CHROMOSOMAL POLYMORPHISM IN DROSOPHILA EURONOTUS[1]

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STUDY of the naturally occurring polymorphism found in the banded polytene chromosomes of Diptera has yielded a great deal of information on the genetic structure of natural populations, and also in some instances has indicated the pattern of evolutionary change which has occurred during species formation. Outstanding examples of studies of the latter sort are those in the *Drosophila obscura* group (LOBZHANSKY and EPLING 1944), in the *D. virilis* group (STONE, GUEST, and WILSON 1960; STONE 1962), and in the *D. repleta* group (WASSERMAN 1960, 1962, 1963).

It was with a view to determining the phylogenetic sequence among the members of the *D. melanica* group that the author began some years ago a study of the chromosomal polymorphism of these species. Inversion polymorphism in one of the member species, *D. melanica*, had been described by WARD (1952); the polymorphism found in *D. paramelanica*, and the "sex-ratio" condition associated with it, has been analyzed by STALKER (1960, 1961). The present paper deals with the naturally occurring polymorphism found in a third species of the group, *D. euronotus*. Papers in preparation will deal with three other species in the group, as well as the chromosomal evolution of the group as a whole.

Drosophila euronotus was at first confused with the sibling species, *D. melanica*, but on the basis of cross-sterility tests was later recognized as distinct, and was described by PATTERSON and WARD (1952).

D. euronotus is found in the southeastern part of the United States in deciduous wooded areas. Its known range, indicated in Figure 1, broadly overlaps the ranges of the related forms: *D. melanica*, *D. paramelanica*, *D. melanura*, *D. nigromelanica* and *D. micromelanica*. On the basis of collection records, *D. euronotus* appears to be relatively rare in the northern part of its range, and increasingly abundant toward the south. It has been the experience of the author that in central and southern Florida it is the most abundant member of the *D. melanica* group.

Males of this species are readily distinguished from other members of the group by their characteristic external genitalia, while females can be recognized by their eggs, which have characteristically long and flattened filaments. Both sexes of adults have rather wide heads, large eyes, and a characteristic stocky body form; they resemble no other group member in these respects except *D. melanura*, from which they can be distinguished by color differences.

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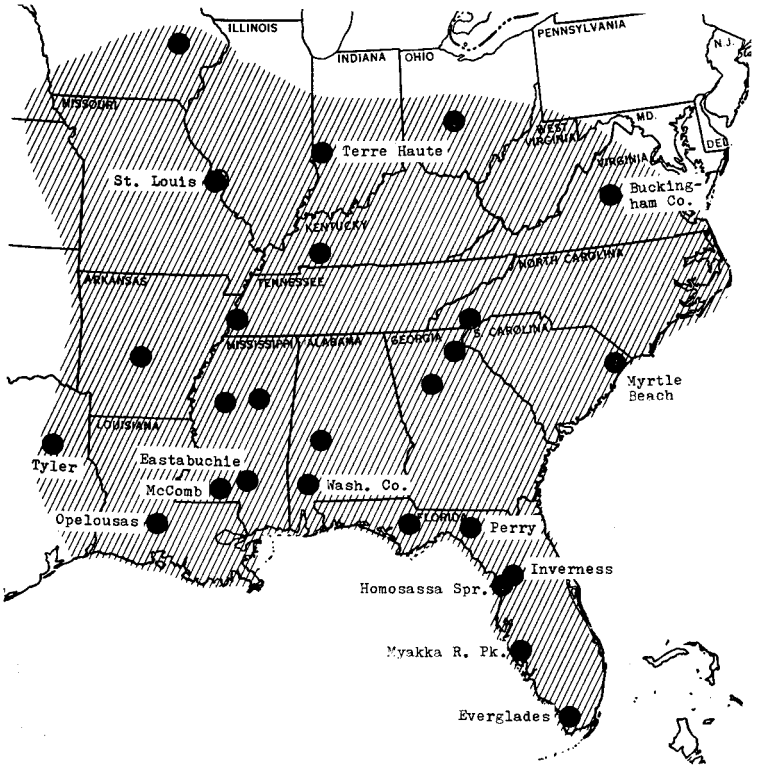

FIGURE 1.—Locations of populations sampled in the cytological survey of *D. euronotus*. The shaded area indicates the known range of the species.

The species breeds well in the laboratory, and at 25°C the life cycle is completed in approximately 18 days. Mating occurs between like-aged individuals three days after eclosion, and oviposition begins on about the fourth day. Uninseminated females oviposit vigorously and continuously, with the result that wild-caught individuals can be proven to carry no sperm, or if shown to be inseminated, can be induced to oviposit until the sperm supply is exhausted, so that they may be used in further matings. At 25°C adults can survive for a period of three months, while at 17°C they have been kept alive for as long as 11 months.

PATTERSON and WARD (1952) and the present author have carried out hybridization tests between *D. euronotus* and other members of the group. The results are summarized in Table 1. In this table, the data of PATTERSON and WARD are identified as "P & W"; the other data are those of the author. All tests involved attempted matings in vials containing up to ten pairs of flies. However, the various tests are not entirely comparable since the present author used divers combinations of light and dark to attempt to induce mating, used a variety of foods in the holding vials, and changed the flies to fresh food every third day of the testing period. All types of female hybrids obtained were at least partially fertile; all male hybrids were sterile.

TABLE 1

Hybridization tests between D. euronotus *and other members of the* D. melanica *species group*

Females	Males	Number pairs tested	Hybrids	Authority
D. euronotus	× *D. melanica*	350	0	P & W
		147	2 ♀♀	Author
D. melanica	× *D. euronotus*	290	0	P & W
		147	0	Author
D. euronotus	× *D. melanura*	20	0	P & W
		126	26 ♀♀, 1 ♂	Author
D. melanura	× *D. euronotus*	30	0	P & W
		147	0	Author
D. euronotus	× *D. paramelanica*	315	115 ♀♀, 110 ♂♂	P & W
		438	1 ♀	Author
D. paramelanica	× *D. euronotus*	355	0	P & W
		1040	0	Author
D. euronotus	× *D. nigromelanica*	260	0	Author
D. nigromelanica	× *D. euronotus*	170	0	Author
D. euronotus	× *D. micromelanica*	155	0	Author
D. micromelanica	× *D. euronotus*	100	0	Author

* PATTERSON and WARD (1952).

