CHROMOSOME INTERACTIONS IN *DROSOPHILA ROBUSTA*[1]

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MOST of the population genetics work on Drosophila species has been done with polymorphism at a single gene locus or different gene arrangements in one chromosome which behave in inheritance as single genetic units. However, little attention has been given to the problem of interactions between unlinked polymorphic loci.

Consider two unlinked autosomal loci *A* and *B* and their variant alleles *a* and *b*, respectively. There will then be nine genotypes *AABB*, *AABb*, *AAbb*, *AaBB*, *AaBb*, *Aabb*, *aaBB*, *aaBb*, and *aabb*. If interactions between the two loci occur, then it is possible to have the relative fitnesses as shown in Table 1, which is a purely hypothetical example. While the *Aabb* genotype shows overdominance to *AAbb* and *aabb* genotypes, the *AaBB* genotype is inferior to the *AABB* and *aaBB* genotypes. Thus, the study of the fitnesses of *AA*, *Aa*, and *aa* genotypes with the *BB* genotype alone will give results different from studies with the *Bb* or *bb* genotypes. Any study which does not consider the fitnesses of the nine genotypes will give results only of doubtful value. However, the knowledge of the marginal fitnesses of *AA*, *Aa*, and *aa* genotypes will be useful if care has been taken to randomize the background at the *B* locus. The single important aspect of interactions is that the fate of one locus is not independent of the others in evolution.

Interactions for linked gene arrangements have been observed in several Dipterans (LEVITAN 1958a). WHITE (1957) presented evidence of interactions between pericentric inversions on two different chromosomes in *Moraba scurra* males. In *Moraba scurra* only one component of fitness, male viability, was studied.

Apart from *Moraba scurra*, there is no organism in which any effort has been made to demonstrate the interactions between naturally occurring unlinked

TABLE 1

Relative fitnesses of different genotypes

	BB	*Bb*	*bb*
AA	1.10	0.90	0.90
Aa	0.80	1.00	1.00
aa	1.00	0.90	0.90

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polymorphisms. The present investigations were carried out on a population of *D. robusta* near St. Louis, Missouri, to test for the presence of interactions between paracentric gene arrangements on different chromosomes. This population is highly polymorphic for paracentric gene arrangements in the three major chromosomes of the species. Two components of fitness, viability in nature and fertility, were studied. In this paper, evidence of interactions between different chromosomes is presented and the role of these interactions in the maintenance of the observed gene frequency equilibria in nature is considered.

METHODS

For viability studies, adult *D. robusta* were collected on fermented banana during the summers of 1963, 1964 and 1965 at Creve Coeur, St. Louis County, Missouri. It has been shown that banana traps attract random samples of *D. robusta* (CARSON 1958). The locality used is approximately three miles west of the Olivette, Missouri, site described by CARSON (1958). The vegetation is similar except that the location is higher and somewhat more exposed. Flies were mated singly to at least two flies of the opposite sex from Standard stock, which is homozygous for all standard gene arrangements. Wild females were fully despermed before mating them to Standard *D. robusta* males. The desperming technique consists of changing the female to well yeasted fresh food vials every two days until no larvae are seen in the last two consecutive vials. On the average, it takes approximately 15 to 20 days before the wild-caught female can be mated to the Standard males. The vigor and longevity of *D. robusta* makes this feasible as a routine procedure. The mortality rate during this technique is about 10%. Salivary gland smears of fully grown third instar larvae were made by the acetolactic orcein technique.

For the sample collected in June, 1963, only one larva from each wild male $\times$ Standard female cross was analyzed. The largest sized male and female samples were collected in June, 1965. At least eight larval smears were prepared to find out the karyotype of a single fly. The chance that a heterokaryotype will be classified as a homokaryotype in any one chromosome arm is then less than $(\frac{1}{2})^7$.

For fertility studies two different experiments were carried out in the summer of 1963 and 1965. The experiment of 1965 was a simplification of the one done in 1963. The methods are described separately for the two experiments.

1. *Summer-1963 experiment:* Forty wild females that had been inseminated in nature were allowed to lay eggs, one female per food vial. F_1 flies emerging from each of these 40 vials were sexed every 24 hours. Male and female offspring of each wild female were stored in different vials and were changed to fresh food every third day. Males were aged to 14 days and the females to seven days. Mating experiments were done using one male with 20 nonsib females. In order to make the 20 females a random sample of karyotypes, only two female offspring were taken from any one of the 40 vials. Thus, 20 females that were mated to a given male came from a random combination of ten vials out of 40, two females from each. The females were etherized at the time of making random combinations of 20 females which was three days before the start of the mating experiment and were stored in two vials (ten per vial). At the time of the experiment, 20 females were put with one male in a well yeasted fresh food bottle which was provided with folded Kleenex tissue. The bottles containing the flies were kept in a constant temperature box, on either side of a 15w fluorescent light. The experiments were begun late in the evening and terminated in the morning after 10 hours.

