X-chromosomal heterosis in *Drosophila melanogaster*

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SUMMARY

Population cages were set up containing an X-chromosome balancer, and either a single wild-type chromosome (homozygous cages) or a mixture of wild-type chromosomes (heterozygous cages). The balancer chromosome was eliminated more rapidly from the heterozygous cages, indicating that chromosome heterozygotes are at an advantage over chromosome homozygotes. The disadvantage of X-chromosome homozygosity in the female is estimated to be about 40%. From earlier studies it is known that the average disadvantage of homozygosity for either of the two major autosomes of *D. melanogaster* is approximately 80%. Since these autosomes are both about twice as long as the X chromosome, the disadvantage per unit length is similar for both chromosomal types.

Both X-chromosomal and autosomal heterosis can be explained by either dominance or overdominance at individual loci. However, a dominance model can only explain the similarity if many of the X-linked loci (about 50%) are limited in expression to the female.

1. INTRODUCTION

The fitness of individuals homozygous for any of the major chromosomes in *Drosophila* is extremely low – of the order of 20% or less. This has been shown by a series of experiments (Sperlich & Karlik, 1970; Sved & Ayala, 1970; Sved, 1971, 1975; Mourão, Ayala & Anderson, 1972; Tracey & Ayala, 1974), using a population cage technique which will be described briefly in the following section. The mean fitnesses of non-lethal chromosomes in these studies ranged from 10% to 35%.

While these results indicate that there is a lot of selection going on at the level of the chromosome (high 'chromosomal heterosis'), we would be more interested in finding out what are the selective patterns at individual loci. Ideally we should like to be able to use the overall fitness estimates to establish both the nature and intensity of selection at individual loci. It is of course not possible to go directly from overall chromosomal fitness to individual locus fitness. However, individual locus selective values can be chosen, and combined under some assumptions to determine what selective intensities are consistent with the overall chromosomal heterosis. In this way it should be possible to obtain some information about the selective values at the 'average' locus. The main problem with this argument is that two single locus selective models are equally consistent with chromosomal heterosis (Crow, 1948). Either heterozygous advantage at individual loci (overdominance) or deleterious recessives (dominance) will produce this result.

Although the long-term consequences of these two models are very different, no results have been obtained in the cited series of experiments which could unequivocally decide between the two. Sperlich & Karlik (1970) have, however, interpreted the results from some of their experiments as favouring the dominance hypothesis.

The X chromosome of *D. melanogaster* was studied principally to provide further evidence on the question of dominance versus overdominance. In particular the consequences of selection against deleterious recessives are expected to be very different for autosomes and sex chromosomes. Autosomal genes can be maintained at relatively high frequencies by a modest mutation rate, provided they are fully recessive in effect. On the other hand, with direct selection against X-linked recessives in the male, the frequency of deleterious X-linked recessives is expected to be only of the order of the mutation rate (Li, 1955, p. 287), so that such genes ought to contribute very little to X-chromosomal heterosis.

We therefore looked upon the experiment principally as a test of the deleterious recessive hypothesis. Our expectation under this hypothesis was that X-chromosomal heterosis should be very low or absent. By comparison, the overdominance hypothesis would predict a substantial level of heterosis, albeit somewhat lower than the comparable estimates from the autosomes. The predictions of the overdominance model are complex for sex-linked loci (Haldane, 1926; Bennett, 1958), but overdominance *per se* in the female should give rise to polymorphism and therefore to detectable heterosis. Absence of X-chromosomal heterosis would thus argue for deleterious recessives as the cause of autosomal heterosis.

Unfortunately the converse result, presence of X-chromosomal heterosis, is not subject to an unequivocal interpretation. There is a class of sex-linked genes not mentioned so far, those which are deleterious recessives but limited in effect to the female. Such genes should contribute disproportionately to X-chromosomal heterosis (Crozier, 1976). The finding of X-chromosomal heterosis can therefore be interpreted as showing either heterozygote advantage or sex-linked, sex-limited deleterious recessives. A more quantitative discussion of the latter possibility is given in the final section.

