CHROMOSOME STUDIES IN WILD POPULATIONS OF *D. MELANOGASTER*[1]

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ABSTRACT

Chromosome studies of wild *D. melanogaster* populations from Missouri, Mississippi, Louisiana and Texas uncovered 58 inversions. Six were common and cosmopolitan; 52 were new, rare and generally endemic. In one of two Missouri populations tested, structurally heterozygous females carried significantly more sperm at capture than did the homozygotes. In both populations comparisons of wild sperms with the females carrying them indicated significant positive assortative mating and an excess production of homozygotes among the F_1 progeny. Wild females structurally heterozygous in up to three major autosomal arms showed no associated nondisjunctional egg lethality; those heterozygous in all four arms produced from 0% to 24% dead eggs, suggesting the presence of intrapopulational gene modifiers of meiosis. Texas populations supported on windfall citrus fruit showed a slight but significant difference in inversion frequencies between flies breeding on oranges and those breeding on grapefruit. Within these populations inversions were not distributed at random among individuals; rather there was an observed excess of individuals carrying intermediate numbers, and a deficiency of those carrying very few or very many inversions. While there was no significant linkage disequilibrium associated with this central tendency, there was a significant interchromosomal interaction: flies carrying inversions in chromosome *2* tended not to carry them in chromosome *3*, and *vice versa*.

IN the cosmopolitan species, *Drosophila melanogaster*, most studies of karyotypic variability in natural populations have led to the conclusion that there is limited variety in the types of inversions present. It has been further shown that the six commonest inversions are cosmopolitan in distribution, with frequencies fairly similar even in widely separated populations, that the "Standard" gene sequences are almost universally commoner than inverted sequences, and that natural populations carry very few *X*-chromosome or pericentric inversions (WARTERS 1944; IVES 1947; MOURAD and MALLAH 1960; WATANABE 1967; YANG and KOJIMA 1972).

The fact that *D. melanogaster* is known to be domestic and regularly associated with human habitations in the higher latitudes might suggest that the observed inversion frequency similarities in various populations could be explained by extensive migration and the assumption that, at least in temperate zones, the number of ecological niches might be limited, with inversion heterozygotes

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having some type of "general vigor" in the sense of CARSON (1959), adapting them to the majority of niches they would be likely to encounter.

The study of natural populations to be reported below was initiated to obtain information relative to the following questions: Is there in fact good evidence for any kind of "general vigor" in nature associated with inversion heterozygosity? Is there evidence for annidation (LUDWIG 1950), i.e., *specific* adaptiveness of certain gene sequences, such as might lead to selectively induced frequency differences in different populations? Do individuals in natural populations breed panmictically, or is there assortative mating, possibly associated with early sexual maturity? Are there adaptive upper and lower limits to the total *number* of inverted sequences that a given fly may carry, and are the various inversions in wild populations distributed randomly among individuals? Are there frequency disequilibria between various types of nonhomologous chromosomes?

Despite the extensive work on this species, there is little information on most of the above points as they apply directly to natural populations.

MATERIALS AND METHODS

Wild flies were collected from pint jars containing fermenting banana (Missouri), with a net in and around garbage cans (Louisiana, Mississippi, Texas), or from windfall fruit (Texas). Mating after collection was prevented by crowding of adults, by cooling, or by prompt sorting for sex. Members of the common sibling species *D. simulans* appeared in all collections but could be readily recognized and eliminated on the basis of the male externalia, wing length and structure of egg filaments in females, and by eye size and color, and cheek width in both sexes.

The cytological "Standard" strain used for comparative purposes was derived from an Oregon-R stock kept in the laboratory for many years, and purged of a short inversion in chromosome *2*, for which it was found to be heterozygous.

Wild-caught males were mated individually to laboratory Standard females, and two or more F_1 larvae scored for inversions. Wild females were treated in various ways; in some cases they were despermed by cold treatment, then mated to Standard males, and seven or eight F_1 larvae were scored for inversions. In other cases they were allowed to reproduce in the laboratory, using the wild sperm they carried, and estimates were made of embryonic (egg) lethality, or of the total number of sperms they carried at the time of capture, or a determination was made of the constitution of a single wild sperm by cytological analysis of an F_1 larva. After the above special tests were made, the despermed females were mated to Standard males, and their karyotypes determined by analysis of seven or eight F_1 larval squashes.

In general, gene sequence determinations were made by observations of pairing patterns following crosses to Standard. In some F_1 larvae produced by structurally heterozygous mothers and utilizing wild sperm, the larval chromosome sequences had to be worked out by reference to a Standard photographic map. All salivary chromosome arms were analyzed except for the microchromosome (*4*), and the short basal section in the left arm of chromosome *2*, which regularly showed knotting due to stickiness, presumably associated with the banding pattern repeats found in this region.

Larvae for chromosome studies were reared on cornmeal-agar-yeast-Karo-Tegosept medium, heavily enriched with live yeast. Salivary glands were dissected in 50% acetic acid. and stained with lactic-acetic-orcein; squashes were made on siliconed slides, coverslips sealed with Vaseline or fingernail polish, and the slides stored at —20°. Slides were read with a light microscope, new inversions were plotted on a Standard photographic map, and then photographed on Eastman High Contrast Copy Film, which was overexposed and underdeveloped to reduce the contrast while retaining the detail.

Special Analytical Procedures

A. *Estimates of chromosome frequencies from incompletely analyzed flies*

The usual method of determining inversion frequencies from wild flies involves crossing to Standard, followed by analysis of a single F_1 larva (yielding one wild chromosome of each type per wild fly), or by analysis of seven or more F_1 larvae, permitting "complete" karyotype determination of the wild fly, and thus the sampling of two wild chromosomes of each type from the population. In those cases in which wild material is abundant, assistance limited, and complete karyotype determination of the wild fly unnecessary, neither of the above methods is economical; the first yields too little information per testcross, while the second involves too much reexamination of the same wild chromosome. Analysis of two to four F_1 larvae per wild fly is often economical and practicable.

While a single F_1 larva from a wild fly samples one chromosome of each pair, two larvae from the same fly will on the average sample 1.5 wild chromosomes per pair, since the wild chromosome in the second larva will be different from that in the first half of the time. Similarly, three larvae will on the average sample 1.75 wild chromosomes. In general, n larvae from the same wild fly will on the average sample $2-(1/2)^{n-1}$ wild chromosomes of each pair. Using this formula, a wild fly showing, for example, three + (Standard) chromosomes among $n = 3$ larvae, would be recorded as having yielded $2-(1/2)^2 = 1.75$ + chromosomes. A fly showing one *A* chromosome and two + chromosomes among three F_1 larvae would be recorded as having yielded $1.75/2 = 0.875$ *A* chromosomes, and 0.875 + chromosomes (since the fly was clearly an *A*/+ heterozygote). Finally, the yields for each type of chromosome from each fly in the sample were summed and rounded to the nearest whole number. In the material reported in this paper the number of F_1 larvae analyzed per wild fly varied according to availability of material and time.

Obviously the above procedure yields unbiased frequency estimates only if the two chromosomes of a given pair are recovered with approximately equal frequency among the gametes from a given wild fly. Counts of the F_1 larvae of 311 wild flies heterozygous for Standard and an inversion showed that among 1772 such larvae, the proportion of chromosomes recovered was 0.524 Standard to 0.476 inversion. This deviation from equality, although probably significant ($\chi^2 = 4.17$, $P = 0.041$), was found to make so little difference in the estimates of inversion frequencies that it was finally ignored. Unequal transmission or recovery rates will introduce some bias into *any* procedure used for estimating chromosome frequencies by gamete samples, whether the procedure involves samples of single gametes, or samples of seven or more gametes, as in the usual "complete" karyotype analysis.

