Genet. Res., Camb. (1975), **25**, *pp.* 267–274
Printed in Great Britain

An experiment on recombination load in *Drosophila melanogaster*

BY BRIAN CHARLESWORTH AND DEBORAH CHARLESWORTH
*Department of Genetics, University of Liverpool**

(*Received* 9 *December* 1974)

SUMMARY

This paper describes the results of an experiment to measure the effect on mean population fitness of recombination in the second chromosome of *Drosophila melanogaster*. There was a small and non-significant effect of recombination in lowering egg-to-adult viability of heterozygotes for wild-type chromosomes. A large (7 %) and significant effect of recombinant chromosomes on the fecundity of *Cy* female carriers was detected.

1. INTRODUCTION

Since the work of Dobzhansky (1951) it has been widely accepted that inversion polymorphisms in organisms such as *Drosophila* are maintained as a result of the selective advantage of genetic factors which (like inversions) reduce the frequency of crossing over between polymorphic genes which interact in their effects on fitness (Fisher, 1930). As we have shown in an earlier publication (Charlesworth & Charlesworth, 1973), the chance of survival of a new inversion under such selection in a large population is roughly $\frac{1}{2}\sqrt{L_r}$, where L_r denotes the genetic load due to recombination in the segment of chromosome within which crossing over is suppressed in inversion heterozygotes. This 'recombination load' is defined here as follows. Suppose we consider a randomly mating population in equilibrium under selection at many loci. Its mean fitness (i.e. the mean fitness of a set of zygotes before selection has acted on the population) is w_0, say. We take a random sample of chromosomes from the population after selection but before recombination has taken place. By random combination of these chromosomes we form a population whose mean fitness is w_1. L_r is defined as $(w_1 - w_0)/w_1$. Provided that the population is approximately in equilibrium under selection pressures maintaining non-zero linkage disequilibrium, we will have $L_r > 0$ (Kojima & Kelleher, 1961; Charlesworth & Charlesworth, 1973).

In this note, we will present the results of an experiment designed to estimate the value of L_r for the second chromosome of *Drosophila melanogaster*. We have made use of the general absence of crossing-over in males of this species in the following way. Measurements of components of fitness on a population formed by combining at random chromosomes derived from unrelated wild-caught males

* Present address: School of Biological Sciences, University of Sussex, Brighton BN1 9QG, Sussex.

will give us an estimate of w_1 as defined above. A population formed by combining chromosomes from males with chromosomes from females will give us an estimate of w_0. It can be shown that a population formed by combining chromosomes derived from unrelated females will suffer about twice the recombination load of the wild population, assuming that the differences between corresponding male and female gamete type frequencies are small enough for their squares to be negligible. We attempted to estimate L_r for egg-to-adult viability by utilizing these genetic properties of *D. melanogaster*. As will be seen, the experiment also yielded information about female fecundity.

2. MATERIALS AND METHODS

Sets of 117 second chromosomes were extracted from wild male and female flies kindly provided for us by Dr P. T. Ives, who trapped them in the Markert Apple Store, Amherst, Massachusetts, in October 1972. These chromosomes thus came from a large population which maintains itself from year to year (Ives, 1970). Each chromosome of the male-derived set (M chromosomes) was extracted from a different wild male by crossing with a stock of *Cy* (*SM5*)/*S ast* by the usual procedure (Wallace, 1968). The female-derived chromosomes (F chromosomes) were obtained by allowing the wild-fertilised females to produce F_1 offspring and extracting a chromosome from a single son of each female. Approximately one-half ($0{\cdot}5 \pm 0{\cdot}046$) of the F chromosomes are descended from a chromosome produced by a female of the wild population, and the other half from a male of the wild population. The M and F chromosomes thus differ in the average amount of recombination to which they have been exposed. Since the F_1 offspring were reared under optimal conditions in the laboratory, the M and F chromosomes should not differ greatly in the amount of selection to which they have been exposed.