It is clear from Table 1 that *D. euronotus* may form fertile hybrids with the three species *D. melanica*, *D. melanura*, and *D. paramelanica*. It is not known to form hybrids with *D. nigromelanica* and *D. micromelanica*. The failure to obtain hybrids with the latter two species is not surprising, since they are morphologically and cytologically rather distant from the first four members of the group. Despite the proven ability to produce some fertile hybrids in the laboratory, no hybrids have been found in wild populations, nor is there any cytological evidence of such natural hybridization having occurred in the recent past.

PATTERSON and WARD concluded (apparently primarily on the basis of their hybridization tests), that *D. euronotus* is more closely related to *D. paramelanica* than to other members of the group. On the basis of the additional hybrids reported in Table 1, as well as a comparison of the morphology, the present author feels that the two closest relatives of *D. euronotus* are *D. paramelanica* and *D. melanura*, but that the latter species is most closely related to *D. euronotus*. As will be indicated in a later publication, cytological analyses seem to bear out this interpretation.

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. It was found that for salivary gland chromosome preparations, larvae which left the food to pupate during the late morning hours were superior to those which appeared mature during the afternoon or evening. 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 smears last for many months if kept in a freezer.

Most of the cytological analyses are based either on wild-caught flies, or on their laboratory-produced F_1 progeny. When laboratory stocks were used in chromosome analyses, this fact is indicated in the tables. All analyses of salivary gland chromosomes involved crosses to a structurally homozygous standard stock derived from the St. Louis area.

Collections of wild flies were made from over-ripe banana bait hung in cups or pint jars in the woods. In most cases, the freshly collected flies were sorted and within a few hours stored in a portable ice chest for transport back to St. Louis. Collecting sites are indicated in Figure 1.

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. Primm Wharton and Miss Jo Beth Kanevsky for technical assistance, and to Marion L. Stalker for her 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. euronotus*, since the various elements are readily homologized to those of *D. melanica* and *D. paramelanica*, the corresponding designations are used.

In *D. euronotus*, preparations of larval neuroblasts from three St. Louis area strains showed ten elements in each nucleus; one pair of large V-shaped chromosomes, a pair of smaller V-shaped chromosomes, two pairs of rods and a pair of dots. 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 rods correspond to salivary chromosomes 2 and 3, and the pair of dots to the short salivary chromosome 5.

In the *D. melanica* group generally, chromosome arms 3, X-left, 4-left and 4-right are conservative; in *D. euronotus*, they show no inversion variability. A single inversion is found in X-right, while chromosome 2 is highly polymorphic, as it is in all members of the species group. Although no structural rearrangements were noted in chromosome 5, the salivary preparations of this chromosome were usually not critical, and minor rearrangements might well have been overlooked.

Plates I and II consist of photographic maps of the standard gene arrangements of all chromosomes. Each of the maps except that for chromosome 5 is a montage, made up of several small sections of photographs mounted together. The composite maps are made by printing to standard size and contrast 35 mm photomicrographs, each showing most favorably a particular chromosome region. The various favorable regions are then cut out with a razor and assembled to make the composite map. It should be emphasized that these maps are designed to represent the chromosomes in their most typical condition, not their most ideal condition. Thus, while an occasional "difficult" section of a chromosome may once in a hundred smears be so stained and stretched as to show large numbers of bands, if it is usually poorly stained, broken or puffed, it is so shown in the

maps. It is felt that the presentation of such "typical" maps may be most useful to other workers engaged in the initially difficult stages of working with a new species. Such maps are useful primarily as bookkeeping devices to facilitate the location of rearrangement break points. For interspecific comparisons they must be supplemented by a large collection of photographs showing the various chromosome regions in greater detail, and also showing the various regions in all possible phases (puffed, contracted, stretched, etc.).

The break points of the various inversions are indicated on the maps. The letter designations for these inversions begin with A for each chromosome arm. The letters used here bear no relationship to those used by WARD in *D. melanica*, or by STALKER in *D. paramelanica*.

GEOGRAPHICAL VARIABILITY IN INVERSION FREQUENCIES

The X-chromosome: Only the Standard gene arrangement was found in X-left. The X-right arm was found to be polymorphic for the simple inversion A (see Plate I). The frequencies and distributions of the X-right arrangements, Standard (+) and A, are shown in Table 2. It will be noted from this table that A is found only in Florida. In view of its presence in the four populations extending southward from Perry to Myakka River State Park, its absence in the rather large sample from Everglades is of some interest, and may be associated with the rather special glade environment.

The second chromosome: As indicated in Plate II, chromosome 2 is highly polymorphic, and shows a total of 31 arrangements differing from Standard. Each of these arrangements differs from Standard by one or more of the 16 different inversions recognized in this chromosome.

The frequencies of most of the arrangements are listed in Table 3. In order to

TABLE 2

Frequencies of gene arrangements, Standard (+) and A, in the right arm of the X-chromosome

Area	Localities	Frequencies (percent)		
		N	+	A
Tennessee, Kentucky, Ohio, Indiana, Missouri, Iowa	6	71	100	0
Louisiana, Mississippi, Texas, Arkansas	7	138	100	0
Georgia, Alabama, North Carolina, South Carolina, Virginia	10	115	100	0
Florida	Blountstown	1	100	0
	Perry	33	88	12
	Homosassa Springs	5	60	40
	Inverness	14	86	14
	Myakka River State Park	24	75	25
	Everglades	62	100	0
Total:	29	463		

