INTROGRESSION BETWEEN CLOSELY RELATED SPECIES OF *DROSOPHILA* IN PANAMA[1]

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INTROGRESSION BETWEEN CLOSELY RELATED SPECIES OF *DROSOPHILA* IN PANAMA[1]

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In a recent paper, Bigelow (1965) argues that closely related but distinct animal species may interbreed in a hybrid zone where their respective distributions overlap, providing the genomes of the two species have become so coadapted that hybridization fails "to make the gene pools progressively more similar." Thus persistence of a narrow hybrid zone over a long period of time demonstrates the biological integrity of the closely related species, and populations of hybrids zones should not be regarded as incipient species. Previously, Dobzhansky (1951) had observed "that populations may continue to diverge despite gene exchange, the real problem being how much gene exchange between diverging populations is possible without arresting and reversing the divergence."

The present work will describe a case which appears to corroborate Bigelow's argument. *Drosophila metzii* Sturtevant (1921) and *Drosophila pellewae* Pipkin and Heed (1964) belonging to the *tripunctata* species group of the subgenus *Drosophila*, are sympatric in the Isthmus of Darien, Panama. These species feed and breed together on fallen fruit and blossoms as part of large multispecific aggregations (Pipkin, 1965). A marked degree of sexual isolation exists, but there is some hybridization. Though the F_1 hybrids between these species are often sterile in crosses *inter se*, female hybrids can backcross with either parental species. Recombination between the genomes of the respective species is limited by translocations involving four pairs of autosomes. However, a carina color polymorphism observed in *D. pellewae* of the Darien, where both species occur, and also present in a strain of *D. pellewae* from Rio Raposo, Colombia, where *D. metzii* is not found, is believed to be the result of introgression between the two species in the region of overlap. It is the purpose of this work to present evidence upon which this interpretation is based and to compare relationships between *D. metzii*, *D. pellewae*, and a third closely related allopatric species, *D. leticiae* Pipkin (1967) from the headwaters of the Amazon River.

DISTRIBUTION AND ECOLOGY

Material includes the following geographic strains of *D. metzii*: 1T9, Turrialba, Costa Rica, from a single founder female; 11A1 3, Almirante, western Panama, 5 founder females; 58B8, Barro Colorado Island, Canal Zone, 8 founder females; 1Tr17, Trinidad, West Indies, 23 founder females; and 5D1, El Real, Darien, eastern Panama, 70 founder females. The strains of *D. pellewae* include 23B6, Barro Colorado Island, from a single founder female; 6D2, El Real, Panama, 109 founder females; 5R3, Rio Raposo, Colombia, 18 founder females. The strain 3L5 of *D. leticiae* derived from 15 founder females was collected at El Marco, Brazil, near Leticia, Colombia. The collection localities are shown on the map in Figure 1.

With the exception of *D. metzii* from Turrialba, Costa Rica, which was taken in a coffee finca at 2200 ft altitude, the three closely related species were all found in lowland forest where they feed and breed on the drier type of fallen fruit such as those of *Clusia* sp., *Bactris* sp., or on fallen

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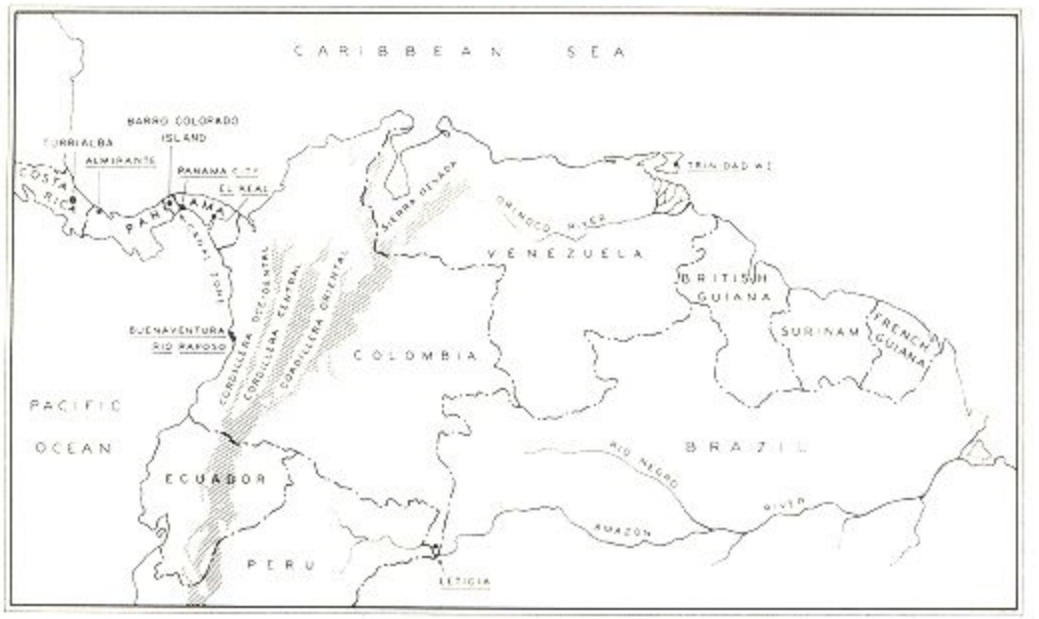

FIG. 1. Map showing collection areas.

blossoms (Pipkin, 1965). When fincas are near forest, these species come out to the orchards and may be netted over "Caimito," Cacao, or coffee beans, and they show a particular liking for breadfruit. They have never been taken in trap cans baited with banana, orange, or pineapple.

Drosophila pellewae is a rare species in central Panama; *D. metzii*, moderately common. Of 88 collections over a 4 year period at Barro Colorado Island, *D. pellewae* was collected twice on dates only a week apart; *D. metzii*, 19 times on dates throughout the wet season. At nearby Madden Forest, Canal Zone, *D. metzii* was collected in 8 of 15 field trips; its sibling, not at all. *D. metzii* only was also taken at Camaron and Chilibre, two other low altitude forest areas near Panama City. Both sibling species were absent from 65 collections at Cerro Campana, near Panama City, at altitudes 2200 to 2800 feet. In the cacao orchards of Almirante, western Panama, *D. metzii* was a relatively common species during week long collecting trips of 1962 and 1964, but *D. pellewae* was absent from collections. Neither species was found on three different collecting trips to El Volcan, Chiriqui, western Panama at an altitude of 4500 feet. Further, only *D. metzii* was caught in 1961, and again in 1962 in a coffee finca of Turrialba, Costa Rica, at altitude 2200 feet. *D. metzii* only was caught in the lowland Bush-bush Forest of Trinidad, where breadfruit bait on the ground was used as a lure, but neither species could be collected using the same method in a cacao orchard 2000 feet in altitude at Arima, Trinidad, over a week long collecting period. Near El Real in the Isthmus of Darien, Panama, 126 individuals of *D. metzii* and 154 of *D. pellewae* were collected in an aggregation together with members of 14 other species of Drosophilidae. *D. metzii* has been collected as far south as Santa Marta, on the northern coast of Colombia by W. B. Heed (personal communication) and as far north as Sonte Comapun near Vera Cruz, Mexico, by A. C. Fabergé (personal communication). In June, 1963, *D. pellewae*, but not *D. metzii*, was netted from fallen breadfruit at five localities, each several miles apart along the Rio Raposo near Buenaventura, Colombia, during a 9 day collecting trip. Of the three sibling species, only *D.*

