THE SALIVARY GLAND CHROMOSOMES OF DROSOPHILA NIGROMELANICA

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DESPITE the great potential value of the salivary gland chromosomes of Drosophila as indicators of exact phylogenetic relationships within species groups, there is in fact a rather limited number of such groups in which these chromosomes have been so used. Notable examples of their use are the *D. obscura* group (DOBZHANSKY and EPLING 1944), the *D. virilis* group (STONE, GUEST and WILSON 1960; STONE 1962), and the very large *D. repleta* group (WASSERMAN 1960, 1962, 1963). The present paper is one of a series dealing with the phyletic relationships within the *D. melanica* species group.

The *D. melanica* group, as now understood, contains seven Nearctic species. One of these, *D. melanissima*, differs markedly from the remainder of the group in having four forked egg filaments, rather than two simple filaments. *D. melanissima* has not been reared in the laboratory, is unknown cytologically and genetically, and will be ignored in the rest of this paper.

Of the remaining six Nearctic species, extensive cytological studies have been reported on *D. melanica* (WARD 1952), *D. paramelanica* (STALKER 1960, 1961) and *D. euronotus* (STALKER 1964). The cytology of *D. nigromelanica* is the subject of this paper, and future papers will cover *D. micromelanica*, *D. melanura* and the relationships of the species within the group.

D. nigromelanica is morphologically distinct from the other members of the group, and is easily distinguished by its dull black color, somewhat elongated shape, and divergent anterior scutellar bristles. Little is known of its ecology. PATTERSON (1943) points out that in Texas it is characteristically a woods species, not living in towns and cities, and has been found on fungi which were growing on the ground or in hollow trees. In the St. Louis area, it has been found feeding on the infected sap of bleeding White Oak (CARSON and STALKER 1951), on Ward's Willow and on windfall pears. The species comes readily to fruit baits, and while not a domestic species in the usual sense, it is found in residential areas which support large trees. The larval food for this species is unknown, but some other members of the group are known to breed on infected tree sap.

The known geographical distribution of *D. nigromelanica* is indicated by the shaded area in Figure 1; this distribution overlaps those of the rest of the group,

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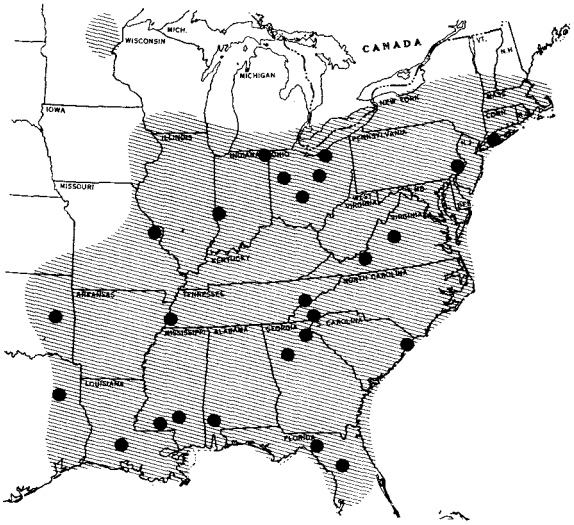

FIGURE 1.—Geographical distribution of *D. nigromelanica*. The shaded area indicates the known range of the species, the black dots the localities which were sampled cytologically in this study.

and with the exception of *D. melanica*, *D. nigromelanica* has a greater north-south range than any other group member. The black dots in Figure 1 show the locations of the 25 populations sampled in the cytological survey.

Hybridization tests: Attempts to cross *D. nigromelanica* with the other five group members have been generally unsuccessful. All possible crossing combinations have been attempted, and are indicated in Table 1. In this table, the term "Author" in the last column indicates new data. All tests reported by the author involved attempted matings in shell vials containing up to ten pairs of flies. A variety of conditions was used to attempt to induce hybridization (different foods, different temperatures, different light intensities, and mutilation of some females by wing-clipping). Except for the hybrids reported by GRIFFEN (1942), there have been no reports of hybridization between *D. nigromelanica* and any other species.

