RELEASE OF GENETIC VARIABILITY THROUGH RECOMBINATION. III. DROSOPHILA PROSALTANS

TH. DOBZHANSKY,[1] H. LEVENE,[2] B. SPASSKY, AND N. SPASSKY

Departments of Zoology and Mathematical Statistics, Columbia University, New York City

Received February 17, 1958

THIS is the third in a series of articles on release of genetic variability through recombination in three species of Drosophila. The previous articles have dealt with *Drosophila pseudoobscura* (SPASSKY *et al.* 1958) and *D. persimilis* (SPIESS 1959). The present article is concerned with *D. prosaltans*. The ecological peculiarities of this species are pertinent. It is native exclusively in the tropics, while the other two species live mainly in the temperate zone. *D. pseudoobscura* is widely distributed, very common, ubiquitous, and ecologically versatile; *D. persimilis* occurs in a much less extensive geographic area, and although it builds very dense populations in favorable habitats it is specialized to live chiefly in cool and humid locations; *D. prosaltans* is a rare form, which reaches considerable population densities only ephemerally in few scattered neighborhoods (DOBZHANSKY and PAVAN 1950). The spontaneous mutation rates for autosomal lethals are, at similar temperatures and in homologous chromosomes, about twice as high in *D. persimilis* and *D. prosaltans* as they are in *D. pseudoobscura* (DOBZHANSKY, SPASSKY and SPASSKY 1952, 1954). The genetic loads carried in the populations (accumulated recessive lethal, semilethal, and subvital gene complexes in the chromosomes) seem nevertheless to be higher in *D. pseudoobscura* than in *D. persimilis* or in *D. prosaltans* (DOBZHANSKY and SPASSKY 1953, 1954). In accordance with this, the loss of fitness produced by inbreeding and homozygosis for naturally occurring gene complexes is greater in *D. pseudoobscura* than in *D. persimilis* or in *D. prosaltans*. It is tempting to speculate that the adaptive norm of *D. pseudoobscura* depends upon balanced heterozygosis to a greater extent than is the case in *D. persimilis* and in *D. prosaltans*. In other words, the genetic architecture of *D. pseudoobscura* approaches that postulated by the "balance" hypothesis, while the genetic architectures of the other two species incline relatively more towards the situation envisaged by the "classical" hypothesis (DOBZHANSKY 1955).

Material and technique in Drosophila prosaltans

The experiments have followed the same plan as those with *D. pseudoobscura* and *D. persimilis* (SPASSKY *et al.* 1958, SPIESS 1959). Ten second chromosomes, permitting normal or subnormal viability in homozygotes, were extracted from

[1] The work reported in this article has been carried out under Contract Number AT-(30-1)-1151, U. S. Atomic Energy Commission.

[2] This research was sponsored by the Office of Naval Research under Contract Number Nonr-266 (33), Project Number 042-034.

a sample of *D. prosaltans* collected in May, 1954, at Pirassununga, in the state of São Paulo, Brazil, by PROF. C. PAVAN and kindly sent by him to our laboratory. These chromosomes will be referred to below, for brevity, as "Pira" chromosomes. Another set of ten normally viable to subvital second chromosomes was extracted from the sample of flies kindly collected for us in April, 1954, in the vicinity of Rio de Janeiro by DRS. H. BURLA and C. MALOGOLOWKIN. These chromosomes will be referred to as coming from "Rio." The techniques of testing the chromosomes of *D. prosaltans* for viability effects have been described by DOBZHANSKY and SPASSKY (1954). The 20 strains containing the original chromosomes used in the present experiments (ten Pira and ten Rio strains) were kept in homozygous condition in a constant temperature room at 19° C.

All the possible intercrosses of the 20 strains were made. Females carrying a given pair of the original chromosomes were then outcrossed to males which carried in one of their second chromosomes the dominant mutant Lobe (eye shape, see SPASSKY, ZIMMERING and DOBZHANSKY 1950). Ten Lobe males were taken from each progeny, and crossed, in individual cultures, to females which carried in one of their second chromosomes the dominant mutants Plum, Star, and Curly (*Pm S Cy*). In each progeny, about five pairs of females and males showing the effects of the genes *Pm*, *S*, and *Cy* were selected and inbred. In the next generation, exactly 100 flies were counted in each progeny, and the numbers of *Pm S Cy* and wild type flies were recorded. The *Pm S Cy* chromosome carries a complex inversion which suppresses most of the crossing over. All the cultures in which counts were made were raised at 25° C.

Viability of the original and the recombination chromosomes

The experimental data are summarized in Tables 1–4, which are constructed exactly like the comparable Tables for *D. pseudoobscura* and *D. persimilis* (SPASSKY *et al.* 1958, SPIESS 1959). The strain numbers are shown on the top and the left margins of the tables. Roman type above and to the right of the diagonals in Tables 1 and 2, and all the figures in Table 3, are the mean numbers of wild type (non-Plum-Star-Curly) flies in the test cultures of the recombination chromosomes. Italic type numerals below and to the left of the diagonals in Tables 1 and 2, and all the figures in Table 4, give variances of the numbers of wild type flies in the ten cultures testing viabilities in homozygous condition of the recombination products of a given pair of original chromosomes. The figures on the bottom and the right margins in the four tables are the mean viabilities, or the mean variances, of the recombination products in which a given original chromosome has participated. Finally, the squares along the diagonals in Tables 1 and 2 give the mean viabilities (Roman) and variances (italics) of the homozygotes for the ten original chromosomes from Pirassununga and from Rio de Janeiro. The original chromosomes were tested (or, rather, retested) at the conclusion of the experiments on the recombination products, each original chromosome in sextuplicate, and the figures in the squares along the diagonals are based on the data obtained in these retests. All the variances are the residual variances

(V), obtained by subtracting the binomial sampling error from the crude variance *(U)*.