B. *Analysis of inversion frequencies in wild sperm*

Karyotype analysis of a single larva produced by a wild-inseminated female will, if the mother's karyotype is later determined, usually permit a determination of the chromosomal constitution of the contributing wild sperm. Since it is highly probable (although apparently not proven) that wild females of this species are frequently multiply inseminated in nature, analyses of more than one sperm per wild female are difficult to interpret, and rarely permit determination of the paternal karyotype.

If a female is observed to be homozygous Standard (+/+) for a particular chromosome, and the F_1 larva +/+ or +/*A*, then the wild sperm clearly must have been + or *A*, respectively. Similarly an *A/B* female producing an *A/A*, *B/B*, *A*/+, or *B*/+ larva permits determination of the sperm type. However, an *A/B* larva from an *A/B* mother could have resulted from either an *A* or a *B* sperm. Such cases, where the mother and the F_1 larva are identically heterozygous, cannot be ignored since to do so would bias the estimates in favor of relatively rare chromosomal types. If in fact an *A/B* female uses an *A* sperm, then since she should contribute her *A* chromosome and her B chromosome with equal frequencies, one-half of the time her offspring would have come from an *A* egg and thus be *AA*, and the other half of the time from a *B* egg and thus be *AB*. Thus the population frequency of *A* sperms carried by *A/B* females may be estimated as twice the frequency of their single *A/A* larvae. Similarly the frequency of *B* sperms from *AB* females would be estimated as twice the frequency of their single

B/B larvae. Such estimates will, of course, reflect differential sperm viability, but they will not be disturbed by assortative mating.

C. *The expected frequencies of recovered chromosome combinations among samples of two gametes per wild fly*

In collections consisting of individuals represented by gamete samples of between one and six per fly, it is necessary to calculate the expected frequencies of samples carrying various chromosome combinations so that they may be compared with observed sample frequencies, and inferences drawn concerning chromosome distributions in individuals in the contributing population. Since two-gamete samples are the most generally useful, the method is outlined for that sample size.

If a particular chromosome arm, such as *2L*, may contain any one of the four gene sequences *A*, *B*, *C* or *D*, with frequencies *p*, *q*, *r* and *s*, respectively (where $p + q + r + s = 1$), then the expected frequency of two-gamete samples (each from a single fly), containing two *A* chromosome arms is:

$$p^2 + (2pq + 2pr + 2ps)/4 = p^2 + p(q + r + s)/2 = (p^2 + p)/2.$$

Similarly, two-gamete samples with two *B* arms would have an expected frequency of $(q^2 + q)/2$, etc.

Two-gamete samples containing say, an *A* and a *B* chromosome arm would have an expected frequency of $2pq \times 1/2 = pq$.

Whenever such predictions of the frequencies of two-gamete samples were made, the observed chromosome arm frequencies (*p*, *q*, *r* and *s*) were based on the two-gamete samples themselves.

Significant deviations from such expected gamete sample frequencies would then indicate a deviation from expectation of the karyotype frequencies of the wild flies. For example, a significant observed excess over expectation of the mixed types of gamete samples, $A + B$, $A + C$, etc., would indicate an excess of heterozygous individuals in the population.

Collection Sites

Webster Groves, Missouri: An area in a residential suburb of St. Louis with large yards, often separated by hedgerows, and generally well shaded by oaks, maples, and a variety of evergreen trees. The warm-weather collecting site was a shaded area under Pin Oaks, kept well-watered during dry periods, and clearly having some of the ecological characteristics of a woods, since 32 species of Drosophilidae were captured there, including nearly all of the species recorded from rural Missouri forests. This more or less linear site was sampled by 18–21 traps hanging from tree limbs. In this area residential garbage is either run through disposal units or packaged and collected; no local garbage cans could be located. The winter collections in this region were made from a grocery store a half mile distant where small numbers of *D. melanogaster*, *D. simulans* and *D. hydei* could often be found on fresh onions, although no flies were breeding in the store trash cans.

In the remaining sites listed below, collections were made from garbage cans or windfall citrus fruit and the composition of the collections was markedly different, with 95%–100% of the collected flies consisting of *D. melanogaster* and *D. simulans*, and with normal woods species generally completely absent.

Collections made in Louisiana and Mississippi in November 1973 were all from garbage cans at roadside rest stops, unless otherwise indicated. The sites were: Louisiana: Highway 80, Tallulah; Interstate-20, Waverly. Mississippi: Interstate-55, Grenada, Norfield, Durant, Canton, Hernando, Wesson, Pope, McComb (grocery store trash cans), and Brookhaven (Holiday Inn trash cans).

Collections from the following south Texas sites were made on March 18–19, 1973. Sites followed by the letter "O" were orange groves; those followed by the letter "G" were grapefruit groves.

Donna: Near Donna, Texas, near Rte. 83 and F.M. 493. Donna-O 500 m. from Donna-G.

Mission: Mission-G ¼ mile south of Rte. 83 on F.M. 492 near Mission, Tex. Mission-O 5600 m. south of Mission-O.

Bass Road: Rte. 83, ½ mile east of Bass Rd. exit F.M. 800, Texas. Bass Road-O 18 m. from Bass Road-G.

La Feria: Rte. 83 west of La Feria, Texas near F.M. 2556. La Feria-G 300 m. from La Feria-O.

The four sites above are near Brownsville, Texas.

S.E. Zapata: Roadside picnic areas ½ mile south of Zapata, Tex. Flies were collected from discarded bananas and oranges on ground near garbage cans. Flies were not breeding on fruit nor in cans, and were apparently drawn from the local resident population. Roadside trash cans along the Laredo-Brownsville axis generally contained few Drosophila as they were equipped with frequently changed plastic liners, leaving the cans themselves appallingly clean.

S.W. Zapata: Trailer camp three miles southwest of Zapata, Texas near the shore of Falcon Lake. Adults were from recently emptied garbage cans which contained larvae and pupae in the garbage residue.

Rio Grande: Residential garbage cans in Fort Ringgold, Rio Grande City, Texas. Larvae and pupae were in garbage residue of cans.

RESULTS

1. *The population frequencies of inversions*

In the present study over 1,000 *X* chromosomes and 3,000 major autosomes were examined from natural populations. In the account below a simplified alphabetical nomenclature is used for each chromosome arm. Where particular cosmopolitan inversions have been described elsewhere the more generally used symbols are also given.

In addition to the six common cosmopolitan autosomal paracentrics, 52 new inversions were found, most or all of them rare endemics. Of this latter group three were autosomal pericentrics; among the paracentrics there were three *X*-chromosomal, 21 on the second chromosome, and 25 on the third chromosome. In the list below the approximate breakpoints are given in terms of Bridges' drawn salivary maps. The approximate nature of the breakpoint designations reflects difficulty in interpreting the drawn maps, a difficulty which was greatly reduced by the use of copies of comparison photographic maps prepared by DR. GEORGE LEFEVRE, and kindly made available to the author. New photographic maps showing the breakpoints of the present series of inversions will be published in the near future. The frequencies and distributions of the commoner inversions are given in Table 1. In the list below frequencies are given only for those inversions which were found more than once in a given locality, and which are not included in the table.

D. melanogaster differs from many other Drosophila species previously studied in the almost complete absence of overlapping inversions occurring on the same chromosome in natural populations. In the present study only one example of such an overlap was found, the rare endemic inversion *2R-J*, which was found on the same chromosome with the overlapping *2R-A* (Nova Scotia) in Tallulah, Louisiana. In all of the other 57 inversions, both breakpoints had occurred in Standard-sequence chromosome sections.

