CHROMOSOMAL POLYMORPHISM IN DROSOPHILA PARAMELANICA PATTERSON

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RECENT work carried out chiefly with Drosophila has revealed a wealth of chromosomal and genetic variability in natural populations, and in addition has begun to indicate some of the evolutionary relationships which exist between species, as well as the complex genetic systems which are responsible for the maintenance of the intraspecific chromosomal and genetic polymorphism.

The *Drosophila melanica* species group appears to offer unusual opportunities for such studies. The members of the group are truly "wild", and unlike some domestic Drosophila species, are relatively little affected by man's activities. They occupy a broad geographical range, and the different species will in many instances cross to form fertile hybrids. Finally, at least some members of the group show a high degree of chromosomal polymorphism.

For a number of years the author has been engaged in a long-term study of the interspecific chromosomal changes which have occurred in the evolution of this group, and as one of the necessary preliminary steps, a study of the intraspecific variation has been undertaken.

Previously the only species in the group which had been thoroughly studied in regard to its chromosomal polymorphism was *D. melanica melanica.* In this species WARD (1952) studied salivary gland chromosomes derived from 65 laboratory strains, and found that the X chromosome showed four inversions differing from Standard, chromosome 4 showed two inversions and chromosome 2 showed 16. No inverted sequences were found in chromosomes 3 or 5. WARD also found that there were some differences in geographical distributions of the various gene sequences. It is the purpose of the present paper to report a series of cytological surveys carried out on *Drosophila melanica paramelanica* Patterson.

PATTERSON (1943) split the species *D. melanica* Sturtevant into the two subspecies *D. m. melanica* and *D. m. paramelanica* on the basis of rather minor differences in morphology, different geographical distributions and a rather high degree of reproductive isolation in the laboratory. It is now known that the males of these two forms show completely distinct differences in genitalia, and in addition there are differences in the egg filaments. It is the opinion of the author, that in the light of the total known differences the two forms constitute distinct species, and they will be so treated in this paper.

In the northeastern part of the United States five members of the *D. melanica* group are encountered; they are: *D. melanica* Sturtevant, *D. paramelanica* Patter-

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son, *D. melanura* Miller, *D. nigromelanica* Patterson and Wheeler and an undescribed species from Nebraska, Iowa, Missouri, Illinois, Indiana, Kentucky, Tennessee and Georgia, "species X".

D. nigromelanica is readily distinguished from the other four species on the basis of adult morphology and color. The remaining four species are easy to confuse in females, but may be separated on the basis of the male genitalia (see MILLER 1944 for diagrams).

D. paramelanica will form hybrids of both sexes with *D. melanica*, *D. melanura*, *D. nigromelanica*, *D. euronotus* (a species apparently confined to the southeastern United States) and "species X" (GRIFFEN 1942; MILLER 1944; PATTERSON and WARD 1952; STALKER, unpublished). GRIFFEN reports that all hybrids obtained in the crosses between *D. paramelanica* and the two species *D. melanica* and *D. nigromelanica* were fertile in both sexes. The author found these hybrids to be male sterile. Hybrids of *D. paramelanica* × *D. melanura* and *D. paramelanica* × *"species X"* are fertile in females but sterile in males.

D. paramelanica has been collected in Nebraska, Iowa, Minnesota, Missouri, Wisconsin, Michigan, Illinois, Indiana, Ohio, Tennessee, Pennsylvania, Maryland, New York, Maine, Vermont, Connecticut, Massachusetts and Southern Ontario. This predominantly northern species shows a broad zone of overlap with the southern *D. melanica:* this zone runs approximately through Nebraska, Missouri, Illinois and eastwards to lower New York.

Ecologically relatively little is known about *D. paramelanica.* CARSON and STALKER (1951) found flies breeding on the slime fluxes of infected Red Oak, Black Locust and Chinese Elm in the St. Louis area, which were identified as "*D. melanica*". Since St. Louis is in the zone of overlap, it is uncertain whether the specimens were *D. melanica*, *D. paramelanica* or "*species X*". It is the author's experience that *D. paramelanica* is most readily collected in low-lying deciduous forests, especially if they contain Oak, Elm or Willow. Stands of Birch in northern Michigan seemed to be free of the species. As in many other Drosophila, collecting is especially favorable near water.

D. paramelanica does not build up large populations in nature early in the year, and even as far south as St. Louis, trapping is difficult before early July. Wild populations in Missouri remain in breeding condition until late fall, and there is no evidence that any of the autumn females go into a reproductive diapause of the sort reported in *D. robusta* (CARSON and STALKER 1948).

MATERIALS AND METHODS

The material used in this investigation was for the most part derived from fresh collections made by the author and others. Some use was made of stocks which had been maintained in the laboratory for a number of years, especially where these were from areas for which fresh material was unavailable.

Wild-caught or stock flies from various areas were mated to one of two laboratory strains, both of which were homozygous for the common "Standard" gene arrangements. The chromosomes of the F_1 larvae were then examined for inver-

sion loops, using acetic-orcein squashes. Various techniques were used to derive the maximum information from the rather limited wild material which could be obtained. Wild-caught males were analyzed by examination of up to seven F_1 larvae, including at least one female, thus indicating the arrangements in their X chromosomes and autosomes. Wild-caught females, if virgin, were analyzed by the same technique, except that up to seven female larvae were examined. Wild-caught inseminated females were allowed to use up their sperm, and then mated to the laboratory Standard stock: when they did not survive the elimination of their sperm one of their sons was analyzed, and treated as if he were a wild-caught fly. Comparison of the wild-caught males, and the F_1 sons of wild-caught females captured at the same time in a large population in Iowa has shown no difference in inversion frequencies between the two classes, so that this treatment is probably justified. In the analysis of any fly, when seven smears of F_1 larvae failed to show more than one chromosomal sequence in either the X or chromosome 2, the fly was considered to be homozygous for the particular chromosome concerned. In many cases examination of fewer than seven F_1 larvae was needed to establish the structural heterozygosity of the wild parent.

All flies were reared on standard cornmeal-karo-agar-tegosept food, seeded with live yeast. Larvae to be used for salivary gland smears were fed a thick live yeast suspension during the latter part of their development to insure large, cytologically favorable salivary gland chromosomes. All rearing was carried out at 25 ± 1.0°C. Adults were frequently stored at 17°C until they could be used.

