COMPARISON OF FREQUENCY DISTRIBUTIONS OF VIABILITIES OF SECOND WITH FOURTH CHROMOSOMES FROM CAGED *DROSOPHILA MELANOGASTER*[1]

ALICE KENYON

Department of Zoology, University of Georgia, Athens, 30601

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TWO properties, small number and lack of detectable intrachromosomal recombination in diploids, have influenced coadaptation of genes on the small fourth chromosome of *Drosophila melanogaster*. To estimate dominance of fitness at theoretically single loci, it has seemed necessary to reason from the viability effects of whole chromosomes to individual loci by making unjustified assumptions about patterns of interaction between genes. This procedure always yields more (for nonlethal loci) or less (for lethal loci) equivocal results and is not only unnecessary, but undesirable in the case of the fourth chromosome. Owing to absolute linkage, the genetic region being subjected to natural selection is not smaller than the entire fourth chromosome. Hence, conditions for heterosis of *entire* fourth chromosomes is the only meaningful theoretical *or* experimental issue. This study shows that nonlethal fourth chromosomes are not, on the average, heterotic with respect to those components of fitness included in the measure, "relative viability".

METHODS

Flies trapped in a grocery store at Syosset, Long Island founded a 16 cup, plastic population cage (MS-1 AK) maintained several years at controlled temperature (24°C) on unsalted SPASSKY (1943) medium. The fourth and second balanced lethal marker stocks, ci^D/ey^D and *Cy L/Pm*, respectively, were reconstituted from segregants (selected for conspicuous expression of dominant markers) of massive and repeated outcrosses to individuals from this cage. From 1961 to 1963 this population provided a continuous source of males for alternating second and fourth chromosome extraction procedures. A caged great-grandfather (+/+) was mated with a cage-outcrossed ci^D/ey^D or *Cy L/Pm* female (P_1). An individual singly marked (F_1) grandfather ($ey^D/+$ or *Pm*/+) was backcrossed to doubly marked females (P_2). Differently marked F_2 were either mass intracrossed ($ey^D/+_1 \times ci^D/+_1$) or intercrossed ($ey^D/+_1 \times ci^D/+_2$) to produce the scored F_3, a four class segregation in which the unmarked class carries test chromosomes either from the same great-grandfather (homozygotes) or from different great-grandfathers (heterozygotes), respectively. The F_3 developed in half pint bottles at 23 to 24°C on killed yeast-enriched, cornmeal/agar medium (WALLACE, personal communication).

The genetic background of test chromosomes was uncontrolled. For a fixed amount of work, it was desirable to have a large number of replicates at the expense of a large number of independently extracted chromosomes since by cytological criteria (COOPER 1950), almost all the genome is in the background in the fourth chromosome experiments. "Relative viability" is the

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number of unmarked flies within a culture divided by number of doubly marked flies within the same culture. For each determination of mean relative viability, eight replicate determinations were attempted. Four replicate crosses employed parents of different identity but identical test genotypes. One transfer to fresh media made replicate cultures having identical parents of different ages. Cultures producing less than 95 total flies were automatically excluded from analysis. The overall harmonic mean numbers of included replicates were:

7.5—chromosome-2 homozygotes
7.8—chromosome-2 heterozygotes
6.6—chromosome-4 homozygotes
6.7—chromosome-4 heterozygotes

In determining relative viabilities, cage test chromosomes were never held in balanced condition longer than the required three generations. Unmarked heterozygotes were identifiable in terms of their midparents and developed under conditions as comparable as possible. Each cross was coded at the time I scored it, typically the 12th, 14th and 17th day of hatching. The total number of independently extracted chromosomes tested as homozygotes and heterozygotes was 125 for fourth chromosomes and 116 for second chromosomes.