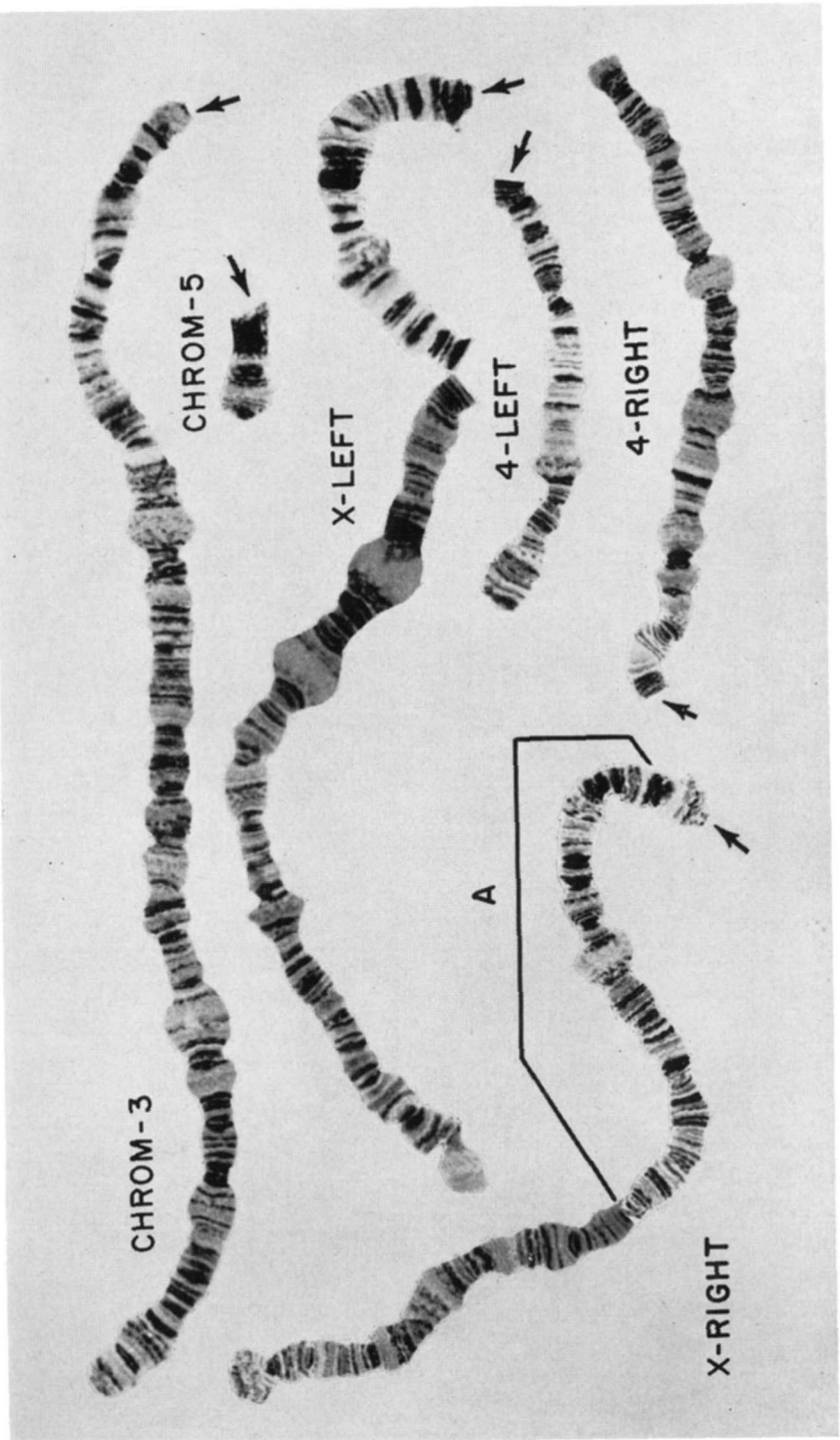

PLATE I.—Salivary gland chromosome maps of X, 3, 4 and 5 of *D. eurontus*. In each case the standard gene sequence is shown and the centromere end of the arm is indicated by an arrow. In X-Right the limits of inversion A are indicated by the bracket. X-Left has been cut in two in order to fit into the plate.

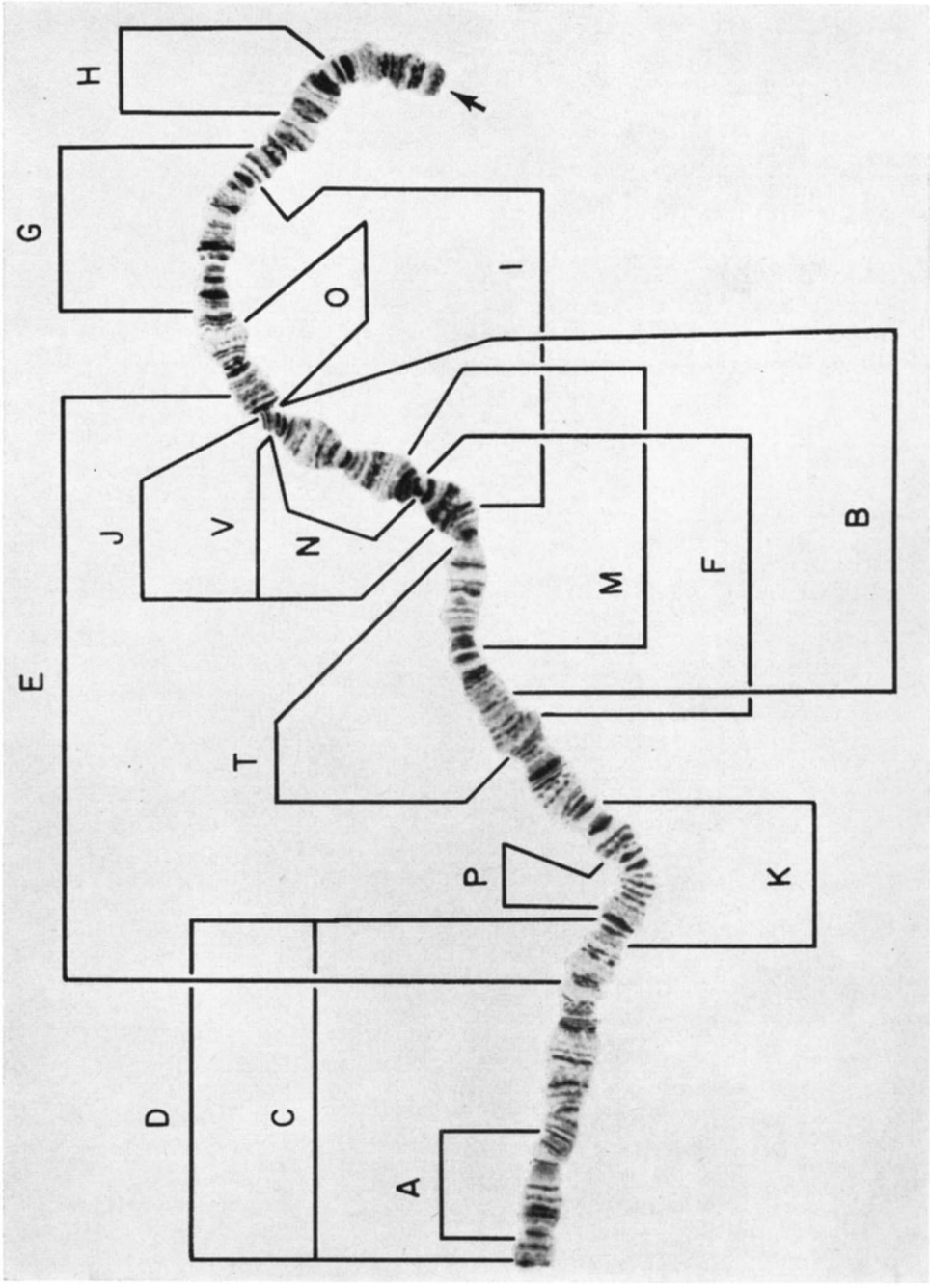

PLATE II.—Salivary gland map of chromosome-2 in *D. euronotus*. The standard gene sequence is shown, and the centromere end of the arm is indicated by an arrow. The limits of the 17 inversions found in this chromosome are indicated by brackets.

shorten the table, chromosome types found in small samples from ten localities are indicated only by number in the last column. The key to these locality numbers is given in the table caption.