RESULTS

The chromosomes of D. paramelanica

In females the oogonial and neuroblast metaphase chromosome complement consists of five pairs of chromosomes: a long pair of approximately equal-armed V-shaped X chromosomes, a shorter pair of V-shaped autosomes (corresponding to salivary gland chromosome 4), a long pair of rods, a somewhat shorter pair of rods, and a pair of very short rods or dots. On the basis of length alone one might suppose that the longer pair of rods corresponds to salivary gland chromosome 2, and the somewhat shorter pair to salivary gland chromosome 3, although this is uncertain.

In male neuroblast cells the Y is a large equal-armed V-shaped chromosome, which in all strains studied by the author is easily distinguished from the X chromosome by the fact that with acetic-orcein it stains more heavily than any of the other chromosomes.

In salivary gland preparations each cell contains seven euchromatic arms, one of them very short. Chiefly on the basis of their tips, the euchromatic arms are easily homologized to those of *D. melanica*, and the author has followed WARD's system of nomenclature, applying the same number and left-right designation to the homologs in *D. paramelanica*. Thus the seven salivary gland arms are designated: XL, XR, 4L, 4R, 2, 3 and 5. In many instances XL·XR and 4L·4R

associations are clearly indicated when the X or 4 breaks free of the chromocenter but retains its interarm attachment.

STURTEVANT and NOVITSKI (1941) have shown that the chromosome arms found in various species of Drosophila may be homologized by comparison of mutant genes found on them. They divide the Drosophila chromosomal material into six "elements", A, B, C, D, E and F. In *D. melanogaster* element A is the rod-shaped X chromosome, B and C are the two arms of Autosome II, D and E the two arms of Autosome III, and the F the dot-shaped Autosome IV. Although it now seems clear that there may have been opportunities for gene exchange between the elements in the course of evolution, it has been demonstrated that in many cases groups of genes associated in a single element in one species are so associated in other species; in other words the breakdown of the six elements has not been extensive. A number of species of Drosophila with V-shaped X chromosomes represent fusion between the regularly sex-linked element A and one of the other elements which is autosomal in *D. melanogaster*. For example in the *D. obscura* group of species, the two-armed X represents a fusion of elements A and D. In the discussion of that species group the authors point out that the "sex-ratio" genotype is regularly associated with the D element of the X chromosome. They also mention that the "sex-ratio" trait is known in *D. melanica*, and that this would lead to the suggestion that the X of *D. melanica* likewise represents fusion of elements A and D. The present author has not been able to demonstrate "sex-ratio" in the species referred to in this paper as *D. melanica*, and the above reference presumably refers to *D. paramelanica*. If we follow STURTEVANT and NOVITSKI's suggestion that the two-armed X of the *D. melanica* species group represents an A-D fusion, then we can go further and designate arm XR as element A, since it carries the sex-linked recessive gene singed near its distal tip. The only other known mutant likely to be confused with singed is forked, which is also on element A of *D. melanogaster*. If the designation of XR as element A is correct, then we might expect to find the genotype associated with the sex-ratio trait on XL, or element D. Preliminary studies indicate clearly that the sex-ratio genotype in *D. paramelanica* is associated with XR. This leads to the rather surprising preliminary conclusion that in this species the sex-ratio genotype is associated with element A, rather than with element D as in the *D. obscura* group. However, as indicated by STALKER (1958) the sex-ratio genotype in *D. paramelanica* is of two types, distinguished by responsiveness to Y chromosome suppression, so it is entirely possible that both XR and XL carry sex-ratio genotypes. If it can be demonstrated that arm XL does *not* carry the sex-ratio genotype, then one might reasonably assume that there had been an interelement exchange in the evolution of either the *D. melanica* group or the *D. obscura* group. This problem is being studied further.

The standard chromosome map

In Figure 1 are shown the relative lengths of the seven euchromatic arms. In this figure all distances are given as percentages of the total length of the longest

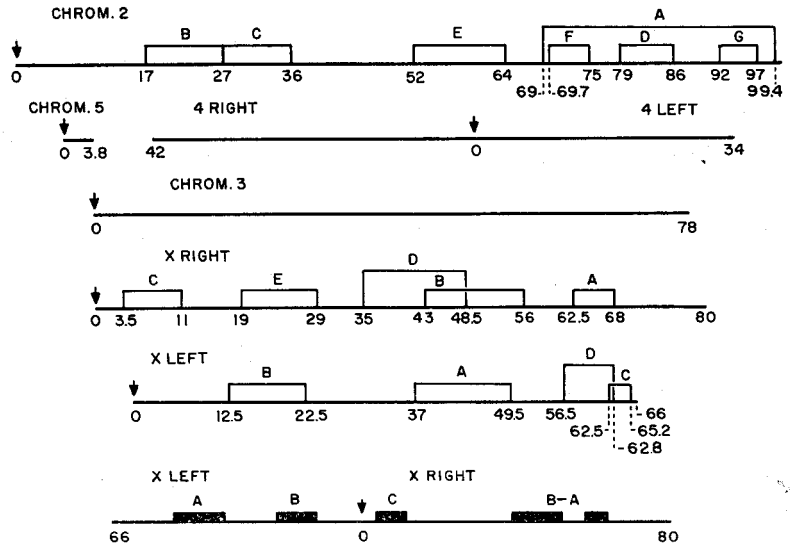

FIGURE 1.—Relative lengths of the euchromatic arms of the salivary gland chromosomes of *D. paramelanica*. In this figure all distances are given as percentages of the total length of the longest single arm, chromosome 2. Locations of known inversions are shown by brackets, and the centromere regions are indicated by arrows. At the bottom of the figure the two arms of the X chromosome are redrawn on a smaller scale to indicate the relative positions of the five sex-linked inversions showing adaptive association.

single arm, chromosome 2. The map distances were obtained from measurements of large numbers of photographs of acetic-orcein squashes of the homozygous Standard stocks: 28–12 and PR944a. In each arm, the position of the centromere, or more precisely the proximal limit of the euchromatin, is indicated by an arrow. The positions of those sections which may be inverted relative to Standard are indicated by brackets, and inversions themselves are given the symbols: A, B, C, etc. within each chromosome arm. In this paper no attempt has been made to homologize the system of inversions in *D. paramelanica* with those described in *D. melanica* by the use of corresponding inversion symbols; in fact it appears that few if any of the inversion differences in one species are homologous to those in the other. At the bottom of Figure 1 the two arms of the X chromosome are drawn on a smaller scale to emphasize the relative positions of the five important inversions which are to be discussed later in relation to the X chromosome inversion associations.

Figure 2 consists of photographs of the chromosome arms, showing the Standard sequence in all cases. Here as in Figure 1, the proximal ends of the chromosome arms are indicated by arrows, and the same numbering scale is used in both sets of illustrations. The limits of some of the inversions indicated in Figure 2 must be taken as approximate, and some may be in error by as much as two or three single bands.

