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High levels of fitness modifiers induced by hybrid dysgenesis in *Drosophila melanogaster*

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Summary

Wild-type chromosomes of *D. melanogaster* mutagenized by passage through a single generation of hybrid dysgenesis have been compared against identical chromosomes passed through a reciprocal, non-dysgenic cross. Fitness of the chromosome in homozygous condition has been examined in population cages using the technique of balancer chromosome equilibration. The results indicate that amongst chromosomes with no lethal or visible mutation, more than 50% have suffered a measurable decline in fitness. The magnitude of this decline is estimated to be in the range 10–20%.

1. Introduction

The elevation of mutation rates is perhaps the best known symptom of the P-M system of hybrid dysgenesis (Kidwell, Kidwell & Sved, 1977*b*). Mutations produced by this syndrome have been studied in several different ways. Best known are the studies on mutational effects at individual loci, e.g. the *white* locus (Simmons & Lim, 1980; Rubin, Kidwell & Bingham, 1982). At a second level, mutation has been studied using the production of lethal mutations, most conveniently on the X-chromosome (Kidwell, Kidwell & Ives, 1977*a*). At a third level, Mackay (1984, 1985) has used mutagenesis to enhance selection for a quantitative character, abdominal bristle number.

Aside from the studies on lethal genes, the first attempt to measure the effects of P factor mutation on fitness has recently been reported by Yukuhiro, Harada & Mukai (1985). These authors measured the effect on homozygous viability of passage of chromosomes through nine generations of dysgenesis, finding that most of the effect could be attributed to lethals and severe detrimentals. In this paper we report an experiment which is similar in principle, but in which the overall fitness of chromosome homozygotes, rather than viability, is measured. Mackay (1986) uses a similar measure of overall fitness. We show that the extra sensitivity of this measure enables detection of the effects on fitness of a single generation of mutagenesis.

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Methods and materials

(i) *Stocks*

Harwich: Classified as P in the P-M system. This stock was obtained from Dr M. G. Kidwell in 1979.

ale: *al cn bw* (II); *e* (III). Classified as M in the P-M system. Also classified as R in the I-R system owing to egg-hatch sterility obtained in crosses to Canton-S males (unpublished results).

Cy/Pm: SM1(*Cy*)/In(2LR)bw^{V1},*ds* ^{33k} bw^{V1}. This stock was synthesized in crosses involving wild-type flies collected from the Hunter Valley district of N.S.W. The stock is classified as Q, since it produces no gonadal dysgenesis when used as either female or male parent.

(ii) *Experimental design*

The crosses used to produce the mutagenized chromosomes are shown on the left-hand side of Fig. 1, while the right-hand side shows the control series. The two series differ only in the direction of the cross producing the G3 females. The experimental series uses a female (G2) with M cytotype, thereby ensuring dysgenesis in the G3 progeny, both male and female (Engels, 1979a). The control series uses the reciprocal cross, which leads to much lower dysgenesis levels. The design is such that there should be no further dysgenesis after G3. Furthermore, mutations occurring in any flies after this stage will not be made homozygous.

Use of single G1 males ensures that all chromosomes in the control and experimental series are iden-

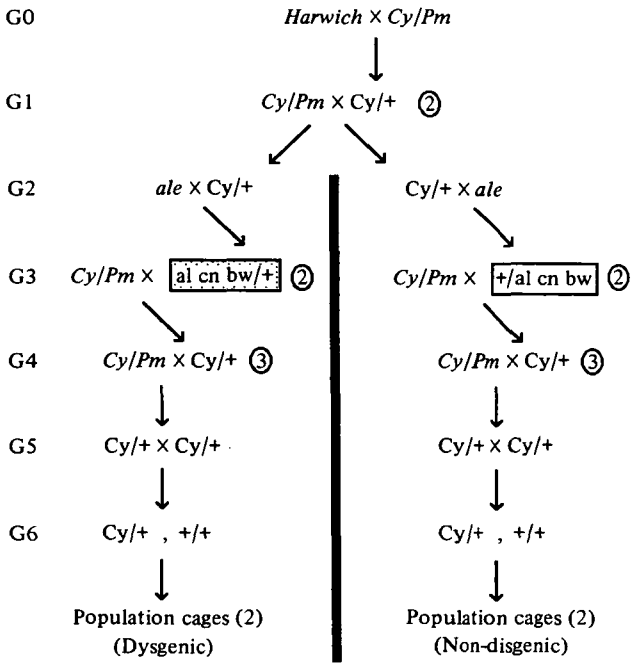

Fig. 1. The crossing program used to produce mutagenized and non-mutagenized replicats of a single wild-type (*Harwich*) chromosome. The female is shown first in all crosses, stock names are shown italicized, and otherwise only second chromosome genotypes are shown. Ringed numbers indicate the number of single males used at each stage, and the dysgenic and non-dysgenic males are boxed, with hatching and non-hatching respectively.

