RELEASE OF GENETIC VARIABILITY THROUGH RECOMBINATION. II. DROSOPHILA PERSIMILIS

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Received February 5, 1958

RECOMBINATION between loci on chromosomes taken from a well adapted population releases an extensive array of variability (DOBZHANSKY 1946; WALLACE *et al.* 1953). To compare the amount of variability provided by recombination of linked genes affecting viability in two sibling species of *Drosophila* the experiments described here on *Drosophila persimilis* recombination follow the experiments in the preceding paper by SPASSKY *et al.* (1958) in basic plan.

D. persimilis is much less widely distributed than *D. pseudoobscura* and is more narrowly adapted since it is found commonly only in cooler and more moist habitats in western U. S. A. and southwestern Canada while the latter species occupies warmer and drier areas as well throughout western North America and Central America south to Guatemala. The populations of both species are highly heterozygous containing a great variety of gene complexes most of which are disadvantageous when homozygous: they do not differ significantly in the frequency of lethal or semilethal chromosomes (33.0 ± 4.5 percent in *pseudoobscura*, 25.5 ± 4.2 percent in *persimilis*) although the mean viability of homozygotes for the second chromosome (which is controlled in these experiments) seems lower in the former (55.2 ± 3.1 percent of control) than the latter species (67.6 ± 2.9 percent of control) (DOBZHANSKY and SPASSKY 1953). On the other hand spontaneous mutation rate seems higher in *D. persimilis* by a factor of nearly two over that in *D. pseudoobscura* (DOBZHANSKY, SPASSKY and SPASSKY 1954).

EXPERIMENTAL METHODS

The plan of the experiments is the same as that described in the preceding paper (SPASSKY *et al.* 1958), namely, second chromosomes which produced "normal" or subvital effects on viability were chosen from samples of flies taken from two different localities, ten chromosomes from each locality. A total of 45 intralocality crosses from each locality and 100 interlocality crosses were made. Single F_1 females resulting from these crosses were tested for viability of recombination homozygotes by a marker technique described below. In addition the twenty balanced chromosomes were retested for their effects in homozygous condition at the termination of the experiment (approximately two and a half years after its initiation). Each of these original chromosomes was tested in six replicate cultures as in the preceding reference.

¹ The work reported here was done under Contract No. AT-(30-1)-1775, U. S. Atomic Energy Commission.

Wild *D. persimilis* were obtained by the author in the summer of 1953 from two localities in the Yosemite region of California. One locality, called "South Fork", is in a dense fir forest at an elevation of 7000 ft. near the south fork of the Tuolumne River (and about 12 miles east of Mather, California); the other locality, called "White Wolf", is seven miles to the east of South Fork at an elevation of 8000 ft. in sparse lodgepole pine woods adjacent to a small pond not far from White Wolf Lodge. These localities are not far from each other but they are ecologically diverse.

The following year male descendants of these flies from 15 SF (South Fork) stocks and from 15 WW (White Wolf) stocks were mated to females homozygous for the recessive mutant cardinal (*cd*) on chromosome II. F_1 males from each cross were mated in single pairs to females in which one second chromosome contains the mutant cardinal, the dominant Delta, and an extensive inversion. The following generation Delta non-cardinal flies were inbred brother to sister. Since Delta is lethal in homozygous condition the progeny should consist of 33.3 percent non-Delta to 66.7 percent Delta if the wild chromosome contains "normal" viability factors. (See DOBZHANSKY and SPASSKY 1953, for description of this method).

From the SF and WW matings ten strains which produced closest to "normal" viability were chosen for the recombination experiment. They were kept in the balanced condition and maintained at 16°C, transferred each generation in the following way: Single pair and mass-mated (10–20 pairs) cultures were kept for each strain; the mass matings were reserved for insurance against loss and consisted of brothers and sisters from the single pair matings.

Intercrosses between strains were made and single F_1 females were mated to *cd/cd* males. Ten F_2 sons heterozygous for *cd*/interstrain chromosome II were then crossed to Delta *cd*/+*cd* females. Finally F_3 brothers and sisters heterozygous for Delta/interstrain chromosome II were mated (from 2–4 pairs per culture) to yield an F_4 of which 100 flies were counted from each culture. For each interstrain cross therefore 1000 flies were counted in F_4 . These F_4 cultures were raised at 25°C.

All stocks including the tester stocks were made homokaryotic for the Whitney arrangement of chromosome III.