RESULTS

A "lethal" chromosome, when homozygous, gave an arithmetic mean proportion of unmarked F_3 flies less than 0.011. The pooled frequency of lethal among all independently extracted second chromosomes was 16 out of 116 or .138. The frequency of noncomplementing compounds among all possible was four out of 120 or .033. The frequency distribution of appearance of at least one lethal gene among 16 independently extracted lethal seconds was 11 once, one twice, one three times and none more than three times. The pooled frequency of sterile second chromosomes was 14 out of 102 or .137. Two of the 14 steriles were also complementing lethals; 13 were male sterile and one was female sterile. The pooled frequency of sterile and/or lethal seconds was 28 out of 116 or .241.

Among 160 fourth chromosomes extracted during 1961–1963, one female sterile, one male sterile and *no* lethals were found. Unconscious selection against slowly developing F_1 or F_2 cultures may have been partly responsible for the relatively low frequency of second lethals and the initial failure to find fourth lethals. A 1964 search in the same cage yielded seven out of 465 or .015 lethal fourth chromosomes. One of these lethals (AKle-82) is poorly viable with ey^D. One of the 21 possible intercrosses between lethals failed to complement. Some 1966 assays have yielded suppressed-lethal fourth chromosomes as anticipated by MAGALHÃES *et al.* (1965). Over all assays of this cage, the ratio of fourth to second frequencies of sterile and/or lethal chromosomes remains about one to 20. One to 20 is also the reported maximum ratio of fourth- to second-chromosome loci capable of mutating to lethality as inferred by LEWONTIN and PROUT (1956). HOCHMAN (personal communication) finds lethal fourth chromosomes from natural populations in the frequency (pooled over time and location) 22 out of 1,721 or .013. The partial genetic load due to lethal fourth chromosomes is similar in magnitude in this cage and in HOCHMAN's natural populations.

Figure 1 gives frequency distributions of mean relative viabilities for second and fourth chromosomes plotted on a common mean relative viability scale. The shift to the right in going from second to fourth distributions is due to lesser likeli-

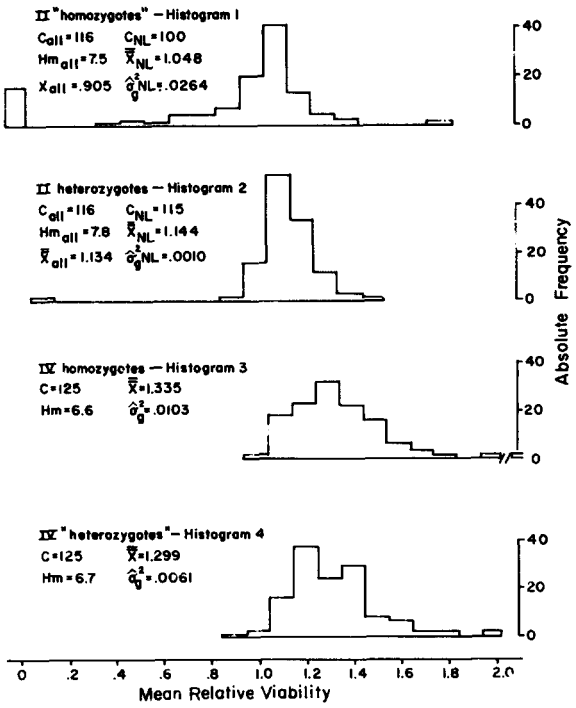

FIGURE 1.—Frequency distributions of mean relative viabilities. $\bar{\bar{X}}_{all}$ is the grand mean of the entire distribution. $\bar{\bar{X}}_{NL}$ is the grand mean of that portion of the entire distribution having a mean proportion unmarked ≥ 0.011. $\hat{\sigma}_g^2$ estimates the "genetic" component of variance of relative viability. C is the number of independently extracted chromosomes. Hm is the harmonic mean number of replicates.

hood of ci^D/ey^D than $Cy\ L/Pm$ flies surviving until scoring time. Tables 1 and 2 give results of hierarchical analyses of variance of relative viability of fourth and second chromosomes, respectively. Since in every case analysis of variance of proportion unmarked leads to qualitatively similar conclusions, those proportions are not reported here. In Figure 1, $\hat{\sigma}_g^2$, the "genetic" component of the distributions' total variance of relative viability, is estimated from the difference of mean squares (MS) divided by the harmonic mean number (Hm) of replicates (Tables 1 and 2). $\hat{\sigma}_r^2$, the average among-replicates, within genotype component of total variance is estimated by the lesser mean square (Tables 1 and 2).