It will be noted from Plate II that the second-chromosome inversion C, which has been found only once, from St. Louis, has indicated break points which are the same as those for inversion D. Whether or not the limits for these two inversions are in fact identical is unknown, since C was seen in a single rather mediocre squash, and D, although seen in many good preparations, always occurs in the AED inversion complex, in which it is difficult to determine exact break points.

The nonrandom associations of inversions in chromosome 2: The concept of balanced polygenic systems is one for which there is now much evidence, particularly in laboratory populations. However, it is difficult to obtain direct evidence of internal polygenic balance under natural conditions without the use of chromosomal markers. The multiple inversion systems known in some Diptera

TABLE 3

Distributions and frequencies for chromosome 2 gene arrangements

Chromosome types	St. Louis, Missouri	Terre Haute, Indiana	Shelby Co., Tennessee	Buckingham Co., Virginia	Atlanta, Georgia	Myrtle Beach, South Carolina	Demopolis, Alabama	Washington Co., Alabama	Eastabuchie, Mississippi
+	18 *23.1*	3 *30.0*	4 *40.0*	16 *53.3*	2 *10.0*	2 *2.1*	. . .	. . .	. . .
A	3 *3.9*	1 *10.0*	1 *10.0*	. . .	. . .	24 *24.7*	. . .	3 *21.4*	1 *10.0*
C	1 *1.3*	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
E	. . .	. . .	. . .	1 *3.3*	. . .	15 *15.5*	. . .	. . .	. . .
G	2 *2.6*	1 *10.0*	. . .	. . .	. . .	. . .	. . .	. . .	. . .
M	. . .	1 *10.0*	. . .	. . .	. . .	. . .	. . .	. . .	. . .
K	. . .	1 *10.0*	. . .	. . .	. . .	. . .	. . .	. . .	. . .
AM	. . .	1 *10.0*	. . .	. . .	. . .	. . .	. . .	. . .	. . .
AB	16 *20.5*	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
AE	12 *15.4*	1 *10.0*	2 *20.0*	. . .	1 *5.0*	. . .	1 *12.5*	. . .	. . .
AJ	2 *2.6*	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
EG	. . .	. . .	. . .	. . .	. . .	46 *47.4*	1 *12.5*	1 *7.1*	1 *10.0*
EV	. . .	. . .	. . .	. . .	. . .	1 *1.0*	. . .	. . .	. . .
AEG	. . .	. . .	. . .	. . .	. . .	. . .	1 *12.5*	. . .	2 *20.0*
AED	. . .	. . .	. . .	13 *43.3*	13 *65.0*	. . .	2 *25.0*	3 *21.4*	. . .
AEV	. . .	. . .	. . .	. . .	. . .	. . .	1 *12.5*	. . .	. . .
EVG	. . .	. . .	. . .	. . .	. . .	2 *2.1*	. . .	4 *28.6*	. . .
ETG	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	1 *10.0*
EGH	. . .	. . .	. . .	. . .	. . .	5 *5.2*	. . .	1 *7.1*	1 *10.0*
AEDP	. . .	. . .	. . .	. . .	1 *5.0*	. . .	. . .	. . .	. . .
AEDG	12 *15.4*	. . .	. . .	. . .	1 *5.0*	2 *2.1*	. . .	. . .	1 *10.0*
AEVG	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
AEDGP	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
AEDFG	11 *14.1*	1 *10.0*	3 *30.0*	. . .	2 *10.0*	. . .	2 *25.0*	2 *14.4*	. . .
AEVGH	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	3 *30.0*
EVGH	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
AEGH	1 *1.3*	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
Total:	78 . . .	10 . . .	10 . . .	30 . . .	20 . . .	97 . . .	8 . . .	14 . . .	10 . . .

TABLE 3—Continued

Distributions and frequencies for chromosome 2 gene arrangements

Chromosome types	McComb, Mississippi	Opelousas, Louisiana	Tyler, Texas	Perry, Florida	Inverness, Florida	Homosassa Springs, Florida	Myakka River State Park, Florida	Everglades, Florida	Additional localities (see text)
+	. . . .	2 *1.4*	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	4, 7, 9
A	. . . .	12 *8.4*	2 *16.7*	16 *25.8*	4 *14.3*	2 *33.3*	4 *12.5*	10 *12.8*	3, 6, 8, 10
C	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
E	4 *9.5*	9 *6.3*	. . . .	1 *1.6*	. . . .	. . . .	. . . .	. . . .	3, 9
G	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
M	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
K	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
AM	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
AB	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	8
AE	6 *14.3*	25 *17.5*	2 *16.7*	. . . .	1 *3.6*	. . . .	. . . .	. . . .	4
AJ	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	4
EG	9 *21.4*	24 *16.8*	3 *25.0*	. . . .	2 *7.1*	. . . .	. . . .	. . . .	. . . . .
EV	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
AEG	9 *21.4*	15 *10.5*	. . . .	. . . .	14 *50.0*	1 *16.7*	28 *87.5*	68 *87.2*	. . . . .
AED	. . . .	. . . .	. . . .	5 *8.1*	1 *3.6*	. . . .	. . . .	. . . .	1, 2, 4, 6
AEV	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
EVG	. . . .	5 *3.5*	. . . .	19 *30.6*	2 *7.1*	2 *33.3*	. . . .	. . . .	. . . . .
ETG	3 *7.1*	9 *6.3*	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
EGH	1 *2.4*	3 *2.1*	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
AEDP	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
AEDG	1 *2.4*	10 *7.0*	2 *16.7*	19 *30.6*	3 *10.7*	1 *16.7*	. . . .	. . . .	4, 9
AEVG	1 *2.4*	6 *4.2*	. . . .	. . . .	1 *3.6*	. . . .	. . . .	. . . .	. . . . .
AEDGP	. . . .	5 *3.5*	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
AEDFG	2 *4.8*	13 *9.1*	3 *25.0*	. . . .	. . . .	. . . .	. . . .	. . . .	4, 5, 7, 9, 10
AEVGH	5 *11.9*	4 *2.8*	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
EVGH	1 *2.4*	1 *0.7*	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	. . . . .
AEGH	. . . .	. . . .	. . . .	2 *3.2*	. . . .	. . . .	. . . .	. . . .	. . . . .
Total:	42 . . .	143 . . .	12 . . .	62 . . .	28 . . .	6 . . .	32 . . .	78 . . .	. . . . .