We estimated the mean viabilities of three populations: $M \times M$, $M \times F$ and $F \times F$. Each M and each F chromosome was given a number between 1 and 117. The $M \times M$ population was constructed by a set of cyclically permuted crosses *Cy*/*M1* × *Cy*/*M2*, *Cy*/*M2* × *Cy*/*M3*, . . . , *Cy*/*M117* × *Cy*/*M1*. Each cross and its reciprocal was replicated once; both reciprocal crosses were set up at the same time, but replicate crosses were carried out many weeks apart. The $F \times F$ population was constructed similarly. The $M \times F$ population was made up of crosses *Cy*/*M1* × *Cy*/*F2*, *Cy*/*M2* × *Cy*/*F3*, . . . , *Cy*/*F117* × *Cy*/*M1*, with reciprocals and replicates. Equal numbers of $M \times M$, $M \times F$ and $F \times F$ crosses were set up at any one time in blocks of about 100 matings, one block being set up each week for the duration of the experiment. Virgins were collected 1 week before being used in three-pair matings in 3 × 1 in. vials containing a standard amount of dried-yeast/agar/sucrose medium. Parents were discarded 4 days after setting up. The numbers of *Cy* and + flies emerging from each vial after 13, 15 and 17 days were recorded and the totals for each genotype over the period of emergence calculated for each vial. All cultures were maintained at 25 ± 1 °C. The experiment extended over about 4 months. The work of counting was divided roughly evenly between the two authors; all cultures were

coded in such a way as to preclude knowledge of their identity at the time of counting.

3. RESULTS

(i) *Egg-to-adult viability*

This component of fitness is measured as the viability of + relative to that of *Cy*/+ heterozygotes. For purposes of statistical analysis this was expressed as the arcsin (in radians) of the square root of the proportion of + in each culture. Table 1 shows the means and the analyses of variance into mean squares for genotypes (excluding reciprocal differences), reciprocal differences and error, for each of the three populations. It will be seen that there are significant nuclear genotypic differences and reciprocal differences only in the $M \times M$ population. The component of variance due to nuclear genotypic differences is estimated as 0·000529, which corresponds to a coefficient of variation of 11·7 %.

Table 1. *Results of viability measurements*

$M \times M$ population

Mean viability 0·620695. Harmonic mean number of flies per vial 134·4.

Analysis of variance

Source	D.F.	Mean square	F
Genotypes	116	0·003017	1·78**
Reciprocals	117	0·002293	1·35*
Error	234	0·001694	

$M \times F$ population

Mean viability 0·619047. Harmonic mean number of flies per vial 130·0.

Analysis of variance

Genotypes	116	0·002479	1·26
Reciprocals	117	0·001529	
Error	234	0·001975	

$F \times F$ population

Mean viability 0·616510. Harmonic mean number of flies per vial 126·3.

Analysis of variance

Genotypes	116	0·002545	1·23
Reciprocals	117	0·002105	1·02
Error	234	0·002067	

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$.

Table 1 also shows that the means of the populations differ in the direction to be expected if there is recombinational load: v_1 (mean of $M \times M$) > v_2 (mean of $M \times F$) > v_3 (mean of $F \times F$). The maximum-likelihood estimate of the loss in mean viability due to recombination is $v_1 - v_3 = 0{\cdot}00418$, with a standard error of 0·00300 (calculated from the joint estimate of the within-population variance in viability from all three populations). The probability of such a large deviation on a one-tailed normal variate test is 8·3 %. This does not take the value of v_2 into account; it is difficult to give a quantitative weight to the fact that v_2 is approxi-

mately half-way between v_1 and v_3 as expected if L_r is non-zero. The experiment thus does not provide any definite evidence for a significant effect of recombination on viability. The estimate of L_r is 0·0068, standard error 0·0048. L_r is thus unlikely to exceed 0·016.

Table 2. *Analysis of numbers of* + *and* Cy *per vial*

Cross		+ Mean no. per vial	+ Standard error*	*Cy* Mean no. per vial	*Cy* Standard error*
M × M	(MM)	49·97	0·674	97·49	1·255
♀M × F♂	(MF)	49·29	0·953	97·29	1·774
♀F × M♂	(FM)	47·54	0·953	93·65	1·774
F × F	(FF)	47·47	0·674	94·35	1·255

* Calculated from the pooled sum of squares from all four crosses.

