Genet. Res., Camb. (1971), **18**, *pp.* 97–105
With 3 text-figures
Printed in Great Britain

An estimate of heterosis in *Drosophila melanogaster*

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(*Received* 20 *April* 1971)

SUMMARY

Twenty-five population cages of *D. melanogaster* were set up, each containing a different wild-type second chromosome and the marker chromosome *Cy*. In all but one case where contamination apparently occurred, the *Cy* chromosome persisted in the population at high frequency, showing a selective advantage of *Cy*/+ heterozygotes over wild-type homozygotes. Overall, the results indicate that homozygosity of the entire second chromosome causes a depression in fitness of the order of 85%.

1. INTRODUCTION

A population-cage experiment to test the fitness of homozygous wild-type chromosomes in *Drosophila pseudoobscura* was carried out by Sved & Ayala (1970). A marker chromosome *Ba*, which is lethal in homozygous condition, was introduced into cages each homozygous for a different second chromosome. In all cases the *Ba* chromosome persisted in the cage, indicating a selective advantage of the *Ba* heterozygote over the wild-type homozygote. Overall, individuals homozygous for the second chromosome were found to have a fitness of approximately 30% compared to individuals heterozygous for this chromosome.

The purpose of the present experiment was to obtain a similar estimate for *D. melanogaster*. The design of the experiment differs from the earlier experiment only in that egg rather than adult samples were taken to give a more direct estimate of fitness, and that some attempt was made to ensure heterozygosity of the genetic background. The recently published experiment of Sperlich & Karlik (1970) using *D. melanogaster* is also similar in principle. The present experiment differs from it mainly in that a range of chromosomes recently isolated from a wild population was studied specifically to obtain a numerical estimate of heterosis in wild populations.

2. MATERIALS AND METHODS

Wild-type flies were collected at five wineries located within a few miles of each other in the Hunter Valley district of New South Wales, Australia. Approximately 50 homozygous second chromosome lines were built up from individual male flies by the *Curly Plum* technique (Wallace, 1956), which is illustrated in Fig. 1. The use of a single *Cy*/+ male in the F_1 backcross ensures that all + chromosomes in a

particular line are descended from the same + chromosome. The F_3 ratio of $Cy/+$ to $+/+$ gives a measure of the viability of the homozygous $+/+$, and the procedure will be referred to as the ratio viability test. In the present experiment all lines giving 5% or less $+/+$ in this test were rejected as being effectively lethal. The population-cage experiment can be expected to give little extra information about fitness in such cases. Five lines from each location were selected at random from the remaining lines for use in the experiment.

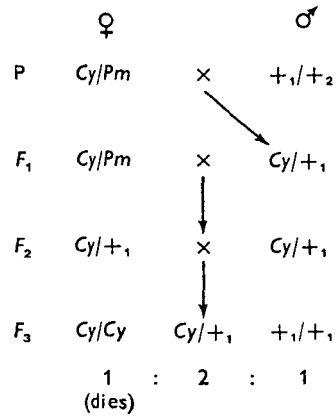

Fig. 1. Series of crosses used in ratio viability test and to obtain homozygous lines.

One population cage was set up for each of the 25 lines with initial populations comprising $Cy/+$ and $+/+$. These initial flies were not, however, those obtained directly from the F_3 generation of Fig. 1. This procedure for extracting + second chromosomes has the undesirable side-effect of inducing homozygosity for other chromosome segments throughout the genome. In order to reduce this effect as much as possible, a Cy/Pm stock was constructed by intercrossing amongst flies of all lines in the F_1 generation and re-extracting Cy/Pm in the progeny. Approximately 50% wild-type genes were thereby introduced into the genetic background. Several $Cy/+$ flies from each line were then backcrossed twice to this stock in an attempt to introduce some background variability into each line. Flies to initiate the populations were then produced by a $Cy/+ \times Cy/+$ cross for each line, so that cages were set up with a starting frequency of $Cy/+$ of 66%, or somewhat higher in some cases.