TABLE 1

Frequencies of autosomal gene sequences (excluding rare endemics)

Locality	N	2L		2R				3L			3R			
		+	B^1	+	A^2	D	O	+	B^3	L	+	A^4	B^5	D^6
Webster Groves, Mo.														
June ♂♂, ♀♀	228	92.98	4.82	88.16	8.33	0.44	—	89.91	6.58	—	79.38	12.72	3.51	2.19
August ♂♂	164	92.68	6.71	87.27	8.48	—	—	94.55	3.64	—	74.55	15.76	5.45	1.82
Sept.-Oct. ♀♀	120	93.33	6.67	84.17	14.17	—	—	93.33	5.83	—	63.33	28.33	5.00	0.83
Winter ♂♂, ♀♀														
1973–1974	32	96.88	3.13	96.88	3.13	—	—	96.88	3.13	—	71.88	21.88	—	—
Grenada, Miss.	175	90.29	9.71	91.43	8.00	—	—	97.14	2.29	—	76.57	20.57	1.71	—
Pope, Hernando, Miss.	52	86.54	13.46	94.23	5.77	—	—	94.23	5.77	—	84.91	13.21	1.89	—
Divers, Miss.*	30	86.67	13.33	86.67	10.00	—	—	93.33	3.33	—	83.33	16.67	—	—
Waverly, Tallulah, La.	55	92.73	3.64	91.07	3.57	—	—	92.73	5.45	—	88.89	11.11	—	—
S.E. Zapata, Tex.	46	78.26	21.74	76.09	23.91	—	—	69.57	30.43	—	65.22	30.43	4.35	—
S.W. Zapata, Tex.	22	86.36	13.64	72.73	18.18	4.55	—	90.91	9.09	—	50.00	40.91	4.55	—
Rio Grande City, Tex.	52	76.92	23.08	71.15	25.00	—	3.85	67.31	30.77	—	53.85	44.23	1.92	—
La Feria, Tex.	181	80.11	18.78	75.69	22.65	—	1.10	72.22	26.11	1.11	35.56	57.78	6.67	—
Mission, Tex.	110	76.36	23.64	65.45	34.55	—	—	76.58	23.42	—	34.23	58.56	7.21	—
Donna, Tex.	146	86.30	13.70	82.19	16.44	—	1.37	75.34	23.29	0.68	53.42	43.84	1.37	—
Bass Road, Tex.	147	85.03	14.97	84.35	13.61	—	1.36	74.15	25.85	—	41.50	51.70	6.80	—

Figures in the body of the table are percentages. Inversion symbols with a superscript represent cosmopolitan gene sequences and are identified at the bottom of the table.

* Canton, McComb, Brookhaven, Norfield, Durant; Mississippi.

1—In(2L)t; 2—In(2R)NS; 3—In(3L)P; 4—In(3R)P; 5—In(3R)C; 6—In(3R)Mo.

As indicated in Table 1 the frequencies of inversions are generally higher in south Texas than in the more northern localities. This is particularly striking in the third chromosome, where in the right arm the total inversion frequency is approximately 25% in the north, and over 50% in south Texas populations generally.

PERICENTRIC INVERSIONS		
In2(LR)1	*24D/E; 60A*	Webster Groves, Mo.
In2(LR)2	*29D; 45B/C*	La Feria, Tex.
In3(LR)1	*77D; 88B*	Webster Groves, Mo.
PARACENTRIC INVERSIONS		
X-Chromosome		
A	*12A; 18F*	Webster Groves, Mo.
B	*6D; 11A*	Hernando, Miss.
C	*15A; 18B*	La Feria, Tex.
Chromosome 2, left arm		
A	*26A; 33E*	Webster Groves, Mo. Freq. 0.74%
B	*22D/E; 34A*	Cosmopolitan *In(2L)t*. See table.
C	*27E; 31A*	Webster Groves, Mo.
D	*24D; 26F*	Tallulah, La.
E	*31B; 34E*	Grenada, Miss.
F	*25E; 30C*	Grenada, Miss.
G	*30A; 34A*	Tallulah, La.
H	*26A; 29F*	La Feria, Tex.
Chromosome 2, right arm		
A	*52A; 56F*	Cosmopolitan *In(2R)NS* (Nova Scotia). See table.
B	*49B; 60B*	Webster Groves, Mo. Freq. 1.10%
C	*48F/49A; 60E*	Webster Groves, Mo.
D	*49A; 51D*	Webster Groves, Mo.; Zapata, Tex. See table.
E	*45D; 49B*	Webster Groves, Mo. Freq. 0.73%
F	*53C; 55F*	Webster Groves, Mo. Freq. 0.37%
H	*50F; 54B*	Grenada, Miss.
J	*55F; 60E*	Tallulah, La. Found on the same chromosome as the overlapping inversion *2R-A* above.
K	*50A; 54B*	Tallulah, La.
L	*42A; 45B*	Tallulah, La.
M	*44C; 57E*	Brookhaven, Miss.
N	*49F; 51D*	Tallulah, La.
O	*49B; 51D*	From four localities in southern Texas. See table.
P	*43E; 47D*	Zapata, Tex.
Q	*54B; 60F*	Bass Road Locality, Tex.
Chromosome 3, left arm		
A	*60F/61A; 67B*	Webster Groves, Mo. Freq. 0.92%
B	*63C; 72E*	Cosmopolitan *In(3L)P* (Payne). See table.
C	*65A; 73B*	Webster Groves, Mo.
D	*65E; 66E*	Webster Groves, Mo.
E	*66C 71C*	Webster Groves, Mo. Freq. 0.55%
F	*67C/D; 76A*	Webster Groves, Mo.
G	*64B; 68F/69A*	Webster Groves, Mo.
H	*62E; 70C*	Webster Groves, Mo.
J	*61C; 64C*	Tallulah, La.
K	*75C; 76F*	Grenada, Norfield, Miss.

L	*66C; 70F*	La Feria, Donna, Tex. See table.
M	*64C; 66F*	Donna, Tex.
N	*77B; 79E*	Rio Grande City, Tex.
Chromosome 3, right arm		
A	*89C; 96A*	Cosmopolitan *In(3R)P* (Payne). See table.
B	*92E; 100F*	Cosmopolitan *In(3R)C*. See table.
C	*87A; 100F*	Webster Groves, Mo. Freq. 1.28%
D	*93D; 98F*	Cosmopolitan *In(3R)Mo*. See table.
E	*86E; 97C*	Webster Groves, Mo. Freq. 0.73%
F	*86D; 88E/F*	Webster Groves, Mo.
G	*96F; 100B*	Webster Groves, Mo.
H	*90B; 98A*	Grenada, Miss.
J	*92D; 93F*	Grenada, Miss.
K	*86E; 87F*	Grenada, Miss.
L	*84D; 86C*	Tallulah, La.
M	*89A; 99D*	Zapata, Tex.
O	*84E; 87C*	La Feria, Tex.
P	*96C; 98E*	Mission, Tex.
Q	*93D; 98B*	Donna, Tex.
R	*86B; 87F*	Bass Road Locality, Tex.

2. *Gametic aneuploidy and structural heterozygosity in wild females*

It has been demonstrated in *D. melanogaster* (COOPER, ZIMMERING and KRIVSHENKO 1955), in *D. pseudoobscura* (TERZAGHI and KNAPP 1960), and in *D. paramelanica* (STALKER, unpublished, cited in RILES 1965) that females heterozygous for inversions in several chromosomes may produce large numbers of dead embryos due to aneuploidy associated with nondisjunction. In the latter two species inversion polymorphism tends to be concentrated in one or two chromosomes, leading to the suggestion by TERZAGHI and KNAPP that the effective reduction of fertility in multiply heterozygous females might result in these species restricting the incorporation of new inversions to one or a few chromosomes, rather than spreading them over all chromosome pairs. In the case of *D. robusta*, a species in which polymorphism occurs in all chromosomes, RILES (1965) found that there was essentially no effect of multiple inversion heterozygosity in reducing the fertility of females. She suggested that possibility in this species genetic modifiers had arisen which essentially suppressed the effects of inversion heterozygosity in the production of aneuploid gametes.