2. THEORY AND METHODS

(i) *Autosomal chromosomes*

There is a very simple rationale underlying the experiments cited earlier in which autosomal heterosis is manifested. Population cages are set up each containing copies of just two chromosomes: one a wild-type chromosome sampled from a natural population, and the second a suitable balancer chromosome which prevents crossing-over and is lethal in homozygous condition. Using as an example a second chromosome balancer in *D. melanogaster*, marked by Curly (*Cy*), the three possible genotypes in the population cage are Cy/Cy, $Cy/+$ and $+/+$, the first of which is lethal. Simple theory predicts that the lethality of the Cy chromosome in homozygous condition will lead to its elimination unless there is a compensating disadvantage of the homozygous $+/+$ genotype. If the three genotypes

are assigned the selective values $0:1:1\text{-}s$ respectively, then for positive values of s an equilibrium of the Cy chromosome is expected at frequency $s/(1+s)$. Inspection of the frequency of the equilibrium therefore allows the magnitude of the disadvantage of the wild-type homozygote, relative to the balancer hetero-zygote, to be obtained.

The experiment involving wild-type chromosome homozygotes must be supplemented by another involving wild-type heterozygotes. In this case population cages are set up containing mixtures of different wild-type chromosomes as well as a balancer chromosome. This experiment allows the selective value of balancer heterozygotes to be obtained relative to wild-type heterozygotes. A comparison between the two types of cages gives an estimate of the fitness of wild-type homozygotes relative to wild-type heterozygotes, the comparison of most interest.

The ideal balancer chromosome for these experiments would be one which suffers no reduction in fitness in heterozygous condition relative to wild-type heterozygotes. In this case, no correction would be required to take account of balancer chromosome fitness. However, every balancer chromosome tested in the studies cited above was found to be rapidly eliminated in heterozygous cages. Balancer heterozygotes were estimated to have fitnesses of around 50 % or less in all cases.

This finding makes it even more striking that balancer chromosomes are maintained at high frequency in most homozygous populations. If, for example, a wild-type homozygote is estimated to have a fitness of 40 % relative to the balancer heterozygote, and the balancer heterozygote has a fitness of only 50 % compared to the wild-type heterozygote, then the overall estimate of fitness for the wild-type homozygote would be only 20 %. It is of course an assumption that the relative fitnesses can be multiplied in this way, but it seems necessary to make such an assumption to derive an overall fitness estimate.

In experiments in which only balancer chromosomes of very low fitness are available (e.g. Mourão *et al.* 1972, in *D. willistoni*), the balancer chromosome may be eliminated in some cases. The problems of estimating fitness in such cases are greater than for cases in which an equilibrium is reached, principally because an estimate of generation length is needed as part of the estimate.

(ii) X *chromosomes*

The theory for the X chromosome is a direct extension of the theory for autosomes. Again an ideal balancer would be one which is lethal in homozygous condition (in the female) but not in heterozygous condition. However, an additional requirement is that the balancer should not possess any disadvantage in hemizygous condition (in the male). Unfortunately it quickly became clear in preliminary experiments that neither of these requirements, particularly the latter, can be met with existing balancer chromosomes. The chromosome FM6 (Lindsley & Grell, 1968, p. 407) though quite viable, is male fertile and female sterile, and therefore appeared to have the right combination of properties. As might have been

anticipated, however, males carrying the balancer chromosome had essentially zero fitness under population cage conditions, and this contributed to the rapid elimination of the balancer chromosome from the population. We also tried the balancer FM1 in a preliminary experiment, but this did not appear to possess any advantages over FM6. In order to estimate X-chromosomal heterosis we were therefore forced to compare the rate of loss of balancer chromosomes from homozygous and heterozygous cages. The statistical problems involved in this comparison are considered in the following section.