For each gene arrangement the number of occurrences, and the percentage frequency (italicized) are given for each sample in which it occurs. In order to shorten the table, arrangements found in small samples from ten localities are indicated only by locality number in the last column. The key to the locality number is given below.

1. Sunrise Peak, near Highlands, N.C. Progeny of single wild female. In addition to AED indicated in the table, progeny carried gene arrangement NO.
2. Bear Camp Gorge, near Highlands, N.C. Progeny of single wild female. In addition to AED indicated in the table, progeny carried arrangements AN and NO.
3. Blountstown, Florida. Two chromosomes.
4. Cornelia, Georgia. Five stocks from single wild females. In addition to the chromosomes indicated in the table, one stock carried arrangements GH and J.
5. Greenwood, Mississippi. Two chromosomes.
6. Mathiston, Mississippi. Two chromosomes.
7. Little Rock, Arkansas. Two chromosomes.
8. Mt. Vernon, Iowa. Two chromosomes.
9. Hopkinsville, Kentucky. Progeny of two wild females. In addition to the arrangements indicated in the table, progeny carried arrangement AI.
10. Columbus, Ohio. Two chromosomes.

are ideally suited to this purpose, and in the Drosophila, as well as in some other forms such as Chironomus, nonrandom associations of inversions in nature form models of polygenic balance (LEVITAN 1958, 1959; LEVITAN and SALZANO 1959, STALKER 1960, 1961). If indeed structurally polymorphic chromosomes in natural populations do form realistic models of internally balanced polygenic

systems, then they would be expected to show interaction between many recognizable segments of the chromosome, not just two, and in addition the adaptive values of the specific segmental combinations would be expected to differ in different populations, due to the variations in the genic content of the segments, the total genotype of the population, and the ecological conditions. Since the internally balanced inversions systems of *D. euronotus* do show these features, it is felt worthwhile to present them in some detail.

Despite the large number of inversions in chromosome 2, the only overlapping pair in the coupling phase is the very common DE. In all chromosomes with the DE complex, the included inversion A is also present (see Table 3). While A frequently occurs without ED, the absence of ED without A suggests that A is unable to separate from the AED complex by crossing over, so that the AED association cannot be considered as a nonrandom one maintained by selection. Similarly, the short basal inversions G and H are rigidly associated. H, found in nine localities, is never found without G, although G occurs in complexes without H in 21 localities. With no proof that crossing over occurs between G and H, GH is taken to represent an unbreakable linkage combination.

Inversion B has been found only in Missouri and Iowa, and although B is far enough from A to permit crossing over between the two (crossing over is known

TABLE 4

Association of inversion A with other independent inversions in chromosome-2

Locality	Inversions compared	N	Coupling		Repulsion		Excess classes	P
			Observed	Expected	Observed	Expected		
Myrtle Beach, South Carolina	A *vs.* E	41	2	19.56	39	21.44	Repulsion	<.0001
	A *vs.* EG	72	2	32.66	70	39.33	Repulsion	<.0001
	A *vs.* EGH	31	2	9.74	29	21.26	Repulsion	<.005
Washington Co., Alabama	A *vs.* EG, EVG, EGH	9	0	4.00	9	5.00	Repulsion	<.02
Perry, Florida	A *vs.* E, EVG, EGH	38	2	18.84	36	19.17	Repulsion	<.0001
Inverness, Florida	A *vs.* E, EG, EVG	24	16	17.33	8	6.67		>.50
Eastabuchie, Mississippi	A *vs.* EG, ETG, EGH, EVGH	9	5	5.66	4	3.33		>.90
Opelousas, Louisiana	A *vs.* E	48	27	29.42	21	18.58		>.40
	A *vs.* EG	53	17	26.73	36	26.26	Repulsion	<.01
	A *vs.* ETG	23	2	11.39	21	11.61	Repulsion	<.0001
St. Louis, Missouri	A *vs.* E	34	31	17.24	3	16.76	Coupling	<.0001
	A *vs.* B	37	34	18.43	3	18.57	Coupling	<.0001

Chi-square tests of significance were used. In the samples from Alabama, Florida and Mississippi, the various classes of chromosomes carrying E (E, EG, EGH, EVG, etc.) were summed and considered as a single class. Yates' correction was used in the Alabama and Mississippi tests.

to occur in the much shorter interval between A and E), B has never been found in the absence of A. The nonrandomness of this association in the St. Louis population is highly significant (see Table 4, last line), and probably represents a selectively maintained nonrandom association in which the BA^+ combination is nonadaptive in nature.

Inversions A and E (in the absence of D, i.e. D^+) are sufficiently separated to permit recombination between them, and indeed all four combinations $AE(D^+)$, $A^+E(D^+)$, $AE^+(D^+)$ and $A^+E^+(D^+)$ are found in nature. However, A and E are not randomly associated in wild populations, as indicated in Table 4. It will be noted that this nonrandomness obtains whether one considers the association of A with $E(D^+)$ by itself, or with $E(D^+)$ in combination with inversions G, H, V or T.

Of the six populations suitable for testing these associations, all but that from Inverness, Florida show significant nonrandomness. In all populations except that from St. Louis, there is an excess of the repulsion classes $A^+E(D^+)$ and $AE^+(D^+)$. Although A and $E(D^+)$ in the St. Louis population are significantly nonrandom in their association, in this population the *coupling* $AE(D^+)$ and $A^+E^+(D^+)$ rather than the repulsion phase is present in excess.

