 × H62.29	Fertile F_2
	H62.29 × H75.11	Sterile	H163.29 × C. Rica A	F_1*
	H62.29 × H206.19	Fertile F_1	H163.29 × H75.11	Fertile F_1
	C. Rica A × H62.29	F_1*	H163.29 × H206.19	Fertile F_1
	C. Rica A × H163.29	Fertile F_1	H206.19 × H62.29	Sterile
	H75.11 × C.Rica A	Fertile F_1	H206.19 × H163.29	Fertile F_2

* = fertility of F_1 not determined at the time of publication.

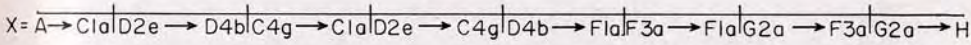

FIG. 4. The X chromosome sequence in *D. nigricruria*.

version overlaps Xa. The *nigricruria* X, in relationship to the *repleta* X, is shown in Figure 4.

2 Chromosome: The basic *nigricruria* 2 chromosome is quite complicated (Fig. 5). The following must be considered highly tentative as far as the existence of $2t^2$, $2u^2$, $2v^2$, $2w^2$, and $2x^2$ are concerned. Possibly intermediate forms will be found which will give a truer picture. Figure 5 shows the postulated changes from *repleta* to *nigricruria*.

3 Chromosome: Inversion 3b is present.

4 Chromosome: The basic 4 chromosome is the same as that for *repleta*. In addition, several of the Mexican strains are homozygous for two inversions: 4c (*repleta* regions F1a to G1a inverted) and 4d (*repleta* regions E3– to E4– inverted).

5 Chromosome: All strains differ from the *repleta* sequence by being homozygous for 5f, regions E2– to G1– being inverted.

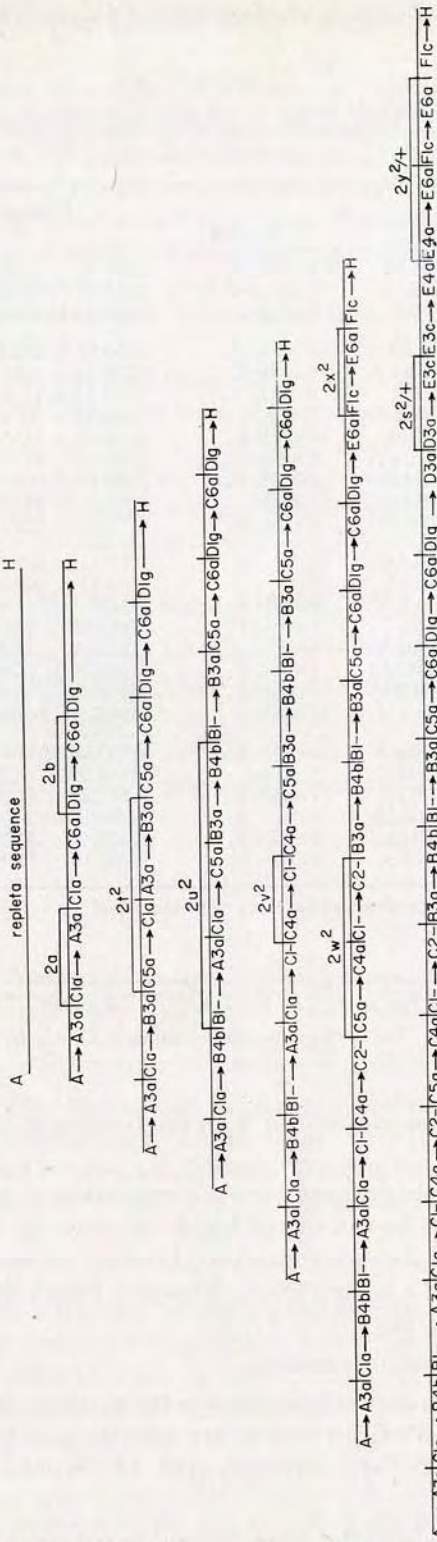

FIG. 5. A tentative evolution of the 2 chromosome from the *repleta* sequence to the *nigricruria* sequences.

Discussion

The basic chromosome configuration of the species is Xa, Xb, Xc, Xk, 2a, 2b, $2t^2$, $2u^2$, $2v^2$, $2w^2$, $2x^2$, 3b, *repleta* 4, 5f. The Central and South American strains appear to be homozygous for this genome. Figure 6 gives the known cytological composition of the strains.

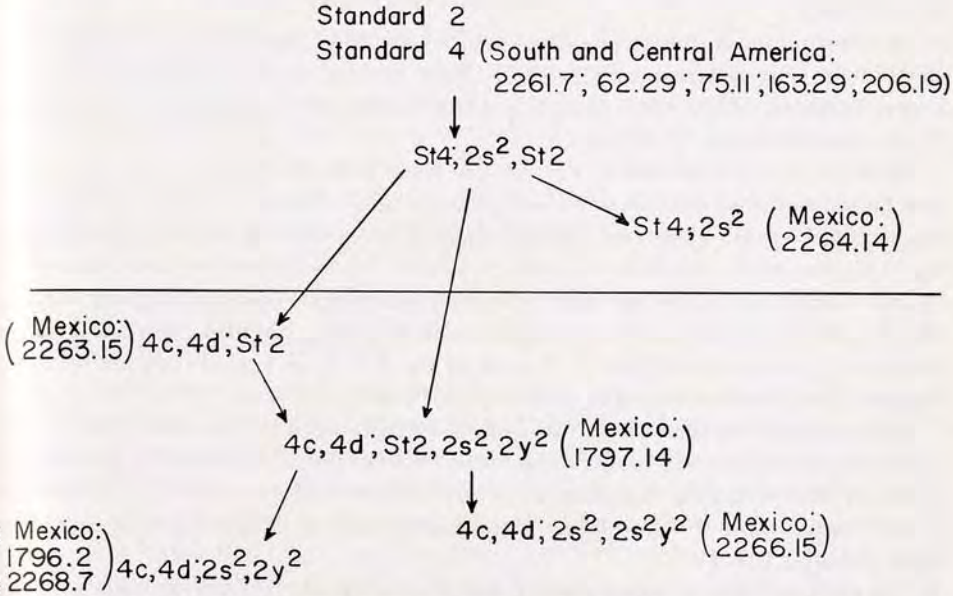

FIG. 6. Distribution of inversion sequences among the strains of *D. nigricruria*. The horizontal line denotes that no intermediate forms have been found between standard 4 strains and the strains homozygous for 4c and 4d.

Our data show that the standard 4 chromosome occurs from Venezuela, Costa Rica, Salvador, and into southern Mexico at Tehuacan, Puebla and San Andres Tuxtla, Vera Cruz. Almost immediately north and west, the standard is completely replaced by the 4c, 4d chromosome in the Tezuitlan, Mexico City, Cuernavaca, Morelia and Sierra Madre del Sur populations. The map distance between Tehuacan and Tezuitlan is of the order of 100 miles. The other distances are not much greater, Mexico City and Cuernavaca being approximately 125 to 150 miles from Tehuacan.

The basic 2 chromosome has a distribution almost identical to that of the basic 4. It also occurs in South and Central America and is either replaced by or coexists with newer sequences in southern Mexico. However, it was not found in the San Andres Tuxtla strain where standard 4 is present, and it is present in the strain from Tezuitlan where the 4c, 4d chromosome is found. The Mexico City strain has 4c, 4d, St 2, $2s^2$ and $2y^2$. The other three Mexican populations are apparently either homozygous or heterozygous for $2s^2$ and $2y^2$.

Thus in this geographical area our data indicates a complete change in the chromosome morphology of the species. Six of the seven populations fall within a semicircle whose radius is 150 miles. There is only one population, from Mexico

City, where the standard 2 chromosome has been demonstrated to coexist with the newer sequences. None of the populations seems to be heterozygous for the 4 chromosome. This may be due to insufficient sampling. Probably more collections in this area would yield populations heterozygous for the 4 chromosome. The size of our samples has been small; the collection data of Wheeler and Heed and Wasserman show that these strains originated from collections of from 2 to 44 individuals.

Another factor is that some of the strains may have become homozygous in the laboratory. The strains numbered 1700 have been in the laboratory for 10 years; those numbered 2200, for 5 years; and the South and Central American strains, from several months to 3 years.

It seems evident, however, that in this area both Standard 2 and Standard 4 are being replaced by the newer sequences. The change in the 2 chromosome appears to be gradual, as one would expect. The validity of the abrupt change in the 4 chromosome should be tested more fully. More collections and an analysis of individual flies should be made. It would be enlightening to discover not only the genetic composition of the populations in this area, but also the genetic constitution of the individual flies. A change in the genotype would indicate a change in selective forces or sexual or geographical isolation.

Our mating tests have shown no lack of fertility between the different chromosomal types. Matings in mass do not yield data as critical as paired or preferential matings. However, the variation as to how far north the standard 2 chromosome exists compared to the standard 4 chromosome indicates that there is some gene flow through this area.

There is no obvious geographical barrier involved. In Mexico, *nigricruria* is mainly a high altitude pine-oak forest form. This habitat is present at Morelia, Tezuitlan, Sierra Madre del Sur and Cuernavaca. San Andres Tuxtla is also forest, but at 1700 feet compared to 5–8000 feet in the above-mentioned sites. Mexico City has an altitude of about 7000 feet. Tehuacan is on the Mexican Plateau, in a dry cactus locality at about 5000 feet. The Central and South American localities are also forested areas.

Cytologically it is interesting to note that $2s^2$ and $2y^2$ are small independent inversions. They are situated very close to each other, there being only about 10 bands separating them. In the Mexico City strain there are three types of 2 chromosomes present, the basic 2, $2s^2$ and $2y^2$. The Morelia strain has $2s^2$ and $2y^2$. A total of 28 chromosomes of the two strains were in combinations where it could be ascertained that $2y^2$ and $2s^2$ were not on the same chromosome. The exact relationship of $2y^2$ and $2s^2$ could not be interpreted in 18 chromosomes. In no instance could it be demonstrated that $2s^2$ and $2y^2$ were on the same chromosome.

In the strain from Sierra Madre del Sur, all of the 2 chromosomes examined (28) had the $2s^2$ sequence. Ten had, in addition to $2s^2$, the $2y^2$ sequence. We cannot determine when these two rearrangements arose in relationship to each other because they are independent inversions. However, in order to get them occurring both singly on different chromosomes and also together on the same chromosome, there must have been at least one crossover in this short region of approximately 10 bands in a cell which was heterozygous for both inversions. This is a

somewhat unexpected finding in view of the fact that a heterozygous inversion tends to suppress crossingover in the adjacent regions. Two adjacent independent inversions practically eliminate the crossingover in the intercalary uninverted region (see Carson, 1953 and 1955 for discussion and references).

The *mulleri* complex

The mulleri complex is composed of a group of species, all members of the mulleri subgroup, showing a very close cytological relationship to each other (Wasserman, 1954). These include *Drosophila arizonensis* Patterson and Wheeler, *D. mojavensis* Patterson and Crow, *D. wheeleri* Patterson and Alexander. *D. aldrichi* Patterson and Crow, *D. mulleri* Sturtevant, *D. ritae* Patterson and Wheeler, and *D. martensis*, new species. Two strains of the new species were obtained in South America. The description follows:

Drosophila martensis Wasserman and Wilson, new species.

External characters of imagines.

♂, ♀. Arista with about 7 branches; antennae yellowish, the base of the second and the third joint dark brown. Front yellowish brown, orbits and ocellar triangle lighter, pollinose; hairs of the anterior orbits, the anterior and posterior orbitals, and anterior verticals with basal blackish spots. Middle orbital about ½ length of anterior and ⅗ length of posterior. Second oral about ½ length of first. Carina broadened below, sulcate. Palpi pale yellow, with one long terminal bristle, two long ventral bristles and a number of smaller weaker bristles. Face brownish yellow. Cheeks yellowish gray, their greatest width about ¼ greatest diameter of eyes. Eyes reddish (Pl. 3-L11) with short black pile.

Acrostical hairs in 8 rows; no prescutellars. Anterior scutellars convergent. Sterno index about 0.82. Middle sternopleural about ⅓ length of posterior. Mesonotum gray, bristles arising from brown spots with a slight tendency to fuse giving a superficial appearance of two brown bands between the dorsocentral rows, separated by a thin pollinose stripe. Some fusion of spots outside of dorsocentral rows. Scutellum dark brown; pollinose spots at angle of the scutellum between the bristles and occasionally at base. Pleurae yellowish gray with indistinct fuscous bands going from the base of the wing to the humerus, from the base of the haltere to the fore coxa, and in the region of the sternopleurals. Legs yellowish gray with indistinct black bands on distal ends of femora; black bands near bases of tibiae. Apical bristles on first and second tibiae, preapicals on all three.

Abdominal segments yellowish pollinose, the second to fifth tergites with an interrupted black band with forward extensions at the interruption, the lateral margins, and the angles of the tergites. The latter extensions widen rapidly at the anterior margin, usually connecting laterally with the lateral extensions enclosing an irregularly shaped yellow area. Dorsally they cut sharply into the yellow region leaving yellow markings as shown in Figure 7. The 6th tergite with an interrupted indistinct black band which fades at the angle.

Wings clear, veins brown; apex of 1st costal section darker. Costal index about 2.8; 4th vein index about 1.9; 5x index about 1.4; 4c index about 2.0. Two well-

developed bristles at the apex of the 1st costal section; 3rd section with heavy bristles on its basal ¼.

Length of body, about 3.0 mm.; wing, about 2.5 mm.

FIG. 7. Dorsal view of abdomen of female *D. martensis*.

Internal characters of imagines.

Testes yellow (Pl. 9-L1), with 2 inner and 2½ outer coils. Sperm pump with short blunt diverticula similar to those figured for *D. longicornis* by Patterson (1943; 143). Spermathecae slightly chitinized, bell-shaped. Ventral receptacle with about 15 loose coils.

Other Characteristics, relationship, and distribution.

Eggs. With 4 filaments of equal length.

Puparia. Tan (Pl. 12-L11); each anterior spiracle with about 14 branches; horn index about 1.5.

Chromosomes. Metaphase chromosomes are 5 pairs of rods and one pair of dots. The Y is a small metacentric chromosome (Clayton and Wasserman, this bulletin).

Relationship. Belongs to the mulleri subgroup of the repleta group of the subgenus Drosophila. Its phylogenetic relationship is discussed elsewhere in this paper.

Distribution. The type locality is Santa Marta, Colombia, from which the name is derived. It has also been collected in Barquesimeto, Venezuela. Collections were made by Dr. H. L. Carson, Mr. Hugo Hoenigsberg, and the senior author. The holotype and 9 paratypes are in The University of Texas collection.

Matings

In addition to the *nigricruria* and *martensis* strains, two strains of *D.* "*aldrichi*" were collected by Dr. W. B. Heed in Salvador and Colombia. Table 6 shows the matings carried out using these new strains. *D. martensis* would not cross with any of the other species tried. The two strains of "*aldrichi*" crossed readily with each other, producing a large number of F_1 and F_2. When crossed to an *aldrichi* standard from Mexico (1781.4) both "*aldrichi*" strains produced a large number of F_1 males and females. However, only the cross between Mexican *aldrichi* females and the Salvador "*aldrichi*" males produced an F_2 (1 male and 2 females). The reciprocal cross was unfortunately not attempted. Crosses between the Salvador strain and the *D. wheeleri* standard from Arcadia, California (1980.1) also yielded some F_1 flies but no F_2.

Dissections were made of several of the F_1 flies. Seven females and five males from the *wheeleri* × Salvador crosses appeared to have normal external and in-

TABLE 6

Crosses Among the Strains of the *mulleri* Complex.

♀ \ ♂	*martensis* Colombia	*martensis* Venezuela	*aldrichi* Mexico	"*aldrichi*" Salvador	"*aldrichi*" Colombia	*mulleri*	*wheeleri*
martensis Colombia	X	F_2	..	(3) St	St	St	..
martensis Venezuela	(2) F_2	X	..	St	St	St	..
aldrichi Mexico	..	..	X	F_2†	(2) F_1*	..	..
"*aldrichi*" Salvador	(2) St	St	..	X	(2) F_2	(2) St	F_1*
"*aldrichi*" Colombia	(2) St	(2) St	(2) F_1*	F_2	X	St	St
mulleri	St	St	..	St	St	X	..
wheeleri	St	St	..	F_1*	St	..	X

St = sterile
* = no F_2
† = 2♀♀, 1♂ only
(2) = number of attempts using 20 pairs in mass.

ternal genitalia but no sperm were seen in either the spermathecae of the females or the testes of the males. Three F_1 females from the *aldrichi* × Salvador cross were also found to lack sperm in the spermathecae; three males, however, had many sperm, but very few of them were motile. This latter cross is the one that produced some F_2 flies.

Morphologically, except for a difference in color, we have not been able to separate the Salvador and Colombian strains from *aldrichi*. Genetically they appear to be closer to *aldrichi* than to any other species tested. The F_1 females of these crosses were not tested for fertility because *aldrichi-wheeleri* crosses produce fertile F_1 females as shown by Patterson and Alexander (1952). Their extensive tests have never yielded any fertile F_1 males. In the Mexican *aldrichi* × Salvador strain, some F_1 male fertility was present. Due to the paucity of our genetic data, it seems best to consider these two strains as "*aldrichi*" for the purpose of this paper.

Cytology

The salivary gland chromosomes of "*aldrichi*" are identical to those of *aldrichi*, *mulleri* and *wheeleri* (Wasserman, 1954).

The chromosomes of *martensis* are as follows:

X Chromosome: The *martensis* X chromosome differs from the *mulleri* X (Xa, Xb, Xc) by being homozygous for inversion Xj, which has not been found in any other species. The chromosome is shown in Figure 8.

2 Chromosome: The evolution of the 2 chromosome is esesntially as shown in Figure 9. This is the first species of this complex which has been found to be chromosomally polymorphic. Both strains are heterozygous for $2g^2$.

3 Chromosome: The *martensis* 3 chromosome has 3b, 3a, and a new sequence, 3k. It is shown in Figure 8.

4 and 5 Chromosomes: These appear to be identical to *repleta*.

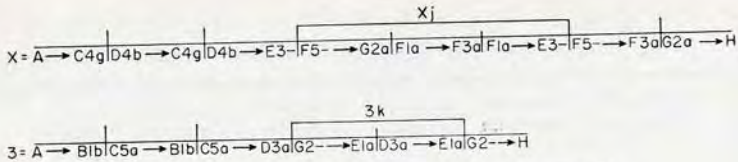

FIG. 8. The X and 3 chromosomes of *D. martensis*.

Discussion

It was shown in 1954 that the six species, *ritae*, *mulleri*, *aldrichi*, *wheeleri*, *mojavensis* and *arizonensis*, although each is apparently homozygous for certain gene sequences, share a series of 5 inversions. The data are such that a simple picture of speciation through geographical divergence could not be constructed. However, the inversions were so placed among the six species that the construction of a linear cline of four chromosomal types (called "subspecies") could be used to explain the data obtained without resorting to hybridization between species. One of the points in question in the hypothetical system was whether two populations could allow a free exchange of certain genes or groups of genes (an inversion) and still not allow other groups of genes to pass from one population to the other. In *nigricruria*, there is an indication that such a phenomenon is taking place. The so-called barrier for the standard 4 chromosome does not prevent the various types of 2 chromosomes from migrating from population to population.

Table 7 shows the gene sequences which are shared among the seven species. In 1954, the proposed linear cline was *ritae; mulleri, aldrichi, wheeleri; mojavensis; arizonensis*. There was no reason to believe that the precursor of this complex necessarily had a linear distribution. However, the chromosome morphology of the six species discussed in 1954 could be interpreted with the linear arrangement as the simplest explanation. The discovery of 2c and 3a in *martensis* complicates the picture. The lack of 2g which is present in the other six species places *martensis* off to one side, possibly south since it is now known only from South America. The gene sequences 2c and 3a both occur only in *mojavensis* and *mulleri*, *aldrichi*, and *wheeleri*. We therefore believe that the precursor of *martensis* probably occurred farther south than the other known species, bordering on either the precursor of *mojavensis* or, more likely, the ancestor of *mulleri*, *aldrichi*, and *wheeleri*. The gene sequences 2c and 3a were fixed in the *martensis*

TABLE 7

Distribution of inversions among the members of the *mulleri* complex.

Inversion	*ritae*	*mulleri aldrichi wheeleri*	*mojavenis*	*arizonensis*	*martensis*
2g	present	present	present	present	absent
3a	present	present	present	absent	present
2c	absent	present	present	present	present
2f	absent	present	present	present	absent
2h	absent	absent	present	present	absent
3c	present	present	absent	absent	absent

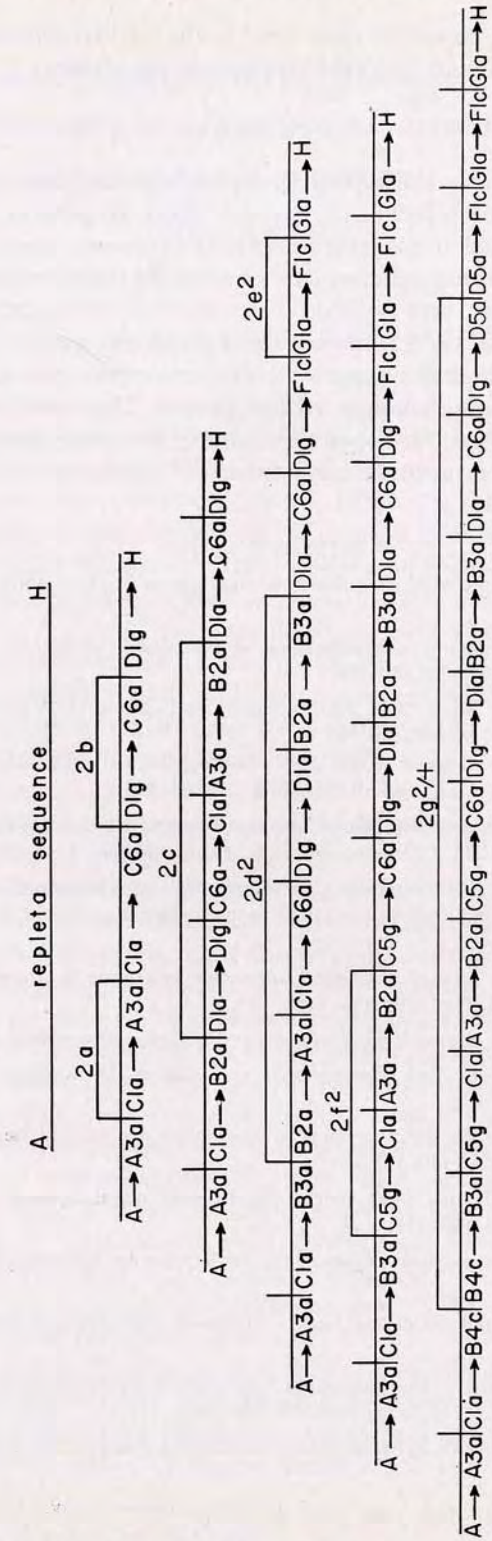

FIG. 9. Evolution of the 2 chromosome of *D. martensis*.

population; 2g, 2c, 2f, 3a and 3c were fixed in the *mulleri-aldrichi-wheeleri* populations; and 2g, 2c, 2f, and 3a in the *mojavensis* populations.

SUMMARY

It is difficult to discuss the repleta group as a whole, therefore the discussion has been given with each form as it was described. In general, the repleta group is not well represented in Central and South America. Mexico, with its large number of cactus-feeding species, can give us the most information as to the evolution of the group.

We now have data on 22 species throughout the group. This includes an analysis of the cytological changes which have taken place, as well as some knowledge of the genetic changes within groups. There are, however, some 60 species in the group, and it is hoped that some of the other forms can be analyzed to give us information on other mechanisms of speciation in this large and interesting species group.

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XII. Studies on Experimental Populations of *Drosophila arizonensis* and *Drosophila mojavensis*

LAWRENCE E. METTLER[1]

INTRODUCTION

In recent years a great deal of research has been done on the study of biotype competition in experimental populations. L'Héritier and Teissier (1934, 1937) found that under particular conditions the frequency of various mutant forms of *Drosophila melanogaster* decreased when in competition with the wild type. Dobzhansky and collaborators (see references in Dobzhansky, 1951) made extensive studies of populations of *Drosophila pseudoobscura*. Studies have also been made using different species as the competing forms, usually with the elimination of one species (Merrell, 1951; Moore, 1952). In these experiments the competition was between the two species only, since hybrids were not formed or were inviable or sterile. Koopman (1950) introduced *Drosophila pseudoobscura* and *Drosophila persimilis*, each marked by mutant genes, into a population cage. In each generation he discarded the hybrids which were phenotypically normal in order to test the increase in sexual isolation due to selection.

The present investigation was made on experimental populations derived from crosses of two species which have been shown to produce fertile hybrids in the laboratory. This was done to determine the results of competition and selection when the hybrids formed were allowed to remain in the population. The only comparable study of this type was made by Bruneau (Unpublished thesis) using species of the virilis group.

Great divergence in the degree of cross fertility has been found between different members of the mulleri subgroup (Crow, 1942; Patterson, 1947), where the prevention of free gene exchange is due largely to sexual isolation. The production of hybrid offspring in only a few crosses where the females are inseminated indicates the presence of other isolating mechanisms (Baker, 1947; Patterson, 1947). Of the various members of the group, *D. arizonensis* and *D. mojavensis* show the greatest possibility for gene exchange by interspecific hybridization. One combination (*mojavensis* ♀ × *arizonensis* ♂) produces fertile hybrids of both sexes, while the reciprocal cross produces fertile females but sterile males. On the basis of current knowledge, these forms are geographically (Patterson and Wagner, 1943) and presumably ecologically (Spencer, 1941; Wagner, 1949) isolated (Fig. 1). The mitotic chromosome configuration of both species consists of 5 pairs of rods and a pair of dots (Wharton, 1942). The two species differ cytologically by inversions in three chromosomes (Wasserman, 1954). The X chromosomes differ by one simple inversion, the 2nd chromosomes by 4 overlapping inversions, and the 3rd chromosomes by 2 overlapping inversions. These two species were chosen for study since fertile hybrids are formed between them and since the composition of the populations could be analyzed cytologically by examining the salivary gland chromosomes for normal and various hybrid combinations.

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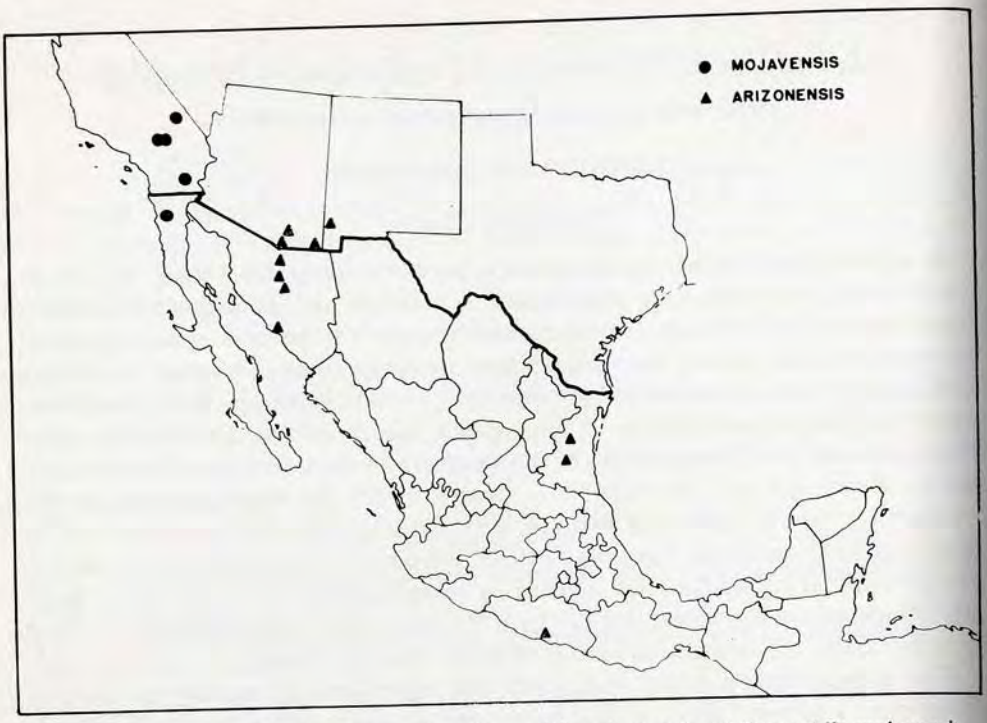

FIG. 1. Distribution of the known records of *Drosophila arizonensis* and *Drosophila mojavensis*.

MATERIALS AND METHODS

Experimental populations derived from crosses involving *D. arizonensis*, *D. mojavensis*, and their F_1 hybrids were maintained in population cages. Crosses were made using males and females of both species to determine if hybridization occurs when there is a choice of mates. This type of cross tests the effects of competition between species and hybrids if hybridization occurs, or solely between the two species if hybridization does not take place. Populations were started with males of one species and females of the other to test the effects of selection between possible gene and chromosome combinations where the F_1 hybrids are necessarily formed. Finally, an experimental population was initiated with F_1 hybrid males and females to determine the effects of competition in a population where the first generation consisted of various F_2 hybrids; here backcrosses, if any, were of necessity between hybrid forms.

Each of these experimental populations was analyzed by taking egg samples from the cage and examining the salivary gland chromosomes of the developing larvae. Population changes were determined by the change in frequency of the chromosome inversions characteristic of each species. The homozygous chromosome combinations for each species could be determined by the banding pattern.

The experimental populations were bred in population cages similar to those used by Wright and Dobzhansky (1946), which in turn were modifications of those devised by Héritier and Teissier (1933). All experiments were carried out at 23 ± 1° C. on a banana-agar-malt-yeast culture medium. The stocks used were

Drosophila arizonensis from Tucson, Arizona (stock no. 928.5) and *Drosophila mojavensis* (strain from Chocolate Mountains, California).

The flies were raised in regular culture bottles. Virgins from these were aged for six days and introduced into the population cages in desired proportions. Seven experiments were initiated. The crosses involved, the proportion of flies, and the date started are summarized in table 1.

TABLE 1

The initial experimental populations

Cage no.	Started	Initial population		Number
1	November 14, 1953	*mojavensis*	♀	500
		mojavensis	♂	500
		arizonensis	♀	500
		arizonensis	♂	500
2	November 14, 1953	*arizonensis*	♀	1,000
		mojavensis	♂	1,000
3	November 14, 1953	*mojavensis*	♀	1,000
		arizonensis	♂	1,000
4	February 14, 1955	*mojavensis*	♀	500
		mojavensis	♂	500
		arizonensis	♀	500
		arizonensis	♂	500
5	February 14, 1955	*arizonensis*	♀	1,000
		mojavensis	♂	1,000
6	February 14, 1955	*mojavensis*	♀	1,000
		arizonensis	♂	1,000
7	April 2, 1955	F_1 hybrid (M X A)	♀	1,000
		F_1 hybrid (M X A)	♂	1,000

Samples were taken from the cages once a month in order to determine the composition of the populations in regard to the three chromosomes mentioned above. A fresh food cup was placed in the cage and was removed after 24 hours. Eggs were taken from various areas of the food and placed in a regular culture vial. The process was repeated for five successive days. Chromosomes were examined from 20 larvae of each of these daily subsamples, giving a total of 100 larvae (600 chromosomes) examined for each monthly sample. Since the X chromosomes were being studied, only female larvae were utilized.

Cytological Technique

The cytological technique differed slightly from the usual method. The larvae were dissected in Drosophila saline solution, the salivary glands were placed in 1*N* HCl for six minutes, and then transferred to a drop of distilled water directly on the slide. The water was drained off and a drop of stain added. After five minutes the stain was drained off, a drop of 40 percent acetic acid was added and the salivary glands covered with a coverslip. Spreading of the chromosomes was done by pressing lightly on the coverslip with a needle. The preparation was then ringed with paraffin-lanolin. The stain used was one percent orcein in 50 percent acetic acid.

RESULTS

Of the seven experiments, no two were exactly alike. Cages four, five, and six were duplicates of cages one, two, and three in regard to the initial composition of the population; but the first analysis was deferred several months in the latter group to test the effects of selection over a longer period of time. Experiments one, two, and three were started November 14, 1953, but the first analysis was not begun until April, 1955. The duplicate experiments were started February 14, 1955, and samples were taken within 30 days. During December of 1954, cage three was found to have a contamination of *Drosophila melanogaster* and was discontinued before samples were taken. Since the male hybrids of the cross *arizonensis* ♀ × *mojavensis* ♂ are sterile, no attempt was made to set up a hybrid cage reciprocal to cage seven.

Tables 2–7 summarize the results of the six experiments. The information is arranged to show the number of heterozygotes and homozygotes for the three chromosomes. Homozygous *arizonensis* is represented by AA, homozygous *mojavensis* by MM, and the heterozygous condition by AM. The initial population composition is given as a percentage (considering both males and females). Otherwise, since exactly 100 individuals were studied per sample, each entry can be read as an absolute number or a percentage. The percentage of *arizonensis* chromosomes is given in the last three columns. Since three chromosomes were followed, each of which may be homozygous or heterozygous, one particular larva could be placed in one of 27 classes of chromosome combination. These will be referred to as larval classes. The number of individuals in these various larval classes is presented in the bottom half of tables 2–7. In this case, homozygous *arizonensis* is denoted by A, homozygous *mojavensis* by M, and the heterozygous condition by H.

The data are presented graphically in figures 2–7. The percentage of homozygous and heterozygous combinations of the three chromosomes is shown for each population. The time ordinate is given in elapsed numbers of days.

Cage 1

(Table 2, Figure 2)

Cage 1 was started with males and females of both species in equal numbers. Following the first analysis samples were taken at 30-day intervals, except between the 638th and 698th day. The population went quite well from its start, with an over-abundance of larvae in each food cup. The experiment was started with a total of 2,000 flies, but within three generations the population increased to over twice this number (by visual estimation).

Only the *mojavensis* X chromosome was present in any of the six successive samples. The *arizonensis* X chromosome was eliminated from the population within 518 days. The 2nd and 3rd chromosomes of both species were still present in the population after 698 days from its initiation.

The *mojavensis* 2nd chromosome homozygotes varied in frequency between 16 and 50 percent and were more frequent than *arizonensis* homozygotes in every sample. The 2nd chromosome heterozygotes were found in higher frequency than either homozygote in all samples except the first, where homozygous

TABLE 2

Results of cage 1, started with females and males of both species

Date	Elapsed Time in Days	Chromosone X			Chromosome 2			Chromosome 3			Percent ARIZONENSIS		
		AA	AM	MM	AA	AM	MM	AA	AM	MM	X	2	3
11–14–53	0	50	0	50	50	0	50	50	0	50	50.0	50.0	50.0
4–16–55	518	0	0	100	4	46	50	0	44	56	0.0	27.0	22.0
5–16–55	548	0	0	100	6	78	16	0	40	60	0.0	45.0	20.0
6–15–55	578	0	0	100	10	51	39	0	32	68	0.0	35.5	16.0
7–15–55	608	0	0	100	8	64	28	0	30	70	0.0	40.0	15.0
8–14–55	638	0	0	100	18	44	38	0	24	76	0.0	40.0	12.0
10–13–55	698	0	0	100	2	64	34	0	14	86	0.0	34.0	7.0

Elapsed Time in Days	(X) A	A	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H	H	M	M	M	M	M	M	M	M
	(2) A	A	A	H	H	H	M	M	M	A	A	A	H	H	H	M	M	M	A	A	A	H	H	H	M	M
	(3) A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M
518																				4			16	28		26	26
548																				6			22	56		12	4
578																				3	7		17	34		12	27
608																				2	6		22	42		6	22
638																				2	16		10	34		12	26
698																					2		10	54		2	32

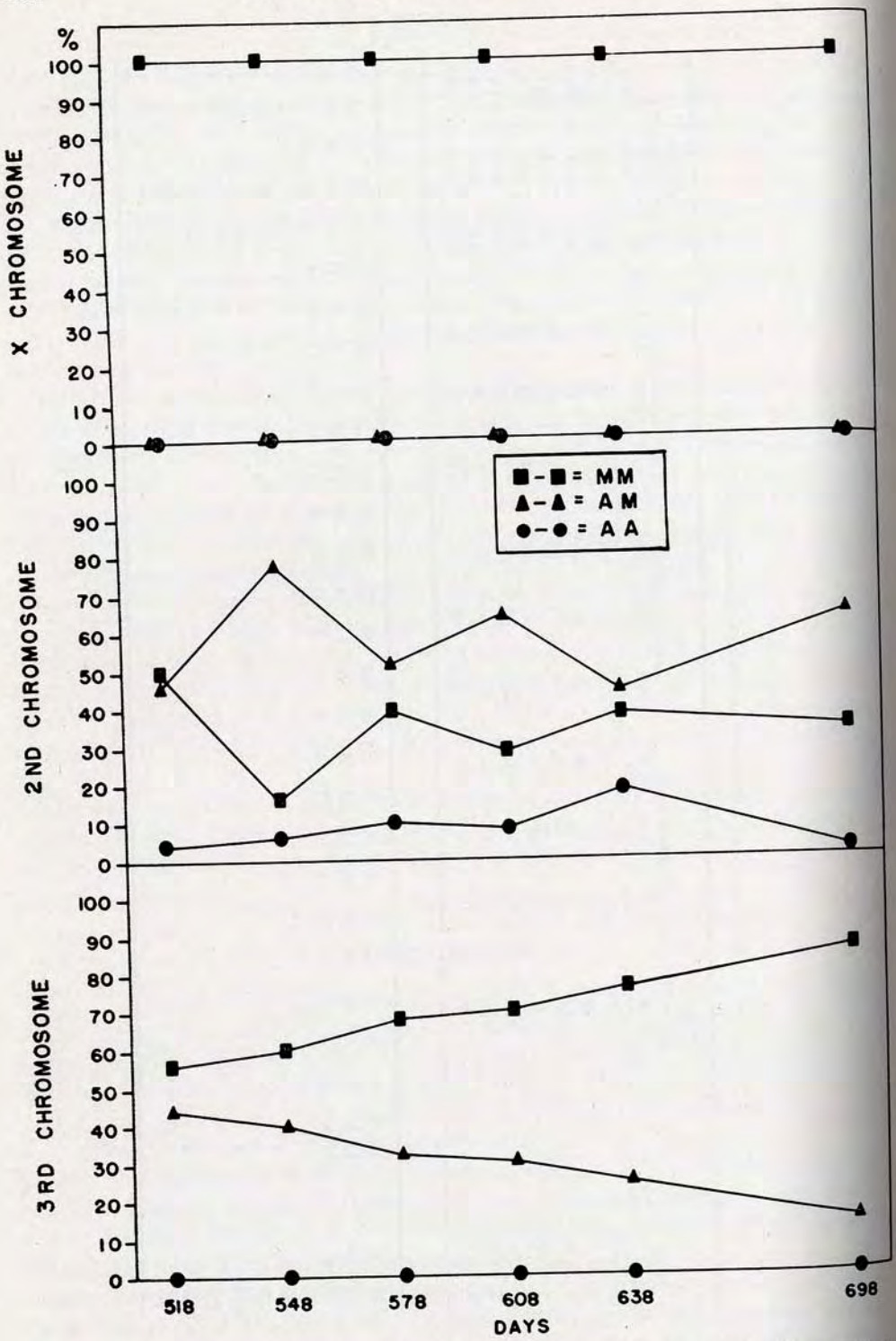

FIG. 2. Cage 1. Started with males and females of both species. The percentage of homozygous and heterozygous combinations of the three chromosomes.

mojavensis was 4 percent greater. The percentage of *arizonensis* 2nd chromosomes varied between 27 and 45 percent.

Third chromosome *arizonensis* homozygotes were not found in any sample. Third chromosome heterozygotes declined steadily from 44 to 14 percent, while *mojavensis* homozygotes increased reciprocally. There was a gradual decrease in the number of *arizonensis* 3rd chromosomes from 22 to 7 percent. A few *arizonensis* homozygotes would be expected from matings between heterozygotes. Either the matings did not produce them or they were selected against in the samples.

Only six of the 27 possible larval classes were represented. These six classes differed from one another in frequency, and individually varied somewhat between samples. The largest class was M-H-M, with over 50 percent of all larvae analyzed in the 548- and 698-day samples. Of the total number of individuals analyzed in cage 1, 77 percent were known hybrids.

Cage 2

(Table 3, Figure 3)

The initial population of the second experiment consisted of *arizonensis* females and *mojavensis* males. Subsequent to the first analysis, samples were taken after intervals of 30, 60, and 60 days. The population went well at first, but its size dropped in the final six months of the experiment accompanied by bacterial and mold infection. At no time did the number of flies present fall below 1,000–2,000.

As in cage 1, only *mojavensis* X chromosomes were present in the samples. Both chromosome arrangements of the 2nd and 3rd chromosomes were still present in the last analysis. The 2nd chromosome *arizonensis* homozygotes were below eight percent in each sample. The heterozygous state ranged between 26 and 48 percent and was lower in frequency than the *mojavensis* homozygotes except in the 578-day sample. The percentage of 2nd chromosomes which were *arizonensis* varied between 15 and 32 percent in the four samples.

The 3rd chromosome percentages were similar in the four successive analyses. Homozygous *arizonensis* 3rd chromosomes were not found in the 400 larvae examined. The heterozygous condition fluctuated in frequency between 16 and 18 percent, and *mojavensis* homozygotes ranged between 82 and 84 percent. The number of *arizonensis* 3rd chromosomes was between eight and nine percent.

Only six of the 27 possible larval classes were represented, and these were the same classes found to be present in cage 1. The class M-M-M was the largest, consisting of 34 to 58 percent of the larvae analyzed in each sample. Considering the four samples, 53 percent of all analyzed individuals were known hybrids.

Cage 4

(Table 4, Figure 4)

Cage 4 was started with males and females of both species. The first analysis was made 10 days after the flies were introduced into the cage. The larvae of this sample were produced by the initial population. Subsequent samples were taken at 30-day intervals. The adult population increased in size, and extremely large numbers of larvae were present in the food cups.

TABLE 3

Results of cage 2, started with *arizonensis* females and *mojavensis* males.

Date	Elapsed Time in Days	Chromosone X			Chromosome 2			Chromosome 3			Percent ARIZONENSIS		
		AA	AM	MM	AA	AM	MM	AA	AM	MM	X	2	3
11–14–53	0	67	0	33	50	0	50	50	0	50	66.7	50.0	50.0
5–16–55	548	0	0	100	1	41	58	0	16	84	0.0	21.5	8.0
6–15–55	578	0	0	100	8	48	44	0	18	82	0.0	32.0	9.0
8–14–55	638	0	0	100	2	44	54	0	16	84	0.0	24.0	8.0
10–13–55	698	0	0	100	2	26	72	0	18	82	0.0	15.0	9.0

Elapsed Time in Days	(X)	(2)	(3)																										
	A	A	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H	H	M	M	M	M	M	M	M	M	M	M	M
	A	A	A	H	H	H	M	M	M	A	A	A	H	H	H	M	M	M	A	A	A	H	H	H	M	A	H	M
	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	M	M	M	M
548																				1			7	34		8	50	
578																					8		8	40		10	34	
638																					2		8	36		8	46	
698																					2		4	20		16	58	

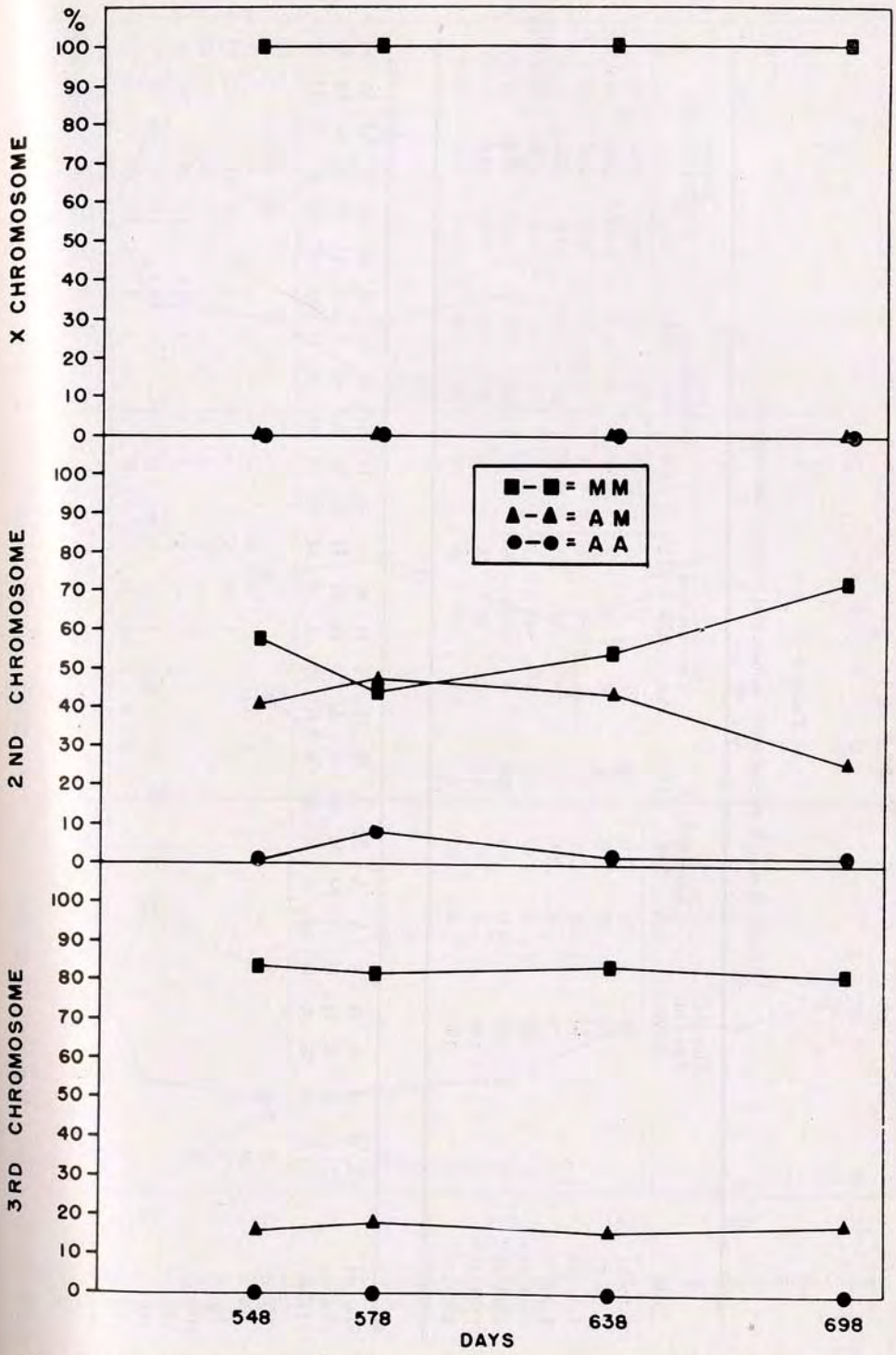

FIG. 3. Cage 2. Started with *arizonensis* females and *mojavensis* males. The percentage of homozygous and heterozygous combinations of the three chromosomes.

TABLE 4

Results of cage 4, started with females and males of both species

Date	Elapsed Time in Days	Chromosone X			Chromosome 2			Chromosome 3			Percent ARIZONENSIS		
		AA	AM	MM	AA	AM	MM	AA	AM	MM	X	2	3
2–14–55	0	50	0	50	50	0	50	50	0	50	50.0	50.0	50.0
2–24–55	10	28	0	72	28	0	72	28	0	72	28.0	28.0	28.0
3–26–55	40	24	24	52	26	16	58	24	20	56	36.0	34.0	34.0
4–25–55	70	5	24	71	7	31	62	8	26	66	17.0	22.5	21.0
5–25–55	100	1	30	69	5	44	51	6	29	65	16.0	27.0	20.5
6–24–55	130	0	24	76	9	52	39	0	42	58	12.0	35.0	21.0
7–24–55	160	0	19	81	16	56	28	2	43	55	9.5	44.0	23.5
8–23–55	190	0	25	75	15	50	35	4	50	46	12.5	40.0	29.0

Elapsed Time in Days	(X) A (2) A (3) A	A A H	A A M	A H A	A H H	A H M	A M A	A M H	A M M	H A A	H A H	H A M	H H A	H H H	H H M	H M A	H M H	H M M	M A A	M A H	M A M	M H A	M H H	M H M	M M A	M M H	M M M
10	28																										72
40	24										2			12	2		2	6						2		4	46
70	5									2			1	7	8		3	3					4	11		12	44
100	1									1			3	6	8		5	7		1	2	1	5	20		13	27
130											2	2		6	6		5	3		3	2		10	30		16	15
160										1	2	4	1	2	4		2	3		5	4		19	29		12	12
190											4	3	1	6	3		6	2		3	5	3	18	19		13	14

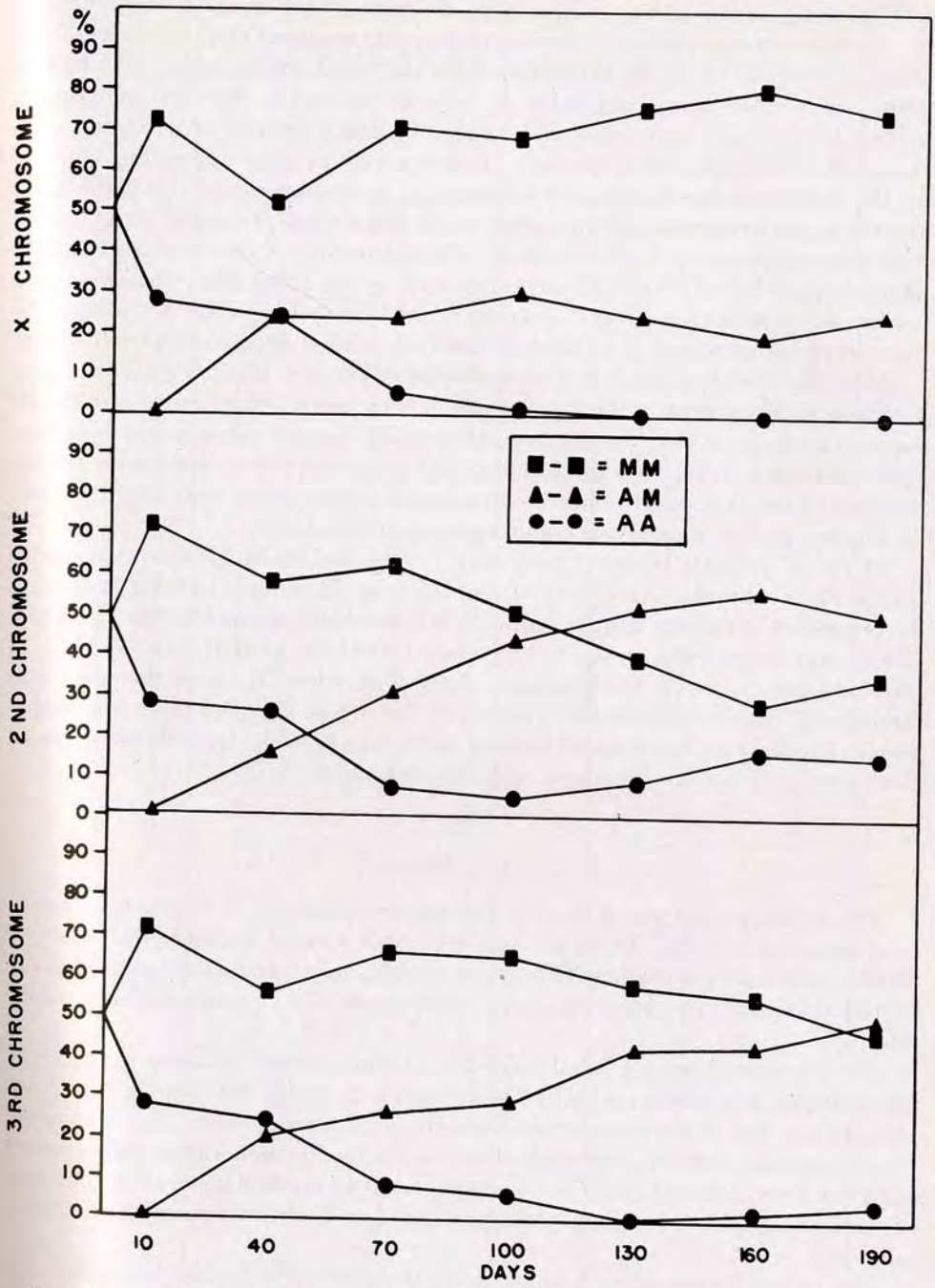

FIG. 4. Cage 4. Started with males and females of both species. The percentage of homozygous and heterozygous combinations of the three chromosomes.

The results of the 10-day sample were the same for all three chromosomes: 28 per cent *arizonensis* homozygotes and 72 percent *mojavensis* homozygotes. Since a few individuals in the 40-day sample were the result of a backcross or F_1 hybrid cross, there must have been some F_1 hybrids present in the first generation, although they were not detected because of chance or time of sampling. The *arizonensis* chromosomes were lower in frequency in all samples; but whereas in the autosomes the decrease of *arizonensis* chromosomes was complete by 70 days and was even reversed thereafter, there was a more prolonged trend toward loss of the *arizonensis* X chromosomes. The *arizonensis* X chromosome homozygotes dropped rapidly from 50 percent to none by the 130th day, while *mojavensis* homozygotes increased to 75 percent in the last analysis. The X chromosome heterozygotes increased to 24 percent and remained at approximately this level.