For nonlethal second chromosomes, the approximate 95% confidence interval for the difference, +.096 (Table 2), between grand mean relative viabilities of unmarked heterozygotes and homozygotes is +.056 to +.136 and does not overlap zero. But fourth chromosomes extracted from the same population at similar times give a qualitatively different result. The approximate 95% confidence interval for the difference, −.036 (Table 1), between grand mean relative viabilities of unmarked heterozygotes and homozygotes is −.079 to +.007 and overlaps zero. An average superiority of fourth chromosome unmarked heterozygotes

TABLE 1

Fourth chromosome analysis of variance of relative viability

Experiment	C	$\bar{\bar{X}}$	Sums of squares: Main	Deviations	Total	No. of replicates	Hm
Homozygotes							
Nov. 1961	24	1.518	9.1181	29.0998	38.2179	126	5.00
Jan. 1962	25	1.344	4.1525	12.2053	16.3578	156	6.20
June 1962	25	1.319	3.3141	13.1987	16.5128	192	7.62
Aug. 1962	36	1.243	3.4117	17.8523	21.2640	274	7.50
Dec. 1962	15	1.273	1.9442	7.8867	9.8309	118	7.81
Pooled Total	125	1.335	21.9406	80.2428	102.1834	866	6.64
Unmarked "Heterozygotes"							
Nov. 1961	24	1.428	2.8884	9.5691	12.4575	125	5.00
Jan. 1962	25	1.360	3.5501	14.4079	17.9580	162	6.44
June 1962	25	1.293	3.0659	10.9365	14.0024	191	7.60
Aug. 1962	36	1.202	3.7895	14.4075	18.1970	275	7.58
Dec. 1962	15	1.231	0.8095	4.8104	5.6199	119	7.94
Pooled Total	125	1.299	14.1034	54.1314	68.2348	872	6.72

Source	SS	df	MS	EMS	F	P
Homozygotes						
Main	21.9406	124	.1769	$\mathrm{Hm}\sigma_g^2 + \sigma_r^2$	1.63	$<.005$
Deviations	80.2428	741	.1083	σ_r^2		
Total	102.1834	865	....	..		
Unmarked "Heterozygotes"						
Main	14.1034	124	.1137	$\mathrm{Hm}\sigma_g^2 + \sigma_r^2$	1.57	$<.005$
Deviations	54.1314	747	.0725	σ_r^2		
Total	68.2348	871	.	..		

more than 0.5% would be inconsistent with these observations. Based on the lower (second chromosome and upper (four chromosome) confidence limits for the grand mean difference between heterozygote and homozygote relative viabilities, the relation $400 : .056 = X : .007$ estimates that 50 is the maximum number of loci on chromosome 4, relative to 400 on chromosome 2, consistent with the observed difference of grand means and a constant contribution to it per locus. It is reassuring that this uncertain maximum estimate of 50 is not less than HOCHMAN, GLOOR and GREEN's (1964) revised minimum of 35. BAND and IVES (1963) summarize the evidence for the generalization that the grand mean of *any* frequency distribution of autosomal "homozygotes' " viabilities is always less than corresponding heterozygotes' in both natural and experimental populations of several species of Drosophila, but the present study shows that the generalization does not necessarily apply to small autosomes.