Failure to obtain hybrids in a particular cross by one worker despite the fact that they have been obtained by another, is not in itself surprising, but GRIFFEN's report of fertile hybrids of *both* sexes is an interesting one, since (as will be demonstrated in a later paper of this series), *D. nigromelanica* and *D. paramelanica* are phylogenetically distantly related; in fact only *D. nigromelanica* and *D. melanica* are more distantly related within the group. Moreover, except for the hybrids reported by GRIFFEN (*D. nigromelanica* × *D. paramelanica* and reciprocals; *D. paramelanica* × *D. melanica* and reciprocals) all species hybrids in this group studied by other workers have been found to be female fertile but male sterile, and this list includes the *D. melanica-D. paramelanica* hybrids reported by GRIFFEN (MILLER 1944; PATTERSON and WARD 1952; STALKER

TABLE 1

Hybrdization tests between D. nigromelanica *and other members of the* D. melanica *species group*

Females	Males	Number of pairs tested	Hybrids	Authority
D. nigromelanica	× *D. paramelanica*	2250	few fertile males and females	GRIFFEN 1942
		240	0	Author
D. paramelanica	× *D. nigromelanica*	2250	few fertile males and females	GRIFFEN 1942
		220	0	Author
D. nigromelanica	× *D. melanica*	900	0	GRIFFEN 1942
		80	0	Author
D. melanica	× *D. nigromelanica*	900	0	GRIFFEN 1942
		94	0	Author
D. nigromelanica	× *D. melanura*	25	0	MILLER 1944
D. melanura	× *D. nigromelanica*	80	0	Author
D. nigromelanica	× *D. euronotus*	310	0	Author
D. euronotus	× *D. nigromelanica*	260	0	Author
D. nigromelanica	× *D. micromelanica*	270	0	Author*
D. micromelanica	× *D. nigromelanica*	300	0	Author*

* PATTERSON and STONE (1952) also indicate that crosses between *D. nigromelanica* and *D. micromelanica* failed to produce hybrids.

1964 and unpublished data). Thus the finding of fertility in both sexes of the hybrids between the distantly related *D. nigromelanica* and *D. paramelanica* is a distinct surprise, and its confirmation would be interesting and important.

Parthenogenesis in D. nigromelanica: In the course of the tests described in Table 1, the author observed parthenogenetically produced females in three instances, all involving attempted crosses between *D. nigromelanica* females (the Standard stock from Terre Haute, Indiana) and *D. euronotus* males. Since the mothers in all three cases had earlier produced quantities of unfertilized eggs, there was no question of nonvirginity. Two of the impaternate females were squashed as mature larvae and studied cytologically. The banding sequences were typical of *D. nigromelanica* Standard, and very different from those of *D. euronotus*. In the third case, an adult female was obtained which was typically *D. nigromelanica* in phenotype, crossed readily to *D. nigromelanica* Standard males, and produced many larvae, of which 11 were studied cytologically and showed the typical *D. nigromelanica* banding sequences. Such parthenogenetically produced progeny have of course been observed in other species of Drosophila (see for example STALKER 1954; CARSON 1961, 1962a,b) although they have never been reported before in the *D. melanica* species group.

MATERIALS AND METHODS

Flies were reared at 25 ± 1°C., on standard cornmeal-karo-agar-tegosept food. Larvae to be used for cytological purposes were reared in vials, the food being enriched daily with yeast paste. Mature larvae which had left the food to pupate were chosen for salivary squashes, and it was found that such larvae, whether they left the food in the morning or in the afternoon, made

equally satisfactory preparations. This is in sharp contrast to the situation in *D. euronotus*, since in that species larvae maturing in the late morning hours make the best squashes. Salivary glands were dissected in 60 percent acetic acid, quickly transferred to a small drop of lactic- acetic-orcein on a silicone-treated slide, allowed to stain for approximately 2 minutes, then squashed under a coverslip. Such unsealed temporary preparations last for many months if kept in a freezer.

Most of the salivary chromosome analyses were based on wild-caught males or females, or their F_1 laboratory-reared sons and daughters. Since gene arrangement frequencies were not demonstrably different in the two sexes, nor in wild-caught as opposed to F_1 individuals, the four kinds of data are lumped within locality. When a locality was represented by a laboratory stock, this fact is indicated in the tables. All analyses of salivary gland chromosomes involved crosses to a structurally homozygous Standard stock derived from Terre Haute, Indiana.