After the 10-day analysis 2nd chromosome *mojavensis* homozygotes gradually dropped to 35 percent in the last sample. There was a reduction of *arizonensis* homozygotes to an even lower level, 16 percent. Second chromosome heterozygotes increased to over 50 percent. The results of the 3rd chromosome were similar to those of the 2nd, except that the *arizonensis* homozygotes were slightly fewer in number and the *mojavensis* homozygotes more frequent.

Of the 27 possible larval classes, only A-A-A and M-M-M were represented in the 10-day sample. Class M-M-M dropped from 72 per cent in the first analysis to 14 percent in the last sample, while A-A-A decreased to none by the 130th day. There was an increase in the hybrid classes M-H-M, M-H-H, and M-M-H. In the last three samples these classes, along with M-M-M, were the ones most abundant. Within 130 days, 85 percent of the larvae sampled were known hybrids. Thus in cage 4, where a choice of mates was possible, hybrids were formed and eventually became the majority in the population.

Cage 5

(Table 5, Figure 5)

The initial population of the 5th experiment consisted of *arizonensis* females and *mojavensis* males. The cage went well, and a large number of larvae were produced in each generation. The cross was obligatory, producing only F_1 hybrids (H-H-H) in the first generation. As stated above, the F_1 males of this cross are sterile.

The *arizonensis* arrangement of the three chromosomes was lower in frequency than *mojavensis* in most samples. As in cages 1, 2, and 4, the trend in cage 5 was toward the loss of the *arizonensis* X chromosome arrangement. The *arizonensis* X chromosome homozygotes dropped to none in the first generation and remained at a low level. The reduction of heterozygotes after the first generation continued to the last sample, in which only one percent of all X chromosomes was *arizonensis*.

The second chromosome heterozygotes were found in higher frequency than either of the homozygotes, at approximately a 50–60 percent level starting with the 40-day analysis. The two homozygous conditions fluctuated somewhat, but *mojavensis* was usually in higher frequency. After the first generation, the two chromosome arrangements of the 3rd chromosome approximated an equilibrium. Third chromosome *arizonensis* homozygotes were found to be less than six per-

TABLE 5

Results of cage 5, started with *arizonensis* females and *mojavensis* males.

Date	Elapsed Time in Days	Chromosone X			Chromosome 2			Chromosome 3			Percent ARIZONENSIS		
		AA	AM	MM	AA	AM	MM	AA	AM	MM	X	2	3
2–14–55	0	67	0	33	50	0	50	50	0	50	66.7	50.0	50.0
2–24–55	10	0	100	0	0	100	0	0	100	0	50.0	50.0	50.0
3–26–55	40	0	46	54	0	49	51	0	58	42	23.0	24.5	29.0
4–25–55	70	0	53	47	13	58	29	2	56	42	26.5	42.0	30.0
5–25–55	100	0	31	69	14	61	25	3	47	50	15.5	44.5	26.5
6–24–55	130	3	24	73	25	57	18	5	50	45	15.0	53.5	30.0
7–24–55	160	0	13	87	11	59	30	2	44	54	6.5	40.5	24.0
8–23–55	190	0	2	98	12	50	38	6	42	52	1.0	37.0	27.0

Elapsed Time in Days	(X) A	A	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H	H	M	M	M	M	M	M	M	M
	(2) A	A	A	H	H	H	M	M	M	A	A	A	H	H	H	M	M	M	A	A	A	H	H	M	A	M
	(3) A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	M	M
40														17	9		13	8					12	10		16	15
70											4	5	2	20	7		10	4		2	2		14	15		6	9
100											5	1	2	10	7		3	3		4	4		18	24	1	7	11
130	1				2					1	3		2	7	5		1	5	1	12	7		20	21		5	7
160											1	1		6	1		1	3	1	3	5	1	20	31	1	13	12
190															2					8	4		20	28	6	14	18

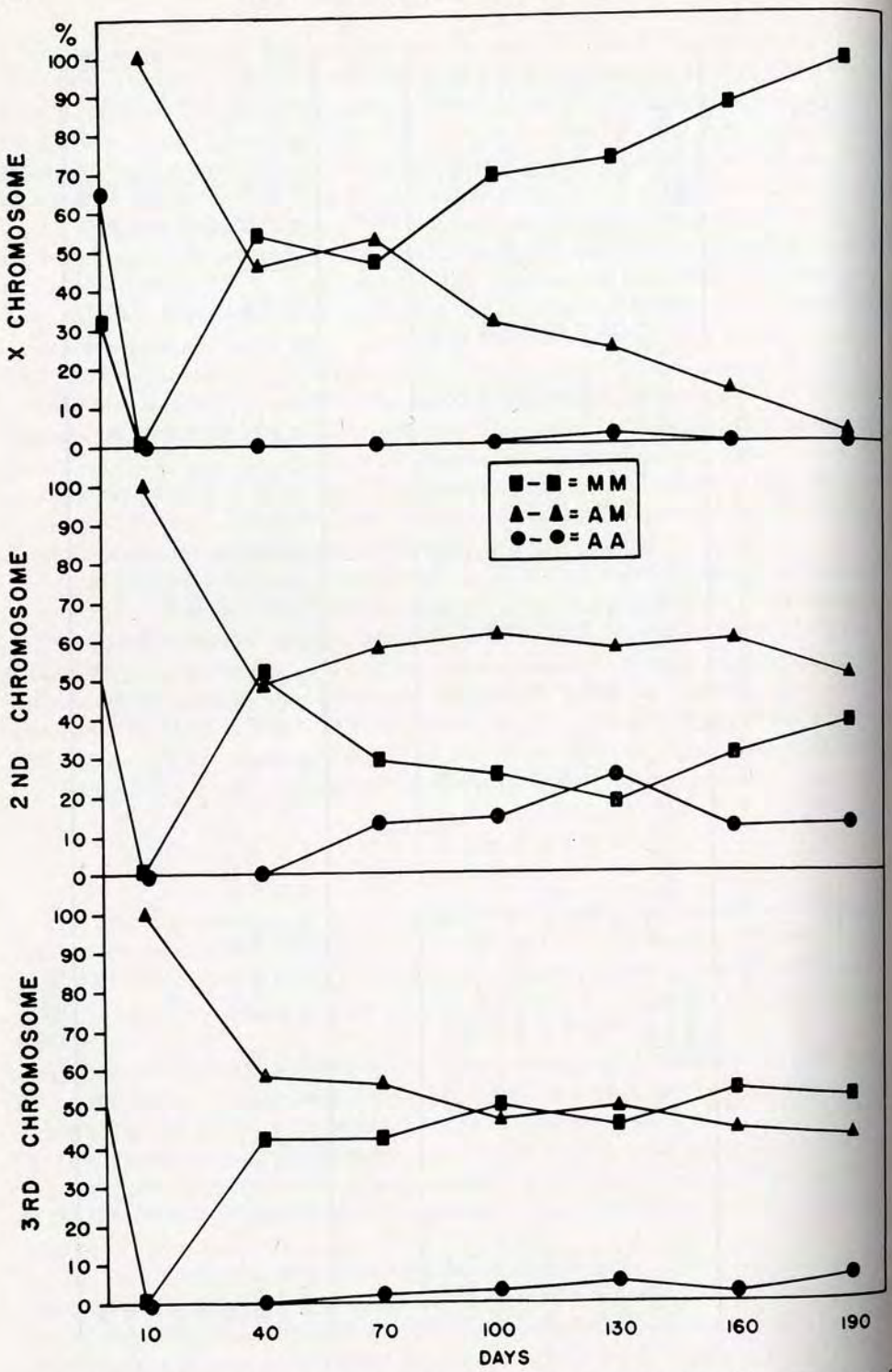

FIG. 5. Cage 5. Started with *arizonensis* females and *mojavensis* males. The percentage of homozygous and heterozygous combinations of the three chromosomes.

cent in all samples. After the 40-day analysis, there was a slight increase of *mojavensis* homozygotes to about 52 percent and a decrease of heterozygotes to approximately 42 percent.

Only H-H-H of the larval classes was represented in the first generation due to the obligatory cross. The number of represented classes increased to 16 by the 130th day but then decreased to eight by the last analysis. Six of these eight classes were the ones found to be present in cages 1 and 2. Classes M-H-M and M-H-H increased rapidly and became the most abundant in the last four samples. Over 82 percent of the larvae analyzed in each sample were known hybrids.

Cage 6

(Table 6, Figure 6)

This cage was started with *mojavensis* females and *arizonensis* males. The cage went very poorly at the beginning, although numerous eggs were laid. Egg samples were taken, but the hatch was too low to yield a significant analysis. It was impossible to make an analysis until the 100th day, when many larvae were produced both in the sample and in the cage. At this time the size of the population increased rapidly until it was substantially larger than the initial number of 2,000 flies. There was a certain degree of fluctuation in each of the zygotic chromosome combination percentages, but no trend was observed. The heterozygous condition of each of the three chromosomes was highest in frequency in most samples, while *mojavensis* homozygotes were the lowest. Thus it may be noted that this was the only experiment having the total number of *arizonensis* chromosomes greater than 50 percent. In the other cages *arizonensis* X chromosomes tended toward less or were eliminated. In all samples of this experiment, the X chromosome heterozygotes were the largest class.

Many of the 27 possible larval classes were represented. Individuals of 22 larval classes were present in the 160-day sample. No noticeable trend in the change of class frequency was observed.

Cage 7

(Table 7, Figure 7)

Experiment 7 was started with male and female F_1 hybrids from the cross *mojavensis* ♀ × *arizonensis* ♂. The cage went poorly for the first 2–3 months, but then the population started to build up slowly. It was impossible to make an analysis until the 104th day. Even at this time the number of larvae in the sample and in the cage was small. The same was true for the 134-day analysis. The 164-day sample vials had a large amount of mold present, and not enough larvae were produced to make an analysis. By the 194th day the cage was going very well, with many larvae present in the food cups and sample vials.

The X chromosome behavior was similar to that in cages 4 and 5. The homozygous *arizonensis* combination dropped to none, the heterozygous condition decreased to 13 percent, and the *mojavensis* homozygotes increased to 87 percent by the 194th day. The 2nd chromosome heterozygotes were far more frequent than the two homozygotes. The *mojavensis* homozygotes decreased to 25 percent, while *arizonensis* homozygotes were even less frequent, 9 percent in the

TABLE 6

Results of cage 6, started with *mojavensis* females and *arizonensis* males.

Date	Elapsed Time in Days	Chromosone X			Chromosome 2			Chromosome 3			Percent ARIZONENSIS		
		AA	AM	MM	AA	AM	MM	AA	AM	MM	X	2	3
2–14–55	0	33	0	67	50	0	50	50	0	50	33.3	50.0	50.0
2–24–55	10	0	100	0	0	100	0	0	100	0	50.0	50.0	50.0
5–25–55	100	44	46	10	52	42	6	48	40	12	67.0	73.0	68.0
6–24–55	130	30	47	23	35	47	18	37	48	15	53.5	58.5	61.0
7–24–55	160	30	42	28	45	48	7	43	47	10	51.0	69.0	66.5
8–23–55	190	35	49	16	38	53	9	47	49	4	59.5	64.5	71.5

Elapsed Time in Days	(X) A (2) A (3) A	A A H	A A M	A H A	A H H	A H M	A M A	A M H	A M M	H A A	H A H	H A M	H H A	H H H	H H M	H M A	H M H	H M M	M A A	M A H	M A M	M H A	M H H	M H M	M M A	M M H	M M M
100	22	6		6	8	1	2			12	6		6	12	6		2	2		4			2	4			
130	11	10		7	1	1					7	6		5	13	3	3	4	6		1	3	9	5	1	4	
160	12	7		7	3		1			10	8	1	5	11	4	1	2		2	4	1	4	11	3	1	1	1
190	10	11		10	5			1		10	7		13	13	2		1	1	1			2	7		1	4	1

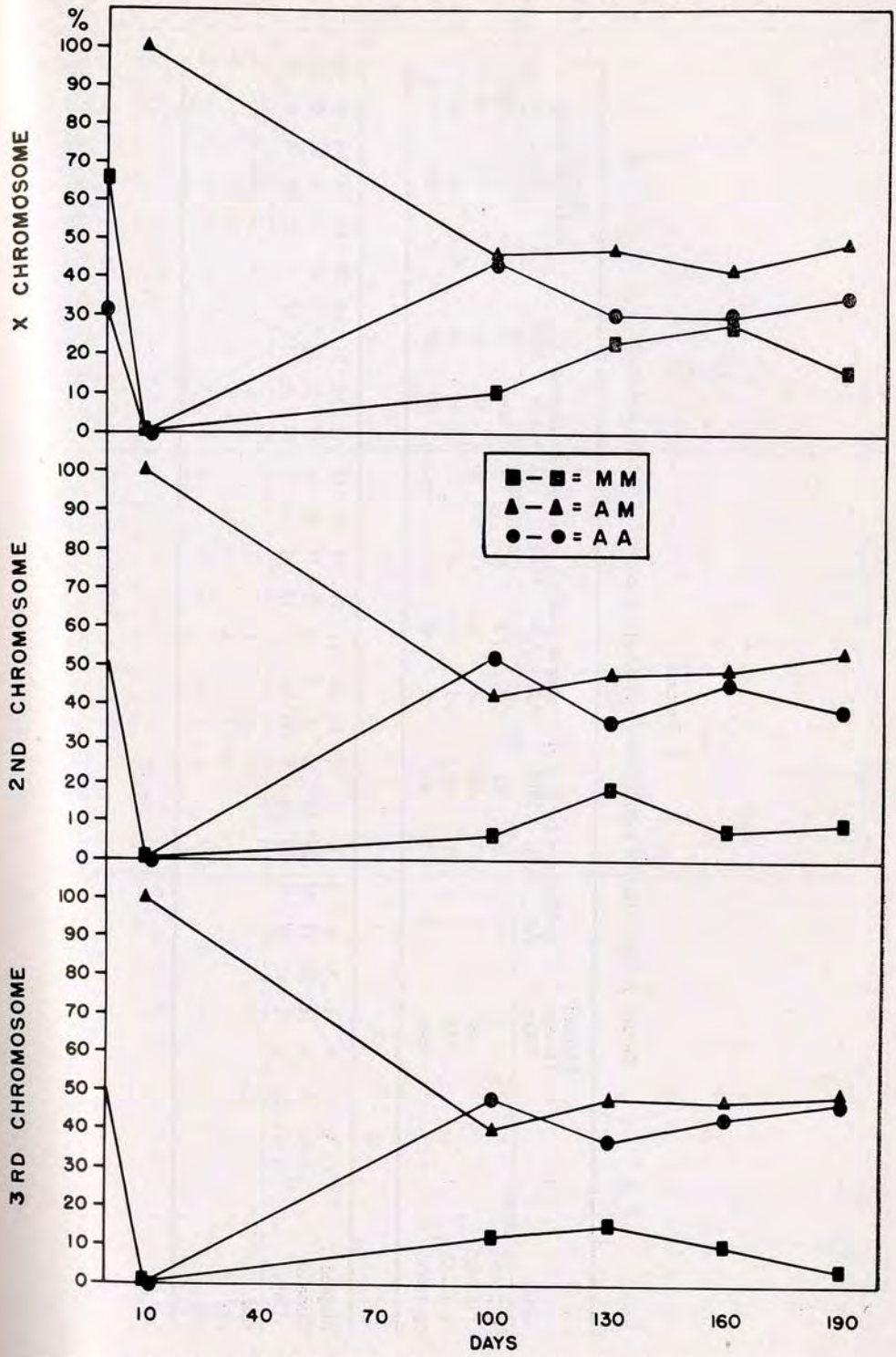

FIG. 6. Started with *mojavensis* females and *arizonensis* males. The percentage of homozygous and heterozygous combinations of the three chromosomes.

TABLE 7

Results of cage 7, started with hybrid (*mojavensis/arizonensis*) females and males.

Date	Elapsed Time in Days	Chromosone X AA	Chromosone X AM	Chromosone X MM	Chromosome 2 AA	Chromosome 2 AM	Chromosome 2 MM	Chromosome 3 AA	Chromosome 3 AM	Chromosome 3 MM	Percent ARIZONENSIS X	Percent ARIZONENSIS 2	Percent ARIZONENSIS 3
4–2–55	0	33	0	67	0	100	0	0	100	0	33.3	50.0	50.0
7–15–55	104	2	30	68	12	53	35	20	55	25	17.0	38.5	47.5
8–14–55	134	0	24	76	15	53	32	23	47	30	12.0	41.5	46.5
10–13–55	194	0	13	87	9	66	25	12	61	27	6.5	42.0	42.5

Elapsed Time in Days	(X) A	A	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H	H	M	M	M	M	M	M	M	M
	(2) A	A	A	H	H	H	M	M	M	A	A	A	H	H	H	M	M	M	A	A	A	H	H	H	M	M
	(3) A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M	A	H	M
104		1		1						4	2		4	7	8		4	1	2	3		4	17	12	4	21	5
134										2	1	1	4	5	2	2	5	1	3	5	2	11	15	16	1	14	9
194										1	1			7			4		1	5	1	7	34	19	3	10	7

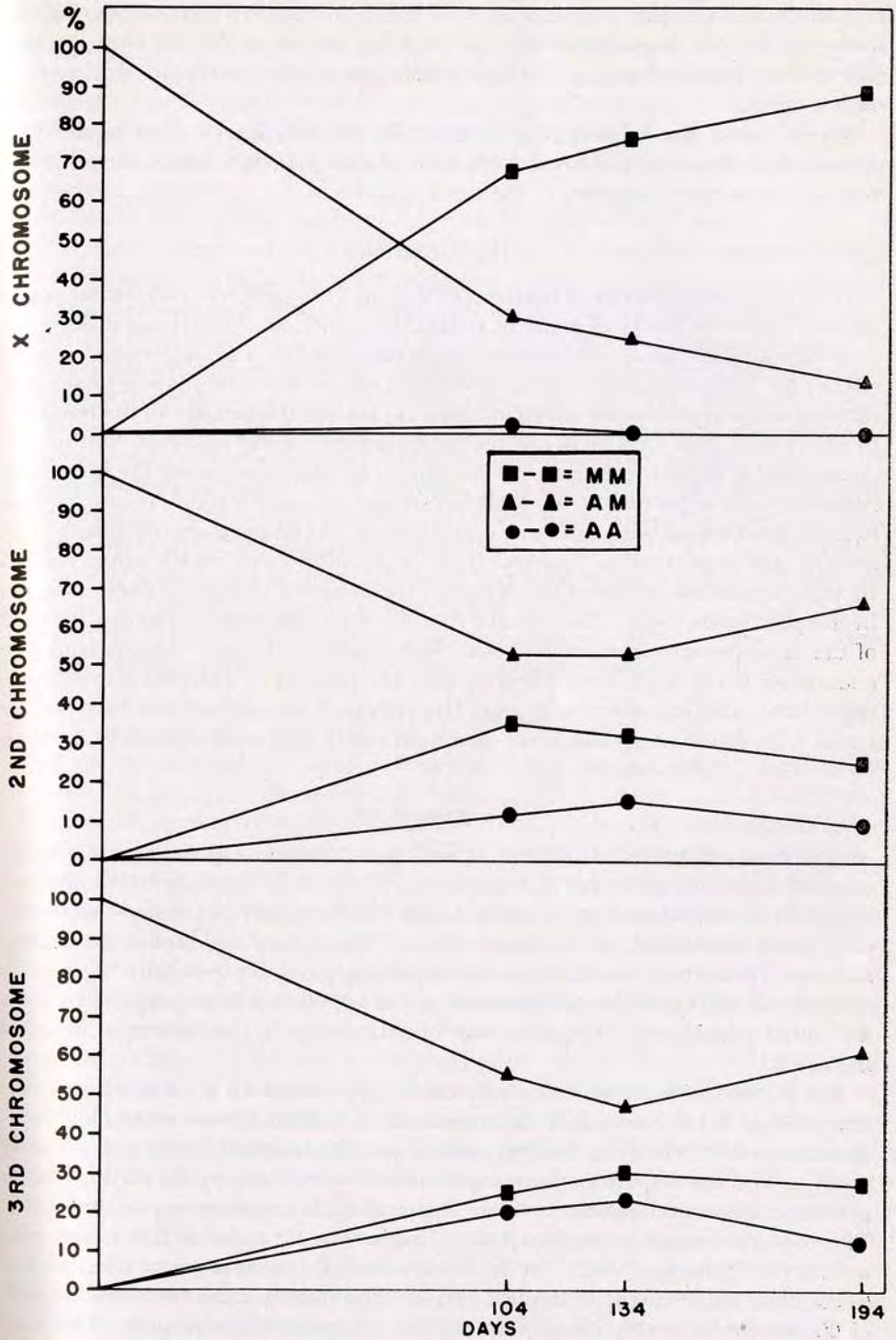

FIG. 7. Cage 7. Started with hybrid *(mojavensis/arizonensis)* males and females. The percentage of homozygous and heterozygous combinations of the three chromosomes.

last analysis. The relative frequencies for the three zygotic chromosome combinations of the 3rd chromosome were very similar to those of the 2nd chromosome. The *arizonensis* arrangements of each chromosome were lower in frequency in each sample.

Several larval classes were represented. There was an increase in frequency of classes M-H-H and M-H-M and a decrease of class M-M-H. These three classes were the ones most abundant in the three samples.

DISCUSSION

Inversions, which serve as markers in this analysis of the experimental populations, represent blocks of genes in particular sequences. Mutational differences exist between the two species utilized. A certain number of these genes are bound within the inversions, since in heterozygotes the free recombination of the different genes is restricted by inversion loops, at least in the vicinity of the breakage points. These gene sequences are the actual entities being followed. Within the experimental populations pure species cannot be identified, since the inversions represent only a portion of the total karyotype. By appropriate crosses, various hybrids are formed with different combinations of the gene arrangements. The hybrids and homozygous "species" have genic differences, which are expressed by the competitive ability of the different forms under stringent selection set up in the population cages. The relative frequencies of the gene sequences for each of the three chromosomes studied sometimes underwent rapid changes from one generation to the next, demonstrating that the carriers of different chromosome types have different selective values. The results of the competition between the forms with different arrangements are most easily discussed separately for each of the three chromosomes.

X Chromosome

On the assumption that a carrier of some particular gene arrangement is better adapted than one with another sequence, it would be expected that the less adapted one should be lost. In cages 1 and 2, *arizonensis* X chromosomes were completely eliminated, and in cages 4, 5, and 7 there was a rapid reduction in their number. The *mojavensis* X chromosome homozygotes are generally superior in competitive ability in the environment of the population cages, depending upon the initial population. The percentage of *arizonensis* X chromosomes increased in cage 6.

The initial cross in cage 6 (*mojavensis* ♀ × *arizonensis* ♂) was reciprocal to that of cage 5 (*arizonensis* ♀ × *mojavensis* ♂). Both crosses were obligatory, producing only hybrids in the first generation. The reciprocal results in the early analyses of these experiments are explicable if the majority of the matings which produced the second generation were F_1 females backcrossing to parental males. The backcross was a necessity in cage 5, since the F_1 males of this initial cross are sterile (Patterson, 1942). In the 40-day analysis (second generation) of cage 5, the observed frequencies did not deviate significantly from the expected ratio of 50 percent heterozygotes and 50 percent *mojavensis* homozygotes. The eight larval classes observed, in approximately equal frequencies, would be expected due to chromosome segregation in the gamete formation of the F_1 females. The

backcross was likewise very probable in cage 6. By the time the F_1 flies emerged the parental *mojavensis* females were too old to produce many eggs which would develop. Males of the mulleri subgroup usually do not mate until 6–7 days after eclosion. Thus the majority of the matings might be expected to be F_1 females backcrossing to parental *arizonensis* males. This would increase the number of *arizonensis* chromosomes in the second generation and yield reciprocal results to those seen in cage 5.

Backcrossing explains the reciprocal results in the early samples of cages 5 and 6, but it is apparent from the results of later analyses that selection strongly favored *mojavensis* chromosomes in cage 5 and did not in cage 6. Only *arizonensis* Y chromosomes were present in the cage 6 experiment. An unbalanced genic system in the carriers of *arizonensis* Y and *mojavensis* X chromosomes could be selected against, giving a higher percentage of *arizonensis* X chromosomes than expected from the results of the other cages. Baker (1947) reported that no progeny was produced when this type of male (*arizonensis* Y, *mojavensis* X) was crossed to *arizonensis*, *mojavensis*, or hybrid females, in pair matings, but progeny were produced in mass matings. He dissected 15 of the males and found that 13 did not contain motile sperm. In cage 7, where only *arizonensis* Y chromosomes are present, the results are similar to those of cages 1, 2, 4, and 5, and not to those of cage 6. It might be necessary to postulate the existence of different genes on the chromosomes of the initial populations of cages 6 and 7 or the production of rare recombinations in cage 7 to eliminate this discrepancy. Alternatively, a *mojavensis* Y might have come in by non-disjunction or error.

Second Chromosome

In the majority of the samples from all cages, the 2nd chromosome heterozygotes are slightly higher in number than expected on the basis of the Hardy-Weinberg formula. After 698 days of selection, both 2nd chromosome arrangements were present in cages 1 and 2. This time period corresponds to approximately 28 generations. The retention of both gene orders can be explained by the theory of balanced polymorphism. When there is heterosis of the heterozygote, selection will not eliminate either chromosome from the population. The population will be polymorphic and will reach an equilibrium. The percentage of *arizonensis* 2nd chromosomes after a few generations reached a level of approximately 40 percent in cages 1, 4, 5, and 7. In cage 2 it was nearer 20 percent. This cage was going poorly during the sampling period (latter six months), and a great amount of bacteria and mold was present in the food cups. It is possible that the selective forces were different in this cage and reduced the level of *arizonensis* 2nd chromosomes. Dobzhansky and Wallace (1953) gave evidence that in populations of *D. pseudoobscura* the different inversions in a particular locality are coadapted to give a superior heterozygote. In the present investigation heterosis is not a matter of coadaptation, since distinct species are involved and not merely strains of one species from the same locality. Each species has genes which are selectively retained for adaptation to its particular environment, and certain combinations of the different genes in hybrids establish a well-integrated physiological system to such a degree that heterosis is apparent.

The results of these experiments show that the selective advantage of a par-

ticular karyotype depends not only on the physical environment but also on the other karyotypes present in the population. In cage 6, with the *arizonensis* Y and a high percentage of *arizonensis* X and 3rd chromosomes and numerous larval classes represented, the frequency of *arizonensis* 2nd chromosomes was about 55 percent after 190 days. In the other experiments, with fewer *arizonensis* X and 3rd chromosomes and fewer larval classes represented, the frequency of *arizonensis* 2nd chromosomes was approximately 40 percent. There was also a difference in the types and frequencies of karyotypes in cage 6 versus the other experiments.

Third Chromosome

The results of the 3rd chromosome are similar to those of the 2nd chromosome in each experiment, in that the heterozygotes show heterosis. Nearly every sample of each cage had slightly more heterozygotes than are to be expected according to the Hardy-Weinberg formula. Both gene arrangements were present in the final samples of cages 1 and 2, with balanced polymorphic equilibrium apparently reached in cage 2.

In cages 1 and 2, where only X chromosome *mojavensis* homozygotes occur, 3rd chromosome *arizonensis* homozygotes did not appear in the samples, and the number of *arizonensis* 3rd chromosomes was between seven and nine percent. There was a high percentage of *arizonensis* X chromosomes (about 60 percent) in cage 6. The number of *arizonensis* 3rd chromosomes was also about 60–70 percent, and numerous *arizonensis* homozygotes were present. A direct relation was also found in the other experiments. In each experiment, very few larvae had one of these chromosomes homozygous for *arizonensis* and the other homozygous for *mojavensis*. Larval classes A-A-M, A-H-M, A-M-M, M-A-A, M-H-A, and M-M-A were low in frequency in every experiment (tables 2–7). Either the crosses failed to produce these classes, which is improbable, or the larvae were selected against in the samples. It cannot be considered that these larval classes are complete lethals, since most of the classes are represented by a few larvae in some samples. In cages 1 and 2, *arizonensis* 3rd chromosome homozygotes were not found among the 1,000 larvae examined, although some would be expected from random mating. The samples were not completely free of selection. Although extra yeast was added to each sample vial, the conditions were still slightly overcrowded. If the individuals of the above classes have an extremely low selective value they could be eliminated by selection in the samples. From these data it seems that a genic unbalance exists in individuals having the X chromosome homozygous for the gene sequence of one species and the 3rd chromosome homozygous for the gene order of the other species. The equilibrium level of the two 3rd chromosome arrangements thus depends not only on the physical environment and the respective selective values of the two gene sequences, but also on the frequency of the X chromosome karyotypes.

The results of a similar investigation by Bruneau (Unpublished thesis), using species of the *virilis* group, are comparable to those reported here. Using the same type of experimental cages, conditions, and method of analysis, he obtained similar results with the species *Drosophila virilis*, *D. novamexicana*, and *D. texana*. The frequency of *virilis* chromosomes was predominant in crosses with

either *texana* or *novamexicana*. In most cases the heterozygotes showed heterosis. In the population started with males and females of *arizonensis* and *mojavensis*, hybrids were formed and replaced the parental types. Hybrids were produced and heterosis was apparent in the same type of population initiated with *texana* and *novamexicana*. There seems, however, to be a greater degree of isolation between *virilis* and either *texana* or *novamexicana*, since hybrids were not formed when there was a choice of mates. A trend toward species replacement resulted in these cages, with *virilis* predominating. In the present report the X chromosome *mojavensis* homozygotes had a greater selective value, as evidenced by the loss or trend toward loss of the *arizonensis* X chromosomes. Only in cage 6 did the X chromosome heterozygotes show heterosis. The results of the *virilis* group crosses indicated that the X chromosome heterozygotes showed heterosis except in the crosses with a choice of mates and in the population started with *novamexicana* females and *virilis* males. Bruneau reports that, for any two of the three species, the reciprocal obligatory populations gave similar results and that little difference existed between these and the F_1 initiated populations. As already discussed, the results of the F_1 *(mojavensis* $\times$ *arizonensis)* population were similar to the one started with *arizonensis* females and *mojavensis* males, but reciprocal results were observed in the cross of *mojavensis* females to *arizonensis* males.

The production of hybrids in nature between populations normally separated by isolating mechanisms frequently leads to an increase in the variation and adaptive versatility of the species involved. For hybridization to occur isolating barriers need to be incomplete. Gene exchange, leading to more variability of a species due to an occasional hybridization with another species without destroying specific identities, is termed introgressive hybridization by Anderson and Hubricht (1938). This process has been extensively studied by Anderson (1949, 1953). Although little is known of the frequency of introgression, most cases studied have been found primarily among plants. The results of the present investigation, with the production of heterotic hybrids, show that there is a possibility that introgression or intergradation might conceivably occur in natural populations of Drosophila. The present investigation, as well as that of Bruneau, shows that occasional crosses between species in nature could produce heterotic combinations. These could survive and continue to contribute genes from one species to another.

SUMMARY

The two closely related species *Drosophila arizonensis* and *Drosophila mojavensis* are not known to be sympatric. They have been shown to produce some fertile hybrids in the laboratory. Populations derived from crosses involving these species were maintained in population cages. They were analyzed frequently to determine the effects of competition between the species and their interspecific hybrids when formed. Seven populations were initiated as follows: males and females of both species (in duplicate); *mojavensis* females and *arizonensis* males (in duplicate, but one was discontinued); *arizonensis* females and *mojavensis* males (in duplicate); and F_1 hybrids from the cross of *mojavensis* females and *arizonensis* males. The latter three crosses forced the production of hybrids. The populations could be analyzed cytologically since the species differed by inversions in the X, 2nd, and 3rd chromosomes.

In the experiments initiated with males and females of both species not only were hybrids formed, but they survived and tended to eliminate the parental species. In all experiments except cage 6, which was started with *mojavensis* females and *arizonensis* males, the *mojavensis* X chromosome homozygotes proved superior in ability to compete. The 2nd chromosome heterozygotes exhibited heterosis in each experiment. The level of balanced polymorphic equilibrium was reached relatively soon and was dependent upon the genetic background of the population. Although *arizonensis* 3rd chromosomes were highly selected against, heterosis was sufficient to retain the chromosome arrangements of both species. A genic balance system was found to exist between the Y, 3rd and X chromosomes. The *mojavensis* arrangements of the three chromosomes became more frequent than *arizonensis* in all populations except that in cage 6. The reciprocal results of this experiment are discussed, and a comparison is made between this study and a similar one by Bruneau.

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XIII. An Attempt to Detect Hybrid Matings Between *D. mulleri* and *D. aldrichi* Under Natural Conditions

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A new isolating mechanism in the genus *Drosophila* was reported by Patterson (1946, 1947) in the form of a prolonged insemination reaction in the vagina of the female resulting from interspecific matings. Patterson examined all possible crosses in the *mulleri* subgroup and found a correlation in the degree of severity of the mass with subsequent success or failure in hybrid production. A reaction mass is usually formed in intraspecific matings also but they are less severe and pass from the vagina in a shorter time. Here then is presented a quantitative method by which one could detect attempted hybrid matings under natural conditions by isolating freshly caught females until all homogamic (intraspecific) reaction masses had been passed and then dissecting out the reproductive parts. Any reaction mass found would presumably be a heterogamic one and would indicate a hybrid mating in nature.

This procedure was followed in respect to *D. mulleri* Sturtevant and *D. aldrichi* Patterson and Crow where they were trapped in the fall of 1951 at the Aldrich Farm near Austin. The two species are closely related and are sympatric in the south central part of Texas. Both larval types utilize the fruit of the cactus *Opuntia* although *D. mulleri* also inhabits other fruits and vegetables. Sexual isolation is high in the laboratory and the cross of *aldrichi* females with *mulleri* males does not produce hybrids. The reciprocal cross will yield a few sterile hybrid males and females (Patterson and Crow, 1940; Crow, 1942). Patterson (1947) identified hybrid males from field collections but only when the proportion of *aldrichi* males was at least one-third of the total *aldrichi-mulleri* male population.

In respect to the reaction mass, Patterson found that when *aldrichi* females were exposed to *mulleri* males for 96 hours and the females dissected, all of the 24 that were inseminated had a reaction mass in the vagina and five had motile sperm in the ventral receptacles. This cross does not yield hybrids. The reciprocal cross showed that of the 36 females inseminated, 20 had a reaction mass and 18 had motile sperm. This cross does produce hybrids.

Wheeler (1947) reported the behavior of the insemination reaction resulting from intraspecific matings from a survey of the genus.

METHODS

The only species tested was *mulleri* females for possible matings to *aldrichi* males. The flies were brought into the laboratory immediately after collection and the female *mulleri* were isolated for at least 12 hours. The data presented by Patterson showed that reaction masses from homogamic matings pass from the vagina in eight to nine hours. The reproductive tracts were then dissected directly into saline after various hours of isolation and examined under the microscope.

Two techniques were used in handling the control material. First the usual method of ether anesthetic was employed. As the experiment progressed it was

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feared that etherization was delaying the passage of the reaction mass. Carbon dioxide was then administered as an anesthetic. The usual mating arrangement for the controls was 20 virgin females and 10 virgin males per large shell vial. The flies were aged about one week before mating. The controls were run on first and second generation individuals originally collected at the Aldrich Farm.

RESULTS

Table 1 shows that 84 reaction masses were found in 1,973 *mulleri* females inseminated in the wild. Table 2 shows that a small proportion of the masses were found up to 47 hours isolation. Most of the masses were of medium to small size in the pouch of the vagina. Two of the masses however differed markedly. One female, dissected after 29 hours isolation, contained a mass which made the whole vagina turgid. The other female, after 24 hours isolation, had a very large refractive dark pouch. Of all the dissections containing a reaction mass, four were

TABLE 1

Collection data

	October (9 Collect.)	November (4 Collect.)	December (4 Collect.)	Total
mulleri ♀	6427	1022	387	7836
mulleri ♂	6561	1060	387	8008
aldrichi ♀	111	187	45	343
aldrichi ♂	193	265	71	529
Percent aldrichi ♂	2.9%	20.0%	15.5%	
No. mulleri ♀ Dissected	1707	750	338	2795
No. Dissected with V.R.	1220	709	319	2248
No. with Sperm in V.R.	1091	617	265	1973
Percent with Active Sperm	89.4%	87.0%	83.1%	
No. Reaction Masses	43	23	18	84
Percent Reaction Mass	3.9%	3.7%	6.8%	

TABLE 2

Proportion of reaction masses in etherized wild *D. mulleri*.

Mating Time	Least Isolation Time (Hours)	Reaction Mass / No. Inseminated	Fiducial Limits of Expectation
	12–14	6/89 –6.7%	2.5–13.8
	15–17	10/159–6.3%	3.1–11.1
	18–20	34/436–7.8%	5.4–10.7
	21–23	9/139–6.5%	3.0–11.7
	24–26	11/331–3.3%	1.7– 5.7
Unknown	27–29	7/85 –8.2%	3.4–16.0
	35–37	1/136–0.7%	0.0– 4.1
	39–41	1/266–0.4%	0.0– 2.1
	42–44	4/237–1.7%	0.5– 4.3
	45–47	1/57 –1.8%	0.0– 9.8
	63	0/32 –0.0%	0.0–11.5

not dissected complete with ventral receptacles. Of the 80 remaining dissections, only two had no sperm in the receptacles.

Table 3 shows that a small proportion of homogamic reaction masses remains in the control females even after four days isloation and in approximately the same proportion as that found in the wild flies. The fiducial limits of expectation are taken at the 95% confidence band.

Table 3 shows that etherization does not slow down passage of the reaction mass to any marked degree. The etherization does however retard mating ability considerably. An average of 15 hours for mating was necessary to obtain a satisfactory number of inseminated females. In Table 4 is listed a random sample of

TABLE 3

Proportion of reaction masses in control homogamic matings of *D. mulleri*

Technique	Mating Time	Isolation Time (Hours)	Reaction Mass No. Inseminated	Fiducial Limits Of Expectation
Ether	12 hrs.	none	14/22–63.6%	
CO_2	10 min.	1– 2	62/85–72.9%	
CO_2	10 min.	5– 6	17/63–27.0%	
Ether	15 hrs.	8– 11	4/49– 8.1%	2.3–19.2
CO_2	10 min.	13– 14	2/63– 3.2%	0.4–10.7
Ether	20 hrs.	18– 20	5/90– 5.5%	1.8–12.1
CO_2	10 min.	19– 20	2/53– 3.8%	0.5–12.7
Ether	22 hrs.	26– 28	2/41– 4.8%	0.6–16.5
CO_2	10 min.	26– 30	3/99– 3.0%	0.6– 8.9
Ether	12 hrs.	33– 37	1/48– 2.1%	0.1–11.6
CO_2	10 min.	40	0/37– 0.0%	0.0– 9.9
CO_2	10 min.	48	1/50– 2.0%	0.5–14.4
CO_2	10 min.	87– 88	2/51– 3.9%	0.5–13.4
CO_2	10 min.	99–102	2/68– 2.9%	0.3– 9.9

TABLE 4

Mating success with CO_2 and ether anesthetized virgin *mulleri*

	Age (days)	Technique	Time for Mating	No.Females Inseminated
CO_2 *Method*	9	19♀, 10♂	10 minutes	16
	10	19♀, 10♂	10 minutes	13
	9	11♀, 10♂	10 minutes	10
	8	17♀, 10♂	10 minutes	16
	9	20♀, 10♂	10 minutes	19
	9	20♀, 10♂	10 minutes	20
	9	18♀, 10♂	10 minutes	15
	10	15♀, 10♂	10 minutes	14
	11	15♀, 10♂	10 minutes	15
	10	15♀, 10♂	10 minutes	12
Ether–CO_2 *Method*	10	19♀, 10♂	33 hours	9
	9	20♀, 10♂	13 hours	9
	10	20♀, 10♂	10 hours	12
	10	21♀, 10♂	1 hour	9
	10	20♀, 10♂	1 hour	10
	8	20♀, 10♂	15 minutes	7
	8	15♀, 10♂	15 minutes	7
	11	20♀, 10♂	10 minutes	0
	10	19♀, 10♂	10 minutes	6
	10	18♀, 10♂	10 minutes	6

the experiment comparing mating success after virgins were anesthetized with carbon dioxide or ether, aged for about 9–10 days, set up for mating with CO_2 and etherized just before dissection after a varying number of hours isolation. These data indicate that given over a week to recover and a much longer time to mate, the etherized flies will not mate as readily as individuals taken virgin with CO_2. Courtship is vigorous with the CO_2 method but poor with the ether method.

In order to observe the behavior of heterogamic reaction masses 379 control *mulleri* females were tested with *aldrichi* males in many different trials under varying mating conditions of light, temperature and proportion of males to females. Both the ether and carbon dioxide techniques were employed and in the latter case the *aldrichi* males courted actively as soon as placed with *mulleri* females. The females, however, refused to accept the males. Only 16 females of nine different trials were found to be inseminated upon dissection. Five females contained sperm in the ventral receptacle but no reaction mass (none of the isolation times was more than nine hours; average mating time was 18 hours). Of the remaining 11 with a reaction mass, four had no sperm in the ventral receptacles. Only one of the masses completely filled the vagina. Four masses were of medium size and six masses were small and usually indistinguishable from homogamic masses.

CONCLUSIONS

The heterogamic reaction mass is not a suitable criterion to detect hybrid mating attempts in nature for the hybrid-producing cross of *mulleri* female to *aldrichi* male under the conditions of the experiment. This is so since a small proportion of homogamic masses remain in the female even after four days isolation and since the less severe heterogamic masses cannot be distinguished from homogamic ones. Of the 84 reaction masses from the wild population, two could be safely distinguished as a severe heterogamic type.

The probable functions of the homogamic reaction mass are discussed by Patterson (1946) and Wheeler (1947). One of the functions stated is to prepare the reproductive tract for the fertilization mechanism. It is believed that even though some homogamic masses do remain for a considerable period after mating, they are of such a nature as not to interfere with normal egg passage and fertilization.

The superior mating ability of flies handled throughout their life with carbon dioxide instead of ether suggests that this method should be seriously considered as a technique for Drosophila workers.

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XIV. Genetic Studies on *Drosophila mulleri*. I. The Genetic Analysis of a Population

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INTRODUCTION

Drosophila mulleri was first recognized as a distinct species and described by Sturtevant (1921). More recently Patterson (1943) has redescribed the species, including a complex of taxonomic characters which makes it possible to separate it from many other closely related forms. Wharton (1942) described the metaphase chromosomes as five pairs of rods and a pair of dots, with the X-chromosomes longer than the autosomes, and the Y-chromosome much shorter than the X. She found that the salivary chromosomes consisted of five long arms and a dot-like element. Patterson and Wagner (1943) published a distribution map of the species and stated: "*Drosophila mulleri* has been found chiefly in Texas and northeastern Mexico. A few specimens have been collected at single points in Oklahoma, Arkansas, Louisiana and Florida. This species is very common in Texas, especially in the central and southern parts of the state." Wagner (1944) studied the food habits of the species. He found larvae in the rotting fruit of the prickly pear cactus, *Opuntia Lindheimeri*, collected at several localities in Texas. Nine yeasts were isolated from these fruits, and it was established that *D. mulleri* would develop to maturity on a cactus juice-agar medium inoculated with each of the yeasts, but would not grow on sterile medium alone. *D. aldrichi*, a closely related species, developed to maturity on only six of the yeasts. Wagner stated: "Collections of *mulleri* have been made in fruit stores, fruit dumps, etc., but in general it may be said that this species restricts itself to cactus provided none of the domesticating influences of man are around to tempt it." The same author (Wagner, 1949) studied the larval development of the five closely related species, *mulleri*, *aldrichi*, *mojavensis*, *arizonensis*, and *buzzatii*, on the eight species of yeasts grown on a synthetic basal medium. He found that *mulleri* and *arizonensis* showed similar growth on the several yeasts, while the others differed in yeast requirements from these two and from each other. He concluded: "The work reported here gives concrete evidence that significant physiological differences exist between five closely related species of *Drosophila* which are of a type that conceivably could have been of importance in the evolution of these species."

The chief interest in *D. mulleri*, however, relates to certain possibilities it offers for comparative genetic and cytological studies. This point can best be documented by a brief review of a few facts concerning the taxonomy and cytology of the genus. Patterson and Wheeler (1949) catalogued 605 species described and assigned to the genus *Drosophila*. While a few of these have since been reassigned to other genera, the collection and description of new species has added considerably to this total. Of the eight subgenera now recognized from material available for study by far the largest is the subgenus *Drosophila*, with over 180 species, followed by the subgenus *Sophophora*, with about a third as many species.

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The subgenus *Drosophila* was first divided into 14 species groups by Sturtevant (1942). Patterson and Stone (1952) have listed additional groups, bringing the total to 22. Each species group contains those forms considered to be most closely related on the basis of comparative taxonomic, cytological and ecological studies. At this stage in the development of our knowledge the most significant contributions to evolution in the genus are likely to be made at the level of intra- and interspecific studies within the species group.

Of the 22 species groups now recognized in the subgenus *Drosophila* by far the largest in described species is the *repleta* group. Recent collections of Dr. William Heed in Central America indicate that the *tripunctata* group may eventually rival the *repleta* group in this respect. A considerable number of the *repleta* group are readily cultured in the laboratory; the flies are large and vigorous and have a high reproductive potential. Of the 51 described species in this group 39 have been further assigned to subgroups as follows: *hydei* subgroup, six species; *melanopalpa* subgroup, 13 species; *mercatorum* subgroup, two species; *mulleri* subgroup, 18 species (Patterson and Stone, 1952; Patterson and Alexander, 1952; Wheeler, 1954).

The cytological picture in this large and successful species group is of unusual interest and significance in relation to an analysis of the mechanism of evolution in the genus. In a number of species of both the *Sophophora* and the *Drosophila* subgenera numerous intraspecific inversions have been found in natural populations. In some species the selective advantage of flies heterozygous for certain of these inversions has been demonstrated both in natural populations and in population cage experiments (Dobzhansky, 1947; Dobzhansky and Levene, 1948). It has been postulated that the selective advantage of inversion heterozygotes may have served in the past and may continue to serve as a potent factor in evolution. However, in this species group no evidence has been found of any considerable frequency of intraspecific inversions. Wharton (1942) reported finding no inversions in strains of *D. repleta* from Japan, Turkey, Guatemala, Texas, and Connecticut when these were crossed. Warters (1944) tested 21 strains of *D. meridiana* from ten areas to a standard strain and found no inversions. She reported no inversions in cross tests of five strains of *D. bifurca* from widely separated localities. In 26 strains of *D. hydei* collected at several points in the United States only one inversion was found by this author. This inversion, about one-third the length of the longest autosome, was widely spread in American population samples and was also found in collections from Hawaii and South Africa.

In an extensive cytological study of the *repleta* group Wasserman (1954a) found no intraspecific inversions in three strains of *D. nigrospiracula* of the *melanopalpa* subgroup, to which *D. repleta* also belongs. In the *mercatorum* subgroup five strains of *D. paranaensis* varied in respect to two inversions in the third chromosome, and three of the 14 strains of *D. mercatorum* tested varied in respect to three inversions in the third chromosome. In the *mulleri* subgroup 39 strains, distributed among 12 species, showed no intraspecific inversions with the exeption of one inversion in three of the seven strains of *D. buzzatii*. On the basis of these and some additional population samplings it may be concluded that this species group contains very few intraspecific inversions in natural populations.

In many of these species such inversions are either absent or extremely rare. This is the situation in populations of *Drosophila mulleri* thus far tested.

Wasserman's major contribution involved the careful comparison of the banding pattern of the salivary chromosomes of 18 species and one subspecies of the *repleta* group to the salivary maps of *D. repleta* and *D. mulleri*. All major interspecific inversions were observed and on the basis of these observations a phylogenetic tree of these species was constructed. His observations indicated that the fourth and fifth salivary chromosomes have remained largely unchanged in their banding pattern, with few interspecific inversions in these 18 species. Others of the chromosomes, including the X, were shown to have identical patterns in several of the species. This elegant cytological study lays the foundation for and serves as added stimulus to comparative genetic studies in this species group, including the construction of genetic linkage maps of certain key species and their comparison against the background of the comparative cytology. Thus, a fairly extensive genetic map of the X-chromosome of *D. hydei* is already available (Spencer, 1949). Wasserman reported no interspecific inversions between the X of *D. hydei* and *D. mulleri*. A genetic map of the X of *mulleri*, including a series of gene loci homologous to those already known in *hydei* would thus make possible a new approach to certain problems in comparative cytogenetics.

For several reasons *D. mulleri* would seem to be the most logical choice as a key representative of the *mulleri* subgroup. In the first place it is relatively easy to culture this species in the laboratory, in contrast to many of the 18 species assigned to this subgroup. Furthermore, collection records indicate that *D. mulleri* is a very successful species over its main geographical range of Texas and northern Mexico, ensuring easily collected and abundant samples for laboratory analysis, in contrast to many of the other species in this subgroup.

In respect to interspecific hybridization *D. mulleri* occupies a favorable position in the subgroup. Patterson and Alexander (1952) reported on hybridization cross-tests between six closely related species in the *mulleri* subgroup. Table 1 is adapted from their table on cross-tests. It will be seen by reference to the table that *mulleri* will produce fertile female hybrids with two of the species and sterile hybrids with the other three species. *Mojavensis* also produces fertile hybrids with two of the species, and in one of these crosses, *mojavensis* females x *arizonensis* males, hybrids of both sexes are fertile. But this species forms sterile

TABLE 1

P_1 crosses between six members of the mulleri subgroup
(Adapted from Patterson and Alexander, 1952)

males	mojavensis	wheeleri	aldrichi	arizonensis	buzzatii	mulleri
females						
mulleri	ft.f;st.m	ft.f;st.m	st.f;st.m	st.m	ab.flies	....
mojavensis	.....	st.m	none	ft.f;ft.m	none	none
wheeleri	st.f	.....	ft.f;st.m	st.f	ab.f	st.f
aldrichi	st.f	ft.f;st.m	.....	st.f	none	none
arizonensis	ft.f;st.m	none	none	.....	larvae	none
buzzatii	none	larvae	none	none	.....	none

*Key to above table. ft.f—fertile females ft.m—fertile males
st.f—sterile females st.m—sterile males
ab.f—abnormal females ab.flies—abnormal flies

hybrids with only two of the remaining three species. *Wheeleri*, *aldrichi*, and *arizonensis* produce fertile hybrids with only one other species and *buzzatii* with none. Thus for experiments on gene transfer from one species to another and in the investigation of genetic factors in F_1 sterile hybrids, *mulleri* and *mojavensis* may be given an approximately equal rating and one distinctly above the other four species. No other species in the *mulleri* subgroup is as favorable as these two for hybridization studies.

The Drosophila mulleri Population

The author spent the academic year 1954–1955 on research leave at The University of Texas and was fortunate to find living quarters within 10 minutes walk of the campus and at the same time in an area which seemed ideal for Drosophila collecting. Using a method of Drosophila collecting first used by Dr. Harrison Stalker and later described by the author (Spencer, 1950) about 50 paper drinking cups containing banana mash with yeast added were hung on the lower branches of trees and bushes, or set in the garage, all within 150 feet of the author's home. All of the traps were set in an oval area 85 feet long by 40 feet wide, with a total area of approximately 3400 square feet. It was estimated that the collecting area was approximately one two-billionth of the area of the state of Texas. No doubt many of the flies taken in the traps were drawn to them from contiguous territory outside this area. Wasserman (1954b) made a study of population dynamics in *D. mulleri*, and discovered that population movement in this species is very rapid.

No attempt was made to list the plants in the collecting area. There was a considerable variety of plants including bushes with ripe berries, trees, vines, and herbs. A small stream flowed along one side of the area, and beyond this stream was a wooded park. Live oaks grew in the vicinity but not in the collecting area. It should be noted that there were no cactus growing within the collecting area, and the nearest cactus plants were a few specimens in a garden over a hundred yards from the area. It seems almost certain that the large *D. mulleri* population, together with that of other species reported below, was not being maintained on cactus. The major food source maintaining the population was not determined. The city maintained a regular garbage collecting service so it seemed unlikely that this could be a chief food source. This observation is in no way a contradiction of Wagner's observations on cactus as the main food plant of *D. mulleri* in natural habitats, but it seems quite apparent that in those habitats where man is a disturbing influence the species may maintain itself prolifically on other food sources.

From November 5, 1954, through December 15, 1954, collections were made twice daily in the morning and evening. On cool or cloudy days collections were also made at noon. Metal cylinders with cheese cloth covering one end were used as collectors. One of these was brought close to the trap; the latter was suddenly picked up and thrust into the lower open end of the cylinder and flies present in the traps or lures flew up toward the lighted upper end, covered with cheese cloth. After collecting from several traps the open end of the collector was plugged with a large cotton plug. These collectors were then taken to the laboratory immediately; the flies were etherized in the collectors and examined. Records

were kept of the numbers of specimens of males and females of each species. In the determination of species the author wishes to acknowledge the invaluable help of Dr. J. T. Patterson, who gave unsparingly of his time and expert knowledge of *Drosophila* species.

Table 2 presents a summary of the collection records for the six-week period. While sexes were recorded separately it was thought unnecessary to record them in the table, except for the *affinis-algonquin* series in which males can easily be

TABLE 2

Numbers of each Drosophila species taken in collecting area at 805 Leonard Ave., Austin, Texas; November 5–December 15, 1954. Species arranged in order of abundance.