An additional set of cages was set up with duplicates of 9 of the 25 chromosomes, starting with high frequency (about 90%) of $+/+$. The cross $+/+ \times +/+$ was attempted for all chromosome lines, but was unsuccessful in a number of cases owing to sterility. Since progeny from these crosses, together with $Cy/+$ flies carrying the same wild chromosomes, were used in setting up these cages, the 9 chromosomes involved here constituted a biased sample of the original 25.

A further four cages were set up as a control on the effect of the Cy chromosome. In these cages a mixture of all 25 wild-type chromosomes was used against the Cy

chromosome, thereby ensuring that most phenotypically wild-type individuals were heterozygous for the second chromosome.

The population cages used were small plastic boxes of size $21 \times 13 \times 7$ cm. Each contained six food vials which were changed twice weekly, giving an overall 3-weekly cycle. Sampling was carried out at weekly or 2-weekly intervals by allowing flies to lay eggs in special food-cups. A sample of approximately 200–300 eggs was then placed in a 5 oz cream jar with excess food. Adults were classified as $Cy/+$ or $+/+$ after 11 or 12 days. The experiment was carried out at 25 ± 1 °C.

3. RESULTS

In all but one of the original 25 homozygous cages the frequency of $Cy/+$ dropped only slightly, and it appeared that an equilibrium had been reached in most cases. Evidence will be presented in the final section that the one exceptional cage (M 47) became contaminated, and this cage will be ignored in the summary of the data. For the remaining 24 cages the results from each sampling have been averaged and are presented in Fig. 2. The frequency of *Cy* appears to have fallen somewhat overall, although not over the last eight or so samples.

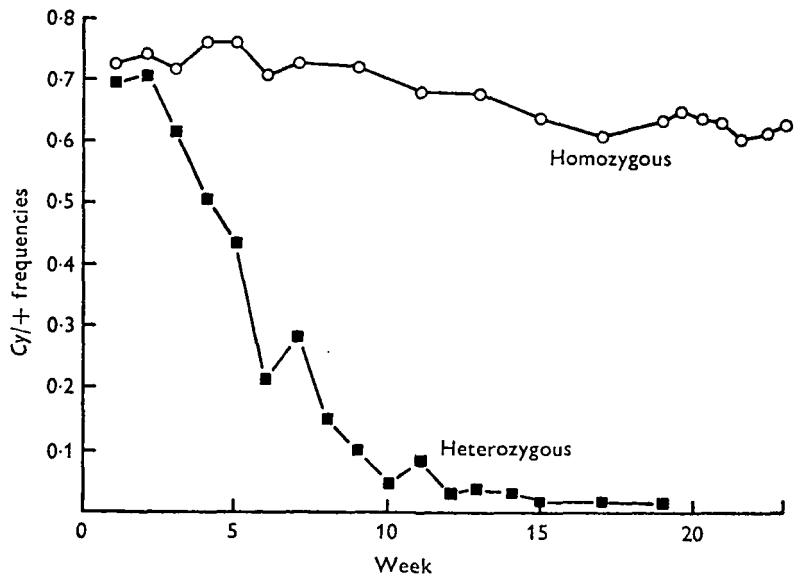

Fig. 2. Comparison of averaged $Cy/+$ frequencies from 24 homozygous cages with results from four heterozygous cages.

Fig. 2 also presents results for the four heterozygous cages. Here the frequency of *Cy* has fallen rapidly from almost the beginning of the experiment. A similar result was also found in two heterozygous cages set up with laboratory stocks of Canberra wild type. This rapid loss of marker chromosomes in a heterozygous (polychromosomal) background was also found by Sperlich & Karlik (1970).

