A POPULATION CAGE TEST FOR HETEROSIS IN *DROSOPHILA PSEUDOOBSCURA**

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THE existence of high levels of heterozygosity in some natural populations, perhaps as high as 10–20% of the genome per individual, seems by now a well-established phenomenon (see review by SPIESS 1968). Given the rather small suspected size of these populations, some explanation of how this variability is maintained seems necessary. In addition, as emphasized by LEWONTIN and HUBBY (1966), such explanation should take into account the high degree of similarity with respect to particular polymorphisms amongst populations, suggesting the likelihood that the polymorphisms are maintained by natural selection (see also PRAKASH, LEWONTIN and HUBBY 1969).

Heterozygote advantage, or heterosis, is one of the principal selective mechanisms at present under consideration. In the present article we will describe an experiment which is designed to investigate the adequacy of this explanation.

Tests for heterosis: KING (1967) and SVED, REED and BODMER (1967) have recently emphasized the rather obvious point that an efficient method for testing whether the homozygote is usually at a disadvantage to the heterozygote is to compare the fitness of ordinary outbred individuals with that of individuals having a greater than normal proportion of homozygous loci, produced through some form of inbreeding. The results of many such tests are of course available, and it was argued that these results were compatible with only a limited amount of heterozygote advantage.

A point which must be noted here is that tests such as the above give information only on the overall homozygous disadvantage. They do not indicate whether at any given locus the heterozygote is at an advantage to both homozygotes, but only whether heterozygotes are at an advantage overall. Either dominance or partial dominance in fitness at individual loci, as well as overdominance, could produce such a result. It is argued later, however, that these considerations may not be as crucial from the point of view of the maintenance of heterozygosity as might at first be thought.

Numerous experiments could be cited which give evidence on the relative fitness, or components of fitness, of inbred offspring. These experiments may be roughly divided into two classes, namely those which test the effect of making a particular chromosome or chromosome region homozygous by mating schemes

* This paper is dedicated to Professor THEODOSIUS DOBZHANSKY on the occasion of his 70th birthday.

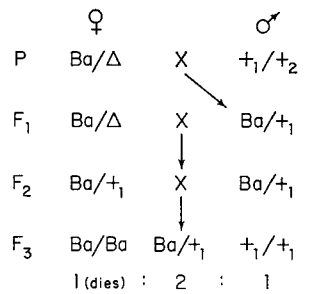

FIGURE 1.—The series of crosses used to obtain homo- and heterozygotes for wild chromosomes of *D. pseudoobscura*. *Ba* represents a marker chromosome which suppresses recombination. Δ is a marker chromosome; $+_1$ and $+_2$ represent wild chromosomes.

such as the one illustrated below, and those which produce throughout the whole genome an increase in homozygosity whose mean overall value is known, such as $F = .25$ for full-sib mating.

Viability tests have been carried out mainly by the first method. Perhaps the most comprehensive such test is that of DOBZHANSKY and SPASSKY (1963). In this experiment over one thousand second chromosomes of *Drosophila pseudoobscura* were extracted from field populations, and the viabilities of individuals homozygous for these chromosomes were compared against the viabilities of individuals heterozygous for each chromosome and a marker chromosome (*Bare*). The general scheme for such a test is summarized in Figure 1. Use of a single male in the F_1 cross depicted in the figure ensures that all chromosomes labeled + in a particular line are identical by descent. The *Bare* chromosome contains an inversion which suppresses recombination over most of the chromosome. Deviations from a 2 *Ba*/+ : 1 +/+ ratio in the progeny of the cross *Ba*/+ × *Ba*/+ are used as indicators of homozygote inviability. In Table 1 are shown the results of all such crosses, together with those from intercrosses of the different wild-type chromosomes, which give control for the effect of the marker chromosome. The results show a bimodal distribution for homozygotes' viability. Approximately thirteen percent of homozygotes are lethal, while a second and much larger group is centered just to the left of the peak for the control group. Chromosomes falling within this group have been designated as quasi-normal. The two groups are by no means distinct, since intermediate ratios are found, although at low frequency. However, the existence of large numbers of chromosomes having little or no reduction in viability in homozygous condition seems scarcely compatible with the hypothesis of heterozygote superiority at large numbers of loci, and it is this class of chromosomes with which we are particularly interested in the present investigation.

Many tests have been made of the fertility of inbred individuals. In one very comprehensive such test, GOWEN (1952) has produced evidence of reduced egg-laying ability of inbred *Drosophila melanogaster* females at all stages. A similar result was found by MARINKOVIC (1967a) using *D. pseudoobscura* females homozygous for the second chromosome. MARINKOVIC (1967b) found further that eggs

TABLE 1

Numbers of chromosomes giving various percentages of wild-type flies in viability ratio test
(from DOBZHANSKY and SPASSKY 1963)

	Lethal	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	Total
Homozygotes	134	20	12	20	21	12	14	23	22	28	24	31	56	70	102	145	137	96	64	20	8	4	1063
Heterozygotes	1	..	..	..	..	..	1	..	..	1	4	6	8	37	69	133	194	231	175	121	33	20	1034