Species	Total	Species	Total
1. D. mulleri	7389	13. D. longicornis	57
2–3. D. melanogaster-simulans	4853	14. D. macrospina	52
4. D. affinis males	1546	15. D. putrida	47
5. D. algonquin males	153	16. D. micromelanica	20
4–5. D. affin.-algon. females	635	17. D. busckii	13
6. D. meridiana meridiana	129	18. D. duncani	3
6. D. meridiana rioensis	25	19. D. tripunctata	3
7. D. hamatofila	132	20. D. ananassae	1
8. D. aldrichi	123	21. D. carbonaria	1
9. D. melanica	107	22. D. cardini	1
10. D. hydei	101	23. D. immigrans	1
11. D. pseudoobscura	93	24. D. repleta	1
12. D. virilis	64	25. D. victoria	1

Total Collection—15,551 Flies.

separated, while females of these two species are not distinguishable. As in other collections from this laboratory *melanogaster-simulans* are listed together. Of the 15,551 specimens of *Drosophila* collected 7389 were *D. mulleri*, 47.5% of the total. The *melanogaster-simulans* collection of 4853 specimens constituted 31.2% of the total, and the *affinis-algonquin* collection of 2331 specimens made up 15.0% of the total. Of the other twenty species, with two subspecies of *D. meridiana*, *meridiana*, *hamatofila*, and *aldrichi* represented approximately one percent each of the population, and other species were represented by still fewer specimens, with six species, *immigrans*, *cardini*, *repleta*, *victoria*, *carbonaria*, and *ananassae* represented by single specimens.

By far the largest collection of *Drosophila* ever made was that of Patterson (1943) and collaborators from the Aldrich plot a few miles from Austin, Texas where collections were made almost continuously from July, 1938 to May, 1941, a total of 141,126 specimens of *Drosophila* distributed among 31 species. Our collection over a six-week period included 25 species. In spite of the fact that cactus grew abundantly on the Aldrich plot the total of *mulleri* and *aldrichi* collected on this plot in the three-year period was 16,203. Our collection showed almost half this number of *mulleri* for the six-week period. The peak collections for *mulleri* and *aldrichi* at the Aldrich plot came in October and November respectively for the two years in which autumn collections were made. It seems likely that our collections were made at about the time of the maximum development of the population. Much more work would have to be done on the ecology of this species together with analysis of population samples from contiguous areas to determine the actual extent of the population from which our collection

sample was derived. In fact the *D. mulleri* populations in the vicinity of Austin, Texas may approach a panmictic state during their peak development in the autumn and early winter.

Genetic Analysis of a Population Sample

A preliminary account of the data presented here was given in a report by Stone (1955) on genetic and chromosomal variability in *Drosophila*. In a few of the later collections of *D. mulleri* the sexes were not recorded separately. In that part of the collection where sexes were recorded there were 3518 males and 3290 females. In order to estimate the kinds and numbers of recessive mutant types carried in the *D. mulleri* population 1000 pair matings of wild flies were made up in small vials on the standard culture medium used in the Texas laboratory. In order to space the work of analysis these matings were made up in small lots during the six-week collecting period. Because of the failure of some cultures and because certain others had to be discarded during the necessary absence of the author from the laboratory, only 736 of these pair matings gave F_1 progeny which were used in the analysis. Undoubtedly many if not most of the females used had already been inseminated before capture. Whether the females used in this study were inseminated by the male provided in the laboratory or by a male in nature, the analysis is based on the assumption that multiple insemination had not occurred. This seems to be a valid assumption in view of the extensive work of Patterson (1947) on the insemination reaction in the *mulleri* subgroup. He states: "We found, however, that in a species in which a strong reaction occurs, the female usually does not remate for a long time. It was further found, both after homogamic and heterogamic matings, that the females do not immediately respond to the vigorous courtship of males." As *D. mulleri* shows a strong insemination reaction it seems valid to conclude that the data presented below are based on pair matings and not multiple inseminations.

Spencer (1947a) has reviewed the different methods of analysis of wild populations for mutants and has pointed out that, when feasible, pair matings of the F_1 progeny give the most accurate statistical results. When pair matings produce a large progeny it is possible to analyse the total mutant load carried by a population sample quite accurately. If, however, many pair matings give no progeny and many others give only a few flies, this method of analysis must be discarded and small mass matings must be used. From the first fifty pairs of wild flies of *D. mulleri* a small mass mating and three pair matings were made up. Because of the disappointing number of failures from the pair matings the author reluctantly abandoned the pair mating technique in favor of small F_1 mass matings. Fortunately the author had run extensive tests on the mass mating versus pair mating technique in the analysis of population samples of *D. hydei*, *D. immigrans*, and *D. melanogaster*, and could estimate that about one-third as many mutants would be recovered by the mass mating technique as by rearing three F_1 pair matings in these species in which pair matings go very well.

The problem of why pair matings do not go well in *D. mulleri* is an intriguing and exasperating one, and should be given further study. It has been this author's experience that six females of the species, if placed together in a small mass mating, will produce many more offspring than will the same six females if

placed separately in six culture vials. If *Drosophila mulleri* is to be used extensively in genetic studies this problem should be solved. This author found that when pairs of *D. mulleri* were placed in large soda straws pushed into culture medium the flies gave a much larger percentage of successful cultures than when pairs were placed in small glass vials. But he is under the impression that *mulleri* in mass cultures produce more offspring than in pair matings, and he feels that more study should be given to the problem of how to rear this species successfully in pair matings.

One small mass mating from each of the 736 F_1 cultures was reared. The F_2 offspring were examined by etherizing the flies and looking for new mutant types under the binocular microscope. Routinely the flies emerging from each culture were examined as they first emerged and twice subsequently at about two or three day intervals. With the culture technique used, in which absorbent tissue paper, soaked in a suspension of baker's yeast, had been placed in each vial, large progenies were generally secured and most of the flies had emerged by the time of the last examination of the progeny. Mutant types were scored and given temporary names at the time they were found. Cultures of the more vigorous and interesting types were established, but many of the weak and poorly expressed mutants were immediately discarded because of lack of time for further study.

From the 736 F_2 progenies of the mass matings 263 mutant types were recorded. These were not all mutants at different loci as shown by the analysis below of the numerous recurrences of bright scarlet-like eye colors. From former experience with the mass mating versus pair mating technique of extracting visibles it was estimated that the population sample of 1472 wild flies carried somewhere between 900 and 1400 mutant genes giving visible phenotypes similar to those reported for *D. melanogaster* in the comprehensive monograph of Bridges and Brehme (1944) on the mutants of this species. This gives an average of 0.6 to 1 mutant gene per wild fly tested. The author (Spencer, 1946, 1947b) analysed two population samples, each of 110 flies of *D. immigrans*, by the pair mating technique and estimated approximately 100 visibles to be present in the sample, approximately 0.5 mutant gene per fly tested. From fairly extensive samples of several populations of *D. melanogaster* and *D. simulans* (Spencer, unpublished) 0.7 mutant per fly was estimated to be present. Large samples of the species, *D. hydei* and *D. robusta* (Spencer, unpublished), have given somewhat lower estimates of visible mutants per fly tested. In the analysis of *D. hydei*, mutants at the bobbed locus, which can be demonstrated in most wild flies, were omitted from the calculations. Alexander (1949, 1952) analysed population samples of several species of *Drosophila*, using the F_1 pair mating technique, and estimated the frequency of mutant genes for *D. hydei* to be 2.38 per fly tested; for *D. limpiensis*, 1.14; for *D. americana*, 1.69; for *D. texana*, 1.32; and for *D. novamexicana*, 0.55 mutants per fly tested. On the basis of the population analysis using the rather crude mass mating technique it appears that this population of *D. mulleri* carried a visible mutation load falling well within the range of the varying mutation loads carried by populations in several other species.

Many of the mutants recovered in such an analysis as the one presented here are obviously semi-lethal types. The flies appear weak and die within a few days of emergence. Such mutants have been discarded although they could be kept

in stock by breeding from heterozygotes, using pair matings. Other mutants which appear vigorous enough phenotypically prove to be sterile in one sex or the other. These also were discarded in most cases. A third type of mutant shows many normal overlaps in homozygous cultures. These are also discarded. Any of the above types could be cultured but when one is starting the genetic analysis of a species such types require too much time for culture and can well be discarded until the genetics of the species has been worked out, using those visible mutants which have a relatively high viability and fecundity.

The Mutants Extracted from the Population

In the procedure of extracting mutants from the population sample the F_1 mass cultures were numbered consecutively from 1 to 736. The mutants found were given a number corresponding to the stock number and a tentative name. A brief description of each mutant was included in the record except for eye colors, where the name was generally considered to be a sufficient tentative description. The names of some mutants were later changed after a more thorough study indicated probable or possible homologies with mutants of *D. melanogaster* (Bridges and Brehme, 1944). After considering several methods of presenting the data it has been decided to include a check list of the mutants in the consecutive order in which they were found, rather than to group them under various phenotypic categories. This procedure has some disadvantages but it does give the reader a clear picture of the variety of mutant types that turn up in the day by day analysis. Perhaps the casual reader may glance through the table and get some concept of the sense of anticipation which the worker develops as he examines culture after culture, knowing that sooner or later new and exotic types never seen before in any species will be found and equally interesting homologues of mutants seen in other species, but for the first time revealed against a new genetic background, will appear.

Table 3 contains a check list of the mutants discovered, including the stock number, tentative name, and except for eye colors, a brief diagnostic description. The eye colors are recorded as scarlet-like, peach-like, etc. Subsequent analysis showed many cases of identity or allelism in eye colors recorded, but this was not known at the time the mutant was found. There are, of course, numerous cases of apparently phenotypically identical eye colors occupying different loci, so-called mimic mutations, in *D. melanogaster* and other species.

TABLE 3

Mutants recovered from the F_2 progeny of 736 pair matings of wild *D. mulleri*. Single F_1 mass matings were made up from progeny of each pair of wild flies and numbered consecutively. These stock numbers precede the name of each mutant extracted from the stock. Tentative names in this table were later changed after further study. Most of the mutants were discarded and names used in this table are to be considered descriptive and not permanent symbols. Short diagnostic descriptions follow the names except in case of eye colors.

(1)	1 *bronze;* eye color.
(2)	1 *small bristle;* late emerging, bristles reduced in size.
(3)	2 *erect;* post-scutellars erect.
(4)	4 *rough-coarse;* eyes rough; wings coarse; more extreme in males.
(5)	5 *scarlet-like;* eye color.

TABLE 3—Continued

Mutants recovered from the F_2 progeny of 736 pair matings of wild *D. mulleri*. Single F_1 mass matings were made up from progeny of each pair of wild flies and numbered consecutively. These stock numbers precede the name of each mutant extracted from the stock. Tentative names in this table were later changed after further study. Most of the mutants were discarded and names used in this table are to be considered descriptive and not permanent symbols. Short diagnostic descriptions follow the names except in case of eye colors.

(6)	5	*lozenge-like-thick;* eyes rough, lozenge shaped; legs thick.
(7)	6	*roof;* wings held at 45 degree angle, roof-like.
(8)	7	*scarlet-like;* eye color.
(9)	9	*scarlet-like;* eye color.
(10)	10	*peach-like;* eye color.
(11)	10	*light;* body color, spots reduced on thorax; only in males when found; sex-linked and carried in P_1 male.
(12)	11	*abnormal abdomen;* extreme in females; tergites very irregular.
(13)	11	*missing-scutellars;* post-scutellars missing; normal overlaps.
(14)	13	*wavy-like;* wing surface wavy.
(15)	20	*abnormal abdomen;* tergites irregular in females; less extreme in males.
(16)	22	*purple-like;* eye color; males sterile.
(17)	23	*rough-small;* eyes small and rough; wings often partly unfolded.
(18)	30	*scarlet-like;* eye color.
(19)	31	*brown-like;* eye color; best in young flies.
(20)	31	*inturned-like;* dorso-centrals turn in; hairs on wing margin stand out.
(21)	31	*wavy-like;* wing surface wavy.
(22)	34	*peach-like;* eye color.
(23)	34	*thin-wing;* wing thin textured; crumpled near distal end.
(24)	36	*rough-small;* eyes small and rough; variable in expression.
(25)	37	*dark;* eye color; difficult to classify.
(26)	38	*light;* body color; spots much reduced on thorax; sex-linked.
(27)	41	*rough-notched;* eyes moderately rough, small; wings notched; sometimes abnormal abdomen.
(28)	42	*peach-like;* eye color.
(29)	42	*spineless;* very small bristles; female sterile.
(30)	42	*cup-wing;* wings form small cup, convex above, or are inflated; wings about one-third normal length and at right angles to body.
(31)	47	*short-narrow;* wings short and narrow; body chunky; flies weak.
(32)	47	*fringe-like;* hairs on margin of wing irregular.
(33)	51	*ragged-roof;* hairs on wing margin ragged; wings roof-like.
(34)	53	*semi-lethal;* eyes very small and rough; bristles small and many missing; wings do not unfold; flies soon die.
(35)	53	*scarlet-like;* eye color.
(36)	53	*silver-like;* wings and frons silver; thorax dark.
(37)	56	*peach-like;* eye color.
(38)	61	*brown-like;* eye color; best in young flies.
(39)	63	*broken;* posterior cross-vein broken; variable.
(40)	65	*light;* body color; spots on thorax reduced; probably sex-linked.
(41)	66	*silver-like;* wings, thorax and frons silvery; variable expression.
(42)	67	*purple-like;* eye color.
(43)	71	*blister;* wings with blisters; very variable.
(44)	71	*rough-like;* eyes medium rough.
(45)	73	*scarlet-like;* eye color.
(46)	76	*broken;* posterior cross-vein broken; very variable.
(47)	77	*rotated;* male genitalia rotated.
(48)	80	*peach-like;* eye color.
(49)	90	*scarlet-like;* eye color.
(50)	91	*small bristle;* bristles about half normal length.
(51)	95	*fringe-like;* hairs on margin of wing irregular.
(52)	97	*scarlet-like;* eye color.
(53)	106	*scarlet-like;* eye color.
(54)	108	*roughest;* eye facets very irregular; some facets enlarged.
(55)	109	*peach-like;* eye color.
(56)	111	*scarlet-like;* eye color.
(57)	115	*shaved;* many bristles missing; wings nicked; eyes rough.
(58)	117	*naked-like;* hairs very sparse on thorax.
(59)	119	*rough-like;* small rough eyes.
(60)	120	*inturned-like;* dorsocentrals turned in; wing hairs stand out on margin of wing.

(61) 120 *reinforced;* margin of wing with a vein-like thickening at distal end of wing.
(62) 122 *wavy-like;* wing surface in waves.
(63) 122 *abnormal abdomen;* abdominal tergites very irregular.
(64) 126 *knotted;* thick knot at end of fifth vein.
(65) 126 *pearly-like;* body color pearly; bristles silvery.
(66) 127 *purple-like;* eye color.
(67) 130 *scarlet-like;* eye color.
(68) 131 *dumpy-like;* wings truncated, often blistered.
(69) 132 *broken;* posterior cross-vein broken; very variable.
(70) 134 *scarlet-like;* eye color.
(71) 135 *dumpy-like;* wings short and truncated; body short; variable but no normal overlaps.
(72) 135 *radius-incompletus;* second longitudinal vein broken near base.
(73) 144 *cut-net;* wing with margin incised and net veins.
(74) 144 *purple-like;* eye color.
(75) 147 *Minute;* one male with very small bristles; lost before testing.
(76) 148 *abnormal abdomen;* tergites irregular; medium expression.
(77) 150 *extra;* small bits of vein between L 2 and L 3 near wing tip.
(78) 153 *tiny-like;* bristles about one-third normal length.
(79) 154 *silver-like;* body and wings silvery; thoracic spots gone.
(80) 156 *anterior-cross veinless;* anterior cross-vein missing.
(81) 158 *brown-like;* eye color.
(82) 160 *two-jointed;* tarsi with only two joints, the basal and distal.
(83) 161 *grooveless-like;* groove between scutellum and thorax missing; etherizes very rapidly; flies weak.
(84) 163 *silver-like;* body and wings silvery; antennae white.
(85) 164 *brownish;* entire thorax brownish; spots almost obliterated.
(86) 170 *bent-down;* wing margin bent down near tip on outer edge.
(87) 174 *peach-like;* eye color.
(88) 175 *rough-cut;* eyes rough; wing margins cut; wings may be blistered and veins thickened.
(89) 175 *pearly-like;* body color like silver but less extreme.
(90) 175 *spineless-like;* very tiny bristles.
(91) 177 *small bristle;* bristles slightly reduced in size.
(92) 178 *pearly-like;* body color.
(93) 181 *pearly-like;* body color.
(94) 183 *scarlet-like;* eye color, but little darker than other scarlets, and with a cherry tinge.
(95) 189 *scarlet-like;* eye color.
(96) 193 *brown-like;* eye color.
(97) 193 *silver-like;* body color.
(98) 199 *scarlet-like;* eye color.
(99) 200 *small bristle;* bristles about two-thirds normal length.
(100) 201 *brown-like;* eye color.
(101) 203 *pearly-like;* body color.
(102) 205 *scarlet-like;* eye color.
(103) 205 *silver-like;* body color.
(104) 208 *mahogany-like;* eye color.
(105) 212 *blackish;* wings and thorax very dark; flies weak.
(106) 214 *scarlet-like;* eye color.
(107) 214 *pearly-like;* body color.
(108) 214 *narrow;* wings long and narrow.
(109) 219 *scarlet-like;* eye color.
(110) 222 *rough-abnormal;* rough eye; abnormal abdomen; wings often nicked.
(111) 224 *grooveless-like;* groove between scutellum and thorax missing.
(112) 230 *serrate-like;* wings cut along both margins; eyes small.
(113) 232 *peach-like;* eye color.
(114) 237 *broken;* posterior cross-vein broken; variable.
(115) 238 *bent wing;* wings bent down near tip.
(116) 240 *scarlet-like;* eye color.
(117) 245 *roughened;* eye large and rough.
(118) 249 *purple-like;* eye color.
(119) 251 *peach-like;* eye color.
(120) 253 *humpy-like;* thorax humped; flies small.
(121) 256 *cross-vein-dome;* posterior cross-vein missing; wings broad and convex dorsally.
(122) 260 *fuzzy-like;* hairs on wing margin stand out, but dorso-centrals not turned in.
(123) 262 *curled;* wings curled up strongly; posterior scutellars erect and sharply crossed; flies dark.
(124) 262 *brown-like;* eye color.
(125) 263 *scarlet-like;* eye color.
(126) 269 *sparse;* hairs on wing margin sparse.
(127) 272 *peach-like;* eye color.

TABLE 3—Continued

Mutants recovered from the F_2 progeny of 736 pair matings of wild *D. mulleri*. Single F_1 mass matings were made up from progeny of each pair of wild flies and numbered consecutively. These stock numbers precede the name of each mutant extracted from the stock. Tentative names in this table were later changed after further study. Most of the mutants were discarded and names used in this table are to be considered descriptive and not permanent symbols. Short diagnostic descriptions follow the names except in case of eye colors.

(128) 276 *rotated abdomen;* right side of abdomen rotated down, left side up; both sexes fertile.
(129) 276 *dachsous;* flies short and chunky; wings short; x-veins close.
(130) 282 *scarlet-like;* eye color.
(131) 288 *broken;* posterior cross-vein broken; variable.
(132) 289 *spread-like;* wings held out; flies very weak.
(133) 290 *tiny-rough;* bristles very small; eyes rough; wings nicked; semi-lethal.
(134) 295 *scarlet-like;* eye color.
(135) 295 *peach-like;* eye color.
(136) 298 *lanceolate;* wings long and narrow; pointed at end.
(137) 303 *dark brown;* eye color.
(138) 303 *Extension;* semi-dominant; in heterozygote extends size of dark spots on thorax; in homozygote thorax color an even black or dark brown; thorax humpy; head bristles lie flat.
(139) 304 *brown-like;* eye color.
(140) 304 *scarlet-like;* eye color.
(141) 305 *scarlet-like;* eye color.
(142) 305 *small;* flies small; pigment of thorax light; eyes rough; bristles small; viability very poor.
(143) 306 *cross-veinless;* posterior cross-vein missing.
(144) 307 *scarlet-like;* eye color.
(145) 307 *blister-like;* large, discolored blisters on wing.
(146) 313 *thick-vein;* veins thickened; particularly strong in L 5.
(147) 321 *thickened;* posterior cross-veins thick; normal overlaps.
(148) 336 *serrate-like;* wing margin serrated.
(149) 339 *small bristle;* bristles about one-half normal length; flies small.
(150) 341 *short;* fifth longitudinal short.
(151) 341 *warped-like;* wing warped in both axes.
(152) 343 *peach-like;* eye color.
(153) 344 *chubby-like;* flies short; cross-veins close together; legs thick.
(154) 346 *erupt-like;* erupted pustule on posterior margin of eye.
(155) 348 *peach-like;* eye color.
(156) 348 *polychaete-like;* extra bristles on thorax.
(157) 348 *crease-eye;* a distinct furrow across eye.
(158) 351 *peach-like;* eye color.
(159) 357 *hoary;* eye pile white rather than dark as in normal eye.
(160) 361 *scarlet-like;* eye color.
(161) 362 *scarlet-like;* eye color.
(162) 364 *broken;* posterior cross-vein broken; variable.
(163) 369 *broad wing;* wings short and broad.
(164) 370 *scarlet-like;* eye color.
(165) 371 *scarlet-like;* eye color.
(166) 372 *thickened;* posterior cross-veins thick; normal overlaps.
(167) 373 *light;* body color; spots on thorax reduced; sex-linked.
(168) 376 *peach-like;* eye color.
(169) 377 *abnormal abdomen;* abdominal tergites slightly abnormal.
(170) 379 *rough-like;* eyes large and rough textured.
(171) 392 *broken;* posterior cross-vein broken; variable.
(172) 396 *short;* fifth longitudinal short.
(173) 398 *serrate-like;* wing margins nicked in several places.
(174) 403 *thick-4;* end of fourth longitudinal thickened.
(175) 404 *delta-like;* all longitudinal veins thickened at ends.
(176) 404 *small bristle;* bristles small; flies weak.
(177) 408 *thick;* anterior and posterior cross-veins thick.
(178) 413 *extra bristles;* extra dorsocentral bristles.
(179) 414 *broken;* posterior cross-vein broken; variable.
(180) 416 *bobbed;* bristles in females very small; abdominal tergites irregular; sex-linked and sex-limited.
(181) 417 *scarlet-like;* eye color.
(182) 420 *spread wing;* wings widely spread and drooping.

(183) 433 *sepia-like;* eye color.
(184) 443 *silver-like;* body color.
(185) 445 *dash-vein;* small piece of vein near wing tip between L 3 and L 4.
(186) 449 *rough-like;* eyes medium rough.
(187) 451 *inturned-like;* dorsocentrals inturned; hairs on wing margin stand out.
(188) 451 *small bristle;* bristles about half normal length.
(189) 454 *thin-wing;* thin textured wing.
(190) 456 *eyeless;* extreme; eyes absent; head greatly reduced in size; flies sluggish but viable and fertile.
(191) 460 *rough;* eyes reduced in size and rough; uniform expression at a given temperature.
(192) 463 *split-thorax;* longitudinal groove at anterior end of thorax.
(193) 464 *scarlet-like;* found in one male; sex-linked vermilion.
(194) 468 *polychaete;* extra bristles on thorax and head; variable but always some extra bristles near mid-line anterior to dorsocentrals; small bits of extra vein in wing.
(195) 470 *serrated;* many small notches in wing near distal margin; variable but no normal overlaps.
(196) 475 *dark eye;* eye color, but difficult to classify.
(197) 482 *orange-like;* eye color.
(198) 483 *scarlet-like;* eye color.
(199) 484 *brown;* a translucent brown eye color.
(200) 490 *thickened;* posterior cross-veins thick.
(201) 496 *spineless-like;* bristles very small, some missing.
(202) 498 *curled-like;* wings turned up, but posterior scutellars normal.
(203) 500 *pearly-like;* body color.
(204) 501 *crippled;* legs twisted and gnarled.
(205) 501 *net-like;* rather extreme netting of veins.
(206) 503 *nicked-like;* wings notched near distal end.
(207) 504 *brown-like;* eye color.
(208) 508 *mosaic;* light areas in eye.
(209) 508 *scarlet-like;* eye color.
(210) 510 *rough-blister;* eyes rough; blisters on wings.
(211) 512 *scarlet-like;* eye color.
(212) 516 *humped-like;* thorax humped and short; posterior scutellars often erect and wings bent down.
(213) 519 *rough-like;* medium rough eye.
(214) 521 *stubble-like;* bristles short and heavy.
(215) 523 *rough-like;* eye rough near posterior border.
(216) 524 *speck-like;* dark speck at wing axil and darkened sutures.
(217) 528 *small bristle;* bristles reduced in size.
(218) 529 *de-pigmented;* pigment lacking along ventral margin of tergites; stronger expression in males.
(219) 530 *purple-slant;* eyes purple; posterior cross-vein oblique; flies weak.
(220) 532 *abnormal abdomen;* extremely abnormal abdominal tergites in females; character less strong in males.
(221) 542 *stubble-like;* bristles short, straight, and heavy; normal overlaps.
(222) 557 *semi-lethal;* wings not unfolded; flies small and weak.
(223) 568 *spineless-like;* bristles very small.
(224) 576 *chubby-like;* flies short and thick-set; cross-veins close together.
(225) 581 *blurred;* spots on thorax not distinct; background color dark.
(226) 584 *garnet-like;* eye color found in several males; sex-linked.
(227) 591 *short-5;* fifth longitudinal vein short.
(228) 592 *delta-like;* heavy deltoid thickening at tips of longitudinal veins; wing margin often incised.
(229) 603 *abnormal abdomen;* extreme expression; very abnormal tergites.
(230) 606 *scarlet-like;* eye color.
(231) 609 *split-thorax;* longitudinal groove at anterior end of thorax.
(232) 615 *gap-vein;* gap in fifth longitudinal near base; fourth and fifth veins close near posterior cross-vein.
(233) 623 *held-out;* wings held out at right angle to body, not drooping.
(234) 625 *inturned-like;* dorsocentrals inturned; hairs on wing margin stand out; males sterile.
(235) 633 *blister-wing;* blisters in wings; good expression.
(236) 635 *rough-like;* medium rough eye.
(237) 635 *extra-bristles;* extra dorsocentrals; variable.
(238) 641 *abnormal abdomen;* medium expression.
(239) 642 *stubble-like;* slightly thickened, stubbly bristles.
(240) 644 *rolled-like;* wings thin textured, crumpled and rolled under at distal outer margin.
(241) 647 *dark eye;* eye color dark.
(242) 647 *pads-like;* wings not unfolded; flies small and weak.
(243) 659 *small-eye;* eyes slightly smaller than normal.

TABLE 3—Continued

Mutants recovered from the F_2 progeny of 736 pair matings of wild *D. mulleri*. Single F_1 mass matings were made up from progeny of each pair of wild flies and numbered consecutively. These stock numbers precede the name of each mutant extracted from the stock. Tentative names in this table were later changed after further study. Most of the mutants were discarded and names used in this table are to be considered descriptive and not permanent symbols. Short diagnostic descriptions follow the names except in case of eye colors.

(244)	663	*grooveless-like;* groove between scutellum and thorax missing; black excrescences on sides of scutellum.
(245)	680	*cherry-like;* eye color.
(246)	681	*grooveless-like;* groove between scutellum and thorax missing.
(247)	682	*javelin-like;* bristles straight; not tapering as in normal; often forked near the end.
(248)	686	*pearly-like;* body color.
(249)	687	*purple-like;* eye color.
(250)	690	*dumpy-like;* body short and chunky; wings truncated.
(251)	692	*rotated genitalia;* in males, which are sterile.
(252)	694	*pads-like;* wings not unfolded; semi-lethal.
(253)	695	*brown-like;* eye color; classify in young flies.
(254)	697	*scarlet-like;* eye color.
(255)	706	*stubble-like;* bristles short and straight; difficult to classify.
(256)	708	*abnormal-tarsus;* tarsal joints short and often fused; flies weak.
(257)	715	*semi-lethal;* rough eyes; grooveless; tumor at base of last leg.
(258)	719	*taxi-like;* wings held at wide angle and elevated; wings slightly darker than normal.
(259)	721	*dull;* eye color.
(260)	722	*sepia-like;* eye color.
(261)	723	*scarlet-like;* eye color.
(262)	726	*brown;* translucent brown eye color.
(263)	730	*peach-like;* eye color; best in young flies.

Analysis of the Scarlet-like Eye Colors

From the standpoint of population structure the most interesting observation on the mutants found was the repeated occurrence of scarlet-like eye colors. A check of Table 3 will show 35 entries for these, all of which appeared identical in phenotype except 183 scarlet-like, which was a little darker than the others, more of a cherry color. Some of the scarlet-like mutants were found in only a few flies from the mass mating and no stock was established. A few of the others were discarded without testing. No. 464 scarlet-like was found as a single male and it was assumed that it might be sex-linked vermilion, which it turned out to be. Twenty of the scarlet-like stocks were retained for testing. On the assumption that there might be more than one locus involved 18 of these stocks were mated simultaneously to 189 scarlet-like and 205 scarlet-like, chosen at random. When the F_1 progeny began to emerge it became apparent that stocks 189 and 205 were indeed different. Nine of the tested stocks turned out to be alleles or identical to 189, and three of the stocks to be alleles or identical to 205. But, in addition, stock 214, which gave only scarlet-like offspring when cross-tested to 189 also gave about half the progeny scarlet-like when tested to 205. Thus both scarlet-like 189 and 205 were carried in the wild parents of 214. It was, of course, impossible to determine whether both mutants were carried in the same wild fly or whether each parent carried a mutant. The fact that none of the offspring of stock 214, after it was established, were wild-type is, of course, corroborative evidence of the point made earlier that many mutants present are not picked up by the mass mating technique. If, in isolating mutants from 214, some homozygous for 189 and others homozygous for 205 had been used to establish stock 214, then one

would have expected some wild-type flies in the next generation. Apparently all of the flies used in establishing this stock were homozygous for 189, but some of them were also heterozygous for 205. In any case it was fortunate that 214 was not chosen as a tester stock. Six of the stocks mated to 189 and 205 gave only wild-type offspring with both testers. One of these, 90 scarlet-like, was chosen as a tester and the other five were mated to it. Four of these proved to be alleles or identical to 90, and as was to be expected, 183 turned out not to be an allele.

Thus five bright scarlet-like loci were represented in the 21 stocks tested: 464 vermilion, 90, 189, and 205 all apparently phenotypically identical and 183, a little darker than the others. However, very careful examination of stock 90 showed a long light shadow running across the eye in young flies, which was not present in 189 and 205. A stock of 90 could be recognized as distinct from the others, but it would be difficult if not impossible to separate flies from a mixture of the stocks, particularly if they varied in age.

If one assumes that the same proportion of the three mutant genes, 90, 189, and 205 were present in the 14 scarlet-like types not tested, and that approximately one-third of the mutants present are picked up by the mass mating technique, then 189 scarlet was present in about 50 of the 2944 genes at this locus in the population sample, a gene frequency of 1.7%. The other two mutants, recovered half as frequently, would each have a gene frequency of about 0.8% in the population sample. The author (Spencer, 1946) recorded gene frequencies of 10% and 4% respectively for the mutants, stubble bristle and brick eye color, in a population sample of *D. immigrans*, and found a frequency of 4% for the mutant, rotund (Spencer, unpublished), in a population of *D. melanogaster*. The finding of high gene frequencies for certain mutant genes in *Drosophila* populations is therefore nothing unique. But it is an amazing coincidence that three mimic scarlet-like eye colors should have attained a high frequency simultaneously in this population. Peach-like eye colors were found 17 times. But several of these were phenotypically different; others proved to be sterile in one or both sexes. It is doubtful if any of the peach-like mutants had attained a frequency as high as the scarlets, although they were not tested as carefully.

When the scarlet-like mutants had been analysed markers for the four long autosomes had not yet been determined. On the assumption that the three mutants, 90, 189, and 205, might represent homologues of cinnabar, scarlet, and cardinal in elements C, D, and E respectively of *D. melanogaster*, it was decided to run immediate tests on whether these three mutants were on the same or separate chromosomes of *D. mulleri*. If any two were in the same linkage group then a cross of these two should give a second generation ratio of 1 wild-type: 1 scarlet; but if in different linkage groups the second generation ratio should be 9 wild-type: 7 scarlet. Table 4 gives the results of the cross-tests, indicating that the three mutant types were in three different linkage groups. Subsequent tests to other markers for the chromosomes have established that 90 scarlet-like is *cardinal* on the second chromosome, homologue of element E, the right end of chromosome III in *melanogaster;* 189 scarlet-like is *scarlet* on the fifth chromosome, homologue of element D, the left end of chromosome III in *melanogaster;* and 205 scarlet-like is *cinnabar* on the third chromosome, homologue of element C, the right end of chromosome II in *melanogaster*.

TABLE 4

F_2 counts of cross-tests of the three scarlet-like eye colors, stocks 90, 189, and 205, indicating by the 9:7 ratios that each mutant lies in a separate chromosome

	F_2 of stock 90 × stock 189		
	Actual Count	Expected	Deviation
9 wild-type	314	317	—3
7 scarlet	249	246	+3
Totals	563	563	
	F_2 of stock 90 × stock 205		
	Actual Count	Expected	Deviation
9 wild-type	213	210	+3
7 scarlet	169	172	—3
Totals	382	382	
	F_2 of stock 189 × stock 205		
	Actual Count	Expected	Deviation
9 wild-type	325	338	—13
7 scarlet	276	263	+13
Totals	601	601	

Visibly Different Alleles in the Population

In the population sample several other cases were found in which mutants which were phenotypically identical proved to be actually identical when cross-tested. Thus 67, 127, and 249 purplish proved identical on test and the same mutant was picked up in later generations from the stock of 256 cross-vein-dome, subsequently designated elbow. Other cases proved by test to be identical or indistinguishable alleles were: 484 and 726 brown; 42 and 295 peach; and 10, 38, and 373 light, a sex-linked body color. However, two phenotypically identical mutants, 47 fringe and 95 fringe, were shown not to be alleles, and in fact subsequent tests showed them to be on chromosomes III and V respectively. For lack of time many cross-tests were not made between certain similar mutants before they were lost or discarded. It seems probable that several other cases of allelism were present among the mutants extracted. Such sporadic cases of the same mutant appearing more than once in the population sample were to be expected on the basis of the population structure revealed by the scarlet-like eye color analysis.

But it was rather surprising to find in this population several cases of non-identical alleles. Spencer (1944) had shown that populations of *D. hydei* carried a large series of distinguishable alleles at the bobbed locus, and other phenotypically indistinguishable iso-alleles, which could be seriated by the use of special genetic tools. One of the primary considerations which led to the present study was to discover whether populations of another species in the *repleta* group also contained such a series of bobbed alleles. As the analysis progressed it soon became apparent that *D. mulleri* contained no such remarkable allelic series. Bobbed, a sex-linked and sex-limited recessive, is one of the mutants which has been found in many species of *Drosophila*. In the present analysis two distinguishable alleles were found. A medium bobbed, with bristles in females reduced to about half normal length, turned up in one of the F_1 pair matings from culture 15. An extreme bobbed, with females having very tiny bristles and abnormal

abdominal tergites, was found in mass culture 416. The finding of two such alleles in an analysis of the same scope would not be surprising in several *Drosophila* species. It seems apparent that the bobbed case in *D. hydei* is unique and quite different from the situation in *D. mulleri*.

The other two cases seem more remarkable. No. 120 reinforced is an excellent mutant, in which the margin of the wing at the distal end is thickened. While the mutant is variable in expression there are no normal overlaps. No. 592 delta-like is more extreme, with heavy deltoid expansions at the tips of the longitudinal veins often accompanied by notching of the wing margin. The heterozygote is identical to reinforced. Thus the allele with weaker phenotypic expression is dominant to the other. No. 170 bent-down is a mutant in which the distal outer margin of the wing is bent down; this mutant shows a few normal overlaps. No. 341 warped-like produces curvatures in both axes of the wing, with no normal overlaps. The heterozygote is identical to bent-down. Again the phenotypically weaker allele is dominant. In general, mutations at any one locus are rare. But certain *Drosophila* species show frequent mutations at some specific locus. Possibly these two loci in *D. mulleri* are unusually mutable. It would be of interest to test samples of different populations of this species by crossing the flies to 120 reinforced and to 170 bent-down.

DISCUSSION

The present analysis has given some information on the structure of the *D. mulleri* population under study. In the first place the mutants recovered indicate in some degree the hidden genetic variability present in the population. Only a fraction of the visibles present in the sample of 736 pairs of flies were recovered. This sample represented less than one-fifth of the *D. mulleri* captured during the collecting period. At no time during this period was there any indication that the total population was being trapped out; fluctuations in numbers of flies taken from day to day were rather obviously due to temperature and other environmental factors. Apparently the total population was much larger than the sample trapped.

Furthermore, a glance at Table 3, which represents roughly a chronological series of mutants extracted from smaller sub-samples taken over the collecting period, indicates that the law of diminishing returns was not operating drastically. Obviously certain of the later mutants extracted were identical to some of the earlier ones, but many new mutant types were being found toward the close of the analysis. It seems apparent that the visibles found represent only a fraction, though perhaps a sizeable fraction, of the total number of different visibles present. When one considers that the visibles of the type to be observed by the investigator constitute only a small fraction of the total mutants, lethals, semi-lethals, iso-alleles, and physiological mutants with no apparent morphological effects, the tremendous mutant load or perhaps better the tremendous evolutionary potential may be surmised. Some idea of the variability present in the population may be gained from the following item. The author carefully checked all of the mutant autosomal eye color loci recorded in the monograph on the mutants of *D. melanogaster* (Bridges and Brehme, 1944) and found that over half this number of separate autosomal eye color loci were represented in

those mutants found in this analysis and shown to be non-alleles. Many other eye color mutants were lost before being tested. While in some respects this was the most interesting population sample this author has analysed, it was by no means atypical in regard to total mutation load in comparison to samples of populations of *D. melanogaster*, *simulans*, *ananassae*, *hydei*, *immigrans*, and *robusta* formerly analysed.

However, the high gene frequencies of mutants at certain loci in the population sample, particularly the *scarlet*, *cardinal*, and *cinnabar* loci, deserve some consideration in connection with the problem of the possible breeding structure of this and presumably other populations of this species. Elton (1927) first called attention to the importance of fluctuation in numbers in populations as a potent factor in evolution. In non-mathematical terms he states the matter thus: "Many animals periodically undergo rapid increase with practically no checks at all. In fact the struggle for existence sometimes tends to disappear almost entirely. During the expansion in numbers from a minimum almost every animal survives, or at any rate a very high proportion of them do so, and an immeasurably larger number survives than when the population remains constant. If therefore a heritable variation were to occur in the small nucleus of animals left at a minimum of numbers, it would spread very quickly and automatically, so that a very large proportion of numbers of individuals would possess it when the species had regained its normal numbers. In this way it would be possible for non-adaptive characters to spread in the population, and we should have a partial explanation of the puzzling facts about closely allied species, and of the existence of so many non-adaptive characters in animals. . . . Finally, what little we know about the regulation of numbers in animals enables us to say that the problem of the origin of species can only be successfully solved by the aid of work on numbers."

In a brilliant series of papers Wright (1931, 1932, 1940, 1942, 1948, and many others) has expressed this idea in mathematical terms, and has discussed its relation to many other factors in a comprehensive theory of the mechanism of evolution at the incipient level. The phenomenon, which Wright prefers to term random fluctuation, has also been designated genetic drift or the Sewall Wright effect. On the matter of fluctuating population size Wright (1931) states in his first paper dealing with these matters: "If the population fluctuates greatly, the effective N (population number) is much closer to the minimum number than to the maximum number. If there is a great difference between the number of mature males and females, it is closer to the smaller number than to the larger. . . . The conditions of random sampling of gametes will seldom be closely approached. The number of surviving offspring left by different parents may vary tremendously either through selection or merely accidental causes, a condition which tends to reduce the effective N far below the actual number of parents or even of grandparents." And again (Wright, 1942) he states: "The effective value of N should often be much smaller than its apparent value. It obviously applies only to individuals that reach maturity. If there is cyclic variation in population size, N is more closely related to the minimum than to the maximum number. It is also reduced if there is excessive variability in the number of mature offspring from different parents."

If one were to base his ideas of population number in *D. mulleri* in the vicinity

of Austin, Texas, on collections made during the season when the populations attain a maximum, the late autumn, he might conclude that N was very large and that genetic drift was a negligible factor. This author made no month by month observations on population size in this species. Fortunately Patterson (1943) and his colleagues had already done so. An examination of the month by month collection records of this group at the Aldrich plot shows that *mulleri* and *aldrichi* build up to very large populations in the late autumn and early winter, then decline rapidly to extremely low populations in the late winter and spring, to build up slowly again through the summer. Thus for the one year when collections were made every month there were 5,715 *mulleri* and *aldrichi* taken out of 15,634 *Drosophila* collected in October and only 61 of these species out of 13,710 *Drosophila* collected the following May. In the interim the proportionate number of these two species had dropped even lower. Thus they have established that *D. mulleri* undergoes tremendous fluctuations in population numbers in an annual period in this area. Presumably there is considerable variation in this fluctuation from year to year, depending upon seasonal rainfall, temperature and other ecological factors.

In view of these fluctuations in population size it is not surprising that some particular mutant gene, such as *scarlet*, had attained a frequency of 1.7% in the population, and others, *cinnabar* and *cardinal*, a frequency of 0.8%. Actually the variety of mutant types found is more difficult to understand, and it probably points to a tendency for the *mulleri* populations in the vicinity of Austin to approach a panmictic state during peak populations, with much migration into and out of a given population. Of course the particular events which led to the high frequencies of the scarlet-like mutants may have occurred many years previously, and a more or less endemic population might have acquired most of the remaining mutation load subsequently.

It should be pointed out that Dr. Wright has always maintained that random fluctuation is only one of many factors involved in the mechanism of evolution, and that over long periods selection, in the final analysis, is the major factor in speciation. It may further be emphasized, as he has done, that selection and genetic drift are not necessarily antithetical. They may on occasion act in the same direction in establishing high gene frequencies and combinations of gene frequencies adaptively favorable to the organism.

No attempt will be made here to evaluate the evolutionary role of genetic drift in this or other populations of *D. mulleri*. The facts seem to indicate that it has played a role in establishing certain high gene frequencies, quite possibly non-adaptive or detrimental, in this particular population. It seems likely that the relative roles of various factors differ in speciation in different species groups and sub-groups in this genus. In those species with few intraspecific inversions in the chromosomes possibly random drift may play a larger significant role. Freer recombinations of genes are possible, so that certain mutant types, possibly with some selective advantage, might reach high frequencies and become fixed without the accompanying load of deleterious genes originally present in contiguous chromosome segments. Possibly freer crossing-over, resulting in longer chromosome maps as found in *virilis* and *hydei* and subsequently to be reported in *mulleri*, is a factor in this mechanism. On the other hand the development of

many intraspecific inversions in a species may well lead that species along the pathway of enforced chromosome heterozygosity. This latter mechanism is certainly not found extensively in the *repleta* group, which is at present represented by more species than any other group in the genus.

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XV. Genetic Studies on *Drosophila mulleri*. II. Linkage Maps of the X and Chromosome II with Special Reference to Gene and Chromosome Homologies

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INTRODUCTION

While some sporadic observations on gene homologies in the early work on the genetics of *Drosophila* species had appeared, Dr. A. H. Sturtevant made the first significant contribution in this field in a series of papers on the mutants of *Drosophila simulans*, culminating in a monograph (Sturtevant, 1929) on the genetics of this species. At that time the only case of species hybridization known in the genus *Drosophila* was the cross of *D. simulans* to *D. melanogaster*, which gave sterile hybrids. Sturtevant demonstrated that 14 recessive mutant types in the X of *simulans* were actual homologues or alleles of similar mutants in *melanogaster* by making the appropriate crosses and observing the recessive character in the sterile female hybrid. He also established homologies for three recessive mutants in chromosome II and five in chromosome III. This work remains the most significant contribution to the study of gene homologies in the genus. It should be noted that Sturtevant was able to predict with a high degree of accuracy which mutants would behave as alleles or homologues.

Sturtevant and Novitski (1941) published a comprehensive report of gene and chromosome homologies in *Drosophila* species, summarizing the data available up to that time. For anyone interested in the subject this report should serve as necessary background material. They present convincing evidence that the chromosome elements of *D. melanogaster*, X, left and right limbs of chromosome II, left and right limbs of chromosome III, and the dot-like chromosome IV have their homologous chromosomes and/or chromosome limbs, as revealed by homologous mutant genes, in the species *simulans*, *affinis*, *algonquin*, *ananassae*, *azteca*, *miranda*, *pseudoobscura*, *montium* and *virilis*.

This author (Spencer, 1949) discussed gene and chromosome homologies in *D. hydei*, comparing certain mutant genes found in this species with those in *melanogaster*, which still serves as a base of reference for such studies. He has also considered the criteria and pitfalls to be avoided in establishing homologies, particularly in the case of autosomal mutants.

In a significant paper dealing with the comparative cytology of the salivary chromosome of species in the *repleta* group Wasserman (1954) reported on the sequence of the banding pattern in these choromsomes. He studied two species in the *melanopalpa* subgroup, one of which was *D. repleta*, the type species of the group, two species in the *mercatorum* subgroup, two species in the *hydei* subgroup, including *D. hydei*, and 12 species in the *mulleri* subgroup. In this thorough cytological analysis Wasserman made a study of the interspecific inversions which had occurred in the course of evolution and worked out the chromosome phylogeny for the X and second chromosomes of the species studied. He pre-

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sented an interesting discussion of the possible course of explosive evolution in the *repleta* group. As Wasserman points out, in the absence of paleontological evidence, these hypotheses remain highly interesting though not conclusive speculations.

Of unusual interest was the fact that Wasserman found that certain chromosomes and parts of chromosomes had retained their identical pattern of banding in certain species which on morphological and ecological grounds had diverged to a point where they are classified in different subgroups. For instance his analysis indicated an identical banding pattern in the X of *hydei* and *mulleri* even though they belong to different subgroups. As the author (Spencer, 1949) had accumulated a considerable body of data on the genetic maps of *D. hydei*, particularly the X, and as he had available a large number of mutant types (Spencer, this bulletin) in *D. mulleri*, it seemed only logical to undertake a comparative genetic study of these two species, which might conceivably supplement the cytological work of Wasserman. The present paper is a preliminary report on the linkage maps and homologous gene loci in *D. mulleri* as compared to *hydei* and *melanogaster*.

THE X CHROMOSOME

The Mutant Loci and Alleles

During the course of analyzing the population sample for hidden recessive autosomal mutants and in the subsequent linkage tests on these mutants, several sex-linked recessives have been found. Of these all are obvious homologues of sex-linked mutants known in *melanogaster*, with the possible exception of carnation eye color and sable body color. In the following section each mutant is given a name, and a symbol corresponding to that used for its homologue in *melanogaster;* these are followed by the record of the origin of the mutant and a short description. The mutants are arranged in the order of their loci in the X chromosome of *mulleri*. No locus number is assigned in the description because this will undoubtedly change with the discovery of new mutants and the accumulation of linkage data on some of the longer as yet unmapped regions. However, the temporary locus number assigned to each mutant may be ascertained by reference to the X chromosome map, Figure 1.

vesiculated (vs). Several males in a stock of the sex-linked mutant, light body color. Wings very abnormal, blistered, often shriveled, and divergent; sometimes shows in only one wing, and some normal overlaps. When character is strongly expressed the flies are small and weak.

vermilion (v). One male in F_2 mass culture from wild pair 464. Evidently arose as a mutant in the laboratory. Brilliant scarlet-like eye color. In the double recessive, vermilion-brown III, the eye color is white.

white-ivory (w^i). One male from the F_2 of a cross of rudimentary male x forked-light female. This male was light-rudimentary. Eye color very light yellowish; vermilion-white-ivory is white in phenotype and easily distinguished from white-ivory alone.

light (li). Recovered three times from wild pairs 10, 38 and 373. Apparently carried in one of the two flies of each pair. As light in *hydei* is an allele or pseudo-

FIGURE I.

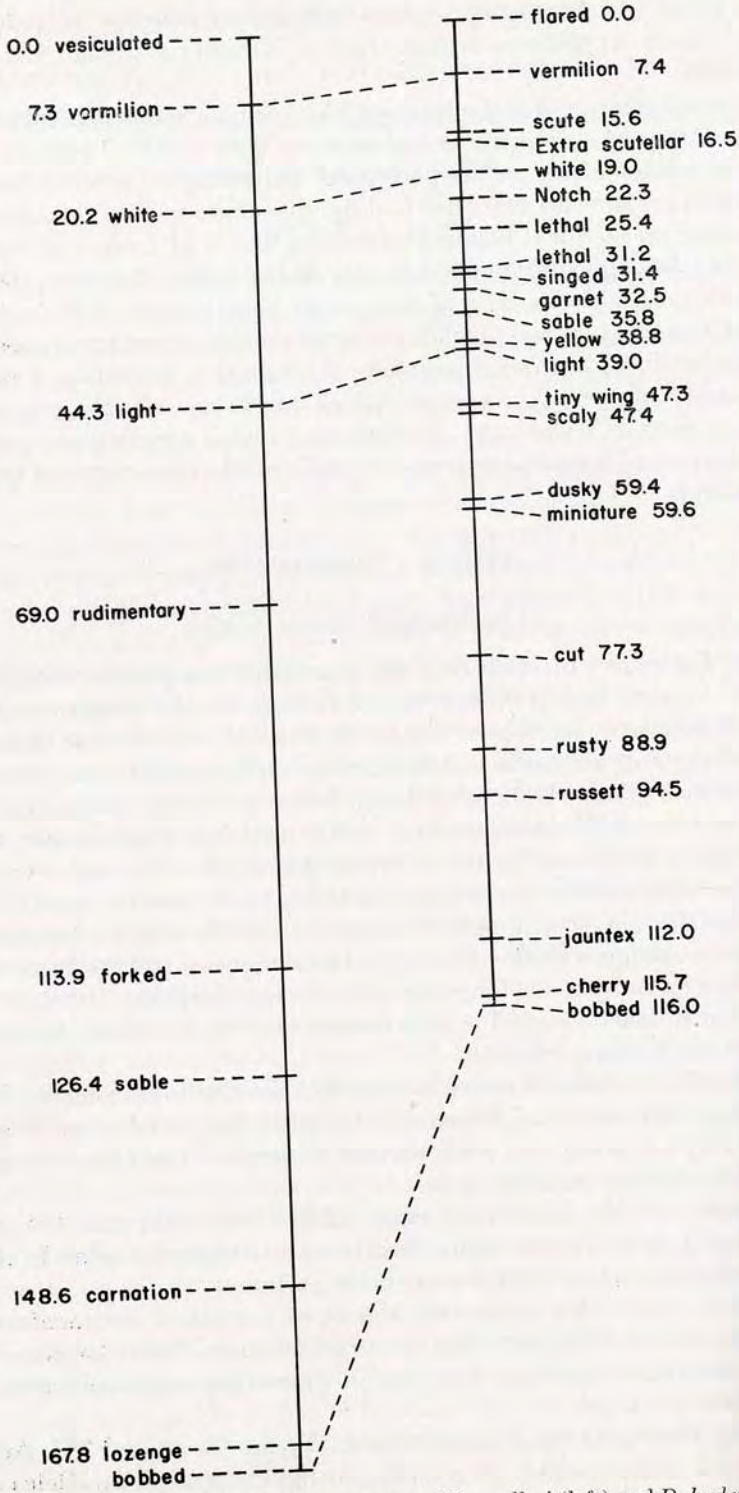

FIG. 1. Genetic maps of X-chromosomes of *D. mulleri* (left) and *D. hydei*.

allele of yellow, the same probably holds for *mulleri*, although no yellow mutant has been found as yet in this species. Spots of dark pigment on thorax much reduced in size; heterozygous females intermediate. An obvious homologue of light in *hydei* and *repleta*.

rudimentary (r). Many males in second chromosome linkage experiment involving cardinal-depigmented-polychaete-two joint. Wings often truncated to one-half to two-thirds normal length. Gaps and irregularities in wing-veins. Apparently temperature sensitive with some flies showing wings of almost normal length, but these can always be classified easily by the sparse and irregular arrangement of hairs on the outer wing margin. Females almost completely sterile.

forked (f). From wild stock 253 as several males. Head and thoracic bristles slightly gnarled or twisted. Variable but with no normal overlaps. Normal viability and fertility in both sexes.

extreme forked (f^e). Recovered repeatedly from forked stock. Forked seems to mutate to extreme forked roughly about 1:1000, but this should be given further study. Apparently extreme forked is stable, not mutating back to the slighter allele. All bristles and hairs extremely gnarled and twisted as in some singed alleles in *melanogaster*. Flies easily mired but both sexes fertile.

carnation (car). Four males in F_2 of wild pair 584. Apparently arose in this laboratory stock. Dark, translucent eye color as in mutant of this name in *melanogaster;* females somewhat infertile. This, of course, may be a homologue of one of the other sex-linked eye colors in *melanogaster*.

lozenge (lz). Several males in a cross of stock 120 reinforced x stock 372 thickened. Eyes lozenge shaped, with many facets tending to be fused, giving eye surface a very rough appearance in some areas and a glassy texture in others. Abnormal pigment distribution in the eye. Lozenge-vermilion has little pigment in the eye. No terminal tarsal claws; females almost sterile and completely so with lozenge males.

bobbed (bb). From an F_1 pair mating from wild pair 15. Medium bobbed allele. Bristles reduced in size in females but abdominal tergites normal. Males do not show the character.

bobbed^2 (bb^2). An extreme bobbed allele recovered from F_2 of wild pair 416. In females bristles much reduced in size and abdominal tergites abnormal.