The mean viability of the homozygotes for the ten original Pira chromosomes (Table 1) is 30.53, and for the ten original Rio chromosomes (Table 2) 33.11. According to DOBZHANSKY and SPASSKY (1954), the mean viability of the homozygotes for a random sample of second chromosomes extracted from natural populations of *Drosophila prosaltans* is 21.3, while the normal (control) viability

TABLE 1

Viability (Roman type numerals above and to the right of the diagonal line) and variance (italic numerals below and to the left of the diagonal) of the recombination products of ten original chromosomes (nos. 13, 15 . . . 55) from Pira. $\overline{M}$ *and* $\overline{V}$ *= mean viability and mean variance of the recombination products of the crosses in which a given original chromosome is a participant. The squares along the diagonal show the viability (Roman) and the variance (italic) of the original chromosomes. Further explanation in text*

	13	15	48	49	68	69	72	78	82	55	$\overline{M}$
13	29.5 *−0.1*	28.9	30.0	29.0	23.0	30.0	27.8	32.0	26.1	30.7	*28.6*
15	*0.4*	32.3 *24.8*	30.5	26.9	28.1	29.7	31.7	33.0	18.9	28.2	*28.4*
48	*40.1*	*26*	23.7 *9.5*	25.8	27.5	31.2	29.2	31.2	27.9	28.7	*29.1*
49	*51.2*	*−4.0*	*14.6*	32.3 *−16.4*	29.2	27.7	13.3	26.1	31.3	20.1	*25.5*
68	*−2 2*	*30 6*	*33 2*	*−3 8*	32.3 *−8.0*	28.3	29.2	25.6	28.3	27.5	*27.0*
69	*13.9*	*58.2*	*10.9*	*23.8*	*35.0*	34.0 *−11.6*	27.7	29.4	31.9	16.2	*27.4*
72	*21.9*	*21.9*	*9.1*	*198.9*	*−6.7*	*36.4*	35.0 *16.8*	25.3	30.5	32.9	*28.0*
78	*16.9*	*28.1*	*10.9*	*170.4*	*16.1*	*−1.2*	*22.9*	29.8 *28.4*	29.7	29.4	*27.5*
82	*11.5*	*160.1*	*27.8*	*97.6*	*14.4*	*7.5*	*12.6*	*50.5*	23.8 *−8.4*	29.1	*29.1*
55	*14.1*	*−0.9*	*35.6*	*204.9*	*44.8*	*49.9*	*28.0*	*8.8*	*24.4*	32.5 *21.6*	*28.2*
$\overline{V}$	18.6	33.0	20.5	83.7	17.9	26.0	38.3	35.9	45.2	45.5	

is 33.85. The original chromosomes utilized in the present experiments are, thus, normally viable to mildly subvital in homozygotes.

The mean viability of homozygotes for the recombination products of these original chromosomes is markedly lower than that of the original homozygotes themselves. The figure for the recombinations of the Pira chromosomes (grand mean) is 27.88, for the Rio chromosomes 28.12, and for the interpopulational crosses (Pira × Rio) 28.27. It is evident, then, that in *D. prosaltans*, as in *D. pseudoobscura* and *D. persimilis*, the recombination between chromosomes which give homozygotes of better-than-average viability leads to depression of the recombinants towards the mean viability of the homozygotes for randomly chosen chromosomes in natural populations of the species. It is interesting to note

TABLE 2

Viability and variance of the recombination products of ten original chromosomes for Rio. For further details see the legend to Table 1 and text

	22	90	105	118	122	127	142	158	190	232	M
22	30.6 -15.6	23.3	24.2	37.3	31.4	23.1	27.0	30.4	27.2	25.8	28.0
90	148 8	35.0 -10.0	33.9	34.4	32.4	30.7	34.9	34.5	11.0	21.4	28.5
105	17.8	38.6	33.0 -8.1	34.9	17.7	24.8	32.7	36.0	16.1	26.8	27.5
118	38.2	41.9	-5.7	37.8 -4.6	19.1	31.8	31.3	31.8	29.3	32.0	31.3
122	74.1	-13.4	269.0	312.0	31.2 -16.8	20.8	31.4	25.9	29.7	29.1	26.7
127	109.0	-1.0	103.3	18.7	200.2	36.3 2.1	34.6	33.5	25.6	33.3	28.7
142	18.5	1.6	0.2	11.0	71.6	32.8	33.0 -10.9	29.7	17.4	28.0	29.7
158	130.2	47.0	0.1	-14.4	47.1	-10.9	3.8	33.3 0.8	22.2	27.4	30.7
190	56.8	200.1	378.8	32.2	22.7	15.7	322.0	40.7	30.3 48.3	27.0	22.8
232	-2.5	154.8	69.2	13.8	34.8	-8.4	4.1	31.5	-8.6	30.5 -16.9	27.9
V	65.7	68.7	96.8	49.7	113.1	51.0	51.7	30.6	117.8	32.1	

that this regression is apparently less pronounced in *D. prosaltans* and in *D. persimilis* than in *D. pseudoobscura* (see below).