Collections of wild flies were made from over-ripe banana bait hung in cups, pint jars or plastic bags in the woods. The author is greatly indebted to other workers for their kindness in supplying stocks and wild-caught individuals. In this regard, he wishes especially to thank Drs. Max Levitan, D. D. Miller, M. R. Wheeler, and H. L. Carson. He is also indebted to Mrs. Jo Beth Webber for technical assistance, and to Marion L. Stalker for help in preparation of the plates. Part of the work was done during the tenure of a National Science Foundation Senior Postdoctoral Fellowship spent at the Genetics Foundation of the University of Texas. It is a pleasure to acknowledge the generous hospitality extended by Dr. Wilson Stone and the members of his staff.

THE CHROMOSOMES

In the numbering and identification of the mitotic and salivary gland chromosomes of *D. nigromelanica*, since the various elements are readily homologized to those of *D. melanica*, *D. paramelanica* and *D. euronotus*, the corresponding designations are used.

In *D. nigromelanica*, preparations of larval neuroblasts from two St. Louis strains showed ten elements in each nucleus: one pair of large V-shaped chromosomes, a pair of smaller V-shaped chromosomes, two pairs of medium length rods, and a pair of very short rods. The large V-shaped chromosomes are the XX or XY pair, and are represented in the salivary chromosomes by the X-left and X-right euchromatic arms. The short pair of V-shaped autosomes is represented by the short salivary chromosome arms 4-left and 4-right. The two pairs of medium length rods correspond to salivary chromosomes 2 and 3, and the pair of microchromosomes to the short salivary chromosome 5.

In *D. nigromelanica* the metaphase microchromosomes are variable in size and shape. Strains from Rhode Island, North Carolina and Virginia have a pair of short rods representing the microchromosomes and Ward (1949) points out that in these strains the microchromosomes are long enough to make it impossible to distinguish them from the other rod-shaped autosomes. In the two St. Louis strains mentioned above, the microchromosomes are so short that it is easy to distinguish them from the other rod-shaped autosomes. In a strain from Texas, Wharton (1943) reports that the microchromosomes are represented by a very short pair of V's. Ward interprets the observed size variability as due to changes in the heterochromatic content of the chromosome pair, and suggests that a pericentric inversion may also be involved.