Natural populations of *D. melanogaster* regularly have inversions in all four major autosomal arms: they rarely occur on the *X* chromosome. The production of aneuploid gametes by females structurally heterozygous in two or more chromosomes would, if it occurred in nature, constitute an important load in some populations, and might be a factor in controlling the total number of inversions carried in heterozygous condition. Since in *D. melanogaster* Standard gene sequences generally have frequencies in excess of fifty percent, this would mean that in this species the effects of gametic aneuploidy would tend to keep the frequency of inverted sequences, and hence the frequency of multiply heterozygous females, low.

Despite the clear results of COOPER, ZIMMERING and KRIVSHENKO (1955) and REDFIELD (1957) on this species, their work involved crosses of laboratory strains of diverse origin, and hence did not duplicate the situation that might arise in a natural population. If, following RILES' suggestion for *D. robusta*, a system of genetic modifiers is available in *D. melanogaster* to suppress production of aneuploid female gametes, populations might be expected to selectively incorporate such modifier systems if they produced enough multiply heterozygous females to make the problem an important one.

In order to study the eggs produced by wild flies, freshly caught inseminated females were allowed to oviposit in the laboratory, the eggs produced being checked for embryonic lethals; afterward the females were despermed with cold, mated to Standard laboratory males, and seven or more F_1 larval squashes were studied to determine the maternal karyotype.

A possible cause of embryonic death in the progeny of wild females is homozygosity for recessive embryonic lethals or semi-lethals. While there is no easy way of recognizing all such cases, a partial correction was made by a program of retesting all females which had produced three percent or more lethals among the eggs fertilized by wild sperm. The second test was made following the desperming of the female and a mating to laboratory Standard males. If the lethality levels observed in the first test were not maintained in the second, it was assumed that recessive lethals or semi-lethals were involved. With few exceptions, the second tests showed lower lethal frequencies than the first, indicating that naturally occurring early-acting recessive lethals were present and functioning in the populations.

In Table 2 the lethal frequency data are presented in two parts. The first (Raw data) gives the frequencies when wild sperm were involved. In the Corrected data the material presented differs only in that for those females that were retested, the data from the retests are substituted for the original Raw data. The correction is incomplete, since only selected females were retested. However it does permit a more realistic estimate of the upper level of lethality associated with various maternal karyotypes. Data for karyotype classes in which none of the females required retesting are simply presented as Corrected data.

The wild females produced some unfertilized eggs; these could be distinguished by color from those containing dead embryos. Unfertilized eggs remain white for many days after being laid, and dissection of many such eggs failed to show any signs of embryonic development. Eggs containing dead embryos turn brown in three to five days after oviposition; dissection of such brown eggs always gave evidence of development. The other class of eggs which may become a light tan color with age are those which are obviously defective at the time of oviposition. Such eggs are undersized, lack normal turgor and chorionic shine, and may have abnormal, short filaments. They are generally produced by females that have temporarily ceased laying and are starting in again. Those defective eggs that become brown are easily distinguished from the embryonic lethal group.

The wild females used in these tests came from one of the Webster Groves collections (57 females), and from all eleven Texas populations (69 females).

TABLE 2

Embryonic lethality and maternal karyotype

Heterozygous autosomal arms	Females tested	Raw data		Corrected data*	
		Fertile eggs	Percent lethal	Fertile eggs	Percent lethal
A. Webster Groves, Mo. Sept.–Oct. 1973					
None	12	1,326	0.82 ± 0.34	1,354	0.58 ± 0.22
One	27	2,810	1.45 ± 0.71	2,874	0.72 ± 0.17
Two (1 chrom.)	4	471	1.12 ± 0.85	493	0.59 ± 0.37
Two (2 chrom.)	12			1,341	0.44 ± 0.25
Three	2			208	0.98 ± 0.98
B. Eleven Texas Populations					
None	4			443	0.67 ± 0.42
One	23	2,589	2.17 ± 1.00	2,630	0.84 ± 0.20
Two (1 chrom.)	11	1,154	2.04 ± 1.13	1,226	1.01 ± 0.22
Two (2 chrom.)	20	2,215	0.70 ± 0.20	2,223	0.61 ± 0.15
Three	7	768	1.97 ± 0.97	815	1.11 ± 0.34
Four	4	391	10.06 ± 5.43	429	10.16 ± 5.04
Four	1	75	24.00	104	24.04
	1	114	13.16	120	9.17
	1	97	3.09	108	7.41
	1	105	0	97	0

* All females showing 3% or more lethals from wild sperm were retested following mating to Standard males. Corrected data column reflects replacement of original data for these females by results of second tests. Categories not listed under Raw data contained no females showing 3% or more lethals.

In the Webster Groves collection (Table 2A) no females were found with all four major autosomal arms structurally heterozygous. Among the groups of females with lesser numbers of heterozygous arms, none of the corrected mean lethal frequencies exceeded one percent, and there is no obvious relationship between the lethal frequencies and the maternal karyotype. In the Texas material (Table 2B) again no females with three or fewer inverted arms showed any marked degree of lethality, and the mean frequencies for each group are not related in any obvious way with the maternal karyotype. However in the four females heterozygous in all four arms the raw and corrected data show mean lethal frequencies around 10%. The four individual female frequencies are shown at the bottom of the table, and it will be noted that there is wide individual variation, with frequencies ranging from 0% to 24% lethality. In these four cases the retest frequencies were comparable to those of the original tests, indicating that recessive lethals were probably not involved, and that the high lethal frequencies in the first two and possibly the first three individuals were associated with their karyotypic peculiarities. All four females were heterozygous for the common inversions: *2L-B*, *2R-A*, *3L-B* and *3R-A*. Outside of this group of four, none of the 126 females tested showed a lethal frequency (which persisted in the retest) of more than three percent. Thus it may be concluded that heterozygosity in all four arms may be associated

with high lethal frequencies, as suggested by the work of COOPER, ZIMMERING and KRIVSHENKO (1955) and of REDFIELD (1957), but that in natural populations the deleterious effects of multiple heterozygosity may be suppressed, presumably by some genetic modifying system such as that suggested by RILES (1965).

3. *Relationship of insemination level to karyotype of wild females*

At the time of capture wild females were placed in an ice box under crowded conditions so that little or no oviposition occurred. In the laboratory, females from three of the collections were placed in separate food vials at approximately 24° and the food was changed every two days until sufficient unfertilized eggs had been produced to indicate sperm exhaustion. These females were then crossed to Standard males and seven or eight F_1 squashes were analyzed from each to determine the karyotypes. The total progeny produced by each female prior to sperm exhaustion was used as a measure of her sperm load, and compared with her karyotype.

Table 3 summarizes the results of these comparisons. In this table females are grouped according to the number of major autosomal arms structurally heterozygous. Student t tests are used in comparing the mean progeny of various groups except in those cases where the group variances differ significantly. In the latter cases t′ values were used and the levels of significance determined following the method described by SOKAL and ROHLF (1969). In the body of the table the numerical values of t′ are followed by the prime superscript.