TABLE 3

Percentage of Ba/+ *flies in control cages (egg samples)*

	Week											
Cage	3	4	5	6	7	8	10	11	12	13	14	15
CON(5)	51.4	22.4	27.7	22.5	32.7	36.4	0	4.3	9.0	0.5	5.5	1.3
CON(6)	50.1	23.0	29.4	23.8	23.8	34.5	23.1	0	2.3	4.7	3.8	1.8
CON(7)	51.2	38.6	40.5	25.6	17.7	33.2	23.7	6.8	7.1	4.7	4.3	2.5
CON(8)	58.5	48.7	33.4	38.3	33.6	23.8	18.3	3.4	8.7	4.4	0.6	2.4
Average	52.8	33.2	32.7	27.6	27.0	32.0	16.3	3.6	6.8	3.6	3.5	2.0

laid by homozygous females, regardless of their own genotype, developed into larvae in a much lower proportion of cases than did eggs laid by heterozygous mothers. He found an overall reduction in female fertility of nearly 50%.

An experiment in which both viability and fertility are taken into account is that of LATTER and ROBERTSON (1962). These authors measured a statistic to which both male and female viability and fertility differences contribute (although not in quite the correct proportions). The level of crowding was made quite high to accentuate these differences. A mean overall fitness of the order of 50% was found for individuals having a mean inbreeding coefficient of 0.25.

A further experiment which should be mentioned in this context is that of Vann (1966). In this experiment a variety of radiation-induced chromosomal rearrangements were introduced into a number of genetic backgrounds. Despite the apparent disadvantage of chromosome breaks, it was shown that paracentric inversions. where they differed in genic content from the non-inverted segment, tended to increase in frequency. Heterozygote advantage was thus indicated for such segments, and since these segments were spread over a large portion of the genome, the heterozygote advantage appeared to be widespread.

In the experiment to be described below we have also attempted to take into account all components of fitness. We have used the second chromosome of *D. pseudoobscura*, the same chromosome tested by DOBZHANSKY and SPASSKY (1963) and by MARINKOVIC (1967a,b), but the use of population cages allows all components of fitness to be combined in a reasonably meaningful way in a single experiment.

THE EXPERIMENT

Collections of *Drosophila pseudoobscura* were made in four areas of the United States: Palo Alto, California (R11-R13); Texas (R21-R30); Strawberry Canyon, California (R41-R44); and Arizona (R51-R55). For these collections we are indebted respectively to Dr. S. KESSLER, Mr. R. KIESTER, Dr. TH. DOBZHANSKY, and Dr. W. HEED. A procedure similar to that given in Figure 1 was followed in order to obtain twenty nonlethal second chromosomes from stocks from the four areas. In producing this sample, only chromosomes which gave complete lethality, or in two cases less than one percent homozygous progeny, were excluded. Although our interest was primarily in those chromosomes which gave little or no reduction in viability in the ratio test, it was decided to include all nonlethal chromosomes in order to give an unbiased estimate of homozygous fitness. In fact only one of the twenty nonlethal chromosomes gave a markedly reduced viability (Table 4, column 9), which is a lower proportion than that found by DOBZHANSKY and SPASSKY (1963). The reason for this discrepancy is not clear, since we used similar procedures for the ratio test. In view of the small number of chromosomes tested in the present experiment, the difference could be due to chance. The twenty stocks were subsequently reduced to eighteen by the loss of two stocks, through sterility of the culture in one case, and recombination of the second chromosome in the other.

Thirty-six small population cages of the type described by AYALA (1968) were set up, two from each of the eighteen stocks. For each stock one cage was started with 40% of the genotype *Ba*/+ (and 60% +/+), and the other with 10% *Ba*/+. In order that the cages be started with reasonably large numbers of flies we used separate crosses for building up stocks of the two genotypes, viz., *Ba*/+ × *Ba*/+ for the former, and +/+ × +/+ for the latter. For reasons which may not be related to genotype, the latter cultures were usually more successful, and owing to the higher initial fitness of the +/+ flies the proportions with which the cages were effectively started were closer to one-half of the stated frequencies of the *Ba*/+ flies. However, as will be

explained later the design of the experiment was essentially to minimize the effects of the starting conditions.

Four control cages were set up at the same time. Two of these were also begun with 40% *Ba*/+ and two with 10% *Ba*/+. In all cases the *Ba*/+ and +/+ flies were equally divided amongst the eighteen stocks. Therefore while in the experimental cages the *Ba*/+ flies were heterozygous for the second chromosome and the +/+ homozygous, in the control cages the +/+ flies were, near the start of the experiment at least, almost all heterozygous.

Samplings from the cages were made every four or five weeks, somewhat irregularly since the main interest was in the final frequencies. The interval between the last two samples was in fact nearly eight weeks. Cages became rather dirty, and therefore were changed concurrently with sampling. At this stage the flies were etherized, and a sample of about 200 adults scored from each cage. The flies and food cups containing larvae and pupae were then moved to a new cage.