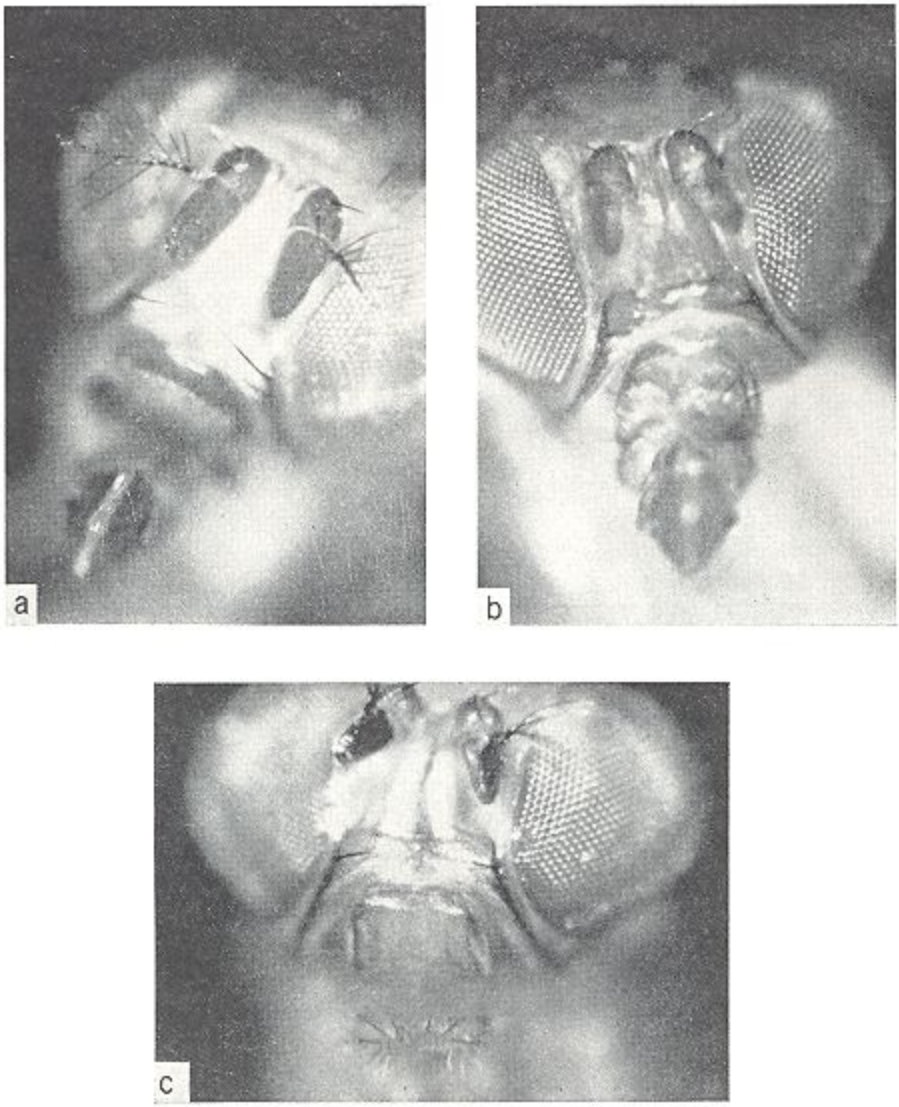

FIG. 2. (a) Head of *D. metzii* male showing chalk white face and carina. (b) Head of *D. pellewae* male showing brown face and carina. (c) Head showing reduced white area on face and carina of a male derived from *D. metzii*/*D. pellewae* female hybrids backcrossed with *D. pellewae* males.

leticiae was collected in forest beside the Amazon River near Leticia, Colombia.

MORPHOLOGICAL DISTINCTION BETWEEN THE SIBLINGS

Drosophila metzii displays two striking sexual dimorphisms: (1) a chalk-white carina and face in the male (Fig. 2a) vs. a whitish carina and face in the female and (2) an almost completely black abdomen in the male vs. a tan abdomen with thin black apical bands in the female. The carina and face of *D. pellewae* males (Fig. 2b) and females are uniformly yellowish brown, but *D. pellewae* shares the sexual dimorphism of abdominal coloration with *D. metzii*. *D. leticiae* possesses a banded abdomen in both sexes, similar to that of females of its two sibling species. Whereas the carina and face of *D. leticiae*

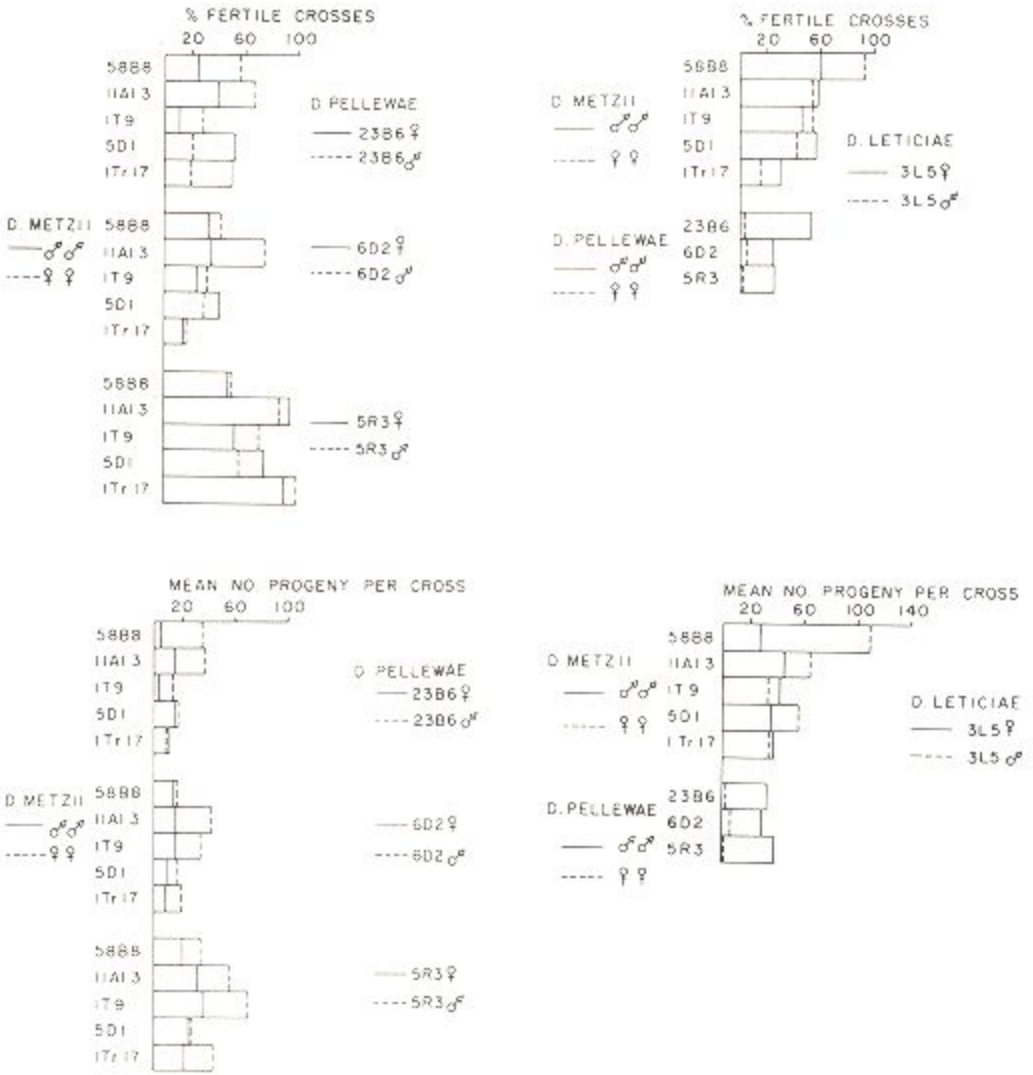

FIG. 3. Interspecific crosses: per cent fertility and mean number of progeny per fertile cross in interspecific matings between geographic strains of *D. metzii*, *D. pellewae*, and *D. leticiae*.

males are white though not so chalky as those of *D. metzii* males, the carina and face of *D. leticiae* females are yellowish brown as in *D. pellewae*. Male genitalia of *D. metzii*, *D. pellewae*, and *D. leticiae* are indistinguishable on inspection, any differences in proportions being of a statistical nature. (Pipkin and Heed, 1964; Pipkin, 1967.)