In good preparations all of the major chromosome arms may be recognized

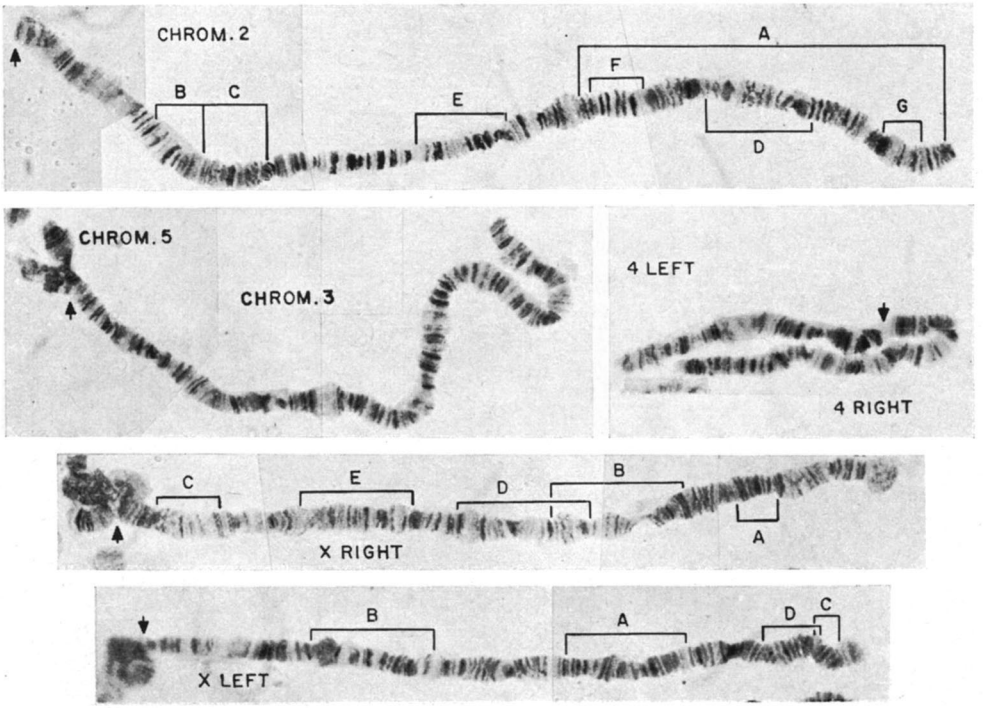

FIGURE 2.—Composite photographs of the salivary chromosome arms of *D. paramelanica*, showing the Standard banding sequence. The proximal ends of the arms are indicated by arrows, and the numbering system is the same as that used in Figure 1. The lettered brackets indicate the positions of the known inversions.

readily by the banding pattern at the tips. In poor preparations the tip of XL is extremely variable, and where difficulty is found in identifying this arm by the tip, the series of puffs found along its length constitute useful auxiliary characteristics.

Chromosome arm XR regularly shows a swelling at the tip, and very frequently exhibits a weak spot which may become stretched out and break, located approximately in the middle of inversion XR B.

Chromosome 2 has a weak spot at the base which is located approximately eight bands proximal to the proximal limit of inversion 2 C. The chromosome often breaks at this point.

In a few cells of some preparations chromosome arm 4L may show peculiar median adhesions resulting in figures superficially resembling inversion loops.

Geographical variability in chromosome 2

The known geographical ranges of the 16 gene sequences of chromosome 2 are indicated in Table 1. In examining the data for possible geographical trends in frequencies, it should be kept in mind that the second chromosome may be divided into two quite distinct regions, the proximal B-C region and distal A-F-D-G

region. That crossing over occurs between these two in nature is not surprising, and is proven by the finding of the reciprocal types such as AB, AC, A, B, C and + (here the symbol + indicates the Standard sequence for the pertinent inversions). The relationship of the rare median inversion E is unclear; it has been found only three times (in northern Michigan). Within the B-C region only the three types B, C and + are known, and no crossing over is expected between B and C. Within the A region, the three short inversions F, D and G which are included would not be expected to cross over with A, and there is no evidence that they have ever done so.

Considering first the proximal B-C region and ignoring for the moment the median and distal regions, the frequencies of the three classes B, C and + may be extracted from Table 1. If the various collecting sites are grouped into three broad zones, Northern, Middle and Southern, then it is clear (see Table 2) that arrangement C is northern, B is predominantly southern, and that + has its lowest frequency in the south.

The second section of Table 2 indicates the general clinal tendencies exhibited by the distal section of chromosome 2. Here the two commonest sequences, + and A replace each other, with A common in the north and nearly absent in the south and + the reverse. As expected, because of the association with A, sequences such as AG, AF, AD and ADG are commonest in the north.

Due to the recombination possible between the proximal and distal series of gene sequences, the phylogenies for the two should be considered separately. The basal +–B–C region could be represented phylogenetically as B⟷+⟷C, and since all three types are common in one region or another, and all but C widespread, it is difficult to guess which sequence was ancestral, although it seems probable that it was not C because of its restricted and northern distribution.

In the tip of chromosome 2 the phylogenies which can be established are illustrated in Chart 1. In this case the step from A to ADG could go through either AD or AG, as indicated by the alternate pathways. Both the + and A sequences are commoner and more widespread than the other four. This would suggest that either + or A is ancestral, and of the two perhaps + would be the likeliest candidate as it is typically southern while A is a northern sequence.

Since both the proximal and distal gene sequences in chromosome 2 show geographical frequency differences, it is not surprising that the proximal and distal ends show an interrelationship, such that the northern second chromosomes are characteristically + or C proximally and A distally (+ A or C A), while the southern chromosomes are B proximally and + distally (B +). Of somewhat more interest is the fact that even *within* a given population the two ends may not be associated at random. This is clearly shown in the sample from Mt. Vernon,

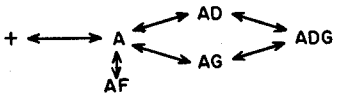

CHART 1.—Phylogeny of gene sequences in the tip of chromosome 2.