The theory behind the experiment is a very simple one. The genotype *Ba/Ba* is known to be lethal. In addition we assume for the moment that the homozygote +/+ is at a disadvantage to the heterozygote *Ba*/+, so that the relative selective values of the two genotypes are, respectively, $1 - s : 1$. Then the genotype *Ba*/+ is at an advantage to both homozygotes, and a classical heterotic equilibrium is expected with the frequencies of the *Ba* and + chromosomes $s/(1+s)$ and $1/(1+s)$, respectively. If an equilibrium is established, the *Ba*/+ genotype must be at an advantage to the +/+, and the higher the equilibrium frequency of the *Ba* chromosome, the greater the advantage. On the other hand if the *Ba* chromosome is lost, this indicates either a disadvantage of the *Ba*/+ genotype or near equality, and recourse must be made to the rate of loss to determine the selective values.

One of the principal advantages of this experiment is that the measurement of fitness is made under conditions which are little affected by the way in which the population cages are set up. By the time the final measurements are made the population has had ample time to adjust to a reasonably stable age distribution, as against the artificial distribution (most individuals of the same age) with which most one-generation measurements must be made. Differences in longevity are thus taken into account in a meaningful way. In one-generation experiments there is also the problem of what level of crowding should be used. This difficulty is obviated in the present experiment where an approximately constant level of crowding is attained in the cages, a level which is appropriate for the conditions of the experiment. The level of crowding attained may in fact be higher than can be produced artificially. Of course we have no guarantee that under natural conditions populations attain levels of crowding or competition as high as this, although if populations are controlled in a density-dependent manner this must happen to some populations at least some of the time. Finally, from a purely statistical point of view, we have the advantage that the final result in the cages is the product of not one but a number of generations of selection. Any fluctuation of selective values in one particular generation is therefore not of crucial importance.

As against these advantages, the present experiment has several disadvantages. There is no way of distinguishing the male and female components of fertility, and indeed fertility and viability may only be distinguished by additional experiments which will be described later. Provided the male and female fitnesses are not too different however, the overall estimate of fitness should be reasonably accurate (WRIGHT and DOBZHANSKY 1946; ANDERSON 1969). The fact that a marker chromosome must be used in the heterozygote is also a considerable disadvantage as will be described below. There is also the problem of newly arising mutations (MUKAI and YAMAZAKI 1968), and of recombination within the chromosome. The first of these is however not strictly speaking a problem, since mutations may be expected at about the same rate as in natural populations whose behavior we wish to simulate. Recombination of the chromosome could reduce the selective differential between homozygote and heterozygote but scarcely increase it, and in any case the chromosome is marked so that excessive numbers of recombinants could be detected. Finally the homozygosity of chromosomes other than the second could affect the results, although provided that these associate randomly with second chromosome homozygotes and heterozygotes little bias should be introduced.

RESULTS

The complete results from the experiment are given in Table 2. The values in the body of the table are the percentages of *Ba*/+ flies at different samplings. The average sample size is 230 adult flies per sample. However, in a number of cases there were few adult flies when the cage was sampled, and those readings based on less than one hundred flies are marked with an asterisk. As seen from the table, a number of cages were discarded early due to contamination.

The most striking feature of the results is that the *Ba* chromosome was not lost from a single cage. In the majority of cages, the frequency of *Ba* increased over the course of the experiment, while in those cases where the frequency decreased it did not appear to be tending towards zero. A rough picture of the overall change in frequency is given by averaging the frequency of the *Ba* heterozygote over the thirty cages for which all six samples were taken. These averages, which are given in Table 2, show that there was a steady increase in the frequency of *Ba*, tailing off somewhat over the last three samples. It must be emphasized that this overall increase in frequency of the *Ba* chromosome has occurred despite the lethality of this chromosome in homozygous condition.

A second noteworthy feature of the results is the close agreement between replicate cages. By the time of the last sample there were no large disparities between replicate cages, despite the fact that most pairs had been started with quite different frequencies. This demonstrates that the final frequency attained in the cage is a function of the chromosomal constitution and is not an artifact of the genetic background or the way in which the cage was set up.

Before considering what selective advantages of *Ba*/+ and +/+ are necessary to produce the frequencies of Table 2, the results from the control cages must be examined. As can be seen, the *Ba* chromosome was rapidly eliminated in all the control cages. The speed of elimination cannot be attributed solely to the known selection against the *Ba*/*Ba* homozygote, since this would take ten generations to reduce the frequency of the *Ba*/+ genotype from 20% to 10%, a further twenty generations from 10% to 5%, and so on. Clearly the frequency of this genotype is being reduced much faster than this. The calculation of the exact disadvantage of the *Ba*/+ genotype is however made difficult by the fact that the generation time is unknown. We can give a rough estimate of the disadvantage as at least 40%, based on an estimated generation time of 3–4 weeks. However, it should be emphasized that this is not as precise an estimate as those obtained from the cages in which *Ba* was not eliminated.