(ii) *Female fecundity*

The experiment gave, somewhat unexpectedly, evidence for an effect of recombination on female fecundity (fertility and egg hatchability were confounded). Table 2 shows the mean numbers of *Cy* and + individuals per culture in the three experiments. The *MF* experiment is broken down into *MF* and *FM* crosses. It can be seen that the mean numbers of both *Cy* and + flies per culture are consistently higher in those crosses where the mothers carried *M* chromosomes. The contrast *MM v. FF* for + is significant with $P < 0{\cdot}01$ on a one-tailed normal test; the contrast *MM v. FM* for + has $P < 0{\cdot}02$, and the contrasts *MM v. FF* and *MM v. FM* for *Cy* have $P < 0{\cdot}05$. All the remaining contrasts are non-significant, but it can be seen from the table that the differences in means between the crosses agree with each other, except that the percentage effect in the contrast *MM v. FF* for + is slightly higher than that for the other contrasts. This reflects the suggestion of an effect of recombination on mean viability described earlier, although the difference is non-significant statistically.

The simplest interpretation of these results is that *Cy* females which carry non-recombinant chromosomes produce either more eggs or eggs with a higher hatchability than *Cy* females with recombinant chromosomes (a maternal effect on egg-to-adult viability, though less likely, cannot be excluded: cf. Charlesworth, 1969). The best estimate of the percentage reduction in fecundity of *Cy* females due to heterozygosity for a recombinant chromosome is obtained from twice the variance-weighted mean of $(MM-FF)/MM$ and $(MF-FF)/MF$, for *Cy* flies. The use of *Cy* flies alone excludes the possible bias due to an effect of recombination on viability. This estimate comes out at 0·0730, with standard error 0·0266. It should be noted that this does not give a direct estimate of the fecundity load due to recombination in the population at large, since the *Cy* chromosome is genetically the same in all female carriers. The load may be either smaller or larger than the effect we have estimated. However, an effect of this size could hardly have been detected if there were not a fairly substantial effect in the base population.

4. DISCUSSION

The results described in section 3(i) show a small and doubtfully significant effect of recombination in lowering the mean viability of the population from which these chromosomes were drawn. Using the formula of Charlesworth & Charlesworth (1973), the chance of survival of a new inversion suppressing crossing over throughout the second chromosome would be 0·047, taking our estimate of L_r at face value, and assuming that viability is the only component of fitness that matters. Since many naturally occurring inversions in *D. melanogaster* effectively suppress crossing over in one chromosome arm (Lindsley & Grell, 1968), a more realistic estimate of the chance of survival of a new inversion would be $0{\cdot}047/\sqrt{2} = 0{\cdot}033$. These estimates should obviously not be taken very seriously.

An experiment with a somewhat similar aim to ours was carried out by Wasserman (1972), who measured components of fitness on the offspring of matings between six different lines of heterokaryotypic females and homokaryotypic males of *D. subobscura* (both types of parent were the F_1 products of crosses between inbred, homokaryotypic strains), and of the reciprocal crosses. The first type of cross allows no recombination to occur in the part of the chromosome covered by the inversion, whereas recombination is free in the reciprocal cross. Wasserman found a significantly lower hatchability of eggs produced by homokaryotypic mothers, which he interpreted as due to the unfavourable effects of recombination, manifested in the offspring. Unfortunately, it does not seem to us that one can exclude the possibility that this effect is due simply to the well-documented superior performance of inversion heterokaryotypes in *D. subobscura* (Philip *et al.* 1944), since hybrid vigour in *D. subobscura* is frequently expressed as a higher hatchability of eggs produced by hybrid mothers (Hollingsworth & Maynard Smith, 1955). Wasserman rejects this interpretation on the grounds that there is no correlation between the performance of the F_1's and the performance of the maternal (homokaryotypic) strains used to produce them, but it is not obvious that such a correlation is to be expected on the grounds of general heterokaryotypic superiority.

In an extensive study of components of genetic variation in viability, Mukai & Yamaguchi (1974) report finding a significant difference between the viability of homozygotes for chromosomes extracted from females and from males in *D. melanogaster*. They also examined the viabilities of chromosomal heterozygotes and found that the progeny of $F \times M$ crosses (where F denotes chromosomes derived from wild females and M denotes male-derived chromosomes) were significantly superior to those of $F \times F$, which had, however, a similar mean viability to the progeny of the $M \times M$ crosses. These results, although not as striking as our finding on female fecundity, seem to point in the direction of an effect of recombination on fitness.