The salivary gland chromosome maps shown in Plate I represent the Stand-

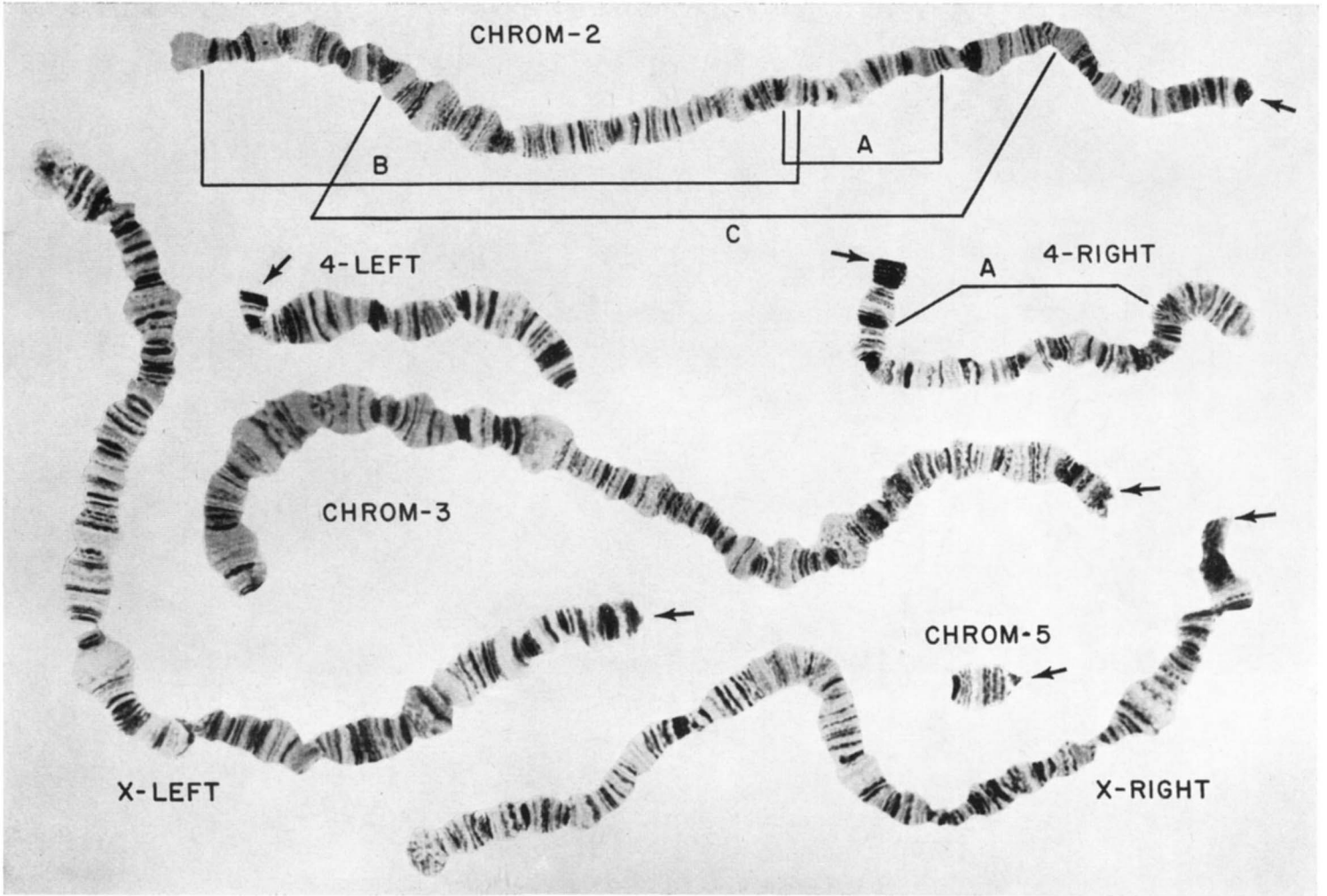

PLATE I.—Salivary gland chromosomes of *Drosophila nigromelanica*. The inversion limits are indicated by brackets, the proximal end of each arm by an arrow. Standard banding sequences are shown throughout.

ard sequence of all arms. Each of these maps except that for chromosome 5 is a montage, made up of several small sections of photographs mounted together. The break points of the various inversions are indicated by the lettered brackets, and the proximal ends of the chromosome arms are indicated by arrows. The letter designations used for the inversions begin with A for each chromosome arm, and bear no relationship to those used by WARD in *D. melanica* or by STALKER in *D. paramelanica* and *D. euronotus*.

THE INVERSION POLYMORPHISM

In *D. nigromelanica* inversions have been found only in chromosomes 2 and 4. Although only a single gene sequence is known for chromosome 5, this chromosome is frequently poorly spread, and small rearrangements in it could easily have been overlooked.

Data to be presented in a later paper will show that *D. micromelanica* is ancestral to *D. nigromelanica*, thus the various gene arrangements in the latter species can properly be related to those found in the former. Chromosome-2 of *D. nigromelanica* has four known sequences: +, B, BA and BC. Of these, B is most closely related to the second chromosome of *D. micromelanica* (from which it differs by at least eight inversions). Hence, B may be taken as the starting point of the intraspecific phylogeny. Both BA and BC differ from B by the single overlapping inversions A and C, while Standard (+) is related to BA and BC only through B. This leads to the simple phylogeny shown in Figure 2.

Of the two sequences in the right arm of chromosome 4, Standard (+) must be considered as ancestral to A because of its greater similarity to chromosome 4 of *D. micromelanica* (from which it differs by at least six inversions). See Figure 2.

Both chromosome 2 and chromosome 4 arrangements show pronounced geographical frequency changes. It will be noted from Table 2, and especially from Figure 3, that populations from the north-central region (Indiana, Ohio) and from the Appalachian chain generally, differ rather strikingly from those in Louisiana, Mississippi, South Carolina, and particularly Florida. More specifically, while sequences AB and + in chromosome 2 show no clearly systematic changes with locality, arrangement B in chromosome 2 is characteristic of southern populations; it shows low frequencies in the north-central region and in the Appalachians generally, high frequencies in the southern states, and reaches fixation in Florida. Conversely, BC in chromosome 2 is characteristic of the

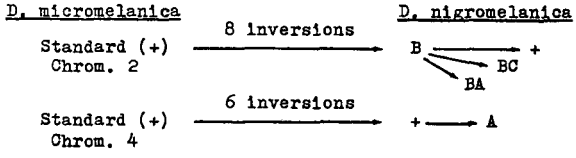

FIGURE 2.—Phylogenetic relationships of gene arrangements in the second and fourth chromosomes of *D. nigromelanica*.