This aspect of the experiment was repeated under slightly different conditions. Four further control cages were set up with a higher initial frequency of *Ba*/+ flies in order to obtain a more accurate assessment of the rate of loss of the *Ba* chromosome. Sampling from these cages was carried out at weekly intervals, starting with the third week, and egg rather than adult samples were taken. The results are given in Table 3. They are essentially similar to the results of the control cages given in Table 2. The estimate of *Ba*/+fitness compared to the wild-type heterozygote is if anything lower than the estimate from the first series of controls.

TABLE 2

Percentages of Ba/+ *flies in the populations at various samplings*

Population	Sample number					
	1	2	3	4	5	6
R11(1)	48.6	72.6	71.9	85.3	86.9	84.3
(2)	49.2	70.8	73.5	80.2	85.4	81.4
R12(1)	6.3	27.0	35.9	49.1	46.7	41.2
(2)	13.9	-	-	-	-	-
R13(1)	21.9	9.1	19.5	15.5	-	-
(2)	0.9	0.7	9.8	14.0	-	-
R21(1)	22.2*	24.5	47.5	33.8	37.6	38.0
(2)	7.2	8.0	22.4	31.6	36.0	34.5
R23(1)	30.4	31.4	51.6	51.1	54.1	54.3
(2)	6.2	12.2	13.9	22.2	37.1	-
R27(1)	8.2	15.6	16.2	18.3	-	-
(2)	3.4	1.2*	11.1	15.1	17.6	-
R28(1)	20.6	21.0	15.1	20.2	29.9	26.9
(2)	3.4	1.9	9.9	13.5	11.1	18.3
R29(1)	21.7	28.0*	32.4	45.1	43.8	34.1
(2)	21.7	27.9	42.8	40.3	44.3	39.7
R30(1)	13.1	19.1	55.3	58.6	55.0	46.5
(2)	4.3	16.7*	49.8	51.4	48.0	46.0
R41(1)	10.0	18.2	25.8	22.0	33.8	36.5
(2)	5.6	12.1	20.9	23.9	41.1	40.6
R42(1)	52.8	54.0	44.5	54.2	50.6	48.7
(2)	25.5	17.6*	39.9	36.0	40.9	42.6
R43(1)	33.3	39.9	65.7	60.6	59.1	60.8
(2)	0.0	0.6	3.3	6.0	25.0	64.2
R44(1)	37.0	53.7	53.9	69.1	75.0*	72.4
(2)	7.0	9.2	13.4	52.9	63.9	68.2
R51(1)	41.1	45.1	47.3	44.7	42.2	56.4
(2)	8.6	10.9	12.2	32.1	45.7	62.6
R52(1)	51.9	52.6	45.2	39.3	40.1	41.0
(2)	2.0	1.0	23.8	27.7	30.4	35.1
R53(1)	31.6	37.3	33.1	22.5	13.7	11.7
(2)	15.5	16.7	10.1	13.4	18.9	13.6
R54(1)	45.9	28.8	72.1	85.0	70.6	72.8
(2)	21.2	13.4	55.1	86.9	79.7	79.4
R55(1)	37.9	60.0	51.3	67.7	70.9	71.3
(2)	21.8	31.6*	44.1	57.7	60.0	71.1
AVERAGE	23.2	28.4	39.0	45.4	48.0	50.1
CON(1)	25.3	23.9	5.7	4.0	0.8	0.0
(2)	4.8	6.4	1.0	0.5	0.0	0.0
(3)	23.5	6.9	6.5	2.3	0.0	0.0
(4)	3.0	3.8	3.9	0.0	0.0	0.0

* Sample size was smaller than 100 flies.

These results indicate that the upper limit for the homozygote fitness as derived from the frequencies in the experimental cages ought to be 0.6 rather than unity. Therefore, as a first approximation all fitnesses from the cages will be multiplied by 0.6 to give the fitness of the homozygote compared to the normal wild-type heterozygote. If the two genotypes, +/+ and *Ba*/+ have equal fitness, it will take 150 generations to reduce the frequency of the *Ba*/+ genotype from 20% to 1.2%. If the fitness of the *Ba*/+ genotype is half the fitness of the +/+ genotype, the frequency of *Ba*/+ will be halved each generation. Thus, for instance, it will take 4 generations for the frequency of *Ba*/+ to go from 20% to 1.2%. This seems to be approximately the rate at which the *Ba*/+ frequency is decreasing in the control populations, both in Tables 2 and 3, assuming generation time to be 3–4 weeks. To be on the conservative side, however, we have estimated the fitness of *Ba*/+ as .6 rather than .5.

ESTIMATION OF SELECTIVE VALUES

Theory: In order to estimate selective values, we need to assume that the experimental cages have reached an equilibrium point, so that the selective advantage of *Ba*/+ over +/+ just cancels out the disadvantage of the *Ba* chromosome due to lethality of *Ba*/*Ba*. While there is some fluctuation of frequencies from one sample to the next, the average results given in Table 2 suggest that there is little systematic change over the last three generations. The assumption of equilibrium thus seems justified, and should if anything lead to an underestimate of the *Ba*/+ fitness, since equilibrium may not quite have been reached.