Viability in intrapopulational crosses

The data on the effects of recombination between chromosomes of single locality origin are given in Table 1 (South Fork) and in Table 2 (White Wolf). Both tables are constructed as in the preceding paper, namely with strain numbers on the top and left margins, Roman type above the diagonal to the right are average numbers of wild type (non-Delta) flies for the ten F_4 cultures arising from recombination between interstrains; italic type below and to the left of the diagonal give variances to be discussed below, and the diagonal gives means and variances for the six replicate cultures of each original chromosome.

In every case in SF the crossover general effect is a lowering of viability (com-

TABLE 1

Viability (Roman type above and to the right of the diagonal) and variance (italic type below and to the left of the diagonal) of the recombination products of ten original chromosomes (nos. 4, 10, 12 . . . 43) from South Fork. $\bar{M}$ and $\bar{V}$ = mean viability and mean variance of recombination products, or the general effect of a given original chromosome. The squares along the diagonal show the viability (Roman) and the variance (italic) of original chromosomes

	4	10	12	21	25	27	29	30	38	43	$\bar{M}$
4	37.2 -6.76	31.5	30.6	30.4	27.8	32.7	27.6	29.7	25.6	22.4	28.70
10	13.36	30.5 14.60	29.9	29.5	27.6	28.9	23.5	32.8	30.7	34.7	29.90
12	-4.31	10.25	31.0 -2.68	31.6	25.7	26.5	29.4	18.1	32.3	23.1	27.47
21	1.73	75.70	4.90	35.3 -12.64	31.8	20.8	29.3	32.8	31.0	34.2	30.16
25	0.15	-3.50	17.13	46.53	27.0 21.49	26.7	26.4	28.7	21.3	25.2	26.80
27	0.90	53.35	-0.98	102.37	20.66	33.7 2.26	31.6	24.5	27.6	27.7	27.44
29	68.95	80.91	-5.60	-3.38	-4.10	17.50	31.5 -6.18	31.0	27.3	11.0	26.34
30	-9.31	12.13	253.95	7.25	42.22	6.67	-8.28	33.0 5.90	30.8	31.9	28.92
38	-4.56	-2.16	-0.53	-3.83	136.80	8.95	20.16	5.09	33.3 15.59	25.6	28.02
43	85.99	6.90	10.56	51.90	2.88	9.54	200.21	-3.18	88.95	30.2 27.12	26.20
$\bar{V}$	16.99	27.44	31.71	31.46	28.75	24.33	40.71	34.06	27.65	50.42	

pare the original chromosome viability with the corresponding average in the right hand column). Except for two strains (WW 3 and WW 11) the same is true for WW. But the average for the controls (original chromosomes' mean SF = 32.1 percent, mean WW = 31.7 percent) is higher than the recombination averages (intracrosses SF = 27.99 percent, WW = 28.74 percent, intercrosses = 27.75 percent). SPASSKY *et al.* (1958) found this lowering of viability except in cases where control viabilities were quite low, and they explain this phenomenon as a regression of recombinants away from the higher viability of the chosen controls

TABLE 2

Viability and variance of the recombination products of ten original chromosomes from White Wolf. For further details see Table 1 legend and text

	3	11	12	13	14	15	18	21	25	31	$\bar{M}$
3	28.3 20.31	31.0	32.7	32.4	32.3	30.9	30.3	28.0	27.7	28.2	30.39
11	23.94	28.5 0.22	32.2	32.4	27.7	31.8	32.4	26.9	29.9	31.0	30.59
12	3.33	9.90	32.8 -2.24	28.2	33.4	18.5	29.7	29.0	31.9	27.2	29.20
13	-10.12	-2.08	-6.96	31.0 3.41	28.4	28.0	31.2	27.6	30.3	27.6	29.56
14	-13.86	6.87	-1.31	9.67	35.2 -6.21	28.4	27.5	28.7	30.3	30.5	29.68
15	-0.36	8.71	360.36	11.62	40.38	32.5 -4.94	27.6	26.5	29.1	30.0	27.87
18	-12.68	10.81	-10.20	-8.63	-7.44	5.58	33.7 20.66	26.0	30.5	13.4	27.62
21	11.17	27.55	-3.92	-4.82	36.21	11.69	-5.24	33.8 -11.38	29.1	25.7	27.50
25	10.43	9.36	-2.74	3.55	1.34	37.93	-4.92	10.80	32.7 -19.01	21.0	28.87
31	6.86	-5.17	-5.58	0.69	6.19	9.90	189.33	52.69	129.18	28.2 -14.85	26.06
$\bar{V}$	2.08	9.99	38.06	-0.79	8.67	53.98	17.40	15.11	21.66	42.68	

towards the mean viability of chromosome homozygotes in the wild population from which they were taken. These data from *D. persimilis* parallel those from *D. pseudoobscura* but show a slightly smaller effect: the average lowering of recombinants with respect to their controls (intralocalities together) in *persimilis* amounts to 3.52 percent, but is 7.38 percent for *pseudoobscura* from Mather and 3.55 percent from Texas.