Although the Florida populations in Myakka and Everglades carry the inversion A in all chromosomes, and thus do not permit an intrapopulation test of association of A and E, the fact that in both of these populations nearly 90 percent of the chromosomes present are AEG, indicates that here, as in St. Louis, the AE combination must be a highly adaptive one. It might, of course, be claimed that the high frequency of AEG in southern Florida simply reflects the absence of other alternate arrangements which could replace it, rather than its own inherent adaptive characteristics. However, this objection is practically eliminated by the fact that in southern Florida sequence A *is* present as an alternative, as it is over most of the range of the species. Thus, it appears that in the northern part of the species range there is a favored AE association, in most of the south the dissociation of A and E is favored, while in Florida the favored dissociation in the north (Perry) is replaced by no association further south (Inverness) and a strong association in the southern half of the peninsula (Myakka and Everglades). A somewhat less complex reversal of associations has been demonstrated in *D. robusta* by LEVITAN (1959).

Inversion G is rigidly associated with inversion E, whether or not the latter is bound into the AED complex. G has been found only three times in the absence of E, twice (as G) in the laboratory-reared progeny of wild-caught St. Louis females, and once (as GH) in a laboratory stock from Cornelia, Georgia. Thus, while G may separate from E by crossing over, it appears that the GE^+ derivatives are nonadaptive in nature. On the other hand, the reciprocal G^+E class is presumably well adapted to natural conditions since it is common in many localities.

Although G (or GH) is virtually always associated with E in some combination, its association with ED in the AED complexes (AED, AEDP, AEDG, AEDGP, AEDFG) as opposed to the ED^+ complexes (E, AE, EG, EV, AEG, AEV, EVG, ETG, AEVG, AEVGH and AEGH) is not always at random. Three

TABLE 5

Associations of second chromosome inversion G with inversion E in the AED complex, as opposed to ED+ (E in chromosomes without the overlapping inversion D)

Locality	N	Inversion association classes: G+AED, GED+ Observed	G+AED, GED+ Expected	G+ED+, GAED Observed	G+ED+, GAED Expected	P
St. Louis, Missouri	36	1	16.33	35	19.67	<.0001
Opelousas, Louisiana	129	67	81.75	62	47.26	<.01
Perry, Florida	46	26	22.26	20	23.74	>.25

The data are drawn from AED complex chromosomes (AED, AEDP, AEDG, AEDGP, AEDFG) and from ED+ chromosomes (E, AE, EG, EV, AEG, AEV, EVG, ETG, AEVG, AEVGH, and AEGH). In the table headings the designation G+ indicates the Standard gene sequence for the region covered by inversion G.

population samples suitable for testing this association are listed in Table 5. It may be seen that while in the Florida population, the association is a random one; in Louisiana and Missouri, G tends to be associated with AED complexes and G+ with ED+ complexes.

Since there is an excess of the G+ED+ and GAED classes as compared to the G+AED and GED+ classes, the question arises as to whether the favored association may depend on the fact that A is ever present in AED, but often missing from ED+ classes. If this were the case, then it might be expected that in chromosomes without D, G would show an excess coupling with AE(D+) as opposed to A+E(D+). Actually, a considerable number of GAED+ and GED+ chromosomes exist which may be used to test this hypothesis. In these chromosomes, G may be associated with AE(D+), or with E in the absence of A, that is A+E(D+). Two populations are suitable for testing this point; the data are presented in Table 6. It will be seen from this table, that while in the McComb sample there is no departure from randomness, in the Opelousas population there is a highly significant association, with GAE(D+) and G+E(D+) below expected frequencies, while GE(D+) and G+AE(D+) are in excess of expectation. Since the observed deviation from expectation is in the opposite direction from that expected according to the hypothesis, it must be concluded that at least in the Opelousas population (Table 5), the excess of the GAED and G+ED+ classes cannot be ex-

TABLE 6

Associations of second chromosome inversion G with inversions AE as opposed to E; in all cases inversion D is absent

Locality	N	Inversion association classes: GE, G+AE Observed	GE, G+AE Expected	GAE, G+E Observed	GAE, G+E Expected	P
McComb, Mississippi	39	20	18.76	19	20.24	>.65
Opelousas, Louisiana	101	67	50.67	34	50.34	<.002

The data are drawn from AE chromosomes (AE, AEG, AEVG, AEVGH) and from E chromosomes (E, EG, EVG, ETG, EGH, EVGH). In the table headings the designation G+ indicates the Standard gene sequence for the region covered by inversion G.

plained by the presence of A in the AED complex, nor by its occasional absence in the ED+ complex, but must instead depend on other genetic differences between these chromosome segments.

One final association remains. Inversion T (included in E) has been found 13 times in ETG chromosomes, and never in any other combination. Whether or not T arose in a chromosome carrying E, its present restriction to ETG chromosomes is rather striking, since ETG occurs in populations in which crossing over between ET and the inversions G, GH or A might be expected to occur. The only sample of sufficient size to test the association of ET and G is that from Opelousas, and in that sample the association is significant ($\chi^2 = 5.88$; $P = <.02$).

The nonrandomness of inversions in chromosome 2 may be summarized by the following series of statements: (1) B is found only with A (Missouri). (2) A and E (in the absence of D) tend to be *associated* in Missouri, tend to be *dissociated* in Louisiana, Alabama, South Carolina and northern Florida, while in southern Florida over 80 percent of the chromosomes are of the *associated* AE type. (3) G is found in nature only in chromosomes carrying E (11 populations). (4) G is associated with AED in preference to A^+ED^+ or AED^+ in Missouri and Louisiana. (5) G (in the absence of D) is associated with E in preference to AE in Louisiana. (6) ET is found only in association with G (Louisiana).

The phylogenetic sequence in chromosome 2: In working out probable intraspecific phyletic relationships of the various gene sequences, use will be made of overlapping or closely linked series of inversions, following the method developed by STURTEVANT and DOBZHANSKY (1936). In addition, evidence based on nonrandom associations of inversions will be used wherever available. It is recognized that such association data are difficult to interpret, since we have proof that the adaptiveness of coupling or repulsion combinations of inversions may be altered in the course of time (see above, and LEVITAN 1959). Despite this fact, it seems inadvisable to completely ignore evidence that may be available, even if it leads to clearly tentative conclusions.

The selection of the most probable ancestral gene sequence within *D. euronotus* is based on the results of studies with other related species. Data will be presented in a future paper to show that *D. melanura* is ancestral to *D. euronotus*, which in turn is ancestral to *D. paramelanica*. It will also be shown that the second chromosome gene sequence in *D. euronotus*, which is most closely related to the second chromosome of *D. melanura*, is Standard (+). Accordingly, in the intraspecific phylogenetic scheme to be developed below, Standard is taken as the starting point for the whole phylogeny (see Figure 2).