In addition to the above mutants, all of which have been established in stock and used in linkage studies, a single miniature or dusky male was found, but died without giving offspring. A dusky-like mosaic with one short dark wing and a singed or forked like mosaic failed to give any mutant offspring when flies from them were inbred. They may have been somatic mutations not affecting the germ cells.

The Map of the X Chromosome

In Table 1 all linkage data on sex-linked genes are summarized and from these data a genetic map of the X chromosome was constructed. Figure 1 shows the genetic maps of the X chromosomes of *D. mulleri* and *D. hydei* drawn to the same scale. The map of the *hydei* X is identical to that published by Spencer (1949) except for the position of dusky which in the earlier map was placed about four units away from miniature. A large scale miniature-dusky linkage experiment

TABLE 1

Summary of all linkage data on the X-chromosome of *D. mulleri*.
Those items in the table preceded by a number in parenthesis are the items actually used in mapping the chromosome, and the numbers refer to the successive map region, beginning at the left or 0.0 end of the map.

Loci	Total Flies	Crossovers	Per-cent
carnation-forked	540	162	30.00
carnation-light	3181	1587	49.89
(7) carnation-sable	2906	646	22.20
carnation-vermilion	2106	1084	51.40
forked-light	3900	1889	48.44
forked-lozenge	5209	2261	43.41
(5) forked-rudimentary	635	285	44.88
(6) forked-sable	2003	250	12.48
forked-vermilion	3277	1511	46.11
forked-vesiculated	563	276	49.00
light-lozenge	5439	2789	51.09
(4) light-rudimentary	1215	301	24.77
light-sable	1421	651	45.81
light-vermilion	3372	1073	31.82
light-vesiculated	82	24	29.30
(3) light-white	249	60	24.10
(8) lozenge-sable	3186	1320	41.40
lozenge-vermilion	3951	1976	50.03
rudimentary-vermilion	580	238	41.04
rudimentary-white	249	102	40.96
sable-vermilion	4046	1940	47.90
sable-vesiculated	136	58	42.70
(1) vermilion-vesiculated	82	6	7.32
(2) vermilion-white	249	32	12.85
Total Linkage Determinations—48,577.			

with marker genes on either side showed that dusky lies about .2 of a map unit to the left of miniature. With the author's permission Stone (1955) published a preliminary report of the work on *D. mulleri* and included a tentative map of the *mulleri* X based on the fragmentary data then available and compared with the *hydei* X. This map placed lozenge at the 0.0 locus, followed in order by carnation, forked, vermilion, light, and bobbed. This order was based on a recombination value of about 32% between forked and vermilion in a small experiment. The true recombination value for forked-vermilion is close to 50%. Our table shows 46.11%, but this is almost certainly low, due to the inclusion of one set of data with an abnormally low value. One experiment in 1865 flies gave a value of 49.17%.

With the recent discovery of rudimentary, which linkage tests show to lie between light and forked, it became apparent that the genes earlier mapped at the left end of the X actually lie in reversed order far to the right of light. This immediately cleared up what had seemed a puzzling situation. Early in this work the author crossed lozenge males to bobbed females, and from the F_2 selected lozenge males and mated them singly each to several lozenge/bobbed females. If lozenge and bobbed were at opposite ends of the chromosome presumably several of these males would be genetically bobbed. But of the 12 males which gave progeny apparently none carried bobbed, for no bobbed females appeared among their daughters. It is obvious that lozenge lies close to bobbed, perhaps less than 10 units away.

Inspection of crossover values in the table may not be convincing for the plac-

ing of vesiculated to the left of vermilion, but in the one small experiment including vermilion, vesiculated, and light the results showed this to be the position of vesiculated. The present map of the *mulleri* X is almost 170 units long, and yet it includes two regions where the contiguous genes used in mapping show recombination values of over 40%. Eventually the total map length from vesiculated to bobbed should be considerably more than 170 units. Actually the recombination value for carnation and lozenge remains to be determined and will undoubtedly result in lengthening the present map. Chino (1936) has published the genetic maps of the *virilis* chromosomes. The X in this species is 170.5 units in length, based on 35 loci with no unmapped regions longer than 15 units. It seems almost certain that the *mulleri* map will eventually be longer than that of any species yet investigated.

On the *hydei* map furnished Stone for publication (Stone, 1955) there is one glaring error. The carnation locus, 50 units to the left of flared on that map is a spurious locus, and should be deleted as we have in the present map. On finding a wild *hydei* male showing an eye color the author assumed that it was sex-linked and set up a curious sequence of matings testing the linkage to mutants all along the X, in no case making a mating which would show that the mutant was a sex-linked recessive or was not. For instance the original stock was established by mating the male to stock *hydei* females and then remating him to his daughters. Mr. Thomas Gregg, a former student of the author, and doing graduate work at Texas, discovered the error and passed the word along by messenger. The case is doubly embarrassing because, having gotten rid of the left end of the *mulleri* X by swinging it in to the right end, it would seem that the author had to get rid of a long section at the left end of the *hydei* X in order to present a consistent story.

As the *hydei* map now stands it will probably not grow much in length between the two ends now mapped, for there are no long regions as yet unmapped as in the *mulleri* X. Certain comparisons between the X chromosome maps of the two species will be made following the presentation of data on chromosome II.

CHROMOSOME II

The Mutant Loci

Of the autosomal recessives extracted from the *D. mulleri* population many were discarded as not suitable for further study; some others were accidentally lost. Of those remaining a few seemed clearly to be homologues of mutants already known in *D. hydei* and *D. melanogaster*. Certain of these were first used in cross-tests to each other and later to other mutants. With over 250 of these cross-tests in addition to the series of tests on the scarlet-like eye colors (Spencer, this bulletin) five autosomal linkage groups were established, as one would have predicted from the chromosome number in the species. Table 2 presents the list of mutants localized to each chromosome. Those clearly homologues of mutants in *melanogaster*, *hydei* and *virilis* are listed separately. It should be noted that many of the mutants in the lower list, particularly eye colors, are probable homologues of mutants known in *D. melanogaster*.

We are using here the same numbering of the linkage groups as that used by

TABLE 2

List of *D. mulleri* mutants located in the five autosomes. The homologous chromosome element of *D. melanogaster* is in parenthesis after each chromosome number of *mulleri*. The numbers preceding each mutant are numbers of wild stocks from which they were derived.

Mutants recognized as homologues in other species				
II (E– 3R)	III (C– 2R)	IV (B– 2L)	V (D– 3L)	VI (F– 4)
90 cardinal	484 brown	276 dachsous	663 ascute	303 Extension
262 curled	205 cinnabar	256 elbow	95 fringe V	456 eyeless
42 peach	298 lanceolate	623 held out	120 inturned	
468 polychaete	644 rolled		682 javelin	
460 rough	470 serrated		276 rotated	
144 sepiaoid			189 scarlet	
719 taxi			433 sepia	
Mutants not clearly recognized as homologues				
61 brown-like	42 cup wing	170 bentdown	158 brown-like	
529 depigmented	37 dark eye	303 dark brown	31 brown-like	
339 small bristled	47 fringe III	224 grooveless	592 delta-like	
160 two joint	482 orange-like	26a narrow w.	135 dumpy-like	
	109 peach-like	341 warped-like	272 peach-like	
	127 purple-like		730 peach-like	
	454 thin wing		269 sparse	
	183 scarlet-like			

Spencer (1949) for *hydei*. The same system has been used by Weinberg (1954) for the linkage groups of *D. macrospina*. It should be noted, however, that Chino's (1936) chromosome III in *D. virilis* corresponds to the *mulleri* chromosome V, and chromome V of *virilis* is homologous to chromosome III of *mulleri*. Chromosomes II, IV and VI are the same in *mulleri* and *virilis*. For other chromosome homologies consult Sturtevant and Novitski (1941).

We shall confine further discussion of autosomal linkage to chromosome II, for which the linkage map has been worked out, and report later on the other autosomes on the completion of work now in progress. The work has progressed to a point where it should be possible to identify the linkage groups with the salivary chromosomes in *D. mulleri* and *D. hydei*. Unfortunately the translocation stocks have not been prepared and tested to date. But the evidence strongly favors linkage group II as corresponding to the longest salivary autosome which has been designated II. Thus Warters (1944) reported finding a heterozygous inversion in salivary chromosome II of *D. hydei*, widespread in wild stocks, and no other inversions in this species. Certain aberrant results of the author (Spencer, unpublished) on linkage in group II of *D. hydei* are best explained on the basis of a heterozygous inversion in this chromosome.

In describing the mutants of chromosome II the same system will be followed as for the X chromosome, the order being determined by the position of the mutant in the chromosome, starting with two joint at the 0.0 end of the chromosome map, Figure 2. This map was constructed on the basis of the linkage data presented in Table 3. It should be noted that all loci have been located on the basis of cross-over values between contiguous genes except for the right end locus, sepiaoid. Only one set of data for sepiaoid cross-overs is available, the cardinal-sepiaoid data. Sepiaoid cannot be worked accurately with rough, a small rough eye. So it was planned to run a sepiaoid-rough crossover experiment on a cardinal background. Cardinal-sepiaoid can easily be distinguished from cardinal in rough

FIGURE 2.

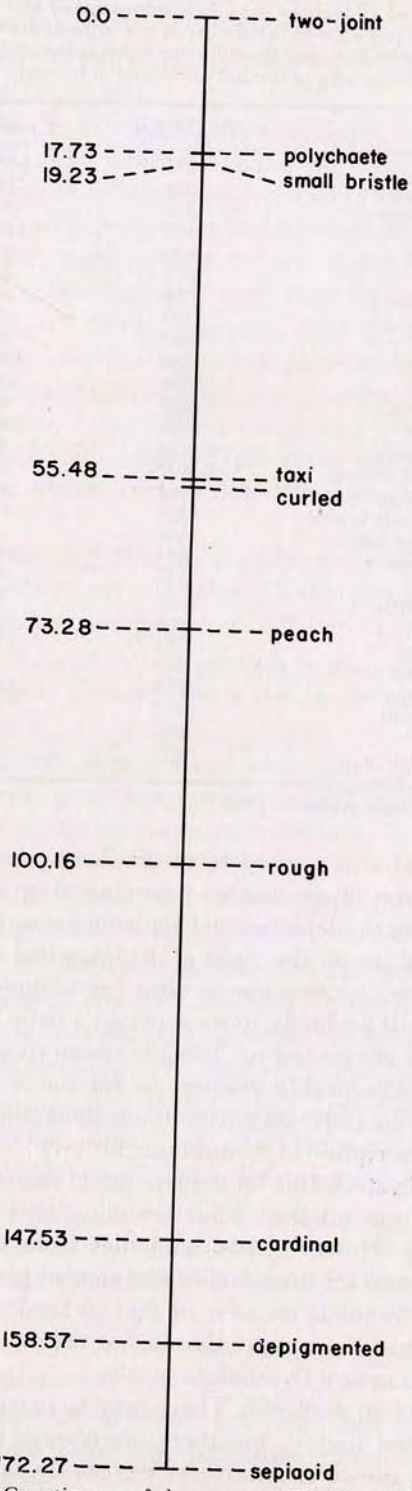

FIG. 2. Genetic map of chromosome II in *D. mulleri*.

TABLE 3

Summary of all linkage data on chromosome II of *D. mulleri*.
Those items in the table preceded by a number in parenthesis are the items actually used in mapping the chromosome, and the numbers refer to the successive map regions, beginning at the left or 0.0 end of the map.

	Loci	Total Flies	Crossovers	Per-cent
	cardinal-curled	4135	2031	49.12
(7)	cardinal-depigmented	951	105	11.04
	cardinal-polychaete	910	473	51.98
(6)	cardinal-rough	4135	1959	47.37
(8)	cardinal-sepiaoid	861	213	24.74
	cardinal-taxi	678	328	48.41
	cardinal-two joint	910	470	51.65
	curled-depigmented	497	269	54.10
(4)	curled-peach	1388	247	17.80
(3)	curled-polychaete	4342	1639	37.75
	curled-rough	8856	3088	34.87
	curled-small bristle	1292	449	34.75
	curled-two joint	4754	2096	44.09
	depigmented-peach	954	462	48.42
	depigmented-polychaete	497	250	50.30
	depigmented-rough	1494	730	48.86
	depigmented-small bristle	1384	671	48.48
	depigmented-two joint	1494	744	49.80
	peach-polychaete	2477	1139	45.98
(5)	peach-rough	3572	960	26.88
	peach-small bristle	1955	801	40.97
	peach-two joint	1388	663	47.77
	polychaete-rough	2954	1352	45.80
(2)	polychaete-small bristle	467	7	1.50
(1)	polychaete-two joint	5719	1014	17.73
	rough-small bristle	1292	594	45.98
	rough-two joint	3696	1756	47.51
	small bristle-two joint	875	179	20.46

Total Linkage Determinations—63,927

eyes. Cardinal-sepiaoid was crossed to cardinal-rough and the ease with which rough-sepiaoid cross-over chromosomes were picked up in the F_3, as compared to the difficulty in picking up depigmented-sepiaoid cross-over chromosomes, leaves no doubt that sepiaoid lies to the right of depigmented and is the terminal gene at the right end of the chromosome as thus far mapped. Eventual data on depigmented sepiaoid will probably move sepiaoid a little further to the right. Certainly if new mutants are picked up, lying between rough and cardinal, the total map length will be considerably greater. As for the X chromosome, the temporary locus number of each second chromosome gene may be found by consulting the map, Figure 2. Description of the mutants follows.

two-joint (tj). From stock 160. Of the five tarsal joints all but the proximal and distal are removed from all legs. Flies are sluggish but show close to normal viability and fertility. However, two-joint flies from moldy cultures are never able to clean mold spores off their bodies and special precautions should be taken to prevent mold by frequent transfer of flies to fresh medium. Dr. J. D. Krivshenko (oral communication) states that he has the same mutant in *D. busckii*. It is otherwise unknown in any *Drosophila* species.

polychaete (pc). From stock 468. There may be extra bristles in different bristle groups on head and thorax; but there are always two or more well defined presutural bristles in the acrostichal rows next to the outer ones as found in wild

D. putrida and *D. testacea.* In well nourished flies there are also bits of unattached venation near the distal end of the wing between L 2 and L 3. An almost certain homologue is known in linkage group II in *D. hydei*, showing both the bristle and venation characters.

small bristle (sb). From stock 339. Bristles about half normal length. Flies small and late emerging. This character masks polychaete in some flies, the presuturals in polychaete-small bristle being reduced to size of acrostichal hairs. In running linkage tests on polychaete-small bristle only non small bristle flies were classified. This procedure, which eliminates one of the reciprocal cross-over and non cross-over classes is necessary where there is epistasis.

taxi (tx). From stock 719. Wings generally held out almost at right angles to the long axis of the body; tend to be convex dorsally. Sometimes the wings are not so spread, but in these flies recognition is possible by the slightly smaller, narrower wings which are dusky. The locus is very close to curled. In several large scale experiments no cross-over chromosome bearing the two mutant loci has been recovered. An almost certain homologue of taxi in *melanogaster* and *hydei.*

curled (cu). From stock 262. Wings strongly upcurved and slightly divergent. Post-scutellar bristles stand erect and cross at a sharp angle. Flies slightly darker than wild-type. An excellent homologue of curled in *melanogaster* and *simulans.*

peach (p). In stocks 42 and 295. A pinkish peach eye color, easily classified. Darkens with age. Probably a homologue of similar mutants in *melanogaster* and *hydei.*

rough (ro). In stock 460. Eye texture very rough with facets disarranged. Eye size reduced depending on temperature. At 20° C. the eyes are very small with few facets. At 30° C. the eyes approach normal size but are rough. A good homologue of mutants in *melanogaster* and *hydei.*

cardinal (cd). In stock 90 and four others. A bright scarlet color in young flies, and with a long, light shadow across the eye. Darkens rather rapidly with age so that it is difficult to classify in old flies. Homozygous cardinal-brownIII is a light lemon color in newly emerged flies, and cinnabar-brownIII is about the same color; whereas vermilion-brownIII is white, and scarlet-brownIII is almost white. Cardinal is a good homologue of cardinal in *melanogaster* and *hydei.*

depigmented (dp). In stock 529. In wild-type *mulleri* the lower apical corners of the tergites of the abdomen show small dark gray triangular areas, very well defined. In depigmented most or all of this dark pigment is absent. The character is, however, more extreme in males and somewhat variable in females. For this reason only males were used in the linkage counts. No homologue known, but mutant resembles wild-type pigmentation in some other species of the *mulleri* subgroup.

sepiaoid (sed). In stock 144. A rich, dark purplish-sepia color. Probable homologue of sepiaoid in *melanogaster.*

brown-like. In stock 61. A dull brown eye color, best classified in young flies. The mutant was lost before its locus in the chromosome could be determined. It is therefore not given a symbol.

The Map of Chromosome II

The map of chromosome II presented in Figure 2 and based on the linkage data in Table 3 is 172.27 units in length. When and if mutants are found between small bristle and curled, and between rough and cardinal many units will undoubtedly be added to the present map. Chino (1936) published the linkage maps of *D. virilis*. Chromosome II was 210.5 units long. Since then he has sent in several records to *Drosophila Information Service* with new mutant loci mapped. His last list (Chino, 1940) in *D. I. S.* includes loci at 256.6 and 257.6 in chromosome II. This is at present the longest map reported in any species of *Drosophila*. Significantly, however, no new loci have been added to lengthen the map of the X in this species, 170.5 units as published by Chino (1936). As our map of the X in *mulleri* is almost this long, with long unmapped regions, it seems likely that the *mulleri* autosomes will also turn out to give linkage maps as long or longer than those in *virilis*. Our map of choromosome II is based on the location of 10 genes. Chino's 1936 map of virilis II, 210.5 units long, is based on the location of 21 genes. As the ratio of length of the map of the X to length of the maps of the autosomes seems to hold fairly well from species to species (with the obvious exception of such species as *pseudoobscura*, where an extra limb has been added to the X) this seems to be a reasonable hypothesis. At least we would predict that the *mulleri* autosomes will be about as long or even longer than the *virilis* autosomes.

DISCUSSION AND CONCLUSIONS

As stated in the introduction it was our purpose to make a comparative study of the genetics of the two species, *D. mulleri* and *D. hydei*, as a supplement to the cytological work of Wasserman (1954). An inspection of the linkage maps shown in Figure 1 reveals this interesting point. The *mulleri* X is definitely longer than the *hydei* X. This is no spurious conclusion based on sampling errors. There is a real difference, *mulleri* 167.2 units, *hydei* 116 units. And the presumption is, based on long unmapped regions, that the discrepancy in length will be greater, not less, with accumulation of more data.

Our data on the linkage maps of the *hydei* autosomes, which are not being published here, but which are based on the location of about as many genes per autosome as for chromosome II of *mulleri*, show *hydei* chromosome II, 103 units, chromosome III, 127 units, chromosome V, 100 units of map distance. These figures are certainly tentative, but they do indicate, on the basis of the *mulleri* II map, that the autosomes in *hydei* give considerably shorter linkage maps than in *mulleri*. We are forced to conclude that identity in banding pattern of the salivary chromosomes in two species does not lead to identity in recombination values.

Of more interest is the matter of the order of the gene loci in two rather distantly related species, *hydei* and *mulleri*, which have retained the same banding pattern in the X. It will be noted that the four homologous gene loci, vermilion, white, light, and bobbed, are in the same order in the two species and spaced about as would be expected on the basis of freer crossing-over in the *mulleri* X. But there is a mutant type, sable, certainly as good a parallel or suspected homologue as one could wish on morphological grounds, which does not fit into the

picture. Sable in *hydei* is between white and light, while in *mulleri* it lies between light and bobbed and closer to bobbed. We shall therefore suspend judgment on the matter of the gene order in the two species until more homologous loci can be compared. Of course there are two mimic loci, miniature and dusky, which have remained close together in all *Drosophila* species in which both have been found. There are also two other mimic loci, forked and singed, which have shown very different relative positions in the few species where both have been found. It is interesting but perhaps idle to speculate at this point on whether there are two mimic sable loci.

We conclude that the evidence is fair, though not incontrovertible, that the gene order has remained essentially the same in the X chromosomes of these two species, but that recombination values have changed in one or the other or both.

ACKNOWLEDGMENTS

The author wishes to thank particularly Mr. James Jolliff, who made many of the linkage determinations on the X chromosome loci. He also thanks Mr. Thomas Gregg for calling his attention to the mode of inheritance of the mutant termed carnation in *D. hydei*. Both to the College of Wooster, which made a year's research leave possible, and to the staff of the Zoology Department of The University of Texas, who provided ample facilities and warm hospitality, go his profound thanks. The latter part of the work was supported in part by a grant from the Wilson Fund, and he wishes to thank Dr. Robert Wilson for making this fund available.

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XVI. Relationships between Species Groups of the Subgenus Pholadoris, Drosophila (Diptera: Drosophilidae)

WHARTON B. MATHER[1]

INTRODUCTION

The subgenus Pholadoris has been divided into five species groups on morphological grounds (Mather 1955): victoria, coracina, maculosa, mirim and levis groups. Further, four species of the coracina group, *Drosophila cancellata* Mather, *D. lativittata* Malloch, *D. enigma* Malloch and *D. novopaca* Mather have been shown to form an interbreeding network (Mather, 1956). The purpose of the present work was to test further the biological reality of these species groups by attempted hybridization, and if possible to throw light on the biological relationships of the groups. The species groups established by Burla (1954) have not been considered here, since stocks of the species concerned were not available.

MATERIALS AND METHODS

The stocks used in this study are listed in Table 1. The methods used are those previously described (Mather 1955, 1956). Ten pair matings both ways, changed weekly, were run in vials for one month and replicated 10 times.

TABLE 1

Stocks of Pholadoris species used in this investigation.

Species group	Species	Stock No.	Origin
victoria	victoria	1865.3	Utah
coracina	cancellata	2372.1	Moggill, Queensland
coracina	enigma	2372.3	Moggill, Queensland
coracina	lativittata	2372.4	Moggill, Queensland
coracina	novopaca	2372.7	Noosa, Queensland
maculosa	novamaculosa	2372.6	Moggill, Queensland
mirim	latifasciaeformis	1975.4	Brazil
levis	bryani	2372.5	Maroochydore, Queensland

RESULTS AND DISCUSSION

The interspecific crosses attempted are set out in Table 2. Unfortunately *D. coracina* Kikkawa and Peng, the fifth member of the coracina species group, was not available for these tests but its biological relationships will be reported on in a subsequent publication. No tests have been carried out between the levis and mirim groups on the one hand and the victoria, maculosa and coracina groups on the other due to their considerable morphological divergence.

D. novopaca is the one member of the coracina group which will cross with the other three available species of the group; therefore crossing was attempted between this species and *D. victoria* Sturtevant in the related victoria group.

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TABLE 2

Attempted crosses between species of the subgenus Pholadoris.

Attempted cross	Sexual Isolation*	Insemination reaction
victoria ♂ × novopaca ♀	15/15	
victoria ♀ × novopaca ♂	23/23	
victoria ♂ × novamaculosa ♀	40/42	present
victoria ♀ × novamaculosa ♂	31/31	
cancellata ♂ × novamaculosa ♀	13/13	
cancellata ♀ × novamaculosa ♂	13/13	
enigma ♂ × novamaculosa ♀	19/19	
enigma ♀ × novamaculosa ♂	15/16	absent
lativittata ♂ × novamaculosa ♀	15/15	
lativittata ♀ × novamaculosa ♂	15/15	
novopaca ♂ × novamaculosa ♀	13/13	
novopaca ♀ × novamaculosa ♂	15/15	
latifasciaeformis ♂ × bryani ♀	6/8	absent
latifasciaeformis ♀ × bryani ♂	24/24	

* Fraction of females dissected which did not show sperm in their reproductive tracts after exposure to males for one month.

Crosses were also attempted between *D. novamaculosa* Mather and *D. cancellata*, *D. lativittata* and *D. novopaca* with which the former species is sympatric.

The failure of hybridization between the species tested lends support to the erection of the groups on morphological grounds. However, there is a lack of complete sexual isolation between *D. bryani* Malloch and *D. latifasciaeformis* Duda, between *D. enigma* and *D. novamaculosa*, and between *D. victoria* and *D. novamaculosa;* as is shown in Table 2, there were a few cases of insemination in crosses involving these pairs of species. Thus a close biological relationship existing between the mirim and levis groups on the one hand, and the maculosa, coracina and victoria groups on the other is indicated.

An insemination reaction in the two cases where *victoria* ♂ ♂ mated with *novamaculosa* ♀ ♀ is interesting since it has been shown that in intraspecific matings of *victoria* there is a strong homogamic insemination reaction (Wheeler, 1947). On the other hand, in the two cases of mating between *latifasciaeformis* ♂ ♂ and *bryani* ♀ ♀, and the single case of mating between *enigma* ♀ ♀ and *novamaculosa* ♂ ♂, an insemination reaction was not observed. However, nothing is known of the intraspecific insemination reactions in these species.

SUMMARY

The failure of hybridization between species groups of the subgenus Pholadoris established on morphological grounds supports the biological reality of these groups. However, sexual isolation is incomplete between the levis and mirim groups, between the maculosa and coracina groups, and between the maculosa and victoria groups, indicating the close biological relationships of the cross mating groups.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to Professor Wilson S. Stone of the Genetics Foundation, University of Texas, where this work was carried out while the author was on sabbatical leave from the University of Queensland, Australia.

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XVII. Genetic Relationships of Four Drosophila Species from Australia (Diptera: Drosophilidae)

WHARTON B. MATHER[1]

INTRODUCTION

Drosophila serrata Malloch, *D. pseudotakahashii, sp. nov.*, *D. bryani* Malloch and *D. buzzatii* Patterson and Wheeler from Australia are not closely related morphologically to any other species so far recorded from this area (Mather 1955). By breeding with conspecific strains or with the most closely related species, morphologically, from other parts of the world, information on the phylogeny of these species occurring in Australia has been investigated.

MATERIAL AND METHODS

The stocks used were those indicated in Table 1. The methods used were similar to those described previously (Mather 1955, 1956b, 1956c). Ten pair matings, both ways, replicated ten times and run for a month with weekly food changes were attempted for *D. serrata* × *D. kikkawai* Burla, and for *D. pseudotakahashii* × *D. takahashii* Sturtevant. Duplicate matings both ways were extended to the F_2 for *D. bryani* × *D. bryani* and for *D. buzzatii* × *D. buzzatii* crosses. The *pseudotakahashii* × *takahashii* hybrid F_1 was back-crossed to the parents as indicated in Table 3.

TABLE 1

Stocks utilized in this study.

Species	Stock No.	Origin
serrata	2372.8	Greenslopes, Queensland
kikkawai	2363.3	Katmandu, Nepal
pseudotakahashii	2372.9	Samford, Queensland
takahashii	2363.4	Katmandu, Nepal
bryani	2372.5	Maroochydore, Queensland
bryani	2370.10	Majuro, Marshall Islands
buzzatii	2372.10	Moggill, Queensland
buzzatii	190	Carpentaria, Italy
buzzatii	2093.11	Beirut, Lebanon

RESULTS AND DISCUSSION

Drosophila serrata Malloch.

D. serrata Malloch 1927:6.

D. serrata, Mather 1955:565.

On morphological grounds this species is most closely related to *D. kikkawai* Burla (Mather 1955), the most obvious points of contrast being the sex-combs and certain features of the internal male genitalia (Table 2). Tests indicated that these species never produce hybrids, nor were sperm detected in the female

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TABLE 2

Chief contrasting characters of *D. serrata* and *D. kikkawai*.

Species	Sex-comb teeth	Penis	Hypandrial bristle length / Length of penis
serrata	34 + 18	rounded	0.5
kikkawai	25 + 18	pointed	1.0

genital tracts of 54 *kikkawai* and 20 *serrata* dissected after exposure to males for four weeks. However, when virgin females were placed with the males, the latter immediately very vigorously attempted to mate. That there was sometimes successful mating was indicated by the fact that sperm were occasionally found in the female genital tract when dissected 30 minutes after placing them together. Thus sexual isolation is not as complete as would be concluded by the results of dissections made after four weeks of exposure. The explanation of this paradox may be that the sperm have a very short life in the alien female and that alien species lose interest in mating after the initial attempts. It is of interest that no sign of an "insemination reaction" was found in any of the flies dissected. Thus, *D. serrata* and *D. kikkawai* are quite distinct species and although closely related morphologically, very effective isolating mechanisms have developed between them.

Drosophila pseudotakahashii, *sp. nov.*

D. takahashii, Mather 1955:568.

The stock designated *Drosophila takahashii* Sturtevant from Australia (Mather 1955) when crossed with *takahashii* from Nepal produces hybrids only when the female parent is from Australia. These hybrids are produced in approximately equal numbers of both sexes, unlike the situation in other interspecific crosses where females far outnumber the males (Mather 1956c). They are intersterile, due apparently to lack of motile sperm in the testes of the hybrid males. The F_1 females, however, can be backcrossed to both of the male parents (Table 3). Further, the salivary chromosomes of the F_1 hybrids show very poor pairing. Thus although some "gene flow" would be possible if these species have a contact zone, the above data would indicate that these two stocks are distinct species.

D. takahashii, *D. lutea* Kikkawa and Peng, and *D. nepalensis* Okada, all members of the takahashii subgroup of the melanogaster species group, can best be separated morphologically by differences in the internal male genitalia (Okada 1955). Accordingly the internal male genitalia of both of the above stocks were examined and it was found that the stock from Nepal was indeed *takahashii* Sturtevant, but the stock from Australia differed in that the basal branch of the posterior paramere was vestigial and the basal conical process of the novosternum was long and serrated instead of being short and conical. Unfortunately stocks of *lutea* and *nepalensis* were not available for hybridization tests but the morphological differences of the Australian stock from the three established species of the subgroup are considered sufficient to raise it to specific rank (Table 4).

In both the F_1 and the backcrosses of the female hybrids to both the male parents , the basal branch of the posterior paramere was present and the basal conical

process of the novosternum was elongated and serrated, although in the backcrosses the degree of expression of these characters was variable. This would indicate that these characters are controlled multifactorially. In hybrids the alleles controlling the basal branch of the posterior paramere from *takahashii* are dominant over the alleles from *pseudotakahashii*. On the contrary, the alleles controlling the basal process of the novosternum from *takahashii* are recessive to the alleles from *pseudotakahashii*. In this case, unlike the case of body markings in the coracina species group (Mather 1956c), outstanding morphological differences between species are controlled multifactorially, similar to the situation found in the machaon group of butterflies where the majority of outstanding differences are also multifactorially controlled (Clarke and Sheppard 1955).

Drosophila bryani Malloch.

D. bryani Malloch 1934:310.

D. levis Mather 1955:561.

D. bryani, Mather 1956a:65.

The synonymy of *D. levis* Mather with *D. bryani* Malloch was suggested on morphological evidence despite the difference in known geographical range

TABLE 3

Results of crosses between D. takahashii and D. pseudotakahashii.

Attempted cross	Fertile vials	Progeny ♂	Progeny ♀	♂ genitalia dissected	Remarks
takahashii ♂ × pseudotakahashii ♀	5/10	96	107	17	giant chromosome pairing poor in hybrids; total progeny of 4 week test.
pseudotakahashii ♂ × takahashii ♀	0/10	0	0		
(takahashii ♂ × pseudotakahashii ♀) ♂ × (takahashii ♂ × pseudotakahashii ♀) ♀	0/2	0	0		10 ♂♂ were dissected; none showed motile sperm in testes.
(takahashii ♂ × pseudotakahashii ♀) ♀ × takahashii ♂	4/4	15	21	10	total progeny is result of first week of attempted hybridization.
(takahashii ♂ × pseudotakahashii ♀) ♀ × pseudotakahashii ♂	3/4	38	47	17	total progeny is result of first week of attempted hybridization.

TABLE 4

Chief contrasting features of the takahashii subgroup.

Species	black patch on wing	decasternum	basal branch, post. paramere	basal conical process of novosternum	common duct of Malpig tubes; post./ant.
takahashii	absent	narrow, pointed	short, serrate	short, conical	1
lutea	absent	narrow, truncate	long, serrate (at tip only)	short, serrate	1
nepalensis	present	broad, truncate	long, serrate	short, serrate	<1
pseudotakahashii	absent	narrow, pointed	vestigial	long, serrate	1

(Mather 1956a). A stock of *bryani* from Australia has been found to be completely cross-fertile to the F_2 with a stock of the same species from the Marshall Islands, thus adding support to the synonymy of these species.

Drosophila buzzatii Patterson and Wheeler.

D. buzzatii Patterson and Wheeler 1942: 97.

D. versicolor Mather 1955:573; 1956a; 1956b.

Despite certain morphological differences a stock of *D. versicolor* (Mather 1955, 1956a, 1956b) will freely interbreed with two stocks of *buzzatii* (Table 1) thus showing that *versicolor* is synonymous with *buzzatii*. Further, the Australian strain has the j inversion in the 2nd chromosome which has previously been recorded only from Lebanon (Wasserman, 1954). It has been assumed that *buzzatii* was introduced into Lebanon and Italy from South America but Wasserman points out that there is insufficient data to show whether or not the j inversion arose in South America or in Lebanon. However, it seems more likely that *buzzatii* was introduced into Australia from South America (possibly on cactus) than from Lebanon, thus giving some support to the hypothesis that the j inversion did in fact arise in South America.

SUMMARY

By breeding tests it has been established that:

1. *D. serrata* Malloch is a distinct biological species from *D. kikkawai* Burla.
2. The fly previously recorded as *D. takahashii* Sturtevant from Australia is in fact a new species, *D. pseudotakahashii*, which is morphologically distinguishable from *takahashii* by two features of the internal male genitalia which are controlled multifactorially.
3. The synonymy of *D. levis* Mather with *D. bryani* Malloch, previously established on morphological grounds, is confirmed biologically.
4. *D. versicolor* Mather from Australia is synonymous with *D. buzzatii* Patterson and Wheeler, and contains an inversion previously recorded only from Lebanon.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to Dr. Wilson S. Stone, Professor of Zoology, Genetics Foundation, University of Texas, where this work was carried out while the author was on sabbatical leave from the University of Queensland, Australia.

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XVIII. A New Drosophilid from Australia (Diptera)

MARSHALL R. WHEELER

Through the courtesy of Dr. F. I. Van Emden of the Commonwealth Institute of Entomology, London, I have had the opportunity of studying four specimens of a beautiful new drosophilid from the C. I. E. collection. The flies were collected on Mt. Tambourine, Queensland, Australia, and were apparently reared from *Endiandra sp.*, a member of the Lauraceae. The holotype and two paratypes are being returned to Dr. Van Emden for deposit in the British Museum (Natural History); the third paratype is in the Drosophila Type and Reference Collection of the Genetics Foundation, University of Texas.

All four specimens are females, but since striking sexual dimorphism is so rare in this family, it is probable that the males will be recognizable from the description and figures given here.

Tambourella Wheeler, new genus

Type species: *Tambourella endiandrae* Wheeler, new species.

Small species with highly marked wings and aberrant wing venation. Arista plumose; carina high and exceptionally narrow; postvertical bristles minute; acrostichal hairs absent; anterior dorsocentral placed at sutural level; no presutural bristle; a single postalar; basal scutellars small; no mesopleurals; two sternopleurals. Wings as in Figure 2; costa reaching 4th vein; 2nd vein bent abruptly to costa apically; 5th vein incomplete at apex but continued by fusion with the long, undulant posterior crossvein. The veins on all specimens show a strong + and — character: costa +, 2nd —, 3rd +, 4th —, 5th, and posterior crossvein +.

The generic name, derived from the name of the type locality, is considered to be feminine.

Tambourella endiandrae Wheeler, new species

♀. Front broader than long, yellowish tan with dense pollinosity, less so along a narrow area between orbit and large frontal triangle; orbits elongate, reaching anterior margin, the orbital bristles placed rather far forward (Fig. 1), the middle orbital minute or absent, the proclinate about 0.4 length reclinate,

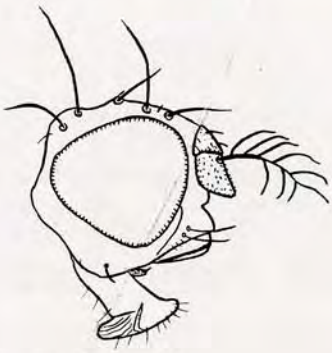

FIG. 1. *Tambourella endiandrae*, head in profile.

the latter about 0.9 length ocellars. Bases of ocellars clearly within the triangle formed by the ocelli. Both inner and outer verticals strong, the latter about 0.6 length of the former. Vertex elevated, mound-like, around bases of vertical bristles. Postverticals minute, cruciate, placed on rear of head.

Antennae tan, 2nd joint with small hairs of which two are more prominent; 3rd joint a little darker, short pubescent. Arista with 5 dorsal and 3 ventral branches in addition to the terminal fork, the branches rather long and bent at their tips. Face glassy hyaline and yellowish on all four specimens; carina very narrow and sharp, ceasing abruptly before level of antennal apex, with the lower margin of face again elevated into two mound-like swellings, leaving a low median crease between them. Two thin orals, nearly of equal length. Cheeks narrow below the eyes, greatly widened behind, mostly tan in color becoming blackish along lower margin. Posterior cheeks strongly pubescent when viewed from certain angles. Clypeus hyaline, narrow; oral opening rather large, ephydrid-like; palpi narrow, brown.

Mesonotum opaque tan, strongly pollinose, the disc with two darker brown areas (variable in extent), one between posterior dorsocentrals and another anterior to it; scutellar disc with a similar brown mark when seen from above. No evident acrostichal hairs, but there are a few scattered hairs in the dorsocentral rows and laterally. Two pairs of strong dorsocentrals, the anterior pair placed at the sutural level; two pairs of scutellars, the basal ones small and thin, about 0.25 length apical ones. No presutural bristle; one small humeral; two notopleurals; two supraalars, one postalar (the 2nd one represented by a small hair). Side of mesonotum with darker brown areas, especially around bases of supraalars; pleura brownish, rather shiny and hyaline, especially over mesopleura and center of sternopleura. A short anterior sternopleural bristle and a long stout posterior one. Knobs of halteres dark.

Abdomen of all specimens shriveled but evidently the basal 3 (or 4) tergites are dark with thick grayish to grayish golden pollinosity, the remaining (apical) tergites being dark and shining. Ovipositor tan with small stout teeth along both upper and lower margins.

Legs mostly collapsed, apparently pale tan with darker coxae and femora. An evident small apical bristle on 1st tibia, apical and preapical on 2nd tibia. First femur with a row of about 15 very small thornlike spines along inner side, each spine associated with a tiny hair.

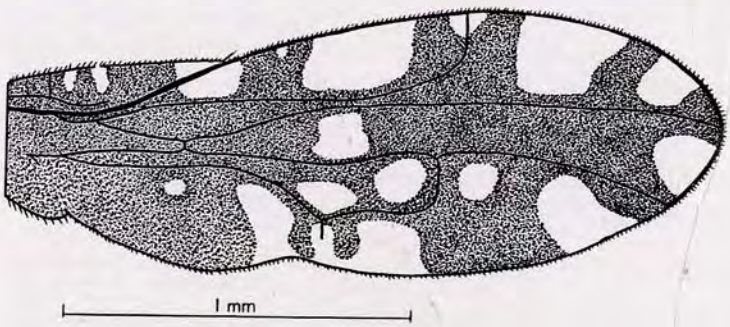

FIG. 2. *Tambourella endiandrae*, wing, semi-diagrammatic.

Wings generally brown with discrete white areas (Fig. 2). Costal index about 1.1; 4th vein index about 0.9. Apex of 1st costal section with a single small apical bristle; 3rd costal section with the small black spines on the basal 0.8. Costa reaching 4th vein; distal costal break shallow, the costa not prolonged as a lappet. Anal vein absent. Wing length about 2.2 mm.; overall length (tip of head to tip of wing) about 3.5 mm.

Types.—Holotype ♀ and three paratype ♀ ♀, all bearing labels as follows: (1) "ex Endiandra sp; Mt. Tambourine; 30–12–52; May"; (2) "731"; (3) "Com. Inst. Ent. Coll. No. 14691." One specimen bears the notation "killed 16–1–53"; if this means that the flies were kept alive for some two weeks, then the glassy hyaline character of the face and clypeus should not be interpreted as teneral peculiarities.

XIX. Genetic Variation of Isoxanthopterin Content in *Drosophila melanogaster*[1]

FORBES W. ROBERTSON[2] AND HUGH S. FORREST

INTRODUCTION

Hadorn and Mitchell (1951) showed that *Drosophila melanogaster* contained a number of fluorescent substances which could be readily separated into more or less distinct spots by paper chromatography of flies squashed directly on to the paper. Several of these compounds have since been identified as pteridines (Forrest and Mitchell, 1954, 1955; Viscontini, 1955), all containing the basic structure I (Fig. 1), but showing various differences in the side chains R_1 and R_2. Be-

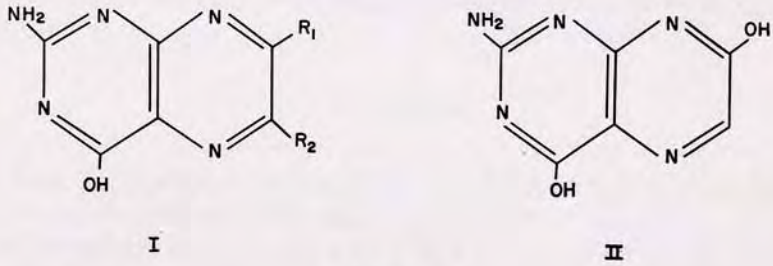

FIGURE I.

cause of their close chemical relationship, Forrest and Mitchell (1955) postulated that these compounds represent individual steps in a biochemical pathway leading to the red eye pigments characteristic of Drosophila. However it seems likely that one of them, isoxanthopterin (II; Fig. 1), is not directly on this pathway but is produced from one of the intermediates on it, namely 2-amino-4-hydroxypteridine (III; Fig. 2). Schematically this hypothesis can be written as shown in Fig. 2. The evidence for this scheme is based on chromatographic examination of mutants of *Drosophila* (Hadorn and Mitchell, 1951; Forrest, unpublished), and also on the demonstration of the virtual absence of isoxanthopterin in the mutants *rosy*, *maroon* and *maroon-like* (Forrest, Glassman and Mitchell, 1956; Hadorn, 1956). These do not lack the red eye pigments, although they appear to have greatly reduced amounts, whereas the *sepia* mutant, for example, lacks the red eye pigments and "accumulates" a yellow pteridine which is therefore assumed to be their immediate precursor.

Since the *rosy*, *maroon* and *maroon-like* mutants have reduced amounts of red pigments and of isoxanthopterin, it would be reasonable to assume that the latter

[1] The work reported here was supported in part by a grant from the Welch Foundation, Houston, Texas.

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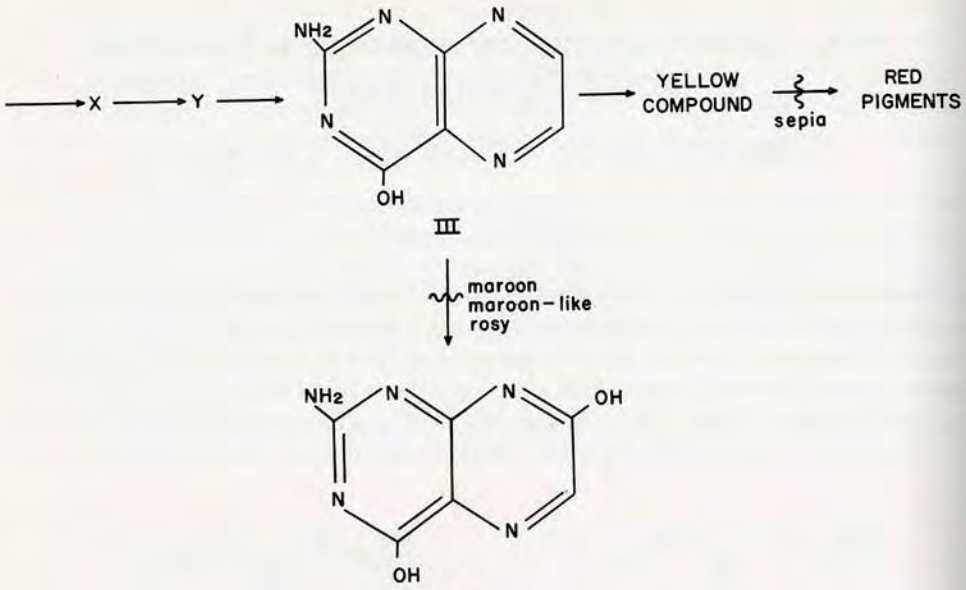


FIGURE 2.

had some effect on the production of the pigments. This has not been demonstrated on an enzymatic level, although some effects of isoxanthopterin on pigment production in other systems have been claimed. Thus Polonovski, Gonnard and Baril (1951) stated that it inhibited the initial oxidation of dihydroxyphenylalanine (DOPA), but accelerated the production of melanin at the expense of the red intermediates, and more recently, Oshira, Seki and Ishazaki (1956) propounded a scheme for its activity in the control of the colour of the elytron in the lady-beetle. It would be possible, of course, to attribute the apparent relationship between red eye pigment and isoxanthopterin in *Drosophila* to the functioning of the enzyme, xanthine oxidase, which is responsible for the production of isoxanthopterin (Forrest, Glassman and Mitchell, 1956) but which could also act as an oxidase or dehydrase in the process of red eye pigment formation.

As is obvious, however, information on the biological function of isoxanthopterin is inadequate, although there are some indications that it may play an important role. Thus it appears to be especially abundant in certain tissues of *Drosophila*, especially the testes, thereby producing a characteristic sex difference (Hadorn and Mitchell, 1951), although females also carry the compound in easily detectible concentration. Also during the life-cycle of *Drosophila melanogaster*, there is a rapid and spectacular rise in concentration during the pupal period. It has also been suggested (Haddow, 1955), that its isomer, xanthopterin, plays an important part in cell division in renal tubular epithelium and, on this basis, that other specific pteridines might also be involved in the growth of other types of tissue.

So far attention has been confined to the isoxanthopterin content in mutant stocks and at least three genes have been shown to influence the content. It is a reasonable inference that many other genes will also intervene in the production

of this compound and it would be interesting to know how far non-mutant, wild stocks differ in isoxanthopterin level, since this would provide a basis for assessing the relative effects of different mutant genes and would indicate how far differences in genetic background are likely to influence the individual content. Indeed, an identifiable chemical compound, subject to the influence of many gene differences, constitutes rather novel material for the study of quantitative inheritance, since there is the ultimate prospect of relating the genetic to the underlying biochemical behaviour. If isoxanthopterin, or some precursor which is related quantitatively to the final isoxanthopterin level, turns out to be important in metabolism or differentiation, evidence of genetic variation and of genetic behaviour will acquire additional interest. Isoxanthopterin is eminently suited for such comparisons since it can be readily isolated from individual flies and then estimated accurately.

The present paper deals with the isoxanthopterin content in a number of non-mutant inbred lines and wild stocks of diverse origin. Also some of the inbred lines have been crossed to determine the relation between the F_1 and the parent lines. The effects of variation in body size have been estimated by comparing the isoxanthopterin content in selected strains which differ in size.

MATERIAL AND METHODS

The technique for chromatography was essentially that of Hadorn and Mitchell (1951). Individual flies were squashed on Whatman No. 1 filter paper on a line drawn 1.5 cm. from one edge and each at a distance of 1.5 cm. The paper was developed, using n-propanol, 1% ammonia (2:1) as the solvent, for about 16 hours, after which the chromatogram was dried and the areas corresponding to isoxanthopterin were located by their fluorescence in U.V. light. These were cut out and the fluorescent material was eluted from them with 0.5 N ammonia. Fluorescent measurements on the eluting fluid were made with a Farrand Model A Fluorometer using the following filters: Primary filter, Corning No. 5860; Secondary filters, Corning Nos. 3389 and 4308. A weighed sample of isoxanthopterin was used as the standard.

Two dimensional chromatograms were occasionally run (see text). In these cases, individual flies were squashed at one corner of a rectangle of filter paper (15x20 cm.). The paper was developed in the shorter direction with n-propanol, 1% ammonia (2:1), and in the longer direction with 5% acetic acid. The isoxanthopterin was located and eluted as described above. The isoxanthopterin content of individual flies obtained from such chromatograms was lower than from one dimensional ones, as would be expected, but the results were consistent within a group. Since the two dimensional technique is much more laborious, especially when handling large quantities of flies, it was therefore abandoned as a general procedure.

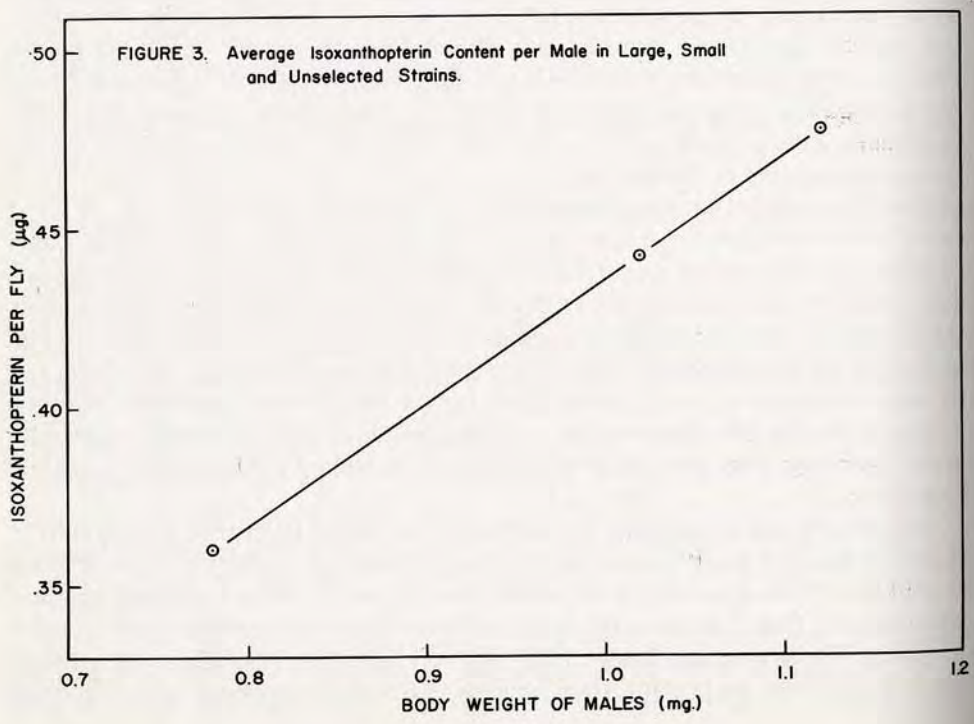
The genetic stocks included 6 wild stocks descended from wild flies caught in widely separated localities and kept in the laboratory in mass culture. Fifteen inbred lines were also compared; these were derived by long-continued brother-sister mating from various wild stocks or from strains selected for large or small body size (Robertson and Reeve, 1952; Robertson, 1956). Five of the inbred lines were intercrossed to provide 10 F_1 groups. All flies were reared under optimal

conditions on the usual cornmeal-molasses medium, well supplied with yeast; 70 eggs per vial were set up to eliminate crowding. The cultures were kept at 22–23° C. The isoxanthopterin content for each genotype is based generally on data from 10 flies. Within about 18 hours after emergence from the pupa, a sample of 20 flies was weighed. The flies were aged about 2½ days before being chromatographed.

The isoxanthopterin content is a good deal higher in males than females, sometimes as much as 15 times greater. Since the coefficient of variation works out at about the same in the two sexes (17–18% in the present determinations) a logarithmic scale is indicated. However, since the analysis is confined to comparisons within one sex or the other, the range is not great enough for such a transformation to make much difference and so the data are expressed in micrograms (μg.) per fly or per milligram weight.

RESULTS

(a) *Isoxanthopterin content and body size.* The first point to consider is whether the total content per fly or the amount per unit weight should be the basis for comparison. To answer this question, isoxanthopterin was determined on selected large and small and also unselected, normal-sized flies from the Pobla de Lillet stock; only males were studied in this test. Mass selection had been carried out for some 10 generations and there was a substantial difference in size between the large and small strains, 0.78 mg. in the former, 1.12 mg. in the latter. Fig. 3 shows the isoxanthopterin content per fly plotted against body weight. The graph falls close to the line of proportional increase, so that isoxanthopterin con-



tent per milligram body weight is virtually the same in the large and small flies. Since appreciable differences in size occur among the inbred lines and wild stocks described below, it would be misleading to deal in terms of total content per fly and so the data are expressed in terms of isoxanthopterin per milligram weight.

(b) *Differences between inbred lines.* Six of the lines used were inbred from various wild stocks; the lines are referred to as Nb_2, R_2, OrR, C_6 and C_9, while one line carries *vermilion* in what was originally an OrR background (OrR v). The other lines, inbred from selected strains, include CL_1, RL_4, IL_4 and LT_9 from different large strains, and CS_1, ES_{12} and IS_2 from small strains. In addition, two lines, EL_1 and ES_1 were homozygous for white-eyed alleles which had arisen by mutation during inbreeding.