As the source of wild-type flies for the experiment, we used two populations, one from the Hunter Valley district of New South Wales and the other from Canberra. Flies from the Hunter Valley, our main source of wild-type collections, show hybrid dysgenesis in crosses to some laboratory strains (Kidwell, Kidwell & Sved, 1977), and the Canberra collection was made to provide an additional guard against the possibility that the observed effects could be attributable to hybrid dysgenesis. The similarity of results obtained from the two stocks provides some assurance against this possibility.

The procedure for setting up homozygous X-chromosome cages is illustrated in Fig. 1. In the parental cross a single wild-type male was crossed to FM6/lethal stock females, thus ensuring that all descendants from that cross contained the identical wild-type chromosome. The FM6/+ female progeny from this cross were crossed to FM6/Y male progeny from other similar parental crosses, the purpose of outcrossing at this stage being to avoid autosomal inbreeding. One further generation of intercrossing was then carried out in order to increase numbers for starting the population cage. Only progeny that had inherited an FM6 chromosome were collected as virgins and added to the cages.

Heterozygous lines were set up at the same time as the homozygous lines by mixing large numbers of homozygous lines at the F_1 stage. In all, 34 different homozygous lines were set up (24 Hunter, 10 Canberra) and 10 heterozygous lines (7 Hunter, 3 Canberra). Duplicate cages were also set up for 6 of the homozygous lines as a small test for consistency.

In addition to the above cages, 20 of the homozygous lines were duplicated with a different genetic background (bw on chromosome II). As well as providing a test for possible effects on background genotype, these cages also provided a check against contamination by wild-type (or non-bw) flies. The production of lines with a bw background was achieved by crossing FM6/+ progeny from the F_1 generation to a v, bw stock. Non-Bar male progeny from this cross were then crossed to an FM6/v stock which contained the bw gene, and B, bw progeny were selected from this cross to start the cages. The extra generation of crossing to v, bw in this programme was balanced by the extra generation of intercrossing needed in the wild-type background lines, so that the two sets of cages were started at the same time. Duplicate cages were also set up for two of these lines, and six heterozygous cages were started with mixtures of the bw background lines.

Out of these 28 cages, 5 were found during the course of the experiment to contain non-bw flies, and these five were consequently rejected. Most of these

were probably not contaminated, but accidentally set up with one or more bw^+ genes. This is also likely to have happened with v alleles in two cages which were rejected for having large numbers of white-eyed flies. We also rejected three cages in which an early sample gave a high proportion of $+/+$ flies, indicating that non-virgin FM6/+ females had inadvertently been used in setting up the cage.
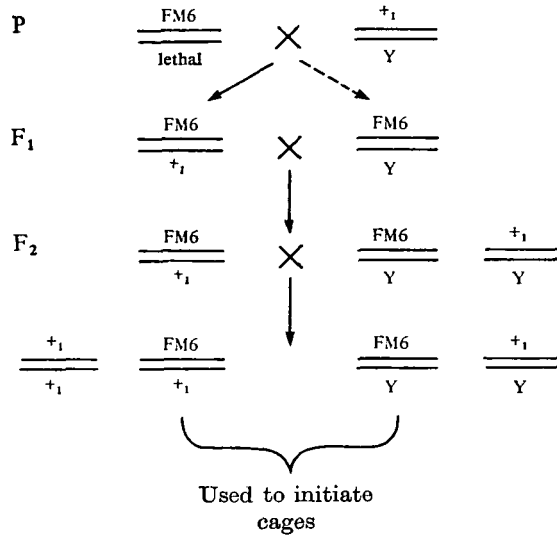

Fig. 1. Series of crosses used to set up the cages. The broken arrow indicates that the flies came from similar crosses in different lines.

Samples from all cages were also tested for the polymorphic sex-linked electrophoretic marker 6-phosphogluconate dehydrogenase (*Pgd*). A 'homozygous'+ background cage was found to be polymorphic for this marker and was also rejected from the analysis. Finally five + background cages were rejected because of cage leaks or the appearance of occasional unexpected markers. Many lines produced occasional unusual males which were feeble, infertile, Bar-eyed, and of a Notch-like phenotype. These flies are expected from crossing-over in FM6/+ heterozygotes (Lindsley & Grell, 1968, p. 406). Hopefully their low fertility prevented them from affecting the results.