A further problem which needs to be considered in some detail concerns separating the effects of viability and fertility. The importance of doing this is readily seen by considering an example in which the equilibrium of *Ba*/+ is 2/3 and of +/+ 1/3, a similar situation to that observed in several of the cages. We can consider two extreme possibilities. The first is that there are no fertility differences, and that all selective differences are due to viability differences between the egg and adult stage (determined by the zygotic genotype, unlike the viabilities determined by the maternal genotype found by MARINKOVIC 1967b). Then the gametes produced from the above adult flies will be 1/3 *Ba*:2/3 +, and the frequencies of the genotypes *Ba*/*Ba*, *Ba*/+ and +/+ at the zygotic stage will be 1/9, 4/9, and 4/9, respectively. The relative viabilities of the *Ba*/+ and +/+ genotypes must be 1:0.5 in order to produce the observed adult equilibrium frequencies. Consider now the opposite extreme, where there are only fertility and no viability differences. Clearly the adult frequencies observed are those expected, in the absence of viability differences between *Ba*/+ and +/+, if the parental crosses are all *Ba*/+ × *Ba*/+. The +/+ flies would need to have zero fertility to produce this situation, so that the fitness of this genotype would be zero, as opposed to one-half under the viability model. This problem arises because we are estimating the frequencies of the two genotypes at the adult stage, rather than at the zygote stage as would be necessary to give the estimate of fitness directly (PROUT 1965).

The estimation of selective values can be carried out using essentially the discrete generation model of selection considered by PROUT. The viability of the homozygote +/+ compared to the heterozygote *Ba*/+ is assumed to be V, corresponding to the early selection coefficient, E, of PROUT, while the fertility of both male and female homozygotes is assumed to be F. The overall fitness of the homozygote is w $(= 1 - s)$ which is equal to $V \times F$. In the following argument we will assume that the frequency of *Ba*/+ has reached equilibrium, at a frequency H amongst adults.

Allowing for fertility differences, the relative frequencies of *Ba*/+ and +/+ contributing to the next generation are

$$H \; : \; (1 - H)\, F$$

The relative frequencies of the gametes *Ba* and + will therefore be

$$\frac{H}{2} \; : \; \frac{H}{2} + (1 - H)\, F$$

Assuming random mating, the relative proportions of *Ba*/+ and +/+ zygotes are

$$2\left(\frac{H}{2}\right)\left[\frac{H}{2} + (1 - H)\, F\right] \; : \; \left[\frac{H}{2} + (1 - H)\, F\right]^2,$$

and, allowing for viability differences, the relative adult frequencies are

$$2\left(\frac{H}{2}\right)\left[\frac{H}{2} + (1 - H)\, F\right] \; : \; \left[\frac{H}{2} + (1 - H)\, F\right]^2 V.$$

Since the frequency of the two genotypes is at equilibrium, this may be equated to the proportion $H : 1 - H$.

The overall homozygous fitness, w, cannot be obtained immediately from this equation, since V and F do not always occur together. As a first approximation therefore, the value of w will be calculated under the assumption of either all fertility or all viability differences. If all selection is attributable to fertility, so that $V = 1$, and $w = F$, then the above equation may be solved to yield

$$w = F = \frac{2 - 3H}{2 - 2H} \tag{1}$$

This equation gives meaningless negative values for w if $H > \frac{2}{3}$. From the example given previously it is clear that the value of H cannot go above $\frac{2}{3}$ unless there are viability differences.

If all selection is due to viability, so that $F = 1$, and $w = V$, then

$$w = V = \frac{2 - 2H}{2 - H} \tag{2}$$

If we assume that neither the viability nor the fertility is higher in the homozygote than in the heterozygote, the values derived above may be given as upper

and lower limits for the fitness. Thus for values of $H \leqslant \frac{2}{3}$, $\frac{2-3H}{2-2H} \leqslant w \leqslant \frac{2-2H}{2-H}$ while for $H \geqslant \frac{2}{3}$, $0 \leqslant w \leqslant \frac{2-2H}{2-H}$.

The value of w may lie outside these limits if either the viability or the fertility is higher in the homozygote than in the heterozygote (see below). However, in general the higher the contribution of fertility differences to fitness the closer w will be to the lower limit, and *vice versa*. As previously mentioned, the calculations assume equal male and female fertilities. Should this assumption be invalid, the true value of w will be slightly underestimated.

If the equilibrium frequencies of genotypes at the zygote stage (i.e., before viability selection) are known, the relative fitnesses of homozygote and heterozygote can be estimated directly. Proceeding as previously it can be shown that if the frequency of *Ba*/+ at the zygote stage is h, then

$$w = \frac{2-3h}{2-2h} \tag{3}$$

Similarly, knowing both H and h it is possible to estimate the two components of fitness, V and F. These come to