The averaged results from the nine duplicate homozygous cages set up with high frequency of + are given in Fig. 3, together with the averaged results from the corresponding nine low + cages. It is seen that the average *Cy*/+ frequencies of the two sets became indistinguishable after about 15 weeks, thereby demonstrating that the starting frequency is not of any systematic importance. Results of the final frequencies attained in these two sets of cages are given in Table 1, based on the last six samples taken of each. The agreement for individual chromosomes is reasonable, although by no means perfect.

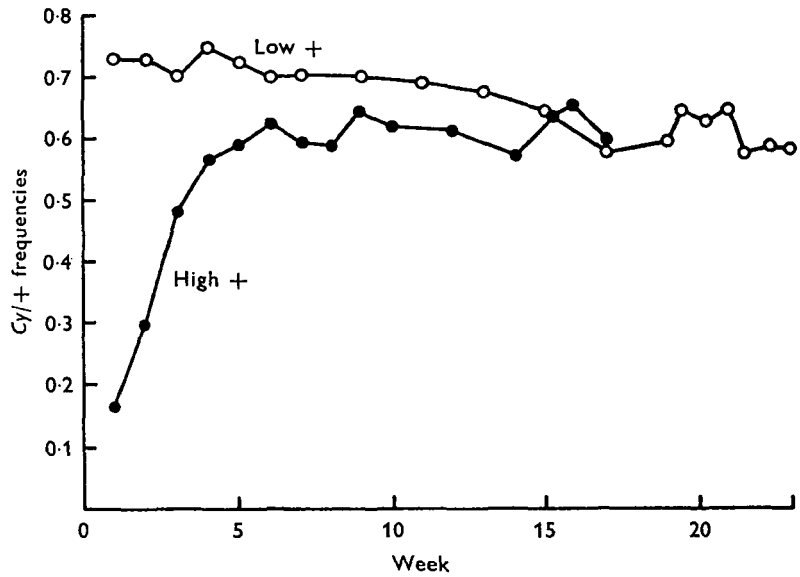

Fig. 3. Comparison of results from nine cages with high frequency of wild-type (high+) with corresponding cages started with excess of *Cy*/+ (low+).

Table 1. *Comparison of* Cy/+ *frequencies attained in duplicate homozygous populations started with low and high frequency of* +

Chromosome	M3	M9	M21	M28	M41	M69	M76	M86	M87
Low+	0·55	0·50	0·71	0·48	0·73	0·49	0·66	0·76	0·64
High+	0·62	0·64	0·54	0·50	0·68	0·49	0·73	0·67	0·67

4. INTERPRETATION AND CONCLUSIONS

The fact that in all experimental cages the *Cy* chromosome was not eliminated is by itself a strong indication of heterosis. Since the *Cy*/*Cy* genotype is lethal, the *Cy* chromosome would be eliminated except in the face of an opposing advantage of *Cy*/+ over +/+. The principal aim of this section is to calculate the magnitude of the disadvantage suffered by +/+ individuals.

We assume that the relative frequencies of *Cy*/+ and +/+ at the zygote stage of one generation are $h':1-h'$. The relative selective values of the genotypes *Cy*/*Cy*, *Cy*/+ and +/+ are taken as $0:1:w$, respectively. Then by definition the

https://doi.org/10.1017/S0016672300012453 Published online by Cambridge University Press

relative frequencies of $Cy/+$ and $+/+$ contributing to the next generation will be

$$h' : (1-h').w.$$

Thus the relative frequencies of Cy and $+$ in gametes will be

$$\frac{\frac{1}{2}h'}{h'+(1-h')w} = p \quad \text{and} \quad 1-p \quad \text{respectively.}$$

The ratio of $Cy/+$ and $+/+$ among the zygotes of the next generation will be

$$2p(1-p) : (1-p)^2.$$

If the population is at equilibrium then this ratio should be equal to $h' : 1-h'$. Solving this equation gives the estimated value of w as $(2-3h')/(2-2h')$.