The average isoxanthopterin content per milligram for the 15 lines is listed in Table 1. Bearing in mind that the standard error of the difference between means, based on the pooled variance, is 0.033, it is obvious that significant and substantial differences exist. Excluding the white-eyed lines the values range from 0.690 μg. in IL_4 to 0.312 μg. in RL_4, with an average of 0.408 μg. for the 13 lines. There is no evidence of characteristic differences between inbred lines derived from large as opposed to small strains.

TABLE 1

Isoxanthopterin Content in Males of Inbred Lines

Line	μg. per mg. body weight	Line	μg. per mg. body weight
C_6	0.434 ± .039	CL_1	0.366 ± .024
C_9	0.413 ± .014	RL_4	0.312 ± .001
R_2	0.374 ± .027	IL_4	0.690 ± .009
Nb_2	0.327 ± .006	LT_9	0.412 ± .012
OrR	0.412 ± .023		
OrRv	0.404 ± .018	CS_1	0.358 ± .028
		ES_{12}	0.407 ± .021
EL_1 (white-eyed)	0.035 ± .006	IS_2	0.392 ± .018
ES_1 (white-eyed)	0.184 ± .014		

The variance of the determinations on individuals of the same genotype is rather high; the average coefficient of variation is approximately 17%. Since the isoxanthopterin content of each fly is divided by the average of the weighed sample, rather than its own weight, the variance will be compounded of individual variation in size, true variation in relative isoxanthopterin content, and also errors of determination. General experience suggests that the last two sources of variation are likely to be the most important.

The lower values recorded for the pair of white-eyed lines are not unexpected (Hadorn and Mitchell, 1951; Forrest, unpublished), but although the value found in ES_1 is well below the wild-type range, it is nevertheless substantially higher than has been found in other tests on stocks carrying a white-eye allele. It has been shown by Judd (unpublished) that this effect is comparatively independent of genetic background, so we appear to have here a new allele at the white locus, characterized, so far, only by its association with a relatively higher isoxanthopterin content.

(c) *Wild stocks and crosses.* In this test determinations were carried out on both males and females of 6 unrelated wild stocks, 5 of the inbred lines from un-

selected stocks, together with the 10 possible crosses between them. All the cultures were set up together and the chromatograms were run at the same time. The average values for the different genotypes are set out in Table 2. Comparison of the values for males of the 5 lines also studied in the earlier test shows fair agreement, although the values in the second test tend to be a little higher. The biggest discrepancy occurs in the R_2 line, with estimated values of 0.374 ± .027 and 0.519 ± .030 in the first and second tests respectively. The reason for this difference is unknown, except that there are indications that this line may be atypical in that the isoxanthopterin content appears to increase to its maximum level during the first day or so after emergence.

TABLE 2

Isoxanthopterin Content in Wild Stocks, Inbred Lines and Crosses

	μg. per mg. body weight	
	Males	Females
Wild Stocks		
Pobla De Lillet	0.507 ± .027	0.032 ± .004
Stephenville	0.297 ± .015	0.059 ± .002
New Mexico	0.549 ± .012	0.058 ± .005
Pennsylvania	0.480 ± .008	0.037 ± .002
Hawaii	0.485 ± .011	0.057 ± .005
Texas	0.449 ± .010	0.049 ± .003
Average	0.461	0.048
Inbred Lines		
C_6	0.485 ± .011	0.068 ± .004
R_2	0.519 ± .030	0.074 ± .004
Nb_2	0.369 ± .023	0.061 ± .005
OrR	0.447 ± .020	0.062 ± .002
OrRv	0.404 ± .015	0.081 ± .003
Average	0.445	0.069
Crosses		
$C_6 \times R_2$	0.372 ± .021	0.050 ± .002
$C_6 \times Nb_2$	0.410 ± .037	0.057 ± .003
$C_6 \times$ OrR	0.462 ± .031	0.057 ± .004
$C_6 \times$ OrRv	0.392 ± .015	0.056 ± .003
$R_2 \times Nb_2$	0.452 ± .020	0.053 ± .003
$R_2 \times$ OrR	0.464 ± .012	0.056 ± .003
$R_2 \times$ OrRv	0.416 ± .013	0.050 ± .003
$Nb_2 \times$ OrR	0.374 ± .016	0.065 ± .006
$Nb_2 \times$ OrRv	0.306 ± .038	0.073 ± .002
OrR × OrRv	0.433 ± .019	0.083 ± .003
Average	0.408	0.060

The standard error of the difference between means, from pooled variances is 0.032 for males and 0.005 for females. It is evident, therefore, that appreciable differences occur between wild stocks. In males the range is from 0.297 in Stephenville to 0.549 in New Mexico, while in females the lowest and highest values are found in Pobla de Lillet and Stephenville: 0.032 for the former, 0.059 for the latter. The average content per mg., is about 9–10 times as great in males as females. The value quoted for males of Pobla de Lillet is appreciably higher than the value found in the first test relating to the large, small and unselected strains: 0.507 versus 0.441. However, these values are not strictly comparable, since the latter was based on two-dimensional chromatograms which may be expected to afford lower estimates of isoxanthopterin and this probably accounts for the apparent discrepancy.

An interesting feature appears in the lack of correlation between the content in males and females. Thus for the entire set of comparisons—stocks, lines and crosses—the correlation works out at —.237, an insignificant value. It might be argued that it is unfair to include the inbred lines which may be atypical; however, the picture is not altered if they are excluded (r —.276). This lack of agreement between the sexes will be considered later.

Turning now to the crosses between lines, we find a distinct tendency for the content per mg. to be higher in progeny than parents. The differences between F_1 and mid-parent values are listed in Table 3; the average difference in males of 0.037 is clearly significant at the 0.05 level, while in females, the average value of 0.009 falls just below significance.

TABLE 3

Difference in Isoxanthopterin Content between Inbred Lines and Crosses Between the Lines

Cross	Mid-Parent Content—F_1 Content	
	Males	Females
$C_6 \times R_2$	+0.130	+0.021
$C_6 \times Nb_2$	+0.017	+0.007
$C_6 \times OrR$	+0.004	+0.008
$C_6 \times OrRv$	+0.052	+0.018
$R_2 \times Nb_2$	—0.008	+0.014
$R_2 \times OrR$	+0.020	+0.012
$R_2 \times OrRv$	+0.046	+0.028
$Nb_2 \times OrR$	+0.034	—0.004
$Nb_2 \times OrRv$	+0.081	—0.002
$OrR \times OrRv$	—0.008	—0.012
Average	0.037	0.009

Finally, the regression of the content in F_1 on the average value for parent lines may be expected to provide some indication of genetic behaviour. With a fully additive situation, the regression will approximate to unity, while non-additive effects will lower it to varying degree, depending on their nature and relative magnitude. In the present instance the regression for males and females works out at $0.73 \pm .42$ and $-0.25 \pm .75$ respectively. Thus in females there is no evidence of correlation between the concentration in progeny and average concentration in parents. It is improbable that any particular significance can be attached to the negative sign. In males, however, there is evidence of positive association between parent and progeny, although the regression is not statistically significant, probably because of non-additive interactions which occur in the various crosses. It seems reasonable to conclude that non-additive genetic effects are important in the control of isoxanthopterin content.

DISCUSSION

The substantial differences in isoxanthopterin content between wild stocks and various inbred lines indicate that many gene differences are involved and so the level of concentration of this compound can be added to the the list of "quantitative characters" available for experimental study. It follows that standardization of the genetic background is essential for comparison of the effects of different mutant genes.

The number of comparisons between parent lines and their crosses are too few for very definite assertions, but there does appear to be a considerable departure from an additive system. The indication of a relatively more additive situation in males as opposed to females is probably related to their higher concentration of isoxanthopterin. The most interesting aspect of the crosses refers to the tendency for relative content to be lower in the F_1 than in the parents. At first sight this might suggest that the isoxanthopterin content was influenced by inbreeding, being higher in the homozygous lines, relatively lower in the crosses, which, of course, will be more nearly like wild-flies in growth and reproduction (Robertson and Reeve, 1955). It is a little difficult to see why the changes in growth which accompany inbreeding should be reflected in a higher isoxanthopterin content, although it may occur. However, there is an alternative and, at present, more attractive explanation. Recent work by Robertson (unpublished) has indicated that the genetic variation of body size in *Drosophila* may be due to changes in either cell number or size or both and that variation of body size originating in different kinds of genetic change are associated with characteristic changes in one or the other of the primary components of body size. Thus changes in size due to selection are due to changes in cell number, whereas the reduction in size due to inbreeding and its restoration to the average level of wild stocks by outcrossing are associated with corresponding changes in cell size. If isoxanthopterin content is primarily correlated with cell number, and the more or less proportional differences in content in comparisons between large, small and unselected strains support this view, and if the F_1 flies have relatively larger cells than the parents, then we could expect isoxanthopterin per unit body weight to be lower in the crosses. Hence a fuller understanding of the inheritance of this compound in crosses involves an understanding of the behaviour of cell size and number as well; experiments are in progress to throw further light on this problem. In view of the somewhat variable degree of heterosis in crosses between lines, it might be thought that the total content per fly rather than per mg. would provide a better basis for comparing parent and progeny. However, since the regression of F_1 on mid-parent value works out at $0.73 \pm .41$ in males and $-0.68 \pm .79$ in females, the situation remains the same.

From the variation in isoxanthopterin revealed in these tests, it appears that the biosynthetic pathways which lead to the formation of this compound are associated with a variety of equilibria which are reflected in the differences between strains. It would be particularly instructive to know how the other related compounds, which are probably involved in the same system, also vary, since this would help us to understand the significance of variation in isoxanthopterin and probably afford further clues to the characteristic sex difference. It is likely that isoxanthopterin is not confined to the testes in males, but may exist in other tissues at about the same concentration as that found in females, but the behaviour of genetic variation in this respect would be obscured. The genetic variation of the content in the testes may differ in behaviour from variation in other tissues and this may be related to the apparent discrepancy between the sexes in the regression of F_1 on mid-parent level in the crosses between lines, and also to the lack of correlation between males and females.

SUMMARY

1. Isoxanthopterin content has been determined by paper chromatography on a number of wild strains, inbred lines and crosses between lines.

2. Increase or decrease of body size by selection is accompanied by more or less proportional changes in isoxanthopterin and so the comparisons are based on the content per mg. body weight.

3. Substantial differences in isoxanthopterin content exist between wild stocks and inbred lines and many gene differences are probably involved.

4. No correlation has been found between the content in males and females.

5. Crosses between lines suggest that non-additive genetic interactions are important in the control of isoxanthopterin level. This is particularly evident in females, less so in males; the apparent sex difference in this respect is probably related to the much higher concentration in males.

6. Content per unit weight tends to be lower in the F_1 of crosses than in the parent lines and it is suggested that this may be due to the relatively larger cells in such crosses, compared with the homozygous lines.

7. An allele of white-eye has been identified which is responsible for a higher isoxanthopterin content than has been previously found for other alleles which at first sight appear to be phenotypically identical.

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XX. The Production of Attached-X Chromosomes in *Drosophila hydei*

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INTRODUCTION

The first known case of attached-X chromosomes in *Drosophila* was discovered in *Drosophila melanogaster* by L. V. Morgan (1922). Later, E. G. Anderson (1925) succeeded in producing an attached-X female which was heterozygous for a number of recessive mutants. This enabled him to study crossing-over in attached-X chromosomes. From this study he determined that crossing-over in attached-X chromosomes occurred with the same frequency as crossing-over in free X chromosomes, and furthermore, that crossing-over takes place in the four strand stage with only two chromatids involved at any level. He also showed that any single cross-over involves two homologous chromatids at random, regardless of which chromatids were involved at a different level.

These results of Anderson were substantiated by Sturtevant (1931), Emerson and Beadle (1933), and Beadle and Emerson (1935). In addition, Beadle and Emerson were able to show that two and four strand double exchanges occurred with approximately equal frequencies, as did two and three strand double exchanges.

Anderson also noted that the frequency with which the heterozygous recessives became homozygous, as the result of non-reciprocal cross-overs, was a function of the distance of each mutation from the spindle attachment, so that the mutations more distant from the attachment appeared homozygous more frequently. Markers approximately 40 units from the centromere became homozygous with a frequency approaching 16.7 per cent. This is the frequency of homozygosis that would be expected if the chromatids at a given locus were assorted at random during meiosis.

L. V. Morgan (1925), M. M. Rhodes(1930) and Beadle and Emerson (1935), however, have data to show that the frequency of homozygosis for mutations near the distal end of the X chromosome is definitely greater than the 16.7 per cent expected on a basis of random assortment of genes. Some of their values are as high as 20.3 per cent. Thus it seems that homozygosis does not increase in a regular fashion with 16.7 per cent as a limit, as Anderson had assumed. Since homozygosis values greater than 16.7 per cent were observed it was concluded that sister strand crossing-over does not occur, or is not equivalent in interference to regular crossing-over, as stated by Beadle and Emerson (1935). The latter workers also showed that there is a discrepancy between actual homozygosis frequencies and expected frequencies, calculated on the assumption that sister strand crossing-over does not occur.

In the present experiment it seemed theoretically possible to produce attached-X chromosomes in a second species of *Drosophila*, *Drosophila hydei*, since one arm of the large metacentric X chromosome is entirely heterochromatic. Attached-X chromosomes in *hydei* should prove very interesting for two reasons.

First, an attached-X has never been found in any species of *Drosophila* other than *melanogaster*. Secondly, crossing-over in *hydei* is over twice as frequent as it is in *melanogaster*. The linkage map of the *hydei* X chromosome is 116 units long, while that of *melanogaster* is only 66 units long. Thus the comparison between the actual and expected frequency of homozygosis could be worked on more profitably in *hydei* attached-X chromosomes.

Welshons (1955) showed that when double crossing-over occurs in a short region of attached-X chromosomes, the types of double cross-overs recovered do not occur in the ratio expected. He finds that this is not inconsistent with the sister strand cross-over theory of Schwartz. More information on this point might be gained in properly marked *hydei* attached-X chromosomes, since double crossing-over within a short region would be more frequent in *hydei* than in *melanogaster*.

Attached-X chromosomes in *hydei* would, therefore, be interesting from the standpoint of comparing crossing-over in the attached-X chromosomes of the two species, and especially interesting from the standpoint of gaining more information about sister strand crossing-over and the frequency of homozygosis of recessive mutations. In addition, the attached-X chromosomes are useful tools in genetic research on other problems.

PROCEDURE AND RESULTS

To begin the experiment, sixty virgin females, homozygous *white*, less than 72 hours old, were subjected to X-ray treatment. The total dose given was 3000 r at the rate of 112 r per minute. The flies were exposed for a total of 26.8 minutes. A beaker suspended in ice water maintained their temperature between 13 and 17 degrees centigrade. During treatment, the flies were contained in celluloid capsules on the bottom of the suspended beaker.

Twenty-four hours after treatment, the females were mass mated to wild type males. The age of the males varied from one to seven days. The flies were changed to fresh food each day so that any X-ray sensitive period in meiosis, as measured by non-disjunction, might be detected, if a large number of non-disjunction offspring were produced from the eggs laid on any particular day or group of days. Larval competition was held at a minimum by spreading eggs and larvae from overcrowded vials on to fresh food. The offspring from the 60 females are summarized in Table 1.

Each non-disjunction male was individually mated to females homozygous for *vermilion*, *scute*, *yellow*, *miniature*, *cherry* and *bobbed*. All such males proved to be sterile as expected, since primary non-disjunctional males are usually XO. The super females were short-lived and inviable.

Subsequent to the treatment of the 60 females just mentioned, 300 *white* females were given the same treatment except that the temperature was held lower, varying from seven degrees centigrade at the start of irradiation, to 13 degrees centigrade at the end of the irradiation period. The same mating procedure was followed for these females as for the first group. The offspring from the 300 females are summarized in Table 2.

Non-disjunction males were again mated to mutant females and changed to fresh food several times. This time 49 of the males bred as normal wild type

TABLE 1

OFFSPRING FROM SIXTY WHITE-EYED IRRADIATED FEMALES

Type of Offspring	Days 1	2	3	4 and 5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totals
Regular ♀	16	63	15	584	283	231	367	403	344	300	374	475	434	366	95	58	67	4375
Regular ♂	30	67	21	571	348	339	405	491	448	256	492	275	408	174	18	95	64	4502
Non-disjunction ♀	0	1 super ♀	0	1 super ♀	0	0	0	0	1 super ♀	0	0	0	0	0	0	0	0	3 super ♀ ♀
Non-disjunction ♂	1	3	2	2	0	6	3	3	0	0	1	3	0	0	0	0	0	22

TABLE 2

OFFSPRING FROM 300 WHITE-EYED IRRADIATED FEMALES

Type of Offspring	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Totals
Regular ♀	0	0	0	0	33	438	851	1926	1948	1330	1255	2731	1594	2297	2283	16686
Regular ♂	0	0	0	0	40	499	891	1546	1264	1472	917	2531	1809	2371	2397	15737
Non-disjunction ♀ (white-eyed)	0	0	0	0	0	1	1	2	3	0	1	0	0	0	0	8
Non-disjunction ♂ (wild type)	0	0	0	0	0	11	13	37	17	19	8	13	3	6	11	138
													Total Offspring			32569

males, thus giving mutant males and wild type females in the F_1 generation. The remaining 89 males were sterile. This strongly indicates that the 49 normal males resulted from a contamination, while the remaining 89 were actually non-disjunction males.

Even with this slight distortion in Table 2, it appears possible that a peak of non-disjunction occurs in eggs laid seven to nine days after X-ray treatment. This possibility is supported by the data in Table 1, although the number of flies in Table 1 is not large.

Non-disjunction females were individually mated to wild type males. Six of the females produced white-eyed sons and wild type daughters, and so were discarded as not being attached-X. The other two, however, continued to produce non-disjunctional offspring; *i.e.*, white-eyed females and wild type males, and proved to be of attached-X constitution. An attached-X stock was started from each of these females.

To check for complications, the salivary gland chromosomes of six F_2 larvae from each original attached-X female were analyzed for inversions, deletions or other chromosomal aberrations that might have been produced concurrently with the attched-X. No such complications were evident, and the normal *hydei* salivary gland chromosome configuration of five arms extending from the chromocenter was present in all cases.

The metaphase configuration of the chromosomes in the males from the attached-X stocks was four pairs of rods, a pair of dots, and a large V-shaped X chromosome, paired with a J-shaped Y chromosome. This is the normal *hydei* male configuration. In the attached-X females, the chromosome configuration is exactly the same as it is in normal *hydei* males. The normal *hydei* female configuration is four pairs of rods, a pair of dots and a large pair of V's. Thus it appears that the heterochromatic arms were lost from the egg which produced each attached-X female.

As a further investigation into the normality of the attached-X stocks, the sex ratios were checked and egg counts were made, in order to determine the per cent emergence. It was also hoped that some idea of the rate of detachment of the attached-X could be gained. This was done in the following way. (The attached-X stocks have been arbitrarily numbered as 1 and 2 so that they can be referred to more easily.)

Ten four- to six-day-old attached-X females from stock 1 were mated individually to wild type males. The flies were changed to fresh food vials daily, the number of eggs laid in each vial was counted, the pupae were counted, and the emerging flies were counted and classified. These data are shown in Table 3. Four of the females produced no offspring and are omitted from the table.

In none of the females which produced offspring was there a significant deviation from the 1:1 ratio of males to females. (Whether or not a given deviation was significant was determined by applying the chi-square test with one degree of freedom.) Likewise, there is no significant deviation from the 1:1 ratio when all of the offspring from the females in Table 1 are totaled.

Of the eggs laid, 31.7 per cent developed into adults. The most eggs that could be expected to develop into adults would be 50 per cent, since YY zygotes are lethal and XXX super females rarely develop beyond the pupal stage. That only

TABLE 3

Fertility and sex ratio checks in attached-X females from stock 1

	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totals
Female A																			
Eggs							44	93	95	63	102	92	95	0	54	0	28	0	666
Pupae							16	62	40	36	60	56	50	0	7	0	0	0	327
White eyed ♀							9	24	18	20	27	19	19	0	1	0	0	0	137
Wild type ♂							5	29	17	10	25	15	8	0	1	0	0	0	110
Other							0	2 super ♀	0	0	0	0	0	0	0	0	0	0	2
Female B																			
Eggs							113	109	57	84	78	0	200	122	48	51	56	132	1050
Pupae							50	35	14	32	50	0	60	53	20	28	40	56	438
White eyed ♀							27	14	5	16	14	0	16	14	12	8	12	21	159
Wild type ♂							17	13	4	12	27	0	24	23	5	7	14	19	164
Other							0	0	0	0	0	0	1+/+ ♀	0	0	0	0	0	1
Female C																			
Eggs							92	143	62	79	62	74	82	95	26	40	21	80	856
Pupae							38	56	36	45	40	30	45	60	22	28	17	48	465
White eyed ♀							17	27	15	21	17	13	13	26	9	7	5	18	183
Wild type ♂							18	18	18	16	20	9	12	20	5	10	8	17	171
Other							0	0	0	0	0	0	0	0	0	0	0	0	0
Female D																			
Eggs							111	121	69	54	80	83	72	128	16	0	96	60	810
Pupae							16	42	26	17	40	25	24	40	0	0	0	0	230
White eyed ♀							2	15	7	8	8	9	9	11	1	0	0	0	69
Wild type ♂							10	12	13	8	14	5	9	15	0	0	0	0	86
Other							0	0	0	0	0	0	0	0	0	0	0	0	0
Female E																			
Eggs							99	0	108	114	101	99	62	106	48	63	82	61	943
Pupae							30	0	46	48	50	40	28	52	33	38	48	37	450
White eyed ♀			16	29	12	24	10	0	16	29	25	13	15	21	8	14	12	13	257
Wild type ♂			15	25	8	19	10	0	11	10	24	13	8	15	9	12	10	14	203
Other							0	0	0	0	0	0	0	0	0	0	0	0	0
Female F																			
Eggs							108	26	95	87	100	54	84	110	41	34	47	144	930
Pupae							43	5	29	33	60	28	50	56	26	23	30	55	438
White eyed ♀				16	19	11	15	1	9	10	30	12	15	11	7	9	9	16	190
Wild type ♂				16	18	14	20	2	14	19	22	4	15	19	9	5	11	18	206
Other							3 super ♀	0	1 super ♀	0	0	0	0	0	0	0	0	0	4 super ♀ ♀

Grand Totals:

Eggs	5335
Pupae	2348
White eyed ♀	995
Wild type ♂	940
Super ♀	6
Wild type ♀	1

31.7 per cent of the eggs developed probably indicates the presence of some lethal genes in the stock.

Among the 1,935 total flies in Table 3, one apparently normal wild type female was produced by female B. This female may have resulted from a detachment of the attached-X chromosomes, but since she died within a few days without offspring, it is impossible to be certain of her origin.

Stock number 2 was tested in the same way as stock 1. The data are shown in Table 4. Only eight females were mated this time. One produced no offspring and is omitted from the table.

Female G showed a significant deviation from the 1:1 ratio of males to females. Unfortunately, the presence of this deviation was not realized until after the offspring had been discarded.

TABLE 4

Fertility and sex ratio checks in attached-X females from Stock 2

		Days												
		7	8	9	10	11	12	13	14	15	16	17	18	Totals
Female A	Eggs	0	0	0	70	170	84	44	0	0	0	0	0	368
	Pupae	0	0	0	34	46	45	28	0	0	0	0	0	150
	White eyed ♀	0	0	0	14	20	15	11	0	0	0	0	0	60
	Wild type ♂	0	0	0	13	15	24	12	0	0	0	0	0	64
	Other	0	0	0	1 super ♀	0	0	0	0	0	0	0	0	1 super ♀
Female B	Eggs	0	47	0	74	0	112	36	68	0	0	46	40	423
	Pupae	0	0	0	40	0	25	0	0	0	0	0	0	65
	White eyed ♀	0	0	0	18	0	10	0	0	0	0	0	0	28
	Wild type ♂	0	0	0	17	0	8	0	0	0	0	0	0	25
	Other	0	0	0	0	0	0	0	0	0	0	0	0	0
Female C	Eggs	24	88	113	85	114	71	72	150	50	36	56	128	987
	Pupae	20	30	36	53	65	26	30	55	20	30	34	58	451
	White eyed ♀	7	16	14	13	23	6	11	22	8	15	18	19	172
	Wild type ♂	10	11	13	21	24	14	8	18	7	13	13	15	169
	Other	0	0	0	0	0	0	0	0	0	0	0	0	0
Female D	Eggs	49	83	118	140	82	87	92	108	33	60	65	80	997
	Pupae	0	39	48	50	50	46	18	52	20	34	44	42	443
	White eyed ♀	0	22	10	21	18	17	8	17	9	15	17	18	171
	Wild type ♂	0	8	20	19	15	15	5	3	7	7	17	14	160
	Other	0	0	0	0	0	0	0	0	0	0	0	0	0
Female E	Eggs	42	0	69	68	90	0	79	147	33	52	73	38	691
	Pupae	0	0	34	30	60	0	45	60	30	30	32	30	351
	White eyed ♀	0	0	16	10	24	0	13	21	14	11	23	9	141
	Wild type ♂	0	0	14	11	25	0	19	23	11	11	14	6	134
	Other	0	0	0	0	0	0	0	0	0	0	0	0	0
Female F	Eggs	109	96	106	46	97	102	93	0	0	0	0	0	649
	Pupae	38	36	37	45	58	45	45	0	0	0	0	0	304
	White eyed ♀	11	20	16	18	17	12	11	0	0	0	0	0	105
	Wild type ♂	12	8	13	21	27	16	13	0	0	0	0	0	110
	Other	0	0	0	0	0	0	0	0	0	0	0	0	0
Female G	Eggs	102	107	72	44	60	88	84	120	21	52	44	96	890
	Pupae	0	34	35	36	45	43	44	46	14	35	29	50	411
	White eyed ♀	0	14	15	16	28	18	16	17	7	15	12	18	176
	Wild type ♂	0	7	15	6	10	5	6	15	4	11	14	15	108
	Other	0	1 w ♂	0	0	0	0	0	0	0	0	0	0	1

Grand totals	
Eggs	5005
Pupae	2175
White ♀	853
Wild ♂	770
Super ♀	1
White ♂	1

Deviations shown by the other six females individually, and by all seven collectively, were not significant. Of the 5,005 eggs laid, 32.4 per cent developed into adults, again indicating the probability of some lethal genes in the stock. One white-eyed male was produced by female G. Although the male died within a few days, he probably originated from a detachment of the attached-X chromosomes.

The fact that four stock 1 females and one stock 2 female gave no offspring is not considered at all significant since individual matings in *hydei* are often incompatible.

Detachment of the attached-X in either stock is at best a rare occurrence. To check this further, 1,079 flies from stock 2 were examined for detachments. One wild type female was found. This female was slightly rough-eyed and sterile, which indicates that she was probably a super female. Of 1,137 flies from stock 1, none showed any possibility of having originated from a detachment.

Since two stocks of attached-X were obtained by irradiation of white-eyed females, an attempt was made to produce an attached-X female heterozygous for a number of markers, suitable for cross-over studies. To do this the necessary mutant stocks were established and females, homozygous *vermilion* but heterozygous for several other mutations, less than 60 hours old were given 3,250 r at the rate of 740 r per minute. This treatment was given to several groups of females at both room temperature and lower temperatures but no cases of attached-X were detected.

It is interesting to compare the X chromosome non-disjunction rate in this experiment with the spontaneous non-disjunction rate in the X chromosome of *hydei*, studied by Spencer (1930). The rate of non-disjunction in irradiated females is considerably higher than in the untreated females studied by Spencer. However this higher rate results in fewer nondisjunction female offspring from the irradiated parent, presumbably due to the tendency of tangled chromosomes to be lost in the polar bodies in meiosis.

ACKNOWLEDGMENTS

The writer wishes to express thanks to Prof. W. S. Stone for advice and consultation during the course of the experiment, to Dr. L. E. Mettler for analyzing the salivary gland chromosomes, and to Prof. W. P. Spencer for providing all the *Drosophila hydei* stocks.

SUMMARY

White-eyed *hydei* females were subjected to 3,000 r of X-radiation. Among the offspring of the irradiated females, two attached X females were recovered.

Analysis of the salivary chromosomes of F_2 larvae from each attached-X female showed that no chromosome abnormalities were present other than the attached-X. The metaphase chromosome configuration of females from the attached-X stocks is the same as the metaphase configuration of a normal male *hydei*. Detachment of the attached-X chromsomes is a rare occurrence.

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XXI. Effects of Irradiation on the Screw-Worm, *Callitroga hominivorax* (Coq.)

GAIL KAUFMAN AND MARVIN WASSERMAN

Since the eradication of the screw-worm, *Callitroga hominivorax*, from the island of Curaçao by Baumhover and co-workers in 1954 (Bushland, 1955), workers in the fields of entomology and genetics have been studying the effects of irradiation on these flies with the intention of carrying out a project which would eradicate the screw-worm population from Florida. The work we have done in these laboratories, in conjunction with the U. S. Department of Agriculture's Bureau of Entomology and Plant Quarantine Laboratory at Kerrville, Texas, was performed in an effort to determine the following: 1) whether irradiated males would inseminate as many females as nonirradiated males when both were presented with a maximum number of virgin females; 2) whether there was any recovery of fertility in the irradiated males after they had used up the irradiated sperm; 3) whether there were any viable offspring from matings between irradiated males and non-irradiated females; 4) if spermatogenesis in irradiated males differed from spermatogenesis in nonirradiated, or normal, males; and, 5) what cytological effects the irradiation had on the cells in the testes. We wish to thank Dr. R. C. Bushland and his colleagues for supplying us with the flies and pupae which were necessary for these experiments and for observing the egg masses laid by the females and the subsequent egg hatches. We also wish to thank Dr. Forbes Robertson for advising us on the best statistical analysis to use on the data compiled, and Dr. Wilson S. Stone for his help and advice throughout the experiments and for reading and criticizing the manuscript.

METHODS

Half a population of five-day old *C. hominivorax* pupae were irradiated in air with 7500r at 250 KV, with a ½mmCu-1mmA1 filter, from a Westinghouse X-ray machine. The irradiation rate was approximately 750r/minute. These pupae were taken to Kerrville and reared at the USDA laboratory there. Upon emergence from the pupae cases, 100 of the irradiated males were numbered and each was put into a carton with 15 non-irradiated, or normal, virgin females from the same population. At the same time, 100 normal males from that population were also numbered and each put into a carton with 15 normal virgin females. At the end of one week, 10 cartons containing irradiated males and their females and 10 cartons containing normal males and females were brought to The University of Texas laboratory and the spermathecae of the females were checked for sperm content. At Kerrville, the remaining males of both groups were put into new cartons, each again with 15 normal virgin females, and the females which had been exposed to these males previously were allowed to lay their eggs. The egg masses were observed and a count was made of the subsequent egg hatches. At the end of the second and third weeks, the same process

was repeated. Three experiments were run in this manner, each one lasting for three weeks only, since most adults live for only three weeks after emergence from the pupae cases.

For the cytological studies of the irradiated male testes, 5-day old pupae were irradiated as described above. Starting with one-half hour after irradiation, the testes were dissected out in saline solution, fixed and stained with aceto-orcien, and squashed. Slides were made every half hour for three hours and then every hour up to 15 hours after irradiation. At 28 hours after irradiation, slides were again made. Some pupae were also given 1160r at 210KV, at a rate of 340r/minute, with the same filter, in order to see what effects a lower dosage had on the testes. Slides of the normal testes were prepared in the same manner as slides of the irradiated testes. For the comparative studies of the testes themselves, we attempted to keep the organs as nearly intact as possible and did not squash them, although they were fixed and stained as were those used in the cytological studies.

EFFECTS OF IRRADIATION ON INSEMINATION RATE

Results and Discussion

The data for the three insemination studies are presented in Tables 1, 2, and 3. The males are identified by their numbers, and the number of virgin and number and per cent of inseminated females is given for each male. We generally received a random sample of the population here, with little effort being made to get ten consecutively numbered males. For each week the per cent of females inseminated was calculated for each individual male and the over-all percentage of inseminated females for that particular group was also calculated. In some cases there were one or two dead females in the cartons and these were not checked for sperm content, since we had no way of knowing how long they had been dead. When there were either two males or a dead male in the carton, none of the females were checked.

It is obvious from a cursory glance at the data that the three populations studied differed from one another, and at times quite significantly so. Also, within each population and within each week's lot of males, there was a great deal of variation among the individual males of both types in insemination activity.

The flies in the first population, experiment 1, did not survive as well as flies of the second and third groups. The irradiated males began dying in large numbers in the second week, and by the third week there were large numbers of males dead in both the irradiated and normal groups.

In the first week of experiment 2, the flies were brought to our laboratory several days in advance of the end of the week and many of the males and females died here in the lab, the greatest percentage of deaths being in the irradiated males. Whether it was the change in environment or a shortage of water, we do not know with certainty, but the high mortality and poor condition of the remaining flies unfortunately skewed our results in this particular phase of the experiments.

In experiment 3, all irradiated males were dead by the end of the third week.

There are several reasons for high numbers of early deaths: a change in the environment, scarcity of food and water, and parasites. Throughout the experi-

TABLE 1

Data from insemination studies, Experiment 1.

	Normal ♂♂	Inseminated ♀♀	Virgin ♀♀	% Insem. ♀♀	Irradiated ♂♂	Inseminated ♀♀	Virgin ♀♀	% Insem. ♀♀
	No. 3	5	9	35.7	No. 3	5	10	33.3
	6	5	9	35.7	6	7	8	46.6
	8	8	6	57.1	10	4	9	30.7
1st	10	(2♂)*	..	...	11	6	8	42.8
week	11	5	10	33.3	16	4	11	26.6
	15	6	9	40.0	21	7	7	50.0
	17	3	12	20.0	22	4	11	26.6
	20	1	14	6.6	24	4	11	26.6
	22	7	8	46.6	26	4	11	26.6
	24	8	6	57.1	27	6	9	40.0
		48	83	Av. 36.6		51	95	Av. 34.9
	4	5	10	33.3	8	5	9	35.6
	13	6	9	40.0	9	2	13	13.3
	23	4	11	26.6	12	0	14	0.0
	23	4	11	26.6	12	0	14	0.0
2nd	33	7	7	50.0	13	D		
week	34	4	10	28.5	18	(3♂)*	..	...
	37	1	12	7.6	36	D	..	...
	38	2	13	13.3	37	D	..	...
	39	2	13	13.3	38	D	..	...
	40	5	9	35.6	44	D	..	...
	48	5	10	33.3	53	D	..	...
	50	6	8	42.8	60	D	..	...
		47	112	Av. 29.5		7	36	Av. 16.2
	74	D	..	...	48	2	13	13.3
	71	D	..	...	74	D		
	35	D	..	...	76	0	15	0.0
3rd	51	0	15	0.0	77	0	15	0.0
week	57	0	13	0.0	82	D	..	...
	65	D	..	...	91	D	..	...
	87	D	..	...	94	D		...
	79	D	..	...	96	D	..	...
	97	0	15	0.0	78	D	..	...
	88	2	12	14.3		2	43	Av. 4.4
	84	D	..	...				
	92	D	..	...				
	93	D	..	...				
		2	55	Av. 3.5				

D = no test; dead male.
* = no test; males in carton.

ments, from time to time there would be an outbreak of parasites at the hatchery in Kerrville, and it is quite possible that these parasites were responsible for some of the mortalities and the general poor condition of many of the adults. It is perhaps significant, however, that those flies which were more quickly affected by adverse conditions and which died more quickly and in greater numbers were the irradiated males.

For a statistical comparison of the effectiveness of the irradiated males against the normal males in the number of females inseminated, we ran chi square tests on our data. The results are presented in Table 4. There were three tests which showed significant differences between the insemination rates of the two sets of males—the first and third weeks of experiment 2 and the second week of ex-

periment 3. The first week of experiment 2 may, due to the high mortality in both groups, be ignored. Also, the data for the third week of the same experiment are based on a small sample of irradiated flies, as compared to the sample of the normal males, which, because of the great degree of individual variation in these animals, may account for some of the difference. For the significance in the chi square value in the second week of experiment 3 we have no explanation other than the fact that the irradiated males died much more rapidly in this experiment.

It is significant, we believe, that the chi square values are the smallest, and the probability range the highest, in the first week of experiments 1 and 3. This would probably be the critical week in competition between released irradiated flies and the normal flies in nature. It is also the week when the insemination activity of the normal flies is the highest. Although we found that during this week the irradiated males could inseminate as many females as the normal males

TABLE 2

Data from insemination studies, Experiment 2.

	Normal ♂♂	Inseminated ♀♀	Virgin ♀♀	% Insem. ♀♀	Irradiated ♂♂	Inseminated ♀♀	Virgin ♀♀	% Insem. ♀♀
	No. 10	D	..	...	No. 2	2	13	13.3
	12	0	14	0.0	3	0	15	0.0
	15	6	9	40.0	4	D	..	...
1st	19	3	4	42.9	5	0	14	0.0
week	20	5	8	38.5	8	4	10	28.5
	21	7	7	50.0	10	D	..	...
	27	7	7	50.0	11	D	..	...
	28	6	9	40.0	13	2	13	13.3
	33	6	8	42.8	14	0	11	0.0
	34	3	11	21.4	15	D	..	...
	36	7	8	46.7	16	D	..	...
	37	D	..	...	18	D	..	...
		50	85	Av. 37.0		8	76	Av. 9.5
	6	8	5	53.8	17	7	8	46.7
	8	6	7	46.1	19	4	10	28.6
	9	3	12	20.0	21	5	9	35.7
2nd	11	9	5	64.3	24	5	10	33.3
week	13	6	9	40.0	27	7	8	46.7
	14	7	8	46.7	28	7	7	50.0
	16	8	5	61.5	29	6	9	40.0
	17	6	8	42.8	31	3	13	18.8
	18	6	9	40.0	34	7	8	46.7
	25	0	15	0.0	38	3	11	21.4
		59	83	Av. 41.5		54	93	Av. 36.7
	3	7	8	46.7	57	2	9	18.2
	5	5	9	35.7	64	1	14	6.7
	23	5	10	33.3	65	2	13	13.3
3rd	30	5	10	33.3	69	4	10	28.6
week	44	0	14	0.0	75	0	15	0.0
	53	0	13	0.0	86	4	11	26.7
	58	3	10	23.0		13	72	Av. 15.3
	61	5	10	33.3				
	67	5	8	38.5				
		35	92	Av. 28.0				

D = no test; dead male.

TABLE 3

Date from insemination studies, Experiment 3.

	Normal ♂♂	Inseminated ♀♀	Virgin ♀♀	% Insem. ♀♀	Irradiated ♂♂	Inseminated ♀♀	Virgin ♀♀	% Insem. ♀♀
	No. 1	10	5	66.7	No. 1	9	3	75.0
	2	6	6	50.0	2	8	4	55.7
	3	4	8	33.3	3	8	5	61.5
1st	4	10	3	76.9	4	7	6	53.8
week	5	7	7	50.0	5	7	6	53.8
	6	3	10	23.0	6	9	4	69.2
	7	7	5	58.3	7	11	4	73.4
	8	5	7	41.7	8	4	8	33.3
	9	12	1	92.3	9	3	10	23.0
	10	8	4	66.6	10	7	5	58.3
		72	56	Av. 56.2		73	55	Av. 57.0
	11	6	9	40.0	12	3	12	20.0
	12	0	12	0.0	14	2	12	14.3
	15	4	10	28.6	16	1	10	9.1
2nd	16	6	9	40.0	17	0	13	0.0
week	17	9	6	60.0	19	2	11	15.4
	18	7	7	50.0	22	4	10	28.6
	20	9	6	60.0	23	0	14	0.0
	24	8	7	53.3	25	2	12	14.3
	25	8	7	53.3	26	3	12	20.0
	26	4	10	28.5	27	3	11	21.4
		61	83	Av. 42.3		20	117	Av. 15.7
	27	2	12	14.2				
	28	2	12	14.2				
	32	5	9	35.7				
3rd	42	9	4	69.2				
week	50	1	12	8.3	All males dead			
	54	3	11	27.2				
	69	6	9	40.0				
	83	0	12	0.0				
	85	0	14	0.0				
	86	3	11	27.2				
		31	106	Av. 29.2				

TABLE 4

Comparison of Insemination Rate of Normal Males Against Irradiated Males Using Chi Square Test

Week of Experiment	Experiment 1 Chi Square	Experiment 1 Probability	Experiment 2 Chi Square	Experiment 2 Probability	Experiment 3 Chi Square	Experiment 3 Probability
1	0.05	0.90>P>0.75	12.39	0.005>P	0.177	0.75 >P>0.50
2	3.03	0.10>P>0.05	0.69	0.50 >P>0.25	25.36	0.005>P
3	0.63	0.50>P>0.25	4.38	0.05 >P>0.02	.....	

when they were isolated with a maximum number of females in individual cartons, our data do not show what the insemination rate of the two groups would be in a situation where both types of males were exposed to the same females at the same time. (For further information on this aspect, see Bushland, 1951.)

The decline in activity of both types of males during the second and third weeks, as they got progressively older, can best be appreciated by the graph in Figure 1. Here we have taken, for experiments 1 and 3, the insemination rate of

the normal males in the first week of each experiment and let this represent 100 per cent efficiency for that experiment. It is obvious from this graph that during the first week the efficiency of the irradiated males in insemination is favorably comparable to that of the normal males. Both groups in both populations decline in per cent of females inseminated in the second and third weeks, with the irradiated males showing the greatest decline. In the third week of experiment 1, the per cent of females inseminated by the normal males shows an unexpectedly steep drop. This could well be a reflection of the extremely high mortality of the three-week old flies in this particular population, indicating that possibly those males which were still alive, when we checked for sperm in the females, were in

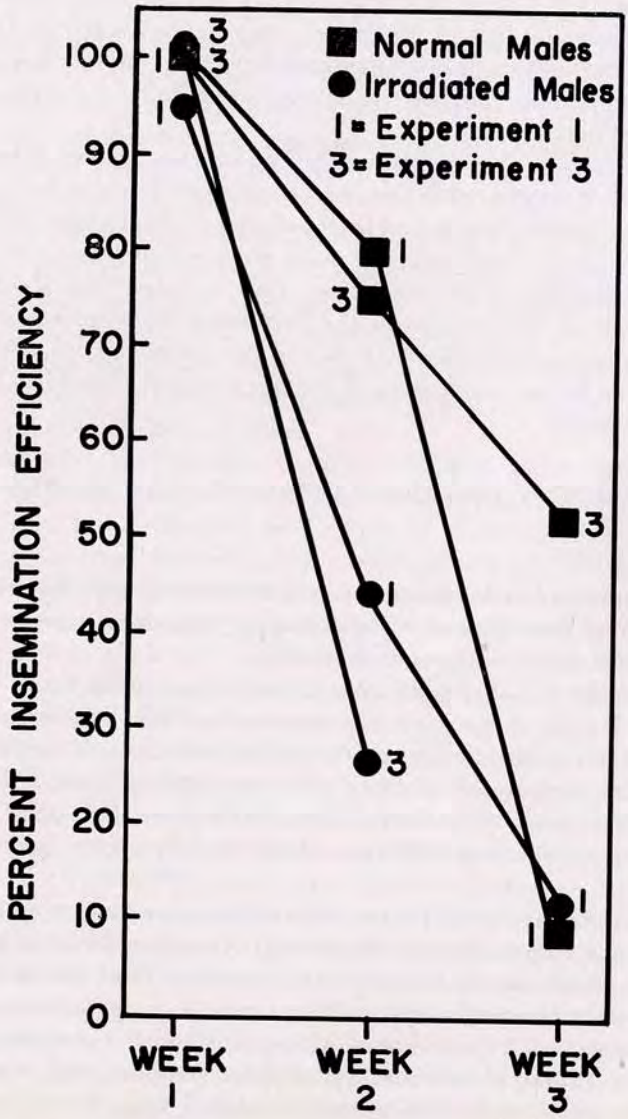

FIG. 1. A comparison of efficiency of insemination between normal and irradiated males.

poor condition. We have no data for the insemination rate of the irradiated males for the third week of experiment 3, since those males were all dead when we received the cartons in the laboratory.

At Kerrville, the egg masses laid each week by the females, and the subsequent egg hatches, were counted. Because several of the females from a particular male laid their eggs in the same cage and quite often one on top of another, the counts were often approximations. Since even virgin females lay, theoretically, a full complement of eggs, we would have expected the percentage of egg masses hatching to follow fairly closely the percentages of females inseminated, for the percentage of egg hatches was figured on the number of egg masses which hatched divided by the total number of females in the cage. There was a discrepancy, however, between our figures for the per cent of females inseminated by normal males and the Kerrville figures for the per cent egg hatch for the normal populations studied. Part of this discrepancy may have been due to the gregariousness of the egg-laying females and the consequent necessitated approximations in the egg mass and egg hatch counts.

The most significant observation on the egg hatches was with females which had been mated to irradiated males. Although these females laid as many egg masses as did the females which had been mated to normal males, no eggs hatched from their egg masses. The irradiated males were completely sterile and showed no recovery of fertility after irradiation. This, coupled with the fact that the insemination rate of these males in the first week after emergence from their pupae cases compares favorably with that of the normal males, adds to the likelihood of possible future eradication of the screw-worm from Florida and Southeastern United States.

EFFECTS OF IRRADIATION ON THE TESTES

Results and Discussion

We have compared the normal testes with the testes of the irradiated flies. There is variation from fly to fly The following account is a general description of the sequence of events in the aging of the flies.

An examination of 5-day old pupae revealed two ovoid testes less than one millimeter in length. Approximately one-tenth of the testis was composed of spermatogonial cells, four-tenths spermatocytes, and one-half sperm and spermatids. During the next two days there was very little growth. A layer of tissue composed of binucleate cells formed and surrounded the testis. This layer of cells persisted throughout the life span of the flies. The testes had begun to turn golden yellow.

Examination of normal adults, ten days after emergence, revealed some spermatogonial cysts. Approximately one-fourth of each testis contained spermatocytes. Sperm and spermatids filled up the remainder. The irradiated testes at this age had much more pigment, were dark orange in appearance, and were elongated rather than ovoid. Only sperm were present in the irradiated testes.

The testes of 17-day old normal males were elongated and orangish, looking somewhat like those of the 10-day old irradiated flies. There were still some meiotic cells, although most of the testis was filled with sperm. The irradiated

males had very elongated, thin, almost collapsed testes, which were highly pigmented. Very few sperm were present.

Normal males at 24 days were found to have some meiotic cells present in the testes. There was essentially no change from the 17-day old males, except that the testes were beginning to empty. Some sperm were still observed in the testes of the irradiated flies at this time.

EFFECTS OF IRRADIATION ON SPERMATOGENESIS

Results and Discussion

The normal karyotype of the male screw-worm is five pairs of autosomes and the X and Y chromosomes. The X is an acrocentric with a large terminal satellite. The Y is a short rod. One of the pairs of autosomes is a metacentric with arms of equal length. Three other pairs are metacentrics with unequal length arms. The fifth pair of autosomes is J-shaped, having one of the arms very short. Figure 2 shows an idiogram of the haploid set. Both the X and the Y are included. Figure 3 shows a metaphase of a spermatogonial cell. A metaphase plate of the female, taken from a brain cell, is shown in Figure 4. The X and Y are indicated in each camera lucida drawing.

In the meiotic divisions a definite equatorial plate was observed in metaphase. In the first division the sex bivalent was seen in the center of the equatorial plate, surrounded by the autosomes. The X and Y were oriented toward the poles, attached by their terminal ends. No chiasmata were seen. The first division was reductional for the sex chromosomes. Figure 5 is a camera lucida drawing of a first division metaphase. An early anaphase is shown in Figure 6. The X and the Y appeared negatively heteropycnotic.

The chromosomes were somewhat smaller in the second meiotic division. One could usually differentiate between those cells with the X chromosome (Figure 7) and those with the Y chromosome (Figure 8) in the second metaphase.

From the pupae irradiated with 7500r, a series of slides was made beginning with one-half hour after irradiation and continuing until 15 hours after. Several slides were made 13 hours after this to see if there were any marked changes in cytological effects with a longer period of time postirradiation. We checked over 1350 cells in anaphase, telophase and daughter cell stage and we also checked about the same number of metaphase plates.

After a dose of 7500r, there were no normal divisions observed in either meiosis I or II. All of the cells seen from the anaphase stage on had at least one chromosome bridge, and many had from 2–4 distinct bridges. The higher number of bridges was generally more evident in middle and late anaphase. In early telophase, quite often bridges were seen which appeared to have broken immediately before, or perhaps, upon fixation (see Figure 9). Camera lucida drawings of several types of bridges are shown in Figures 9–13. In Figure 10, a typical anaphase cell, it will be noted that there is no clear line of demarcation between the chromosomes towards the poles and throughout part of the bridge area. The vast majority of anaphase cells, in both meiosis I and II, exhibited this characteristic, which we attribute to the phenomenon termed "stickiness" by various authors (Sax and Swanson, 1941; Carlson, 1954). At no time did we see an acentric fragment in the 1350 cells that we studied. This does not, however,

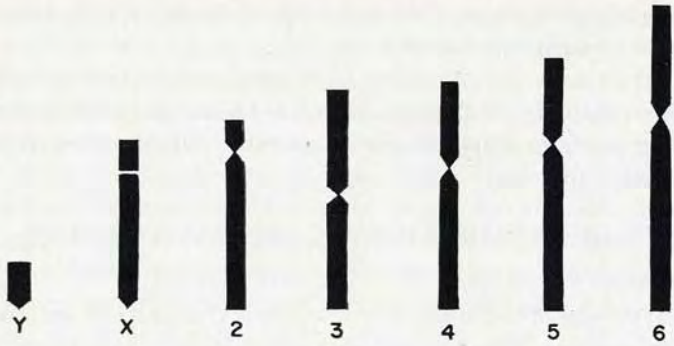

FIG. 2

FIG. 3

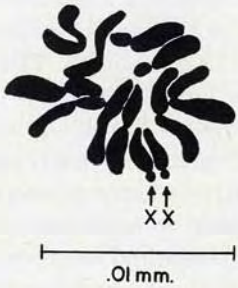

FIG. 4

FIG. 5

FIG. 6

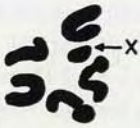

FIG. 7

FIG. 8

FIG. 9

FIG. 10

FIG. 11

PLATE 2

The post-irradiation chromosomes of *Callitroga hominivorax*.

FIG. 9. Cell in telophase, showing bridge formation. 9 hrs. after irradiation with 7500r of X-rays. FIG. 10. Cell in late anaphase, showing stickiness effect. 13 hrs. post-irradiation, 7500r. FIG. 11. Unequal division, late telophase cell. 28 hrs. post-irradiation, 7500r.

invalidate the proposal by Sax and Swanson (1941) that the acentric fragments which they observed in *Tradescantia* were due to stickiness and subsequent breakage of sister chromatids. It is quite likely that our material was given such a high dosage that not only the chromatids and chromosomes exhibited stickiness (Carlson, 1954), but that any fragments which were broken off also tended to adhere to the centric chromatids or chromosomes and were taken along to the poles in the division process. Muller (1954) suggested that in spermatocytes there are spatial difficulties involved in both recombination and restitution of broken chromosomes or chromosome fragments. While it is true that the spermatocyte chromosomes appear to be more widely separated than those in the spermatogonial cells, we feel that acentric fragments were able to overcome the spatial difficulties of such recombinations, as were centric fragments, possibly because of the fact that a dosage as high as 7500r would speed up the molecular motion to a very great extent. There would thus be a greater chance for recombination between centric fragments, along with a greater chance of the acentric fragments coming into contact with centric portions of chromatin and being taken along to the poles in this way. Whether any given fragment would thus go to the right pole would probably be a matter of chance. Obviously unequal divisions were evident in many of the cells (Figure 11). These, coupled with the fact that we ob-

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PLATE 1.

The chromosomes of *Callitroga hominivorax*.

FIG. 2. Idiogram of the chromosomal complement. FIG. 3. A metaphase of a spermatogonial cell. The X and the Y are shown. FIG. 4. A metaphase plate of a female ganglion cell showing two X chromosomes. FIG. 5. Metaphase I of the male. The X and Y show terminal association. FIG. 6. Early anaphase I of the male. FIG. 7. Polar view of metaphase II. This cell has an X chromosome. FIG. 8. Polar view of metaphase II. This cell has a Y chromosome.

served, on several occasions, three nuclei joined by bridges, as in Figure 12, have led us to believe that perhaps a primary spermatocyte had undergone a division which resulted in two aneuploid nuclei joined by a bridge, and that the bridge remained while one of these nuclei divided again, with a bridge formed in the second division also. Figure 13 illustrates an intermediate stage in this process. Here are shown two meiotic cells, the nuclei of which are dividing, but which are also joined by a definite bridge from the previous meiotic division. Although the final division of these two cells, with bridge formation in each, would give four nuclei connected by bridges, we unfortunately never observed cells which portrayed the final stage of this particular phenomenon. Another explanation of the phenomenon of 3 or 4 nuclei joined by bridges would be the formation of a multipolar spindle. As far as we know, however, only extremely high dosages of X-irradiation have been known to produce this effect on the spindle (Henshaw, 1940 and 1941).

FIG. 12

FIG. 12. Three nuclei joined by chromosome bridges. 11 hrs. after irradiation, 7500r.

The cause of the bridges is in some cases undoubtedly chromosome stickiness. We do feel, however, that even though we never saw an acentric fragment in any of these cells, there was probably a certain amount of recombination, especially in those cells which were in the early stages of division when irradiated. At a lower dosage of irradiation, which will be discussed later in this paper, we did find acentric fragments with bridge formation.