$$F = \frac{H\,(2-3h)}{2h\,(2-H)} \tag{4}$$

and

$$V = \frac{h\,(1-H)}{H\,(1-h)} \tag{5}$$

Analysis of adult frequencies: The estimated equilibrium frequencies for all chromosomes are given in column 2 of Table 4. These have generally been obtained by averaging the frequencies in replicate cages at the last sample. Fitnesses of homozygous individuals for each chromosome have then been calculated according to the fertility model (1) (column 4), and according to the viability model (2) (column 5). For the fertility model, the fitnesses range from zero, for chromosomes R11, R44, R54, and R55 to 0.93 for R53. The mean value over the eighteen chromosomes is 0.50. Similarly for the viability model the fitnesses range from 0.29 for R11 to 0.93 for R53, with an overall mean of 0.68. The estimated range for the mean fitness in the population, $\bar{w}$, is thus from 0.50 to 0.68. If, as argued above, these values are multiplied by 0.6 to give the fitness compared to wild-type rather than *Ba* heterozygotes, the range is 0.30 to 0.41. These values moreover must be reduced proportionately by about 15% to give the mean homozygous fitness for all chromosomes including lethals. The range for this parameter is 0.26 to 0.35.

Estimation of components of fitness: We attempted to get evidence in two ways on the question of what component of the selection is due to fertility and what to viability. The first approach was to take egg samples from the cages just before the last adult sample, and allow these to grow to the adult stage under uncrowded conditions which reduce the death of larvae as much as possible. The difference between these adult frequencies and those observed in the cage

TABLE 4

Estimates of fitness and fitness components

Chromosome	$H=(Ba/+)$ freq. (Adults)	$h=(Ba/+)$ freq. (Eggs, corrected)	$w=\frac{2-3H}{2-2H}$ (Fertility model)	$w=\frac{2-2H}{2-H}$ (Viability model)	$w=\frac{2-3h}{2-2h}$ (Egg sample)	$F=\frac{H(2-3h)}{2h(1-H)}$	$V=\frac{h(1-H)}{H(1-h)}$	Viability (Ratio test)
R11	.83	.47	0	.29	.56	3.06	.18	.50
R12	.41	.37	.65	.74	.71	.86	.82	1.00
R13	.15	..	.91	.92	..	..	..	1.00
R21	.36	.33	.72	.78	.75	.86	.87	1.00
R23	.54	.53	.41	.63	.43	.44	.96	.91
R27	.18	..	.89	.90	..	..	..	1.12
R28	.23	.41	.85	.87	.66	.28	2.33	1.00
R29	.37	.43	.71	.77	.60	.45	1.32	1.00
R30	.46	.51	.57	.70	.47	.39	1.22	1.00
R41	.39	.35	.69	.76	.72	.83	.88	1.06
R42	.46	.33	.58	.71	.76	1.30	.58	1.00
R43	.62	.45	.17	.54	.59	1.19	.49	1.00
R44	.70	.63	0	.46	.16	.21	.71	1.00
R51	.59	.50	.27	.58	.51	.76	.67	1.12
R52	.38	.37	.70	.77	.71	.73	.97	1.00
R53	.13	.07	.93	.93	.96	1.84	.52	..
R54	.76	.16	0	.39	.90	14.88	.06	1.00
R55	.71	.57	0	.45	.34	.63	.54	.94
Average	..	..	.50	.68	.61	..	..	..

should therefore give a measure of the effect of crowding on relative viabilities. The second test was to study the viability under uncrowded or slightly crowded conditions by the ratio test described previously, in which both parents are $Ba/+$. In the absence of gametic disturbances such as meiotic drive, the frequencies of homozygotes and heterozygotes are known exactly in this test. These two tests therefore complement each other well, and any viability difference occurring under cage conditions should be detected by either one test or the other, or possibly both.

We did not carry out any systematic tests for male or female fertility. However none of the stocks was completely male or female sterile, since as mentioned previously all gave satisfactory numbers of progeny from crosses of the type $+/+ \times +/+$.

Few significant deviations from a 2:1 ratio were found in the ratio test, despite the fact that some of the tests were carried out under a variety of conditions of crowding, and that over sixty thousand progeny were scored altogether. Of the eighteen chromosomes, only R11 gave a large reduction in viability (Table 4, column 9). Several of the other chromosomes gave marginally significant deviations from a 2:1 ratio, in both positive and negative directions.

The results from the ratio test provide a control for the egg sample test, provided that individuals in the two tests are raised under the same conditions. What the ratio test results suggest is that except for R11, the ratios observed at

the adult stage reflect with reasonable accuracy the ratios at the zygote stage. Therefore except for R11, for which a correction has been made, the observed frequency of *Ba*/+ among the adults developing from the egg samples has been used as a measure of the zygotic frequency (h).

Unlike the ratio test, the egg sample test showed considerable evidence of viability effects. Unfortunately this test suffers from statistical disadvantages. A number of egg samples were taken for each cage, and there was considerable heterogeneity between such samples, as is to be expected if each female contributes on the average more than one offspring to the sample. As we were unable to obtain sufficient samples to give an estimate of error for each cage, it is difficult to tell for which chromosomes the egg frequencies, which are given in column 3, Table 4, are significantly different from the adult frequencies. Values of F and V were calculated for each chromosome according to formulae (4) and (5) (columns 7 and 8, Table 4). In 11 out of 16 cases the value of F is less than unity, suggesting a homozygote fertility disadvantage, while in 13 out of 16 cases V is less than unity, suggesting reduced homozygote viability. The extreme values of F and V estimated for chromosome R54 are presumably somewhat spurious, although the consistently reduced frequencies of *Ba*/+ in the egg samples for both cages of this chromosome, as was also true for R53, suggest the possibility that the homozygote possesses a fertility advantage over the heterozygote which is counterbalanced by a stronger viability disadvantage.