The two western strains of *D. metzii*, 1T9, from Turrialba, Costa Rica, and 11A1 3, from Almirante, Panama, possess a dark brown mesonotum and pleura which are tan in the strain 58B8 from Barro Colorado Island, Canal Zone, and paler in strains 5D1 from the Darien and 1Tr17 from Trinidad. The absence of much melanin pigment from the latter two strains causes the face and carina of the females to appear whiter, though still not so intensely white as in males. A cline in body color similar to that described for *D. metzii* has been observed in certain Panamanian flower-feeding *Drosophila* (Pipkin, Rod-

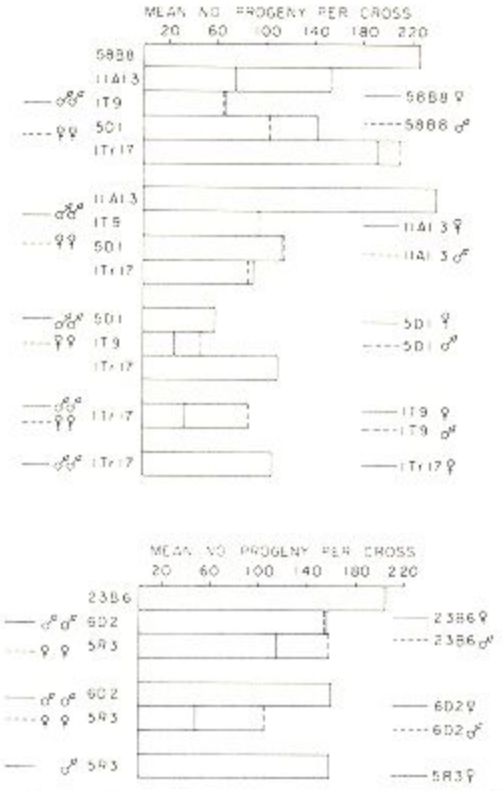

FIG. 4. Intra-specific crosses: mean number of progeny per fertile cross in matings between geographic strains of *D. metzii* or of *D. pellewae*.

ríguez, and León, 1966) only distantly related to the *tripunctata* species group.

LABORATORY INTROGRESSION BETWEEN THE SIBLING SPECIES

1). *Methods.*—To study capacity for laboratory introgression between the three species, reciprocal matings between them, using all combinations of strains of each species were prepared. Twenty virgin males and 20 virgin females, aged 1–3 days, were used per cross, with a total of 26 crosses for each kind of mating. Three crosses of each possible mating between strains of each single species as well as of males and females of the same strain were prepared similarly as controls. Parents were transferred every two days until a total of 10 transfers of each initial cross had been made. The progeny yield of a cross included the flies hatching from all the transfers of the initial 20 males and 20 females. A cross was classified as fertile if at least one imago hatched. The nonproducing vials usually contained only eggs; only rarely did hybrids die as larvae or pupae.

2). *Introgression between* D. metzii *and* D. pellewae.—The percentage of fertile crosses and mean number of F_1 progeny per fertile cross between two sibling species indicate the potential capacity for introgression to occur in the laboratory. Figure 3 shows that crosses between all five strains of *D. metzii* with the three of *D. pellewae* are possible, though the percentage of fertility is reduced compared with crosses involving strains of the same species, which always yield progeny (see Fig. 4). The mean number of progeny per fertile cross was with few exceptions markedly less in crosses between *D. metzii* and *D. pellewae* than in control crosses between strains of the same species. Thus only 4 of 31 intraspecific crosses; i.e., 5D1 ♀ × 5D1 ♂; 1T9 ♀ × 5D1 ♂; 5D1 ♀ × 1T9 ♂; and 1T9 ♀ × 1Tr17 ♂, yielded a lower mean number of progeny than the cross yielding the highest number of progeny among 30 interspecific crosses; i.e., 1T9 ♀ × 5R3 ♂, as a comparison of Figure 3 with Figure 4 shows.

In crosses between *D. metzii* and *D. pellewae*, the F_1 females were fertile in 38% of 76 mass matings with *D. pellewae* males and in 63% of 52 mass matings with *D. metzii* males. In contrast, F_1 hybrid males were fertile only in 2 of 66 backcross mass matings with *D. pellewae* females. It was certain that no non-virginity had occurred here since the progeny were polymorphic for carina color. F_1 hybrid males produced progeny in 5 of 72 backcross matings with *D. metzii* females. A higher per cent of fertility was obtained in crosses between F_1 hybrid males and females, particularly those involving certain strains of each species. Table 1 shows the number of sterile F_1 ♀ × F_1 ♂ crosses and the number of crosses classed fertile by the production of a few adult progeny. F_1 hybrids with a *D. metzii*

TABLE 1. *Outcome of mass matings of* F_1 ♂ × F_1 ♀ *hybrids between* D. metzii *and* D. pellewae.

D. metzii ♀ parent of hybrids			*D. pellewae* ♀ parent of hybrids		
F_1 ♂ × F_1 ♀	Fertile	Sterile	F_1 ♂ × F_1 ♀	Fertile	Sterile
58B8/23B6	1	7 + 5 dd as pupae	23B6/58B8	0	3
1T9/23B6	2	2	23B6/1T9	0	2
11A1 3/23B6	5	7 + 3 dd as pupae	23B6/11A1 3	2	5
5D1/23B6	0	3	23B6/5D1	1	8
1Tr17/23B6	2	1	23B6/1Tr17	0	5
58B8/6D2	3	4	6D2/58B8	0	6
1T9/6D2	3	6	6D2/1T9	0	4
11A1 3/6D2	8	10	6D2/11A1 3	0	4 + 1 dd as pupae
5D1/6D2	1	1	6D2/5D1	1	13
1Tr17/6D2	0	3	6D2/1Tr17	0	2
58B8/5R3	1	6	5R3/58B8	2	6
1T9/5R3	11	2	5R3/1T9	4	6
11A1 3/5R3	9	4 + 2 dd as pupae	5R3/11A1 3	5	9
5D1/5R3	1	7	5R3/5D1	1	14
1Tr17/5R3	2	7 + 1 dd as pupae	5R3/1Tr17	4	11
Total	49	81		20	99

female parent (left half of Table 1) participated in more fertile crosses than F_1 hybrids with a *D. pellewae* female parent (right half of Table 1). F_1 hybrids with a *D. metzii* female parent belonging to strain 1T9 or 11A1 3, from Costa Rica or western Panama, respectively, produced a higher proportion of fertile crosses than F_1 hybrids with female parent belonging to the Darien strain, 5D1, or the Trinidad strain, 1Tr17, of *D. metzii*. The Costa Rica and western Panama strains of *D. metzii* can be distinguished from the Darien and Trinidad strains also by the possession of a dark mesonotum in the former and a pale mesonotum in the latter. No matter whether the *D. pellewae* strain 5R3 from Rio Raposo, Colombia, was the male or female parent in crosses with *D. metzii*, the F_1 hybrids mated *inter se* produced a higher proportion of fertile crosses than hybrids with their *D. pellewae* genome belonging either to strain 23B6 (Barro Colorado Island) or 6D2 (Darien).