In Figure 1, the ranges of histograms 3 and 4 are about the same. For both relative viability (Table 1) and proportion unmarked (not reported), in both homozygotes and unmarked heterozygotes, the probability that MS I = MS II is less than .005. In each of these four analyses there is a small but real effect of independently extracted fourth chromosomes on total observed variation that cannot be entirely accounted for by variation among replicates within test geno-

TABLE 2

Second chromosome analysis of variance of relative viability

Experiment	C	$\bar{X}$	Sums of squares: Main	Deviations	Total	No. of replicates	Hm
Nonlethal "Homozygotes"							
April May 1962	21	1.060	3.9628	4.6023	8.5651	152	7.09
Sept. Nov. 1962	27	1.009	6.4469	7.4862	13.9331	204	7.48
May Aug. 1963	37	1.089	9.4128	22.7331	32.1459	286	7.68
Sept. 1963	15	0.999	5.5262	3.6672	9.1934	118	7.81
Pooled Total	100	1.048	25.3487	38.4888	63.8375	760	7.52
Nonlethal Heterozygotes							
April May 1962	26	1.127	1.3180	6.9309	8.2489	192	7.28
Sept. Nov. 1962	28	1.142	2.2356	12.0775	14.3131	222	7.89
May Aug. 1963	44	1.162	3.7758	22.0818	25.8576	349	7.91
Sept. 1963	17	1.124	0.3181	5.2532	5.5713	134	7.83
Pooled Total	115	1.144	7.6475	46.3434	53.9909	897	7.75

Source	SS	df	MS	EMS	F	P
Nonlethal "Homozygotes"						
Main	25.3487	99	.2560	$\mathrm{Hm}\sigma_g^2 + \sigma_r^2$	4.39	$<.005$
Deviations	38.4888	660	.0583	σ_r^2		
Total	63.8375	759	....	..		
Nonlethal Heterozygotes						
Main	7.6475	114	.0671	$\mathrm{Hm}\sigma_g^2 + \sigma_r^2$	1.13	$>.10$
Deviations	46.3434	782	.0593	σ_r^2		
Total	53.9909	896	....	..		

type. The "genetic" variance component of homozygotes' relative viabilities is ostensibly, but not significantly, higher than unmarked heterozygotes' ($F = 1.69$ with 17.6 df in numerator, 15.3 df in denominator; $P > .20$). Both "genetic" components of variance of relative viability are significantly different from zero (Table 1). Excluding lethals, there is no significant difference in magnitude between concealed and expressed "genetic" components of variation of fourth chromosome relative viability. This lack of distinction was anticipated by HOCHMAN (1961).

There is one respect in which fourth chromosomes obey previous generalizations for large autosomes. Higher replicate variance for "homozygotes" than heterozygotes has been observed in this and other species of Drosophila (DOBZHANSKY and WALLACE 1953; BAND and IVES 1963). For both relative viability and proportion unmarked, the "replicate" component of variance, $\hat{\sigma}_r^2$ (Table 1), is significantly higher in homozygotes than in unmarked heterozygotes ($F = 1.49$ with 741 df in numerator and 747 df in denominator; $P \leq .02$). Binomial sampling variance accounts for about 3/5 the "replicate" component of total variance of proportion of unmarked flies in both homozygotes and heterozygotes. The near equality is due in part to the ostensibly greater, but not significantly different, mean number of flies (398.4 *vs.* 373.6) in cultures that give unmarked heterozygotes as opposed to homozygotes, respectively ($t = 1.46$ with 248 df; $.20 > P > .10$).

DISCUSSION

Lack of fourth chromosome recombination offers a good chance for the accumulation of linked genes producing pseudo-overdominant supergenes; these were not, on the average, found. Trisomy would offer an extra protection from elimination by natural selection, of detrimental fourth chromosomes. To the probably slight extent that Triplo-4 flies can live to reproduce in this cage, the experiment is biased toward seeing excess viability variation. Haplo-4 flies cannot explain deficient variation since they are of negligible fertility. With one possible reservation, there is no obviously biased feature of design which would, *a priori*, render this study incapable of showing average heterosis of viability for fourth chromosomes. The reservation is that presumptive background heterozygosity may be a restrictive agency for manifestation of overdominance as argued by MUKAI, YOSHIKAWA and SANO (1966).