TABLE 1. *Geographical variability in gene arrangements of chromosome 2*

Locality	N	Standard	A	AC	AB	AD	ACF	ABD	ABE	ACD	ACE	ADG	AG	AE	B	C	ACDG
St. Louis, Mo.	44	6	..	..	..	..	..	..	..	..	..	..	..	..	38	..	..
		13.6	..	..	..	..	..	..	..	..	..	..	..	..	*86.4*	..	..
Watseka, Ill. / Terre Haute, Ind.	10	6	1	..	..	..	..	..	..	..	..	..	..	..	3	..	..
	..	*60.0*	*10.0*	..	..	..	..	..	..	..	..	..	..	..	*30.0*	..	..
Tennessee: Sevierville, / Gatlinburg	10	..	..	..	..	..	..	..	..	..	..	..	..	..	10	..	..
	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*100.0*	..	..
Iowa City, Iowa / Mason City, Iowa	23	10	3	..	..	..	..	..	..	..	..	..	..	..	9	1	..
	..	*43.5*	*13.0*	..	..	..	..	..	..	..	..	..	..	..	*39.1*	*4.4*	..
Mt. Vernon, Iowa	251	140	17	19	..	..	..	..	..	..	..	..	1	..	66	8	..
	..	*55.8*	*6.8*	*7.6*	..	..	..	..	..	..	..	..	*0.4*	..	*26.3*	*3.2*	..
Plainwell, Mich.	36	11	11	6	2	2	..	..	..	1	..	..	..	..	1	2	..
	..	*30.6*	*30.6*	*16.7*	*5.6*	*5.6*	..	..	..	*2.8*	..	..	..	..	*2.8*	*5.6*	..
Little Falls, Minn.	95	3	38	39	11	2	1	1	..	..	..	..	..	..	..	..	..
	..	*3.2*	*40.0*	*41.1*	*11.6*	*2.1*	*1.1*	*1.1*	..	..	..	..	..	..	..	..	..
Lincoln, Humbolt, / Nebraska	16	6	2	..	1	..	..	..	..	..	..	..	..	..	7	..	..
	..	*37.5*	*12.5*	..	*6.3*	..	..	..	..	..	..	..	..	..	*43.8*	..	..
Iron River, Wis.	8	..	4	3	..	1	..	..	..	..	..	..	..	..	..	..	..
	..	..	*50.0*	*37.5*	..	*12.5*	..	..	..	..	..	..	..	..	..	..	..
Iron Mt., Mich.	37	..	30	3	2	..	..	..	..	..	1	..	..	1	..	..	..
	..	..	*81.1*	*8.1*	*5.4*	..	..	..	..	..	*2.7*	..	..	*2.7*	..	..	..
Petoskey, Mich.	13	..	10	1	..	1	..	..	1	..	..	..	..	..	..	..	..
	..	..	*76.9*	*7.7*	..	*7.7*	..	..	*7.7*	..	..	..	..	..	..	..	..
Cold Spring Hbr., N.Y.	28	14	2	..	3	..	..	..	..	..	..	..	..	..	9	..	..
	..	*50.0*	*7.1*	..	*10.7*	..	..	..	..	..	..	..	..	..	*32.1*	..	..
Millersburg, Penn. †	†	..	..	..	..	..	..	..	..	..	..	*	..	..	*	..	..
	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Guarette, Maine †	†	*	..	..	*	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
South Hero, Vt. †	†	..	..	*	..	..	..	..	..	..	..	..	..	..	..	*	..
	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Bridgewater, Vt.	49	12	18	6	..	4	..	..	..	1	..	1	..	..	..	3	4
	..	*24.5*	*36.7*	*12.2*	..	*8.2*	..	..	..	*2.0*	..	*2.0*	..	..	..	*6.1*	*8.2*
Total chromosomes	620	208	136	77	19	10	1	1	1	2	1	1	1	1	143	14	4
Total localities	16	10	11	8	6	5	1	1	1	2	1	1	1	1	9	5	1

In the body of the table the actual numbers of chromosomes of each kind from a given locality are shown by the upper figure; the lower italicized figure gives the percentage frequency.
* Indicates the presence of a given gene sequence.
† In the left column of the table shows that one or more laboratory stocks were the source of the chromosomes examined.

TABLE 2

Frequencies of selected gene sequences in chromosomes 2 and X

Zone	Chromosome 2							X chromosome	
	Proximal			Distal					AB·CBA CAB·CBA
	+	B	C	+	A	AG-AF	AD-ADG	+ · +	AB·CB
North: Minn., Wis.,	125	15	62	18	168	1	15	109	11
N. Mich., Vermont	*61.9*	*7.4*	*30.7*	*8.9*	*83.2*	*0.5*	*7.4*	*90.8*	*9.2*
Middle: Iowa, N.Y.,	219	98	37	286	64	1	3	144	37
S. Mich., Nebraska	*61.9*	*27.7*	*10.5*	*80.8*	*18.1*	*0.3*	*0.8*	*79.6*	*20.4*
South: Mo., Ill.,	13	51	0	63	1	0	0	17	11
Indiana, Tennessee	*20.3*	*79.7*	*0*	*98.4*	*1.6*	*0*	*0*	*60.7*	*39.3*

In chromosome 2 the proximal and distal inversions are treated separately. In the X chromosome both arms are listed, in the order: X-Left·X-Right. For each cell in the table the upper figure indicates the number of chromosomes studied, the lower italicized figure the percentage within the particular geographical zone.

Iowa (see Table 3). In this table the computations of the expected numbers of proximal-distal combinations are based on the inversion frequencies within the population and the assumption of random association between the two ends of the chromosome.

From the left-hand section of Table 3 it will be noted that the agreement with expectation is not close: the χ^2 value of 81.0 leads to a P value of less than 0.001. In the right-hand section of the table the typically northern and typically southern associations, and the recombinant associations are lumped for a single comparison with their expected frequencies. Here again the discrepancy between observed and expected appears to be significant, and indicates that there is a tendency for northern and southern associations to persist even in intermediate populations where both northern and southern gene sequences occur with high frequencies.

Although other populations do not show significant non randomness of association in the second chromosome, the smaller sample sizes and relative lack of heterogeneity in the two ends of the chromosome make the detection of such non randomness unlikely in these populations.

GRIFFEN (1942) in discussing a preliminary cytological study made of *D. paramelanica,* states that using a Madison, Wis. strain as a Standard (he does not state that the strain was structurally homozygous, and the context suggests that it was not) he made a cross with a Wooster, Ohio strain and the F_1 showed two short overlapping inversions at the base of the long autosome (apparently chromosome 2). The position of these inversions certainly suggests 2-B and 2-C, and the most reasonable explanation of GRIFFEN's observation seems to be that his Madison Standard carried one inversion, the Wooster strain the other, so that he was observing a 2-A/2-B heterozygote. If this is the correct explanation, then the inversions 2-B and 2-C should perhaps be shown as overlapping. In the course of the present work 2-B/2-C heterozygotes have not been observed, and since comparisons of each of them with the Standard indicate they are adjacent rather than overlapping, they are shown in the former relationship. In addition to the two short inversions in the base of "the longest autosome" GRIFFEN reports that

Madison and Wooster strains differed by "two small inversions in another autosome". Another autosome would mean chromosome 3 or chromosome 4, and thus GRIFFEN's report would indicate heterozygosity in one of these autosomes. The material on which the present report is based does not include strains from Ohio, and the author has found no inversion heterozygosity in either chromosome 3 or 4 from other areas.