tical in origin. The experiment reported below is based on just two such G1 males, whose descendant second chromosomes will be referred to as C-1 and C-2. Use of single males is also critical at the G4 stage, where each male carries an individually mutagenized chromosome to be made homozygous. Single males were also used at the G3 stage, although only as a test for homogeneity of mutation rates in different males of the same series and chromosomal constitution.

(iii) *Choice of test chromosomes*

An inherent difficulty of the design, and of other designs we could envisage, concerns the choice of chromosomes to be mutagenized. Ideally, a range of wild-type chromosomes should be used. However, the necessity of using a known P strain constrained us in our choice of chromosomes. All strong P strains, the *Harwich* strain included, are inbred owing to the use of a limited number of founding parents and also to many years of laboratory storage. In fact, the results given below suggest that there is by no means complete homogeneity in the *Harwich* strain.

Looking at the genotypes of the G3 males in Fig. 1, it can be seen that there is, in principle, the possibility of using the *al cn bw* second chromosome derived from the laboratory M strain rather than the wild-type chromosome. Variants of the present design using M balancer chromosomes would have to be used in such a case to ensure identity of the chromosomes used in the dysgenesis and non-dysgenesis series. It should be noted that the necessity for distinguishing the two chromosomes in the G4 male means that a genetically marked laboratory strain must be used as the M strain. The *al* and *cn* markers are convenient ones for this strain, in that they are present in the SM1(*Cy*) balancer chromosome.

The decision to use *Harwich* rather than *al cn bw* chromosomes was based on a desire to avoid the possibility of complicating fitness effects of the visible markers. However, the results of the experiment given below suggest that the mutagenic fitness effects may be sufficiently large to justify an experiment using laboratory marker chromosomes. As discussed later, the use of laboratory chromosomes would also be desirable if the chromosomes are to be analysed for insertional changes at the molecular level.

(iv) *Estimation of fitness*

To measure the fitness of chromosomes which have been subject to P factor mutagenesis, we chose to use the population cage method introduced by Sved & Ayala (1970), which may be described as the 'balancer chromosome equilibration' technique. The technique uses a continuously breeding population set up with just two chromosomes, the chromosome whose fitness is to be measured and a suitable balancer chromosome. Using as an illustration the balancer chromosome labelled *Cy*, and designating the wild-type chromosome as +, the three possible genotypes are *Cy*/*Cy*, *Cy*/+ and +/+. Most balancer chromosomes contain lethal genes, so that the relative fitnesses of the these genotypes may be written as $0:1:1-s$, where s is the selective advantage, or disadvantage, of the chromosome homozygote compared to the heterozygote. If, as was found by Sved & Ayala (1970), the +/+ homozygote has a lower fitness than the balancer heterozygote, then the parameter s in the above formulation will be positive, leading to a situation equivalent to heterozygote advantage. This ensures that the balancer chromosome comes to an equilibrium in the cage, and the magnitude of s can be calculated from the equilibrium frequency.

The population cages and procedures were as described in Sved (1971). Sampling was carried out by taking eggs and allowing adults to develop under uncrowded conditions. After setting up, three cages were monitored at intervals of two weeks to test for attainment of equilibrium. All cages were then sampled at 7 weeks, and again at 10 weeks. The average sample size per cage was 595, and although there was considerable variation in individual sample sizes, all readings were based on counts of more than 150.

Details of the estimation of s have been given by Sved (1971). The estimation is complicated by two factors. First, for an unbiased fitness estimate, frequencies need to be estimated at the zygote stage (Prout, 1965). In practice, such frequencies cannot be obser-

ved, and they must therefore be inferred using viability estimates obtained in separate experiments. Secondly, the parameter s estimates the chromosome homozygote fitness relative to the balancer heterozygote, a genotype which is not of inherent interest. Any estimate relative to the wild-type heterozygote requires a separate control experiment involving mixtures of wild-type chromosomes rather than single wild-type chromosomes (Sved & Ayala, 1970).