(iii) *Methods of sampling*

In order to obtain a fitness estimate which correctly incorporates components from different stages of the life-cycle it is necessary that the frequencies be estimated at the zygote rather than at the adult stage (Prout, 1965). This poses a problem, since all phenotype observations must be made at the adult stage. This problem was overcome as follows (cf. Sved, 1971).

Samples from the cages were taken by allowing flies to lay for 24 h in a food cup. Eggs were then transferred from these to vials in batches of 100–200 and thereby allowed to grow to adulthood under conditions which minimize crowding. Adults obtained from these vials were scored for phenotype.

The estimates obtained in this way reflect the frequencies at the zygote stage as

modified by egg-to-adult viability. This viability was estimated in separate experiments as follows. Crosses of the type FM6/+ × +/Y were set up with 20–30 flies of each sex. Eggs were obtained from these crosses and placed in vials in batches of 100–200 to duplicate the conditions of rearing of the cage samples. The zygote frequencies in these crosses are known, assuming that gametic selection is absent, to be 1 FM6/+ : 1 +/+ female and 1 FM6/Y : 1 +/Y male. Comparison of the observed adult frequencies within each sex (line 3, Table 1) allows the relative egg-to-adult viability to be estimated. The zygote frequencies in the cage samples were then inferred from their adult frequencies by using this estimate of viability. The procedure assumes that there are no frequency-dependent effects on viability.

Table 1. *Summary of symbols for frequencies and relative fitnesses of X chromosome genotypes*

Genotype	Females		Males	
	+/+	FM6/+	+/Y	FM6/Y
Relative frequency in egg sample adults	$1-h$	h	$1-k$	k
Relative frequency in viability ratio test (optimal conditions)	$1-r$	r	$1-s$	s
Relative frequency at zygote stage (inferred)	$1-H$	H	$1-K$	K
Inferred relative fitness in cage (zygote to zygote)	w	1	1	0 (see text)

The population cages and the procedures for the experiment were similar to those of Sved (1975). Egg samples were taken at 3-weekly intervals, the first sample being at $2\frac{1}{2}$ weeks after the cages were set up. After the fifth sample, taken at $14\frac{1}{2}$ weeks, the cages were terminated since the frequencies had reached an uninformatively low level. Egg samples were also taken twice weekly from eight cages in order to follow more closely the shape of the elimination curve.

3. RESULTS AND ANALYSIS

In all but one of the homozygous cages, and in all of the heterozygous cages, the FM6 chromosome was eliminated or reduced to very low frequency. No FM6/FM6 females were found after the $5\frac{1}{2}$-week sample. Since this genotype is quite viable in test crosses, its absence from the samples constitutes strong evidence that the FM6/Y male genotype has essentially zero fitness under cage conditions. This undoubtedly contributed to the rapid loss of the FM6 chromosome.