Three possible pathways exist to explain the origin of sequences A and E. A and E could have arisen independently by inversions in Standard, or the pathway might have been either +→A→AE→E (the last step by crossing over), or +→E →AE→A. The relatively high frequencies and extensive range of A in natural populations (found in 17/27 of the populations) might suggest it was ancestral to the much more restricted E (found in 7/27 of the populations). However, the fact that Standard, which is ancestral to both A and E is found in only 10/27 of the populations, indicates the danger of choosing an ancestral sequence solely on

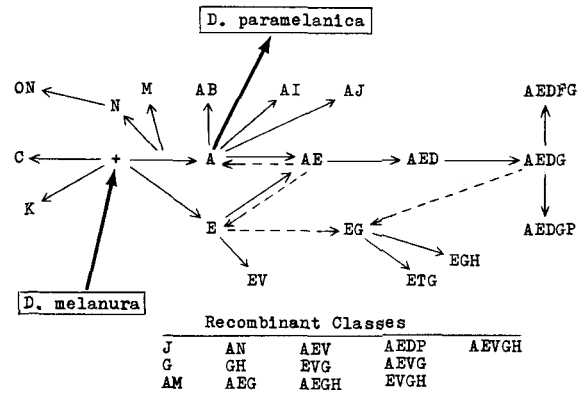

FIGURE 2.—Phylogenetic scheme of chromosome-2 gene arrangements in *D. euronotus*. Solid arrows indicate development of new arrangements by production of new inversions. Dashed arrows indicate origin by recombination. Chromosome 2 of *D. melanura* differs from + of *D. euronotus* by two inversions. Sequence A of *D. euronotus* differs from chromosome 2 of *D. paramelanica* by three inversions.

the basis of its population frequencies and range. Taking all these facts into account, the three possible origins are all indicated in Figure 2.

Inversion D must have arisen in a chromosome carrying both A and E, since A and E do not recombine in chromosome pairs heterozygous for D. It would be expected that if the newly formed AED complex were to survive, then D would have arisen in an AE chromosome which was already a well adapted complex. Association data for A and E, and Table 3, indicate that such well adapted AE chromosomes occur in the north (St. Louis) and in the extreme south of Florida (Myakka and Everglades). In the large area in between, the AE compound is a nonadaptive one. Thus, it would be expected that D should arise and persist either in the extreme north or extreme south of the species range. The fact that the AED family of chromosomes is unknown in the extreme south, and is common in the north, suggests that D arose in some northern population, such as that presently in St. Louis.

Inversion F is unknown outside of the AEDFG complex, where it has been found 46 times. It seems highly improbable that F could recombine with the rest of the AED complex by crossing over, but even if such recombinations could occur, the absence of the derived F chromosome indicates that the latter is not well adapted in natural populations. If F had arisen in some chromosome which did not carry the AED complex, it would have had to survive long enough to allow for introduction into the complex by rare double crossing over. The facts above indicate that F arose in an AED complex.

Inversion P has been found only six times, once as AEDP and five times as AEDGP. This suggests its origin in an AED complex (following the arguments for F, above), although the small number of cases here weakens the case somewhat.

The short inversions V and T are included within the break points of E, and

are in fact found only in chromosomes of the ED$^+$ family. The previously noted dissociations of A with V and T suggest the origin of V and T in E chromosomes, rather than in AE chromosomes.

Inversion G shows an association with AED rather than with ED$^+$ in two of the three testable populations. It is partly for this reason, but primarily because of the presence of G in all 46 of the chromosomes carrying the complex AEDF, that G is considered to have arisen in an AED rather than in an ED$^+$ chromosome.

Inversion H, as indicated above, occurs only with G, and might have arisen in a chromosome such as AEDG. The chromosome AEDGH has never been found in natural populations, and while the noted deficiency is not statistically significant in the single testable population (Opelousas), the widespread association of H with ED$^+$G chromosomes, and its absence in AED chromosomes strongly suggests its origin in an AED$^+$G or ED$^+$G chromosome resulting from crossing over between chromosomes such as AE or E with AEDG.

Inversion B is found only with A (in AB chromosomes) and, on the basis of this rigid association, is presumed to have arisen in an A chromosome.

The origin of the inversions: C, I, J, K, M, N and O can be specified only to the extent of pointing out that they probably did not arise in any chromosome carrying inversion E, since they are covered by, or overlap E, and are not now found with that inversion. Their position in the phylogenetic diagram is based on presently known associations with inversion A, with Standard, or with both.

Finally, 13 chromosome types are known which might have been produced by recombination between chromosomes included in the phylogenetic diagram. These are listed below the diagram as "Recombinant Chromosomes."

DISCUSSION

The relationship of *D. euronotus* to the other members of its species group is an interesting one. As indicated above, there is evidence that the *D. euronotus* chromosome 2 Standard (+) is most closely related to the second chromosome of *D. melanura*, from which it is derived phyletically. *D. euronotus* arrangement A, on the other hand, is most closely related to the second chromosome of *D. paramelanica*, to which it is ancestral. However, the passage from *D. melanura* through the *D. euronotus* phylogeny and on to *D. paramelanica* requires only a single change (+ → A) within the *D. euronotus* phylogeny. This indicates that the phylogenetic changes:

D. melanura → (+ → A) → *D. paramelanica*

occurred before the development of the presently existing *D. euronotus* phylogeny. If the first step in the above series had been followed by extensive chromosomal evolution within what is now *D. euronotus* before the step to *D. paramelanica*, it would be highly probable that the *D. euronotus* sequence that became ancestral to *D. paramelanica* would be much further removed from the origin (+), than is the actual ancestral sequence A. It is thus suggested that the complete chromosome 2 changes from *D. melanura* through to *D. paramelanica*

occurred relatively rapidly, followed by later elaboration of the chromosomal phylogeny within *D. euronotus*.

D. euronotus is a good example of a species which is chromosomally highly polymorphic, but in which the chromosomal polymorphism is almost entirely restricted to a single chromosome. In this respect, it resembles other members of the species group, especially *D. melanica*, and more distantly related forms such as *D. pseudoobscura* and *D. persimilis*. Many of the gene arrangements in chromosome 2 have very wide geographical ranges, with the result that individual populations may carry a very large number of alternate arrangements and karyotypes (see Table 7).