We chose to bypass both of these complications in the present experiment, by simply comparing the cage frequencies of mutagenized versus non-mutagenized chromosomes. This does not lead to an estimate of homozygote fitness for either chromosome type. However, the main point of interest in the experiment is the overall level of detectable mutation, rather than an absolute measure of its magnitude. In practice, also, it is doubtful whether the extra uncertainties introduced by the side experiments do not outweigh the gain in information on the absolute fitnesses. Recent experiments of Pascoe (1985) have shown that such side experiments may give biased results unless the conditions of rearing of both parents and offspring are made comparable to those of the cage samples.

The experiment is specifically suited to detecting recessive deleterious effects. In each case, the homozygous mutagenized or unmutagenized chromosome is being compared to the heterozygote involving the same chromosome. It is readily seen that any mutation which reduces the heterozygote fitness by the same proportion as the homozygote fitness will have no effect on the equilibrium. Conversely, if there is a change in the equilibrium frequency, the assumption of recessivity gives a minimum estimate of the magnitude of the selective effect.

Results and Discussion

(i) *Sterility and lethal production*

Preliminary tests for sterility showed that the strains used in the experiment give high levels of dysgenesis. When female *ale* were crossed to male *Harwich*, female progeny raised at 29 °C yielded 100% gonadal sterility. The reciprocal cross gave no sterility.

Under the conditions of the experiment, in the crosses of Fig. 1 the sterility of female sibs of the G3 males was 64%. The lower sterility is presumably a reflextion of the lower number of *Harwich* chromosomes involved. Again there was zero sterility in progeny from the reciprocal cross, confirming the expected difference in dysgenesis between the two reciprocal series.

Although the sterility test provides a useful guide to the incidence of dysgenesis, a more direct guide for the present experiment is given by the rate of production of lethal mutations. The procedure for this test is similar to that given in Fig. 1, with the existence of a lethal mutation being indicated by the non-appearance of homozygous progeny at the G6 stage. Much larger numbers of G4 males were used for this test than for the population cage test, and also both chromosomes of the hybrid, *al cn bw* and + were examined for the production of new lethals.

Table 1. *Frequency of lethals produced by crossing program of Fig.* 1

Chromosome	Dysgenic lines	Non-dysgenic
Harwich	8/90 (9·8%)	3/88 (3·5%)
al cn bw	9/115 (8·5%)	2/111 (1·8%)
Total	17/205 (9·0%)	5/199 (2·6%)

Results from the test are given in Table 1. A significantly higher ($\chi^2 = 6{\cdot}55$, $P < 0{\cdot}05$) rate of production of lethals in the dysgenic series is seen overall. There were five cases where more than one lethal was detected in a progeny group, and although allelism was not tested, the differences between series are still significant even if it is assumed that all such cases represent single mutational events ($\chi^2 = 4{\cdot}08$, $P < 0{\cdot}05$).

The results show no significant differences in lethal production between the two chromosome types *al cn bw* and +. This result is in accord with Kidwell *et al.* (1977*a*), who found that the mutation rate in females for X chromosome lethals was comparable to that of the P and M chromosomes of the hybrid. The result is noteworthy in one respect, in that one of the traits of hybrid dysgenesis is a deficiency of the P chromosome in progeny of the hybrid parent (Hiraizumi, 1977). An increased mutation rate to dominant lethals in the P chromosome would explain this deficiency, and it is surprising that this increase is apparently not accompanied by an increased mutation rate to recessive lethals.

One further consideration from Table 1 concerns the lethals produced in the non-dysgenic series. Five such lethals were produced altogether, representing four independent events. The accepted rate of occurrence of 'spontaneous' second chromosome lethals is 0·5% (Simmons & Crow, 1977). On this basis, the probability of observing at least four independent mutation in 199 progeny is only 1·8%. Therefore the results indicate a significant production of mutations in the 'non-dysgenic' series. Using the 'singed-weak' test, Engels (1979*b*) has previously shown that a low but significant level of dysgenesis is expected in the cross between P female and M male. This cross is described for simplicity in Table 1 and elsewhere as 'non-dysgenic', but this is clearly only an approximate description.