The important information for this study concerns the relative rates of elimination of the FM6 chromosome from heterozygous and homozygous cages. A summary of this information is provided in Fig. 2, which gives the combined results for the + background cages 1–20. From both female and male frequencies it can be seen that the rate of elimination is higher in heterozygous than in homozygous cages. This demonstrates the existence of X-chromosomal heterosis. The estimation of its magnitude is considered in the following section.
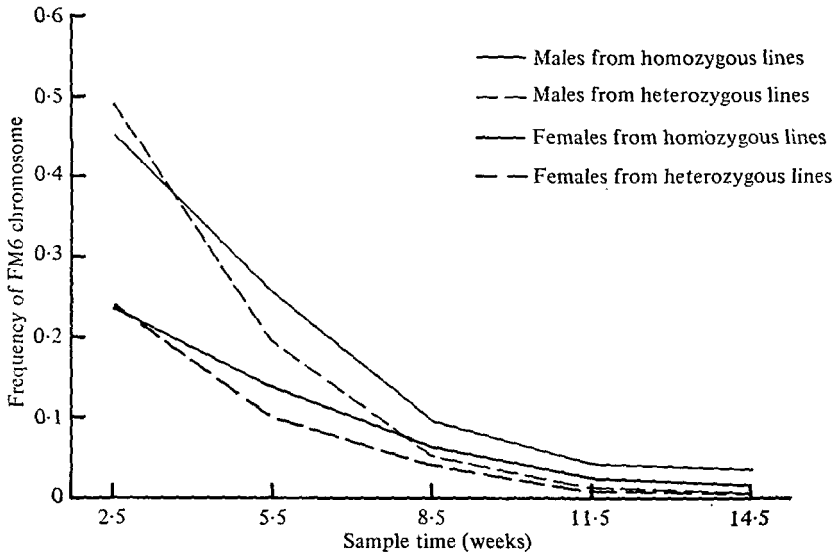
Fig. 2. Mean frequency of the FM6 chromosome in males and in females from homozygous (+) lines (1–20) and from the corresponding heterozygous lines.

(i) *Analysis*

The notations used for the frequencies and fitnesses of the four fertile genotypes are given in Table 1. By applying the relative viabilities of the female genotypes from the ratio viability test to the egg samples, the frequency in the zygotes of FM6/+ (H) can be readily determined in terms of the frequency in the adults (h).

$$H = \frac{(1-r)h}{(1-r)h+(1-h)r} \qquad . \tag{1}$$

Similarly, in males the zygote frequency of FM6/Y,

$$K = \frac{(1-s)k}{(1-s)k+(1-k)s}. \tag{2}$$

Assuming the relative fitness of the $+/+$ female is w, the expected frequency of the next generation at the zygotes stage in the male is

$$K' = \frac{\tfrac{1}{2}H}{(1-H)w+H} \qquad , \tag{3}$$

which is equivalent to the frequency in the female (H') when the FM6/Y male fitness is zero. (Primed frequencies represent those one generation later than the unprimed frequencies.) Due to differences in relative viabilities between the sexes the frequency in the adult males is not equal to that in the females.

Instead, $$\qquad k' = \frac{(1-r)h}{(1-s)[2r(1-h)w+h(1-r)]+(1-r)h} \qquad , \tag{4}$$

while $$\qquad h' = \frac{h}{2r(1-h)w+(1-r)h+h} \qquad . \tag{5}$$

These equations are obtained by substituting (1) and (2) in equation (3) and rearranging.

Equations (4) and (5) were used to produce curves describing the expected rate of elimination of the FM6 chromosome. A computer programme was written to generate trial values for the two parameters, frequency of zygotes at the start of the simulation, and $+/+$ fitness (w). Chi-square values were calculated for agreement of observed and expected values in both females and males, and a minimum chi-square criterion was applied to obtain the parameters giving the best fit (cf. Tracey & Ayala, 1974).
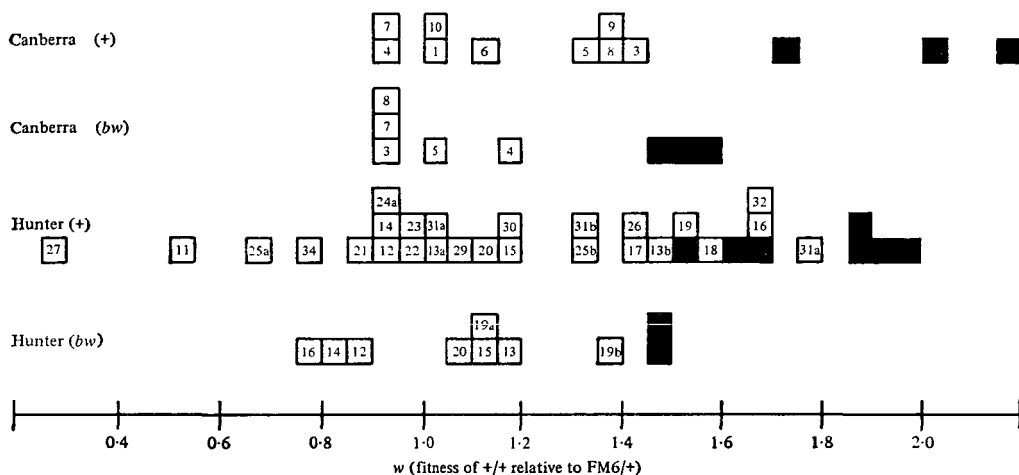