This formula must be corrected before it can be used with the raw frequency data. While egg samples were taken, the $Cy/+$ frequencies are those estimated at the adult stage rather than at the egg stage. All egg samples were grown under optimal conditions to minimize mortality, but some differential mortality between $Cy/+$ and $+/+$ must nevertheless be expected. This effect can be controlled, provided frequency–dependent effects are not too strong, by comparison with results from the cross $Cy/+ \times Cy/+$ grown under similar conditions. This was done for each chromosome in the present experiment by allowing inseminated females from such a cross to lay eggs in sampling cups which were then treated in the same way as eggs from cage sampling cups.

If the frequency of $Cy/+$ adults from such a cross is r, then it is readily shown that the egg-to-adult viability of $+/+$ relative to $Cy/+$, which will be designated as v, is estimated as $[2(1-r)]/r$. Then in terms of h, the frequency of $Cy/+$ adults in cage samples, and of v, the frequency of $Cy/+$ zygotes in cage samples, h', is estimated as $(vh)/(1-h+vh)$. Then substituting for h' the estimate of w, $(2-3h')/(2-2h')$ becomes $(r-h)/[r(1-h)]$. A more direct derivation of this formula is obtained by direct comparison of the ratio of $Cy/+ : +/+$ from cage samples with that from the ratio viability test.

A further correction must be also made, to take account of the fact that the estimate of fitness of $+/+$ homozygotes is obtained relative to $Cy/+$ rather than to wild-type second chromosome heterozygotes. If the Cy chromosome had no effect on fitness this would not be important, but the results from the heterozygous cages where Cy is eliminated very rapidly show that in fact $Cy/+$ is at a considerable disadvantage to the normal wild type. Unfortunately it is not easy to calculate the selective disadvantage of this genotype, nor to make accurate allowance for it if it is known. The generation interval in this experiment, a crucial feature in calculating the expected rate of loss of the Cy chromosome, is unknown. However, if the estimated generation time of $2\frac{1}{2}$ weeks given by Crow & Chung (1967) is accepted, then graphical methods can be used to show that the rate of loss of Cy in Fig. 2 is consistent with a selective value of $Cy/+$ relative to wild-type heterozygotes of 50 % or slightly less.

Accepting the figure of 50 %, then it will be taken that a homozygous fitness of

100% relative to $Cy/+$ corresponds to a fitness of 50% compared to wild-type heterozygotes. This is of course an assumption which cannot be tested directly in the present experiment. Furthermore, as a first approximation the selective values of all homozygotes relative to $Cy/+$ may be reduced proportionately by 50% to give the estimated selective value relative to wild-type heterozygotes.

Table 2. *Fitness estimates for individual chromosomes*

Chromosome	h (freq. of $Cy/+$ adults in cages)	r (freq. of $Cy/+$ adults in ratio test)	v, viability (ratio test) $= \frac{2(1-r)}{r}$	w (fitness of $+/+$ rel. to $Cy/+$) $= \frac{r-h}{r(1-h)}$
M3	0·54	0·70	0·87	0·49
5	0·54	0·71	0·88	0·49
7	0·77	0·83	0·40	0·34
9	0·50	0·74	0·70	0·65
11	0·80	0·79	0·53	−0·03
21	0·71	0·73	0·75	0·10
23	0·56	0·69	0·92	0·42
24	0·62	0·66	1·03	0·15
26	0·40	0·71	0·81	0·73
28	0·45	0·68	0·93	0·61
41	0·72	0·79	0·52	0·32
43	0·76	0·71	0·80	−0·25
44	0·49	0·69	0·91	0·56
48	0·68	0·67	0·96	−0·02
64	0·97	0·93	0·14	−0·92
66	0·73	0·75	0·67	0·08
67	0·51	0·70	0·87	0·56
69	0·51	0·72	0·79	0·59
76	0·66	0·78	0·55	0·47
81	0·85	0·83	0·41	−0·16
86	0·72	0·79	0·53	0·32
87	0·66	0·72	0·79	0·23
91	0·56	0·73	0·73	0·54
94	0·51	0·66	1·03	0·47
Average			0·73	0·28