The June sample from Webster Groves (Table 3 A) shows no obvious systematic relationship between karyotype and total sperm load. In the case of the September-October sample from the same locality (Table 3 B), there is a clear trend, with larger numbers of structurally heterozygous arms associated with larger sperm loads. This trend is significant at the two percent level; homozygous females or those with one heterozygous arm carry significantly less sperm than those with two heterozygous arms, and homozygous females carry significantly less sperm than those with two heterozygous arms.

In the sample from Grenada, Mississippi (Table 3 C) the trend is the same as in part B above, with a positive association between sperm load and number of structurally heterozygous arms. While the Grenada data taken by themselves do not show a significant association (since the critical P values are slightly below 0.09), considered along with the more significant association in the second Webster Groves collection, they are suggestive.

Somewhat similar results have been obtained in a number of populations of *D. euronotus* (STALKER, unpublished) in which females heterozygous at a sex-linked acid phosphatase locus tend to carry more sperm than the corresponding homozygotes, despite the fact that in that species no *X*-chromosome inversions are involved.

In the laboratory, females of *D. melanogaster* which are well supplied with sperm produce more eggs per day, and a higher proportion of fertilized eggs, than do females with a depleted sperm supply. The rapid production of fertile eggs and the avoidance of the wasteful production of sterile eggs are reproductive

TABLE 3

Comparisons of structural heterozygosity in wild females with total progeny produced prior to sperm exhaustion

A. Webster Groves, Mo. June, 1973

	No. arms heterozygous	Females tested	Total progeny $\bar{x}$ $s_{\bar{x}}$
(*a*)	0	17	361.4 ± 31.67
(*b*)	1	17	296.6 ± 28.22
(*a*+*b*)	0 or 1	34	329.0 ± 21.63
(*c*)	2 or 3	10	361.0 ± 31.54

Comparison	t	d.f.	P
(*a*) *vs.* (*b*)	1.527	32	0.14
(*b*) *vs.* (*c*)	1.460	25	0.16
(*a*+*b*) *vs.* (*c*)	0.736	42	0.47
(*b*) *vs.* (*a*+*c*)	1.778	42	0.08

B. Webster Groves, Mo. September–October, 1973

	No. arms heterozygous	Females tested	Total progeny $\bar{x}$ $s_{\bar{x}}$
(*a*)	0	13	335.4 ± 30.72
(*b*)	1	26	361.7 ± 25.83
(*a*+*b*)	0 or 1	39	352.9 ± 19.90
(*c*)	2	16	422.8 ± 18.02

Comparison	t or t′	d.f.	P
(*a*) *vs.* (*b*)	0.617	37	0.54
(*b*) *vs.* (*c*)	1.940′	40	0.08
(*a*+*b*) *vs.* (*c*)	2.605′	53	0.02
(*a*) *vs.* (*c*)	2.564	27	0.016

C. Grenada, Miss. November, 1973

	No. arms heterozygous	Females tested	Total progeny $\bar{x}$ $s_{\bar{x}}$
(*a*)	0	16	193.75 ± 26.89
(*b*)	1	20	250.45 ± 15.06
(*c*)	2 or 3	5	239.60 ± 15.76
(*b*+*c*)	1, 2 or 3	25	248.28 ± 12.36

Comparison	t or t′	d.f.	P
(*a*) *vs.* (*b*)	1.840′	34	0.09
(*a*) *vs.* (*b*+*c*)	1.843′	39	0.09
(*b*) *vs.* (*c*)	0.345	23	0.73

t values marked with a prime superscript are t′ values calculated for cases in which sample variances differ significantly. See text.

advantages which are presumably important in nature as well as in the laboratory. Heterozygous females in small or scattered populations, where mating contacts are limited, would be expected to have a decided advantage over the homozygotes, and this heterozygote advantage would of course tend to preserve the structural or genic polymorphism.

The mechanisms by which heterozygotes maintain an adequate sperm supply are presently unknown. The conjecture that they might involve a lowered fecundity, and thus a simple failure to use up available sperm, can probably be rejected because of the positive association between egg production and sperm supply. There may be a greater sexual receptivity or attractiveness on the part of such females (possibly associated with increased vigor), resulting in more matings, and thus the transmission of more sperm per mating. Finally, heterozygous females may preserve sperm more effectively, due to physiological characteristics of the sperm receptable.

Laboratory studies on *D. robusta* carried out by PRAKASH (1967, 1969) showed an association between mating speed and structural heterozygosity in both males and females. These results differed from those of SPIESS (1970) and KAUL and PARSONS (1965) in *D. pseudoobscura*; in that species, while structural heterozygosity was associated with increased mating activity in males, this was rarely the case for females. Laboratory studies of *D. melanogaster* carried out by WATANABE and WATANABE (1973) showed an increased productivity in females heterozygous for the inversion *In(2L)B* probably *2L-B*, *In(2L)t* in the present paper), but no increased productivity for heterozygous males. In that study "productivity" included both mating success, and in the case of females, fecundity. PARSONS (1965) points out that differences between strains of identical gene arrangements may be so great that genetic backgrounds may give contrasting results from separate laboratories, even though all strains may have originated from the same natural population and locality. Clearly, with mating ability under such complex genetic control, it is difficult to apply laboratory results to nature, and more direct study of natural populations is badly needed, despite the obvious technical difficulties.

4. *Relationship between karyotype and food niche*

Eight collections of flies breeding on windfall Red Blush grapefruit and Valencia oranges were made at four localities in Texas. In most of the Texas groves observed by the author grapefruit and oranges were grown in separate but immediately adjacent areas. Damaged and fermenting windfall fruits were abundant, and usually contained breeding *D. melanogaster* and *D. simulans*. Adults were readily collected in quantity as they poured out of the holes of the disturbed fruits. Each collection, whether from grapefruit or oranges, consisted of a mixture of flies taken from at least four sites within an area approximately ten meters square.

Flies breeding on the two types of fruit showed a tendency to differ in the frequency of Standard chromosome arms, the Standard frequency being higher in the collections from oranges. This tendency was most consistent in the autosomal arms *2L*, *3L* and *3R*. The final comparisons shown in Table 4 are based on the total of these arms for each collection, and the numbers of Standard and inverted arms found in that total. Although not shown in the table, similar results were obtained from a consideration of all four major autosomal arms, from arms *2L* and *3R* taken together, and from chromosome *3* considered by

TABLE 4

Comparisons of frequencies of Standard and inverted arms 2L, 3L *and* 3R *in flies breeding on Red Blush grapefruit and Valencia oranges in four Texas localities*

Locality	Food	Distance	*N*	Standard %
A.				
Bass Road	Grapefruit	18 m.	150	52.0
	Orange		291	58.1
La Feria	Grapefruit	300 m.	322	50.3
	Orange		219	50.7
Donna	Grapefruit	500 m.	192	56.3
	Orange		246	70.7
Mission	Grapefruit	5600 m.	114	46.5
	Orange		218	49.1

	All 4 localities			First 3 localities		
Statistical comparisons	d.f.	χ^2	P	d.f.	χ^2	P
B.						
Food *vs.* localities	3	83.040	<0.001	2	65.664	<0.001
Chrom. *vs.* localities	3	26.465	<0.001	2	19.142	<0.001
Food *vs.* chromosomes	1	6.405	0.011	1	8.402	0.004
Interaction	3	5.079	0.166	2	3.786	0.151
Total	10	120.989	<0.001	7	96.993	<0.001

Distances given in meters are between orange and grapefruit collections in each locality. *N* refers to sum of *2L*, *3L* and *3R* arms in each collection.

itself. As shown in Table 4B the interaction χ^2 values are not significant, and accordingly the association χ^2 from the pooled data was used as a test of significance of the food-chromosome relationship. Under the heading "All 4 localities" it is seen that for the four localities taken together, the food-chromosome association is significantly non-random ($P = 0.011$). The inclusion of the Mission locality in this test is questionable, however, because of the 5600 meter distance between the orange and grapefruit collections. If this locality is excluded, and only the first three localities are considered, the food-chromosome association remains significantly non-random ($P = 0.004$). Despite the highly significant non-randomness, and despite the fact that all four localities are consistent in showing a greater frequency of Standard arms in the orange collections, these results must be viewed with some caution since most of the observed frequency difference occurs in the Donna locality. More data are needed, and the conclusion that there is a consistent karyotype difference in flies breeding on the two types of food must be regarded as preliminary.