Populations derived from mating of any *D. metzii-D. pellewae* hybrids *inter se* often died out in the F_2 or F_3 generations at most (imagines failed to hatch or were sterile), but 10 of these populations have been carried for 36 to 60 generations. Populations derived from backcrosses of F_1 hybrid females with either *D. metzii* or *D. pellewae* males also tended to die out in early generations, but 14 of these populations have been carried for 36 to 60 generations.

3). *Carina color polymorphism in hybrid populations.*—Regardless of whether the female parent was *D. metzii* or *D. pellewae*, F_1 males hybrid for the *D. metzii* and *D. pellewae* genomes possessed a carina and face as white as in *D. metzii*, but female hybrids had a brown face and carina as in *D. pellewae*. Thus the carina color character appears to have an autosomal but sex-limited inheritance (white carina is dominant in males; recessive in females). When females hybrid for the *D. metzii* and *D. pellewae* genomes were backcrossed to *D. pellewae* males, some of their male offspring had a less extensive white area on the face and carina and often a pigmented line down the center of the carina (Fig. 2c).

TABLE 2. *Loss of dominance modifiers of white carina in males of early generations of a population derived from a backcross of F_1 females* (D. pellewae *5R3*/D. metzii *5D1*) *to* D. pellewae *(5R3) males.*

Generation Number	White carina ♂	Reduced area white carina ♂	Whitish carina ♂	Brown carina ♂	Brown carina ♀
1	7	0	4	7	10
2	Not counted				
3	6	0	4	6	22
4	1	0	10	13	36
5	1	2	9	9	31
6			1	20	15
7			2	21	33
8			1	2	3

The variable reduction in intensity and extent of the white area on the carina in some of the back cross male progeny is apparently due to loss of modifier(s) reinforcing the dominance of the white carina character which were present in the *D. metzii* genome but absent from that of *D. pellewae*. In other males of the first back cross generation an intense white covered as much area on carina and face as in *D. metzii* males, while in still others the face and carina were brown as in *D. pellewae* males. A segregation for dominance modifier(s) was also observed in the F_2 progeny of F_1 hybrids for the *D. metzii* and *D. pellewae* genomes.

After 36 to 60 generations, the carina color polymorphism was found to be persistent in 7 of 14 back cross populations. With the *D. pellewae* genome twice as frequent as the *D. metzii* genome in initial populations, it is not surprising that four back cross populations became monomorphic for brown carina, and in three such populations, brown appeared to be replacing white carina. Similarly, a persistent polymorphism was present in 7 of 10 surviving populations derived from the progeny of F_1 hybrids, mated *inter se*, after 36 to 60 generations. In two such populations, brown appeared to be replacing white carina, and in one population, white carina had replaced brown carina in both sexes.

The face and carina of non-brown males in hybrid populations after 36 generations was whitish, not intensely white as in *D. metzii*. Therefore, it was of interest to learn how soon a hybrid laboratory population loses the modifier(s) which extend and intensify the white area on carina and face. In Table 2 appear counts of "white" vs. "brown" carina in males for the first 8 generations following mating *inter se* of back cross progeny of the cross 5R3/5D1 ♀ × 5R3 ♂. After the fifth generation, the dominance modifier(s) were lost since the white area on face and carina was restricted and less intense (i.e., whitish, not chalky-white) in all non-brown males. Similarly in five other backcross populations involving different strains of *D. metzii* and *D. pellewae*, the dominance modifiers were lost in the 4th to 7th generation. In one population, derived from 5R3/11A1 ♂ ♀ × 5R3 ♂, half of the males did show a chalky white face as late as the 12th generation (see Table 3). Here there was a persistent polymorphism for modifier(s) of the white carina character as well as for the white vs. brown carina color character itself.

4). *Introgression between* D. leticiae *and its sibling species.*—Figure 3 shows that *D. leticiae* crossed readily with four geographic strains of *D. metzii* and with more difficulty with the Trinidad strain; 1Tr17. In contrast, *D. pellewae* females of all three strains produced few or no progeny in crosses with *D. leticiae* males. When *D. leticiae* was used as the female parent, fertility was 54% in crosses with males of *D. pellewae* strain 23B6 and 25% in crosses with *D. pellewae* strains 6D2 and 5R3. *D. leticiae*/*D. pellewae* F_1 females backcrossed with *D. pellewae* males of the strain contributing its genome to the female hybrid parent, were fertile in 7 out of 9 such mass mating trials. Two of these populations derived from backcrossing to 23B6 and 6D2 males, respectively, and continued by mating *inter se*, were carried for 20 generations. Table 4 shows the outcome of mass matings of F_1 ♂ × F_1 ♀ hybrids between *D. metzii* and *D. leticiae*. Of the 17 fertile out of 59 mass mating trials of *D. metzii*/*D. leticiae* hybrids, five became sterile or

TABLE 3. *Persistent polymorphism for dominance modifier(s) of white carina in males of a population derived from a backcross of F_1 hybrid females* (D. pellewae *5R3*/D. metzii *11A1 3*) *with* D. pellewae *males (5R3).*

Generation Number	White carina ♂	Reduced area white ♂	Whitish carina ♂	Brown carina ♂	Brown carina ♀	Whitish carina ♀
Backcross progeny	8	0	3	1	15	0
1	3	3	0	4	13	0
2	7	18	11	0	40	2
3	20	9	5	0	36	3
4	8	17	10	1	64	0
5	6	6	0	0	30	3
6	25	9	7	0	17	22
7	3	30	13	0	31	1
8	7	16	6	0	31	0
9	0	15	6	0	22	0
10	7	9	10	0	21	0
11	9	6	3	0	21	0
12	29	19	1	0	70	0

failed to hatch in F_2 or F_3 and none survived for 20 generations. Similarly, *D. leticiae*/*D. metzii* F_1 hybrids crossed *inter se*, were fertile in 12 of 39 mass mating trials, but seven of these were either sterile or died out in the F_2 or F_3 generation, and only one has survived for 20 generations. Table 4 shows that the use of neither *D. leticiae* nor of any of the five strains of *D. metzii* as the female parent of the F_1 hybrids has a marked influence on the fertility of the F_1 ♂ × F_1 ♀ cross.

When either *D. metzii* or *D. pellewae* was crossed with *D. leticiae*, F_1 hybrid males showed the extent of abdominal tergite pigmentation to be intermediate between that of *D. metzii* or *D. pellewae* (with solid black tergites), and that of *D. leticiae* males (with tan tergites marked by narrow black apical bands). Reduction of pigment in hybrid males compared with the solid black abdominal coloration of *D. metzii* males occurred at the lateral margins of all tergites, and more extensively of the sixth. All males hybrid for the *D. metzii* and *D. leticiae* genomes had white carinas, like both parent species, but all F_1 females had brown carinas like *D. leticiae*. Mating *inter se* of F_1 hybrids for the *D. metzii* and *D. leticiae* genomes resulted in an F_2 progeny segregating for abdominal coloration in males and carina color in females. Males hybrid for the *D. pellewae* and *D. leticiae* genomes had white carinas as in *D. leticiae*, and F_1 hybrid females had brown carinas as in both parent species. Mating *inter se* of F_1 hybrids for the *D. pellewae* and *D. leticiae* genomes yielded an F_2 progeny seg-

TABLE 4. *Outcome of mass matings of F_1 ♂ × F_1 ♀ hybrids between* D. metzii *and* D. leticiae.