Random samples of chromosome II homozygotes obtained from Mather 1951 populations of *pseudoobscura* and *persimilis* give overall viabilities of 19.82 percent and 23.32 percent respectively with more than 100 chromosomes tested for each (DOBZHANSKY and SPASSKY 1953). In the 30 strains of *persimilis* tested in preparation for these experiments before choosing 20 normals, the overall average

Viability in interpopulational crosses

The grand mean of 27.75 percent is very close to those means of the intrapopulational crosses, namely, 27.99 percent for SF and 28.74 percent for WW. It has

$\bar{M}$ = the mean viability of the recombination products from crosses in which a given original chromosome participated

			S	O	U	T	H		F	O	R	K
		4	10	12	21	25	27	29	30	38	43	M
W O L F	3	28.9	32.4	32.5	32.5	22.9	32.1	29.2	26.9	27.0	31.1	29.55
	11	31.0	33.5	30.3	31.8	22.1	30.9	35.6	27.2	26.0	24.4	29.28
	12	28.3	24.3	25.0	31.7	31.2	32.7	33.0	23.5	33.1	26.0	28.88
	13	32.1	15.2	24.1	30.7	23.6	15.9	29.4	10.8	30.7	25.9	23.84
	14	27.1	31.9	24.8	31.8	27.5	31.1	30.5	30.6	29.0	22.7	28.70
T E	15	30.0	26.3	25.1	32.8	23.5	23.8	27.6	31.1	30.2	26.2	27.66
	18	29.9	32.9	27.8	29.1	26.7	30.1	30.0	29.8	29.2	20.0	28.55
W H I	21	29.0	28.1	27.2	26.7	25.7	31.0	36.5	27.8	29.4	29.4	29.08
	25	26.1	29.3	28.7	29.8	12.2	30.2	32.5	34.4	21.4	20.4	26.50
	31	24.8	30.3	26.8	28.8	28.4	21.8	25.5	32.2	25.8	10.4	25.48
	M	28.72	28.42	27.23	30.57	24.38	27.96	30.98	27.43	28.18	23.65	27.75

been demonstrated (SPIESS 1950, and unpublished data) that populations of *D. persimilis* occupying the Yosemite transect may differ markedly in their adaptive genotypes on chromosome III though populations may have come from localities only 30 miles apart. It is then to be expected that even this short distance separating SF and WW and the diversity of ecology in those areas allows for some genetic divergence between local populations. Nevertheless recombination products of chromosomes from the same or from different populations are not significantly different in viability, a fact which agrees completely with the situation in *D. pseudoobscura*.

It is also interesting that in all cases except strains WW 3 and WW 11 the inter-locality recombination average effects were lower than their original chromosome viabilities. These two strains displayed slight rise in their average effects in the intralocality effects as well. Of course both are relatively low in the control in comparison with other WW strains. In other words, average effects that are displayed in intra- and interlocality recombination hold true for particular strains but not for others. This will be discussed in more detail below in the analysis of variance.

Variance in the intrapopulation crosses

Variances given in Tables 1 and 2 in italics have been computed for each group of ten cultures with recombination chromosomes and for each group of six cultures with original chromosomes. As in the preceding paper the crude mean square (U) is obtained as the sum of squared deviations from the mean of each group divided by nine or by five, as the case may be, and is decreased by the binomial sampling error to give a residual variance (V) which represents that contributed by environmental plus genetic differences between cultures.

In SF the average residual variance for each chromosome's general effect on recombination is greater than the corresponding variance for replicate cultures of original chromosomes. The grand mean variance is 31.35 for recombination chromosomes while control variance average is 5.87.

In WW the grand mean variance (20.89) is greater than the control mean variance (-1.40) also and by nearly the same amount, but there are three chromosomes here which do not show any detectable increase in variance after recombination (WW3, 13, and 18).