In his 1954 paper, Carlson mentioned the fusion of *Chortophaga* chromosomes, in prometaphase, metaphase or anaphase, if they were irradiated at those stages. Evans (1956) produced this same clumping, in *Chortophaga*, by cooling the material he studied. Carlson also stated that the chromosomal mass thus formed "elongates in the direction of the poles and appears to be divided by a pressing inward of the cleavage furrow". Although our experimental design eliminated observation of material immediately after irradiation, we did find clumping, or fusion, of the chromosomes in most of the metaphase plates seen, even up to 28 hours postirradiation (Figure 14). We also noticed that there were many divisions, as illustrated in Figure 15, in which the chromosomal mass seemed to be elongating toward the poles with a general thinning out between the two poles. We did not see a cell in which a noticeable cleavage furrow was apparently responsible for the ultimate constriction and division of such a mass.

The chromosomes in metaphase and anaphase, even at 28 hours postirradiation, looked coagulated. That is, instead of the arms being the same diameter

throughout, in places they seemed pinched in and in other areas, swollen. This is, perhaps, a similar phenomenon to that noted by Carlson (1940) in his studies on mitosis in the *Chortophaga* neuroblast, in which he saw chromosomes with a "beaded" appearance after an X-ray dosage of 250r. Although he states that, since these chromosomes went through mitosis in an apparently normal fashion afterwards, this was a reversible change as opposed to a degenerative one, we feel that the coagulated chromosomes we saw in *Callitroga* pupal testes represent a degenerative change and that the constricted areas were possibly breakage points. This latter has previously been suggested by White (1937). We base our

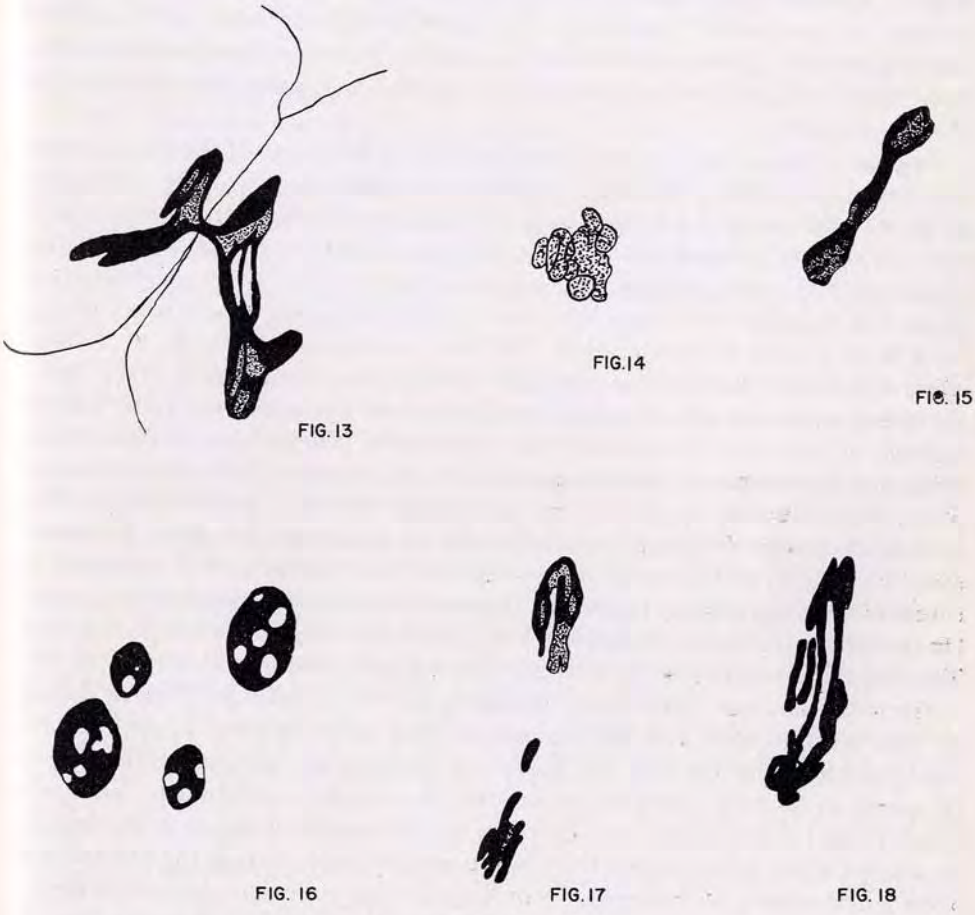

PLATE 3

The post-irradiation chromosomes of *Callitroga hominivorax*.

FIG. 13. Meiosis II division, showing bridge remaining from meiosis I and further bridges in the current division. 13 hrs. post-irradiation, 7500r. FIG. 14. Metaphase plate, showing clumping of chromosomes. 28 hrs. post-irradiation, 7500r. FIG. 15. Elongation in late anaphase of previously clumped chromosome mass, ½ hour after irradiation, 7500r. FIG. 16. Degenerating nuclei, 28 hrs. post-irradiation, 7500r. FIG. 17. Chromosome fragment in late anaphase cell, 2½ hrs. after irradiation with 1160r. FIG. 18. Chromosome fragments and bridges in anaphase cell, 2½ hrs. after irradiation with 1160r.

belief that the change was a degenerative one on the fact that we never found a normal meiotic division in process in all of the cells examined.

There were also many degenerating nuclei evident at all postirradiation stages studied. The number seen seemed to increase at about 4½ hours after irradiation, and at 5½ hours there were large numbers of such cells. After that there was neither an apparent increase nor decrease in their number. A camera lucida drawing of several of these nuclei is shown in Figure 16. It is also evident in Figure 12 that the 3 nuclei joined by bridges are becoming vacuolated and are disintegrating. In addition, there were no more sperm in the slides made 28 hours after irradiation than there were in the half-hour slides, as far as noticeable increase in numbers is concerned. The individual sperm were not counted. It seems quite likely, from both these slides and the studies of the testes themselves, that the dividing cells and spermatogonial cells disintegrated before the formation of mature sperm.

Because of the absence of fragments and great stickiness of the chromosomes after irradiation with 7500r of X-rays, we irradiated several dozen pupae with 1160r in order to see if a lower dosage had similar effects. As in those irradiated with 7500r, we noticed metaphase clumping, distortion of the chromosomes, stickiness, and many bridges. We examined about 100 cells in anaphase, telophase and daughter cell stage. We found three cells in late anaphase which may have been normal divisions. They had faint indications, however, of a "halo" effect which was found surrounding the bridges seen in other cells. This "halo", appearing as a light area running linearly between the poles, may have been the spindle, or may have been caused by the spindle. There were no chromosome bridges or indications of bridges present. We also found several cells in late anaphase which showed possible deletions (Figure 17), and several others which contained acentric fragments, probably due to either translocations, isochromosome formation, or deletions. An example of this type of cell is shown in the camera lucida drawing in figure 18. There were quite a few degenerating nuclei. On the whole, the visible chromosome distortion and damage seemed less marked. The clumping was not as extreme nor was the stickiness as widespread.

In conclusion, the 7500r X-ray dosage apparently caused permanent damage to both meiotic cells and spermatogonia. The large number of degenerating nuclei accounts for the fact that there was no apparent increase in the number of sperm in 28 hours postirradiation over the number seen half an hour after irradiation. It also explains the decrease in the amount of sperm in the testes of irradiated males much sooner than in the normal males during the insemination tests. Furthermore, it is obvious from the fact that no meiotic cells were seen in the testes 12 days after irradiation, while normal males still had quite a few cells undergoing meiosis at this time, that the spermatogonia were adversely affected by the X-rays. The chromosomal aberrations and stickiness as late as 28 hours postirradiation furnish further evidence for this fact.

That the irradiated sperm themselves carried a dominant lethal effect is indicated by the complete sterility of all matings in which the irradiated males were involved. This dominant lethal effect was probably due to broken or fused chromosomes which, upon union with the normal chromosomes of the egg, were unable to organize normal development.

SUMMARY

1. Screw-worm flies, *Callitroga hominivorax*, were irradiated with a dosage of 7500r of X-rays.

2. From the data compiled in our insemination studies, it was seen that in the first week after emergence from the pupae cases the irradiated males could inseminate as many females as could the normal males when both types were isolated with a maximum number of normal virgin females.

3. No larvae developed from matings between irradiated males and normal females, even after three weeks of matings. This dosage produced complete sterility, with no recovery of fertility, in the male screw-worm.

4. The dominant lethal effect in the sperm was probably due to induced chromosome breakage.

5. The irradiation caused permanent genetic damage to both spermatogonial cells and spermatocytes, as well as to spermatid and sperm, as evidenced by the presence of stickiness and bridge formation in all of the dividing cells seen, the great number of degenerating nuclei observed, and the fact that there was no apparent increase in the amount of sperm in the testes after irradiation.

6. The bridges seen in the dividing cells could have been due to recombinations, or chromosome stickiness, or both.

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XXII. Genetic Studies of Irradiated Natural Populations of Drosophila[1]

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The genetic structures of populations and their changes through time are important in the evolution of living forms. Few populations are sufficiently isolated from neighboring populations that they can be regarded as isolated units. Some islands or similarly isolated areas are of this kind. Very few of these isolated populations are satisfactory for the study of changes in the genetic structure of the populations. The *Drosophila ananassae* populations of the Marshall Islands and of the eastern Carolines provide this opportunity. In order to determine the changes in genetic structure of the populations through space and time, collections were made in 1955 and 1956 and these were analyzed in a number of ways. These analyses are given in the several sections of the paper.

ACKNOWLEDGMENTS

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Section I

GEOGRAPHY, ECOLOGY AND RADIATION

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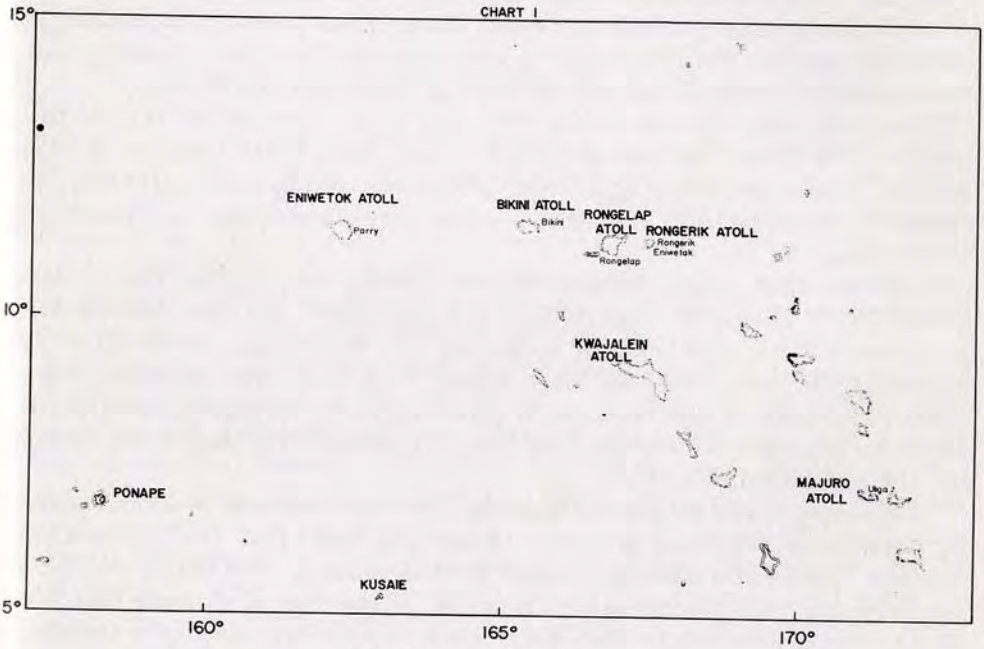
and Wilson S. Stone

Drosophila ananassae has a broad circum-tropical distribution and has been reported occasionally from more temperate climates. In the Micronesian islands it is clearly the dominant species of Drosophila. We have collected this species in the Marshall Islands and on Ponape, a "high island" in the eastern Carolines. Chart 1 gives the general location of the collecting areas in relation to Eniwetok



The Marshall Islands area.

and Kwajalein atolls. Our collections were made working out of the Atomic Energy Commission Pacific Proving Ground base on Parry Island, Eniwetok Atoll. The flies were shipped airmail or were carried directly to Austin for laboratory analysis. Collections were made in August and early September, 1955, on Bikini, Rongelap, Rongerik and Majuro atolls; and in August, 1956, collections were made on Bikini, Rongelap, Rongerik and Majuro atolls, and on Ponape Island. Only *Drosophila ananassae* was found in the northern Marshall Islands, while in the south (Majuro) and on Ponape, several other species occur. On Majuro, a few *D. melanogaster* and *D. bryani* were taken, but *D. ananassae* was extremely common. On Ponape *D. ananassae*, *D. hypocausta*, and *D. spinofemora* were all common, and *D. melanogaster*, *D. bryani*, and *D. kikkawai* occurred in much smaller numbers.

The natural food sources varied from island to island. On Bikini the *ananassae* population probably maintains itself primarily on the fallen fruits of *Morinda citrifolia*, with less extensive breeding occurring in fruits of papaya and possibly pandanus. On Rongelap, where large populations of *ananassae* were found, papaya and Morinda were common, and there were also several fruiting breadfruit trees (*Artocarpus* sp.). As has been pointed out by Gressitt (1954) the productivity of one rotten breadfruit in terms of individual Drosophila flies is enormous. In 1955 on Eniwetok Island, Rongerik Atoll, the only available food appeared to be the small fruit of *Guettarda speciosa*, and the population of *ananassae* was very small. In 1956 we were unable to collect the species on this island, but on the nearby island, Rongerik, a few hours collecting over Morinda fruits yielded a moderate number of flies.

Majuro Atoll, in the southern Marshalls, had Morinda, papaya and breadfruit of which Morinda fruits which had fallen into a pigpen proved to be the best for collecting purposes. On Ponape the opportunities for Drosophila breeding were more extensive, with considerable amounts of breadfruit and banana.

There was some variation in the two years in the amount and type of fruit available. On Bikini, for example, papaya fruits were fairly common in 1955, while in 1956 there were very few and collections could be made only from Morinda and Pandanus fruits although some flies were taken at baits of bananas and yeast mixtures.

In the Marshall Islands the morning and evening temperatures have a mean around 78° F. The usual range is 77° to 87° (at midday) but more extreme temperatures do occur (data provided by the A.E.C.). This range corresponds to that reported by Pipkin (1953) for Moen Island, Truk Atoll. The humidity is generally high, and although rains are frequent and heavy throughout the area, the northern Marshalls are clearly drier than the southern portions, while Ponape has the highest rainfall of all.

Availability of food for natural breeding is seriously reduced on several islands by competitors, consisting primarily of rats and land crabs. On Eniwetok, for example, there was a small population of Drosophila in 1955 but by August of the following year the increase in numbers of rats was so extreme that little fruit remained available for flies. Three kinds of predators were fairly common: skinks, toads and spiders. On Ponape, toads *(Bufo marinus)* were especially common, and often congregated in a circle around the fruit being used as bait. On all of the islands, small web-building spiders were extremely numerous and often seriously hampered our collecting.

We did not record the actual numbers of Drosophila collected on the various islands. On Rongerik we were able to collect a few hundred flies in about three hours, collecting over fallen fruit and yeast bait. On Rongelap (1956) we collected well over 6000 flies within an hour, primarily under breadfruit trees, and there seemed to be no diminution of flies as we collected. The population on Bikini was somewhat larger than that of Rongerik; in 1956 we collected approximately 2000 flies in four mornings using several collecting stations. On both Majuro and Ponape, it was possible to collect 6000 flies within an hour at one locality; on one occasion, on Ponape, we collected a large number of flies in an open room containing bananas and other fruit.

Pipkin (1953) collected for a year (1951–52) on Moen Island, Truk Atoll. *D. ananassae* was the most numerous of the four species she collected. Using protected trap cans baited with fruit and placed close to breadfruit and papaya plants, she observed that the *ananassae* population increased considerably when the breadfruit matured twice a year. The population of *ananassae* on Moen was neither minimum nor maximum during August, being about one-eighth the size of the population at that locality during the two months when breadfruit was available. The two other species numerous enough to score, *hypocausta* and *anuda*, were also relatively low in August, so that this seems to be a less favorable season of the year for Drosophila. Patterson (1943) and Dobzhansky and Pavan (1950) showed that in both temperate and tropical localities, various species have cycles of abundance which do not necessarily coincide with the cycles of other species. At the present we are unable to say when the peak population of *ananassae* occurs in the Marshall Islands.

The amount of radiation to which the various tested Drosophila populations have been exposed is unknown, although certain minimum amounts can be surmised from other data (Cronkite, Bond and Dunham, 1956). The Bikini Island population received considerable direct radiation during the Castle Operation in the spring of 1954 as well as in tests of previous years. It received a relatively small amount in the Redwing test series of 1956. Following the explosion of a thermonuclear device March 1, 1954, the wind deposited radioactive materials not only on Bikini but also on Rongelap and Rongerik atolls. Control *ananassae* populations of Majuro and Ponape received negligible radiation from fallout as far as our measures of genetic effects on the populations are concerned.

Sondhaus *et al.* (1956) estimated that at three feet above ground level the cumulated gamma radiation from fallout (assumed completed in about 12 hours) gave a dose of approximately 200 r in 48 hours (or about 4000 mr/hour) on both Rongelap and Rongerik atolls. This decayed so that five days later the rate was about 375 mr/hour and in two more days about 280 mr/hour. On Bikini the fallout (over and above the direct radiation) cumulated to about 800 r in the first 48 hours. We cannot, however, even approximately guess the dose received by the Drosophila. Most adult and immature stages are at ground level where intensity is greatest, and their small size also makes beta rays effective. Furthermore, they and their descendants remained on the islands and obtained their food from sources contaminated with radio-active materials. Cohn *et al.* (1956) state as follows:

"The importance of ingestion as a continuing source of contamination is evidenced by the level of internal contamination of the pigs from Rongelap. These animals had about ten times the body burden of the human population in the same locality. As the air-borne activity had already dropped to a low value at the time of evacuation of the humans, the contamination of the pigs during their prolonged stay on the island necessarily derived from ingestion of radio-active food and water.

"Radioanalysis of water and soil samples from Rongelap indicated high levels of contamination from the fallout at early times following detonation.

"It appears that during the first month a limited amount of fission products was available to plants growing on the contaminated soil. Significant amounts of beta activity as well as small amounts of alpha activity were present on the external surface of plants at 42 days post detonation. Only very small amounts of beta

activity and no alpha activity were detected in the edible portions of fruits such as pandanus, papayas and coconuts. However, high levels of activity were found in the coconut tree sap, and the isotopic concentration was very similar to that of water."

They list as biologically hazardous internally deposited fission products Sr^{89}, Y^{91}, Zr^{95}, Ru^{103}, Ru^{106}, I^{131}, Ba^{140}, La^{140}, Ce^{141}, Pr^{143}, Ce^{144} which produce beta or beta and gamma radiations and suggest that Sr^{89} is the most hazardous. Since Drosophila larvae live in fruit and sap and since these are also food for the adults, such sources could keep the level of radioactivity available internally to Drosophila high even after the air level of radiation had fallen.

These authors report on their studies of animals from the fallout islands. They point out that a much greater amount of the activity from internal deposition of radionuclides is removed from the body of the chicken through egg laying than the amount excreted in urine and feces during their study. They state:

"Studies made on egg production of contaminated hens gave no evidence of any effect of radiation. The rate of production and the eggs produced were both normal. The extraordinary ability of fowl to mobilize calcium in shell formation resulted in the presence of very high activity in the shells of the first few eggs. The activity was associated with the fission products of the alkaline earth group. A significant amount of activity was found in the yolk, and lesser amounts in the albumen. The removal of activity from the body of chickens by egg production provides an effective natural decontamination process."

If Drosophila concentrate radioactive materials in their gonads—and the storage products in their eggs have many similarities to the hen's egg yolk—then the amount of effective soft radiation to the germ cell chromosomes would be much higher than the radiation in the air around them. All these facts would seem to indicate that we can only estimate a minimum amount of radiation damage to the genetic structure of a population such as Drosophila from measurements of the radiation in air and other sources.

Section II

COMPETITION TESTS WITH DROSOPHILA ANANASSAE

Florence D. Wilson, June T. Neuenschwander, and Wilson S. Stone

One of the interesting problems of these island populations is why *ananassae* is the dominant or a major species. A second and related problem is the effectiveness of *ananassae* in larval competition, which must be a major factor in their survival. Some of these factors were studied in population cages of the type devised by L'Heritier and Teissier (1934). In the tests the cages were started with equal numbers of each species—1000 *ananassae* plus 1000 of another species. Under these circumstances the population builds up so that a great excess of eggs is laid on the food soon after a new container is added. Preliminary experiments in the spring of 1955 showed that *D. funebris*, *D. busckii*, *D. novamexicana* could not survive long in competition with the more rapidly reproducing *ananassae* (a strain from Central America). *D. virilis* survived for several generations but *D. melanogaster* (*ebony*^{11} mutant) was a very effective competitor.

The data on competition in Table 1 were obtained by placing four fresh containers of food in the cage and leaving them about five days. A sample was taken every thirty days approximately. Two of these food masses with many larvae and eggs were transferred to bottles and the flies allowed to develop (restricted food). Two were spread into two added bottles of the yeast-banana-agar food so that the larvae had about three times as much food available as the others (increased food). These spread bottles reduced the intensity of competition and provided increased opportunity for the less effective competitors to develop. In the cages of *ananassae* and *melanogaster*, two fresh containers of food (banana, yeast, agar, propionic acid) were added Tuesdays, Thursdays and Saturdays, so that all the food in the cage was replaced every 16 days. In competition with *virilis* and the other longer life cycle forms, one fresh container was added Monday, one Wednesday and two Friday so a full food change for a cage required 28 days.

Table 1 and Figure 1 show that *ananassae* can displace *virilis* at both temperatures. The points on the figure represent the percent of *ananassae*. The replacement occurs more slowly at 71° ± 1 (tests 1–4) than at 77° ± 1 F (tests 5–8). In the figure the numbers refer to the tests given in Table 1, solid lines to changes measured on restricted food, and dotted lines to changes measured when the food in the cups was supplemented. A much smaller percent of the flies that emerge from the supplemented food are *Drosophila ananassae*. The data show that somewhat more *ananassae* emerge on better food conditions but a great many more of the competitor emerge. This differential is lost near 0 and 100% *ananassae*. Test 7 is an exception but the food was bad in this test and spreading improved the development of all flies.

The tests with *melanogaster* were similar except that *melanogaster* is a much more effective competitor. At 71° F it very rapidly replaces *ananassae* but at 77° *ananassae* usually displaces *melanogaster*. The tests at 74° ± 1 F show the two species nearly equal. *Drosophila ananassae* first falls, then increases. Nevertheless there was still a considerable *melanogaster* population in the cages at the end of three months as indicated by the number that emerged on spread food. Both tests illustrate the tremendous effectiveness of *ananassae* in larval competition under limited food conditions. The more strenuous competition probably approached more nearly the conditions on the islands. At temperatures close to that of the islands, they can compete with other species most effectively.

Zimmering (1948) tested competition between *D. pseudoobscura* and *D. melanogaster* in population cages. He showed that strains of the former of two origins (Mather and Pinon) differed in their competitive ability. Four strains of *melanogaster*, one wild type and three mutant stocks, differed also. One with six second chromosome recessive mutants was displaced by *pseudoobscura*, but the other three displaced *pseudoobscura*. Timofeef-Ressovsky (1933, 1935) demonstrated that different strains of *D. funebris* were of different competitive abilities, which was related to the temperature of their origin. His tests differed in that a certain number of eggs from *funebris* and *melanogaster* were placed together under crowded conditions. Merrell (1951) also tested competition between *funebris* and *melanogaster*. Most of the competition was between larvae. The species could coexist in population bottles for long periods since the larvae of *melanogaster*

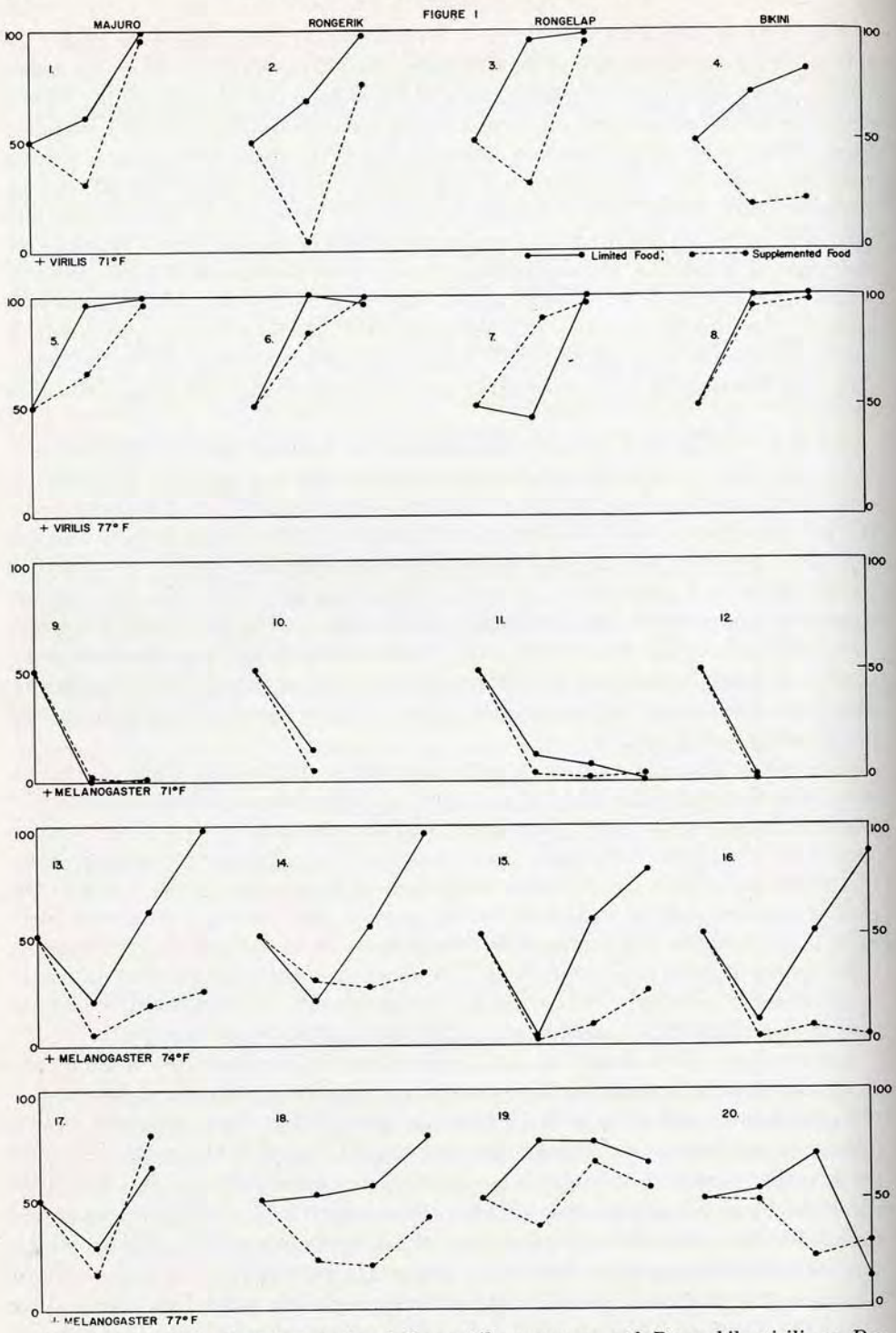


FIG. 1. Competition between stocks of *Drosophila ananassae* and *Drosophila virilis* or *Drosophila melanogaster* in population cages. Solid line shows percent *ananassae* in the sample recovered from cage on restricted food, dotted line shows percent *ananassae* on supplemented food. Numbers correspond to tests in Table 1.

TABLE 1

Competition between Drosophila ananassae and Drosophila virilis or Drosophila melanogaster

Stock of *ananassae*	Competitor	Temperature ° F	Date Started	Date Tested	*Restricted Food* Total flies	*ananassae* number	*ananassae* %	*Increased Food* Total flies	*ananassae* number	*ananassae* %
1. Majuro	*virilis*	71°	12/23/55	1/25/56	283	173	61.1	1599	502	31.4
				2/20/56	1104	1094	99.1	2449	2354	96.1
2. Rongerik	*virilis*	71°	12/14/55	1/13/56	485	331	68.2	2051	101	4.9
				2/20/56	582	571	98.1	1146	871	76.0
3. Rongelap	*virilis*	71°	12/ 9/55	1/ 7/56	475	452	95.2	2693	858	31.9
				3/ 2/56	637	629	98.7	1518	1455	95.8
4. Bikini	*virilis*	71°	12/13/55	1/13/56	421	305	72.4	2700	584	21.6
				2/20/56	491	407	82.9	1065	246	23.1
5. Majuro	*virilis*	77°	2/ 6/56	3/ 7/56	1295	1251	96.6	1574	1026	65.2
				4/ 6/56	1638	1630	99.5	3280	3178	96.9
6. Rongerik	*virilis*	77°	2/27/56	2/25/56	1462	1462	100.0	2108	1778	84.3
				4/29/56	1370	1337	97.6	2158	2137	99.0
7. Rongelap	*virilis*	77°	2/ 7/56	3/11/56	500	223	44.6*	2207	1982	89.8
				4/11/56	557	557	100.0	2407	2320	96.4
8. Bikini	*virilis*	77°	2/ 2/56	3/ 7/56	1745	1737	99.5	2535	2386	94.1
				4/ 8/56	2110	2110	100.0	3127	3073	98.3
9. Majuro	*melanogaster* (e^{11})	71°	12/ 7/55	1/ 5/56	313	1	0.3	1617	16	1.0
				3/ 9/56	746	7	0.9	2606	16	0.6
10. Rongerik	*melanogaster* (e^{11})	71°	12/ 2/55	1/ 5/56	550	79	14.4	1990	96	4.8
11. Rongelap	*melanogaster* (e^{11})	71°	12/12/55	1/13/56	525	58	11.5	2390	94	3.9
				2/12/56	427	30	7.0	2051	40	1.9
				3/ 7/56	287	13	4.5	2483	62	2.5

TABLE 1—Continued

Competition between Drosophila ananassae and Drosophila virilis or Drosophila melanogaster

Stock of *ananassae*	Competitor	Temperature °F	Date Started	Date Tested	*Restricted Food* Total flies	*ananassae* number	*ananassae* %	*Increased Food* Total flies	*ananassae* number	*ananassae* %
12. Bikini	*melanogaster* (e^{11})	71°	12/ 2/55	1/ 5/56	486	9	1.9	2035	2	0.1
13. Majuro	*melanogaster* (e^{11})	74°	4/23/56	5/27/56	809	164	20.3	3636	205	5.6
				6/24/56	820	500	61.0	1878	354	18.8
				7/28/56	1064	1046	98.3	1879	483	25.7
14. Rongerik	*melanogaster* (e^{11})	74°	4/23/56	5/27/56	1296	263	20.3	2803	825	29.4
				6/24/56	827	451	54.5	2296	621	27.0
				7/26/56	943	866	91.8	2505	796	33.7
15. Rongelap	*melanogaster* (e^{11})	74°	4/25/56	5/27/56	1843	71	3.9	3868	141	3.6
				6/24/56	487	277	56.9	2513	245	9.7
				7/26/56	726	579	79.8	2805	688	24.5
16. Bikini	*melanogaster* (e^{11})	74°	4/25/56	5/27/56	485	53	10.9	3982	127	3.2
				6/24/56	781	402	51.5	2602	211	8.1
				7/26/56	669	586	87.6	2154	86	4.0
17. Majuro	*melanogaster* (e^{11})	77°	2/ 6/56	4/11/56	564	160	28.4	2099	341	16.2
				5/11/56	1202	814	64.5	2271	1691	74.5
18. Rongerik	*melanogaster* (e^{11})	77°	3/ 7/56	4/ 8/56	1095	575	52.5	3077	728	23.7
				5/10/56	756	419	56.9	2772	567	20.5
				6/ 6/56	884	695	78.6	2591	1076	41.5
19. Rongelap	*melanogaster* (e^{11})	77°	2/ 1/56	3/ 7/56	995	758	76.2	1756	667	38.0
				4/ 6/56	2192	1564	71.4	3027	2008	66.3
				5/ 6/56	1608	1071	66.6	2724	1499	55.0
20. Bikini	*melanogaster* (e^{11})	77°	3/12/56	4/12/56	1156	625	54.1	2968	1455	49.0
				5/16/56	758	535	70.6	2043	629	23.8
				6/ 9/56	1251	185	14.7	2776	837	30.2

* Bad food.

developed more effectively on fresh food whereas *funebris* larvae could develop in old food which was not usable by *melanogaster*. L'Heritier and Teissier (1935) had noted that mixed populations of *funebris* and *melanogaster* came to equilibrium with a small proportion of *funebris*. Merrell showed that the balance between the competitors was precarious since two populations became all *funebris*, another all *melanogaster* in addition to the group which ended as *funebris* because no fresh food was added.

Moore (1952) reported a series of studies of competition between the sibling species *D. melanogaster* and *D. simulans*. He showed that at 25° C with rapid food replacement (up to 8 cups per week) *melanogaster* displaced *simulans* but that at 15° C with slow food replacement (2 or 3 cups per week) *simulans* replaced *melanogaster*. In addition *simulans* will utilize an "undesirable" food surface for laying much more effectively. *Drosophila ananassae* is in the *melanogaster* species group, but is not as closely related as *simulans*. In our tests, which usually stopped short of complete replacement, *melanogaster* displaced *ananassae* rapidly at 71° F but *ananassae* had the advantage at 77° F (25° C). Even at 74° the superior competitive ability of *ananassae* larvae gives the latter species an advantage. This factor and greater temperature tolerance must account for the dominant role of *ananassae* in the Marshalls and even at Ponape.

Section III

CYTOLOGICAL ANALYSIS

Robert L. Seecof

INTRODUCTION

This report describes the results of sampling the chromosome arrangements of populations of *Drosophila ananassae* from the Marshall Islands. Populations were sampled from three of the islands that had been exposed to radiation from atomic weapons, Bikini, Rongerik and Rongelap, and from two of the islands that received no irradiation, Majuro and Ponape.

Previous studies on Drosophila have already established the kinds of chromosome rearrangements which are successfully transmitted after irradiation. The variety and incidence of X-ray induced aberrations have been used as a basis for estimating breakability among parts of chromosomes, physical arrangements of chromosomes during cell processes, and efficiency of the radiation employed. Such studies were made by Bauer *et al.* (1938), Helfer (1941), Koller and Ahmed (1942), Kaufmann (1946) and Haldane and Lea (1947). Later experiments on *D. melanogaster* by Lüning (1952) and Auerbach (1954) demonstrated a pattern of X-ray sensitivity through the stages of spermatogenesis which was confirmed for *D. virilis* by Alexander and Stone (1955).

In a recent article Stone (1955) has reviewed the extensive changes in chromosome pattern that have accompanied Drosophila speciation. Evidence is summarized to show that gene sequences, as preserved by chromosome rearrangements, have adaptive value which is dependent on the nature of the rearrangement, the gene pool, and the habitat of the species.

In order to establish perspective for evaluating the rearrangements recovered from irradiated wild populations, a control experiment relating the radiation

sensitivity of *D. ananassae* to other Drosophila has been performed. Information concerning the distribution of chromosome breaks and the differential sensitivity of stages of spermatogenesis was gathered from analyses of the salivary gland chromosomes of F_1 larvae from X-rayed males. The results of the laboratory control and the wild population survey are presented together as subsections A and B respectively.

MATERIALS AND METHODS

A. *The Control X-ray Induced Rearrangements*

All X-ray treatment was applied with a Westinghouse "Quadrocondex" machine through a filter of 1 mm. of aluminum and ½ mm. of copper. Dosage was calibrated with a Victoreen-made Condenser r-Meter, model 70. Two sixty second r-meter readings were taken before each irradiation and one afterwards. The three readings fell within a 15 r range to be considered acceptable. In all tests, X-rays were delivered at 250 kv., 15 ma., at about 800 r per minute and at 24° C.

D. ananassae shows four metacentric chromosomes at mitosis and six chromosome arms in salivary nuclei. In larval ganglion cells the three autosomes are of different sizes and the sex chromosome is intermediate in length (Kaufman, 1937; Kikkawa, 1938). In salivary nuclei the largely heterochromatic fourth chromosome is buried in the chromocenter. The few euchromatic bands representing the fourth are described by Kikkawa (1938) and Kaufman (1937). These bands are indistinct and have been ignored in the present study. The base of 1R (numbered 12 on the included map, Chart 2) is often indistinguishable and breaks were not localized within it.

The flies used in this experiment were derived from a stock collected on the Pacific island of Majuro. They were selected, by the author, to be homozygous for a "standard" chromosome arrangement. Kaufmann (1939) showed that a homozygous inversion did not influence the chromosome breakage pattern in *D. melanogaster*. In common with all other *D. ananassae* strains examined, several extra chromatin bands were present heterozygously in the salivary chromosome set. These give no detectable phenotypic effect.

All of the tests were made by X-raying adult males, mating them to virgin females, and examining male and female F_1 larvae for chromosome aberrations which could be detected in the salivary gland nuclei. The salivary glands were prepared as paraffin sealed aceto-orcein squashes and then examined within twelve hours. A salivary chromosome map was drawn, using a camera lucida, from these preparations (Chart 2).

Series 1500. Males received 1500 r 12 to 24 hours after eclosion. After irradiation, a mass mating was set up of 20 to 30 males and an equal number of mature virgin females. New virgins were supplied to these males, at 48 hour intervals, for twelve days. The number of pairs per vial was adjusted to the expected egg hatch in an effort to keep the number of larvae at an optimum. The ratio of males to virgin females was established at 1:1 in every 48 hour interval tested.

Males were etherized once before and once after irradiation, and then again at 48 hour intervals when being transferred. Matings were made and larvae were raised on heavily yeasted banana food. This sequence, from X-raying through raising larvae, was repeated eight times during a period of seven months. The

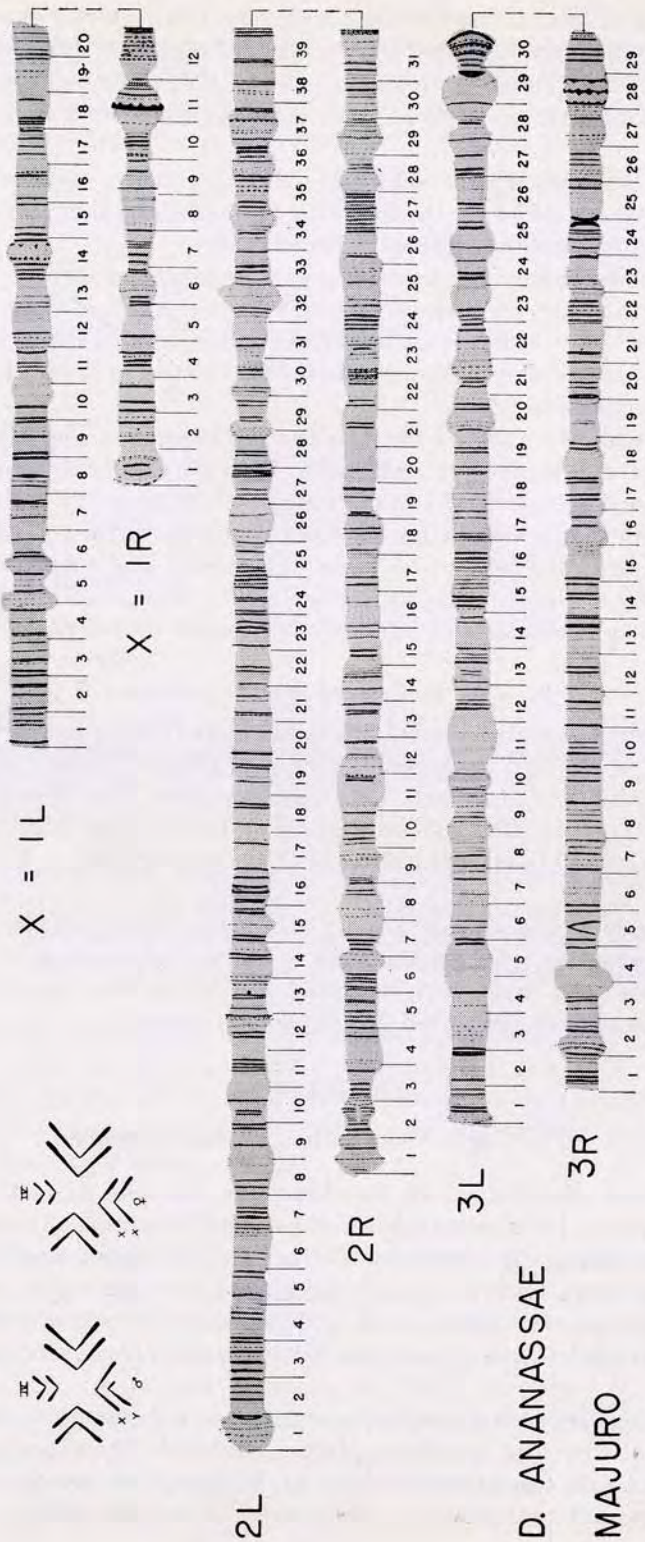


CHART 2: Chromosome map of *Drosophila ananassae*.

combined score of one hundred larvae for each 48 hour interval of series 1500 is made up by contributions of from four to seven of these sub-series.

Series 1000. Series 1000 was similar to series 1500 in all respects except that the dose given to the males was 1000 r. Series 1000 and series 1500 were run concurrently.

Series 1570. In series 1570, seven-day-old virgin males were given 1500 r, placed with virgin females for the following 24 hours, and then discarded. Other details were similar to those in series 1500 and 1000.

The larvae derived from the successive 48 hour periods of series 1500 and 1000 represent sperm which were in successively earlier stages of maturation when irradiated. The larvae from series 1570 represent irradiated mature sperm from seven-day-old males and are compared to the first offspring of the 12–24 hour old males irradiated in series 1500.

The aberrations were scored for type of rearrangement, identity of chromosomes involved, and location of each break. Very small rearrangements and deletions were not detected. Breaks were located with an accuracy of two numbered sub-divisions on the included map. Five to ten percent of the squashes were unanalyzable because too few nuclei were well spread, and such slides were not included in any of the results. Catcheside (1938), in a similar study, found no indication that greater doses of X-rays induce more unanalyzable glands.

B. *The Analysis of Wild Populations*

Island populations were sampled in the summers of 1955 and 1956. The 1955 sample males were taken in the wild or were F_1 offspring from females inseminated in the wild, one male from each female parent. The 1956 sample males were either captured in the wild or were taken from a large population of flies, from the respective island, maintained in the laboratory during four months of sampling.

These males were crossed individually to females homozygous for an arbitrary standard chromosome arrangement. The salivary chromosomes of four female F_1 larvae from each male were analyzed for chromosome abnormalities. Six chromosome arms were inspected, the dot fourth ignored.

RESULTS

A. *The Control X-ray Induced Rearrangements*

Over-all break distribution. In the three series, a total of 1100 sperm were analyzed, of which 137 showed some chromosomal aberration. In all, 321 breaks were detected among six chromosome arms and the chromocenter. Break frequencies are given in Table 2. The distribution of 284 breaks within the numbered divisions of the salivary chromosome map is shown. These represent all of the breaks from the three series which were located with a precision of two numbered divisions.

Total breakage for each chromosome arm is seen to be roughly proportional to the relative salivary arm lengths as given in Table 2. The expected number of breaks for each arm was calculated from the lengths of the arm as shown on the salivary chromosome map. Again, 1R interval 12 was not considered. This cal-

TABLE 2 (III)

Chromosome Arm Map Interval	1L	1R	2L	2R	3L	3R
1–2	3	3	3	3	3	15
3–4	4	4	6	8	6	2
5–6	2	2	4	2	5	2
7–8	2	1	3	2	6	5
9–10	2	3	1	6	1	0
11–12	3	2	1	3	3	2
13–14	1	C	5	1	8	4
15–16	0		3	6	1	4
17–18	2		6	0	5	2
19–20	2		7	1	3	4
21–22	C		9	2	3	3
23–24			7	3	5	5
25–26			4	2	1	0
27–28			3	1	1	7
29–30			4	4	12	3
31–32			2	7	C	C
33–34			0	C		
35–36			4			
37–38			3			
39–40			1			
			C			
Total number of observed breaks	21	15	76	51	63	58
	× 2	× 2				
	42	30				
Expected number of breaks	39	23	78	62	60	58
Arm length ratio from salivary map	1.7	1	3.4	2.7	2.6	2.5

Table 2 lists the number of breaks recovered and their locations for six chromosome arms. The intervals are identical to those shown on the included salivary chromosome map. C means centromere.

culation was made after doubling the total number of observed sex chromosome (1L and 1R) breaks, an irradiated sex chromosome having been present in only half the larvae examined (in female larvae only). A small error is caused by this procedure. Table 6 gives the ratios of males and females examined in each interval.

It should be remembered, when considering salivary chromosome measurement, that some distortion is caused by stretching of interband regions. Mitotic arm lengths have not been established for *D. ananassae* but the arms of the second and third chromosomes are roughly equal. See the metaphase drawings on the salivary chromosome map.

The distribution of breaks within arms is shown in Table 2. A chi-square test indicates that breaks are distributed to chromosome arms in proportion to their relative salivary lengths ($.5 > p > .4$). The low number of breaks per map division necessitates arbitrary grouping to test the within-arm break distribution. Chromosome lengths of 6 map divisions, from tip toward centromere with extra divisions included in the proximal grouping, were compared. Breaks totals within the resultant 25 chromosome segments, tested by chi-square against the overall average number of breaks expected per segment, indicate that within-arm distribution is not other than random ($.05 > p > .01$).

Relative frequencies of multiple break rearrangements. Table 3 gives the proportion of intra-arm to inter-arm rearrangements for chromosomes 1, 2, and 3.

Two-break rearrangements confined to one arm are listed as 2. Two-break rearrangements between different arms are listed as 1, 1. Higher break rearrangements are listed accordingly.

The ratios of "2" to "1, 1" breaks recovered is 1:5 to 1. These are comparable to the values obtained by Bauer *et al.* (1938), Helfer (1941), and Koller and Ahmed (1942) which are listed in Table 3 along with data from the present study. Bauer's figures were obtained from *D. melanogaster* at 1000 to 5000 r. Helfer and Koller worked with *D. pseudoobscura* using 4500 to 5000 r.

TABLE 3 (III)

No. of Breaks	Distribution	No. of Cases	Ratio	Ratio A. Bauer (1938) 1000–5000r B. Helfer (1941) 5000r C. Koller (1942) 4500r		
				A	B	C
2	1, 1	65	1.5	1.8	1.9	1.2
	2	42	1	1	1	1
3	1, 1, 1	1				
	2, 1	3				
	3	. .				
4	2, 2	1				
5	2, 1, 1, 1	1				
Alterations involving heterochromatin		26				
Kind of alteration not determined		6				

Table 3 lists the recovered rearrangements by number of breaks and number of chromosome arms involved. The first four columns show data from the present study, the last three from previous studies.

The 107 two-break rearrangements shown in Table 3 are made up of 41 paracentric inversions, 23 pericentric inversions, 42 translocations and one deletion. Six of the paracentric inversions include more than half of their chromosome arm. This is not significantly different from the 10.25 expected on the basis of random breakage and fusion. Chi-square test gives $p > .05$.

Because breaks in the heterochromatic fourth chromosome could not be distinguished from breaks in the Y, no direct measure of Y breakability is available. However, among the total of 26 two-break rearrangements involving heterochromatin, 17 occurred in males and 9 in females. A 17.3 to 8.7 ratio is expected from equal numbers of male and female larvae if the fourth and Y chromosomes are considered equal in length and were breaking and recombining at random. This is because an irradiated fourth chromosome was present in all examined larvae (ignoring non-disjunction), but an irradiated Y was present only in male larvae.

Variation of damage with the course of spermatogenesis. Table 5 shows the numbers of damaged sperm and the total chromosome breakage. Periods B-F represent successive 48-hour intervals during which males inseminated fresh virgins. In all three series, 100 sperm were analyzed for each 24-hour interval. Figure 2 illustrates the information in Table 5. The stages of spermatogenesis are inferred from studies on *D. melanogaster* which are discussed later.

Comparison of mature sperm to the most sensitive stage of spermatogenesis. Because of the similarity in number of breaks and number of larvae showing aberrations (Table 5 and Figure 2), both series 1500 interval B, and series 1570

are considered to be derived from mature sperm. Similar results were obtained by Khishin (1955) who found that the sensitivity of maturing spermatozoa in newly emerged *D. melanogaster* imagines is the same as that in mature sperm.

Too few breaks were scored to compare period B against period D for distribution of breaks along chromosome arms. Table 4 gives the relation between

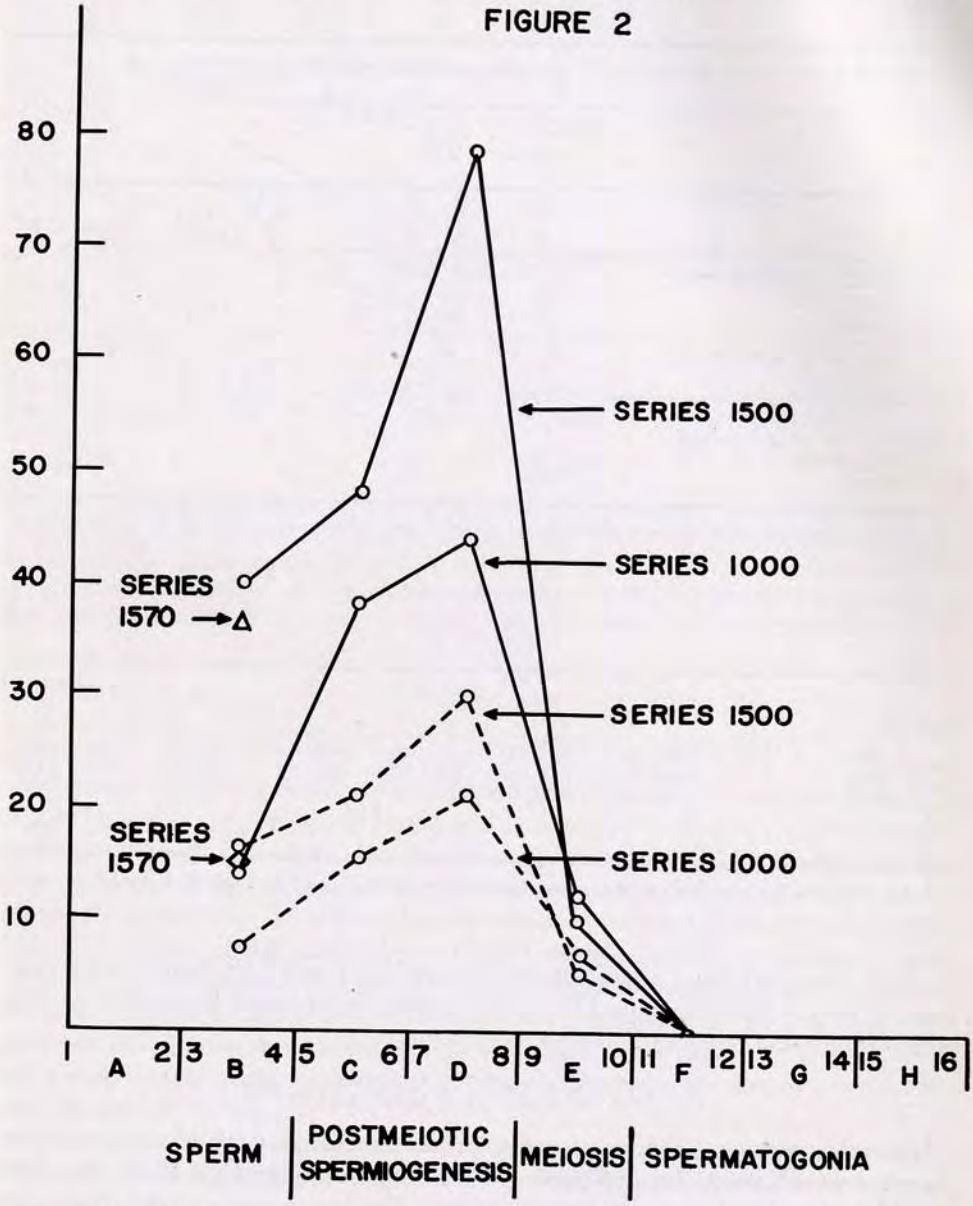


FIG. 2. Variation in recovered X-ray damage from successive brood intervals. The abscissa is marked off in two-day intervals A-H. A is the first two days after eclosion. The stages of spermatogenesis are inferred from work on *D. melanogaster* which is discussed above. Ordinates for the triangle and the solid lines are to be read as "number of breaks." Ordinates for the diamond and the dotted lines are to be read as "% of sperm showing a rearrangement."

TABLE 4 (III)

No. of Breaks	Distribution	No. of Times Recovered Interval B	D	B-F	Ratio Interval B	D	B-F
2	1, 1	19	20	65	1.58	1.33	1.55
	2	12	15	42	1	1	1

Table 4 lists the recovered two-break rearrangements among chromosomes 1, 2 and 3.

TABLE 5 (III)

Day Interval	3, 4 B	5, 6 C	7, 8 D	9, 10 E	11, 12 F	total B-F
Series 1500, no. of larvae with rearrangements	16	21	30	5	0	72
No. of breaks	40	48	79	10	0	177
Series 1000, no. of larvae with rearrangements	7	16	21	6	0	50
No. of breaks	14	38	44	12	0	108
Series 1570, no. of larvae with rearrangements	15					15
No. of breaks	36					36

Table 5 shows the chromosome damage recovered from 100 larvae sampled in each 48-hour period.