(ii) *Population cage results*

The complete population cage results for the experiment are shown in Table 2, in terms of the frequency of the +/+ genotype. Two chromosomes coming from different G1 males were tested. Each series used two

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Table 2. *Population cage frequencies for the +/+ genotype*

	Dysgenic Count 1	Dysgenic Count 2	Non-dysgenic Count 1	Non-dysgenic Count 2
	26·5	22·1	21·5	18·9
	25·1	24·1	22·2	15·7
	20·6	21·2	30·4	28·6
	24·9	22·7	30·7	30·6
	26·7	26·4	27·0	28·3
	23·9	25·5	26·4	27·8
	30·1	27·8	30·7	28·9
	26·3	28·9	29·3	29·5
	22·6	27·7	28·1	31·3
	24·0	26·0	29·1	29·0
	27·6	22·7	—	—
	25·8	25·9	—	—
C−1 average	25·4	25·1	27·5	27·0
	26·1	24·5	35·3	35·9
	26·0	23·1	33·4	40·6
	20·9	24·3	35·1	35·6
	24·1	26·0	38·2	36·8
	33·9	37·1	36·2	39·9
	37·4	34·7	31·7	39·9
	33·4	39·0	38·3	33·5
	35·6	38·5	36·7	35·3
	23·9	26·5	37·1	33·5
	23·0	29·6	34·1	28·5
	16·4	14·9	34·4	31·6
	17·3	15·9	37·7	35·3
C−2 average	26·5	27·8	35·7	35·5
Overall average	25·9	26·4	31·6	31·2

different males at the G3 stage. Three G4 sons were used for each such G3 male, each representing an independently sampled gamete. Finally, two duplicate cages were set up for each such chromosome. Thus there should have been altogether $2 \times 2 \times 3 \times 2 = 24$ cages for each of the dysgenesis and non-dysgenesis series, although this number was reduced by 2 for the non-dysgenesis series through the absence of one sampled chromosome.

The low frequency of the +/+ genotype in Table 2, despite the lethality of the *Cy/Cy* homozygote, is an illustration of the expected low homozygote fitness under population cage conditions (see e.g. Lewontin, 1974, p. 62; Haymer & Hartl, 1982). The agreement between the two counts indicates that the population cage frequencies are in approximate equilibrium by the time of the first count. Also preliminary indications that dysgenesis is having an effect on the frequencies come from a comparison of the averages from the two series. The chromosome frequencies are consistently lower in the dysgenesis series.

Table 3. *Analysis of variance of population cage frequency results of Table 2*

Source of variation	D.F.	M.S.	*F*
Dysgenesis	1	696·6	8·0 *
Chromosome	1	594·0	6·8 *
Dysgenesis × chromosome	1	218·4	2·5 n.s.
Between G3 males	4	39·7	0·4 n.s.
Between G4 males	15	99·3	20·9 ***
Between cages	23	2·9	0·5 n.s.
Count	1	0·2	
Dysgenesis × Count	1	4·9	
Chromosome × Count	1	5·3	
Dysgenesis × chromosome × count	1	2·6	
Error (counts)	42	5·9	

An overall analysis of variance confirms these indications (Table 3). The last five components in the table show that there is no overall difference between the two counts, in either series or chromosome. The next component shows that the variability between duplicate cages is comparable to the variability between counts. Based on the overall variance generated by these six components, there is a highly significant difference between gametes from the dysgenic (and non-dysgenic) males, labelled as the G4 component in Table 3. Different males within the same series produce no extra variability (G3 component), as expected given the genetic identity of different males within each series. Finally, based on the pooled G3–G4 classes, the (dysgenesis × chromosome) interaction is high but not significant, while the between-chromosomes and between-treatments classes are each significant at the 5% level. It is worth noting that the high value of the between-chromosomes component, and also the (dysgenesis × chromosome) interaction, are inconsistent with the notion of complete homogeneity of the Harwich stock from which the two chromosomes were extracted.

The results of Table 2 are presented graphically in Fig. 2. Each point in Fig. 2 designates the fitness of one mutated chromosome, and is obtained from pooling both counts of the two duplicate cages for each sampled gamete. The difference between the two original chromosome types C-1 and C-2 is apparent, as is the difference in the manner in which dysgenesis affects the two chromosomes.

Perhaps the most interesting insight into the data comes from a comparison of the variances of the dysgenic and non-dysgenic lines. The C-2 lines give a clear picture in this respect, since the variability is clearly, and significantly ($P < 0{\cdot}001$), greater amongst the dysgenic lines. Furthermore, the pattern is one in which all four lines which depart from the non-dysgenic lines show reductions.