Variance engendered by recombination

The mean residual variance *(V)* for the original chromosomes turns out to be 5.6 for the Pira chromosomes, and –3.6 for the Rio chromosomes. In *D. prosaltans*, like in the other species, this residual variance is contributed to by the environmental differences between the six replicate test cultures, and by the possible accumulation of mutations in the strains in which the original chromo-

TABLE 3

Viability of the recombination products of chromosomes from Pira and from Rio. $\overline{M}$ *= the mean viability of the recombination products of the crosses in which a given original chromosome is a participant*

		PIRA									
		13	15	48	49	68	69	72	78	82	55	$\overline{M}$
	22	33.3	30.9	27.4	18.2	30.6	25.1	16.7	30.5	30.3	26.2	*26.9*
	90	30.4	33.9	25.9	27.3	31.7	29.7	30.1	31.2	30.4	37.4	*30.8*
	105	34.8	24.4	31.2	32.2	34.6	34.8	35.0	20.6	32.4	29.2	*30.9*
	118	31.0	32.9	34.1	26.1	31.4	32.7	33.5	33.0	33.6	31.0	*31.9*
O	122	26.4	7.6	31.0	20.0	31.3	9.1	24.8	24.2	27.4	29.3	*23.1*
—	127	29.4	34.5	23.3	14.2	29.5	32.9	34.6	31.7	15.8	27.5	*27.3*
R	142	33.3	33.8	23.2	20.5	32.8	34.9	33.6	36.5	33.6	34.1	*31.6*
	158	34.2	35.4	32.2	31.9	28.4	12.9	16.6	36.9	20.3	17.8	*26.7*
	190	35.6	29.9	26.8	25.1	27.2	23.4	28.2	14.4	27.2	27.0	*26.5*
	232	19.0	31.6	30.2	30.6	29.8	30.8	29.5	31.4	26.4	9.6	*26.9*
	$\overline{M}$	*30.7*	*29.5*	*28.5*	*24.6*	*26.9*	*30.7*	*26.6*	*28.3*	*29.0*	*27.7*	

somes were perpetuated during the course of the experiments. It is evident that this residual variance for the original chromosomes is not significantly different from zero.

The variances for the recombination chromosomes is considerable. The mean for the recombination products of Pira chromosomes turns out to be 36.5, and for Rio chromosomes almost twice as large, 67.7. The interpopulational crosses give a variance intermediate between the two groups of the intrapopulational crosses, namely 53.4. Inspections of Tables 1, 2, and 4 shows that some of the original chromosomes seem to yield recombination products more varied in viability than do other chromosomes. Thus, the mean variances of the recombina-

TABLE 4

Variance of the viabilities of the recombination products of chromosomes from Pira and from Rio. $\bar{V}$ = *mean variance in the crosses in which a given original chromosome participates*

	PIRA										
	13	15	48	49	68	69	72	78	82	55	$\bar{V}$
22	24.9	23.2	17.9	17.5	4.1	49.5	225.2	62.9	6.0	24.4	45.6
90	31.1	19.7	76.5	10.8	30.4	9.8	64.2	11.4	19.3	-5.6	26.8
105	17.9	51.6	47.8	31.9	27.9	33.9	3.9	195.2	19.0	4.8	43.4
118	21.7	-4.9	13.4	-1.6	4.3	-14.2	0.2	0.3	17.5	28.2	6.5
0122	0	154.1	13.9	178.4	3.4	154.0	83.3	35.0	23.9	8.2	65.4
— 127	-6.0	-13.7	62.4	231.3	-8.3	-5.5	3.6	-11.9	285.8	113.7	64.9
RC 142	17.6	5.4	84.1	66.6	2.4	-16.6	-6.0	11.3	10.2	8.3	18.3
158	25.2	1.8	26.1	16.6	0.4	194.9	310.2	3.7	336.5	341.7	125.7
190	18.7	38.7	33.2	51.1	155.3	153.0	97.9	172.2	45.5	24.5	79.0
232	169.0	36.2	10.2	4.4	32.6	18.2	-1.4	122.3	27.5	170.5	59.0
$\bar{V}$	32.0	31.2	38.6	60.7	25.2	57.7	78.1	60.0	79.1	71.9	

tion products in which the chromosomes Pira-13 and Pira-68 participate are 18.6 and 17.6 (Table 1), while Rio-122 and Rio-190 yield respectively 113.1 and 117.8 (Table 2). Since the residual variance for the original chromosomes is very low, almost the entire variance observed among the recombination products is engendered by recombination.

The release of variability

The statistical analysis of the data for *Drosophila prosaltans* is given in Tables 5 to 8. The organization of these tables is the same as that in the papers dealing with *D. pseudoobscura* and *D. persimilis*, and does not require any special commentary. Accordingly, in this section and the following one the results for all three species will be compared.

The real residual variability as given by $\overline{V}$ for the original chromosomes is in all cases negligible, with a grand average for all three species of 2.4. The variability released by crossing over can be estimated either from $\overline{V}$ for the crosses or from the component of variance within crosses, which differs from $\overline{V}$ only by the subtraction of the negligible $\overline{V}$ for the original chromosomes. For *D. prosaltans* the value of $\overline{V}$ is 68 for the Rio intrapopulational crosses, 36 for the Pira intrapopulational crosses, and the intermediate value of 54 for the interpopulational crosses. The problem of interpretation of such tests as that for the difference between the two intralocality crosses is discussed in the fourth paper of this series (LEVENE 1959). For testing whether the two particular sets of chromosomes used differ significantly, the F ratio of the two sets of $\bar{U}$'s is 1.53 which is highly significant. However, for testing whether there are real differences between the localities themselves a Student's t test is more appropriate and gives a P value of about 0.05.

Taking *D. pseudoobscura* and *D. persimilis* alone, there would seem to be a very significant difference between the amount of recombination obtained. However, we must consider this in the light of the difference found betweeen the two localities for *D. prosaltans*. It should be noted that *D. prosaltans* from Rio gives a $\overline{V}$ closely comparable to that for *D. pseudoobscura*, whereas *D. prosaltans* from Pira is comparable to *D. persimilis*. Accordingly it seems evident that the essential thing is which particular localities were chosen for each species and the rigorous test for differences between species would then be an F test based on two observations (two intrapopulational crosses) for each species. It is not clear how the interpopulational crosses could be used in this test since they do not represent an independent third locality; but since the interpopulational crosses give results usually intermediate between the two intrapopulational crosses, it seems better to ignore them for the present purpose. The test using the intralocality crosses gives $F = 3.53$ with 2 and 3 degrees of freedom which is not significant, P being between 10 and 25 percent.