D. metzii ♀ parent of hybrids			*D. leticiae* ♀ parent of hybrids		
F_1 ♂ × F_1 ♀	Fertile	Sterile	F_1 ♂ × F_1 ♀	Fertile	Sterile
58B8/*leticiae*	6	11	*leticiae*/58B8	2	5
1T9/*leticiae*	2	13	*leticiae*/1T9	0	6
11A1 3/*leticiae*	3	7	*leticiae*/11A1 3	4	6
5D1/*leticiae*	3	9	*leticiae*/5D1	4	9
1Tr17/*leticiae*	3	2	*leticiae*/1Tr17	2	1
Total	17	42		12	27

TABLE 5. *Elimination of* D. metzii *characters from a population derived from* F_2 *hybrids between* D. metzii *strain 58B8 and* D. leticiae.

Generation	Abdominal coloration ♂♂			Carina color ♀♀	
	Intermediate	Black	Pale with black bands	Brown	Whitish
F_1	38			45	
F_2	9	7	8	18	2
F_3	10	0	11	7	10
F_4	0	0	20	34	

Sterile in F_4

TABLE 6. *Elimination of* D. leticiae *characters from a population derived from* F_1 *hybrids between* D. metzii *strain 58B8 and* D. leticiae.

Generation	Abdominal coloration ♂♂			Carina color ♀♀	
	Intermediate	Black	Pale with black bands	Brown	Whitish
F_1	13			13	
F_2		24		22	8
F_3		102		8	76
F_4		21		8	11
F_5		90		0	105

Sterile in F_5

regating for both abdominal coloration and carina color in males.

The *D. metzii* abdominal or carina color characters were replaced by *D. leticiae* characters in the third to fifth generations of two populations derived from F_1 hybrids but in two other such populations, *D. metzii* characters became fixed. Table 5 shows an example of four generations of a population derived from mass mating of hybrids between *D. metzii* and *D. leticiae* in which the *D. metzii* characters (solid black abdomen in males and whitish carina in females) were eliminated three generations later. Table 6 shows the converse situation in which *D. leticiae* characters (pale banded abdomen in males and brown carina in females) were lost in generation F_5. F_1 hybrid males for the *D. metzii* and *D. leticiae* genomes, backcrossed to *D. metzii*, were sterile in 17 of 18 mass mating trials. The one fertile backcross was carried two generations when it showed all *D. metzii* characters and was discarded. F_1 hybrid males backcrossed to *D. leticiae* were sterile in 18 of 20 mass mating trials. One fertile backcross population became sterile in the next generation and one was not tested past the initial cross. Females hybrid for *D. metzii* and *D. leticiae* genomes, backcrossed to *D. leticiae* males, were fertile in 16 of 27 mass mating trials, but 11 of these became sterile in F_2 or F_3; two were not tested past the first backcross; and three died out before the 20th generation. When such F_1 hybrid females were backcrossed to *D. metzii* males, 19 of 26 trials were fertile, but five of these became sterile or died out in F_2 or F_3, and the remaining became fixed for *D. metzii* characters by the fourth backcross generation and were discarded.

EVIDENCE FOR INTROGRESSION BETWEEN *D. METZII* AND *D. PELLEWAE* IN NATURE

In November, 1962, a mixed population of 144 *D. pellewae* and 128 *D. metzii* individuals was collected in a multispecific aggregation together with 399 individuals of 14 other species of Drosophilidae by sweeping over an unknown fruit in lowland forest near Uroseke (near El Real), Darien, Panama. Females were separated according to carina color. Among the progeny of the mass culture of 109 brown-faced females classed as *D. pellewae*, five males were observed, each with a chalky white carina as in *D. metzii*. These were discarded in the mistaken belief that somehow a *D. metzii* female accidentally had been included in the original mass culture of the *D. pellewae* strain from Darien. After it was clear from crossing the two sibling species in the laboratory that introgression could take place, a search was made in the stock cultures of all three *D. pellewae* strains for evidence that such introgression might have been taking place in nature. The male population of the 23B6 strain from Barro Colorado Island, Canal Zone, never exhibited polymorphism of carina color. An obscure polymorphism of carina color was found in the stock strains of *D. pellewae* from Darien (6D2), where both

siblings occur, and in the *D. pellewae* strain from Rio Raposo, Colombia, (5R3), where *D. metzii* was not found after a thorough search. Counts of males of strain 6D2 showed 42% males with all brown face and carina and 58% with whitish on the sides of the carina and at the base of the clypeus. In the 5R3 strain, 62% of the males had a brown face and carina and 38% possessed whitish areas on the carina and clypeus. All females of both 6D2 and 5R3 strains possessed typically brown carinas and faces as in the 23B6 strain of *D. pellewae*. In a second trip to the Darien in November, 1963, no large population of *D. metzii* or of *D. pellewae* was found, but two males and six females of *D. metzii*, with white carinas, and one male and two females of *D. pellewae* with brown carinas were netted in a mixed population on a logging path in forest a few miles west of El Real. In addition, a male with a reduced amount of white on face and carina was collected; the male was apparently part of a hybrid population. Three of the *D. metzii* females were bred singly and gave rise only to white faced male and female descendants for three generations. One *D. pellewae* female died without producing progeny, but one female, No. 10D10a, classified as *D. pellewae*, since she had a brown face, was mated in nature and gave rise to a male population showing polymorphism for carina color. Since not all the male progeny of female 10D10a had the chalky white face characteristic of *D. metzii*, the female herself must have belonged to a hybrid population, and her unknown wild mate either similarly belonged to a hybrid population, or he was *D. pellewae*. The polymorphism in the descendants of female 10D10a was maintained at least six generations, as Table 7 shows. A re-examination of this strain three years later showed it to be monomorphic "brown faced."

Original field notes taken in June, 1963, at Rio Raposo, Colombia, recorded that one of the eight *D. pellewae* males taken during a 9-day collection trip at the Rockefeller Field Laboratory had a whitish carina, whereas the other seven had typically brown carinas and faces. Since no *D. metzii* was collected at Rio Raposo, though 18 females of *D. pellewae* were taken from breadfruit at five different localities from 4 to 6 miles apart along the river, the aberrant male is thought to have been the result of introgression between the two species further to the north.

TABLE 7. *Polymorphism for carina color in a population derived from single female #10D10a taken from a natural population in the Darien, Nov., 1963.*

Generation	Reduced white area on carina ♂	Whitish carina ♂	Brown carina ♂	Brown carina ♀
1	3	7	4	13
2	7	0	9	21
3		1	9	18
4	not counted			
5		7	55	59
6		2	54	38

Three years later the population is monomorphic for brown carina.