The simplest possible explanation for the viability effects is that they are due to crowding. But this explanation is not favored by results from the ratio test. For some chromosomes this test was carried out under a variety of conditions of crowding, but this produced no noticeable effect. Tests for three chromosomes were carried out using counted numbers of eggs, so that the increase in overall mortality was accurately defined. R11 was one chromosome tested in this way, but even for this chromosome there was no significant effect of crowding on relative homozygote viability. TEMIN *et al.* (1969) have also recently reported a failure to influence homozygote viability by increased crowding. Nevertheless there is still the possibility that the level of crowding of larvae attained in the food cups of the population cages is still higher than the greatest crowding used in the ratio test.

Another possibility which must be considered is that the ratios in the cages reflect a reduced longevity of adult homozygotes. This component would not influence the ratio or egg samples tests since in these cases adults are usually scored soon after emergence and in any case are not subjected to the conditions of the population cage. It is not easy to discuss longevity in the present context, since we have been considering a discrete generation model. While it might be considered as a component of fertility rather than viability, here it is properly included in the V component (or the early component of selection of PROUT 1965), since selection for longevity has taken place before the observation of gene frequencies are made.

The data from the egg samples can be used to give a direct estimate of homozygous fitness according to formula (3). This is given in column 6 of Table 4.

The mean homozygous fitness comes to 0.61, which is a little closer to the value 0.68 calculated for the viability model than to the 0.50 calculated for the fertility model. It would be still closer to the viability estimate if we take account of the fact that egg samples were not taken for two of the chromosomes with the highest homozygous fitness.

Overall the results suggest that viability and fertility differences are probably both important. As might have been expected, they also suggest that the relative magnitude of these two components is different for different chromosomes.

DISCUSSION

It seems reasonably clear that individuals completely homozygous for second chromosomes in *D. pseudoobscura* are at a considerable disadvantage to heterozygous individuals. The results give us no grounds for unequivocally rejecting the hypothesis of generalized heterozygote advantage. Ideally however, we would like to be able to go further than this and make some quantitative statement about whether the amount of heterozygote advantage found is sufficient to allow the maintenance of the high levels of heterozygosity found in natural populations. It will be seen that the answer to this question requires a much greater undertanding than is available at the moment of the theory of the maintenance of linked polymorphisms. It also depends on having more accurate measurements of such parameters as population size and structure, and rates of mutation. However we believe that a first attempt at answering this question can legitimately be made. It is important that this be done in view of the fact that inbreeding methods are at the moment one of the most powerful techniques for investigating questions concerning heterosis and variability. The discussion may at least serve to draw attention to those areas where additional information is required.

An essential part of the theory needed in this discussion concerns the effect of linkage. In order to see the importance of linkage effects it is convenient to present an argument which effectively ignores these (for instance, SVED, REED and BODMER 1967). Consider a model chromosome having 200 loci, each of which has a symmetrical 1% heterozygote advantage: Then asuming a multiplicative interaction of selective values, the selective value of a completely homozygous chromosome compared to the average heterozygote would be $(.99)^{100}$. This comes to approximately 0.36, close to the average value found in the experiment. If it is assumed that 1% heterozygote advantage is necessary to ensure the maintenance of a polymorphism, then the results of the present paper would seem to indicate a maximum of 200 such polymorphisms in the second chromosome. Furthermore, from one point of view this calculation gives an upper limit for the expected number, since it assumes symmetrical heterozygote advantages. ROBERTSON (1962) has shown that the stability of polymorphisms drops off rapidly as the selection coefficients against the two homozygotes become unequal. As pointed out earlier, this experiment, in common with all inbreeding experiments, tells us nothing about the symmetry of the selective advantages. The

conclusion from this argument is that the number of polymorphisms which can be maintained by heterozygote advantage is about one order of magnitude lower than the numbers which appear to exist in most natural populations. For instance, natural populations of *Drosophila pseudoobscura* have been estimated to be polymorphic at about 40 percent of all loci (PRAKASH, LEWONTIN and HUBBY 1969).

A crucial assumption which is implicitly made in the above calculation is that genes at the 200 loci are associated at random, i.e., in linkage equilibrium. However recent results obtained by HILL (1968), HILL and ROBERTSON (1968), KARLIN and MCGREGOR (1968), OHTA and KIMURA (1969 a,b), and SVED (1968, 1970), suggest that linkage equilibrium is not to be expected in finite populations. Further FRANKLIN and LEWONTIN (1970) have shown that linkage disequilibrium may be expected in each chromosome even in infinite populations if selective values are sufficiently high and there is a multiplicative interaction. The effect of linkage disequilibrium will not be discussed in detail here. However, roughly speaking the effect is to cause heterozygotes at linked loci to be associated, and thus to cause the heterozygote advantages to reinforce each other. The net effect is therefore in the direction of increasing considerably the estimate of the number of polymorphisms which could be maintained by heterosis. Perhaps a more important possibility which these findings open up is that the crucial quantity in discussions of maintenance of variability by heterozygote advantage might not be the amount of heterozygote advantage per locus, but rather the overall amount of heterozygote advantage per unit of chromosome length.