Results of the analysis of variance

The results of the analysis of variance are given in Tables 5 and 6, of which Table 6 is the more interesting. We will first discuss the analysis of variance for

the means. For *D. pseudoobscura*, there is, in every case, a moderate but significant additive component between crosses, and a somewhat larger, also significant, nonadditive component between crosses, but the total between crosses variance component is only about one third the size of the within crosses component. On the other hand, for *D. prosaltans* none of the additive between crosses components

TABLE 5

Analyses of variance

Description	d.f.	On means F	P (%)	On variances F	P (%)
		Pirassununga intrapopulational cross			
Main effect	9, 35	0.69	50 –75	0.70	50 –75
Interaction	35,405	3.11	≪0.1	3.14	≪0.1
Int. of int.	9, 35	1.26	25 –50	2.29	2.5 – 5
Within	405, 50	2.19	≪0.1	...	.....
		Rio intrapopulational cross			
Main effect	9, 35	1.72	10 –25	1.06	25 –50
Interaction	35,405	3.63	≪0.1	5.60	≪0.1
Int. of int.	9, 35	0.70	50 –75	4.28	0.05– 0.1
Within	405, 50	4.26	≪0.1	...	.....
		Interpopulational cross			
Pira. main	9, 81	0.94	50	0.47	75 –90
Rio main	9, 81	2.18	2.5– 5	1.65	10 –25
Interaction	81,900	5.25	≪0.1	5.00	≪0.1
Pira. int. of int.	9, 81	0.36	90 –95	1.54	10 –25
Rio int. of int.	9, 81	0.40	90 –95	0.81	50 –75
Within	900,100	3.44	≪0.1	...	.....

are significant; but the nonadditive components are somewhat larger so that the total variance between crosses is about the same as in *D. pseudoobscura*. On the other hand the within crosses component for Rio is about the same as *D. pseudoobscura*, while for Pira this component is much smaller. Finally, for *D. persimilis* only one of the additive components of variance is significant, and even that one is extremely small, while the nonadditive components are comparable in size to those obtained for *D. pseudoobscura*. Consequently the total between crosses variance for *D. persimilis* is somewhat smaller than that for the other two species. Futhermore the component within crosses is relatively even smaller and is on the average only about twice the component between crosses. The real residual component of variance is, of course, small for all localities tested, while the binomial component, being a function of the mean, whose total variation for the crosses is only between about 20 percent and 30 percent, is more or less constant throughout. The component of variance between chromosomes for the original chromosomes has no biological meaning since these chromosomes were chosen arbitrarily, but has importance only for purposes of comparison with the additive between crosses components. These two components are usually of comparable

TABLE 6

Components of variance for means, M, *and read variances,* V, *bold face figures are estimates. Figures above and below them are approximate upper and lower 95 per cent confidence limits respectively. Values of within crosses and residual components for* V *are for comparison only (see text). All values of* V *have been divided by 100. All values have two significant figures*

	Description	Additive between crosses	Non-additive between crosses	Total between crosses	Within crosses	Total genetic	"Real" residual	Binomial sampling	Total residual
MEANS (*M*)	Intra P	2.9 **0** 0	24 **12** 5.8	.. **12** ..	41 **31** 16	.. **43** ..	20 **5.6** 0	22 **20** 18	40 **26** 18
	Intra R	19 **2.8** 0	45 **23** 12	.. **25** ..	80 **66** 52	.. **91** ..	6.6 **0** 0	23 **20** 18	27 **20** 18
	Inter P	8.3 **0** 0	 46	.. .. ..	 60	.. .. ..	.. .. 8.8	 22	 29
	Inter	———	**31**	**36**	**52**	**88**	**1.0**	**20**	**21**
	Inter R	24 **4.5** 0	21	.. .. ..	43	.. .. ..	0	18	18 .. ..
	Orig P	44 **10** 2.1	.. .. ..	.. **10** ..	.. .. ..	.. **10** ..	20 **5.5** 0	23 **21** 19	41 **27** 19
	Orig R	19 **3.1** 0	.. .. ..	.. **3.1** ..	.. .. ..	.. **3.1** ..	6.6 **0** 0	23 **22** 21	29 **22** 21
VARIANCES (*V*)	Intra P	13 **2.0** 0	31 **15** 7.4	.. **17** ..	8.5 **5.6** 3.5	.. **23** ..	.. .. ..	.. .. ..	3.6 **1.5** 0.75
	Intra R	30 **1.0** 0	140 **76** 44	.. **77** ..	22 **16** 11	.. **93** ..	.. .. ..	.. .. ..	1.6 **0.91** 0.70
	Inter P	7.8 **0** 0	 71	.. .. ..	 14	.. .. ..	.. .. ..	.. .. ..	.. .. 1.9
	Inter	———	**48**	**54**	**11**	**65**	..	..	**1.0**
	Inter R	33 **5.7** 0	33	.. .. ..	8.0	.. .. ..	.. .. ..	.. .. ..	0.76 .. ..
	Orig P	6.6 **0** 0	.. .. ..	.. **0** ..	.. .. ..	.. **0** ..	.. .. ..	.. .. ..	6.9 **2.9** 1.5
	Orig R	10 **1.7** 0	.. .. ..	.. **1.7** ..	.. .. ..	.. **1.7** ..	.. .. ..	.. .. ..	3.3 **2.0** 1.7

size except in a few cases where the variation between original chromosomes is greater than the additive variance for the derived chromosomes.