TABLE 6 (III)

Interval	B ♂	B ♀	C ♂	C ♀	D ♂	D ♀	E ♂	E ♀	F ♂	F ♀	Total B-F ♂	Total B-F ♀
Series 1500	49	51	48	52	55	45	44	56	44	56	240	260
Series 1000	56	44	61	39	43	57	48	52	48	52	256	244
Series 1570	55	45									55	45
											551	549

Table 6 shows the numbers of male and female larvae examined in each 48-hour interval.

kinds of two-break rearrangements in intervals B, D, and B through F. This comparison fails to show any qualitative differences for interval B (mature sperm) versus interval D (interval showing most damage.)

B. *The Analysis of Wild Populations*

Ten paracentric inversions represent all the rearrangements discovered in the sampled populations. Two of these were presumed identical to those described as CII L-O and CIII L by Kikkawa (1938). The frequency and distribution of these two and the eight previously undescribed inversions, *a* through *h*, are listed in Table 7. The approximate break points associated with each inversion are shown in Figure 3. Inversions *a*, *b*, and *c* were observed in 1955 before the chromosome map was completed and are not accurately located.

FIGURE 3

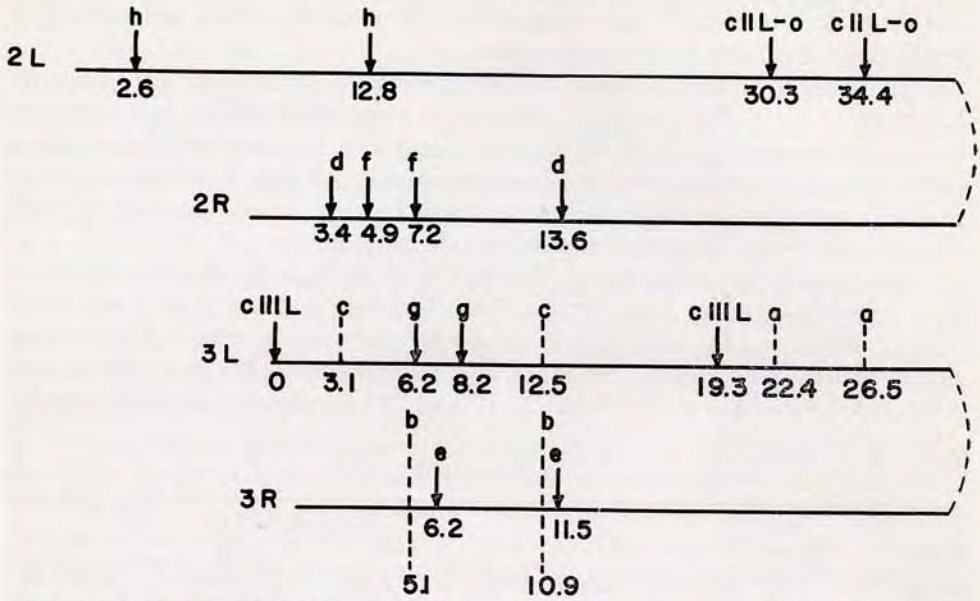


FIG. 3. An illustration, drawn to scale, of the inversions recovered from the wild population samples, each break being located with a number. Digits to the left of the decimal point give the map interval. Digits to the right give the band closest to the break, counting bands from left to right within the interval. All break points are located to within ± two map bands except the breaks for inversions *a*, *b* and *c*, shown dotted, which are located within ± one map interval.

DISCUSSION

A. *The Control X-ray Induced Rearrangements*

There are a number of disadvantages in using the salivary analysis technique in radiation studies. Primarily, radiation is administered to adult flies, but the scoring of damage is postponed to the following generation of larvae. During this interval, chromosome breaks may restitute or reunite in new combinations.

Developing germ cells in male Drosophila are protected from germinal selection after the spermatogonia enter the synchronously dividing cyst stage (Pontecorvo, 1944; Auerbach, 1954). Breaks in mature sperm do not heal and rearrangements are not effected until after fertilization (Kaufmann, 1941, and others). Recovered aberrations are restricted to those which prove viable in the developing larva and, therefore, may not reflect accurately the radiation-induced break frequency and distribution.

However, the salivary analysis technique does permit a comparison of chromosome arms with regard to recovered breakage. Further, the relative frequencies of recovered rearrangements indicate the kinds of chromosome damage that are successfully transmitted after irradiation.

The apparently random break distribution recovered in *D. ananassae* is in agreement with that found by Bauer *et al.* (1938) among *D. melanogaster* salivary chromosomes. In both studies the number of breaks per chromosome was

roughly proportional to salivary and mitotic lengths. Bauer's data, although too scant to be statistically significant, indicated preferred breakage at the proximal and distal ends of certain chromosome arms. More data are required in order to resolve these speculations for either species.

A more detailed study of the *D. melanogaster* X-chromosome was made by Kaufmann (1946). He was able to show that recovered breakage is not random in this chromosome but is found most frequently in the heterochromatin. Data presented above indicate that the heterochromatic 4th and Y chromosomes are probably being broken in proportion to mitotic lengths. It is not possible to determine absolute break frequencies in the two chromosomes.

Theoretical expectations for the distribution of multiple-break rearrangements have been advanced by Bauer *et al.* (1938), Haldane and Lea (1947) and Baker (1949). Bauer and his collaborators compared inter-arm to intra-arm rearrangements by assuming random breakage and recombination. For five chromosome arms, a 4:1 ratio was expected for "1, 1" and "2" rearrangements, respectively.

TABLE 7 (III)

Stock	No. males	1L 1R	Chromosome Arm 2L	2R	3L	3R
Bikini 1955, 1956	171	+	CII L–O=22/89,30/82	*f*= 0,2/82	*a*=1/89,0 *g*=0,1/82	*e*=0,2/82
Majuro 1955, 1956	93	+	CII L–O= 7/47,0	*d*= 1/47,3/46	CIII L=1/47,0 *c*=1/47,0	*b*= 1/47,0
Ponape 1956	8	+	CII L–O= 3/8 *h*=4/8	*d*= 4/8	+	+.
Rongerik 1956	7	+	+	+	+	+
Rongelap 1956	5	+	+	+	+	+

Table 7 lists the frequency of each rearrangement (all inversions) detected in the Marshall Island samples. The expression: *a*=1/89,0 means that inversion *a* was found in 1 of 89 larvae examined from the 1955 Bikini sample, but was not detected in the 1956 sample.

Their results and similar results by Helfer (1941) and Koller and Ahmed (1942) are listed in the last three columns of Table 3. The expected ratio for six arms of chromosomes 1, 2 and 3 in *D. ananassae* would be 5:1.

These theoretical ratios have been criticized by Pontecorvo in Koller and Ahmed (1942) and by Baker (1949) because contributions from dominant lethal chromosome arrangements to the F_1 generation were not considered. Preliminary calculations by L. S. Lockingen (personal communication) indicate that the expected proportion of "1, 1" to "2" rearrangements is greater than 2:1 for doses inducing a mean of 2 breaks per recovered rearrangement, and increases rapidly with dosage. It is impossible to calculate the true expected ratio at this time because the efficiency of X-ray induced breakage in sperm is unknown.

Information presented above and inspection of Table 3, however, show that the ratios recovered in this and previous studies for "1, 1" versus "2" rearrangements are consistently below 2:1. As would be expected from the delay in healing of spermatid chromosome breaks, recovered rearrangements from different spermatogenic stages show the same low ratio (Table 4). Thus, *D. ananassae* behaves like other Drosophila in that the recovered rearrangements after X-raying sperm

do not follow a random expectation. This may be due to preferred patterns of breakage and recombination, or to differential viability conferred by rearrangement types.

Variability in the rate of sperm emission causes uncertainty in interpretation of induced damage versus germ-cell-cycle curves. This was overcome in part by Khishin (1955) who irradiated larvae and pupae of *D. melanogaster*. He determined cytologically the most advanced stage in spermatogenesis at irradiation, and correlated this information with the variation of mutation rate from sperm recovered in future test intervals. Premeiotic stages were further identified by recovered bunches of like mutations.

He found that mutation rate is low up to the onset of meiosis and increases suddenly in meiosis. It rises to a peak in late spermatids and then drops to a level below meiosis in mature sperm. This is in close agreement with the results of Auerbach (1954) who also mentioned that rearrangements reach their peak simultaneously with recessive lethals.

Due to variation of sexual activity among the males, all of the intervals shown in Figure 2 may have received contributions from more than one stage of sperm development. For these experimental conditions, however, Figure 2 does show contributions in each post-irradiation interval from stages of widely differing sensitivity. The 48-hour interval following first sperm elimination from young males already contains contributions from more sensitive stages. By the twelfth day of adult life chromosome damage was not recovered in larval samples.

These curves are in close agreement to those obtained by Alexander and Stone (1955) who measured recovered translocation frequency from *D. virilis* spermatogenesis. The male germ cell cycle is more rapid in the shorter lived *D. ananassae*, but the variation of sensitivity within stages is similar in both species.

B. *The Analysis of Wild Populations*

From a study of natural populations of *D. ananassae* near Japan, Kikkawa (1938) described one reciprocal translocation and five paracentric inversions. Each arm of the second and third chromosomes showed at least one inversion and two of the inversions involved more than half of their chromosome arm. According to Freire-Maia *et al.* (reported in the discussion following the article by Stone, 1955), Brazilian populations have yielded at least 19 different paracentric inversions, one translocation, one deletion and two transpositions. This shows *D. ananassae* to contain a highly polymorphic chromosome pool but not unusually so. See Stone (1955) for comparisons. In addition, Freire-Maia reported finding five different pericentric inversions, a very high frequency.

We cannot accurately estimate the levels of direct radiation and subsequent fall-out radiation to which Drosophila populations in the Marshalls were subjected. Bikini has received radiation from an unknown number of "atomic" tests and from the thermonuclear test in March 1954 and other tests in 1956. Figure 2 indicates that numerous rearrangements must have been induced in the germ cells of contemporary *D. ananassae* populations on Bikini. However, the number of recovered new rearrangements, peculiar to the island, was not appreciably higher from Bikini populations (4/171) than from control Majuro populations (2/93).

It is unlikely that test explosions ever destroyed the Bikini *D. ananassae* population so that our samples were taken from an immigrant population. Bikini is about 100 miles from Rongelap, the closest *D. ananassae* source and, moreover, an apparently equivalent fauna was observed on the island at each collection.

It is possible that the *D. ananassae* population was regenerated mainly from the primary sex cells of larvae and pupae, blast exposed adults being largely sterilized. Figure 2 shows that spermatogonia are comparatively resistant and transmit few rearrangements at the X-ray levels of the control. However, post-irradiation flies were certainly subjected to beta, gamma and alpha radiation from fallout deposited on vegetation and from contaminated food.

The elimination of induced rearrangements could be effected by chance and selection pressure. Translocations, deletions and other changes that lead to future aneuploid gametes would be lost rapidly. Paracentric and small pericentric inversions, which this control study indicates were induced in high proportion, do not have this mechanical disadvantage.

Such inversions may have been eliminated due to harmful effects associated with the rearrangement *per se*, that is, with break points and position effects. In addition, through crossing over suppression, inversions may have become disadvantageous by preserving groups of radiation induced deleterious mutations. Ordinarily, fortuitously grouped detrimental genes are separated by meiotic crossing over to yield combinations of differing selective value. The variability of the population is thus enhanced and individual mutations proven against various genetic backgrounds. Newly formed inversions or any that had been present in low frequency in the pre-irradiation population would rarely become homozygous so as to allow this recombination. Chromatid exchange on either side of a heterozygous inversion will usually be reduced (Sturtevant and Beadle, 1936), adding to the effect for small inversions. Selection against such rearrangements would represent a mechanism, in Drosophila and all other forms exhibiting crossing over, for the elimination of inferior gene associations.

Certainly, very few if any rearrangements capable of establishment through positive selective value have been recovered from these irradiated populations.

SUMMARY

A. *The Control X-ray Induced Rearrangements*

Drosophila ananassae males were X-rayed at dosage levels of 1000 and 1500 r. Recovered salivary chromosome aberrations were scored in successive broods of F_1 larvae.

Analysis of 321 breaks indicated that recovered break points were distributed at random along chromosome arms in rough proportion to salivary and mitotic arm lengths.

A higher proportion of intra-arm to inter-arm rearrangements was recovered than would be expected from a hypothesis of random chromosome breakage and reunion.

The susceptibility of *D. ananassae* germ-cell chromosomes to X-ray damage was shown to vary during the course of spermatogenesis. Very few breaks were

recovered from premeiotic spermatogonia, more from mature sperm and the greatest number from stages of spermiogenesis.

A map of the salivary chromosomes of *D. ananassae* is included.

B. *The Analysis of Wild Populations*

Populations of *Drosophila ananassae* from radiation exposed and non-exposed Marshall Islands were sampled for chromosome rearrangements. Four F_1 female larvae were examined from each of 284 sample males. The number of previously undescribed rearrangements recovered from both irradiated and control islands was low.

Examination of laboratory conducted experiments and field conditions indicates that numerous rearrangements were probably induced in the radiation exposed populations and subsequently eliminated. Factors contributing to the negative selective value of chromosome aberrations are mentioned. A possible role for inversions in trapping unfavorable gene sequences through crossing-over suppression, thus leading to their elimination, is suggested.

Section IV

VISIBLE MUTATIONS IN POPULATION SAMPLES OF DROSOPHILA ANANASSAE FROM BIKINI, MAJURO, AND PONAPE

Warren P. Spencer

Various methods have been employed by different workers in the analysis of population samples of Drosophila for both visible and lethal mutations. These have been reviewed by the present author (Spencer, 1947). For autosomal lethals the best method is to cross the wild fly to a testor stock containing a genetic marker and crossover suppressor in the chromosome which is being studied for the incidence of lethals in natural populations. By the proper breeding technique, which will not be outlined here, all lethals present in that particular chromosome in the population sample can be determined. At first glance this might seem to be the best way to analyze populations for visibles. However, many wild flies will contain both a lethal and a visible in the same chromosome; therefore the visible will never be observed by the above method, as all flies carrying the visible in homozygous form will die because of the lethal present.

On the other hand, if one uses either of the two methods outlined below visibles may be recovered even if there is a lethal in the same chromosome, unless this lethal is sufficiently close to the visible so that few crossovers occur. The methods depend upon the recovery of a certain percent of the visibles and an estimate of the remaining visibles present by simple statistical analysis.

Method 1. A pair of wild flies may be mated and from the F_1 of this mating a mass culture may be made and the F_2 generation inspected for visibles. Theoretically, if one of the P_1 parents carried a recessive autosomal visible in heterozygous form then in the F_2 one out of 16 of the flies should show this visible. Unfortunately the author has found from considerable experience that the F_1 mass mating method is not very reliable. The F_1 flies may not form an actual random

mating population; furthermore some visible mutant types may have a viability much lower than wild type or may be linked to a lethal or semi-lethal.

Method 2. A pair of wild flies may be mated and from the F_1 any number of pair matings may be made. The F_2 flies emerging may be examined for visible mutations. Theoretically these F_2 visibles should appear in one-fourth of the flies in any culture in which both parents are heterozygous. But even if such a visible is linked to a lethal or has low viability it will still show up in a larger proportion of the F_2 flies than with the mass mating method, and therefore will be less likely to be overlooked in scoring visibles. The number of visibles present in the population sample can be estimated rather accurately for comparative purposes, if some consistent method of scoring is used. The author has arbitrarily set up the criterion of the appearance of three flies in an F_2 culture, showing the same marked deviation from the wild-type as evidence that a visible was present in one of the P_1 parents. If this criterion is used consistently, then samples from different populations may be compared objectively. Some of the visibles which appear are sterile in one or both sexes, and even though further test of such mutants would be possible, the comparison of population samples may be made with some accuracy without this laborious check. In the analysis below such elaborate tests were not run on sterile mutant types. In any case the estimates for the several populations are minimum estimates. The actual number of visibles present was probably somewhat higher than that reported. In those F_2 cultures where only a few flies appeared no correction factor was introduced for the chance of a visible not appearing even if present in heterozygous form in both F_1 parents. As the criterion of analysis remained the same for the three population samples it would seem that the comparison of these populations by the method used presented a consistent and fairly accurate picture.

Difficulties in the Analysis of Drosophila ananassae Populations

This author has analyzed population samples of *D. melanogaster*, *simulans*, *busckii*, *hydei*, *mulleri*, *immigrans*, *robusta*, *subquinaria*, *suboccidentalis*, and *montana*. In none of these has he found the problems of culturing the flies as difficult as in *D. ananassae*. But unfortunately for laboratory study this is the species which is found in largest number in the Marshall and Caroline Islands, and therefore the species which must be studied in this investigation. *D. ananassae* is a fragile fly, easily mired in the food medium. We have found that it can be cultured better in pair matings in small vials, but even so it has a lower fecundity than any of the above mentioned species.

Flies collected in the Marshall and Caroline Islands in the summers of 1955 and 1956 were sent first to the Texas laboratory and then shipped on to the Wooster laboratory, either as F_1 cultures from pair matings or as the original wild flies. Many of these F_1 cultures gave no offspring at all. Small pupae were formed, but no adult flies emerged. Well over half of the P_1 pair matings made from wild flies sent to our laboratory gave no offspring. Many of these became mired in the food or were apparently sterile. In the tables below the varying numbers of F_1 cultures reported from different P_1 parents were due to the failure of many F_1 pair matings to reproduce, and not to varying the number of these

cultures set up. The failures of P_1 and F_1 cultures did not appear to be markedly different in the three population samples.

If only one F_1 pair mating is made from each P_1 pair of flies, then one-fourth of the autosomal visible recessives present in the flies should be recovered; for two F_1 pairs 44% of the mutant types should be recovered; for three pairs 58%; for four pairs 68%; for five pairs 76%; for six pairs 82%; and for seven pairs 87%. In the tables below the number of visibles present in the population sample analyzed has been estimated, on the basis of the above figures, from the visibles actually recovered.

Results of the Analysis of Population Samples

In Tables 8, 9, and 10 the results of the analysis of population samples are given respectively for Bikini, Majuro, and Ponape. The Bikini population in the test area had been subjected to radiation, while the populations from Majuro in the Southern Marshalls and from Ponape in the Eastern Carolines serve as controls, hundreds of miles from the test area and therefore subject to very little radiation.

Table 8 shows an estimated 128 visible autosomal recessive mutations present in the population sample of 113 pairs or 226 flies from Bikini, an average of .566 mutants per fly tested. Table 9 shows an estimated 35 visibles present in the sample of 22 pairs or 44 flies from Majuro, an average of .795 mutants per fly tested. Table 10 shows an estimated 40 visibles present in the sample of 38 pairs or 76 flies from Ponape, an average of .529 mutants per fly tested. When the two small control samples are combined, out of a population sample of 120 flies it is estimated that 75 autosomal recessives were present, an average of .625 mutants per fly tested.

TABLE 8 (IV)

Number of Visibles Recovered and Estimated Number Present in Sample of Flies from Bikini

	P_1 Pairs of Wild Flies	F_1 Pairs per P_1 Pair	No. Mutants Recovered in F_2	No. Mutants Estimated Present
	48	3	25	43
	35	2	18	41
	30	1	11	44
Totals	113 pairs		54	128

TABLE 9 (IV)

Number of Visibles Recovered and Estimated Number Present in Sample of Flies from Majuro

	P_1 Pairs of Wild Flies	F_1 Pairs per P_1 Pair	No. Mutants Recovered in F_2	No. Mutants Estimated Present
	18	3	14	24
	3	2	3	7
	1	1	1	4
Totals	22 pairs		18	35

TABLE 10 (IV)

Number of Visibles Recovered and Estimated Number Present in Sample of Flies from Ponape

	P_1 Pairs of Wild Flies	F_1 Pairs per P_1 Pair	No.Mutants Recovered in F_2	No. Mutants Estimated Present
	7	7	10	11.47
	9	6	7	8.54
	8	5	6	7.90
	3	4	1	1.47
	3	3	0	0.00
	5	2	3	6.82
	3	1	1	4.00
Totals	38 pairs		28	40.20

Discussion of the Results of Analysis

While the samples analyzed are disappointingly small, they are almost certainly of sufficient size to have shown large differences in the visible mutation load carried by the populations of control and test areas, had such large differences been present. Actually the visible mutation load in the population sample from Ponape was slightly but not significantly smaller than that from the Bikini sample, but in the other control population sample from Majuro the visible mutation load was higher, though possibly not significantly higher, than that from Bikini. The combined population samples from control areas give .625 mutants per fly tested, while that from Bikini gives slightly less, .566 mutants per fly tested.

Since it has been well established by a very large number of laboratory investigations on various plant and animal forms that radiation produces mutations, the apparently anomalous finding here reported on visible mutation loads carried by a natural population subjected to radiation and those in control areas requires some explanation.

In a very extensive study of mutation rate in controls and in flies subjected to low dosages of radiation Spencer and Stern (1948) report a ratio of 5.2 lethals: 1 visible in the X chromosome of *D. melanogaster*. Muller (1954) reviews the work on semi-lethal and deleterious mutations and gives a ratio of 3 to 5 semi-lethals and deleterious : 1 lethal. The overall ratio would seem to be approximately 1 visible : 5 lethals : 20 semi-lethals and deleterious. In addition there are a large number of mutations, invisible morphologically, but which reduce fertility and fecundity. While visible mutations have advantages for certain types of study, when one is investigating samples of control populations and populations subjected to low dosages of radiation, at least low in comparison to standard laboratory experiments on mutation rate, one may well expect to find significant differences in the mutation load of lethals, semi-lethals, deleterious mutants, and mutants affecting fertility and fecundity, even when the visible mutation picture shows no significant differences. It must be remembered that by the best estimates not more than one visible mutation occurs to 30–50 of the other types mentioned. These facts need to be kept in mind in any apparent discrepancy between the data on visible mutations and the data in other sections of this report.

However, in any investigation of the mutation loads carried in natural populations some attention must be given to the breeding structure of these populations, their size at any given time, and possible fluctuations in population size.

It might be thought that on the atoll islands of the Marshalls, if a population of some species of Drosophila became established, it would show little fluctuation in population size from season to season and from year to year, owing to the uniform climate throughout the year, with some food plants bearing fruits at all seasons. This is not necessarily the case. In the summer of 1955 a small, but widespread, population of *D. ananassae* was found on one of the larger islands of Rongerik Atoll. The fly was collected on the ripe and rotting fruits of *Guettarda speciosa*, a large shrub quite abundant on this island. These fruits were white berries about two centimeters in diameter. At approximately the same time in the summer of 1956 four collectors, working intensively, were unable to collect a single fly of this species, although the fruits were again quite abundant. *Pandanus* and *Morinda*, two other plants whose fruits were found on other islands to attract these flies, were also present on this island, but in very small numbers. Thus the Drosophila population of this island had undergone marked fluctuation in size in a year's time.

On Bikini *D. ananassae* were collected on fruits of *Papaya*, *Morinda*, and *Pandanus*. However, the population of this fly was quite small here, probably only a few thousand individuals, a rough estimate on the basis of several day's collecting and the difficulty of securing an adequate sample for analysis. Such a small population, particularly if it fluctuated in size from season to season, might be expected to lose by chance many of the visible mutant factors which had arisen either from radiation or spontaneously. Conversely one might expect a high incidence of a few visibles. Of the 54 visibles actually recovered from the Bikini sample by inbreeding in the F_2 generation 11 were rough eye mutants and 11 were small bristle mutants. It seems likely that some of these were actually the same mutant, spread through the population by random drift, although no tests for allelism were made. Thus for a given small population, particularly one fluctuating in size, the visible mutation load might be increased or decreased markedly by random drift.

On the other hand, for the 30–50 fold more abundant load of lethals, semi-lethals, lowered viability and sterility mutants in the same population, the effect of random drift on overall numbers of these mutants would be much less marked. Random drift would, of course, be expected to have much the same effect on a sample of these mutant types comparable in size to that of the total number of visibles arising in the population. Furthermore, if exposure of different segments of the population to radiation or other mutating factors has been uneven, then one might expect the visibles to be more abundant in chromosomes carrying lethal, semi-lethal and sterility factors, and therefore often not be recovered even when present.

In any apparent discrepancy in the findings on visibles and lethals, semi-lethals and other deleterious factors in the populations the above facts must be borne in mind. Certainly the control populations on Majuro and Ponape were many times the size of the Bikini population and would presumably not be subject so markedly to the effects of random drift. However, of the 28 mutants actu-

ally recovered from the small Ponape sample six were a distinct type showing tiny scutellar bristles, and probably representing a widespread mutant which at some time in the past had become spread through the populations of this island by random drift. This particular mutant was not recovered from either Bikini or Majuro and therefore is probably not a species specific type such as has been recorded for certain other species of the genus.

The Visible Mutants Actually Recovered

The primary purpose of this investigation was to make a comparative quantitative study of the numbers of visible mutations carried in the population samples. However, it seems worthwhile to put on record a brief description of all the visibles recovered and a somewhat more elaborate description of certain of these visibles. These data may prove of value to others working on population samples of *D. ananassae* from these islands or on other populations of this and other species of Drosophila.

Table 11 includes a check list of all visibles recovered in the F_2 generation from the Bikini, Majuro, and Ponape samples. Mr. Richard Dickerman, a senior stu-

TABLE 11

Check List of Mutants Recovered from Bikini, Majuro, and Ponape Samples in F_2 Generation

Bikini Mutants Affecting the Eye
bronze—eye color
brown—eye color distinct brown, accompanied by very minute bristles
brownish—eye color slight brown
burnt—an area in the eye has a burnt appearance
peach—eye color recovered three times, not necessarily alleles
purplish—eye color
rough—facets of eye irregular, recovered seven times
very rough—extremely rough eyes accompanied by small bristles, recovered three times
rough-coarse—eye rough; wings coarse textured, margin nicked
mosaic—eyes with mosaic spotting; wings coarse, L4–5 short
Bikini Mutants Affecting the Wings
abrupt—L4 and L5 short
short—L3 and L4 short
extreme net—see text for description
crossveinless—crossveins missing
broken—posterior crossveins broken; recovered four times
arch—wings convex dorsally, good expression
club—wings club-like, very abnormal
crumpled—wings crumpled and folded
eagle—wings held out at sides and elevated
folded—wing edge folded up
nick—cut in margin of wing
pads—wings not unfolded
shredded—wings light textured, wrinkled and shredded
warped—wings warped and dark in color
Bikini Mutants Affecting the Bristles
erect—post scutellars stand erect
fuzzy—see text for description
ruffled—hairs on thorax ruffled

silver—some dorso-centrals and scutellars silvery; variable
small—bristles reduced in size; ten recoveries, probably not all alleles, though tests were not made
spineless—bristles very tiny; eyes rough
stubby—bristles short and stubby

Bikini Mutants Affecting the Legs

twisted—legs twisted and misshapen

Majuro Mutants Affecting the Eye

brownish—slight eye color deviation from wild; recovered twice
dubonnet—similar to mutant eye colors in *simulans* and *immigrans*
dull—eye color slightly darker than normal
peach—eye color
sepia—very dark eye color

Majuro Mutants Affecting the Wings

blister—blisters on one or both wings
roofed—wings held roof-like, sloping
branch—a branch on posterior crossvein
extra—small bit of vein between 2L and 3L near wing margin
net—medium expression of net veins; recovered twice
triangle—small vein connecting 2L to margin near wing tip; recovered four times

Majuro Mutants Affecting the Bristles

small—bristles reduced in size
vortex—hairs on thorax in whorls

Ponape Mutants Affecting the Eye

brownish—slight brownish eye color
cinnabar—brilliant red eye color, recovered twice
dark—eye color slightly darker than normal
mahogany—dark reddish eye color, recovered twice
peach—eye color, recovered twice
sepia—very dark eye color
translucent brown—very easily classified eye color
rough—facets of eye irregular
rough-short—eyes rough textured; all bristles short
rough-shrunken—small rough eyes; wings shrunken

Ponape Mutants Affecting the Wings

net—extra venation, slight to medium expression
roof—wings held roof-like, sloping
roof-inturned—wings held roof-like; hairs on abdomen turned in
squatty—see text for description
warped—wings small and dark, warped downward

Ponape Mutants Affecting the Bristles

fuzzy—see text for description
tiny scutellars—scutellar bristles very much reduced in size; recovered six times

Ponape Mutants Affecting Body Color

ebony—dark body color, recovered three times; see text for description

Note: The names applied here are meant to be descriptive and are not to be considered as implying homologies with mutants of the same name in other species. No symbols have been assigned as the stocks are not being maintained. This check list is included to give an overall picture of mutants recovered from Bikini, Majuro, and Ponape.

dent at the College of Wooster was assigned the project of extracting mutants from the F_2 Bikini cultures by further inbreeding. By small mass matings he reared F_3, F_4, and in some cases still later generations of many of these Bikini flies and picked up many of the mutants which by the nature of the F_2 analysis were not then recovered. In Table 12 the mutants which Dickerman recovered in this way are recorded, 34 in all of the 74 mutants estimated to be present in the P_1 Bikini flies and not picked up in the F_2 analysis. It should be noted that Dickerman did not work on all of the F_2 cultures from Bikini and that by the mass mating technique he would not be expected to uncover all mutants present in the

TABLE 12

Check List of Mutants Recovered from Bikini Sample in F_3, F_4, and Subsequent Generations

Mutants Affecting the Eye
maroon—reddish-brown eye color difficult to classify
brown—eye color garnet brown
rough-1—facets of compound eye irregular
rough-2—facets of compound eye irregular
rough-3—facets of compound eye irregular
rough-4—facets of compound eye irregular
Mutants Affecting the Wings
roof—wings sloping in position, normal overlaps
eagle-rotated—see text for description
thickened—wings heavy and coarse textured
opaque—wings dull colored and opaque
inflated—two plates of wing separated and containing fluid
blistered—fluid filled blister on one or both wings
incised—wing margin notched between L2–L3 and L4–L5
scalloped—wing margin has cut to beaded appearance
xasta-like—see text for description
curled—see text for description
lance—wings about one half normal width and convex dorsally
upturned—see text for description
drooping—wings extended at right angles, down curved; small body
rumpled—wings rumpled
bent—tip of wing bent
pinched—wings slightly rumpled
crumploid—wings not fully expanded
rippled—wings slightly rumpled
rumploid—wings rumpled; normal overlaps
abrupt-1—L4 and L5 short
radius incompletus—L2 short
veinlet—see text for description
delta—L2 divides into two veins before reaching margin
Mutants Affecting Bristles
barbed—tips of posterior scutellars bent
broken posterior scutellar—post-scutellars broken or missing
missing—posterior scutellars missing
Mutants Affecting the Legs
bent tarsi—tarsi bent; expression variable
no-joint—see text for description

Note: The names applied here are meant to be descriptive and are not to be considered as implying homologies with mutants of the same name in other species. No symbols have been assigned as the stocks are not being maintained. This check list is included to give an overall picture of mutants recovered from the Bikini sample.

material on which he did work. Of course a very few of the mutants which he extracted may have arisen in the laboratory, but almost certainly the vast majority of them were present in the original population sample collected on Bikini.

Descriptions of Some of the More Interesting Mutants Recovered

no-joint—Bikini—(Dickerman)—autosomal recessive. This was certainly the most interesting and unique mutant recovered. All five tarsal joints are missing from all legs. The terminal claws present normally at the distal end of the fifth tarsal joint are present on the distal end of the tibia. Males are apparently completely sterile, but from females a few offspring were secured by mating them to wild-type males. No mutant of this type has been recorded for any other species of Drosophila. It is certainly not a homologue but may be compared to *four-jointed* in *D. melanogaster*, two alleles of which were found by Schultz and Ives and described by Bridges and Brehme (1944), and to *rotund* in the same species, alleles of which have been found by Glass and reported in Bridges and Brehme (1944) and by Spencer (unpublished). In *rotund* there are three joints in the tarsus. This author has found a mutant, *two-joint*, in *D. mulleri*, in which the three middle joints of the tarsus are missing, and Krivshenko (personal communication) reports the same type of mutant in *D. busckii*.

ebony—Ponape—(Spencer)—autosomal recessive. Recovered from three different pairs of Ponape flies, this mutant darkens the spiracle sheaths of the larva; pupa case is dark; in adult flies body, legs, and wings are very dark. There seems little doubt but that this is a homologue of *ebony* in chromosome III of *D. melanogaster*, many alleles of which have been reported in Bridges and Brehme (1944). *Ebony* has also been reported by Spencer (1949) in *D. hydei* and by Chino (1936a) in *D. virilis*. Moriwaki (unpublished) also reports finding *ebony* in the second chromosome of *D. ananassae*, but as Sturtevant and Novitski (1941) have pointed out this chromosome is actually homologous to chromosome III of *D. melanogaster*.

eagle-rotated—Bikini—(Dickerman)—autosomal recessive. The wings are held out at an angle of 90 degrees from the body and rotated through 90 degrees on the long axis. Thus, seen from above, the wings appear as two narrow bars held at right angles to the body. No such mutant has been been reported in any other species.

curled—Bikini—(Dickerman)—autosomal recessive. The wings show the posterior one-third curled upward and are held somewhat divergent, with posterior scutellars sharply crossed. Apparent homologues of this mutant have been found in *D. melanogaster*, *simulans*, *psuedoobscura*, *subobscura*, *montium*, and *mulleri*.

up-turned—Bikini—(Dickerman)—autosomal recessive. The posterior fourth of the wing is upturned. The wings are held out at a 30 to 45 degree angle. Acrostichal hairs are brownish, giving the thoax an ashen appearance. Scutellars are often disarranged; deep depressions between tergites of abdomen; fertility greatly reduced. A mutant with marked manifold or pleiotropic effects.

veinlet—Bikini—(Dickerman)—autosomal recessive. Longitudinal veins 2, 4 and 5 quite short; posterior cross-vein often broken or missing. Apparently a

homologue of mutants of the same name and description in *D. melanogaster* (Bridges and Brehme, 1944), in *D. pseudoobscura, affinis,* and *willistoni* (Sturtevant and Novitski, 1941), and in *D. virilis* (Chino, 1936b).

fuzzy—Bikini and Ponape—(Spencer)—autosomal recessive. Hairs on the wing margin stand out at a wide angle; hairs on the abdomen are irregularly arranged; dorso-central bristles tend to turn in. A mutant of this description was discovered once from the Bikini sample and once from the Ponape sample and is similar in phenotype to *fuzzy* in Chromosome II of *D. melanogaster* and to *frizzled* and *inturned* in Chromosome III of this species. Mutants of similar type have also been described under the name *irregular* in *D. virilis* by Chino (1929) and in *hydei* by Spencer (1949), and have been found in *mulleri* and *simulans* by Spencer (unpublished).

extreme net—Bikini—(Spencer)—autosomal recessive. Wings show an extreme net venation in homozygous form; some flies show slight net venation when heterozygous. Similar mutants under the name of *plexus* or *net* have been described in *D. melanogaster, simulans, peseudoobscura, affinis, virilis, robusta, subobscura, ananassae* and *immigrans.* In fact this is one of the commonest parallel or homologous autosomal recessives to be found in various species of the genus. A net vein mutant of medium to slight expression was also found twice in the Majuro population sample.

squatty—Ponape—(Spencer)—autosomal recessive. Wings are broad and short; cross-veins very close together; legs short and thick; thorax short. This is a good parallel of *dachsous,* a common second chromosome mutant in *D. melanogaster* (Bridges and Brehme, 1944), *dachsous* in the fourth chromosome of *D. virilis* (Chino, 1929), and *squatty* in the fourth chromosome of *D. hydei* (Spencer, 1949).

xasta-like—Bikini—(Dickerman)—autosomal recessive. Wings shortened and very deeply notched in region of second longitudinal; this mutant is similar in appearance to the dominant, *Xasta,* associated with a II–III translocation in *D. melanogaster* (Bridges and Brehme, 1944).

The Autosomal Eye Colors

In a list of 71 autosomal visibles in *D. ananassae* compiled from reports of Moriwaki, Kikkawa and others in *Drosophila Information Service* and from the published papers of Moriwaki (1935, 1938) only three autosomal eye colors are reported, *cardinal* in Chromosome II, *Plum* in Chromosome III and *purploid,* chromosome undetermined. But from the small Majuro sample of 18 autosomals recovered five distinguishable eye colors were found: *brownish, dubonnet, dull, peach* and *sepia.* From the small Ponape sample of 30 autosomals recovered six distinguishable eye colors were found: *translucent brown, brownish, cinnabar, dark, mahogany,* and *peach.* From the 88 autosomals recovered from the Bikini sample seven distinguishable eye colors were found: *bronze, brown, brownish, burnt, maroon, peach,* and *purplish.* The *translucent brown* and *cinnabar* of Ponape and the *dubonnet* and *sepia* of Majuro were sufficiently different from the Bikini eye colors to be distinguishable, so that in all at least eleven distinguishable eye colors were found. It seems likely that some of the other cases were mutants

at different loci, though not easily distinguished phenotypically. Tests for allelism were not run, nor were the linkage groups determined.

With the number of known eye color mimics, where different loci give phenotypically identical mutant eye colors in *D. melanogaster*, it is useless to suggest possible homologies of these eye colors found in the samples analyzed. The names have been used as descriptive and are not to imply supposed homologies with mutants of the same name in *D. melanogaster* or other species. The overall phenotypic picture of the mutants recovered in this study is similar to that for numerous other species of this genus.

SUMMARY

Wild *Drosophila ananassae*, collected on Bikini in the summer of 1955, were inbred for two generations by pair matings. On the basis of autosomal visible mutants recovered it was estimated that there were 128 such mutants present in the population sample of 226 flies, an average of .566 mutants per fly tested.

Similar procedure for flies collected on Majuro Atoll in the summer of 1955 gave an estimate of 35 such mutants present in the population sample of 44 flies, an average of .795 mutants per fly.

The sample of 76 flies collected on Ponape in the summer of 1956 and tested carried an estimated 40 such mutants, an average of .529 mutants per fly tested.

Many of the visibles in the Bikini sample, not recovered in the F_2, were subsequently found by further inbreeding the stocks.

Factors which might explain the fact that the apparent mutation load of visibles from the Bikini test area was no greater than that from the control areas were considered: such factors as population structure, random drift, and the masking effect of lethals and sterility mutants carried in the same chromosome with visibles.

Check lists of visibles recovered and fuller descriptions of some of the more striking visibles were included.

Section V

FACTORS AFFECTING VIABILITY IN DROSOPHILA ANANASSAE POPULATIONS FROM THE MARSHALL ISLANDS

Wilson S. Stone, Florence D. Wilson, June T. Neuenschwander and Thomas G. Gregg

The study of the genetic composition of populations of any species is always difficult, particularly when one wishes to measure factors which modify the evolutionary fitness of these populations. There exist in crossbreeding populations a number of genetic differences which influence their fertility, fecundity, and viability. Many of them are recessive, others cause such characteristics as heterosis. Some are lethal, others reduce the viability and in combination may act as lethal equivalents, while still others increase the viability singly or in combination. We tested for such factors by comparing results from crossbreeding and inbreeding.

PROCEDURES

The females captured on the islands were placed in individual food vials and allowed to lay. Usually they were fertilized in nature and produced progeny but sometimes no offspring were produced within a few days and a male captured at the same locality was added to the vial. Ordinarily a Drosophila female will produce offspring from one male but some mate more than once and a few of the offspring from these matings may have been half sibs although most were full sibs. [In the following tests we often use an abbreviation to represent a locality: Bikini = B, Rongelap = A, Rongerik = K, Majuro = M, Ponape = P.] The progeny of these isolated females were tested in pair matings in the following ways: (a) random matings between individuals from different parents in the same population, (b) several brother-sister matings between the sibs of those mated at random, (c) crosses between individuals from populations on different islands (for example, B × M). The progeny of (c) were inbred in brother-sister matings and their sibs were crossed to the progeny from other crosses in three-way (*eg.* MP × BP) or four-way crosses (*eg.* BA × MP). Finally the progeny of these three- or four-way crosses were tested by random matings. All of these tests were used to get information on the effect of the several genotypes and their heterozygous and homozygous combinations on fertility measured as percentage of pairs fertile, fecundity measured as average number of eggs laid per day per female, and viability measured as percentage of eggs laid that developed into adults. This is an absolute measure of viability against the upper limit of 100 per cent rather than a comparison of classes so it can be used to compare any tests. Ordinarily the egg count for the first day that eggs developed was not used in order to insure that the females were inseminated. Most of the tests were run at 77° F. (76°–78° F.) but a few in 1955 were run at 71° F. (70°–72° F.). The inbreeding and crossbreeding tests compare the range of effects of the different homozygous and heterozygous genetic combinations on characteristics vital to the species. As Spencer pointed out in Section IV, we miss some of the possible genotypes by the limited size of our tests but this is unavoidable. The nature of the tests involves a succession of generations for each year's collections. General experience has demonstrated that an appreciable part of the culture-to-culture variation in viability within any test will be environmental and due to variations in micro-flora, consistency of the medium and other factors. Some evidence on this point was provided by obtaining two or more cultures of eggs from the individual females on successive days; the pooled variation between such replicates will arise chiefly from such uncontrollable environmental differences.

RESULTS

The results of the tests are summarized in Tables 13 and 14 and Figures 4 through 13. Table 15 and Figures 14 through 16 show data from two other species, *Drosophila novamexicana* and *Drosophila hydei*, which are included for comparison.

As part of the routine, the eggs laid per day per female were recorded for the period during the egg development tests. There is no evidence for consistent or general differences for the different series for the two years. The heterozygous

females from population crosses did not lay more than females from the parent populations. There is no evidence for heterosis in egg laying. This agrees with the tests with *novamexicana* and *hydei* (Stone, Alexander, and Clayton, 1954).

There is a considerable variation in fertility, the proportion of pairs which produced offspring, in the populations of *ananassae* and in heterozygotes between them. Table 16 shows that the average fertility of all stocks available both years (A, B, K, M) was low in 1955 and improved slightly by 1956. The heterozygotes between populations were significantly more fertile than their parents and were alike for the two years. The F_2 averaged higher in fertility than the F_1, which must be due to the heterozygosity of their female parents. The differences imply a genetic basis for part of the sterility. In setting up pair matings between individuals within populations or their heterozygotes, such as three-way crosses, approximately equal numbers of parents were chosen from different parent cultures to avoid as far as possible any bias, that is, the more fertile matings did not contribute disproportionately to the flies chosen for the next series of matings.

The tests for factors influencing egg development proved satisfactory under laboratory conditions. In such a test it is not possible to separate point lethals from lethal combinations due to interaction or to an accumulation of genes which reduce viability. The data on egg counts and development are not given in detail. The zero egg hatch is not included since a great many of the females in pairs which produced no offspring laid eggs. All the pairs which produced no offspring are included in the sterile classes, Table 13. Table 14 gives the egg development results grouped in classes. These are one to nine per cent of the eggs developed, 10 through 19, etc. These are diagrammed by twenties in Figures 4 through 13 which give viability class profiles of the populations and their different combinations. In the figures, the per cent of females having from 1 to 19 per cent of their eggs develop are plotted as 10, those with 20 to 39 per cent as 30, etc. In these tests egg development from 80 to 100 per cent is considered normal. When less than 80 per cent of the eggs laid by the female develop, the flies are considered to carry one or more lethals or lethal equivalents. With a maximum upper limit of 100 per cent development, we cannot demonstrate supervital factors even though they may influence the viability of many gene combinations.

When brother-sister matings were made, we could determine how often their parents carried lethals. These were detected by the fact that at least one of the one to four brother-sister matings had one or more lethals or lethal equivalents; that is, less than 80 per cent of the eggs laid developed into progeny. These tests indicate that the original females, usually fertilized in nature but sometimes mated to males from the same lot, almost always produced offspring that carried one or more lethal equivalents. In 1955, over 90 per cent of the original pairs carried lethals, with Rongerik the exception (75.6% detected). In 1956, about 75 per cent of the pairs from Rongelap and Rongerik carried lethals, about 85 per cent of those from Ponape and Majuro, while only Bikini was extremely high with 96 per cent of the original pairs carrying lethals. Because of the small number of F_1 pairs inbred, the frequency of lethals and lethal combinations was underestimated (see Spencer, Section IV). The brother-sister matings provided tests for all autosomal lethals, lethal combinations, and cumulative subvital forces. Sex linked lethal factors would have been rapidly eliminated by natural selection.

TABLE 13

Egg Development on Inbreeding and Crossbreeding *Drosophila ananassae*

Cross ♀ × ♂	Total Pairs Tested	% Sterile	P_1 Females Tested* Number	% with lethal	Fertile Pairs Tested Number	% with lethal	Days eggs Counted	Eggs/day ♀	Egg Development Number	% Developed	% Based on Angular Transformations
1955 Tests											
1. Rongerik (K)	170	43.5	41	75.6	93	49.5	219	17.2	3760	71.3	75.4
2. Bikini (B)	500	27.2	117	98.3	297	91.2	716	29.9	21464	46.4	46.1
3. Rongelap (A)	216	57.4	44	90.9	90	83.3	227	17.9	4058	40.8	45.0
4. Majuro (M)	288	46.5	61	91.8	139	82.7	359	24.1	8640	56.6	56.3
5. B × M	101	51.5	..	..	43	69.8	79	29.9	2361	67.8	65.7
6. A × M	96	54.2	..	..	48	64.6	91	31.4	2856	64.3	65.3
7. B × A	91	48.4	..	..	39	64.1	77	33.3	2563	70.2	69.0
8. BM × BM	75	25.3	20	90.0	52	67.3	142	34.2	4853	62.5	65.0
9. AM × AM	79	31.6	23	95.7	51	76.5	137	31.8	4351	59.8	61.9
10. BA × BA	77	10.7	22	100.0	58	81.0	155	22.9	3560	52.4	56.6
11. BA × BM	87	25.3	..	..	94	31.3	162	32.8	5320	81.1	84.7
12. AM × BA	101	41.6	..	..	59	30.5	151	31.1	4703	84.3	86.4
13. BM × AM	102	49.0	..	..	48	14.6	125	31.8	3970	89.4	93.4
14. $(BA \times BM)^2$	112	33.9	..	..	73	40.5	188	22.3	4194	78.7	83.8
15. $(AM \times BA)^2$	94	26.6	..	..	69	34.8	165	28.3	4668	80.8	83.5
16. $(BM \times AM)^2$	106	20.8	..	..	83	47.6	196	32.8	6429	77.8	82.8
1956 Tests											
17. Ponape (P)	615	32.2	145	85.5	339	61.7	748	27.5	20532	67.6	72.0
18. P (different P_1's)	91	28.6	..	..	47	21.3	128	21.8	2791	88.3	89.8
19. Majuro (M)	495	45.1	106	85.8	217	70.5	569	20.9	11880	66.4	67.8
20. M (different P_1's)	110	40.0	..	..	55	38.2	146	23.7	3463	80.8	82.6
21. Bikini (B)	421	34.0	101	96.0	256	69.5	653	22.9	14953	60.3	63.4
22. B (different P_1's)	45	17.8	..	..	34	41.2	83	27.7	2303	75.4	79.6
23. Rongerik (K)	458	49.3	84	75.0	173	58.4	393	26.3	10343	68.2	73.5
24. Rongelap (A)	403	27.0	98	74.5	263	42.2	651	28.2	18349	78.9	83.7
25. M × P	123	49.6	..	..	52	59.6	118	13.6	1603	58.2	64.0
26. A × M	125	76.0	..	..	26	19.2	60	22.2	1329	84.6	88.5
27. M × B	142	36.6	..	..	76	50.0	188	23.9	4489	75.4	79.7

28. P × A	104	62.5	..	..	32	21.9	73	26.3	1918	84.9	90.0
29. B × P	138	43.5	..	..	74	52.7	184	33.9	6237	72.7	75.3
30. B × A	112	41.1	..	..	66	45.5	168	30.3	5091	76.5	78.6
31. AM × BP	211	36.5	..	..	124	17.7	366	23.6	8638	89.7	93.3
32. BA × MP	143	55.2	..	..	50	24.0	112	17.7	1987	87.6	89.0
33. MB × PA	70	61.4	..	..	23	4.3	45	16.6	748	91.7	95.4
34. AM × AM	90	44.4	21	81.0	47	59 6	133	21.1	2811	71.1	72.4
35. BA × BA	177	31.1	42	76.2	116	60.3	285	27.8	7930	70.3	72.8
36. MB × MB	215	32.6	54	92.6	143	81.8	415	21.0	8695	58.6	60.1
37. BP × BP	177	54.2	31	83.9	79	62.0	235	18.9	4442	68.4	
38. MP × MP	96	37.5	24	75.0	50	60.0	136	18.0	2454	67.4	
39. PA × PA	38	31.6	11	72.7	24	62.5	66	20.9	1379	74.7	
40. MB × BA	86	12.8	..	..	73	27.4	208	22.1	4587	85 2	90.7
41. BA × PA	85	38.8	..	..	39	33.3	98	16.8	1645	80.5	
42. MP × AM	68	36.8	..	..	43	27.9	109	17.4	1901	83.7	
43. AM × MB	75	54.7	..	..	29	31.0	72	23.2	1668	83.7	85.6
44. PA × AM	83	30.1	..	..	56	12.5	171	19.1	3261	89.1	
45. PA × MP	38	57.9	..	..	14	21.4	37	10.1	375	89.9	
46. AM × BA	75	12.0	..	..	65	24.6	194	20.1	3904	82 8	89.5
47. PA × BP	65	47.7	..	..	29	3.4	73	15.4	1124	91.7	
48. MP × BP	48	70.8	..	..	12	0	27	14.6	395	93.9	
49. BP × BA	87	39.1	..	..	50	20.0	124	20.8	2580	90.8	
50. (AM × BP)[2]	80	37.5	..	..	50	54.0	143	23.6	3381	67.4	
51. (BA × MP)[2]	85	34.1	..	..	50	44.0	123	25.6	3145	73.9	
52. (MB × PA)[2]	63	19.0	..	..	45	24.5	109	14.6	1591	84.2	
53. (AM × BA)[2]	100	30.0	..	..	59	55.9	120	16.3	1961	73.9	73.5
54. (PA × AM)[2]	88	18.2	..	..	64	20.3	178	17.6	3141	84.9	
55. (MP × BP)[2]	50	24.0	..	..	35	37.1	80	27.2	2177	78.8	
56. (BP × PA)[2]	60	26.7	..	..	42	42.8	124	27.5	3413	80.9	
57. (PA × MP)[2]	40	40.0	..	..	33	30.3	80	18.1	1445	78.8	
58. (AM × MB)[2]	86	22.1	..	..	60	33.3	171	13.8	2362	81.0	83.2
59. (BP × BA)[2]	120	15.8	..	..	94	30.8	249	21.9	5453	78.8	
60. (AM × BP)[2]	12	00.0	..	..	12	66.7	31	12.8	396	70.7	
61. (BP × MB)[2]	41	26.8	..	..	22	40.9	60	21.1	1267	77.4	
62. (MB × MP)[2]	39	35.9	..	..	19	15.8	45	16.6	748	83.8	
63. (BA × PA)[2]	113	16.8	..	..	89	33.7	216	24.3	5259	82.4	
64. (MP × AM)[2]	99	15.2	..	..	81	43.2	199	14.4	2875	79.8	
65. (MB × BA)[2]	108	29.6	..	..	71	36.6	184	14.9	2755	81.6	85.0

* Crosses in which values are given in this column are those in which F_1 brother-sister matings were made from pairs. All others were mated at random.