Fig. 3. Fitness of wild-type females (w) from each line. The lines have been grouped by sample locality and background genotype. Numbered squares represent homozygous lines. Shaded squares represent heterozygous lines.

As mentioned previously, sampling from the cages was carried out at 3-weekly intervals. In the above calculations it was assumed that the generation interval was the same as the sampling period, an assumption which is shown below not to be critical. The eight cages sampled twice weekly showed irregular frequency changes for the first 3–4 weeks. Therefore the first sample, taken at $2\frac{1}{2}$ weeks, was ignored in the calculations, on the grounds that the cage had not had time to attain equilibrium numbers or a reasonably stable age distribution.

Estimated w values for all cages are shown in Fig. 3. Homozygous lines are shown as squares containing line numbers, while heterozygous lines are shown shaded. Results for the Hunter and Canberra stocks are shown separately, and the results from $+$ and bw duplicates are given separately within these. The Hunter $(+)$ results contain a set of cages numbered 21–34, for which bw duplicates were not made. These lines were set up as a pilot experiment prior to the main experiment, with sampling starting at a slightly different time. Since there was no obvious difference between these and the Hunter $(+)$ results from the main experiment the two sets are shown together.

The most obvious conclusion from the results of Fig. 3 is that the faster rate of elimination of FM6 from heterozygous cages is consistent for most cages. No homozygote is fitter than the average of the corresponding heterozygotes. The fitness is somewhat lower in the *bw* than in the + background cages, but this reduction applies to both homozygous and heterozygous cages.

The agreement between duplicate cages, i.e. those started with the same chromosome lines, is not particularly good. This shows that individual *w* estimates must have a high standard error. An analysis of variance testing for variability between chromosome lines against variability between duplicates gives $F_5^{27} = 1\cdot35$, which is not significant. Thus the results cannot be said to show significant differences between homozygous lines. On the other hand, the differences between homozygous and heterozygous lines are clearly significant.

The figure of greatest overall interest is the relative fitness of homozygotes to heterozygotes, which we have estimated as the ratio of each homozygous *w* value to the mean *w* value for comparable heterozygotes. These values have been averaged, giving means and calculated standard errors $0\cdot59\pm0\cdot05$, $0\cdot65\pm0\cdot04$, $0\cdot63\pm0\cdot05$ and $0\cdot68\pm0\cdot05$ for the four classes Canberra (+), Canberra (*bw*), Hunter (+) and Hunter (*bw*) respectively. In calculating the fitness over all lines of homozygotes relative to heterozygotes, we averaged over + and *bw* duplicates (relative to the averages of all + and all *bw* heterozygotes respectively) so that each line contributed one value regardless of how many duplicate cages were set up. This gave an overall estimated fitness of chromosome homozygotes of $0\cdot62\pm0\cdot03$.

The calculation was repeated to test the importance of the estimate of generation length. Assuming an unrealistically low generation time in the cages of 2 weeks, and interpolating to derive expected frequencies, the average fitness increased only slightly to $0\cdot65\pm0\cdot03$. We also tested the effect of relaxing the assumption that FM6/*Y* has zero fitness in the cage. Assigning a fitness of 5% to all FM6/*Y* males, the overall fitness dropped slightly to $0\cdot60\pm0\cdot03$. Overall, therefore, we conclude that homozygosity reduces the fitness by approximately 40%.