The comparability of the variance component between chromosomes for the original chromosomes and the additive component between chromosomes for the crosses suggests that the correlations between the means for chromosomes under different conditions be examined. Table 7 gives these correlations for *D. pro-*

TABLE 7

Correlation between the means in different experiments for Pirassununga (above the diagonal) and Rio (below the diagonal) chromosomes

	Original chromosomes	Intra-populational	Inter-populational
Original chromosomes		—0.46	—0.15
Intrapopulational	0.67		0.65
Interpopulational	0.59	0.49	

saltans. The general picture of these correlations is the same for all three species, but does depend somewhat on the kind of comparison being made. Accordingly, all the correlations for the six localities (two for each species) have been combined using Fisher's z transformation. The average correlation of means of the original chromosome with their effects in intrapopulational crosses is 0.03 with confidence limits −0.24 and +0.29. The average correlation of the means of the original chromosomes with their effects in interpopulational crosses is 0.26 with confidence limits of 0 and 0.49, while the average correlation between the additive effects of chromosomes in intra- and interpopulational crosses is 0.57 with confidence limits of 0.37 and 0.73. The average of all the correlations is 0.30 with confidence limits of 0.16 and 0.47. The difference between the correlation of the original with intra- and the correlation of the original with the interpopulational crosses is not significant, but each of these is significantly different from the correlation between the intra- and interpopulational crosses. Nevertheless, even this latter correlation is not very large. Thus the general picture is that there is very little connection between the way a chromosome behaves originally and its average effect in crosses with other chromosomes, but that there is a greater, though not very striking, relationship between the way a chromosome behaves in intra- and interpopulational crosses.

We come now to the analysis of variance of the variances, V. The additive between crosses variance is small and not significant, except for the effect of White Wolf in intrapopulational crosses and of South Fork in interpopulational crosses in *D. persimilis*, and a significant effect of moderate size for Texas in intrapopulational crosses for *D. pseudoobscura*. On the other hand, there is a significant non-additive component of variance between crosses, of moderate to large size, for every cross except the Texas intrapopulational cross for *D. pseudoobscura*. In other words, there are substantial differences in the amount of variability released for different crosses, but there is no particular pattern to these differences.

The observed differences are considerably larger than could be explained merely by the sampling variability of the estimates themselves.

Lethal recombination chromosomes

Some of the recombination chromosomes, both in the intra- and in the interpopulational crosses, were lethal in double dose. A list of the crosses which produced such lethals, with numbers of the lethal chromosomes in each cross which

TABLE 8

Numbers of synthetic lethal chromosomes obtained in different crosses, and means and variances of viability (percentages of wild type flies) in cultures containing nonlethal chromosomes

P = Pirassununga, R = Rio

Cross	Lethals	Viability Mean	Viability Variance	Cross	Lethals	Viability Mean	Viability Variance
P 15 × P 82	3	27.0	—12	P 49 × R 122	3	28.6	—14
P 49 × P 55	3	28.7	22	P 49 × R 127	5	28.4	23
P 49 × P 72	5	26.6	12	P 55 × R 127	1	30.6	24
P 49 × P 78	1	29.0	98	P 55 × R 158	5	35.6	—13
P 55 × P 69	1	18.0	20	P 55 × R 232	5	24.0	58
R 22 × R 127	1	25.7	49	P 68 × R 190	1	30.2	73
R 22 × R 158	1	33.8	20	P 69 × R 122	6	22.8	55
R 90 × R 190	6	27.5	7	P 69 × R 158	5	25.8	29
R 90 × R 232	2	26.8	37	P 69 × R 190	2	29.3	4
R 105 × R 122	4	29.5	72	P 72 × R 22	4	27.8	38
R 105 × R 127	1	27.6	32	P 72 × R 122	1	27.6	9
R 105 × R 190	5	32.2	213	P 72 × R 158	4	27.7	196
R 118 × R 122	4	31.8	81	P 72 × R 190	1	31.3	1
R 122 × R 127	3	29.7	—5	P 78 × R 105	1	22.9	161
R 142 × R 190	5	34.8	—7	P 78 × R 190	4	24.0	37
P 13 × R 232	3	27.1	—1	P 78 × R 232	1	34.9	2
P 15 × R 122	7	25.3	33	P 82 × R 127	5	31.6	27
P 48 × R 127	1	25.9	—4	P 82 × R 158	4	33.8	63

yielded them, is given in Table 8. A test culture which contained a lethal chromosome produced, of course, 100 *Pm S Cy* flies and no wild type flies. Table 8 shows also the mean viability (the mean percentages of wild type flies) of the recombination chromosomes which did *not* act as lethals, and the variances of these viabilities. These figures may be compared with those in Tables 1–4, which show viabilities and variances of recombination chromosomes *including* the lethals.

In all, 45 out of 900 recombination chromosomes in the intrapopulational, and 64 out of 1000 in the interpopulational crosses, were lethal in double dose; 15 intrapopulational and 20 interpopulational crosses produced at least one lethal. With all the crosses combined, we have 5.7 percent lethals among the 1900 chromosomes tested, and 18 percent of the crosses giving at least one lethal recombinant.

The question now arises as to whether all the observed lethals can be explained

as point mutations or whether some, at least, must be synthetic lethals arising purely by recombination between nonlethal chromosomes. For *D. pseudoobscura* synthetic lethals were proven by an excess of crosses with two or three lethal recombinant chromosomes. This was very improbable if all the lethals were point mutations, but *could* occur with synthetic lethals, since the percentage of synthetic lethal derived chromosomes in a given cross is not subject to hard and fast rules.

The distribution of crosses with lethal chromosomes for *D. prosaltans* is 12 crosses with one lethal, two with two, five with three, six with four, seven with five, two with six, and one with seven lethals, out of a total of 190 crosses. This distribution does not differ significantly from what could be expected from point mutations with a suitable mutation rate; on the other hand there is nothing to prevent such a distribution if, in fact, some of the lethals were synthetic. From this data an estimate of about 0.01 for the point mutation rate per generation can be obtained. Since most of this mutation would be occurring at 19°C, this estimate is quite high compared to the estimate of 0.0047 at 25°C obtained by DOBZHANSKY, SPASSKY and SPASSKY (1952). Such a comparison between experiments carried out at different times and under different conditions must be viewed with caution, but it suggests that synthetic lethals may be contributing to the apparent excess of lethals in the present experiment.