Figure 2 is a scatter diagram of 125 independent fourth chromosome unmarked heterozygotes' mean relative viabilities (y) on mean relative viabilities of their midparents (x). The correlation coefficient is +.47. For 123 degrees of freedom, the probability that the true correlation is zero is less than .001. However, Table 1 shows the (partial) grand mean relative viabilities ($\bar{\bar{X}}$), of heterozygotes and homozygotes go up and down together from experiment to experiment raising the suspicion of an environmental influence on slope other than strong, constant partial "dominance" of test chromosome viability effects.

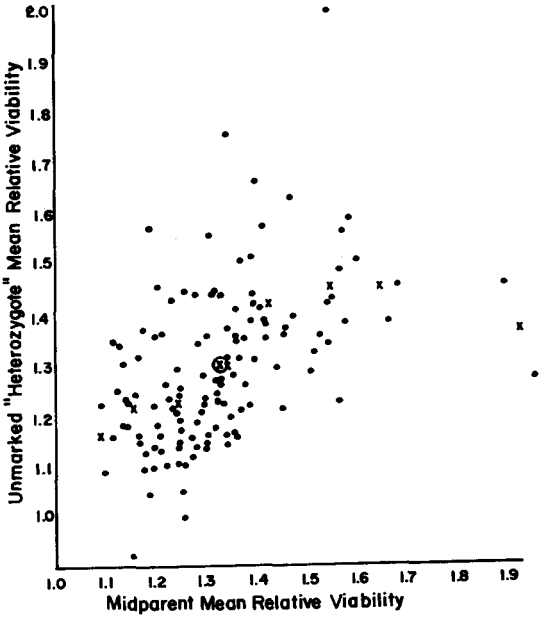

FIGURE 2.—Dots are mean relative viabilities of fourth chromosome unmarked "heterozygotes" (y) on that of their midparents (x). Crosses are means of means within successive tenths of midparents. Encircled cross is the grand mean midparent, grand mean heterozygote (1.335, 1.299) relative viability. Correlation coefficient = +.47 with 123 df; P ($\rho = 0$) <.001.

A procedure suggested by PROFESSOR B. WALLACE (personal communication) estimates the strength of this non-"dominance" influence on slope. By mispairing y with x (e.g., letting $y =$ mean relative viability of unmarked heterozygote 39/40 paired improperly with $x =$ mean relative viability of midparent of 37/37 and 38/38) within the same cycle of crosses made within any one week, the correlation coefficient of improper unmarked fourth heterozygotes' with fourth midparents' mean relative viability is +.41 (123 df; $P < .001$) which is obviously *not* due to "dominance". The total correlation of proper heterozygotes with midparents, +.47, is more correctly accounted for by a strong partial correlation of both with test environment plus a weak partial correlation due to "dominance", the latter not significantly different from a true correlation of zero. It may be heterogeneous (over time) total yields of F_3 flies and density dependent relative viabilities that cause the correlation of all viabilities with environment. The lack of average heterosis suggested partial dominance which could (only if constant, independent of value of viability effect) be revealed by a positive correlation of heterozygotes with midparents. Yet the observed positive correlation cannot be used as evidence against overdominance or against variable partial dominance since the correlation has been shown largely spurious with respect to any hypothesis about the viability effects of extracted chromosomes.

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SUMMARY

Flies homozygous for fourth chromosomes extracted from caged *D. melanogaster* show higher viability than individuals carrying unmarked fourth chromosomes from different caged great-grandfathers when tested in an uncontrolled genetic background. Although the viability effects studied are relatively slight, they are statistically significantly varied effects. There is a lack of inbreeding depression among fourth chromosomes whereas nonlethal second chromosomes from the same population show about 8% inbreeding depression. Although an average superiority of fourth chromosome unmarked "heterozygotes" more than 0.5% would be inconsistent with these observations, the statistically highly significant positive correlation of viabilities of fourth unmarked "heterozygotes" with midparents does *not* imply constant parital dominance as opposed to variable semidominance or "overdominance" of viability effects.

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