TABLE 2

Frequencies of gene arrangements in chromosome 2 and chromosome 4 of D. nigromelanica

Locality	Chromosome 2					Chromosome 4		
	N	+	AB	B	BC	N	+	A
Tyler State Park, Texas	12	2 *16.7*	9 *75.0*	1 *8.3*	.	12	12 *100.0*	.
Checotah, Oklahoma	2	.	2 *100.0*	.	.	2	2 *100.0*	.
St. Louis, Missouri	19	12 *63.2*	6 *31.6*	1 *5.3*	.	19	19 *100.0*	.
Shelby Co., Tennessee	2	1 *50.0*	.	1 *50.0*	.	2	2 *100.0*	.
Terre Haute, Indiana (1 $)		*	*		*		*	*
Pokagon State Park, Indiana	28	10 *35.7*	10 *35.7*	3 *10.7*	5 *17.9*	15	9 *60.0*	6 *40.0*
Lima, Cleveland, Wooster and Columbus, Ohio	19	11 *57.9*	6 *31.6*	.	2 *10.5*	19	16 *84.2*	3 *15.8*
Swarthmore, Pennsylvania	6	.	2 *33.3*	3 *50.0*	1 *16.7*	6	5 *83.3*	1 *16.7*
Cold Spring, Harbor, N.Y. (1 $)		*	*				*	
Buckingham Co., Virginia	10	3 *30.0*	.	7 *70.0*	.	10	10 *100.0*	.
Montgomery Co.,Virginia	94	33 *35.1*	48 *51.1*	9 *9.6*	4 *4.3*	93	85 *91.4*	8 *8.6*
Gatlinburg, Tennessee (1 $)		*	*				*	
Highlands, North Carolina	64	22 *34.4*	26 *40.6*	10 *15.6*	6 *9.4*	64	58 *90.6*	6 *9.4*
Myrtle Beach State Park, South Carolina	58	25 *43.1*	.	33 *56.9*	.	58	58 *100.0*	.
Cornelia, Georgia (1 $)		*		*			*	
De Kalb Co., Georgia	54	11 *20.4*	4 *7.4*	37 *68.5*	2 *3.7*	54	54 *100.0*	.
Opelousas, Louisiana	9	7 *77.8*	.	2 *22.2*	.	9	9 *100.0*	.
Jones Co., Mississippi	2	.	1 *50.0*	1 *50.0*	.	2	2 *100.0*	.
Eastabuchie, Mississippi	8	1 *12.5*	5 *62.5*	2 *25.0*	.	8	8 *100.0*	.
Wagarville, Alabama	2	1 *50.0*	1 *50.0*	.	.	2	2 *100.0*	.
Inverness (47) & Perry (8), Florida	55	.	.	55 *100.0*	.	55	55 *100.0*	.

For each gene arrangement and locality the number of occurrences is given in Arabic numerals, the percentage frequency for that locality in italics. For localities represented by a single laboratory stock (1 $), the presence of a given gene arrangement is indicated by an asterisk.

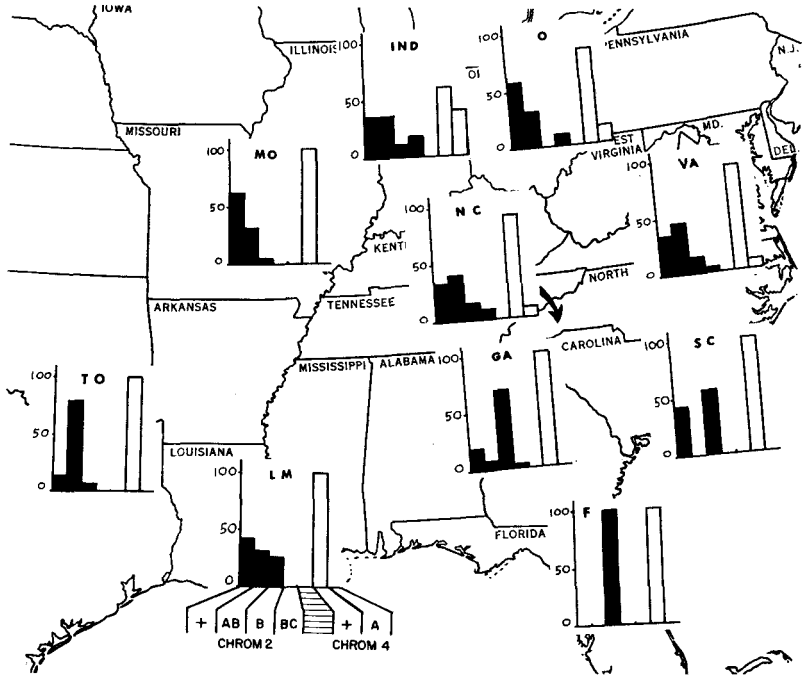

FIGURE 3.—Geographical variability in frequencies of gene arrangements in *D. nigromelanica*. The states from which the data came are indicated by the abbreviations at the tops of the frequency polygons. The data from Louisiana and Mississippi are combined, as are those from Texas and Oklahoma. The data from Virginia include both populations sampled from that state.