Variance in the interpopulation crosses

The residual variances due to genetic and environmental differences between interpopulational recombination are given in Table 4. The grand mean of 28.36 falls between the two variance values of intrapopulational crosses. All the mean variances ($\bar{V}$) are higher than their controls including the three WW chromosomes mentioned above which produced less variance in their intralocality recombination. Especially noteworthy is WW13 which had produced no positive genetic-environmental variance when crossed to other WW chromosomes, but produces the highest variance of any WW chromosome, when crossed to SF

chromosomes. Such an effect emphasizes the nonpredictability of a recombination product based on one set of data. A chromosome in recombination with one population produces an effect entirely different from what it produced with a second population. In contrast there are quite a few chromosomes that are consistently variance producers or uniformity producers between these experiments: SF43, 25, 30, and WW31 seem to produce relatively high variances consistently while WW18, 14, and 21 consistently produce relatively low variances both intra- and interlocally.

There is then no significant difference in the amount of variance released by intralocal chromosomes in contrast with interlocal chromosomes.

TABLE 4

Variance (V) of the viabilities of recombination products of chromosomes from South Fork and White Wolf. $\bar{V}$ = mean variance within crosses involving a given original chromosome

		S	O	U	T	H		F	O	R	K	
		4	10	12	21	25	27	29	30	38	43	$\bar{V}$
F	3	11.77	4.14	26.12	14.78	169.33	-16.14	-0.05	-0.89	38.29	0.89	24.82
J	11	41.28	9.05	0.45	-2.80	99.32	24.53	122.18	-10.18	-1.46	4.71	28.71
O	12	24.59	-7.96	5.47	-2.53	-7.69	-12.01	3.67	3.13	13.52	127.87	14.81
W	13	64.08	169.78	41.38	15.61	11.53	70.29	-8.32	128.32	8.95	77.48	57.91
	14	1.19	-1.16	6.64	9.15	22.78	6.67	-1.37	101.03	-0.15	13.57	15.83
E	15	16.78	-0.94	23.97	25.03	88.24	18.70	1.58	17.34	4.92	60.66	25.63
T	18	-3.30	4.46	72.77	20.80	0.44	5.73	14.11	17.92	-0.94	51.11	18.31
I	21	48.97	18.57	-4.73	31.76	18.90	-10.28	18.65	-1.89	17.68	39.02	17.66
H	25	27.48	8.85	33.77	18.15	243.91	-13.35	-6.11	-9.86	6.56	63.80	37.32
W	31	-6.65	7.77	13.94	45.45	61.94	8.02	55.06	17.46	77.53	145.84	42.64
	$\bar{V}$	22.62	21.26	21.98	17.54	70.87	8.22	19.94	26.24	16.49	58.49	28.36

Release of variability

The magnitude of "real" variance in *persimilis* is of considerable interest for comparison with that observed in *pseudoobscura*: SF control chromosomes give a crude variance ($\bar{U}$) between replicate cultures of 27.56. Their mean viability (32.10 per cent) gives a binomial sampling variance of 21.80, the difference of 5.76 being a measure of "real" variance for SF controls. The average real variance ($\bar{V}$) for the ten control chromosomes is 5.87 ± 4.21 , which is the figure preferably used in later analysis. The original WW chromosomes gave the values: $\bar{U} = 20.16$, mean viability = 31.67, binomial variance = 21.64, and $\bar{V} = -1.40 \pm 4.23$. The $\bar{V}$ values for controls are not significantly different from each other or from zero.

On the other hand recombination produces an average real variance in each experiment which is significantly greater than the control variances, namely South Fork intra $\bar{V} = 31.35 \pm 8.3$, White Wolf intra $\bar{V} = 20.89 \pm 6.5$, and inter-locality $\bar{V} = 28.36 \pm 5.3$. While this increase in variance over the controls is significant, there seems to be no significant difference between the three values. Nevertheless in comparison with values obtained by SPASSKY *et al.* (1958) for *pseudoobscura* variances *persimilis* produces significantly less: the former shows an increase in variance of about 16 times its control average variance, while the latter shows an increase of only six times its control average variance.

In terms of standard deviation and the range of viabilities obtainable, *persimilis* original chromosomes have an average σ of approximately two and a grand mean viability of 31.8. In contrast recombinant chromosomes have a standard deviation close to five, a grand mean viability of 28.1, so that most recombinants are likely to range from semilethal (about 18) to supervital (about 38), or a range about two to three times that of chromosomes before recombination. The distribution of viabilities for chromosomes tested is however bimodal, a fairly symmetrical distribution around a mode of approximately 29.0 and a second small mode just about zero (lethals and extreme semilethals). The increment in range, then, is not so great and the probability of producing extreme phenotypes such as a lethal by recombination is much less than in *pseudoobscura*.