CYTOLOGY

Aceto-orcein-lactic acid smears using a modification of the method of Wasserman (1954) were made of salivary chromosomes of hybrids between *D. metzii* and *D. pellewae*, using various strains of each and also of hybrids between *D. metzii* and *D. leticiae*. Larvae were taken from uncrowded cultures reared at 70 F on corn meal medium enriched with the addition of Fleischmann's yeast. The difficulty in breaking the nuclear membrane in these hybrid cells was overcome by using the suggestion of H. Takada (personal communication) that a drop of 1N HCl be added to the freshly dissected salivary glands for half a minute, followed by washing in distilled water and adding the stain. The slides were made permanent by using alcohol replacement in a desiccator overnight, dipping in absolute alcohol for half a minute, and mounting in euparol. The cells were studied with phase contrast microscopy, magnification 970 X, oil immersion. Figure 5a shows a photograph of the salivary chromosomes of a female hybrid between *D. pellewae* strain 23B6 and *D. metzii* strain 11A1 3. An interpretation of

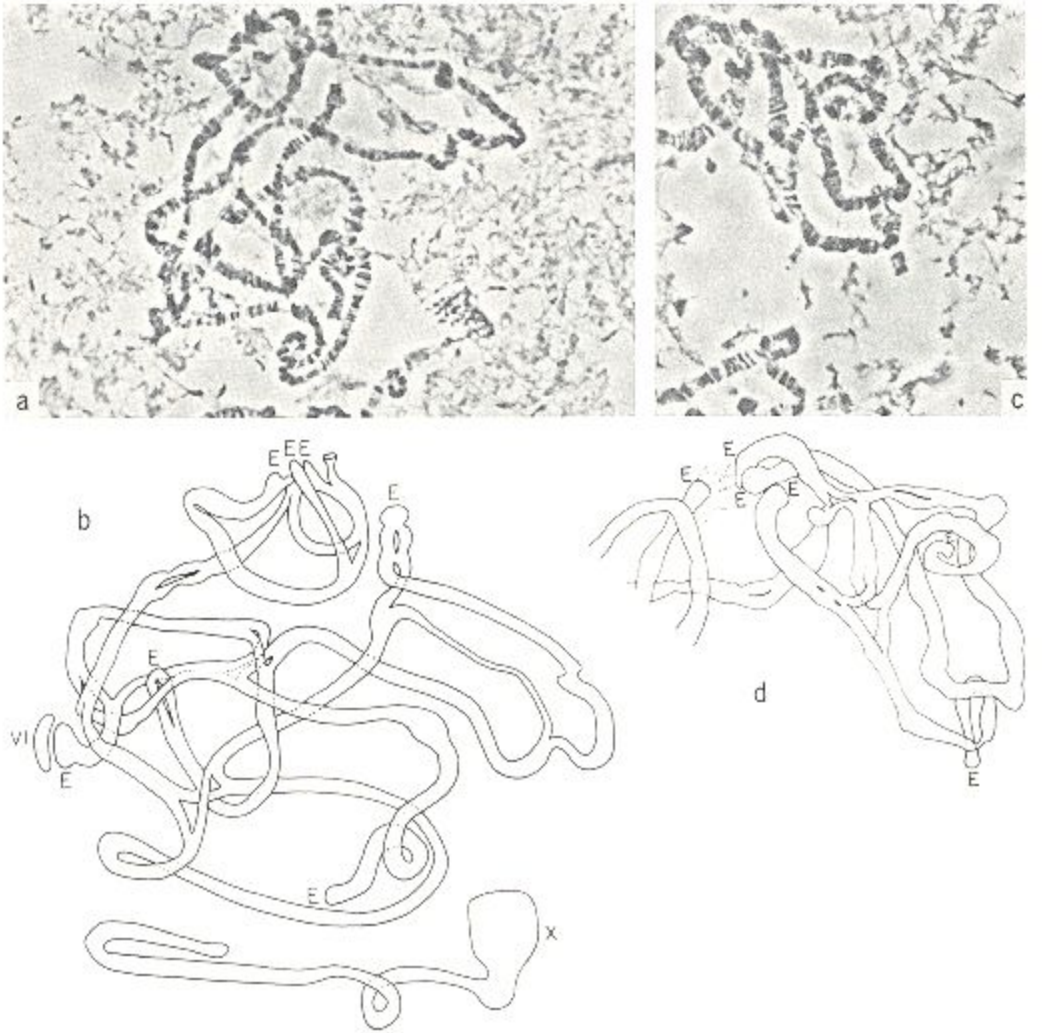

FIG. 5. (a) Photograph of salivary chromosomes of a female hybrid for *D. metzii* and *D. pellewae* genomes (11A1 3/23B6) showing four pairs of autosomes involved in a complex translocation figure. The small chromosome VI and the X chromsome lie free from the autosomes. (b) Interpretation of Fig. 5a; ends of chromosomes are marked "E." (c) Photograph of a portion of the karyotype of a female hybrid for the *D. metzii* and *D. pellewae* genomes showing two cross-shaped heterozygous translocation interchanges. (d) Interpretation of Fig. 5c; ends of chromosomes are marked "E."

the configuration is given in Fig. 5b. The X chromosome, with the right heterochromatic end shaped like the bowl of a pipe, lies free from the autosomal mass. The two homologues of the X chromosome are fully paired, a fact that agrees with the observation that the X chromosome of *D. metzii* as mapped by Kastritsis (1965) corresponds band for band with the X chromosome of *D. pellewae* strains. In Fig. 5a the small dot-shaped chromosome VI appears as an oval spot close to the other autosomes in the leftmost part of the figure. The four pairs of rod-shaped long autosomes, with eight ends marked "E," form a labyrinth of chromosome arms due to pairing between homologous portions of genomes distinguished from one another by translocations. Inversions also distinguish the two genomes; a large inversion loop is visible in the lower middle part of the figure above the free-lying X chromosome. In Fig. 5c

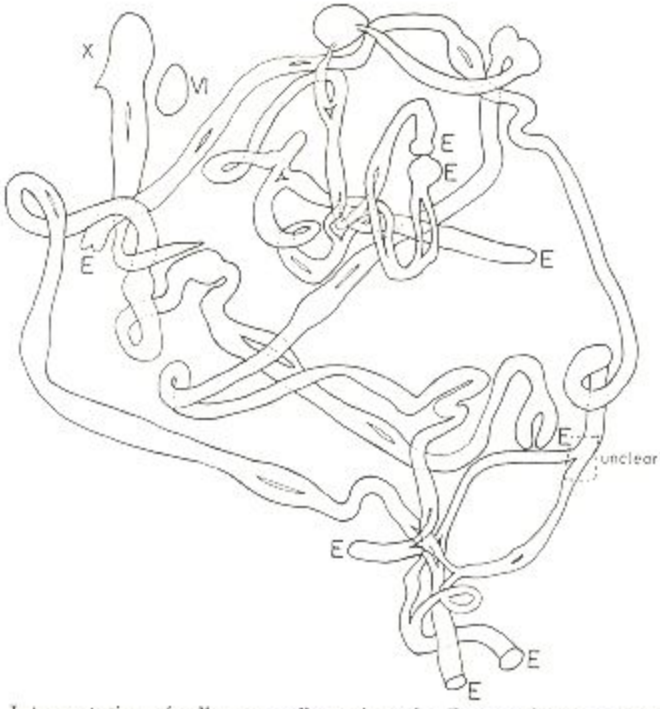

FIG. 6. Interpretation of salivary configuration of a *D. metzii*/*D. leticiae* hybrid.

appears a photograph of a portion of the karyotype of another hybrid derived from the same two strains of *D. metzii* and *D. pellewae*, respectively, in which two interchanges involving translocated chromosomes can be seen. The ends, labeled "E" in the interpretation of the photograph (Fig. 5d) can be seen in Fig. 5c, showing that the interchanges involve translocations rather than inversions. Lack of close pairing of homologues is seen both in Fig. 5a and in 5c, where certain short sections of the chromosome appear abnormally wide. A similar pairing figure of connected autosomes with a free-lying X chromosome has been observed for hybrids between *D. pellewae* strain 23B6 and *D. metzii* strain 58B8; *D. pellewae* strain 6D2 and *D. metzii* strain 5D1; *D. pellewae* 6D2 and *D. metzii* strain 11A1 3, and *D. pellewae* strain 5R3 and *D. metzii* strain 1T9. Figure 6 shows an interpretation of the salivary configuration of female hybrids between *D. metzii* strain 58B8 and *D. leticiae*. Unfortunately, the slide was broken as preparations for photography were being made. The X chromosome and small chromosome VI also lie free from the mass of long autosomes; a portion of the X has been torn off in this cell. Eight ends, marked "E" of the four long autosomes which are connected in the hybrid by the pairing of homologous chromosomes involved in translocations are visible. This hybrid shows a more extensive lack of pairing as well as a greater complexity of structural rearrangements than the *D. metzii*-*D. pellewae* hybrid.