The X chromosome

In the discussion of variability in the X chromosome which follows, the two arms will be treated together. In all listings of gene sequences the sequence in the left arm (XL) will be given first: thus +·CBA represents a chromosome carrying the Standard sequence in the left arm and inverted sequences C, B and A in the right. The raised dot represents the centromere region.

The 13 types of X chromosomes found in wild populations are listed and their frequencies given in Table 4. This table does not include Standard (+·+) chromosomes from the following: Nebraska (2); Mason City, Iowa (4); Terre Haute, Ind. (1); Watseka, Ill. (4); Lincoln, Neb. stocks (2); Guarette, Me. stock (1); Hero, Vt. stock (1); Millersburg, Pa. stock (1).

A fourteenth type of X chromosome not listed in Table 4 has been found in an old laboratory stock from Cold Spring Harbor, N.Y. This chromosome, which may have been derived by crossing over in the laboratory, is B·CB (i.e., carries inversion B in XL and C and B in XR).

It is clear from examination of Table 4 that the commonest types of X chromosomes are: the Standard sequence +·+, and the *family* of sequences that include the inversions AB·CB (AB·CBA, CAB·CBA, AB·CB). Except for the three rare types DAB·CBA, +·D and +·E, all other chromosomes observed may be derived from Standard and the AB·CB family by crossing over.

Considering for the moment only +·+ and the AB·CB family, it is clear (see Table 2) that +·+ is relatively more frequent in the north, while the AB·CB family has higher frequencies in the south. It is worth noting here that in all cases tested (42 to date), the AB·CB family of chromosomes have been shown to carry the "sex-ratio" gene or gene-complex. Males with a "sex-ratio" X chromosome may under certain circumstances produce primarily X-bearing sperm and thus, daughters, but very few sons (STALKER 1958). Similar genes are known in the *D. obscura* group of Drosophila, and in this group as in *D. paramelanica*, X chromosomes carrying the sex-ratio gene have a characteristic gene sequence not found in normal X chromosomes. Likewise in these species it has been found that the frequency of the sex-ratio chromosomes is higher in the south than in the north (see WALLACE 1948).

With the exception of D and B in the right arm, there are no overlapping inversions known in the X chromosome. However, certain inversions are found only in association with others: thus A in XR has not been found unless associated with the adjacent inversion B, although XR B may be found without XR A. This suggests that crossing over may not occur between them and that their relationship is of phylogenetic origin. In XL the small distal inversion C has been found

TABLE 3

Chromosome 2 associations of proximal and distal gene sequences from a population in Mt. Vernon, Iowa

	Typically northern + A	C A	Typically southern B +	+ +	Recombinants B A	C +	Total northern-southern	Total recombinants
Observed	17	19	66	140	0	8	102	148
Expected	22.6	3.9	56.5	134.4	9.5	23.1	83	167
Difference	−5.6	+15.1	+9.5	+5.6	−9.5	−15.1	+19	−19
		$\chi^2 = 81.0$					$\chi^2 = 6.5$	
		$P = <0.001$					$P = 0.01$	

In the table the sequence at the proximal end is listed first, the distal last. Thus, chromosome type: + A has the standard proximal sequence and the inversion A distally. The expected frequencies are computed on the basis of the frequencies of the proximal and distal gene sequences within the Mt. Vernon sample, and on the assumption of random association between sequences in the two ends of the chromosome.

TABLE 4

Geographical variation in frequencies of X chromosomes from wild populations

Chromosome type XL · XR	St. Louis, Mo. N	%	Iowa City, Iowa N	%	Mt. Vernon, Iowa N	%	Little Falls, Minn. N	%	Iron River, Wisc. N	%	Iron Mt., Mich. N	%	Petoskey, Mich. N	%	Plainwell, Mich. N	%	C.S.H., N.Y. N	%	Bridgewater, Vermont N	%
+ · +	12	46	6	86	117	75	53	76	2	50	17	81	11	79	5	63	12	63	26	96
AB · CBA	11	42	1	14	26	17	7	10	..	..	1	5	..	..	1	7	..	..	..	..
CAB · CBA	..	..	..	..	5	3	2	3	..	..	2	10	..	..	..	..	..	..	..	..
DAB · CBA	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
+ · CBA	1	4	..	..	3	2	..	..	..	..	..	..	..	..	1	7	..	..	..	..
AB · CB	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4	21	1	4
+ · BA	..	..	..	..	1	1	2	3	..	..	..	..	..	..	..	..	1	5	..	..
AB · C	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
+ · C	1	4	..	..	..	..	..	..	..	..	..	..	..	..	1	7	1	5	..	..
A · +	..	..	..	..	..	..	1	1	..	..	1	5	..	..	..	..	..	..	..	..
AB · +	1	4	..	..	..	..	..	..	1	25	..	..	2	25	..	..	1	5	..	..
+ · D	..	..	..	..	1	1	5	7	1	25	..	..	1	13	..	..	..	..	..	..
+ · E	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Totals:	26	..	7	..	156	..	70	..	4	..	21	..	8	..	14	..	19	..	27	..

This table does not include + · + chromosomes from the following: Nebraska (2); Mason City, Ia. (4); Indiana (1); Illinois (4); and from the following laboratory stocks: Lincoln, Neb. (2); Guarette, Me. (1); Hero, Vt. (1); Pennsylvania (1).

nine times in three different localities, but always in the association CAB CBA. In this case XL C and XL A appear to be sufficiently separated so that crossing over between them might be expected to occur, and the probable explanation of the absence of other combinations of XL C is that such combinations are genetically nonadaptive and are eliminated as they are produced by crossing over in the +·+/CAB·CBA females.

Associations within the AB·CB family of X chromosomes

In order to clarify the section to follow, a slight change in symbols will be employed. The + symbol will be used to represent the Standard sequence for *each* pertinent inversion, rather than the Standard sequence for the whole arm. Thus a female heterozygous Standard/AB·CBA will be represented as + +·+ ++/A B·C BA.