Analysis of variance for mean viability

Computation and tests of significance for variances are parallel with those in the accompanying papers. The results of the analysis of variance are summarized in Tables 5 and 6. Statistical methods are given in LEVENE (1959).

The total variability of viability is separated into genetic and residual components. The former is further subdivided into genetic variability between crosses (between average effects of recombination arising from particular pairs of chromosomes) and genetic variability within crosses (magnitude of variance between ten recombinants from any pair of chromosomes). The remaining variability is subdivided into environmental variance and residual genotype variance (which can be estimated by control), and binomial sampling variance.

The genetic component can be further subdivided into that produced by dif-

TABLE 5
Analyses of variance

Description	On means			On variances	
	d.f.	F	P (%)	F	P (%)
South Fork intrapopulation cross					
Main effect	9, 35	0.96	40 -50	0.63	75
Interaction	35,405	4.04	≤0.1	6.23	≤0.1
Int. of int.	9, 35	1.06	25 -50	1.63	10 -25
Within	405, 50	1.85	<0.1	3.86	≤0.1
White Wolf intrapopulation cross					
Main effect	9, 35	1.79	10 -25	2.67	1 - 2.5
Interaction	35,405	2.83	<0.1	10.83	≤0.1
Int. of int.	9, 35	4.05	0.1- 0.5	1.54	10 -25
Within	405, 50	2.04	≤0.1	4.69	≤0.1
Interpopulation cross					
SF main	9, 81	2.89	0.1- 1.0	2.44	1 - 2.5
WW main	9, 81	1.92	5 -10	1.25	25 -50
Interaction	81,900	3.87	≤0.1	3.55	≤0.1
SF int. of int.	9, 81	2.55	1 - 2.5	3.37	0.1- 0.5
WW int. of int.	9, 81	2.17	2.5- 5	2.77	0.5- 1
Within	900,100	2.02	≤0.1	4.67	≤0.1

ferences between average effects which are additive and those which are non-additive, that is acting in "epistatic," or nonadditive, ways upon recombination averages.

In no case (except possibly the SF intercross) are the additive (main) effects significantly greater than zero, while in *pseudoobscura* the main effects, though slight, are sufficient to be significant. Nonadditive effects (interactions) are all significant in *persimilis*, on the other hand, and are of about the same magnitude as in *pseudoobscura*.

SPASSKY *et al.* (1958) point out that even though additive effects are significant for *pseudoobscura* (that is, that "good chromosomes give good recombination products"), correlations between values for chromosomes under control, intra- and interconditions show little significance except that "chromosomes which give good recombination products in intrapopulation crosses tend to do so in interpopulation crosses also." From Table 7 it can be seen that these correlations in *persimilis* are not significant (with the possible exception of the SF original—SF intercross correlation) a fact which underlines the unpredictability of the recombinational effect.

The within crosses component ($\bar{V}$ minus [residual genotype + environmental effects], or $\bar{V}$ minus the control variance) is very highly significant and is larger (but not always significantly larger) than that between crosses, but as mentioned above, it is about one half to one third that found in *pseudoobscura*. In other words, chromosome recombination products are varied but less so in *persimilis*.

TABLE 6

Components of variance for means, M, and real 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 have two significant figures