The low fitness of the *Cy* chromosome is an unfortunate aspect of the experiment, and was somewhat unexpected in view of the fact that the viability of $Cy/+$ is usually quite satisfactory in ratio tests where it has been used extensively (e.g. Mukai, 1964). This was confirmed for the *Cy* chromosome used in the present experiment by a small control ratio test (2365 *Cy*: 1167 +, $0{\cdot}7 < P < 0{\cdot}8$). A pilot population-cage experiment was also carried out prior to the main experiment which suggested that the *Cy* chromosome was superior in population cages to the *Pm* chromosome, although Sperlich & Karlik (1970) found *Pm* to be somewhat superior to *CyL*.

The estimated fitnesses for all 24 chromosomes are given in Table 2. The estimated equilibrium frequency of $Cy/+$ (h) is given in column 2, and is obtained by

averaging over the last nine samples of the experiment. $Cy/+$ frequencies from the ratio test (r) are given in column 3, and egg-to-adult viabilities (v) are calculated from these in column 4. The estimated selective values of $+/+$ homozygotes (w) are then given in column 5.

The overall mean value of w averaged over all 24 chromosomes is 0·28. However, several negative values are included in this estimate. These occur in cases where the frequency of $Cy/+$ in cages exceeds that found in the ratio test, non-significantly in all but one case (M 64). A more conservative although probably biased estimate is obtained if these chromosomes are assigned a fitness of zero. This gives a mean fitness of 0·34.

It is not easy to give standard errors for the individual chromosome estimates. On the average, somewhat over 500 progeny were scored in each of the ratio tests, and standard errors could readily be assigned to values of r. On the other hand, the estimates of h are subject to significant fluctuations between samples, each based on counts of around 150 flies, which makes it difficult to assign standard errors. However, the major source of error in the overall estimate of mean fitness comes from the small number of chromosomes sampled. The estimate of mean fitness of 0·28 has a calculated standard error of 0·075, while the estimate of mean fitness ignoring negative values has a standard error of 0·05.

As argued previously, the fitnesses compared to $Cy/+$ should be halved for an estimate of fitness compared to the wild-type heterozygote. This gives a mean value of 0·14 if the negative values are included, or 0·17 if they are excluded. Thus it seems reasonable to give the overall estimate as approximately 0·15, corresponding to an inbreeding depression of 0·85. It must also be remembered that the lethal chromosomes were excluded from the sample taken, and if these are included the mean fitness would be reduced proportionately by about 20%.

(i) *Components of fitness*

Comparison of the values of v (Table 2, column 4) with the values of w (column 5) shows that while there is a reasonably high correlation between the two sets ($r = 0{\cdot}55$, $P < 0{\cdot}01$), nevertheless there are several examples of extreme dissimilarity. Furthermore, if M 64 is removed from the analysis, as seems appropriate in view of the near lethality of this chromosome and of the spuriously low value of w, the correlation falls to 0·27, which is not statistically significant. This suggests that there is not a strong relationship between the performance of a chromosome in the viability ratio test and in cages, and shows that considerable caution must be exercised in extrapolating from the results of viability ratio tests to the overall fitness of *Drosophila*.

This conclusion is strengthened if it is noted that, for each chromosome, the overall fitness w is less than the egg-to-adult viability v, even before the correction factor 0·5 is applied. Two reasons may immediately be suggested for this discrepancy. First the fertility of $+/+$ genotypes could be a significant factor in all cases. Secondly, the conditions under which flies are raised in cages are much

'harsher' than those in the ratio test, which might be reflected in a lower homozygous fitness.

It is by no means surprising that fertility should play an important role in this experiment. Numerous experiments (e.g. Gowen, 1952) have shown that inbred flies are at a disadvantage to outbred flies in all aspects of female fecundity. Similarly, inbred male flies have been shown to be at a considerable disadvantage in mating (Fulker, 1966). However, it is difficult to make a quantitative prediction of the effect of fertility in the present experiment from the results of these experiments.