The females were spread out singly in well yeasted food vials, and were changed to fresh food every two days. The male was mated to three Standard females and seven salivary gland smears were studied to determine the male's karyotype. Generally, a total of four changes was enough to fully desperm the females. In a few cases, though, the females had to be changed once more since they were still laying fertilized eggs. The developing larvae were well yeasted and when third instar larvae began appearing in the vials, Kleenex tissue was provided for pupation of the larvae.

Counts were made of the emerging flies and the vials were discarded when it was certain that no more flies would emerge.

Data of two parameters of male performance were collected: (1) number of females inseminated by the male in ten hours as judged by the number of females which laid fertile eggs, (2) fertility of the male—number of sperm deposited by the male in the females with which he mates. This was assessed from the number of offspring produced by the females with which the male mated.

2. *Summer-1965 experiment:* The F_1 flies born to 40 wild-inseminated females caught in June, 1965, were used for the experiments. Both male and female offspring from each of the 40 vials were matured to 12 days. On the 12th day, one male was given the choice to mate with three nonsib random females in a 2-hr period. In order to make the three females a random sample, only one female was taken from any of the 40 vials, thereby, the three females for the experiment were chosen from a random combination of three out of 40 vials. Females and the male were transferred, in that order, to the mating vial by aspiration, so that flies were not etherized after the initial "sexing."

After the mating period of 2 hours, the females were spread out in fresh food vials. The karotype of the male was inferred by studying at least eight and up to ten salivary gland smears of F_1 larvae obtained from the male $\times$ Standard female cross. The rest of the experimental techniques remained the same as for the summer-1963 experiment. The experiments were run between 2 and 6 PM.

RESULTS

Gene arrangement frequencies over a three-year period: Table 2 presents the data of the percentage frequencies of various gene arrangements of *D. robusta* populations in the Creve Coeur locality for a period of three years. The September–October 1964 sample represents an overwintering adult population (CARSON and STALKER 1948). There are no significant differences in the frequencies of different gene arrangements between June 1963 egg samples, September–October 1964 male samples, and June 1965 male and female samples. The September–October 1964 data, however, show a differential selection for 3R-1 in the females. The females have a higher frequency of 3R-1 than do the males ($P < 0.015$). LEVITAN (1951) found unequal seasonal changes in the gene arrangement frequencies in the two sexes.

Karyotype frequencies: In a random-mating population with no seasonal fluctuations in the frequencies of different gene arrangements, calculation of the Hardy-Weinberg equilibrium should yield information about viability differences between different karyotypes (WALLACE 1958; NOVITSKI and DEMPSTER 1958). The September–October 1964 and June 1965 data show that the Hardy-Weinberg equilibrium holds for the homo- and heterokaryotypes.

Chromosome interactions affecting viability: Intrachromosomal associations: Linkage disequilibria have been observed in many natural and laboratory populations of Drosophila species for marker genes and chromosome inversions (e.g., CANNON 1963; STALKER 1960, 1964; LEVITAN 1958a).

In a random-mating population, the time required for the establishment of equilibrium for linked loci is dependent on the amount of recombination. Thus, in a population which has had enough time for the attainment of equilibrium for the linked polygenic complexes, any departure from the linkage phase equilibrium would be indicative of differential selection.

TABLE 2

Frequencies (in percent) of the various gene arrangements in population samples from Creve Coeur, St. Louis, Missouri

Chromosome arms		XL			XR			2L				2R		3R			
Date	Sex	XL	XL-1	XL-2	XR	XR-1	XR-2	2L	2L-1	2L-2	2L-3	2R	2R-1	3R	3R-1	Nx*	Na†
June 1963	Egg sample	98.9	0.0	1.1	53.3	44.4	2.2	51.8	37.1	10.5	0.7	88.8	11.2	73.4	26.6	90	143
Sept-Oct 1964	Male	100.0	0.0	0.0	57.5	40.4	2.1	53.2	42.6	4.3	0.0	90.4	9.6	71.3	28.7	47	94
	Female	96.5	3.5	0.0	62.0	34.5	3.5	58.6	34.5	5.2	1.7	84.5	15.5	51.7	48.3	60	60
June 1965	Male	97.9	1.1	1.1	52.7	42.6	4.8	60.9	32.4	6.3	0.5	86.0	14.0	68.2	31.8	189	386
	Female	98.5	0.7	0.7	50.7	46.0	3.3	59.9	31.4	8.4	0.4	88.9	11.1	70.6	29.4	136	272

* Nx = total number of X chromosomes examined.
† Na = total number of autosomes examined.