For *D. persimilis* very few lethals were observed, and thus little could be said about their distribution; however the fact that so few lethals were observed suggests that there were few if any synthetic lethals in this species, particularly as DOBZHANSKY, SPASSKY and SPASSKY (1954) found a spontaneous mutation rate in this species at 25°C of 0.013, the highest of the three species.

Finally it may be noted that there is still other evidence for the presence of synthetic lethals in *D. prosaltans*. If a chromosome produces many lethal recombination products when it undergoes crossing over with other chromosomes, it seems simplest to suppose that the strain in which this chromosome is perpetuated has acquired a lethal by point mutation. Yet even such lethals may be shown to be synthetic. Table 8 shows that the chromosome Rio-122 of *D. prosaltans* has yielded 28 lethal recombination products in seven out of the 19 crosses in which it participated; one of the crosses, Rio 122 × Pira 15, gave seven lethal chromosomes out of the ten tested. It was suspected that the Rio-122 strain carries a recessive lethal mutant. Flies from this strain were, accordingly, crossed to Lobe and Plum Star Curly flies, a new viable homozygous Rio-112 strain was isolated, and all the interpopulational Pira × Rio-112 crosses were repeated. The numbers of lethal recombination products out of the ten examined in the original test and in the retests were as follows:

Cross	*Test*	*Retest*	*Cross*	*Test*	*Retest*
Pira 13 × Rio 122	6	0	Pira 69 × Rio 122	6	0
Pira 15 × Rio 122	7	7	Pira 72 × Rio 122	1	0
Pira 48 × Rio 122	1	0	Pira 78 × Rio 122	0	0

Pira 49 × Rio 122	3	6	Pira 82 × Rio 122	0	0
Pira 68 × Rio 122	0	4	Pira 55 × Rio 122	0	2

Since the retests were made immediately following the reisolation of the homozygous viable chromosome Rio-112, the 19 lethal recombination products must certainly be synthetic lethals. PROFESSOR C. PAVAN informs us that he had a similar situation in a strain of *D. willistoni*, which gave numerous recombination lethals in the original tests and also in retests.

DISCUSSION

For convenience of reference for this discussion the main results already given in SPASSKY *et al.* (1958), SPIESS (1959), and in the present paper, as well as some additional quantities which will be discussed below are summarized in Table 9. According to the classical theory of population structure (see discussion in DOBZHANSKY 1955) most loci will possess a typical or "wild type" allele and, in addition, certain chromosomes may contain one or a few major mutant alleles which are usually deleterious. Under such conditions when two chromosomes of high viability, presumably containing "good" genes, are crossed, the resulting recombination chromosomes should also have good viability. Another model might be one in which chromosomes contain a great many polygenes which have mostly additive effects. Under such conditions two chromosomes having polygenes that give them reasonably good viability will yield upon recombination

TABLE 9

Mean viabilities and variances of homozygotes for chromosomes of three species. "Natural" refers to means and variances for samples of wild chromosomes obtained directly from natural populations. "Recombination" refers to mean viabilities and variances (V) *for chromosomes which were recombination products of pairs of quasi-normal original chromosomes obtained from nature*

Species and chromosomes	Mean viability		Variance		Recombination: Natural variance (%)	
	All chromosomes	Quasi-normal	All chromosomes	Quasi-normal	All chromosomes	Quasi-normal
D. pseudoobscura						
Natural	20.29	24.26	140	65		
					43	74
Recombination	22.92	23.93	60	48		
D. persimilis						
Natural	23.54	28.00	110	60		
					24	27
Recombination	28.16	28.76	26	16		
D. prosaltans						
Natural	21.17	25.98	200	85		
					25	28
Recombination	28.14	29.72	50	24		

chromosomes with a large array of different viabilities. However, if the effects are additive, the mean viability of all recombination chromosomes should be the same as the average of the viabilities of the two original chromosomes. Two types of interaction can change this picture, dominance, and epistasis. Since in the present case we are dealing with chromosomes in the homozygous state, only epistasis can play any part. It was pointed out in the discussion of the first paper (SPASSKY *et al.* 1959) that the nonadditive component of variance for the means represents such epistatic interaction. It has been suggested by LEWONTIN (personal communication) that the appearance of the nonadditive effect may be due to the use of the wrong scale of measurement. He suggests that viability may be multiplicative rather than additive, and that the use of the logarithm of viability rather than viability itself would be more appropriate. While this argument has a certain appeal, the use of a logarithmic transformation in our data would accentuate the nonadditivity rather than remove it. Additivity, by its very nature, can only refer to some particular scale of measurement, and in many genetic experiments, it is possible to obtain additivity by a suitable monotonic transformation. With complete additivity, the mean for recombinant chromosomes would be exactly halfway between the means for the two parental chromosomes; if this mean lay between the two parental values but not at the half way point, it might be possible to find a suitable transformation that would bring it to the half way point. However, in the present data, not only is the mean for the recombinant chromosomes very much below the mean for either set of parental chromosomes but the same is true for the median, and no monotonic transformation could bring this median to a point between the two parental medians. This is the strongest kind of evidence that epistatic interaction plays a major role in natural populations. Evidently the original chromosomes, chosen to have fairly high viabilities when homozygous, carried genes which interacted epistatically in a harmonious way, whereas the random recombination products in general contained genes that are on the average less harmonious in their epistatic interaction. It should, of course, be remembered that in natural populations the gene complexes in the chromosomes are not present in the homozygous state, but in a heterozygous state and the present evidence does not permit evaluation of the relative importance which dominance interaction and epistatic interaction in heterozygotes may have in determining a quantitative character, such as the kind of viability studied here.