An analysis of karyotype frequencies requires complete analysis of individual flies by seven or more larval smears. Such analyses of reasonably large samples of wild-caught flies (as opposed to their F_1 laboratory-reared progeny) have been made from only two populations, those from Myrtle Beach, South Carolina and Opelousas, Louisiana. Due to the large numbers of karyotypes in these populations, nothing can be said about how well individual karyotype frequencies fit expectation; however, a comparison of the frequencies of the lumped homokaryotypes with expectation is possible. It will be seen from Table 7 that in both populations there is an observed excess of homokaryotype classes, and that in the Myrtle Beach population this excess is highly significant.

The reason for such high homokaryotype frequencies is not clear. Two sorts of explanations suggest themselves. First, it is possible that maintenance of chromosomal polymorphism within such populations depends on ecological adjustments of different gene arrangement frequencies to different specific ecological requirements, the homokaryotypes being adaptively superior to the heterokaryotypes in such an adjustment, and thus occurring in proportionally higher frequencies. A

TABLE 7

Karyotype frequencies for X and second chromosomes in selected populations

Population	Expected karyotype number	X-Chromosome homozygotes expected	Second chromosome homozygotes			Complete homozygotes expected
			Expected	Observed	N	
St. Louis, Missouri	55	1.000	0.166			0.166
Terre Haute, Indiana	36	1.000	0.160			0.160
Buckingham Co., Virginia	6	1.000	0.473			0.473
McComb, Mississippi	66	1.000	0.145			0.145
Myrtle Beach, South Carolina	36	1.000	0.314	0.680	25	0.314*
Opelousas, Louisana	120	1.000	0.104	0.159	63	0.104†
Perry, Florida	24	0.789	0.262			0.207
Inverness, Florida	39	0.759	0.296			0.225
Myakka State Park, Florida	6	0.625	0.781			0.488
Everglades, Florida	3	1.000	0.777			0.777

In this table the expected number of karyotypes is based on the number of gene sequences present in the population. The expected homozygote frequencies are based on an assumed Hardy-Weinberg equilibrium. The observed homozygote frequencies are based on fully analyzed, wild-caught flies.

* Highly significant deviation; $P = <0.001$. Observed frequency of complete homozygotes $= 0.680$.
† Nonsignificant deviation; $P = >0.15$. Observed frequency of complete homozygotes $= 0.159$.

somewhat more orthodox explanation would be that in such populations positive assortative mating for karyotype occurs, possibly through a general tendency for adults to breed with individuals reared in the same micro-habitat. Obviously these two explanations are not mutually exclusive. Whatever the correct explanation, the finding of significant homokaryotype excess in one of the two populations which could be checked for it is of interest, since it raises the possibility that such deviation from Hardy-Weinberg equilibria might be a general phenomenon in *D. euronotus* populations.

Even if the true homokaryotype frequency is generally higher than expectation, it is clear from the data at hand that inversion polymorphism decreases qualitatively in southern Florida, and presumably the frequency of heterokaryotypes decreases too.

MAYR (1945) and DOBZHANSKY (1951) have suggested that the degree of chromosomal polymorphism in a population is associated with the ecological opportunities open to the species; populations living in restricted habitats showing less polymorphism than those living in areas with many ecological opportunities. Evidence for this viewpoint comes from species such as *D. willistoni* (DA CUNHA and DOBZHANSKY 1954).

CARSON (1955a, b, 1958, 1959) has demonstrated a marginal reduction in polymorphism in *D. robusta*, and interprets it somewhat differently. According to his hypothesis, marginal reduction in polymorphism is associated with small populations (which could ill afford the cost of maintaining structural heterozygosity), drift, inbreeding, and the free recombination associated with structural homozygosity. 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. euronotus*, the very marked reduction in polymorphism apparent in southern Florida may be associated with an ecologically marginal and restricted habitat, although the nature of the association is unclear. If indeed such an association exists in southern Florida, then it might be expected that similar associations would occur near the northern limits of the species range. It will be noted that in the sample from Buckingham County, Virginia, something of this sort may exist; only three gene arrangements have been found in this sample, and the expected frequency of homozygotes is exceeded only by those in southern Florida.

However, in the two populations from St. Louis and Terre Haute, a high degree of polymorphism is clearly retained, with about 16 percent homozygotes expected in each population (Table 7). It is possible that this difference between the eastern and western areas in the north is related to differences in their ecological variability, but in the present state of ignorance concerning the ecology of this and other species of Drosophila, little more can be said.

SUMMARY

A survey is presented of the chromosomal polymorphism in *Drosophila euronotus*, a North American member of the *D. melanica* species group. *D.*

euronotus overlaps the ranges of four other species in the group and will form female-fertile hybrids with three of them: *D. melanica*, *D. melanura* and *D. paramelanica*.

Twenty-seven samples from widely separated populations have uncovered two gene sequences in the X chromosome and 32 gene sequences in chromosome 2. All other chromosomes exhibit only one gene sequence each. The very extensive polymorphism of chromosome 2, and the concentration of variability in that chromosome, is characteristic of the species group as a whole.

Many of the sets of inversions in chromosome 2 capable of recombination show a nonrandom association in nature. Six different types of such nonrandomness have been demonstrated. In one instance (inversions A and E), the coupling phase predominates in the north, the repulsion phase predominates in the middle zone, and in the far south the coupling phase again predominates.

A phylogeny is presented for the gene sequences in chromosome 2. In this phylogeny, it is pointed out that Standard (+) which is derived from chromosome 2 of *D. melanura*, is ancestral for the *D. euronotus* phylogeny as a whole, while gene sequence A, differing from (+) by a single inversion, is ancestral to chromosome 2 of *D. paramelanica*. The fact that most of the *D. euronotus* phylogeny is by-passed in the ultimate development of *D. paramelanica* from *D. melanura*, suggests that the elaboration of the *D. euronotus* phylogeny occurred after the development of the *D. paramelanica* type of chromosome 2.

Since many of the gene sequences in *D. euronotus* chromosome 2 are widespread, many populations show a high degree of structural variability; for example, populations from Louisiana and Mississippi are expected to show 120 and 66 different karyotypes respectively. Comparison of total homokaryotypes with expectation according to Hardy-Weinberg equilibrium frequencies in South Carolina and Louisiana populations, shows an excess of homokaryotypes in both, the excess being highly significant in the former.

Two populations from southern Florida show a marked restriction in their degree of chromosomal polymorphism, suggesting the marginal homozygosity of the sort found in *D. willistoni* and *D. robusta*. In the north, however, in populations near the limits of the species range, the picture is not so clear. The population from Virginia does show a marked reduction in chromosomal polymorphism, but the two populations from Missouri and Indiana do not.

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