TABLE 3

Associations of left and right arms of second chromosome in double heterokaryotypes of males

Karyotypes	Locality and date of sample: Olivette, Missouri June 1948	Olivette, Missouri June 1949	Olivette, Missouri June 1956	Creve Coeur, Missouri Sept-Oct 1964	Creve Coeur, Missouri June 1965	Total
2L 2R–1 / 2L–1 2R	6	10	5	6	15	42
2L–1 2R–1 / 2L 2R	4	5	2	2	6	19

$\chi^2_{(1)} = 8.7$ $\quad P < 0.005$

Since the X and the second chromosome in *D. robusta* are metacentric and both arms in each chromosome have alternate gene arrangements, one can test to see if any linkage associations are favored over others.

Linkages in the X chromosome: In both sexes, the XL-2 gene arrangement in the left arm is always found with the XR-2 gene arrangement in the right arm. In the June, 1965, female samples there were two XL-2 gene arrangements (gene frequency = 0.007) and both were linked to XR-2 (gene frequency = 0.033). Similarly, in the male sample of June 1965, the only two XL-2 gene arrangements found in the entire sample were also linked to XR-2. In the June 1963 samples, only one XL-2 gene arrangement was present and this too was linked to XR-2. Since the frequency of XR-2 is very low, the expected frequencies of these XL-2 and XR-2 linkages are very low. Such XL-2—XR-2 linkages have been demonstrated by LEVITAN (1958a) and CARSON and STALKER (1949) in other *D. robusta* populations.

Linkages in the second chromosome: The second chromosome has four alternate gene arrangements, 2L, 2L-1, 2L-2, and 2L-3 in the left arm and two gene arrangements, 2R and 2R-1 in the right arm. Since 2L-2 and 2L-3 gene sequences are rare, they have not been considered owing to lack of sufficient data. Thus, considering 2L, 2L-1, and 2R and 2R-1 gene arrangements, there will then be four linkage types in the second chromosome—2L 2R and 2L-1 2R-1 (coupling linkages) and 2L-1 2R and 2L 2R-1 (repulsion linkages). Evidence for linkage disequilibrium comes from the double heterokaryotype males. At equilibrium, the frequencies of the coupling and repulsion type double heterokaryotypes should be equal. Table 3 presents the data of double heterokaryotype males. The data for locality Olivette, St. Louis, for the 1948, 1949, and 1956 samples were collected by PROFESSORS H. L. CARSON and H. D. STALKER and were made available for my use, for which I am very grateful to them. The gene arrangement frequencies in Olivette are like those in Creve Coeur. In all the five samples, there is an excess of repulsion heterokaryotypes over the coupling type ($P < 0.005$). In the Creve Coeur samples alone there are 21 (2L-1 2R)/(2L 2R-1) males as opposed to eight (2L 2R)/(2L-1 2R-1) males. In the female samples collected at Creve Coeur, there is no evidence of linkage disequilibrium. LEVITAN (1958a) found that males evidence linkage disequilibria more frequently than females. As might be expected, in all of these male samples there is an excess of repulsion linkages and a deficiency of coupling linkages (see Table 4).

Interchromosomal associations: Natural selection acts on the phenotype, which is the product of the entire genotype. As stated earlier, inversion polymorphism in three major chromosomes of *D. robusta* provides a good material to test the interactions between different chromosome combinations. For example, if one considers two pairs of nonhomologous chromosomes, A and E, and their homologues designated as a and e, then there will be three possible genotypes with respect to chromosome A, namely, *AA*, *Aa*, and *aa*. Similarly, there will be the *EE*, *Ee*, and *ee* genotypes for chromosome E and nine possible genotypes when both chromosomal conditions are considered. These are *AAEE*, *aaEE*, *AAee*, *aaee*, *AaEE*, *AaEe*, *Aaee*, *AAEe*, and *aaEe*. The expected frequency of these genotypes

TABLE 4

Associations of gene arrangements in the left and right arm of the second chromosome in males

Locality, year, and type of sample		Chromosome frequencies 2L 2R	2L-1 2R	2L 2R-1	2L-1 2R-1	$\chi^2_{(1)}$
Olivette	Observed	65	68	17	8	
June 1948						2.358
male	Expected	69.02	63.98	12.98	12.02	
Olivette	Observed	64	67	16	13	
June 1949						0.416
male	Expected	65.5	65.5	14.5	14.5	
Olivette	Observed	40	51	5	4	
June 1956						0.100
male	Expected	40.95	50.05	4.05	4.95	
Creve Coeur	Observed	28	25	7	2	
June 1963						1.066
male*	Expected	29.92	23.08	5.08	3.92	
Creve Coeur	Observed	46	37	7	4	
August 1964						0.038
male	Expected	46.80	36.20	6.20	4.80	
Creve Coeur	Observed	179	108	37	12	
June 1965						3.147
male	Expected	184.5	102.5	31.5	17.5	
	All six samples:	$\chi = 5.5$	$df = 6$	$r = 2.2$	$P < 0.03$	

* Only one larva from each wild male × Standard female cross was analyzed. The Yates correction was used in the Olivette 1948, 1956 and Creve Coeur 1963, 1964 samples. The expected frequencies for each sample are computed from the marginal totals of a 2 × 2 contingency table.

can be calculated from the marginal totals of 3 × 3 contingency table with 4 df. Any significant deviations from expectation would indicate differential viability of different genotypes. The Creve Coeur, June 1965, samples of both sexes have been exhaustively analyzed to check for the chromosome interactions.