The diversity of viability effects among chromosomes resulting from recombination of genes carried in pairs of apparently similar original chromosomes is very great. It is of interest to compare the variance which arises from such recombination with the total variance observed between different chromosomes obtained in nature. The distribution of viabilities for the Texas (*D. pseudoobscura*) chromosomes, of which the ones in the present study are a subsample, is given in DOBZHANSKY, PAVLOVSKY, SPASSKY and SPASSKY (1955), the distributions for *D. pseudoobscura* and *D. persimilis* from California are given in DOBZHANSKY and SPASSKY (1953), and those for *D. prosaltans*, from localities other than those

presently under study, are given in DOBZHANSKY and SPASSKY (1954). The mean viabilities and variances for these distributions have been calculated in two ways, namely for all chromosomes studied and for quasi-normal chromosomes (i.e., excluding chromosomes which were lethal or semilethal to homozygotes). The results are shown in Table 9. These two papers have reported the observed variances for quasi-normal chromosomes, and also the total residual variances. By subtracting the total residual variances from the crude variances, we obtain the "real" or "genetic" variances, corresponding to the components of variance arising through recombination within the crosses described in the present series of papers on *D. pseudoobscura*, *D. persimilis*, and *D. prosaltans*.

Table 9 also gives the ratio of the variance within chromosomes obtained by recombination in the present experiments to the variance of "natural" chromosomes, expressed as a percentage. This ratio varies from 24 percent and 25 percent (for all chromosomes in *D. persimilis* and *D. prosaltans*) to 74 percent (for quasi-normal chromosomes in *D. pseudoobscura*). This is a very remarkable result. We have selected as our "original" chromosomes groups of chromosomes which yield homozygotes of quasi-normal viability, in fact on the average above the mean viability of a random sample of quasi-normal chromosomes found in nature. And yet, we find that recombination of the gene contents of these chromosomes produces an amount of genetic variability that is a substantial fraction, between one quarter and three quarters, of the total genetic variability found among all chromosomes in natural populations. The chromosomes obtained through recombination include, in our experiments, the whole range of viabilities, from lethal, semilethal, through subvital, normal, and supervital chromosomes.

The above observations throw some light on one of the basic problems of evolutionary genetics, namely that of the mechanisms which maintain the immense genetic diversity which we find in the natural populations of sexually reproducing species. According to the classical theory of population structure, this diversity is, in any one environment, due primarily to the presence of more or less recently arisen mutants which have not yet been eliminated by natural selection. The data described in the present series of three articles militate against the classical theory. The genetic diversity which we find in the populations is evidently exceeded by the concealed or potential variability stored in the linked gene complexes in naturally occurring chromosomes, which can be released by recombination.

Indeed, the variance which is released in one generation by recombination of parts of chromosomes selected for a relative uniformity amounts to at least one quarter of the total expressed variance. The fact that some of the recombination products are semilethal or lethal when homozygous is particularly illuminating. The frequency of such synthetic lethals will be maintained in a population not by newly arising mutations, but rather by an equilibrium between the frequencies of their being "synthesized" and "desynthesized". Such a situation is compatible with the balance theory of population structure (DOBZHANSKY 1955). The genetic diversity is maintained primarily not by new mutants, but by the advantages of

heterozygosis for gene alleles and gene complexes which are kept up by natural selection, and also by environmental fluctuations in space and in time which alter the signs and the magnitudes of selective advantages and disadvantages. With a population structure of this sort, a total suppression of the mutation process would probably fail to change the evolutionary plasticity of the species for very many generations.

How widespread is the genetic population structure of the sort we have found is quite another matter. Even the three Drosophila species investigated are perceptibly, and meaningfully, different in this respect. It can be seen in Table 9 that the ratio of the variance released by recombination to the total natural variance observed in nature is higher in *D. pseudoobscura* than it is in *D. persimilis* and *D. prosaltans*. As stated in the introduction, *D. pseudoobscura* is a far more common, successful, and versatile species than *D. persimilis*, and especially than *D. prosaltans*. It has the greatest amount of potential genetic variability stored in the chromosomes, and presumably approaches most closely the population structure visualized by the balance theory.

The fact that the ratio of recombination : total variance is apparently equal in *D. persimilis* and *D. prosaltans* may seem unexpected. The latter species has also a higher expressed natural variance than *D. persimilis* and even than *D. pseudoobscura*. The answer is that the second chromosome of *D. prosaltans* used in the experiments is not a homologue of the second chromosomes of the two other species, and, contains, in fact, almost twice as much chromatin as the latter (SPASSKY, ZIMMERING and DOBZHANSKY 1950). The data for *D. prosaltans* were meant to be compared with those for the homologous second chromosome of *D. willistoni*, a very common and successful tropical species. Unfortunately, the experiments with the latter were not completed. It may also be noted that the recombination : total variance ratio in *D. pseudoobscura* appears to be higher in the California than in the Texas populations. This may be a reflection of the fact that the Texas population studied came from an extreme margin of the distribution area of the species, while the California population is subcentral.