north-central region and Appalachians, and is found nowhere else. In chromosome 4 the distribution of arrangement A roughly parallels that of chromosome 2 BC as a north-central and montane arrangement.

The population from De Kalb Co., Georgia, occupies an intermediate position, both chromosomally and geographically. The population samples came from elevations of 840 to 1010 feet in the foothills of the southern Appalachians, and as such might be considered as southern semi-montane. Chromosomally, the population might be classified as southern (because of its high frequencies of chromosome 2 B and chromosome 4 Standard), except that it also carries chromosome 2 BC, a typically north-central montane arrangement.

The meagerly sampled populations from Missouri and Texas resemble neither each other nor any other population.

DISCUSSION

In many ways *D. nigromelanica* is a rather distinctive member of the *D. melanica* group. In its general appearance, it is more different from each of the other five species than they are from each other (a detailed study of the comparative morphology of the group has not been completed). In its geographical range, *D. nigromelanica* is neither clearly northern nor southern, in contrast to

the other five species, which can be classified as relatively northern or southern without hesitation. For a species which has been so widely sampled geographically, the number of inversions known is surprisingly small; the four inversion differences from Standard in this species may be compared with the 17 inversions known in *D. euronotus*, 22 in *D. melanica*, 16 in *D. paramelanica*, ten in *D. micromelanica* and seven in *D. melanura*. Moreover, it should be pointed out that the last two species listed are rather imperfectly known cytologically because of the difficulty in getting sufficient material, and may in fact carry many more inversions.

Despite the cytological differences between *D. nigromelanica* and the other group members, it has two features in common with them. First, in this species, as in all others of the group, the second chromosome is the most variable. Secondly, in *D. nigromelanica* as in *D. euronotus*, the extreme southeast populations show a pronounced shift towards homozygosity for gene arrangements.

A rough measure of such population structural homozygosity is the expected frequency of individuals homozygous for arrangements in all chromosome arms, this expectation being based on an assumed Hardy-Weinberg equilibrium.

In *D. euronotus*, expected frequencies of homozygous individuals show no marked tendency to southeast homozygosity even in northern peninsular Florida (Perry = 21 percent, Inverness = 23 percent). However, approximately 100 miles to the south of Inverness at Myakka State Park, the expected frequency of structural homozygotes rises to 50 percent, and again rises to 78 percent in the Everglades in the southern tip of Florida.

In *D. nigromelanica*, although a similar trend exists, it becomes apparent much further to the north. Thus, while the population from Montgomery County, Virginia has an expected homozygote frequency of 33 percent, and that from North Carolina 27 percent, the frequency rises to 53 percent in northern Georgia, 51 percent in South Carolina, and finally to 100 percent in Perry and Inverness, Florida.

Structural homozygosity characteristic of marginal populations has been found in Drosophila belonging to other species groups. In *D. willistoni* DA CUNHA and DOBZHANSKY (1954) have attributed it to restricted ecological opportunities in marginal habitats; in *D. robusta* CARSON (1959) has attributed it to small populations (which could ill afford the cost of maintaining structural heterozygosity), drift, inbreeding, and free recombination associated with structural homozygosity. According to this author, large central populations are little affected by drift or inbreeding, and the retention of their structural heterozygosity promotes heterotic buffering of a general nature. In the case of *D. nigromelanica* the marginal Florida populations are apparently small, and may well suffer from an ecologically restricted habitat. However, while populations from South Carolina and northern Georgia may reflect ecological restrictions, they are apparently not small, and would not be expected to show the genetic characteristics specifically related to small population size. The problem of marginal homozygosity in *D. euronotus* is briefly discussed by STALKER (1964).