In certain sections of both XL and XR proof exists that crossing over may occur in nature between adjacent inversions in heterozygous individuals. This proof is based on the finding of all four reciprocal classes involving any two adjacent sections of chromosomes. For example, the existence in natural populations of the four chromosome types: + +·+ ++, A B·C BA, + +·+ BA and A B·C ++ indicates that crossing over may occur between XR C and XR BA. Similar evidence of crossing over exists between XL A and XL B and between XL B and XR C.

In order to obtain crossover X chromosomes for laboratory study, females with one Standard X chromosome, and the other X carrying either the inversions A B·C BA or A B·C B+ were prepared. The system of inversions in chromosome 2 of these females was not controlled; some were homozygous for the short basal second chromosome inversion 2-B, some were heterozygous 2-B/ Standard. The females were all heterozygous for two or more of the sex-linked recessive mutants: singed, garnet, carnation and brown, which were well distributed over the two arms. By picking out sons of the heterozygous females which showed recombination of the mutant genes, and subsequently analyzing their chromosomes, a number of crossovers were obtained. The approximate rates of crossing over for each region are given below, with the figures in parentheses indicating the number of classified individuals leading to each value: XL-A — XL-B = 0.06 percent (4,872); XL-B — XR-C = 0.10 percent (4,184); XR-C — XR-BA = 0.09 percent (9,915). There is no evidence that crossing over occurs between XR-B and XR-A, and thus they are treated as a single segment, XR-BA. On the basis of the data above it is possible to divide the X chromosome into the four separable segments: A B·C BA.

If in a given population, the indicated segments may be separated by crossing over, then the population might be expected to show random associations of them with the frequency of each type of association determined by the population frequency of the given segments. Any significant departure from random association between the various chromosome segments might be accounted for by drift, natural selection, or by recent phylogenetic origin of certain associations and insufficient time for their breakdown by crossing over. In the data to be ana-

lyzed below it will be shown that the four segments of the X chromosome are not randomly associated within populations and that this cannot be attributed to drift, since the associations studied show the same kind of departure from randomness in all populations. The explanation involving recent origin likewise seems unsatisfactory because the broad geographic range of most of the inversion associations indicates a very old, not a very recent origin.

In determining whether the associations of inversions within populations were random, expected numbers of each chromosome type were calculated; these numbers were based on the segmental frequencies within each population, the assumption of random association between segments and the assumption of no selective elimination of any particular chromosome type. The comparison between observed and expected chromosome types is summarized in Table 5. In the body of the table are listed the data from the seven largest population samples; included in the totals in the last two columns, but not given in the body of the table, are the similarly handled data from Petoskey, Mich., Iron River, Wis., and Iowa City, Iowa (24 chromosomes in all).

Table 5 shows that for the chromosome associations: + +·+ ++, A B·C BA and A B·C B+ there is a striking excess of observed over expected in every population sampled, and for all populations taken together. The above three associations were expected to occur in 151 chromosomes and were actually found in 322. On the other hand the remaining 21 classes of X chromosome associations listed in the table were expected to occur in 191 chromosomes and were actually found in only 20. All of the 21 associations which are either absent, or present in less than the expected frequencies, may be derived from the three associations present in excess by crossing over. Since it is clear that crossing over between the four segments actually occurs, then the low observed frequency of the 21 deficient types of chromosomes indicates their nonadaptive nature, and their continued removal from the population as they are produced by crossing over. It may be noted that while some of the deficient types may be derived by single crossovers from the three excessively frequent chromosome types, others would require a number of successive crossovers. The situation is presented in Chart 2 below. In this chart only combinations derived from the Standard + +·+ ++ and the common western A B·C BA are considered. A similar series may be derived from Standard and the eastern chromosome type A B·C B+, and differs from the series below only in the substitution of XR B+ for XR BA in each case.

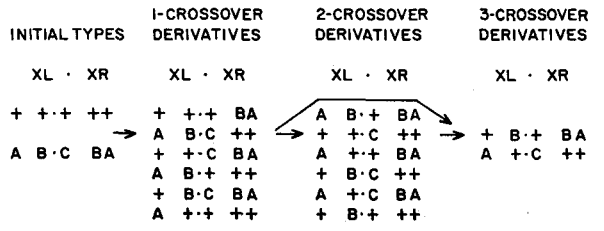

CHART 2.—Scheme to show the origin of recombinant X chromosomes from the Standard + +·+ ++, and the common western A B·C BA types. See text.

TABLE 5

Summary of X chromosome recombinations

Chromosome type XL · XR	St. Louis, Missouri Exp.	Obs.	Mt. Vernon, Iowa Exp.	Obs.	Plainwell, Mich. Exp.	Obs.	Iron Mt., Mich. Exp.	Obs.	Little Falls, Minn. Exp.	Obs.	C.S.H., N.Y. Exp.	Obs.	Bridgewater, Vermont Exp.	Obs.	Totals Exp.	Obs.
+ + · + ++	2.03	12	56.91	117	8.13	11	10.70	17	33.92	53	5.60	12	23.22	26	149.19	261
A B · C BA	1.28	11	0.35	31	0.002	1	0.01	3	0.03	9	0.02	0	...	..	1.70	56
A B · C B+	...	..	...	..	...	..	...	..	...	..	0.08	4	0.0001	1	0.07	5
A B · + BA	1.28	0	1.18	0	0.01	0	0.07	0	0.21	0	0.05	0	...	..	2.82	0
A B · + B+	...	..	...	..	...	..	...	..	...	..	0.20	0	0.001	0	0.21	0
A + · C BA	1.49	0	1.33	0	0.03	0	0.07	0	0.20	0	0.05	0	...	..	3.18	0
A + · C B+	...	..	...	..	...	..	...	..	...	..	0.20	0	0.001	0	0.21	0
+ B · C BA	1.49	0	1.33	0	0.03	0	0.05	0	0.18	0	0.05	0	...	..	3.14	0
+ B · C B+	...	..	...	..	...	..	...	..	...	..	0.20	0	0.001	0	0.21	0
A B · C ++	1.49	0	1.18	1	0.01	0	0.07	0	0.16	0	0.26	0	0.001	0	3.19	1
+ + · C BA	1.74	1	5.01	3	0.37	1	0.30	0	1.11	0	0.14	0	...	..	8.77	5
+ + · C B+	...	..	...	..	...	..	...	..	...	..	0.57	0	0.04	0	0.61	0
A + · + BA	1.49	0	4.52	0	0.10	0	0.42	0	1.23	0	0.14	0	...	..	8.01	0
A + · + B+	...	..	...	..	...	..	...	..	...	..	0.57	0	0.04	0	0.61	0
+ B · + BA	1.49	0	4.47	0	0.10	0	0.30	0	1.11	0	0.14	0	...	..	7.72	0
+ B · + B+	...	..	...	..	...	..	...	..	...	..	0.57	0	0.04	0	0.61	0
A + · C ++	1.74	0	4.51	0	0.17	0	0.42	0	0.99	0	0.71	1	0.04	0	8.68	1
A B · + ++	1.49	1	3.98	0	0.05	0	0.42	0	0.99	0	0.71	1	0.04	0	8.69	5
+ B · C ++	1.74	0	4.47	0	0.17	0	0.30	0	0.88	0	0.71	0	0.04	0	8.40	0
+ + · + BA	1.74	0	16.88	1	1.36	0	1.79	0	6.91	2	0.40	1	...	..	29.70	4
+ + · + B+	...	..	...	..	...	..	...	..	...	..	1.60	0	0.89	0	2.49	0
+ + · C ++	2.03	1	16.88	0	2.22	1	1.79	0	5.45	0	2.00	0	0.89	0	31.89	2
+ B · + ++	1.74	0	15.05	0	0.63	0	1.79	0	5.45	0	2.00	0	0.89	0	30.27	0
A + · + ++	1.74	0	15.05	0	0.63	0	2.52	1	6.17	1	2.00	0	0.89	0	31.72	2
Totals:	26.00	26	153.10	153	14.00	14	20.99	21	65.00	65	19.00	19	27.00	27	342.08	342