TABLE 14

Frequency of Classes with Different Egg Development Ranges on Inbreeding or Crossbreeding *Drosophila ananassae*

Cross ♀ × ♂	Number of Pairs with Indicated Percentage Range of Progeny Developing 1–	10–	20–	30–	40–	50–	60–	70–	80–	90–100
1955 Tests										
1. Rongerik (K)	1	0	4	6	4	6	15	10	24	23
2. Bikini (B)	9	25	45	43	52	36	34	27	17	9
3. Rongelap (A)	8	13	13	6	9	8	9	9	12	3
4. Majuro (M)	4	6	12	11	20	21	30	11	12	12
5. B × M	0	4	0	2	4	5	1	10	8	5
6. A × M	3	3	2	1	4	7	5	7	7	10
7. B × A	0	2	2	1	4	3	7	6	7	7
8. BM × BM	0	1	6	5	5	9	5	4	8	9
9. AM × AM	0	1	2	6	8	8	7	7	8	4
10. BA × BA	1	3	4	8	10	10	3	8	7	4
11. BA × BM	0	2	0	2	2	4	8	2	16	28
12. AM × BA	1	1	2	2	1	3	5	3	7	34
13. BM × AM	0	0	0	1	1	1	2	2	5	36
14. (BA × BM)[2]	0	0	1	1	5	5	10	8	11	33
15. (AM × BA)[2]	0	0	1	1	3	7	6	6	14	31
16. (BM × AM)[2]	1	1	0	2	4	8	11	13	9	35
1956 Tests										
17. Ponape (P)	2	4	17	30	24	38	50	44	50	80
18. P (different P_1's)	0	0	0	1	1	2	4	2	7	30
19. Majuro (M)	1	4	10	19	20	31	33	35	23	41
20. M (different P_1's)	1	0	3	0	2	4	4	7	10	24
21. Bikini (B)	13	11	16	15	26	36	30	31	36	42
22. B (different P_1's)	0	2	0	0	2	3	5	2	7	13
23. Rongerik (K)	1	6	3	8	12	25	20	26	28	44
24. Rongelap (A)	2	2	2	8	14	17	23	43	43	109
25. M × P	3	9	4	3	4	3	1	4	3	18
26. A × M	0	0	1	0	1	1	0	2	6	15
27. M × B	0	0	2	4	4	11	6	11	11	27
28. P × A	0	0	0	1	1	2	2	1	6	19
29. B × P	2	1	2	4	5	5	6	14	13	22
30. B × A	0	3	3	1	4	8	4	7	11	25
31. AM × BP	0	1	2	1	4	1	5	8	15	87
32. BA × MP	0	0	0	0	0	3	5	4	11	27
33. MB × PA	0	0	0	0	0	0	0	1	5	17
34. AM × AM	0	1	2	3	6	4	6	6	6	13
35. BA × BA	1	1	5	7	9	13	19	15	14	32
36. MB × MB	1	2	7	15	21	35	23	13	15	11
37. BP × BP	0	1	2	6	9	9	11	11	15	15
38. MP × MP	0	3	4	0	6	6	3	8	8	12
39. PA × PA	0	0	1	1	1	5	4	4	1	8
40. MB × BA	0	0	1	1	2	5	4	7	9	44
41. BA × PA	0	0	0	1	1	0	6	5	11	15
42. MP × AM	1	0	0	1	1	0	3	6	10	21
43. AM × MB	0	1	1	1	0	1	2	3	7	13
44. PA × AM	0	1	1	0	0	0	1	4	9	40
45. PA × MP	0	0	0	0	0	0	2	1	4	7
46. AM × BA	0	1	2	1	2	3	2	5	6	43
47. PA × BP	0	0	0	0	0	0	1	0	6	22
48. MP × BP	0	0	0	0	0	0	0	0	3	9
49. BP × BA	0	0	0	1	1	2	0	6	4	36
50. (AM × BP)[2]	0	5	1	2	6	2	3	8	10	13
51. (BA × MP)[2]	1	1	3	2	2	4	4	5	11	17
52. (MB × PA)[2]	0	1	0	0	0	3	1	6	13	21
53. (AM × BA)[2]	2	0	2	1	4	6	8	10	11	15
54. (PA × AM)[2]	0	0	1	3	0	1	5	3	13	38
55. (MP × BP)[2]	0	0	1	1	2	0	5	4	8	14
56. (BP × PA)[2]	0	0	0	0	1	3	6	8	13	11
57. (PA × MP)[2]	0	1	1	2	1	0	1	4	9	14
58. (AM × MB)[2]	0	0	2	2	2	1	8	5	15	28
59. (BP × BA)[2]	0	3	2	2	1	3	9	9	28	37
60. (AM × BP)[2]	0	0	0	0	0	4	0	4	2	2
61. (BP × MB)[2]	0	1	0	1	1	1	3	2	3	10
62. (MB × MP)[2]	0	0	0	0	1	0	2	1	6	9
63. (BA × PA)[2]	0	0	2	3	4	4	7	10	27	32
64. (MP × AM)[2]	0	1	1	2	4	4	8	9	10	36
65. (MB × BA)[2]	0	0	1	1	1	2	5	16	20	25

TABLE 15

Frequency of Classes with Different Egg Development Ranges on Inbreeding or Crossbreeding
Drosophila novamexicana and *Drosophila hydei*

Cross	Number of Pairs with Indicated Percentage Range of Progeny Developing											% show Lethal	Sterile	% Sterile	Average† % of Eggs Developed
	1–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–100	Total				
D. novamexicana															
1. 2351.1*	0	0	0	0	0	3	2	0	3	1	9	56	3	25	67.7
2. 2354.4*	0	0	0	2	0	4	5	5	2	10	28	57	1	3	73.1
3. 2358.6*	0	0	0	0	0	1	2	8	10	20	41	27	39	49	86.7
4. 8.6 × 8.6 random	0	0	0	0	0	1	2	1	2	17	23	17	27	54	91.7
5. 4 × 1	0	2	0	0	0	4	3	2	4	3	18	61	7	28	69.2
6. 4 × 6	0	0	0	3	0	1	3	1	5	10	23	35	9	27	79.8
7. 1 × 6	0	0	0	0	1	0	0	0	7	16	24	4	6	20	90.9
8. (4 × 1) F_1*	0	0	2	0	5	6	8	4	5	3	33	76	16	33	66.0
9. (4 × 6) F_1*	0	1	1	6	6	1	5	7	6	5	38	71	37	48	67.5
10. (1 × 6) F_1*	0	0	0	0	1	3	7	8	5	18	42	45	13	24	82.8
11. (4 × 1) F_2 a × b	0	0	2	1	0	1	1	1	2	2	10	60	5	33	70.7
12. (4 × 6) F_2 a × b	0	0	0	0	1	0	1	4	2	3	11	55	6	35	77.8
13. (1 × 6) F_2 a × b	0	0	0	0	0	0	1	3	5	8	17	24	10	37	85.5
14. (4 × 1) F_2 × 6 three-way cross	1	2	0	0	0	1	0	1	9	3	17	29	4	19	64.3
D. hydei															
15. 2354.5*	0	0	0	1	1	0	0	1	5	3	11	27	0	0	78.5
16. 2357.5*	0	0	0	0	2	2	4	3	4	3	18	61	1	5	74.3
17. 2358.9*	0	0	0	0	0	5	2	3	8	9	27	37	4	13	77.0
18. 2360.3*	0	0	0	0	0	0	2	1	5	5	13	23	8	38	83.0
19. 4.5 × 8.9	0	0	0	2	0	0	1	3	7	11	24	25	4	17	82.2
20. 7.5 × 8.9	0	0	0	0	0	0	3	2	3	8	16	31	14	47	82.2
21. 7.5 × 0.3	0	0	0	0	0	0	0	1	6	2	9	11	11	55	84.8
22. 8.9 × 0.3	0	0	0	0	0	0	1	1	6	9	17	12	6	26	87.1
23. (4.5 × 8.9) F_1*	0	0	0	0	1	1	4	10	11	8	35	46	10	22	80.1
24. (7.5 × 8.9) F_1*	0	0	0	0	0	1	2	7	11	15	36	29	11	24	83.4
25. (7.5 × 0.3) F_1*	0	0	0	0	0	1	1	3	2	4	11	46	6	36	80.2
36. (8.9 × 0.3) F_1*	0	0	0	0	0	2	1	3	13	15	34	18	10	23	87.6
27. (4.5 × 8.9) × (7.5 × 0.3) four-way cross	0	0	0	0	0	0	1	2	15	19	37	8	37	50	88.0

Stocks used in crosses are indicated by the last one or two numbers.
* Inbred.
† Based on 1370 to 10,825 eggs in a test.

In view of the uncertainty of the origin of lowered viability which may be due to the presence of one or more completely or partially recessive lethals, semi-lethals, or effects arising from interaction of a number of genes, we cannot evaluate lethal progeny except in very general terms. Therefore, we have calculated the mean viability on inbreeding and crossbreeding, in addition to the egg development classes for each test. We wish to measure the reproductive efficiency of these populations. Inbreeding shows the lower limit and crossbreeding the upper limit measured as egg viability within the sampling limits set by our procedure.

Since the data are expressed in percentages, the *arc sine* transformation has been applied to all records before analysis; variance analysis has been used to test for differences between contrasted groups, samples tested on successive years or at different temperatures, etc. (Means have been restored to percentages in the tables.) The variance of viability between pair matings is compounded of genetic and environmental difference, but the status of both sorts of effect may be expected to differ in different categories. Thus the genetic variance between sib matings might be expected to be lower than in matings between unrelated individuals, although this may not be the case if there is a high frequency of recessive lethals or semi-lethals in the population. Also inbreeding, which lowers the chance of survival, probably increases the sensitivity to environmental variation so that environmental effects may be relatively more important than is true of the random matings from a population and especially of three-way and four-way crosses, which are particularly vigorous.

Some tests were run at 71° F. in 1955; the rest of the tests in 1955 and all tests thereafter were run at 77° F. Samples from the untreated Majuro population, which is used as control, were run at both temperatures although at different times; viability was 10.6% lower at 77° F.—a difference which is significant at the .05 level ($p > .01 < .05$, Table 17). We might infer that higher temperature is adverse, but we cannot be certain that flies from different populations will behave in the same way with respect to such a temperature difference. In fact two collections were made at Bikini in 1955, one at the end of July and the second at the end of August. The first was tested at 71° F., the second at 77° F. The change is in the opposite direction from that with the Majuro population so no conclusion can be reached on the effect of temperature on egg development. The Bikini population may have been improving during the month between collections but it is improbable that it improved so markedly. The two samples at different temperatures were combined in other tests. The differences of this origin will be reflected in higher Standard Errors.

In order to see if the inbred populations differ, the others were compared to Majuro as the control available each year. Table 18 shows the comparisons. In 1955 Rongerik was significantly higher than Majuro while Rongelap and Bikini were significantly lower in per cent development. In 1956 only Rongelap differed significantly from Majuro.

Table 19 shows the reduction in egg viability on inbreeding. In each case inbreeding significantly reduced the number of eggs that developed. The random mated individuals show that egg development is good despite the appreciable number of lethal and lethal combinations demonstrated on inbreeding. However, it may be noted that the differences between Bikini and Ponape random-mated

TABLE 16

Average Fertility of Stocks and their Heterozygotes

	Percent Fertile 1955	1956
P_1	53.0	55.8
F_1	69.4	68.7
F_2	72.9	72.8

TABLE 17

Comparisons Between Temperatures

			% Viability	N	Difference (71–77)	p
1955	Majuro	71°	63.1	44		
					10.6*	>.01 <.05
1955	Majuro	77°	52.5	95		
1955	Bikini	71°	40.9	179		
					—13.2*	>.01 <.05
1955	Bikini	77°	54.1	117		

TABLE 18

Comparison of Different Inbred Populations with Majuro

1955 Population	Deviation from Majuro in % viability	p	1956 Population	Deviation from Majuro in % viability	p
Rongerik	+19.1*	<.05	Ponape	+ 4.2	>.1
Rongelap	—11.3*	<.05	Rongerik	+ 5.7	>.05
B_{71+77}	—10.2*	<.05	Rongelap	+15.9*	<.05
			Bikini	— 4.4	>.05

TABLE 19

Showing Effects of Inbreeding with Individual Populations (1956).

Percentage development	Majuro	Ponape	Bikini
Random mated	82.6	89.8	79.6
Inbred	67.8	72.0	63.4
Decline with inbreeding	—14.8*	—17.8*	—16.2*

* Indicates significance at .05 level.

and also between Bikini and Ponape inbred are statistically significant, so, if Ponape were our standard, Bikini was still low in 1956.

One simple demonstration of the existence of genetic differences within the stocks is a comparison of the variability between sib matings within families with that between families. This is shown in Table 20 for pooled data for 1955 and 1956. In addition to the tests of brother-sister matings within the populations, the brother-sister matings of the F_1 heterozygotes between the stocks is included. There is a significantly greater variation between families in all cases but one.

Table 21 was made to show what kind of factors accounted for the differences between similar tests in 1955 and 1956. The starred differences are all significant at the .05 level. The sexes in the cross between stocks from Majuro and Bikini were reversed for the two years. This should make little difference as sex-linked detrimental factors should be eliminated very rapidly and therefore would have been eliminated from the populations. Therefore, most reduction in egg development must result from autosomal factors. The equivalent crosses are placed together with test values given in the appropriate year. Rongerik is not included in the averages for the populations as it was not tested in crosses.

The inbreeding tests showed that egg development improved in 1956 compared with 1955 in the brother-sister matings from Bikini, Majuro and Rongelap (which were all low in the first test), although in Rongerik there was no difference between the years. The heterozygotes from crosses between different populations also show a higher level in 1956, and there is a similar trend in the effects of inbreeding the heterozygotes from crosses between different populations, except for the MB $\times$ MB tests. Such a trend from one year to the next naturally raises the question as to whether it is due to genetic differences in composition of the stocks in successive years or whether it really reflects improved environmental conditions in 1956. There are several indications that environmental differences cannot be regarded as primarily responsible. Firstly, technique and general treatment of the flies was the same on the two years, secondly the Rongerik population when inbred gave the same value on the two years, and thirdly the performance of three-way crosses and also their crossbred offspring was the same in 1955 and 1956. Thus the average egg development for the former was 88.2 and 88.6% in 1955 and 1956, while for the latter the corresponding figures were 83.4 and 80.6%. While is it quite possible that such crosses may be generally more resistant to environmental variation than either random mated individuals derived from individual population or especially the inbreds, it is unlikely that such a difference in sensitivity could be reflected so dramatically in a hypothetical general environmental difference between 1955 and 1956. Therefore, it is reasonable to conclude that composition of all the populations, other than Rongerik, differed in the two years, presumably due to a different level of recessive genes or gene combinations.

The evidence from three-way crosses suggests that if there are semi-dominant detrimental factors present they are about equal in effect the two years. Crosses between stocks produce all heterozygous progeny but egg developement in 1955 was below that in 1956. As there is no difference in semi-dominant detrimental factors, then the factors responsible for the low egg development in 1955 must have been due to recessives or more probably to combination effects. A difference

TABLE 20

Environmental and Genetic Variation.

Data Pooled from 1955 and 1956	*Between Sib Matings within Families* Sum of Squares	Degrees of freedom	Mean Square	*Between Families* Sum of Squares	Degrees of freedom	Mean Square	F test
Populations Inbred							
Majuro	43,787.93	193	226.88	40,185.40	164	245.03	1.08
Bikini	72,672.45	335	216.93	80,848.18	218	370.86	1.71‡
Rongerik	20,003.07	144	138.91	46,877.29	122	384.24	2.77‡
Rongelap	45,262.60	214	213.50	50,141.23	140	358.15	1.68‡
Ponape	43,118.80	184	234.34	59,870.90	153	391.31	1.67‡
Sum	224,844.85	1070	210.14	277,923.00	797	348.71	1.66‡
F_1 of Crosses between Populations Inbred							
AM × AM	10,835.32	56	193.49	11,720.49	42	279.06	1.44*
BM × BM or MB × MB	22,234.49	122	182.25	18,763.29	73	257.03	1.41†
BA × BA	20,692.03	109	189.84	24,691.91	63	391.94	2.06‡
Sum	53,851.84	287	187.64	55,175.69	178	309.98	1.69‡

*Indicates significance at .05, † at .01, ‡ at .001 level.

TABLE 21

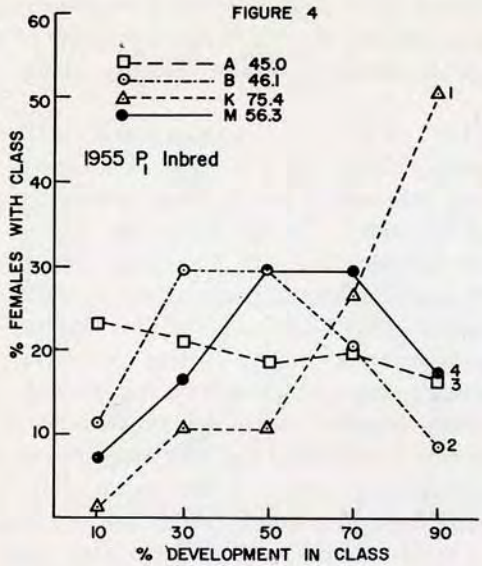
Inbreeding Versus Crossbreeding.

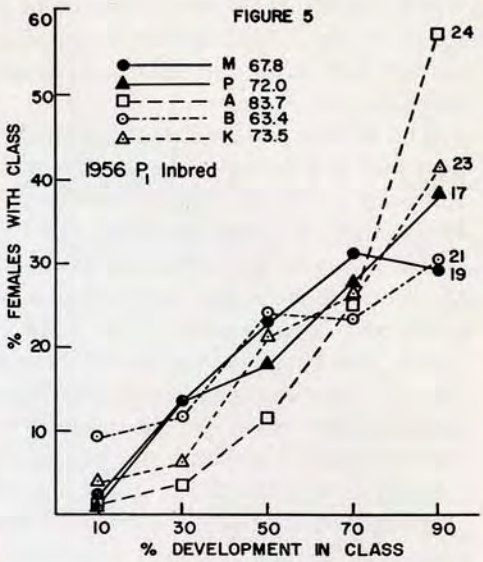
Type of Test	Cross ♀ × ♂	1955	N	1956	N	Difference 1956–1955
Inbreeding, brother-sister matings from population stocks	Rongerik	75.4	93	73.5	173	— 1.9
	Bikini	46.1	297	63.4	256	+17.3*
	Rongelap	45.0	90	83.7	263	+38.7*
	Majuro	56.3	139	67.8	217	+11.5*
	Average, A, B, M	49.1		71.6		+22.5
Crossbreeding, initial crosses between populations	B × M	65.7	43			
	M × B			79.7	76	+14.0*
	A × M	65.3	48	88.5	26	+23.2*
	B × A	69.0	39	78.6	66	+ 9.6
	Average	66.7		82.3		+15.6
Inbreeding, brother-sister matings of F_1 from crosses between populations	BM × BM	65.0	52			
	MB × MB			60.1	143	— 4.9
	AM × AM	61.9	51	72.4	47	+10.5
	BA × BA	56.6	58	72.8	116	+16.2*
	Average	61.2		68.4		+ 7.2
Crossbreeding, F_1 of crosses between populations inter-mated (three-way crosses)	BA × BM	84.7	64			
	MB × BA			90.7	73	+ 6.0*
	AM × BA	86.4	59	89.5	65	+ 3.1
	BM × AM	93.4	48			
	AM × MB			85.6	29	— 7.8*
	Average	88.2		88.6		+ 0.4
Crossbreeding, random matings between non-sib progeny of three-way crosses	$(BA \times BM)^2$	83.8	73			
	$(MB \times BA)^2$			85.0	71	+ 1.2
	$(AM \times BA)^2$	83.5	69	73.5	59	—10.0
	$(BM \times AM)^2$	82.8	83			
	$(AM \times MB)^2$			83.2	60	+ 0.4
	Average	83.4		80.6		— 2.8

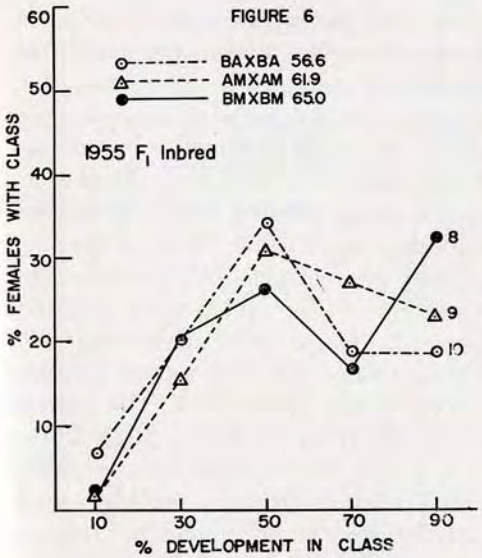
* Indicates significance at .05 level.

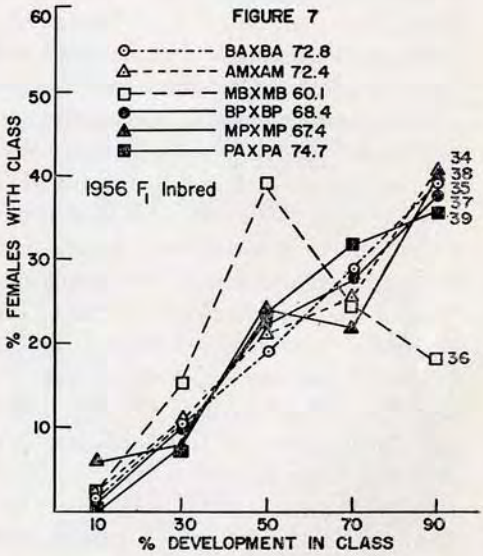
between adverse sex-linked characters for the two years should have been detected in differences between the three-way crosses. It would seem that the amount of heterosis present in three-way crosses was about the same for the two years. Seecof showed that gross chromosomal abnormalities that would produce inviable gametes at meiosis were absent or very infrequent both years. As an additional test for induced chromosomal abnormalities, Ward examined the testes of grasshoppers collected on Bikini in 1956. He found no inversions, bridges or fragments, translocations or supernumerary chromosomes in this material.

Figures 4 to 13 give profiles of egg development with inbreeding (4–7) and crossbreeding (8–13). Similar tests for the two years are together. These are plotted for 20% intervals of egg development so only the class at 90 (80–100% range) may be regarded as free of lethals or summed lethal equivalents. The classes plotted at 70 may be regarded as having one lethal or lethal equivalent, those at 50, 30 and 10 as having two or more lethals or their equivalents. Classes with such values as those plotted at 10 must be regarded as having six or eight lethals or lethal equivalents. As the number of lethal equivalents increases, the actual numerical reduction becomes smaller for each factor reduces the residual survivors by some fraction. Alternately, a gene or genes which caused few functional sperm to be present or shortened the life of the sperm or produced eggs









FIGS. 4–7. Profiles showing the capacity of eggs to develop using brother-sister matings. Progeny of wild females collected in 1955 inbred (4), and 1956 inbred (5); offspring of crosses between the stocks collected in 1955 inbred (6), and 1956 inbred (7). The numbers for each profile correspond to test numbers in Tables 13 and 14; the mean is given with the symbol representing each profile.

which usually failed to develop could give such a result. It is better to regard the mean (which is included on the figures) and the distribution of egg development classes (the profile of the test) as giving us measures of the viability of the breeding population.

The inbred tests show that the stocks in 1955 (Figure 4) produced fewer viable eggs and had much worse profiles of breeding classes than in 1956 (Figure 5), Rongerik excepted. This is shown again on inbreeding the F_1 from crosses between these populations (compare Figures 6 and 7). In the latter, the MB $\times$ MB cross gives a very different profile from the other five tests. The initial crosses between the stock did not lead to a good egg development, especially in 1955 which was significantly below 1956 (compare Figures 8 and 9). This demonstrates that there existed in 1955 a greater number or more effective concentration of (homozygous or adversely interacting) genes which reduce egg development. These acted on the parents or their gametes since the zygotes were heterozygous. The similarity of the three-way crosses and the four-way crosses (Figures 10, 11 and 12) rules out semi-dominant factors as the cause of the difference in viability shown by comparing Figures 8 and 9. Even the heterozygous three- and four-way crosses have a residue of pairs which have a low egg development. These classes with low productive viability are present in random crosses between progeny of the original females, which simulate the crosses under natural conditions (Figure 13).

Table 15 and Figures 14 through 16 give data on the viability and fertility characteristics of some populations of *novamexicana* and *hydei* (Stone, Alexander and Clayton, 1954). The former lives in very small linear populations along desert streams and the latter occurs as a small part of the desert *Drosophila*. The population studied came from widely separated localities in the Southwest. The *novamexicana* came from smaller populations than any of the *ananassae*.

Comparisons between the results with *ananassae* and those with *novamexicana* and *hydei* are very enlightening. Because of the small numbers we combined similar crosses of *hydei* in Figure 14. All the *hydei* tests show these populations have a much more favorable structure in terms of egg viability than even the best counterparts in *ananassae*, except perhaps Rongelap in 1956. None of the very low egg development classes appeared and very few with 20–39% development. Two of the *novamexicana* stocks (2351.1 and 2358.6) resembled *hydei* and were as good or better than Rongelap 1956, Figure 15. No pair with egg development below 40 per cent occurred in tests with these stocks and only six per cent had less than 60 per cent of the eggs laid develop. (Data in Table 15. The average egg development in this table was taken directly from the data.) Stock 2354.4 and its crosses showed a different condition, Figure 16, for these tests resembled tests of *ananassae* stocks and crosses in 1956. This stock carried a seemingly semi-dominant factor(s) which caused death of the gametes or zygotes in crosses—contrast 1 $\times$ 6 in Figure 15 with 4 $\times$ 1 plus 4 $\times$ 6 in Figure 16. Even with this

FIGS. 8–11. Profiles showing the capacity of eggs to develop after crossbreeding. Progeny of wild females collected in 1955 (8) and 1956 (9) crossed to other stocks; offspring of crosses between stocks collected in 1955 (10) and 1956 (11) tested in three-way crosses. The numbers for each profile correspond to test numbers in Tables 13 and 14; the mean is given with the symbol representing each profile.

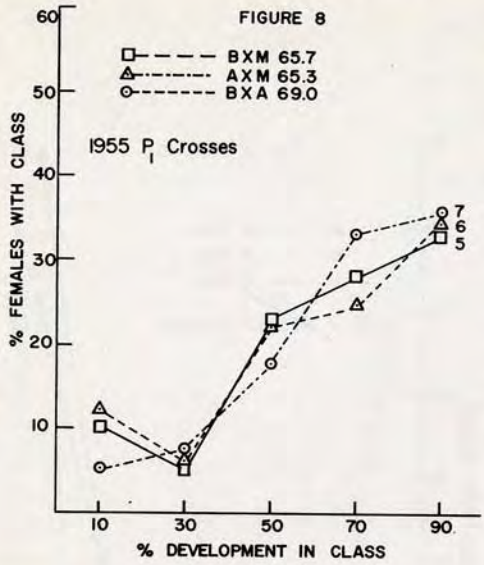


FIGURE 8
BXM 65.7
AXM 65.3
BXA 69.0
1955 P1 Crosses
% FEMALES WITH CLASS
% DEVELOPMENT IN CLASS
7
6
5

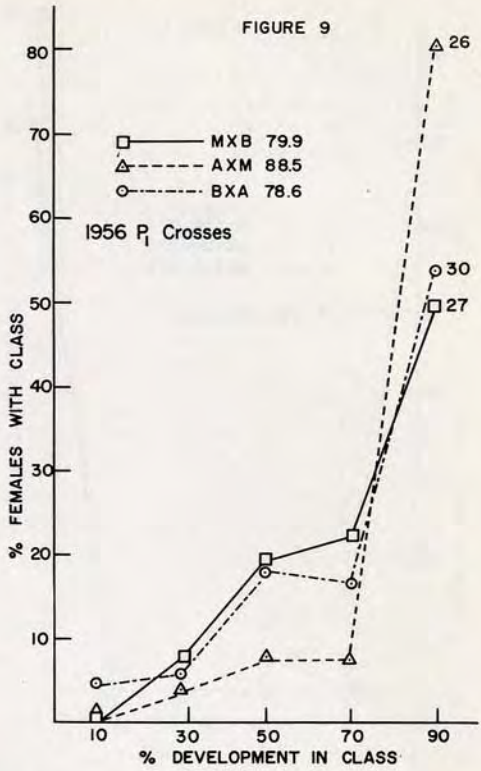


FIGURE 9
MXB 79.9
AXM 88.5
BXA 78.6
1956 P1 Crosses
% FEMALES WITH CLASS
% DEVELOPMENT IN CLASS
26
30
27

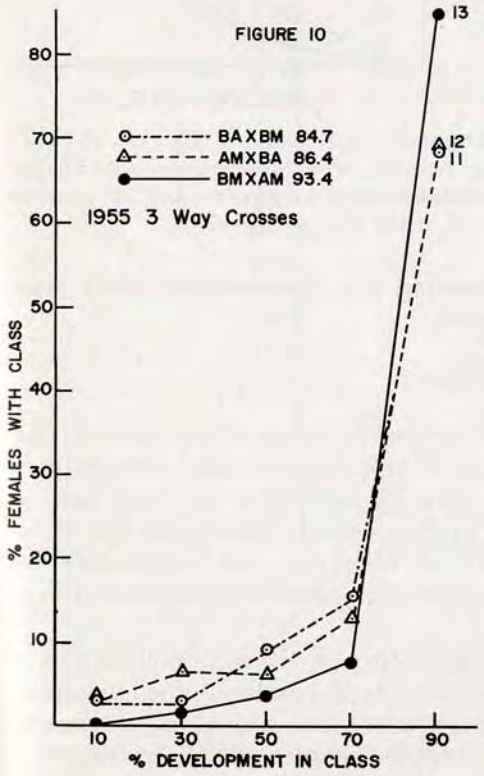


FIGURE 10
BAXBM 84.7
AMXBA 86.4
BMXAM 93.4
1955 3 Way Crosses
% FEMALES WITH CLASS
% DEVELOPMENT IN CLASS
13
12
11

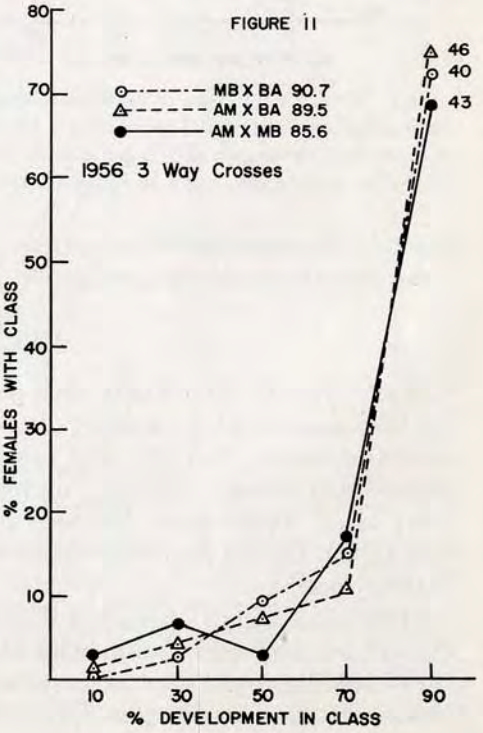
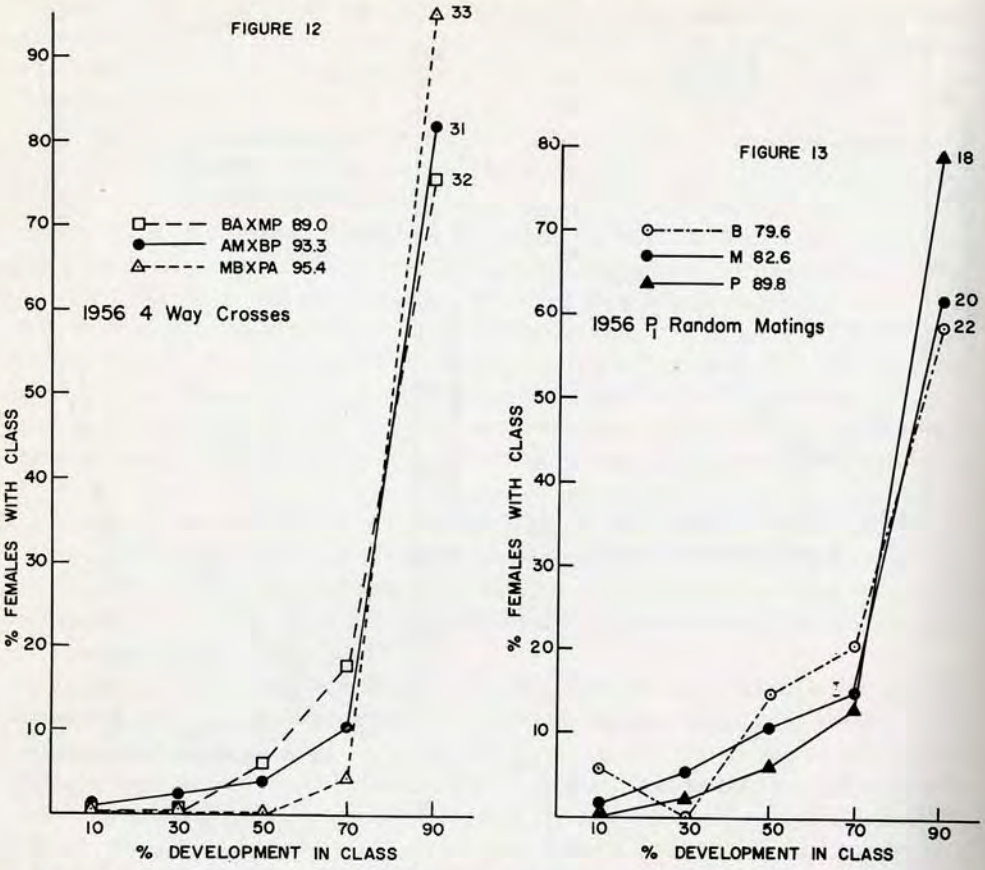


FIGURE 11
MB X BA 90.7
AM X BA 89.5
AM X MB 85.6
1956 3 Way Crosses
% FEMALES WITH CLASS
% DEVELOPMENT IN CLASS
46
40
43



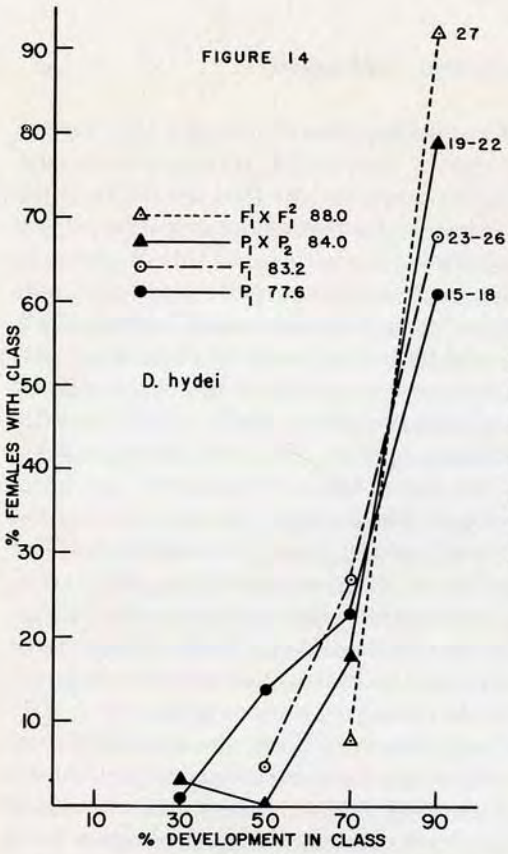
FIGS. 12–13. Comparison of profiles of egg development after crossbreeding (12) and after random matings within the populations (13). This contrasts forced heterozygosity with random (*i.e.*, natural) matings within a population. The contrasts between inbreeding and crossbreeding or random mating are shown in Figures 5 *versus* 13, and in Figures 7 *versus* 12.

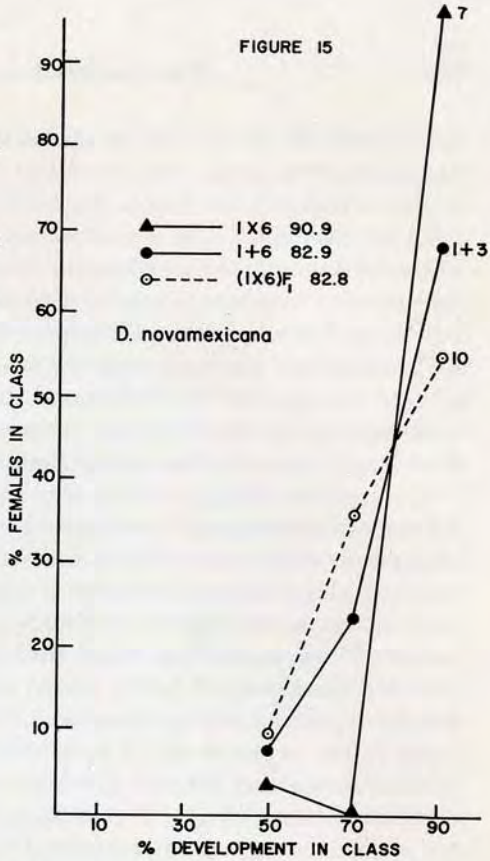
seriously detrimental factor, crosses involving this *novamexicana* stock were better than those involving *ananassae* in 1955.

DISCUSSION

Many types of studies have been made on the variability of populations. This has been measured for a number of categories: gross chromosomal abnormalities, visible mutations, fertility, and factors affecting viability or heterosis such as lethals, semi-lethals, subvitals, normals and supervitals. Sometimes the variability of all chromosomes has been measured, often only one chromosome has been tested. These *Drosophila ananassae* populations have been analyzed for most of these factors.

Spencer (Section IV) showed that the frequency of visible mutations was in the frequency range found in other *Drosophila*. As he points out, the large number of lethals present must have reduced the probability of obtaining a recessive visible mutation homozygous. This would cause an underestimate in frequency





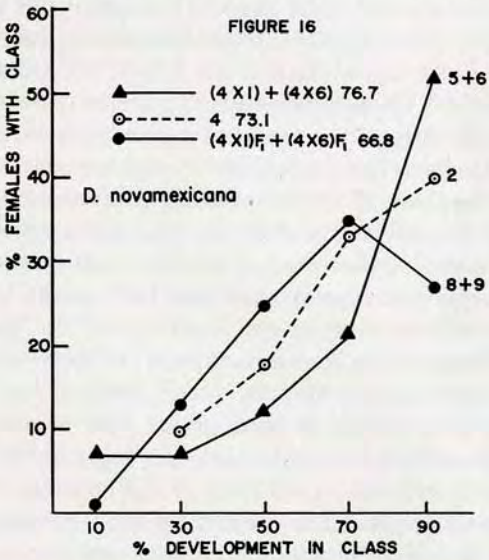


FIG. 14. Profiles of average egg development on inbreeding or crossbreeding *Drosophila hydei*. Each line is the average of tests with several stocks (see Table 15). The mean is given with the symbols representing each profile. Zero points are not plotted unless necessary.

FIGS. 15–16. Profiles of average egg development on inbreeding or crossbreeding *Drosophila novamexicana*. Figure 15 shows tests involving only stocks 2351.1 and 2368.6 and their crosses. Figure 16 shows tests involving 2354.4 and its crosses to 2351.1 or 2358.6 (See Table 15). The mean is given with the symbol representing each profile. Zero points are not plotted unless necessary.

but this would not change the essential conclusion. Seecof (Section III) showed that there were some new rearrangements in Bikini and Majuro populations. These were small and low in frequency. We must assume that the rather small CII L-O inversion which is widespread in *ananassae* populations must be present in these island populations because of heterosis. The survival of this inversion in considerable numbers, where recombination of the homozygotes could eliminate lethals, and the fact that all new surviving inversions were small, supports Seecof's suggestion that rearrangements would be lost not only by chance but also by selection against the detrimental factors present which could not separate by crossingover. In these tests chromosomal abnormalties could not have contributed largely to reduction in egg development because of meiotic abnormalities.

The number of eggs laid per day by the fertile females during the egg count for the egg development tests were recorded. The average eggs per day for the stocks and crosses varied but no consistent relation to genotype was detected. The heterozygotes from crosses between members of different populations did not consistently lay better than their parents. Gowen (1952) demonstrated a marked increase in egg production when inbred lines of *Drosophila melanogaster* were crossed. Robertson and Reeve (1955) compared body size characters and egg production in inbred and crossbred individuals, arranging their experiments so they could follow replacement of individual chromosomes. They demonstrated very marked interaction between different heterozygous and homozygous gene combinations on wing length, thorax length and egg output. Inbred lines produced fewer eggs per day and when inbred lines were crossed, the egg production level approached that of the normal wild stock. They point out that egg production may be regarded as far more sensitive than body size to both environmental and genetic changes. We did not produce and test inbred lines of *ananassae* but tested and crossed wild strains. These possess great genetic variability. We could not make these tests at the same time and under similar environmental conditions (same batch of food, etc.). The variability in eggs per day per female recorded in Table 13 reflects the effect of the genetic and environmental variability.

The percentage of pairs fertile and the percentage of egg development have a defined range. Our tests for the effect of genotype on fertility were consistent. When the stocks and their crosses available both years were compared, the heterozygotes from crosses were more fertile than the parent stocks, Table 16. There was no difference for the same type of test between years. There were genes present in the several populations which reduced fertility in the original combinations below that of the heterozygotes. This increase of fertility may partially result from an increase in viability and vigor of the heterozygotes.

There are two alternatives, in addition to differences between tests in the laboratory, which may explain the differences in percentage egg development between stocks and their combinations and from year to year. On the one hand the changes may be only the normal types of population fluctuation due to local conditions, cyclic changes or chance deviations perhaps due to poor sampling of the populations. The second alternative is that, superimposed on these variables, there exists genetic damage due to direct and fall-out radiations in the northern Marshalls. Many different studies of populations have been made that provide comparisons useful in analysis. Dobzhansky and Spassky (1954) compared the

frequency of autosomal lethals and semi-lethals in *Drosophila prosaltans* with that in *Drosophila willistoni*, *Drosophila melanogaster*, *Drosophila pseudoobscura* and *Drosophila persimilis* for a number of localities. The authors use their own tests and those published by other investigators in this comparison. All these investigators examined the ratios of normal to dominant in crosses between individuals heterozygous for the particular wild chromosome and an inversion (marked by one or more dominants) which reduced crossingover to a minimum. This thoroughly tests one chromosome at a time for the presence of lethals or semi-lethals. The investigators tested samples of second chromosomes and for some species the third and fourth chromosomes as well. There was great variation in the percentage of lethals and semi-lethals (usually classed together), the subvitals and the male and female sterility factors present in different species and even in collections of a species from different localities. The second chromosomes are equivalent in *prosaltans*, *willistoni* and *melanogaster*. Dobzhansky and Spassky report 32.6% lethals in the second chromosome of *prosaltans* from Cavalcanti's and their work and from 28.4% to 31.2% lethals in the second chromosome of *willistoni* from different localities from work by Pavan and collaborators (1951) and Cordeiro and Townsend (Townsend, 1952). Dobzhansky and Spassky report the per cent of lethals in the third chromosome of *willistoni* to be close to that of the second ranging from 25.6 to 32.8%.

Dubinin (1946) reports a low frequency of 7.9% lethals in the second chromosome of *melanogaster* from Gelendzhik in July, 1933, and a high frequency of 29.8% in Uman in September, 1938, for tests of populations in the U.S.S.R. Dubinin also reports a change of around eight per cent within a month at one locality and about an equal change in lethal frequency for the same month from one year to the next.

Ives (1954) reported a considerable variation in per cent of lethals in the second chromosome of *melanogaster* from a low of 28.2% in a stock from Cannonsburg, Pa. in 1952 to a high of 65.3% for strains from Winter Park, Fla. in 1940, 1942. The incidence of lethals in 1951 had dropped to 51.1% at the last locality. Ives reported the frequency of lethals from natural populations at South Amherst, Mass. for 10 years. The range was from a low of 30.3% to a high of 59.3%. The largest change for any two consecutive years recorded was 6.7%.

Goldschmidt *et al.* (1955) made a two-year survey of *Drosophila melanogaster* from Qiryat' Anavim, Israel. There was some slight variation in lethal ($32.16 \pm 1.34\%$) and semi-lethal ($39.12 \pm 1.40\%$) frequency over the two years. The maximum difference between two collecting periods was 7.2%. They conclude that there was no difference in incidence of lethals at different seasons of the year.

Wallace (1956) reviewed his studies of irradiated populations of *Drosophila melanogaster*. He and his colleagues ran a series of population cage tests on large (10,000 or more adults per cage) and small (1,000 or less adults per cage) populations. The large population control increased from zero to around 30% lethal frequency in the second chromosome in 45 generations and fluctuated around this level for perhaps 80 generations. Some samples five generations apart differed by around 10% as did the experimental populations. A large population which received a single radiation (7,000 r to males, 1,000 r to females) began with about 20% second chromosome lethals, and after some slight increase ad-

justed to about this level by the 50 generation and remained there for an additional 75 generations. A large population with a low chronic gamma radiation of 0.9 r per hour was begun 20 generations after the control but in 25 generations had achieved about the same level and 80 generations later was perhaps slightly above the control. A large (Wallace number 6) and a small (Wallace number 5) population which received 5.1 r per hour of chronic gamma radiation reached a level with around 80% of the second chromosomes carrying lethals in less than 60 generations and remained at this high level although the lethal frequency measured in the samples from the small population fluctuated more. Three subpopulations were made from the two populations that received 5.1 r per hour radiation after the populations had received some 250,000 r of gamma radiation. Both a large and small subpopulation were made from the small population (number 5) at an initial level of 85.7% of the second chromosomes carrying lethals. The small subpopulation fell to a level of 56.2% and the large to 28.0% lethal second chromosomes within twenty-five generations. The large subpopulation made from the large chronic irradiated population (number 6) started at 79.7% lethal second chromosomes and ended at 76.7% twenty-four generations later, although at the fifteenth generation only 62.1% lethal second chromosomes were found in the sample. Wallace's study allows us to draw several conclusions. *Drosophila melanogaster* populations can recover completely from a single heavy radiation and can tolerate low chronic radiation, at least in large populations where tremendous egg mortality due to crowding is present. Higher chronic radiation raises the frequency of second chromosome lethals to a high frequency—around 80% of the chromosomes have lethals or lethal equivalents in his tests 5 and 6. If radiation is then stopped, the population may recover even to the control level, although one population apparently did not recover. Wallace has demonstrated that *melanogaster* can recover from several times the L.D. 50, if the radiation is spread over a large number of generations.

The egg development technique is not as accurate a measure of the number of lethals and semi-lethals present in a particular chromosome. Rather it provides a measure of the possible actions and interactions of all autosomal factors on development, even assuming the X chromosome can be ignored because of the effect of selection on sex-linked factors. The data on the P_1 inbred where one to four brother-sister pairs were made give us some measure of the number of lethals or lethal equivalents present on the two major and small minor autosome. If we assume that half the autosomes carry lethals, then about one-sixteenth of the time neither one should have a lethal or lethal equivalent. We have pointed out that our test underestimated the number of lethal complexes present. It would appear (taking the average egg development into consideration also) that the Bikini, Rongelap and Majuro populations of *ananassae* had at least as many lethal complexes in August, 1955, as the Winter Park, Florida population of *melanogaster* described by Ives. This was about 17 months—perhaps 35 to 50 generations—after the March 1, 1954 thermonuclear test. Wallace tested 70 males drawn from his population number 6, the 130th generation. In these males 87% of the second and 92% of the third chromosomes carried lethals.

The considerable changes in percentage egg development from 1955 to 1956, even the extensive change of 45.0% to 83.7% at Rongelap, are of an order of

magnitude predictable from Wallace's results in subpopulations which were removed from radiation. The number of lethals present and the change in the Majuro population is not excessive when compared to the Winter Park, Florida populations described by Ives.

We have compared these *ananassae* to populations of *hydei* and *novamexicana* analyzed in the same way. These populations were collected in the Southwest where they are respectively small but widespread and small linear populations along desert streams. All four *hydei* and two of the three *novamexicana* (Table 15 and Figures 14, 15 and 16) populations gave much better percentage egg development than these *ananassae* populations either year (Tables 13 and 14 and Figures 4 through 13). *Drosophila novamexicana*, stock 2354.4, resembled the *ananassae* in 1956 in both stocks and crosses. An easy way to stress the gross differences is to lump all tests of this strain, those with the other two strains and those involving *hydei*. *Drosophila hydei* is at some disadvantage because of the tremendous number of eggs laid, some days over 200, which will not give a good egg development test for those days. Of the 288 pairs of *hydei* and the 156 pairs of *novamexicana* involving only the high lines, only 3 pairs of *hydei* fell in the 30–39% egg development as contrasted to 23 pairs in all classes below 40% egg development out of 178 pairs of the low line of *novamexicana* (2354.4) and its crosses. This latter population of *novamexicana* resembles the 1956 populations of *ananassae*. Only among the best four-way crosses of *ananassae* do we find egg development equivalent to the good *novamexicana* and *hydei* crosses. The stock of *novamexicana* (2354.4) gave the lower egg development classes due to a semi-dominant factor(s). These may have caused zygotic or gametic mortality. From the residue of egg mortality even in three-way and four-way crosses, we presume that *ananassae* has such factors present or that this species, even when heterozygous, is particularly susceptible to effects of an unfavorable environment. As pointed out in discussing Table 21, there were perhaps homozygous or interacting semi-dominant factors in the Rongelap, Bikini and Majuro strains in 1955 which caused gametic or zygotic mortality in the initial crosses between strains but were lost from the populations by 1956 (compare Figures 8 and 9). Stern and Novitski (1948) and Stern *et al.* (1952) have shown that most of a large series of so-called recessive sex-linked lethals in *Drosophila melanogaster* had a measurable effect on viability and probability on fertility when heterozygous. A few increased viability but most caused a reduction in viability indicated by too few lethal-bearing females in fertile offspring and an appreciable group of sterile females. Russell (1957) showed that fast neutrons from a nuclear detonation reduced the life expectancy of F_1 of irradiated male mice. This presumably is another genetic effect on viability. These experiments suggest that the presence of combinations of (semi-dominant) lethal genes reduced the viability of these *ananassae*, particularly in 1955.

If we assume that the changes in these populations are the result of their natural fluctuations plus an effect of radiations, we can offer the following suggestions. Pipkin (1953) showed that the low point in the population cycles of *ananassae* came in August on similar atoll islands. Our collections in August may have sampled the Marshall Islands populations at their low point for the year. The small Eniwetok and Rongerik populations recovered to a normal level

between the fall-out radiation in the spring of 1954 and the collection in August, 1955 (or 1956). Selection on Eniwetok and Rongerik was strong enough that, with the inbreeding because of small size, the population recovered most rapidly. The larger populations, Rongelap and the more heavily irradiated Bikini, had not recovered to the level of the control population in 1955. By 1956 Rongelap had recovered even above the two controls, Majuro and Ponape. Bikini, which received some additional radiation in 1956 before the collection was made, was still low as compared to Ponape or Rongerik but only insignificantly lower than the Majuro control. We have no explanation for the low Majuro control population in 1955, but perhaps it was due to sampling from a low point in the natural cycle. Perhaps the Majuro population in 1955 resembled the Winter Park, Florida population of *melanogaster* in 1940 and 1942.

The tests we used measure certain variables that are of consequence to an evolving population. The percentage of fertile pairs is one measure of the breeding effectiveness of a population. The ability of larvae to compete for food is another. The temperature studies relating to the normal range of environmental temperatures of the islands show certain interspecific competitive capacities. Crossbreeding (or random breeding between non-sibs from a population) establishes the upper limit of egg development. Inbreeding (brother-sister mating) establishes the lower limit of egg development for the genetic combinations present and shows the maximum potential sacrifice of egg production when inbreeding occurs due to reduction in population size. Also inbreeding gives some measure of the load of detrimental mutations. The greater the load of mutations already present the greater the effect on the egg mortality of a population for the same mutation rate, and the greater the number of alleles that must be replaced, recombined or kept heterozygous for maximum efficiency in offspring production. Figures 4 through 16 allow a quick estimate of the breeding structure of the population in terms of egg development.

SUMMARY

1. The *Drosophila* on different atolls of the Marshall Islands are isolated populations. Some of them (on Bikini, Rongelap, Rongerik) have been subjected to direct and/or fallout radiations. Others (on Majuro) have received very little fallout and together with a population from the Eastern Carolines (Ponape) serve as control populations. The populations at Ponape and Majuro are large and continuous for they have fruit and other food available all year. The population at Rongelap is large but probably fluctuates seasonally. The population at Rongerik is small and probably varies with the season. These last two populations received fallout from the thermonuclear test of March 1, 1954. The population at Bikini is medium and presumably varies with the season. It has received direct and fallout radiation from a number of tests including that on March 1, 1954, and those in the summer of 1956. Our collections at these islands were made in late summer which is near the minimum population period for *Drosophila* on a similar atoll at Truk.

2. We investigated in a number of ways the genetic constitution of the *Drosophila ananassae* populations found at the five named localities (see chart 1).

This was the only species of *Drosophila* found in the northern Marshalls although others are found at Majuro and Ponape.

3. A number of visible mutations were found in the three tested populations (Bikini, Majuro and Ponape). Their frequency was comparable to that found in other studies of members of this genus. Some unique mutation types were recovered.

4. Two inversions known from Japanese and other Pacific area material were present in several populations. In addition there were several new small paracentric inversions. No new large inversions or other chromosomal abnormalities were found.

5. The populations were tested for fertility, rate of egg laying, and egg development with both inbreeding and crossbreeding tests. Fertility was variable with only a little over half the pairs fertile when stocks were tested. The F_1 were considerably more fertile and the F_2 somewhat more fertile than the F_1. This indicated that heterozygous (heterotic ?) parent females produce more fertile offspring. There was no significant difference between years when the stocks tested both years were compared.

6. There was no consistent difference in average number of eggs laid per day during the period of peak fecundity. The values varied so that no consistent change could be claimed for heterozygosity or year to year. We can only assume that uncontrollable differences in microflora and such environmental conditions caused some fluctuations. In these *ananassae* populations there seems to be an established optimum rate of egg laying and crosses between populations did not give an increase through heterosis. These populations resembled *hydei* and *novamexicana* in this characteristic.

7. Comparisons of inbreeding and crossbreeding tests demonstrate the presence of a large number of mutants and gene combinations that influence viability measured as ability of eggs to develop to adults. Three-way crosses which were made with offspring of two crosses involving three stocks averaged the same in egg development the two years. Since food conditions, etc. of the environment were the same the two years, and since we have demonstrated marked genetic differences in stocks, and since the three-way crosses were equivalent the two years, therefore we assign a major part of the differences measured between tests and from year to year to genetic differences.

In inbreeding tests between years, populations from Rongerik atoll did not change in per cent egg development. Majuro, Rongelap and Bikini populations were low in 1955 and all improved significantly by 1956. The change at Majuro was of the order of magnitude reported from other natural populations in a population cycle. The changes at Bikini and especially at Rongelap were larger in magnitude than might be expected in normal population cycles from available reports. They resemble the changes Wallace (1956) found in *melanogaster* populations carried in population cages after they had been separated from sources of direct radiation through which they had received about 250,000 r of gamma radiation over many generations.

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