Twenty preparations each of *D. pellewae* strains 23B6, 6D2, and 5R3 showed no inversion figures, but two cross-shaped translocation figures were seen in two preparations of larvae taken from *D. pellewae* strain 6D2 from the Darien. Inversion figures were not seen in seven individuals heterozygous for *D. pellewae* strains 23B6 and 6D2. Neither 10 individuals of *D. metzii* strain 58B8 nor 10 of strain 1T9 showed heterozygous inversions, nor were inversion figures seen in four individuals heterozygous for these strains. The other strains of *D. metzii* have not yet been exam-

ined for inversion polymorphism. Work is in progress to determine if further heterozygous translocation figures can be found in salivary gland cells of larvae taken from the Darien strains 10D10a and 6D2 of *D. pellewae* and 5D2 of *D. metzii*. The hybrid populations derived from laboratory introgression between *D. metzii* and *D. pellewae* will also be studied to see if any heterozygous translocation figures are present.

Discussion

The observation of segregants showing a carina color polymorphism following crossing between *D. metzii* and *D. pellewae* in natural populations in the Isthmus of Darien is the first of its kind reported in the genus. Ross (1957) presented evidence of the hybrid origin of four members of the leafhopper genus *Erythroneura* and summarized past literature on the subject of hybrid animal populations. Lewontin and Birch (1966) have argued that introgression has occurred between the Queensland fruit fly *Dacus tryoni* and the closely related *Dacus neohumeralis*. Using species specific salivary chromosomal banding patterns as a criterion of species hybrid detection, Carson (1954) found no evidence of crossing between *D. bocainensis* and *D. bocainoides* of a rain forest of Brazil though the two species feed together over fallen fruit and can be made to hybridize in the laboratory. With a similar criterion, no crossing in natural populations between *D. arizonensis* and race B of *D. mojavensis* was found by Mettler and Nagle (1966); between *D. pavani* and *D. gaucha* by Koref-Santibañez and del Solar (1961); between eastern and western strains of *D. athabasca* by Miller and Westphal (1965); between the Orinocan and Amazonian strains of *D. paulistorum* by Dobzhansky and Spassky (1959); or between "light" and "dark" *D. ananassae* (Futch, in Johnson et al., 1966). Although *D. persimilis* and *D. pseudoobscura* will introgress in the laboratory when no choice of mates is given and temperature is held at 25 C, species hybrids in nature are extremely rare (Van Valen, 1963). Natural sterile male hybrids between *D. aldrichi* and *D. mulleri*, detectable by the reduced size of their testes seen through the ventral abdominal wall, are found only in regions where *D. aldrichi* males exceed one-third the male population of both species (Patterson and Stone, 1952). F_1 hybrid females between these species are not detectable in nature, but these have been obtained in the laboratory and have been found to be likewise sterile (Patterson and Stone, 1952).

Drosophila metzii and *D. pellewae* of the present study have met in a hybrid zone in the Isthmus of Darien for a maximum of three million years (Woodring, personal communication), since the formation of the Panama Land Bridge, and neither species has lost its identity nor developed sufficient sexual isolation to totally prevent a certain amount of intercrossing in nature. This is the oldest animal hybrid zone which can be dated in an approximate fashion of which the author is aware. Mayr (1963) cites the 5000 year old hybrid zone occupied by *Corvus c. corone* and *Corvus c. cornix* in Europe.

Since the *Drosophila* species of the present study belong to the typically South American *tripunctata* species group, *D. metzii* is thought to have evolved from a wind spread migrant from South America some time in the Tertiary when there was an oceanic separation of the Americas. The species that became *D. pellewae* was isolated on the west coast of Colombia by the Atrata Trough, a marine waterway extending from the north southward into what is now central Colombia (Schuchert, 1955) and/or by the orogenesis of the Andes Mountains. The species on the headwaters of the Amazon River that became *D. leticiae* shows a greater difference in salivary chromosomal banding pattern from *D. metzii* than distinguishes the latter from *D. pellewae*.

The finding that autosomal translocations have served to keep distinct two closely related species which evolved in isolation but are now reunited in the Darien Isthmus was unexpected in view of the absence of translocation structural differences

distinguishing members of the large *repleta* group studied by Wasserman (1960) and the *melanica* group studied by Stalker (1965). However, translocations of the insertional type may play a role in distinguishing members of these species groups since there are "foreign" regions of corresponding chromosomes of any two related species that are not due to inversions in addition to regions that show a remarkable correspondence in banding pattern. Stone (1962) contrasted the number of paracentric inversions with the virtual absence of translocations, excluding fusions, in *Drosophila*. He pointed out that this is understandable since paracentric inversions may have a positive selective value by holding together favorable gene combinations owing to their reduction of recombination, whereas heterozygous translocations are strongly selected against when they first arise because of the loss of about 50% zygotes in crosses with individuals free of translocations. In the present work the high rate with which populations derived from hybrids for any two of the three sibling species died out in early generations following laboratory introgression was believed to be partly due to heterozygosity for complex autosomal translocations which distinguish the species. That heterozygous translocations may occasionally possess a strongly positive selective value is suggested by the finding of White (1963) of a male *Moraba scurra*, heterozygous for a translocation involving four autosomes in a natural population. Naturally occurring individuals heterozygous for a translocation involving two autosomes also have been described for Brazilian *D. ananassae* (Freire-Maia, 1961). A complex translocation eliminating random assortment of most of the chromosomes might survive and become homozygous in the population if the chromosomes held in linkage are favorably coadapted. This may account for the fixation of carina or abdominal color characters belonging to a given parental species in early generations of populations derived from *D. metzii*/*D. pellewae* or *D. metzii*/*D. leticiae* hybrids, respectively, or in populations derived from backcrossing female species hybrids with one of the parental species.

Although specific isolating mechanisms have not been examined with respect to whether or not a reinforcement of these exists in the area of sympatry of *D. metzii* and *D. pellewae*, data on crossability and mean number of offspring per fertile cross between sympatric and allopatric strains of these species do not support the idea that such a reinforcement has taken place. Instead these factors seem to depend on intrinsic strain characteristics without regard to allopatry or sympatry. For example, the *D. pellewae* strain from Rio Raposo, Colombia, where *D. metzii* was not found, does cross more readily and produces more offspring per fertile cross with four of five strains of *D. metzii* than do *D. pellewae* strains 6D2 and 23B6 which come from areas also occupied by *D. metzii*. On the other hand, the per cent fertile crosses between *D. metzii* strain 58B8 females and sympatric *D. pellewae* strain 23B6 males (57%) is approximately the same as with males of allopatric *D. pellewae* strain 5R3 (52%). Since *D. pellewae* on Barro Colorado Island, Canal Zone, represented by strain 23B6, is an extremely rare species, the argument could be made that no reinforcement of isolating mechanisms should be expected between *D. metzii* (58B8) and *D. pellewae* (23B6) here. In the Darien Isthmus, where *D. pellewae* and *D. metzii* occurred in a 3:2 ratio in a natural population, it might be expected that isolating mechanisms would be strengthened most; i.e., reinforcement of isolating mechanisms is expected to have occurred between *D. pellewae* strain 6D2 and *D. metzii* strain 5D1, both derived from a single mixed population. Reciprocal matings between *D. pellewae* strain 6D2 and allopatric *D. metzii* strains 11A1 3 and 1T9 do indeed show a distinctly higher mean number of offspring per fertile cross than crosses between sympatric *D. pellewae* (6D2) and *D. metzii* (5D1). On the other hand, reciprocal crosses between *D. pellewae* strain 6D2 and allopatric *D. metzii* strains 1Tr17 or 58B8 show as low a mean number of off-