Interactions between the X and second chromosome: Table 5 presents the data for interactions between XR and 2R karyotypes. In the females, the 2R-1/2R-1 column has not been included in the table since the 2R-1 gene arrangement is rare. There were only two flies in the entire sample with the 2R-1/2R-1 karyotype. The deviations are significant at $P \cong 0.03$. As there are only four flies of XR/XR 2R/2R-1, the XR/XR 2R/2R and XR/XR 2R/2R-1 karyotypes can legitimately be pooled with the corresponding heterokaryotype classes to raise the cell frequencies, so that the 2 × 3 contingency table is reduced to a 2 × 2 table of the form: 80/17 | 17/11. The χ^2 for the reduced 2 × 2 contingency table with 1 df is 5.90 and $P < 0.02$. Table 5A includes all 2L karyotypes. The interactions between XR and 2R karyotypes are, however, more obvious when only 2R karyo-

TABLE 5

Interactions between XR and 2R karyotypes of females

Karyotype	$\frac{2R}{2R}$	$\frac{2R}{2R\text{-}1}$	Total
A. Including all XL, 2L and 3R karyotypes			
$\frac{XR}{XR}$	30 (26.38)	4 (7.62)	34
$\frac{XR}{XR\text{–}1}$	50 (48.88)	13 (14.11)	63
$\frac{XR\text{–}1}{XR\text{–}1}$	17 (21.72)	11 (6.27)	28
Total	97	28	125
	$\chi^2_{(2)} = 6.9$	$P \simeq 0.03$	
B. Including all XL 3R karyotypes and only 2L homokaryotype			
$\frac{XR}{XR}$	16 (12.5)	1 (4.5)	17
$\frac{XR}{XR\text{–}1}$	23 (22.1)	7 (7.9)	30
$\frac{XR\text{–}1}{XR\text{–}1}$	9 (13.4)	9 (4.6)	18
Total	48	17	65
	$\chi^2_{(2)} \simeq 11.3$	$P < 0.005$	

Expected numbers in parentheses are calculated from the marginal totals. Observed numbers exclude the rare 2R-1/2R-1 homokaryotype.

types, which are homokaryotypic in the 2L chromosome, are considered. The interactions, then, are highly significant ($P < 0.005$; see Table 5B).

Interactions between autosomes: Interactions between 2L and 3R karyotypes have been tested in a 3×3 contingency table with 4 df (see Table 6A). The data for both sexes have been pooled since the deviations are in the same direction and the gene frequencies are similar. However, the deviations are not significant. Since the frequencies of the rare 2L and 3R karyotypes are low, the table can be reduced to a 2×2 contingency table by pooling the low frequency karyotypes with the corresponding heterokaryotypes. Thus the 2×2 contingency table is of the form: 67/66 | 55/94. The deviations are significant ($P < 0.025$) and seem to establish the presence of interactions between 2L and 3R karyotypes. Interactions between second chromosome double heterokaryotypes and 3R karyotypes were observed (see Table 6B). Flies with coupling phase linkage are more often heterokaryotypic in the 3R chromosome than expected ($P < 0.0003$).

Studies on mating speed and fertility in males: No differences were observed in the mating speed and fertility of 2L/2L, 2L/2L-1 and 2L-1/2L-1 and 2R/2R, 2R/2R-1 and 3R/3R, 3R/3R-1 and 3R-1/3R-1 males. In other words, there was no evidence of marginal overdominance for any of the heterokaryotypes. SPIESS and LANGER (1964) found no heterosis for mating speed in the heterokaryotypes of *Drosophila persimilis*. In *D. pseudoobscura*, however, heterokaryotype males showed overdominance for mating speed (SPIESS *et al.* 1966).

Chromosome interactions affecting male fertility: The data were classified

TABLE 6

Interactions between second chromosome and 3R karyotypes of both sexes

Karyotype	3R/3R	3R/3R-1	3R-1/3R-1	Total
A. Interactions involving 2L karyotypes, including all XL, XR, and 2R karyotypes				
2L/2L	67(57.54)	43(51.92)	12(12.55)	122
2L/2L-1	47(58.01)	62(52.34)	14(12.65)	123
2L-1/2L-1	19(17.45)	15(15.74)	3(3.80)	37
Total	133	120	29	282
	$\chi^2_{(4)} = 7.3$	$P > 0.1$		
B. Interactions involving second chromosome heterokaryotypes				
2L-1 2R / 2L 2R-1	15(10.43)	7(13.50)	5(3.07)	27
2L 2R / 2L-1 2R-1	2(6.57)	15(8.50)	0(1.93)	17
Total	17	22	5	44
	$\chi^2_{(2)} = 16.6$	$P < 0.0003$		