The picture in the C-1 lines is complicated by the single non-dysgenic line, marked with an asterisk in Fig. 2, in which the fitness is markedly reduced from

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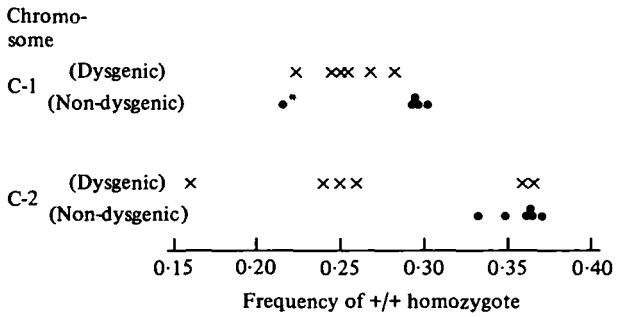

Fig. 2. Population cage frequencies of the + / + genotype for the dysgenic (X) and non-dysgenic (0) series. Each point in the table is based on the average of two samples for each of two duplicate cages.

the other lines. It is not surprising that one out of eleven from the non-dysgenic lines should carry a mutation. As mentioned previously, a significant level of dysgenesis in the 'non-dysgenic' cross is also indicated by the data on lethals. Furthermore, the results obtained by Mackay (1986) indicate the possibility of high levels of mutation induction in such crosses, especially in generations following the initial intercross.

Compared to the remaining four non-dysgenic lines, the dysgenic lines all have lower fitness. The fitness differential between dysgenic and non-dysgenic lines is lower for the C-1 than for the C-2 lines, although non-significantly so, as seen from the non-significant interaction in Table 3.

The results from C-2 can be interpreted as showing that 4 out of 6 gametes from G3 males have reduced fitness. The proportion is less clear from the C-1 lines, but it is probably no lower, and possibly as high as 100%. Overall, a conservative estimate would be that at least half of the chromosomes show a marked depression in fitness. Thus many, and conceivably a majority of, mutational events anywhere in the genome have detectable effects on fitness, provided that the conditions are set so as to enhance fitness differentials. In retrospect it is surprising that mutation systems other than P-M dysgenesis have not been investigated in analogous population cage experiments.

The estimation of fitness values corresponding to the population cage values depends on the extent to which the depression in fitness is attributable to viability or to fertility. For a population cage frequency of the + / + genotype of p, the maximum fitness of the + / + genotype is $2p/(1+p)$, a value which occurs under a pure viability model (see Sved & Ayala, 1970; Sved, 1971). Thus for chromosome C-1, ignoring the one outlying non-dysgenic chromosome, the mean fitness has fallen from 0·45 to 0·40. For chromosome C-2, the reduction is from 0·53 to 0·43. On a proportionate basis, the reduction is approximately 10% for the C-1 series and 20% for the C-2 series. These values may be an underestimate, owing to the use of a viablity rather than fertility model.

We did not attempt a molecular characterization of the mutated chromosomes to estimate how many P-factor excision or insertion events may have been involved in the mutation process. The design of the experiment essentially precludes such an analysis, since we would have had to characterize chromosomes which already contain a large number of P factors. New insertion sites or complete excisions could theoretically be detected by *in situ* hybridization, but only with some difficulty against a background of large numbers of pre-existing P-factor sites. Incomplete excision events could not be detected. Bingham, Kidwell & Rubin (1982) estimated one new insertion site per X-chromosome of M origin, in males of a similar constitution to those we used. The relevance of this figure to a second chromosome of P origin is debatable. There remains also the possibility, as mentioned previously, that dysgenesis in the I-R system could have contributed to the rate of mutation observed.

The results of Mukai (1964) are in many ways comparable to the results we have found. Mukai showed that mutations with partial effects on fitness, in this case viability, occur more frequently than do mutations to lethality. Mukai estimated a rate of mutation to viability polygenes of 14%, somewhat lower than estimated in our experiment. However, the contribution of dysgenesis may not have been as high. Based on a considerably lower estimate for the mutation rate in structural genes, Mukai & Cockerham (1977) suggested that the majority of mutations affecting viability may be located outside structural loci. It would be of considerable interest to use molecular methods, in essence 'transposon tagging' (Bingham *et al* 1982), to attempt a direct confirmation of this result for the mutations affecting fitness detected by the population cage methods of the present study.

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