spring per fertile cross as the crosses between sympatric *D. pellewae* strain 6D2 and *D. metzii* strain 5D1. It is worth noting that the pale eastern *D. metzii* strains 5D1 and 1Tr17 are distinguishable from the dark western strains 11A1 3 and 1T9 and also from strain 58B8, which shows an intermediate mesonotal coloration corresponding to its intermediate geographical position. Thus it appears that the low mean number of progeny per fertile cross of *D. pellewae* strain 6D2 and *D. metzii* strain 5D1 does not necessarily result from a reinforcement of isolating mechanisms in the area of sympatry but could equally be considered a strain dependent phenomenon due to isolating mechanisms developed in allopatric populations.

Since dominance modifier(s) which extend and intensify the white carina color in *D. metzii* are lost in early generations from hybrid laboratory populations, they must be located on chromosomes that undergo random assortment at meiosis; i.e., on the X chromosome or on the small chromosome VI. The X is ruled out as a possible location of these dominance modifier(s) because reciprocal crosses of *D. metzii* and *D. pellewae* each yield F_1 males with a chalky white carina and face; i.e., no reduction in the extent or intensity of the white area which would be expected if the modifier(s) were on the X chromosome of *D. pellewae* in progeny of the cross between *D. pellewae* females with *D. metzii* males. Thus it is believed that the dominance modifier(s) must be located on the small chromosome VI.

The findings with respect to the sibling species *D. metzii*, *D. pellewae* and *D. leticiae* present both similarities and differences with those of Dobzhansky and his colleagues on members of the superspecies *D. paulistorum*. The siblings of the *tripunctata* group and those of the *paulistorum* superspecies occupy about the same area in Central and South America. In consequence their respective evolutionary divergence has been influenced by like topographic features and geological history. Both groups of species produce fertile females and sterile or practically sterile males in laboratory crosses where these succeed. Translocations have not played a role as an isolating mechanism in the *paulistorum* siblings, but sexual isolation and hybrid sterility are important. Barro Colorado Island, Canal Zone, appears to be near the limit of range of the Amazonian and Orinocan siblings of the *paulistorum* set since these are extremely rare here compared with *D. equinoxalis* and *D. tropicalis*, also siblings of the same *willistoni* species group (Spassky, in Pipkin, 1965). The Central American *paulistorum* species has not yet been collected on Barro Colorado Island, but has been taken on the mountain Cerro Campana, some 35 miles west of Panama City (Dobzhansky, personal communication). There are signs that introgression between *paulistorum* species has occurred in northern Colombia since a Transitional strain there shares species specific chromosomal banding patterns with an Andean-Brazilian race (Dobzhansky et al., 1964). There is no evidence on this basis that such introgression has occurred on Barro Colorado Island, and in fact the Amazonian and Orinocan species here will not even cross in the laboratory (Dobzhansky and Spassky, 1959). The sexual isolation which characterizes the Amazonian and Orinocan species of Barro Colorado Island is believed not to have developed there as a response to unfavorable introgression since both species are so rare in this area that little or no crossing between them is to be expected.

Although the *tripunctata* siblings of the present study and the *paulistorum* species are both ground-feeding *Drosophila*, the *paulistorum* species use a wider range of fallen forest fruits and blossoms for feeding and breeding, including both drier types and also fleshy fruits (Pipkin, 1965). Thus it is to be predicted that their populations in non-marginal areas of their distribution would be maintained in larger numbers throughout the year and would undergo expansions in the dry season when the fleshy fruits are dropping. The *tripunctata* siblings on the other hand, feed almost wholly on drier type of fruits and never enter traps

baited with fleshy cultivated fruits which do attract the *paulistorum* species. The *tripunctata* siblings are subject to great reduction in population size during the dry season because of the desiccation of their fallen fruit and blossom breeding sites. They survive at this time by breeding in small numbers in living flowers (Pipkin, 1965). Owing to their breeding habits the *tripunctata* siblings are not expected to build as large populations the year round as the *paulistorum* siblings in non-marginal areas.

Speciation among the *paulistorum* members has progressed farther in Central America than among the *tripunctata* siblings of the present study. Possibly the ancestral *paulistorum* moved into the area earlier or was able to colonize earlier because of its versatility in food habits. The Central American *paulistorum* sibling is distinct from the Amazonian and Orinocan siblings, whereas *D. metzii*, with a similar distribution has only differentiated into a dark western Panama (and Costa Rica) strain and a pale eastern strain from Darien and Trinidad, each group possessing a characteristic crossability with a given *D. pellewae* strain. The differentiation of a Central American *paulistorum* species and a dark strain of *D. metzii* from western Panama and Costa Rica suggests that these evolved as insular forms during the Tertiary when Central America existed as islands. The *tripunctata* sibling from the Amazon River, *D. leticiae*, has not reached the Isthmus of Darien, and nothing is known of its northern margin of distribution. The *tripunctata* sibling from the southwest coast of Colombia is *D. pellewae* which has reached Darien in numbers but is extremely rare at Barro Colorado Island. The *paulistorum* sibling from Rio Raposo, Colombia also shows characteristics of its topographic isolation (Dobzhansky and Pavlovsky, 1967).

Summary

The sibling species of *D. metzii* and *D. pellewae* were found feeding in the same multispecific aggregations on fallen fruit in a forest of the Isthmus of Darien, Panama. *D. metzii* only was collected in western Panama, Costa Rica, and Trinidad. Only *D. pellewae* was found at Rio Raposo on the southwest coast of Colombia. The species differ in the possession by *metzii* males of a chalk white face and carina (whitish in females) which is brownish in both sexes of *pellewae*. Though a degree of sexual isolation exists, some F_1 hybrids between the two species are produced both in nature and in laboratory crosses. Backcrosses of female hybrids to males of either parental species are usually fertile; backcrosses of male hybrids, usually sterile. F_1 hybrids crossed *inter se* are usually sterile. Laboratory populations derived from backcross progeny often die out in three or four generations, but several have been carried as long as from 30 to 60 generations. F_1 hybrid females backcrossed to *pellewae* produce male progeny exhibiting various dilutions of the extent and intensity of the white area on face and carina owing to loss of dominance modifers present in *metzii* but absent in *pellewae*. These laboratory backcross populations display a carina color polymorphism which can also be seen in natural populations. Examination of salivary chromosomes of hybrids reveals the presence of translocations involving four pairs of autosomes. These serve as the major selective mechanism for keeping the species separate in spite of the limited introgression resulting in the carina color polymorphism. It is speculated that the two species have been introgressing for a maximum of almost three million years since the formation of the Panama Land Bridge. *D. leticiae*, from the headwaters of the Amazon River, is an allopatric species closely related to *D. metzii* and *to D. pellewae*. Laboratory introgression is possible between *D. leticiae* and its sibling species. Complex autosomal translocations also distinguish *D. leticiae* and *D. metzii*.

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