In Table 6B, observed numbers include Sept.-Oct. 1964 and June 1965 samples.
Expected numbers in parentheses are calculated from the marginal totals.

according to the karyotype in the right arm of the X chromosome, namely, XR and XR-1. (In cases where the karyotype of the left arm of the X chromosome is not mentioned, it is XL for both males and females.) In both experiments, XR-1 males inseminated significantly more females and produced more offspring (see Table 7). But the differences in the productivity of XR and XR-1 males are not significant at the 5% level. This nonsignificance could be accounted for because the positive correlation between the number of females inseminated by a male and the number of offspring produced is of a medium order ($r = 0.620$) (PRAKASH 1967a). The variability of mating performance of XR-1 males is lower than that of XR males which means that males with higher average mating capacity do so more uniformly than the ones with low average.

Interactions between XR and second chromosome: Table 8 presents the mating speed and fertility data of XR and XR-1 males which are homo- and heterokaryotypic in the second chromosome from the summer 1965 experiments. As there was no evidence of interactions with the third chromosome, only XR and second chromosome karyotypes were considered. Since there were no differences in the number of females inseminated and the number of offspring produced by the XR and XR-1 males which are homokaryotypic in the second chromosome, the

TABLE 7

Mating speed and fertiliity (number of offspring) data of XR and XR-1 males

Male karyotype	Number of males tested	Percentage of males mated	Average number of females inseminated	Variance of number of females inseminated	Average fertility	Coefficient of variation for fertility
A. Summer 1963 experiment*						
XR	23	100.00	10.17	6.41	1312.17	52.35
XR-1	30	100.00	11.50	6.41	1634.00	39.26
			$P \simeq 0.03$		Not significant	
B. Summer 1965 experiment†						
XR	89	84.27	1.876	1.285	233.66	79.33
XR-1	91	91.21	2.176	0.780	253.58	64.71
			$P < 0.01$	$P < 0.05$	Not significant	

* One male was given the choice to mate with 20 random females for 10 hours.
† One male was given the choice to mate with three random females for 2 hours.

two are combined into one group (Group 1). XR-1 males which are heterokaryotypic in the second chromosome (Group 3) have faster mating speed, inseminate more females, and produce more offspring than do the XR males with similar autosomal backgrounds (Group 2). The mating activity of Group 3 males is greater than of Group 1 males. The variance for both parameters of mating activity varies inversely with the mean performance.

Mating speed of homo- and heterokaryotype females:

Method: Mating speed of the females homo- and heterokaryotypic in the XR chromosome was studied. The flies for the experiments were obtained by interstrain and interkaryotype crosses

TABLE 8

Mating speed and fertility (number of offspring) data of different karyotypes of males in the summer 1965 experiment

Group	Karyotype X	Karyotype 2	Number of males tested	Percent of males mated	Average number of females inseminated	Variance of number of females inseminated	Average fertility	Coefficient of variance of fertility
1	XR and XR-1	Homokaryotype	62	85.48	2.00	1.13	226.74	75.77
2	XR	Heterokaryotype	64	82.81	1.75	1.33	210.89	81.34
3	XR-1	Heterokaryotype	54	96.30	2.37	0.44	285.68	56.95

Number of males mating in 2 hours χ^2 test	Mating speed (average number of females inseminated) χ^2 test	Variance of mating speed F test	Average fertility t test
Group 3 *vs.* Group 2	Group 3 *vs.* Group 2	Group 3 *vs.* Group 2	Group 3 *vs.* Group 2
$P < 0.05$	$P < 0.0001$	$P < 0.01$	$P = 0.016$
(Yates' correction)	Group 3 *vs.* Group 1	Group 3 *vs.* Group 1	Group 3 *vs.* Group 1
	$P < 0.01$	$P < 0.01$	$P > 0.10$

One male was given the choice to mate with three random females for 2 hours.

of the following selected homokaryotypic strains: four strains, namely, A_1, A_2, A_3, and A_4 of $\frac{XL\ XR}{XL\ XR}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R}$ karyotype; four strains, namely, B_1, B_2, B_3, and B_4 of $\frac{XL\ XR\text{–}1}{XL\ XR\text{–}1}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R}$ karyotype; and three strains, namely, D_1, D_2, and D_3 of $\frac{XL\ XR}{XL\ XR}\ \frac{2L\text{–}1\ 2R}{2L\text{–}1\ 2R}\ \frac{3R}{3R}$ karyotype. Each strain was started from a single-pair mating of homokaryotypic flies caught at Creve Coeur in June, 1965. Thus each strain had three wild X chromosomes and four wild autosomes.