In this table a comparison is made between the observed and expected population frequencies of X chromosomes carrying the XL inversions A and B, and the XR inversions C and BA (thus A B · C BA), and chromosomes carrying none of these inversions (+ + · + ++) and all possible combinations between them. Chromosomes carrying the rare XL D and XR D and E inversions were not included. In order to shorten the table 24 X chromosomes from Petoskey, Iron River and Iowa City are not listed in the body of the table although they are included in the totals in the right hand column. The expected frequencies are worked out separately for each population on the basis of its own observed inversion frequencies and on the assumption that the four segments: *A B C B.A* are separable by crossing over and should be associated randomly.

If the extremely unlikely double or triple crossing over is ruled out, then the 2-crossover and 3-crossover classes can only be derived via the 1-crossover classes. Thus adaptive inferiority of the 1-crossover classes would in itself tend to prevent production of the other derived classes. The observed and expected frequencies of the Initial types and the three derived classes from eight localities are listed in Table 6. The eight localities were chosen because of large sample size, and because of the fact that the samples studied carried at least two alternates in each of the four segments. The data in this table indicate that while all the derived classes are below expectation, this deficiency is particularly striking in the case of the 2- and 3-crossover classes. If the 17 derived types actually observed were randomly distributed among the three derived classes, then 47 percent or eight should have been 1-crossover types, and the remaining nine should have been 2- and 3-crossover types. The observed distribution of 14 1-crossover types and only three in the other two classes differs significantly from expectation. Using YATES' correction for continuity, $\chi^2 = 7.14$ and $P = < 0.01$.

DISCUSSION

The data and conclusions presented above on inversion associations within chromosomes may be briefly summarized. In both the second and X chromosomes the inversions show non random associations. This non randomness is especially striking in the X, and it is concluded that it is due to the relative nonadaptedness of certain inversion associations, which are eliminated as they are produced by crossing over. One may arrive at a rough estimate of the rate at which elimination of nonadaptive chromosomes must occur by a consideration of the rate of their production by crossing over within populations. For example, in the St. Louis, Mo., population approximately 50 percent of the females should be hetero-

TABLE 6

Frequencies of X chromosomes showing "initial types, 1-crossover, 2-crossover and 3-crossover derivatives" starting with Standard/A B · C BA or Standard/A B · C B+ as the initial types. Data from all sites showing two alternates in each of the four segments

Site	N	Initial types		1-crossover derivatives		2-crossover derivatives		3-crossover derivatives	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
St. Louis, Mo.	26	3.31	23	9.69	2	9.77	1	3.23	0
Plainwell, Mich.	14	8.13	12	2.44	1	3.15	1	0.27	0
Iowa City, Iowa	7	3.78	7	1.50	0	1.50	0	0.21	0
Mt. Vernon, Iowa	153	57.26	148	43.43	5	43.43	0	8.97	0
Little Falls, Minn.	65	33.95	62	15.52	3	13.43	0	2.10	0
Iron Mt., Mich.	21	10.71	20	5.14	1	4.42	0	0.72	0
C.S.H., N.Y.	19	5.69	16	7.94	2	3.94	0	1.43	1
Bridgewater, Vt.	27	23.22	27	1.85	0	1.85	0	0.07	0
Total:	332	146.05	315	87.51	14	81.49	2	17.00	1
Observed / Expected		2.157		0.160		0.025		0.059	

zygous + +·+ + +/A B·C BA and crossing over in such females leads to the production of the deficient types of X chromosomes. From the laboratory crossover data given above, the total proportion of single-crossover chromosomes produced by such a female would be the sum of the crossover values for each of the three sections of the X lying between inversions, thus 0.25 percent. Taking into account the noncrossover chromosomes produced by males, a population such as the one in St. Louis would be expected to produce nonadaptive X chromosomes at the rate of about 1/1,200 per generation. These would all be of the 1-crossover derived type, and might either be eliminated as such, or by further crossing over produce 2- or 3-crossover types which would be eliminated in their turn. In the northern part of the species range, where the frequency of the A B·C BA chromosome is lower, the rate of production of nonadaptive X chromosomes would be only about 1/2,400. Thus the price of maintaining Standard or A B·C BA chromosomes intact in the population is relatively low.

The cause of the relative nonadaptedness of the observedly deficient inversion associations is not clear at present, however one possible factor suggests itself. The A B·C BA and A B·C B+ X chromosomes carry the "sex-ratio" genotype. As has been pointed out by other workers, chromosomes carrying such a genotype should benefit from the effects of meiotic drive, since males carrying the sex-ratio chromosome tend to transmit it to all of their progeny (their daughters) while males with Standard X chromosomes transmit them to only the male half of their progeny. The fact that such sex-ratio chromosomes do not completely replace the Standard chromosomes (with possible extinction for the population) is due to the opposite effects of other selective forces (at present not well understood). Crossover X chromosomes carrying only part of the A B·C BA complex might be expected to lack the sex-ratio genotype, and thus lose the advantages of meiotic drive characteristic of the intact chromosome. Preliminary studies of crossover X chromosomes carrying part of the sex-ratio inversion complex indicate that some (but not all) of them do lack the sex-ratio genotype, and thus should suffer a reduction in their over-all adaptive value.