		Additive between crosses	Non-additive between crosses	Total between crosses	Within crosses	Total genetic	"Real" residual	Binomial sampling	Total residual
MEANS (M)	Intra	5.7	30	..	35	..	21	21	41
	SF	0	16	16	25.0	41	5.9	20	26
		0	8.3	..	11	..	0	19	19
	Intra	7.3	16	..	30	..	9.6	21	30
	WW	1.2	7.6	8.8	22.2	31	0	20	20
		0	3.5	..	12	..	0	20	20
		16	..	..	..	..	..	..	..
	SF	3.5	..	..	..	..	..	..	..
		0.58	21	..	32	..	12	21	32
	Inter	..	14	19	30	49	2.2	20	22
		10	9.2	..	18	..	0	20	20
	WW	1.7	..	..	..	..	..	..	..
		0	..	..	..	..	..	..	..
	Orig	..	..	..	..	..	21	23	44
	SF	3.7	..	3.7	..	3.7	5.9	22	28
		..	..	..	..	..	0	21	21
	Orig	..	..	..	..	..	9.6	22	32
	WW	3.1	..	3.1	..	3.1	0	22	21
		..	..	..	..	..	0	21	21
VARIANCES (V)		0	56	..	6.2	..	..	..	3.7
	Intra	0	30	30	4.3	34	..	..	1.5
	SF	0	18	..	3.0	..	..	..	.80
		9.1	65	..	4.2	..	..	..	2.0
	Intra	0	37	37	3.0	40	..	..	0.89
	WW	0	23	..	2.1	..	..	..	.89
		11	..	..	..	..	..	..	..
	SF	2.05	..	..	..	..	..	..	..
		0	20	..	5.2	..	..	..	2.3
	Inter	..	13	15	4.1	19	..	..	1.1
		4.6	8.6	..	3.3	..	..	..	.89
	WW	0.094	..	..	..	..	..	..	..
		0	..	..	..	..	..	..	..
	Orig	0	..	0	..	0	..	..	4.3
	SF	..	..	..	..	..	..	..	3.0
		..	..	..	..	..	..	..	.98
	Orig	0.16	..	0.16	..	0.16	..	..	2.3
	WW	..	..	..	..	..	..	..	1.6
		..	..	..	..	..	..	..	.98

TABLE 7

Correlation between the means in different experiments for South Fork (above the diagonal) and White Wolf (below the diagonal) chromosomes

	Original chromosomes	Intra-population	Inter-population
Original chromosomes		.50	.62
Intra-population	— .14		.47
Inter-population	.17	.31	

Analysis of variance for variances

As far as the components of variance for the real variances (V) are concerned (lower half of Table 6) the additive (main) component is again nonsignificant except for SF interpopulational crosses. On the other hand the nonadditive component in all three experiments is highly significant. It can be seen that the between crosses components for variance are all much higher than the corresponding theoretical within crosses components, due to the magnitude of this interaction component in every case. In *pseudoobscura* the nonadditive components were not consistently significant and the total between crosses variance component nearly equalled the within crosses theoretical variance component. In other words while the amount of variability released is not so great in *persimilis* as in *pseudoobscura* the amount that is observed can be largely attributed to real differences between chromosomes in determination of interaction effects.

The additive F values for variances of Table 5 were calculated by nonparametric methods, while the components given in Table 6 were calculated in the usual way. In general, agreement in nonsignificance between these two holds, except for the WW main effect F (2.67) which is significant yet that component in Table 6 is not significantly greater than zero. Apparently there is a significant component but its magnitude cannot be estimated by the usual methods, yet it probably is not very far from zero in any case.

In Table 5 the interaction of interactions, or a measure of differences in the size of nonadditive effects for different chromosomes, or the predictability of the results for different chromosomes, is significant for viabilities WW intra- and both SF and WW intercrosses; and in both cases the nonadditive effects are significant. They are also significant for intercross variances.

It is useful to make comparisons between inter- and intrapopulational cross components of variance in viability: There is a slightly smaller genetic component for WW intra- than either SF or the interpopulational crosses; and this drop is chiefly due to a smaller interaction (nonadditive) component in WW (7.6). WW chromosomes when recombined with other chromosomes from the same population produce slightly fewer differences in nonadditive viability although their average ability to produce recombination effects within crosses is not significantly less than other intra- or interpopulational chromosomes. It is certainly evident that no marked change occurs in the magnitude of recombina-

tional viabilities when chromosomes from different populations are crossed in contrast with crosses of chromosomes from the same population.

In the components of variance for variances however there seems to be a slightly significant greater lowering of variance between crosses of chromosomes from different populations than between crosses from the same population (values of 15 *vs.* 30 and 37 respectively). The theoretical within crosses components are very close so that consequently the total genetic component for interpopulational variance remains less than for intrapopulational variances. In other words the genetic variances which come about through specific recombinations of particular chromosome pairs are slightly more different when the chromosomes come from one population than when they come from two different populations. SPASSKY *et al.* (1958), however, found that there were no significant differences either for the means of *M* and *V* or the components of variance of *M* and *V* in *pseudo-obscura*.

Whether this minor difference between the results of *persimilis* and *pseudo-obscura* recombinational effects is of fundamental importance, it is premature to say. If it is important the proximity of localities from which flies were taken in *persimilis* might be a possible clue, though by no means an explanation of this difference.