Whether this possibility is a realistic one seems at the moment still speculative. It requires in particular that genes with asymmetrical overdominance or even just dominance or incomplete dominance contribute to the stability of the system. Single locus considerations would suggest just the opposite, e.g., that a gene which is recessive and disadvantageous would be rapidly lost. The only evidence supporting the argument that closely linked loci with dominance rather than overdominance might contribute to the stability of the system comes from the work of MARUYAMA and KIMURA (1968) and OHTA and KIMURA (1969b) who have shown that pseudooverdominance does apparently build up in such cases.

We must still define what is a realistic length of a homozygous chromosome segment in a natural population. In the experiment described in this paper an inversion has been used to ensure that whenever one region of the chromosome is homozygous, the whole chromosome will be homozygous. Of course this will in general not be the case in natural populations. Occasionally we might expect a whole chromosome to be homozygous by descent, but more commonly the segment of a chromosome becoming homozygous by descent must be much smaller. For example SVED (1970) has calculated that for a population of effective size 1000, any locus which is homozygous by descent will be surrounded on the average by slightly less than one-half map unit of chromosome which is completely homozygous by descent. We may then ask the question: What is the average selective disadvantage of a chromosome segment of this size? Since the average size of a Drosophila chromosome is approximately 50 map units (allow-

ing for recombination in one sex only), 0.5 map units would be 1/100 part of the total chromosome. Assuming again a multiplicative interaction of selective values, if each such region is assumed to have a 1% disadvantage, the selective value of an individual homozygous for a chromosome would be $(.99)^{100}$, close to the average value observed in the experiment.

Whether a 1% average disadvantage for a homozygous chromosome region would be sufficient to produce a significant stabilizing effect seems at present a moot point. Clearly there is a need for much more quantitative information on this and similar points than is available at the moment. The effect on the argument given above of the subdivision of the population also needs to be considered in some detail, as does the question of what is a realistic effective population size.

We have so far only been concerned with the mean fitness of individuals homozygous for second chromosomes. However the results of the experiment are in terms of fitness of individual chromosomes, and this allows us to speculate further on the distribution over the chromosome of genes affecting fitness in homozygous condition. Perhaps as argued above the question of gene number may not be crucial for questions concerning the maintenance of variability, but it is still of considerable interest from a descriptive point of view.

Alternative extreme possibilities which can be considered are that there are many such genes spread over the chromosome, each with a small effect, or that there are few such genes, each with a large effect. The results seem to indicate a mixture of these two situations. Obviously for a given mean fitness the variance in fitness among chromosomes would be greater for the latter model. The relatively large variance observed among chromosomes inclines us to the view that there are probably a number of genes each of large effect. But in another sense the results are quite uniform in that even the best of the eighteen chromosomes shows an estimated 40–50% disadvantage in homozygous condition. This would suggest that there may be many genes, each of small effect, a conclusion similar to that reached by VANN (1966). The likelihood that there is a considerable spectrum of gene effects makes any calculation of the total number of genes involved very difficult.

Note added in proof. Similar results to those described in this paper have been recently obtained in our laboratory with *D. willistoni* (MOURÃO and ANDERSON, in preparation). The experimental design in the new experiment was similar to the one described here, except that the experimental populations were maintained by "serial transfer". The average fitness of flies homozygous for 15 quasi-normal second wild chromosomes was 0.34, relative to the fitness of flies heterozygous for random combinations of the same chromosomes. In a recent paper SPERLICH and KARLIK (1970) have also found that flies homozygous for second chromosomes from a wild population of *D. melanogaster* have considerably lower fitness than flies heterozygous for the same chromosomes. The fact that separate experiments with different species give very similar results strengthens the conclusion that heterosis plays a major role in maintaining the high levels of heterozygosity observed in natural populations of Drosophila.

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SUMMARY

Population cages have been set up each containing the chromosome *Ba*, a marked second chromosome of *Drosophila pseudoobscura* which is lethal when homozygous, and one of 18 nonlethal wild-type second chromosomes. In all cases the *Ba* and wild-type chromosomes appeared to reach an equilibrium, indicating a selective advantage of the *Ba*/+ heterozygote over the wild-type homozygote. Furthermore results from control cages indicated that the *Ba*/+ heterozygotes were themselves at a considerable disadvantage to the normal wild-type heterozygote. The homozygous fitnesses of these 18 chromosomes compared to the normal heterozygote were estimated to range from about 50% down to nearly zero, with a mean of about 30–40%. Both viability and fertility appeared to be important in producing these selective differences. It is argued that these results may not be inconsistent with the heterozygote advantages required to ensure the maintenance of high levels of heterozygosity.

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