The method of measuring viability which we have used can be criticized on the grounds that we are comparing $+/+$ with $Cy/+$, and the $+$ chromosome in the latter varies from cross to cross (Wallace, 1956). If non-recombinant chromosomes

have some effect in increasing the survival rates of their *Cy* as well as + carriers, L_r could be badly underestimated by our experiment. Although it is difficult to take this source of bias into account in any one case, there is some evidence to suggest that the effects of + chromosomes in combination with *Cy* may be much lower than in combination with other + chromosomes. Bonnier (1957) found a 90 % correlation between viability as measured by this technique and as measured by Wallace's *Cy/Pm* method, which is free from this particular source of bias. Wallace (1968, p. 230) found that the difference in mean viability between heterozygotes for wild-type chromosomes from two populations was approximately the same for both methods. Charlesworth (1969) tested this question by crossing heterozygotes for *Cy* (*SM5*) with 15 different + chromosomes to *Cy/Sp*, and measuring the genetic component of variance in the proportion of *Cy*/+ in the progeny. The experiment was of sufficient size to detect a coefficient of variation due to genetic causes of about 11 %, which is of the same order as that found within each population in the present experiment for +/+, but no effect was found. These pieces of evidence suggest that this bias may not be serious, but the fact that we have detected a significant effect of recombinant chromosomes on the fecundity of *Cy* carriers tends to make this conclusion somewhat suspect. It would clearly be desirable to repeat this type of experiment using methods of measuring viability which are unbiased, and making measurements of female fecundity directly on + flies, as well as on *Cy* carriers. The fact that we appear to have detected a substantial effect of recombination on one component of fitness makes this type of experiment seem promising as a tool for investigating the general problem of the importance of linkage and gene interaction in population genetics.

We should like to thank Professor J. Maynard Smith and Professor P. M. Sheppard for their comments on the manuscript, and Doctor P. T. Ives for supplying us with wild flies.

REFERENCES

BONNIER, G. (1957). *Cy L* versus *Cy L–Pm* technique in *Drosophila melanogaster*. *Evolution* **11**, 369–371.

CHARLESWORTH, B. (1969). Genetic variation in viability in *Drosophila melanogaster*. Ph.D. Thesis, University of Cambridge.

CHARLESWORTH, B. & CHARLESWORTH, D. (1973). Selection of new inversions in multi-locus genetic systems. *Genetical Research* **21**, 167–183.

DOBZHANSKY, T. (1951). *Genetics and the Origin of Species*. New York: Columbia University Press.

FISHER, R. A. (1930). *The Genetical Theory of Natural Selection*. Oxford: Clarendon Press.

HOLLINGSWORTH, M. J. & MAYNARD SMITH, J. (1955). The effects of inbreeding on rate of development and on fertility in *Drosophila subobscura*. *Journal of Genetics* **53**, 295–314.

IVES, P. T. (1970). Further genetic studies of the South Amherst population of *Drosophila melanogaster*. *Evolution* **24**, 507–518.

KOJIMA, K. & KELLEHER, T. M. (1961). Changes of mean fitness in random mating populations when epistasis and linkage are present. *Genetics* **46**, 527–540.

LINDSLEY, D. I. & GRELL, E. H. (1968). *Genetic Variation in* Drosophila melanogaster. Washington: Carnegie Institute Publication 627.

MUKAI, T. & YAMAGUCHI, O. (1974). The genetic structure of natural populations of *Drosophila melanogaster*. XI. Genetic variability in a local population. *Genetics* **76**, 339–366.

PHILIP, U., RENDEL, J. M., SPURWAY, H. & HALDANE, J. B. S. (1944). Genetics and karyology of *Drosophila subobscura*. *Nature* **154**, 260–262.

WALLACE, B. (1956). Studies on irradiated populations of *Drosophila melanogaster*. *Journal of Genetics* **54**, 280–293.

WALLACE, B. (1968). *Topics in Population Genetics*. New York: W. W. Norton.

WASSERMAN, M. (1972). Factors influencing fitness in chromosomal strains in *Drosophila subobscura*. *Genetics* **72**, 691–708.