Lethal chromosomes

From inspection of Table 8 it can be seen that 19 out of 900 "intrarecombination" chromosomes and 26 out of 1000 "interrecombination" chromosomes were lethal in homozygous condition (or 2.1 percent and 2.6 percent respectively).

TABLE 8

Numbers of lethal chromosomes obtained in different crosses together with mean viabilities and variances in cultures when the presence of lethals is disregarded

Cross	Lethals	Mean viability	Variance (residual)
SF4 × SF43	1	24.9	27.92
SF12 × SF30	4	30.2	25.90
SF29 × SF43	5	22.0	152.84
SF38 × SF43	1	28.4	9.17
WW12 × WW15	4	30.8	198.09
WW18 × WW31	3	19.1	157.71
WW25 × WW31	1	23.3	84.88
SF10 × WW13	4	25.3	1.76
SF25 × WW 3	2	28.6	32.72
SF25 × WW25	6	30.5	-1.53
SF27 × WW13	5	31.8	34.51
SF30 × WW13	5	21.6	1.3
SF38 × WW31	1	28.7	-4.21
SF43 × WW12	1	28.9	51.08
SF43 × WW13	1	28.8	-5.01
SF43 × WW31	1	23.7	49.56

The means and variances of nonlethal cultures remaining out of ten cultures counted are also given in that table and can be compared with those in Tables 1-4. Only seven intrapopulational and nine interpopulational crosses produced at least one lethal (or 8.4 percent of the 190 crosses made).

It might be emphasized at this point that all control counts were made towards the end of the experiment. In one strain (WW3) which had been nearly finished at the time, the entire control count showed lethality and the strain had to be reextracted from the wild strain, tested, and crossed out again for recombination to all other strains. Undoubtedly such a change to lethality was due to point mutation, since the chromosomes were maintained in the balanced condition. On the other hand, since SPASSKY *et al.* (1958) have shown that it is likely that recombination between nonlethal complexes may engender homozygous lethal compounds in *pseudoobscura*, or synthetic lethals, it is important to weigh the evidence in *persimilis* for the likely origin of these lethal homozygotes.

It has been shown that even if all interstrain F_1 heterozygous females carried a recessive lethal, the maximum number of crosses giving one lethal culture out of ten cultures counted would be two crosses out of 190. Since there were seven such crosses it is more likely that if the lethality is due to point mutation it must have occurred subsequently, namely in the F_2 males tested in those seven crosses. On the other hand there are one cross with two lethal cultures, one cross with three lethal cultures, three with four, three with five and one with six or a total of nine crosses which produced nearer expected 0.5 probability of sons carrying the lethal from a heterozygous mother. This distribution can be compared with an expected distribution among nine samples of ten each with $p = \frac{1}{2}$ (disregarding the samples with one or no lethals). A chi-square test gives $\chi^2 = 2.44$ with $p = 80$ percent. These can therefore be accounted for on the distribution following heterozygosity of the strain cross F_1 mother.

The other seven single lethal crosses are more easily explained by mutation in the F_2 sons tested. Out of the total number of sons tested how many are likely to have point lethals produced? Mutation rate on chromosome II in *D. persimilis* was measured by DOBZHANSKY *et al.* (1954) at room temperature to be 1.31 percent per chromosome generation. There were 1900 F_2 chromosomes tested in which mutation at that rate was likely to produce an average of 25 lethals appearing as one lethal containing culture out of ten. Perhaps one contributory factor to the low number of observed lethals might be the fact that numerous F_1 and F_2 crosses were made at 16°C, and lowered temperature slows the mutation rate.

Lethality among the recombination chromosomes of *persimilis*, then, is not of any magnitude nor is it distributed in any way which cannot be explained by point mutation. From the general discussion of range (see "Release of variability" above) of viability it is not expected that many extreme phenotypes would be engendered by recombination in this species.

Nonlethal cultures

If all observed lethal chromosomes (2.4 percent of the total) were produced by point mutation, the main changes in viability and variance would still be more or less the same. If these lethal chromosomes are disregarded in computation of means and variances, it can be seen that the general averages show little change. The new values would be as follows:

	Mean Viability ($\bar{M}$)	Variance ($\bar{V}$)	
SF intralocality	28.63%	22.17±	5.58
WW intralocality	29.19%	15.60±	6.00
Interlocality	28.52%	17.86±	3.96

The mean viabilities have all increased less than one percent, while the variances have decreased in parallel but are still significantly greater than their controls.

A recalculation of variance analysis on these data excluding lethals has not been done since they amounted to such a low percentage of the total.