The author is unaware of other published data in Drosophila showing a specific association of this sort.

5. *The composition of the sperm carried in wild females*

One larva was analyzed from each of 102 wild-inseminated females from two of the Webster Groves collections. Following analyses of the F_1 larvae, and of the karyotypes of the females which produced them, inversion frequencies among the

sperm were determined by the method outlined in MATERIALS AND METHODS. Such sperm frequency data may be used in two ways: first, to determine whether the chromosome types found in the wild males in the population are proportionately represented in the sperm which is actually transmitted to the females; secondly, to determine whether assortative mating is taking place.

Both sperm and males were sampled only in the June 1973 Webster Groves collection and the Grenada, Mississippi collection. In these collections no significant inversion frequency differences existed between wild males and the sperms carried in wild females.

In order to reduce and simplify the number of different classes of sperm-to-female comparisons, the largest class of females, those homozygous in all four autosomal arms (26/29 were homozygous Standard) were classified for the sperm which they carried. They were said to be "identical" if the one sperm analyzed carried the same gene sequences as the female in all four arms, and "different" if the sperm and the female carrier differed by inversions in one or more arms. The determination of the expected frequency of the various sperm types was in each case based on the arm frequencies found in the total sperm sample from that collection and the necessary assumption that the various gene sequences in the four autosomal arms were associated at random within the sperm. Evidence supporting this last assumption is summarized in the APPENDIX.

In Table 5 the numbers of homozygous wild females carrying identical and different sperm are given for each collection along with the expected numbers based on the assumption of random association of females and the sperm they carried. In both collections there is an observed excess of females carrying structurally identical sperms. Since the heterogeneity χ^2 is not significant, the

TABLE 5

Observed and expected frequencies of homozygous females carrying genetically identical wild sperm

Population	No. wild females	Sperm		χ^2
		Ident.	Diff.	
Webster Groves	17	15	2	3.2925
June, 1973		*11.50*	*5.50*	
Webster Groves	12	8	4	2.8892
Sept.–Oct. 1973		*5.09*	*6.91*	
Total	29	23	6	6.1817
		16.59	*12.41*	

	χ^2	d.f.	P
Total	6.1817	2	0.045
Pooled	5.7876	1	0.016
Heterog.	0.3941	1	0.530

Expected numbers are italicized. See text.

data from the two collections are pooled, yielding a highly significant χ^2 ($P = 0.016$) and indicating significant positive assortative mating.

One consequence of positive assortative mating might be an observed excess of homozygous F_1 larvae. The data on the larvae are presented in Table 6 for all 102 cases. The larvae are divided into two classes, those homozygous in all four major autosomal arms, and those structurally heterozygous in one or more arms. The expected frequency of homozygous larvae cannot be safely estimated on the basis of their own inversion frequencies, since if this were done, and a frequency difference existed in fact between eggs and sperm, the expected frequencies of homozygous larvae would be overestimated. Accordingly the expected frequencies of homozygous F_1 larvae were calculated for each arm by multiplication of the female inversion frequencies and the corresponding sperm frequencies, and the expected frequencies of wholly homozygous larvae calculated as the product of the four single arm frequencies. As expected, the collections show an observed excess of homozygous F_1 larvae; the pooled data yield a suggestive χ^2 value ($P = 0.055$).

The positive assortative mating observed in both of the Webster Groves collections sampled probably involves mating between close relatives. In this species it may be associated with markedly precocious sexual maturity and limited vagility of young adults.

A small but comparable survey made of sperm from 40 wild females in the Grenada, Mississippi collection gave no indication of positive assortative mating. On the basis of random mating 15 females homozygous in all arms were expected to carry identical sperm in 7.6 cases; in fact 7 females did carry identical sperm. Among the 40 F_1 larvae from wild sperm 15.1 were expected to be homozygous

TABLE 6

Observed and expected frequencies of completely homozygous F_1 larvae from wild females and wild sperm; one larva per wild female

Population	No. F_1 larvae	Homozygous	Heterozygous	χ^2
Webster Groves		26	21	
	47			2.7363
June, 1973		*20.38*	*26.62*	
Webster Groves		18	37	
	55			1.2046
Sept.–Oct. 1973		*14.42*	*40.48*	
Total	102	44	58	3.9409
		34.80	*67.20*	

	χ^2	d.f.	P
Total	3.9409	2	0.139
Pooled	3.6917	1	0.055
Heterog.	0.2492	1	0.618

Expected numbers are italicized. See text.

in all arms if mating were at random, and 17 were in fact homozygous. While such a small study certainly does not disprove assortative mating, it does suggest that if it occurs here it is not comparable to that found in the Webster Groves populations. A possible reason may be the ecological conditions. The Grenada collection came from four loosely closed garbage cans that were in the shade most of the day. Each contained an estimated 300 to 400 *D. melanogaster* adults which tended not to leave the cans, since jars of highly attractive bait set around them attracted no flies, while opening and kicking the cans vigorously resulted in clouds of flies rising up and immediately returning to the cans. Under such crowded conditions it might be expected that sexual precocity and limited vagility would be of little significance in promoting matings between close relatives, but rather, that young females would be mated by any available mature male, whether closely related or not.

It is of interest to compare these results with those obtained by WALLACE (1970), in experiments in which homozygous cultures of wild-type and sepia (*se/se*) were exposed in a yard in separate but closely adjacent sites, and observations made on dispersion of the two types of flies and the subsequent mating patterns. In these studies it was found that there was a marked tendency for emerging flies to stay in the immediate area of origin, rather than to migrate to adjacent culture vials. It is of course possible that this limited vagility may have been due in part to the fact that the yard was apparently not a suitable site for *D. melanogaster*, as indicated by their rarity in general collections. The observed limited dispersion of adults might have been expected to result in the production of an excess of wild-type or *se/se* homozygotes. In fact an observed excess of heterozygotes was observed. WALLACE suggested that the few migrant individuals, possibly due to their relative rarity in their new sites, may have benefited from the "minority effect" (EHRMAN 1966), and thus been more effective as mates than the non-migrant resident flies.

It is possible that the results presented in this paper differ from those of WALLACE for the following reasons. (1) In the Missouri population all individuals were closely related; in WALLACE's Bryant Park experiments the three strains of flies used came from widely distant localities. Thus migrants in his experiments were probably more recognizably distinct than those in the Missouri population, and more likely to achieve mating success because of their rarity. (2) Twice-daily collections of flies in the Missouri population would be likely to result in capture of young females that had mated prior to migration, and had had no opportunity to remate with more distantly related individuals. Of course in the Missouri populations all studied individuals were migrants (to the traps); whether this migration resulted in mixing of distantly related individuals is not known, but since little mating occurred in the traps, this would have had little effect on the experiments.