4. DISCUSSION

The *X* chromosome is about half as long as the major autosomes II or III. The combined earlier work on autosomal homozygotes gives an average loss in fitness for homozygosity as approximately 80% for either autosome. Thus a 40% fitness loss for a chromosome of half the length would, on an additive model, indicate a fitness loss per unit length of chromosome which is comparable for the *X* chromosome and autosomes.

A more realistic calculation can be made if we assume a multiplicative rather than an additive fitness model. The unreality of the additive model is readily seen by extrapolating to higher chromosome lengths, in which case negative fitness estimates are obtained. If we interpolate from the autosomal results on a multiplicative rather than additive scale, the expected fitness for a chromosome of half the

length is $\sqrt{0.2}$, which is 0.45. This is below the 0.62 estimated above for the X chromosome, although not substantially so.

These results would seem to rule out the possibility of simultaneously explaining the autosomal and X-chromosomal results in terms of simple deleterious recessives. To show this, we can consider a model in which all loci have the same mutation rate to deleterious recessives, u, and the same selective value of the deleterious phenotype, s. Furthermore the selective values as estimated in the population cage are assumed to be the same as those responsible for keeping the genes at low frequency in nature. The number of loci on the autosomes is taken as N and the number on the X chromosome as $N/2$. We assume further a multiplicative interaction of selective values.

At equilibrium, the expected frequency of deleterious recessives is $\sqrt{(u/s)}$, although this may be an overestimate if effective population sizes are low (e.g. Li, 1955, p. 342). The average number of deleterious genes per autosome is thus $N\sqrt{(u/s)}$. The fitness of autosomal homozygotes is

$$w_A = (1-s)^{N\sqrt{(u/s)}}. \tag{6}$$

For X-linked recessives, on the other hand, the frequency of deleterious recessives at equilibrium is only $(3u)/s$. The homozygous fitness is then

$$w_X = (1-s)^{\frac{1}{2}N.(3u)/s}.$$

Substituting for N from (6) we get

$$w_X = (w_A)^{\frac{3}{2}\sqrt{(u/s)}}. \tag{7}$$

Substituting 0.2 for w_A, and an unrealistically high value of 10^{-3} for u/s, this gives $w_X = 0.93$. More realistic lower values of u/s would give even higher values for w_X, considerably higher than the 0.62 estimated earlier.

Using the model as outlined above, it is possible to estimate the value of w_X in a different manner, independently of the autosomal fitness, w_A, and of the selective value per locus, s. Standard theory for genetic loads (e.g. Crow & Kimura, 1970, p. 299) shows that homozygosity for the X chromosome in an equilibrium population should give a depression of fitness equal to approximately three times the mutation rate for the X chromosome. This would presumably not be much higher than a few per cent. This estimate is potentially sensitive to the form of the selective interaction assumed, as is the estimate from equation (7).

The estimate from equation (7) is also sensitive to the assumption that deleterious alleles are completely recessive. It is possible to adapt the dominance model to fit the observations by including a high level of partial dominance for the deleterious alleles in the derivation of equation (7). The level needed has been calculated at about 37% (Wilton, in preparation) which is unrealistically high in comparison to the estimate given by Temin (1966). Also, the high observed value for w_X would, on the load argument, still imply an unrealistically high overall mutation rate for the X chromosome.

Sex-limited genes

Although simple deleterious recessives can apparently be ruled out as the cause of X-chromosomal heterosis, the same cannot necessarily be claimed for deleterious recessives which are limited in effect to the female. Crozier (1976) has pointed out that if loci can be divided into two classes, those with effects on both sexes and those affecting only the female, the latter will contribute to inbreeding depression out of proportion to their numbers. This is because deleterious genes at such loci can reach much higher equilibrium frequencies, since they are not subject to the effects of selection in hemizygous condition in the male. In the following calculation we will try to estimate what fraction of loci would need to be of this latter class in order to reconcile the heterosis estimates for autosomes and for the X chromosome.