Interstrain crosses $A_1 \times A_2$, $A_2 \times A_3$, $A_3 \times A_4$, and $A_4 \times A_1$ and $B_1 \times B_2$, $B_2 \times B_3$, $B_3 \times B_4$, and $B_4 \times B_1$ were made to provide XR/XR and XR–1/XR–1 homokaryotypic females. Four interkaryotype crosses of $A_1 \times B_1$, $A_2 \times B_2$, $A_3 \times B_3$, and $A_4 \times B_4$ provided XR/XR–1 females. The double heterokaryotypic females XR/XR–1 2L/2L–1 were obtained by interkaryotype crosses of the type $B_1 \times D_1$, $B_2 \times D_2$, and $B_3 \times D_3$.

Ten pairs of virgin, unetherized flies were allowed to mate for a period of one hour in a plexiglass chamber (10.2 × 10.2 × 3.3 cm) with an opening of 2.5 cm. A strip of wet blotting paper (6.5 × 2.0 cm) was put in the chamber to provide moisture. As copulatory pairs formed, they were removed by aspiration and the time of mating was recorded. All of the mating experiments were done between 11:00 AM and 5:00 PM in November 1965 and February 1966. Since genotypic differences will be expected to cause mating activity differences, nearly equal numbers of flies of different groups obtained by strain crosses were tested. The data of mating speed of flies of similar karyotype but from dierent groups were pooled, since in all cases the χ^2 heterogeneity test indicated a nonsignificant difference at $P \geq 0.10$.

Homogamic and heterogamic mating runs were performed to find if any differences exist in the mating speeds of $\frac{XL\ XR}{}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R}$ and $\frac{XL\ XR\text{-}1}{}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R}$ males and the females of the same karyotypes. In total, 500 pair matings were tested. No differences, whatsoever, were observed in the mating speed of these homokaryotypes in both sexes. Results of homogamic homokaryotype $\frac{XL\ XR}{XL\ XR}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R} \times \frac{XL\ XR}{XL\ XR}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R}$ and $\frac{XL\ XR\text{-}1}{}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R} \times \frac{XL\ XR\text{-}1}{XL\ XR\text{-}1}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R}$ matings and homogamic $\frac{XL\ XR}{}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R}$ homokaryotype males $\times \frac{XL\ XR}{XL\ XR\text{-}1}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R}$

TABLE 9

Mating speed at various age levels of females homo- and heterokaryotypic in the XR chromosome and homokaryotypic in the rest of the chromosomes

Age in days		Karyotype of the female	Percent mating in one hour	Number of pairs tested	χ^2
Male	Female				
15	6	Homokaryotype	48.75	240	
		Heterokaryotype	55.00	160	1.5
Both sexes					
12		Homokaryotype	49.20	250	
		Heterokaryotype	54.74	190	1.3
15		Homokaryotype	63.33	240	
		Heterokaryotype	67.06	170	0.8
30		Homokaryotype	73.08	260	
		Heterokaryotype	81.87	160	4.3
		$\chi = 5.3$ df $= 4$	$\tau = 2.67$	$P < 0.01$	

heterokaryotype female matings are presented in Table 9. No differences or presence of a trend in the mating speed of homokaryotype females were observed. At all age levels, however, the mating speed of females heterokaryotypic in the XR chromosome is greater than the corresponding homokaryotype females. None of the differences for each sample are significant except for the matings on the 30th day of age ($P < 0.04$). For all samples, when considered as a whole, the differences are significant at $P < 0.01$.

Table 10 presents that data on mating speeds of double heterokaryotype $\frac{XL\ XR}{XL\ XR\text{-}1}\ \frac{2L}{2L\text{-}1}\ \frac{2R}{2R}\ \frac{3R}{3R}$ females in heterogamic matings with $\frac{XL\ XR}{}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R}$ males. More double heterokaryotypic females have mated by the end of 30 minutes and one hour than the homokaryotypic females in $\frac{XL\ XR}{}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R} \times \frac{XL\ XR}{XL\ XR}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R}$ and $\frac{XL\ XR\text{-}1}{}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R} \times \frac{XL\ XR\text{-}1}{XL\ XR\text{-}1}\ \frac{2L\ 2R}{2L\ 2R}\ \frac{3R}{3R}$ homogamic matings. If double heterokaryotype females (XR/XR-1 2L/2L-1) are compared with single heterokaryotypes (XR/XR-1), the differences are not significant. These data show that XR/XR-1 females have a faster mating speed than XR/XR and XR-1/XR-1 females throughout life.

DISCUSSION

Although equilibria for gene arrangement and karyotypic frequencies exist, disequilibrium for the linked gene arrangements was observed. The nonrandom associations could be explained by selection acting against those linkages which are less frequent than expected. In a population with free recombination, very large selection indices would be required to maintain such disequilibria. However, it has been shown by CARSON (1953) and LEVITAN (1958b) that the recombination fraction between linked inversions in polymorphic *D. robusta* populations is lower than would be expected from the length of free chromosome segments in between two linked inversions.