Recombination effects in two sibling species

The more narrowly distributed and adapted species, *D. persimilis*, consistently displays less variability after recombination than its related species, *D. pseudoobscura*. To what phenomena can this contrast be ascribed?

Before discussing intrinsic differences between these species, we should look for contrasts between the experiments reported herein and in the paper of SPASSKY *et al.* (1958). The *persimilis* control chromosomes chosen were higher in viability than those in *pseudoobscura*. Very often those strains which showed lowest original viability gave greatest variance in *persimilis*; though that correlation was not tested for significance, it may be that if slightly lower viability chromosomes were chosen in *persimilis* a greater similarity with *pseudoobscura* might result.

According to DOBZHANSKY (1946, 1955) and WALLACE *et al.* (1953) who discuss the balance theory of fitness which depends on high heterozygosity in a population, such heterozygosity must be maintained by providing a variety of alleles at each locus on structurally homozygous chromosomes. Alleles preserved by natural selection must give high fitness not only by allelic interaction (heterosis) but by nonallelic interaction, or compatibility between heterozygous loci as well. If a small sample of such chromosomes is allowed to recombine and homozygotes measured phenotypically, it may be reasonable to infer a relative estimate of heterozygosity from the magnitude of variability obtained. However, a measure of heterozygosity can be obtained simply by getting the frequency of recessives in a population. California populations of *pseudoobscura* and *persimilis* did not differ significantly in the frequency of lethals and semilethals (although the latter's frequency was slightly lower than the former's). Rather the interpretation of less variability produced by recombination in *persimilis* may mean less epistatic interaction of nonallelic loci, though heterozygosity may be equal to that

in *pseudoobscura*, that is, recombination's effect is less drastic and produces less extreme viabilities. If that is so, *persimilis* either has not yet evolved epistatic systems as great as *pseudoobscura* or they are not as adaptively valuable to *persimilis* in the area sampled.

It is important to remember that the epistatic effects on viability are selected for their superiority when most loci are heterozygous. The proper balance between heterozygosity and epistatic effects between loci must be maintained. If *persimilis* can maintain heterozygosity equal to *pseudoobscura* without evolving equal epistatic interaction, either a higher mutation rate must exist in that species or recombination must be suppressed to preserve heterozygous blocks. The mutation rate is higher in *persimilis*; from the lack of structural heterozygosity on chromosome II in these species we may assume equal crossing over for them. On the other hand, if *persimilis* is really less heterozygous than *pseudoobscura*, less change would be expected from recombination: if more homozygosis exists, natural selection should eliminate deleterious recessives and bad epistatic interactions in homozygous recombinants. Consequently from the observation that *persimilis* shows a less drastic recombination effect, a proportional decrease in heterozygosity is not necessarily to be inferred.

SUMMARY

Two samples of ten second chromosomes each of *Drosophila persimilis* were chosen for their near normal viability in homozygous condition from populations occupying localities in Yosemite National Park, California.

A total of 1900 recombinant chromosomes from these original 20 were tested for viability effects in homozygous condition by an experimental plan exactly parallel with the preceding and the following papers.

Recombinant viabilities are lower on the average than control viabilities, that is they regress toward the average viability of homozygotes in the populations from which they come. The range of viability observed among recombinants extended from lethality to supervitality, but neither the number nor distribution of lethals is greater than or unaccountable by point mutation. This fact is in marked contrast with *D. pseudoobscura* which certainly produced some lethals "synthetically", that is by recombination.

Analysis of variance shows a significant increase in the genetic components of variability especially in interaction (nonadditive) effects on viability and variance differences over their controls. None the less, the magnitude of this array of variance ($6 \times$ the controls) is not as great as in the sibling species, *pseudoobscura* ($16 \times$ the controls).

While it is known that both species are highly heterozygous, it is possible that the difference between them in these recombinant effects may be due to less epistatic interaction of nonallelic loci in *persimilis* than in *pseudoobscura*. On the other hand a real difference in amount of heterozygosity between these species may account for their differences in recombination effects.

ACKNOWLEDGMENTS

The author is very grateful for the opportunity to share in this extensive experiment on recombination reported in accompanying papers. We are indebted to Professor TH. DOBZHANSKY for inspiration and to Dr. HOWARD LEVENE for guidance through the statistical analysis, to Mr. BORIS SPASSKY for providing tester stocks, and to Miss MARY E. ROWAN and Mr. JACK CAPENOS for assistance in conducting the experiments.

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