The equilibrium frequency of deleterious recessives at a sex-limited locus is $\sqrt{[(3u)/(2s)]}$ (Crozier, 1976). If a fraction, P, of loci on the X chromosome belongs to this class, and a fraction $(1-P)$ to the class with effects on both sexes, the average number of deleterious genes per chromosome, n, is

$$P \cdot \frac{N}{2} \cdot \sqrt{\frac{3u}{2s}} + (1-P) \frac{N3u}{2s}.$$

The value of w_X for this case is then $(1-s)^n$. The value of w_A is taken to be the same as derived previously (equation 6), assuming that sex-limitation for autosomal loci has a comparatively small effect on the equilibrium frequency.

Substituting for w_A from (6) into the equation for w_X, and rearranging, gives

$$P = \frac{2 \cdot \ln(w_X)/\ln(w_A) - 3 \cdot \sqrt{(u/s)}}{\sqrt{\frac{3}{2}} - 3 \cdot \sqrt{(u/s)}}. \tag{8}$$

Since u/s is small for all reasonable values of u and s, P is almost independent of their values. Assigning the estimates of $w_A = 0 \cdot 2$ and $w_X = 0 \cdot 6$, gives a value for P of approximately 50 %. With a similar calculation for an additive interaction model under which $\ln(w)$ in equation (8) is replaced by $(1-w)$, the value of P rises to approximately 80 %. These calculations suggest, therefore, that the proportion of sex-limited loci on the X chromosome would need to be very high in order to reconcile the autosomal and X-chromosomal results in terms of deleterious recessive genes.

It is not easy to decide whether sex-limited loci could play a role as substantial as is needed to explain the above figures. Since the X chromosome plays a major role in determining the female phenotype, it might be supposed *a priori* that many of the loci on the X chromosome would be restricted in effect to the female. This expectation is not borne out by an examination of the known X-linked loci. Only about 10 % of these appear to be limited in effect to the female (Lindsley & Grell, 1968, p. 433). There are of course possible biases in extrapolating from loci ascertained as having large effects on fertility or observed phenotype to the type of gene which might be responsible for reducing female fitness in the present experiment.

We should consider a second possibility – that genes having effects restricted to one sex have a greater bearing on fitness than genes with effects on both sexes. Since viability accounts for only a relatively small proportion of the overall inbreeding load for the autosomes (e.g. Sved & Ayala, 1970), fertility clearly plays an important role in determining overall fitness. This suggests that genes which affect the reproductive systems (independently in females and males) may be of greater than average importance. However, such an argument fails to take into account the probable importance of genes which do not affect the reproductive processes directly, but which diminish vigour and competitive ability and therefore contribute to reduced fitness through the sexual components (Bundgaard & Christiansen, 1972).

A study which gives a more direct estimate of the population incidence of sex-limited genes of large effect is that of Drescher (1964). Drescher estimated that 8·5 % of a sample of 234 X chromosomes from natural populations contain female-limited genes with major effect on viability or fertility. If the 40 % loss in fitness estimated in the present study is to be explained in terms of sex-limited genes, this means that such genes of small effect must be 3–4 times as important in determining overall fitness as genes of large effect. Direct estimates for autosomal loci rank the contribution of genes of small effect as comparable to or less important than genes of large effect (Temin, 1966). However, under population cage conditions, genes of small effect appear to make the major contribution to inbreeding depression (Sved & Ayala, 1970), so the same could possibly also be true for sex-limited genes.

If the X-chromosomal heterosis cannot realistically be explained in terms of sex-limited genes, this leaves overdominance as the most likely explanation. Overdominance *per se*, in the absence of other selective forces, will lead to polymorphism in both the autosomal and X-chromosomal cases. Since only one sex possesses the heterozygote advantage, the level of polymorphism which can be maintained under a selection/drift balance would be lower in the X-chromosomal case. Any asymmetry of selective values in the male would presumably accentuate this difference, except in the unlikely event of an opposing asymmetry of selective values in the female. The level of heterosis in the X chromosome therefore seems to be comparatively high even for a pure overdominance model.

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