What is the role of newly arising mutation in the maintenance of the genetic diversity in the populations of the three species studied remains unclear. The lack of reliable data on spontaneous mutation frequencies in polygenic systems is, in general, one of the greatest gaps in our understanding of the mechanisms of origin of raw materials of evolution. DOBZHANSKY and SPASSKY (1947) submitted to severe selection 14 strains of *D. pseudoobscura* propagated in laboratory cultures; the conditions of the experiment were such that the measured success of the selection could depend only on newly arisen mutants. In 50 generations, the selection was effective in 11 out of 14 strains. On the other hand, the evidence on mutation is largely negative in the present experiments. As stated above, 60 chromosomes were tested from the cultures of the original chromosomes at the end of the experiments, after they had had chance to accumulate mutants for some 10–20 generations. In only one instance (in *D. persimilis*) was the presence of a lethal ascertained. More to the point is that the study of the variance leads

to an essentially negative conclusion. As estimated from our data, the variance observed may be the sum of genetic and environmental components, and this sum, although usually ostensibly greater than zero, is quite small in replicate tests of the original chromosomes compared to the variance released by recombination.

Comparisons of the population structures in the species of Drosophila with that in the human species would obviously be devoid of basis at present. It may however be noted that MORTON, CROW, and MULLER (1956) have inferred that the genetic structure of human populations agrees best with that envisaged by the classical theory. Their method of analysis is most ingenious, involving comparison of mortality rates in the offspring of marriages between cousins and other relatives and in the general population. Unfortunately, their methods are based on several assumptions, one of which is that the loss of fitness stemming from homozygosis for genes which are heterotic in heterozygotes is due to genes each represented by only two alleles in the population. This assumption is not a necessary one. In Drosophila populations heterosis results frequently from interaction not between alleles of a single locus but between "supergenes", i.e., linked complexes of polygenes. The present series of three papers has shown that a very great variety of such "supergenes" arise in the chromosomes of every species studied by recombination. Natural selection will, in every population, select those "supergenes" which interact favorably in heterozygous combinations with other "supergenes" present in the same population.

SUMMARY

Like in the experiments of SPASSKY *et al.* (1958) and SPIESS (1959) on *Drosophila pseudoobscura* and *D. persimilis*, two groups of ten second chromosomes of *D. prosaltans* were chosen from population samples taken in two localities, both in southern Brazil. The chromosomes chosen yielded homozygotes of subnormal to normal viability. All possible intercrosses (190) between the strains carrying the 20 original chromosomes were made; from each intercross ten chromosomes, which were probably products of recombination between pairs of the original ones, were taken and tested for viability in double dose. Like in the other species, a great mass of genetic variability is released by recombination of the gene contents of the original chromosomes. The viability of the recombination products in homozygous condition ranges all the way from normal to lethal (synthetic lethals). Much of this variability released by recombination is attributable to epistatic interactions between the different polygenes contained in the original chromosomes.

Comparison of the species suggests that recombination releases more variability in *D. pseudoobscura*, a widespread, common, and ecologically versatile species, than in *D. persimilis* and *D. prosaltans*, which are more specialized forms adapted to probably narrower ranges of habitats. Even so, the amount of the variability released by recombination between pairs of quasi-normal chromosomes is astonishingly great in all three species. In *D. pseudoobscura* it amounts to about 43

percent, and in *D. persimilis* and *D. prosaltans* to about 24 percent and 25 percent of the total variance present in the natural populations from which the original chromosomes were taken (see Table 9). It is concluded that the genetic variance of the viability in natural populations depends only to a slight extent on newly arisen mutants. Most of the variance is due to recombination within the accumulated store of genetic variants.

ACKNOWLEDGMENTS

DR. ERNEST DUPRAW and MRS. O. PAVLOVSKY have made most of the extensive calculations needed for the analysis of the data here reported. MRS. GLENDA SLOANE has prepared all the typescripts. DRS. BRUCE WALLACE and J. A. BEARDMORE have made many useful suggestions and criticisms.

LITERATURE CITED

DOBZHANSKY, TH., 1955 A review of some fundamental concepts and problems of population genetics. Cold Spring Harbor Symposia Quant. Biol. **20**: 1–15.

DOBZHANSKY, TH., and C. PAVAN, 1950 Local and seasonal variations in relative frequencies of species of Drosophila in Brazil. J. Animal Ecol. **19**: 1–14.

DOBZHANSKY, TH., O. PAVLOSKY, B. SPASSKY, and N. SPASSKY, 1955 Genetics of natural populations. XXIII. Biological role of deleterious recessives in populations of *Drosophila pseudoobscura*. Genetics **40**: 781–796.

DOBZHANSKY, TH., and B. SPASSKY, 1947 Evolutionary changes in laboratory cultures of *Drosophila pseudoobscura*. Evolution **1**: 191–216.

1953 Genetics of natural populations. XXI. Concealed variability in two sympatric species of Drosophila. Genetics **38**: 471–484.

1954 Genetics of natural populations. XXII. A comparison of the concealed variability in *Drosophila prosaltans* with that in other species. Genetics **39**: 472–487.

DOBZHANSKY, TH., B. SPASSKY, and N. SPASSKY, 1952 A comparative study of mutation rates in two ecologically diverse species of Drosophila. Genetics **37**: 650–664.

1954 Rates of spontaneous mutation in the second chromosomes of two sibling species, *Drosophila pseudoobscura* and *Drosophila persimilis*. Genetics **39**: 899–907.

LEVENE, H., 1959 Release of genetic variability through recombination. IV. Statistical theory. Genetics **44**: 93–101.

MORTON, N. E., J. F. CROW, and H. J. MULLER, 1956 An estimate of the mutational damage in man from data on consanguineous marriages. Proc. Natl. Acad. Sci. U. S. **42**: 855–862.

SPASSKY, B., N. SPASSKY, H. LEVENE, and TH. DOBZHANSKY, 1958 Release of genetic variability through recombination. I. *Drosophila pseudoobscura*. Genetics **43**: 844–867.

SPASSKY, B., S. ZIMMERING, and TH. DOBZHANSKY, 1950 Comparative genetics of *Drosophila prosaltans*. Heredity **4**: 189–200.

SPIESS, E. B., 1959 Release of genetic variability through recombination. II. *Drosophila persimilis*. Genetics **44**: 43–58.