6. *The distribution of inversions among individuals within populations*

In this species, populations are regularly polymorphic for inversions in all four major autosomal arms, although with few exceptions the Standard sequence in

each arm has a frequency above fifty percent. A balancing mechanism for this polymorphism might involve a selective elimination of males and females which either carried no or few inversions, or which carried large numbers of inversions; such selection could be related to reduced viability, reduced fertility, or both. If in fact the extremes in the possible range of 0 to 8 inverted autosomal arms per fly are relatively non-adaptive, then one might expect to find a non-random distribution of flies carrying various numbers of inversions, since inviability would directly eliminate such extreme individuals, while partial sterility of such flies would result in the production of fewer progeny which, like the parents, carried very few or very many inverted arms.

It can be readily shown that for each of the 100 possible autosomal karyotypes, the total number of inversions carried by the wild fly is identical to the mean number of inversions in the two-gamete samples it will produce; this is true regardless of any linkage relationships which may exist. Thus, non-random distribution of inversions among the two-gamete samples reflects a similar non-randomness among the flies producing these samples.

Tests of non-random distributions of total inversion number were carried out in two ways. In three of the four Missouri collections, "completely" analyzed flies (seven or more gametes per fly) were used, and the number of inversions carried in such flies compared with an expectation based on a random distribution of inversions in each collection. In one of the Missouri collections, in the collection from Grenada, Mississippi, and in all eleven Texas collections, two-gamete samples from each wild fly were treated as the observed data.

Tests of the four Missouri collections and the one from Grenada, Mississippi, showed no significant deviation between observed and expected inversion distributions. Since the overall inversion frequencies in these populations were low, the tests were rather inefficient, and the results somewhat inconclusive. In the case of the Texas collections, the inversion frequencies were considerably higher, and the results more interesting.

The tests of the Texas populations are summarized in Table 7A. It will be noted that the pooled data from the eleven populations show a non-random distribution of inversions among two-gamete samples, with an observed excess of samples with one, two or three inversions, and a deficiency of samples with none, or more than three inversions. Tests of significance are shown in Table 7B. For such tests the nine possible kinds of two-gamete samples are combined into two groups; one group with one, two or three inversions, the other with samples containing none or more than three inversions. The expected sample frequencies in each collection were based on the observed frequencies of inverted arms in the two-gamete samples, and on the assumption of random association for all inverted arms. Goodness of fit x^2 values were calculated for each collection, and from these, positive or negative x values (square roots of x^2) were obtained. The x values were arbitrarily listed as positive for collections showing an *excess* of one, two, or three inversion samples, and *vice versa* for the negative x values. The algebraic sum of the eleven x values was divided by the square root of the eleven degrees of freedom to yield the standardized normal deviate 2.76, corresponding to a two-

TABLE 7

Observed and expected frequencies of two-gamete samples carrying 0 to 8 inversions per sample

A. Pooled data from all populations

	Number of inversions per sample								
	0	1	2	3	4	5	6	7	8
Obs.	35	63	132	73	61	9	9	0	0
Exp.	50.63	53.88	125.28	63.25	61.82	15.35	10.28	0.92	0.58
Diff.	—	+	+	+	—	—	—	—	—

B. Population comparisons for lumped inversion classes

Population	Inversions per sample		χ^2	χ
	0, 4 . . . 8	1, 2, 3		
La Feria-O	10	31	1.2721	+ 1.1279
	13.39	*27.61*		
La Feria-G	22	38	0.1947	+ 0.4413
	23.67	*36.33*		
Mission-O	18	21	0.5912	— 0.7689
	15.65	*23.35*		
Mission-G	6	14	0.7949	+ 0.8916
	7.95	*12.05*		
Donna-O	13	31	0.9047	+ 0.9512
	16.04	*27.96*		
Donna-G	4	30	9.4549	+ 3.0749
	12.67	*21.33*		
Bass Road-O	15	39	1.0598	+ 1.0295
	18.59	*35.41*		
Bass Road-G	5	22	1.4188	+ 1.1911
	7.81	*19.19*		
S. E. Zapata	5	19	2.5407	+ 1.5940
	8.76	*15.24*		
S. W. Zapata	4	7	0.0042	— 0.0651
	3.90	*7.10*		
Rio Grande	12	16	0.1038	— 0.3222
	11.17	*16.83*		
Totals:	114	268		+ 9.1453
	139.60	*242.40*	$9.1453/\sqrt{11} = 2.76$	P = 0.0058

Based on 11 Texas populations; ♂ ♂ and ♀ ♀ combined. Expected frequencies are italicized.

tailed P value of 0.0058 (see SIMPSON, ROE and LEWONTIN 1960). The result indicates a significantly non-random distribution of inverted arms among the eleven populations and suggests a lowered adaptive value for flies with very few or very many inversions.

While the χ value from the Donna-G population contributes heavily to the $\Sigma\chi$, the heterogeneity χ^2 for the eleven populations is only 10.95, with 10 d.f. and a P value of 0.36. The χ^2 data for the pooled data is 7.39, with 1 d.f. and a P value of 0.0066, a value very close to that obtained from the standardized normal deviate.

7. Association of numbers of inversions within chromosome pairs

The non-randomly distributed inversions in the Texas populations are located on the second and third chromosomes. If the two pairs of autosomes are considered individually, will a non-randomness for inversion number be found within the individual chromosome pairs?

Again using two-gamete samples, the observed distribution of inversions among samples was compared with that expected on the basis of random distribution; in this case the comparisons were carried out individually for each chromosome pair. The results of the comparisons are summarized in Table 8. The statistical tests are similar to those presented in Table 7B, involving a calculation of χ^2 and signed χ values for each population. While both second and third chromosomes show an observed excess of two-gamete samples with one or two inversions, and a deficiency of the other three classes, in neither chromosomes is this deviation significant. Thus if intrachromosomal non-randomness exists, it is as yet unproven.

8. Association of numbers of inversions among chromosome pairs

If flies carrying inversions in the second pair of autosomes tended to have very few or none in the third pair, and *vice versa*—i.e. if the numbers of inversions found in the two pairs of autosomes in a given fly tended to be negatively correlated—then this should result in an observed reduction in the frequencies of flies carrying very few or very many inversions. In testing this possibility, the two-gamete samples from each fly were classified into four groups (Table 9), (a) with

TABLE 8

Comparisons of observed and expected two-gamete samples carrying 0–4 inversions per chromosome pair

A.

Complete distributions	Inversions per sample 0	1	2	3	4
Chromosome *2*	207	82	84	3	6
	213.04	*73.58*	*83.76*	*4.57*	*7.04*
Chromosome *3*	81	68	185	24	24
	88.28	*65.97*	*170.73*	*27.53*	*29.50*

B.

Grouped samples	Inversions per sample 0, 3, 4	1, 2	$\Sigma\chi$*	d.f.	P*
Chromosome *2*	216	166	+ 3.3041	11	0.317
	224.66	*157.34*			
Chromosome *3*	129	253	+ 5.1751	11	0.119
	145.30	*236.70*			

Based on combined ♂♂ and ♀♀ from 11 Texas populations. Figures shown are sums of 11 individual comparisons for each chromosome. Expected frequencies are italicized.

* The sum of 11 Chi values; each χ value positive when samples in the 1, 2 group exceeded expectation. $\Sigma\chi/\sqrt{11}$ yields normal deviate used to determine two-tailed P value.