The question of the effects of crowding in cages needs to be studied in more detail. While it might be predicted that increased crowding could increase the advantage of heterozygotes through soft selection (Wallace, 1970), nevertheless it appears that crowding has had little or no effect on the results of ratio viability tests so far carried out (Temin *et al.* 1969; Sved & Ayala, 1970). Such experiments do not, however, take into account the possibility that the crowding might be insufficient to influence viability ratios but might nevertheless sufficiently handicap inbred flies during development to reduce potential fertility. The range of size variation amongst flies in cages is considerably greater than normally found in bottle cultures, and it seems likely that morphological differences such as these might be correlated with fertility differences.

Finally, it should be emphasized that the results of this experiment tell us only that there is heterosis or heterozygote advantage at the level of the chromosome. They do not reveal whether this is due to dominance or over-dominance at individual loci. Results from cages having a mixture of two different wild-type chromosomes have been interpreted by Sperlich & Karlik (1970) as favouring the dominance hypothesis.

(ii) *Analysis of chromosome M 47*

As previously mentioned, the results from one cage, M 47, have been ignored in the analysis. In this case the frequency of the *Cy* chromosome dropped throughout the course of the experiment, and it was assumed that this could be attributed to contamination. Sperlich & Karlik (1970) also found one such chromosome, and contamination was also suspected by these authors. It is important that these assertions be justified, since the appearance of even a single + chromosome of high fitness in homozygous condition would have serious implications for the hypothesis of generalized heterosis.

A sample of + / + flies was extracted from the cage at the end of the experiment, and examined by gel electrophoresis. I am indebted to Dr I. R. Franklin of C.S.I.R.O. Division of Animal Genetics who carried out this work. It was shown that flies in the cage were polymorphic for one second chromosome marker (alcohol dehydrogenase), and also probably for a second (amylase). Flies from one of the other cages (M 64) on the other hand were monomorphic as expected. Thus contamination by one or more outside second chromosomes either at the start of the experiment or very early during the experiment is strongly suggested.

Evidence from a second source also favoured this view. The viability of M 47 as measured by the ratio test at the beginning of the experiment was approximately 0·5. This was confirmed at the end of the experiment by testing a replicate of the stock which had been kept in bottles throughout the experiment. However, the viability of the chromosome when extracted from the cage at the end of the experiment was indistinguishable from unity.

I am indebted to Miss Penelope Quirk for assistance and to Professor J. S. F. Barker for comments on the manuscript. The study was supported by a grant from the Australian Research Grants Committee.

REFERENCES

Crow, J. F. & Chung, Y. J. (1967). Measurement of effective generation length in *Drosophila* population cages. *Genetics* **57**, 951–955.

Fulker, D. W. (1966). Mating speed in male *Drosophila melanogaster:* A psychogenetic analysis. *Science, N.Y.* **153**, 203–205.

Gowen, J. W. (1952). Hybrid vigor in *Drosophila*. In *Heterosis* (ed. J. Gowen), pp. 474–493. Ames: Iowa State College Press.

Mukai, T. (1964). The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. *Genetics* **50**, 1–19.

Sperlich, D. & Karlik, A. (1970). The genetic conditions in heterozygous and homozygous populations of *Drosophila*. I. The fate of alien chromosomes. *Genetica* **41**, 265–304.

Sved, J. A. & Ayala, F. J. (1970). A population cage test for heterosis in *Drosophila pseudoobscura*. *Genetics* **66**, 97–113.

Temin, R. G., Meyer, H. U., Dawson, P. S. & Crow, J. F. (1969). The influence of epistasis on homozygous viability depression in *Drosophila melanogaster*. *Genetics* **61**, 497–519.

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

Wallace, B. (1970). *Genetic Load: Its Biological and Conceptual Aspects*. New Jersey: Prentice-Hall.