SUMMARY

A survey is presented of the chromosomal polymorphism in *Drosophila nigromelanica*, a Nearctic member of the *D. melanica* species group. The range of *D. nigromelanica* overlaps those of all other members of the group. Except for GRIFFEN's (1942) report of hybrids with *D. paramelanica*, this species has not been found to form hybrids with any other species.

Photographic maps of all salivary gland chromosome arms are presented, showing the Standard gene sequences and the inversions break points.

Samples from 25 widely separated populations have uncovered only four gene sequences for chromosome 2 and two gene sequences for chromosome 4; all other chromosomes are represented by a single sequence each. This structural conservatism is in marked contrast to the situation in other group members.

In *D. nigromelanica*, as in the related *D. euronotus*, populations from the southeastern part of the species range show a striking decrease in intrapopulational chromosomal variability. In *D. nigromelanica* this decrease in variability is evident in Georgia and South Carolina, and complete structural homozygosity is reached in the marginal populations of north peninsular Florida.

LITERATURE CITED

CARSON, H. L., 1959 Genetic conditions which promote or retard the formation of species. Cold Spring Harbor Symp. Quant. Biol. **24**: 87–105.

CARSON, H. L., 1961 Rare parthenogenesis in *Drosophila robusta*. Am. Naturalist **95**: 81–86. —— 1962a Fixed heterozygosity in a parthenogenetic species of Drosopihla. Univ. Texas Publ. **6205**: 55–62. —— 1962b Selection for parthenogenesis in *Drosophila mercatorum*. Genetics **47**: 946.

CARSON, H. L., and H. D. STALKER, 1951 Natural breeding sites for some wild species of Drosophila in the eastern United States. Ecology **32**: 317–330.

DA CUNHA, A. B., and TH. DOBZHANSKY, 1954 A further study of chromosomal polymorphism in *Drosophila willistoni*. Evolution **4**: 212–235.

DOBZHANSKY, TH., and C. EPLING, 1944 Contributions to the genetics, taxonomy and ecology of *Drosophila pseudoobscura* and its relatives. Carnegie Inst. Wash. Publ. **554.**

GRIFFEN, A. B., 1942 Relationships in the melanica species group. Univ. Texas Publ. **4228**: 68–73.

MILLER, D. D., 1944 *Drosophila melanura*, a new species of the melanica species group. J. N.Y. Entomol. Soc. **52**: 85–97.

PATTERSON, J. T., 1943 The Drosophilidae of the Southwest. Univ. Texas Publ. **4313**: 1–203.

PATTERSON, J. T., and W. S. STONE, 1952 *Evolution in the Genus Drosophila*. Macmillan, New York.

PATTERSON, J. T., and C. L. WARD, 1952 *Drosophila euronotus*, a new member of the melanica species group. Univ. Texas Publ. **5204**: 158–161.

STALKER, H. D., 1954 Parthenogenesis in Drosophila. Genetics **39**: 4–34. —— 1960 Chromosomal polymorphism in *Drosophila paramelanica* Patterson. Genetics **45**: 95–114. —— 1961. The genetic systems modifying meiotic drive in *Drosophila paramelanica*. Genetics **46**: 177–202. —— 1964 Chromosomal polymorphism in *Drosophila euronotus*. Genetics **49**: 669–687.

STONE, W. S., 1962 The dominance of natural selection and the reality of superspecies (species groups) in the evolution of Drosophila. Univ. Texas Publ. **6205**: 507–537.

STONE, W. S., W. C. GUEST, and F. D. WILSON, 1960 The evolutionary implications of the cytological polymorphism and phylogeny of the virilis group of Drosophila. Proc. Natl. Acad. Sci. U.S. **46**: 350–361.

WARD, C. L., 1949 Karyotype variation in Drosophila. Univ. Texas Publ. **4920**: 70–79. —— 1952 Chromosome variation in *Drosophila melanica*. Univ. Texas Publ. **5204**: 137–157.

WASSERMAN, J., 1960 Cytological and phylogenetic relationships in the repleta group of the genus Drosophila. Proc. Natl. Acad. Sci. U.S. **46**: 842–859. —— 1962 Cytological studies of the repleta group of the genus Drosophila: Parts III, IV, V and VI. Univ. Texas Publ. **6205**: 63–134. —— 1963 Cytology and phylogeny of Drosophila. Am. Naturalist **97**: 333–353.

WHARTON, L. T., 1943 Analysis of the metaphase and salivary chromosome morphology within the genus Drosophila. Univ. Texas Publ. **4313**: 282–319.