The direction of interaction between XR and 2R and 2L and 3R chromosome karyotypes is such that it cannot be explained either by inbreeding or assortative mating. The results of interactions between 2L and 3R chromosome karyotypes

TABLE 10

Mating speeds of homokaryotype and double heterokaryotype (in XR and 2L chromosomes) females

Karyotype of the female	Percent mated in 30 minutes	Percent mated in one hour	Number of pairs
Homokaryotype	43.75	48.75	240
Double heterokaryotype	45.71	61.43	70
	$\chi^2_{(2)} = 10.2$ $P < 0.01$		

Ten pairs of 15-day old males and 6-day old females were allowed to mate for one hour in a plexiglass chamber.

TABLE 11

Estimates of the relative viabilities of karyotypes, calculated from the deviations from expectation on the basis of the Hardy-Weinberg ratios by the method of HALDANE (1956)

	2R/3R	2R/3R-1	2R-1/3R-1	Percent† Percent frequencies§ karyotypes	Marginal viabilities
A. 2L and 3R karyotypes in both sexes*					
2L/2L	1.4370	1.0000	1.2103	42.34	1.2256
2L/2L-1	0.9390	1.3431	1.3153	45.46	1.1510
2L-1/2L-1	1.4143	1.2107	1.0501	12.20	1.2900
Percent frequencies† of 3R karyotypes	46.84	43.20	9.96		
Marginal viabilities	1.2078	1.1816	1.2384		

B. XR and 2R karyotypes in females‡

	2R/2R	2R/2R-1	2R-1/2R-1	Percent§ frequencies of XR karyotypes	Marginal viabilities
XR/XR	2.1622	1.0000	0.00	27.41	1.8720
XR/XR-1	1.9805	1.7862	3.8128‖	49.90	1.9666
XR-1/XR-1	1.4803	3.3225	0.00	22.69	1.8627
Percent frequencies§ of 2R karyotypes	76.39	22.03	1.58		
Marginal viabilities	1.9168	1.9192	1.9025		

* $\frac{2L\ 3R}{2L\ 3R\text{-}1}$ arbitrarily taken as 1.0000.

† Percent frequencies of 2L and 3R karyotypes were based on the gene arrangement frequencies calculated from the observed frequencies of nine karyotypes.

‡ $\frac{XR\ 2R}{XR\ 2R\text{-}1}$ arbitrarily taken as 1.0000.

§ Percent frequencies of XR and 2R karyotypes were based on the gene arrangement frequencies calculated from the observed frequencies of nine karyotypes.

‖ Observed sample consisted of only two flies of this karyotype.

are further strengthened by the findings of CARSON and STALKER (unpublished). In their experiments, one F_1 third instar larva (which had grown in an optimal environment in the laboratory) from each wild caught *D. robusta* female (Olivette locality) which had been inseminated in nature was analyzed for karyotype. They found that homozygosity in 2L is associated with homozygosity in 3R and heterozygosity in 2L is associated with heterozygosity in 3R and possibly with homozygous 3R-1/3R-1. However, their results do not reach a formal significance level ($\chi^2_{(1)} = 3.3841$, $P < 0.08$), which most probably is

due to small sample size ($N < 100$). Since the 2L 3R chromosome associations in nature and in optimal conditions in the laboratory are similar, it appears that the frequent associations provide the fly with a superior physiology in these environments.

Table 11A presents the estimates of relative viabilities of the 2L and 3R karyotypes. The male mating activity data of summer-1965 do not show any significant interactions between 2L and 3R chromosomes, which is because of fragmentation of the data of 180 males into nine classes. In separate experiments done with the derived strains (PRAKASH 1967b), evidence for interactions between 2L and 3R chromosomes was obtained. While no overdominance for mating speed was observed in $\frac{XR}{-}\ \frac{2L}{2L\text{-}1}\ \frac{2R}{2R}\ \frac{3R}{3R}$ flies, $\frac{XR}{-}\ \frac{2L}{2L\text{-}1}\ \frac{2R}{2R}\ \frac{3R\text{-}1}{3R\text{-}1}$ flies showed strong heterosis for mating speed.

The adaptive topography based on viabilities in Table 11A shows that the present population is located on a saddle (see LEWONTIN and WHITE 1960, for a discussion of the calculation of topographies). Viability here denotes differential mortality between the egg and the adult stage in nature. The adaptive value of the population is highest if it consists entirely of 2L/2L 3R/3R genotypes. The population should then eventually become monomorphic. A large amount of evidence, however, shows that these polymorphisms are indeed stable. CARSON and STALKER in their study of Olivette wood population of *D. robusta* which is only three miles east of Creve Coeur and shows similar gene arrangement frequencies, observed equilibrium for the gene arrangement frequencies over a period of 17 years (CARSON 1958 and unpublished results).