TABLE 9

Comparisons of numbers of inversions carried in chromosome 2 with those carried in chromosome 3

	Inversions per sample						
Chrom. *2* Chrom. *3*	0 0 (*a*)	1–4 0 (*b*)	0 1–4 (*c*)	1–4 1–4 (*d*)	χ^2	χ	*D* freq. (*ad* — *bc*)
La Feria-O	2	17	3	19	0.0921	— 0.3035	— 0.0077
La Feria-G	3	28	7	22	2.2558	— 1.5019	— 0.0361
Mission-O	4	13	4	18	0.1682	+ 0.4101	+ 0.0132
Mission-G	0	9	4	7	4.0909	— 2.0226	— 0.0900
Donna-O	11	21	4	8	0.0042	+ 0.0649	+ 0.0021
Donna-G	1	18	1	14	0.0298	— 0.1727	— 0.0035
Bass Road O	4	29	5	16	1.2623	— 1.1235	— 0.0278
Bass Road-G	2	18	2	5	1.4171	— 1.1904	— 0.0357
S. E. Zapata	2	8	8	6	3.3110	— 1.8196	— 0.0903
S. W. Zapata	3	3	1	4	1.0607	+ 0.0299	+ 0.0744
Rio Grande	3	8	7	10	0.5623	— 0.7499	— 0.0332
Totals	35	172	46	129		$\Sigma\chi = -7.3792$	$\overline{D} = -0.0213$
						$7.3792/\sqrt{11} = 2.33$	$P = 0.0198$

Two gamete samples; ♂ ♂ and ♀ ♀ combined; data from 11 Texas populations.

no inversions in either pair of chromosomes, (b) with one or more inversions in chromosome *2* and none in chromosome *3*, (c) with no inversions in the second, and one or more in the third chromosome, and (d) with inversions in both chromosome pairs. A negative correlation between the two pairs of chromosomes would be indicated when the product of the (b) and (c) frequencies exceeded that of the (a) and (d) frequencies. This frequency-product difference is listed as a disequilibrium coefficient *D*, in the last column of the table. Tests of significance involved calculation of 2×2 contingency χ^2 and χ values. The latter were arbitrarily listed as negative for those populations showing a deficiency of classes (a) and (d), i.e. when $bc > ad$. The algebraic sum of the χ values divided by the square root of the degrees of freedom yields the standardized normal deviate 2.33, which is associated with a two-tailed P value of 0.0198. These results indicate that in the Texas populations flies tend to have inversions either on the second or third pairs of autosomes, with an observed deficiency of flies either carrying no inversions, or having inversions in both pairs of autosomes. Although numerous cases of inversion *linkage* disequilibrium are known in Drosophila (e.g. LEVITAN 1958; LEVITAN and SALZANO 1959; STALKER 1960, 1961, 1964) and in other flies such as Chironomus (MARTIN 1965), the above type of interaction between *unlinked* inversion systems is apparently unknown in any other dipteran.

A somewhat similar non-random association of unlinked inversions has been reported in the grasshopper *Moraba scurra* (LEWONTIN and WHITE 1960). In this latter case the authors were able to demonstrate a relationship between the inversions involved and the size and viability of the organism.

If, as it appears from the observations above, flies showing a non-random association between inversion numbers in the two pairs of autosomes are adaptively superior in some way, then this would result in a recombinational load for the populations involved. A similar non-random association which was less costly to the population might be achieved if individual populations tended to go one way or the other, i.e. have high inversion frequencies on the second chromosome, and low frequencies on the third, or the reverse. In this case the populations themselves might be expected to show a negative correlation for the mean frequencies of inversions carried on the second and third chromosome pairs.

To check this possibility two-gamete samples from the eleven Texas populations were compared for the average numbers of inversions carried in the second and in the third chromosomes. The data are inappropriate for testing by Pearson's product-moment correlation coefficient, r, and instead Spearman's rank correlation coefficient, r_s, was calculated for the eleven population samples. Although the positive sign of the derived coefficient ($r_s = +0.2364$) suggests a positive correlation (i.e. populations with many inversions on the second chromosome tending to have many on the third as well), the coefficient is not significant ($P > 0.20$). Thus it may be concluded that the negative correlation of numbers of inversions in the two pairs of chromosomes is an intra-population but not an inter-population phenomenon.

The failure to find negative correlation among populations might be explained in two ways. One, there is sufficient inter-population migration to prevent local differentiation. Two, the second and third chromosome inversions are not interchangeable, and the presence of both types confers adative advantages to the population which outweigh the associated recombinational load.

DISCUSSION

Although the adaptive significance of the non-random distribution of inversions is not clear, some preliminary suggestions might be made. The presence of inversions, aside from conferring certain general heterotic advantages (WATANABE and WATANABE 1973), seems to involve a more specific advantage in this species, since it was shown in one of the two collections tested from Missouri that there was a very clear positive association between structural chromosome heterozygosity and the amount of sperm carried by wild-caught females. So far it has not been possible to test Texas populations for this relationship. Since the number of heterozygous arms is maximal for flies carrying a total of four autosomal inversions, the overall advantages of inversion heterozygosity should of themselves tend to cause selective clumping around the four-inversion level, i.e. when half of the eight major autosomal arms carry inversions. However, it must be recalled that the presumed heterotic advantages of the four-inversion class of flies may be tempered by the fact that some flies of this sort are females with inversion heterozygosity in all four arms, and such females, as shown above, are liable to high levels of sterility due to nondisjunction and production of aneuploid lethal zygotes.

The discussion above has dealt primarily with the Texas populations. Clearly there must be specific disadvantages associated with inversions not considered above, since the Missouri, Mississippi and Louisiana populations generally show low inversion frequencies, and in such populations sterility of females heterozygous in all four arms can be of little significance because of their low frequencies. The special adaptive value of the Standard sequences in these natural populations is not presently understood.

In summary, special features demonstrated in wild populations of *D. melanogaster* include: non-random mating; association of inversions with specific (food) niches; a disadvantage of very low numbers of inversions per fly (reduced sperm capacity in females), or of high numbers of inversions per fly (nondisjunction in females); and a non-random distribution of inversions among individuals within populations, with an observed excess of flies carrying intermediate numbers. This central tendency is associated with an interchromosomal interaction for inversion number, but apparently not with linkage disequilibrium.

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APPENDIX

Twenty-six of the twenty-nine completely homozygous females listed in Table 5 are Standard. Thus the largest and most important class of identical sperm are themselves Standard. If the Standard sequences in various arms tend to occur together in the sperm, rather than being associated at random, the estimated frequency of Standard sperm based on arm-frequency products would be too small. The overall frequency of such sperm cannot be measured directly because of the uncertainties in cases where a wild female and the larva she produces are identically heterozygous in one or more arms (see MATERIALS AND METHODS). However, an estimate of the randomness of the Standard sequences in sperm may be made by a consideration of the F_1 larvae they have produced, since all larvae homozygous Standard in a given arm must have come from a sperm carrying the Standard sequence in that arm. If the Standard sequences in sperms tend to be associated together in a non-random way, then the F_1 larvae would likewise tend to show a positive association of homozygous Standard arms. (This would be true except in the unlikely situation where the Standard sequences are positively associated in the sperm and negatively associated in the eggs, of *vice versa*, the two non-random associations tending to cancel each other.)

To investigate this situation, in each collection the expected frequencies of larvae homozygous Standard throughout were calculated as the product of the observed frequencies of homozygosity for Standard in each larval chromosome arm. An observed excess of wholly homozygous larvae over this expected frequency would indicate a tendency of standard sequencies to be associated together in the eggs, sperm, or both. The first Webster Groves collection shows a non-significant observed excess of homozygous Standard larvae (Obs. = 20, Exp. = 19.7, P = 0.93). The second collection shows a non-significant deficiency of such larvae (Obs. = 14, Exp. = 14.8, P = 0.81).

With a heterogeneity χ^2 yielding a P value of 0.07, the pooled data (Obs. = 34, Exp. = 34.5, P = 0.92) show no significant excess of homozygous Standard larvae over expectation. Thus it appears that the assumption of random association of the Standard sequences in the various arms of the sperm is not an unreasonable one. It is of some interest that such an apparently random association does not exist in the Texas populations, as indicated elsewhere.

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