Non random association of inversions has been demonstrated, or is suspected for other species of Diptera, especially Drosophila (see LEVITAN 1958 for review). The species which has been most intensively studied in this respect is *D. robusta*, in which LEVITAN has presented data conclusively showing non random associations in the X and in chromosome 2. In this species the associations are of a somewhat simpler sort than those found in the X of *D. paramelanica*, and are in most respects comparable to those in chromosome 2 of the latter species, that is they involve adaptive associations between two segments of a given chromosome.

In *D. melanica* WARD (1952) has found that some of the inversions on chromosome 2 and on the X chromosome regularly occur together. LEVITAN cites this species as offering examples of non random associations, but it is the opinion of the author that he fails to prove his point in this particular instance. In addition to overlooking some rather crucial parts of WARD's data, he has listed examples

that are either represented by too few population data, or are tied up in inversion complexes that may not be separable by crossing over.

The very complex inversion associations demonstrated in the X chromosome of *D. paramelanica* bear some resemblance to the situation found in species of Drosophila in which adaptive associations of linked genes are maintained even without the help of inversion heterozygosity. In these cases the cost of maintenance of such associations is the elimination of individuals carrying nonadaptive chromosomes produced by recombination. Associations of this sort have been demonstrated in *D. melanogaster* (MISRO 1949; WALLACE *et al.* 1953), in *D. pseudoobscura* (DOBZHANSKY 1946; SPASSKY *et al.* 1958), in *D. prosaltans* (DOBZHANSKY *et al.* 1959) and in *D. persimilis* (SPIESS 1959).

In such species it might be expected that systems capable of producing lethal or semilethal recombinant chromosomes would not occur on the X chromosome. As stated by SPASSKY *et al.* (1958), "—one third of the X chromosomes in a population are carried in males which would die if they had a sex-linked lethal. Therefore, X chromosomes capable of giving sex-linked lethals by recombination with other X's in the same population would be discriminated against by natural selection. This is not necessarily the case with autosomes, since the lethals produced by recombination would be carried mostly in heterozygotes, and might be removed by another recombination. Only synthetic lethals that would kill homozygous females, but not males, are expected in the X chromosomes."

It is of course clear that any argument based on lethals will apply also to semilethals and gene combinations causing moderate reductions in viability or fertility. In the X chromosome of *D. paramelanica* recombinants are clearly eliminated. It is not clear in this case whether the elimination occurs predominantly in the male or the female, and the available data on wild populations are not sufficiently extensive to clear up this point.

One may think of the *D. paramelanica* X's as showing two types of adaptive genetic organization. On the one hand, X's which when in structural *homozygotes* are able to produce recombinant lethals or semilethals should be discriminated against, and might be expected to be absent from the population. However, X chromosomes which produce lethals or semilethals following rare crossing over in the structural *heterozygotes* would suffer so little from selective elimination of males, that they would be expected to survive in a population. It is not surprising, therefore, that adaptive inversion associations occur on the X chromosomes of a number of other species of Drosophila. They are found in the *D. obscura* group (where they are associated with the sex-ratio trait), in *D. americana* (BLIGHT 1955) and in *D. robusta* (CARSON and STALKER 1949; LEVITAN 1958).

SUMMARY

1. The paper describes a survey of chromosomal polymorphism in *Drosophila paramelanica*, a member of the *D. melanica* species group found in northeastern North America. Samples of this species were taken from 18 widely separated localities, and the chromosomes from the various populations were classified by

means of crosses with a laboratory Standard strain originating in St. Louis, Mo. Approximately 370 X chromosomes and 600 of each kind of autosome were analyzed from wild populations.

2. Only the X chromosome and chromosome 2 were found to be structurally polymorphic. In the rod-shaped chromosome 2 seven inversions differing from Standard were discovered, and a total of 16 gene sequences, including Standard, have been found in nature. In the V-shaped X chromosome four inversions were found in the left arm and five were found in the right. For the whole chromosome 13 gene sequences including Standard have been found in nature.

3. In chromosome 2 the known inversions are located primarily at the base or tip. The gene sequences in either region show clinal changes in frequency, with certain patterns predominantly northern, others southern. In an Iowa population lying between the northern and southern parts of the species range, where both northern and southern arrangements occur together, the distal and proximal arrangements are not associated at random, but tend to be associated: North-North or South-South.

4. In the X chromosome a series of short inversions tend to be associated; they are inversions A and B in X-left and C and B in X-right, thus: A B·C B. It has been shown that these inversions are all separable by rare crossing over. The commonest kinds of X chromosomes in all populations are either Standard (+ +·+ +) or those with inversions A B·C B. The X's which might be produced by crossing over and subsequent separation of these four inversions are either absent, or present in frequencies significantly below expectation. This relationship holds in all populations studied.

5. Of the X chromosomes showing only some of the four inversions, those which might be produced from A B·C B/+ +·+ + females by a single crossover (such as A B·C + or A +·+ +) are much more frequent than those which would require a succession of single crossovers (such as A +·C B or A B·+ B).

6. It is concluded that natural selection is eliminating the deficient types of X chromosomes as rapidly as they are produced by crossing over (predominantly in ++·++/A B·C B females). Since even the types of chromosomes which may be produced by a single crossover are deficient in the population, it is suggested that the greater deficiency of types that must be produced by two or more successive crossovers can be ascribed to the initial nonadaptedness of the 1-crossover types which makes subsequent production of the 2- and 3-crossover types highly improbable.

7. Preliminary laboratory tests of crossing over in heterozygous A B·C B/ ++·++ females indicate that in a population in which the A B·C B chromosomes have a frequency of 40 percent, as in Missouri, approximately 1/1,200 of the X-bearing gametes formed should show some type of recombination for the four inversions. More northern populations would be expected to produce about 1/2,400 such gametes. Thus the cost to the species of maintaining the adaptive association is a low one.

8. All A B·C B X chromosomes tested carry the sex-ratio trait, of the sort found

in the *D. obscura* group, and thus males carrying such chromosomes tend to produce only daughters. In *D. paramelanica*, as in the *D. obscura* group, sex-ratio X chromosomes have a higher frequency in the southern part of the range (40 percent) than in the north (nine percent).

9. The V-shaped chromosome of *D. paramelanica* apparently includes STURTEVANT and NOVITSKI's Element A (corresponding to the rod-shaped X of *D. melanogaster*) and some other element which is autosomal in *D. melanogaster*. Since in *D. paramelanica* X-right carries the mutant singed apparently this arm should correspond to Element A; the identity of the other element is not clear.

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