Table 11B presents the estimates of relative viabilities of the XR and 2R karyotypes in the females. Table 12 gives the mating speed data of XR and 2R karyotypes of the males. Since XR-1/XR-1 2R/2R-1 females have highest relative viability and XR-1 2R/2R-1 males have greatest mating speed, it would seem that the population should show an increase in the frequency of these genotypes.

TABLE 12

Mating speed data of XR and 2R karyotypes of males in the summer 1965 experiment

Group	Karyotype		Number of males tested	Percent of males mated	Average number of females inseminated
	X	2R			
1	XR and XR-1	$\frac{2R}{2R}$	139	87.05	2.01
2	XR	$\frac{2R}{2R\text{-}1}$	18	83.34	1.50
3	XR-1	$\frac{2R}{2R\text{-}1}$	23	95.66	2.43
					χ^2 test with 1 df: Group 3 *vs.* Group 2, $P < 0.005$ Group 3 *vs.* Group 1, $P < 0.025$

One male was given the choice to mate with three random females for 2 hours.

Estimates of viabilities obtained by LEWONTIN and WHITE (1960) for CD and EF chromosome karyotypes in grasshopper populations were such that the equilibrium is not stable. These authors tentatively suggested that equilibrium in natural populations is maintained by selection which is dependent on changes in gene frequencies and fluctuations in the environment. In the calculation of adaptive topographies, LEWONTIN and WHITE assumed panmixia, which as noted by these authors, need not be strictly true, since these insects do not have wings to fly, and they live and die within a few meters of the place where they hatched from eggs. ALLARD and WEHRHAHN (1963) constructed adaptive topographies assuming different levels of inbreeding, namely, $F = 0.00$, 0.05, 0.10 and 0.25. Values of F in the range of 0.10 to 0.15 produce topographies which show that populations occupy an adaptive peak rather than saddle points as was observed by LEWONTIN and WHITE (1960), in the topographies constructed by their assuming panmixia. The adaptive peak obtained by ALLARD and WEHRHAHN for $F = 0.10$ and 0.15 is in agreement with the gene arrangement frequencies in these grasshopper populations. However, unpublished experiments of PROFESSOR M. J. D. WHITE (R. C. LEWONTIN, personal communication) suggest that inbreeding probably is not the explanation for the observed fitness landscape. In this experiment, all the grasshoppers from a given locality were collected, mixed in a pillow case and then put back randomly at different sites within the same locality. Karyotype analysis of the grasshoppers one generation later showed that the population still occupies a saddle in the adaptive topography. If inbreeding were the probable cause, then one generation of random mating would be expected to bring the population to adaptive peak. Although it seems unlikely, one can argue that the grasshoppers migrated to their original homes and this resulted in the persistence of inbreeding.

It can be argued that these *D. robusta* populations are undergoing inbreeding, and that there is indeed overdominance for viability. While there seems to be no way of satisfactorily answering this question, evidence seems to point against it. The excess of double heterokaryotypes in 2L 3R interactions cannot be explained on the assumption of inbreeding. In *D. robusta*, males reach sexual maturity at the age of eight days while females reach it at four days. Such a difference in the age of maturity in the two sexes will favor outbreeding. It also seems unlikely that the flies emerging out of a brood will not disperse away from their sibs before they reach sexual maturity. Also, the effective population size in *D. robusta* must be large, since matings are short, about 37 seconds, and both sexes repeatmate frequently (PRAKASH 1967a). Multiple inseminations are of frequent occurrence in *D. robusta* males in nature. This conclusion is reached by studying the F_1 egg samples laid by the females which were inseminated in nature. Moreover, the assumption of different levels of inbreeding, namely, $F = 0.00$, 0.05, 0.10, 0.15 and so on up to 0.50 does not give a stable equilibrium point for the XR 2R and 2L 3R interacting systems.

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SUMMARY

Chromosome studies were made on a single population from locality Creve Coeur, St. Louis County, Missouri. This population is polymorphic in both arms of the X and second chromosome and the right arm of the third chromosome.—Gene arrangement frequencies showed no changes over a three year period. There were no differences in the gene arrangement frequencies of the two sexes.—Homo- and heterokaryotypes for different chromosome arms occur in proportions expected by the Hardy-Weinberg equilibrium.—Linkage disequilibrium of X and second chromosome linkages were observed. XL-2 and XR-2 linkages were more frequent than expected. In the male samples there was an excess of (2L/2L-1 2R-1/2R) over (2L/2L-1 2R/2R-1) double heterokaryotypes.—Interactions for viability between XR and 2R chromosome karyotypes in the females were observed. Interactions between 2L and 3R karyotypes were observed in both sexes.—Interactions between XR and second chromosome affecting male fertility were observed.—There was no marginal overdominance for mating activity and fertility in the males. However, XR/XR-1 females appear to show heterosis in mating speed.

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