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SHORT PAPER
Sterility-mutability correlation

On the correlation between sterility and mutability during P-M hybrid dysgenesis in *Drosophila melanogaster*

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Summary

Twelve isogenic *X* chromosome lines from a single natural population of *Drosophila melanogaster* were tested for their potential to induce gonadal dysgenesis and *singed-weak* mutability in *P-M* hybrid dysgenesis. The correlation between sterility and mutability was significantly positive for Cross A, confirming the results reported by Engels (1984) and Kocur, Drier & Simmons (1986). In Cross A* cytotype tests, however, two of the lines gave strikingly different results when measured by the gonadal dysgenesis test as compared to the *singed-weak* test. Positive correlations between traits within a given line were generally not observed. The results suggest that the relationship between gonadal dysgenesis production and the mobilization of *P* elements in *singed-weak* mutability is more complicated than that proposed by Engels (1984). The two phenomena may be separable under certain conditions. Neither test can be taken as an adequate characterization of the hybrid dysgenesis 'profile' of a line of flies.

1. Introduction

Germline hypermutability in *P-M* hybrid dysgenesis generally involves the insertion or excision of *P* elements at specific loci. In crosses of *P* males to *M* females, *P* elements are mobilized in the germ cells of the F_1, presumably due to the action of 'transposase', the protein product of a complete *P* factor (Rio *et al.* 1986). The same $P \times M$ crosses often produce a sterile F_1 at high temperature (above 27 °C), due to failure of the germ cells to develop (gonadal dysgenesis). However, the production of gonadal dysgenesis has not been related directly to the movement of *P* elements or the action of their protein product(s) (for review see Bregliano & Kidwell, 1983; Engels, 1983).

Engels (1984) and Kocur, Drier & Simmons (1986) have examined the correlation between gonadal dysgenesis and hypermutability in inbred isofemale lines isolated from natural populations. Using the *singed-weak* assay developed by Engels (1984), both groups found highly significant positive correlations between the two traits when males from the wild strains were crossed to females of a laboratory tester strain (Cross A). However, both Engels and Kocur *et al.* found exceptional cases in which particular lines of flies gave too much or too little gonadal dysgenesis given the levels of mutability they produced. Neither group examined the correlation between sterility and mutability in Cross A*, which tests the cytotype of a line – its ability to suppress the action of hybrid dysgenesis in a cross of females from the inbred wild lines to *P* strain males.

I have examined the correlation between sn^w mutability and gonadal dysgenesis in twelve isogenic *X* chromosome lines extracted from a natural population of *D. melanogaster*. My results confirm those of Engels and of Kocur *et al.* for Cross A, giving strong positive correlation between gonadal dysgenesis and *singed-weak* mutability. However, in cytotype tests (Cross A*) there are striking cases of non-correspondence between sn^w mutability and gonadal dysgenesis. Gonadal dysgenesis potential and *singed-weak* mutability may therefore be separable under certain conditions.

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2. Materials and methods

(i) *Stocks*

The *X* chromosome lines studied here were those described by Simmons (1986), made by extracting single *X* chromosomes from a wild population into a laboratory background free of *P* elements. These lines, which were initially isogenic, had been maintained in the laboratory for about one and a half years at the time the present experiments were performed. The following stocks were used to test the lines simultaneously for gonadal dysgenesis potential ('GD sterility') and for *singed-weak* mutability potential:

(1) Oregon-R: a wild type *M* strain, completely lacking *P* elements and having no ability to produce gonadal dysgenesis.

(2) Harwich: a wild type *P* strain, containing many *P* elements and having the potential to produce nearly 100% gonadal dysgenesis when crossed to Oregon-R at 28 °C.

(3) *y* sn^w (*M*)/y^+ *Y*; *bw*; *st*: an *M* strain used to measure *singed-weak* mutability. The stock contains the double *P* element insertion in the *singed* locus (O'Hare, Roiha & Rubin, personal communication), and these elements are incapable of transposition except in the presence of a functional *P* factor introduced in a cross to another strain (Engels, 1984).

(4) *C(1)DX*, *y f*/sn^w (ii) π_2: an attached-X *P* strain that contains autosomes derived from the π_2 strain. Males carry the *singed-weak* mutation, which is stable due to the *P* cytotype of the stock. When introduced into the *M* cytotype, however, the *singed-weak* allele may revert (Engels, 1984).

(5) w^c sn^3: a laboratory stock that when heterozygous with the various alleles of *singed-weak* enhances their expression and makes scoring easier (G. M. Simmons, unpublished observation).

All stocks were maintained on standard laboratory medium in bottles at room temperature (21–25 °C).

(ii) *Crossing schemes*

In Cross A, single males were first crossed to three virgin *y* sn^w (*M*); *bw*; *st* females in a vial at 25°. After three days, the male was transferred to a fresh vial containing three virgin Oregon-R females for the gonadal dysgenesis test at 28°. Parents were cleared from both crosses on the seventh day after the cross was initiated. From each vial at 25° I collected five virgin females. These females carried the *y* sn^w (*M*); *bw*; *st* chromosomes from their mothers along with a wild *X* and autosomes from their fathers. Any transposition activity due to *P* factors on the fathers' chromosomes could cause the sn^w allele to mutate to sn^+ or sn^e in the germline of the females. To measure the extent of germline mutability of sn^w I crossed these females to 3 w^c sn^3 males in bottles and scored up to 150 female progeny for the sn^e phenotype. Mutations of sn^w to sn^+ could not be observed since half the progeny inherited the wild-type *X*, which is indistinguishable from sn^+.

Meanwhile, the progeny of the cross to Oregon-R females at 28° were allowed to emerge until day 15 and were then transferred to fresh medium at room temperature. After five days, 25 females from each vial were dissected in distilled water and scored for the number of ovaries present. I thus obtained a measure of the ability of a single male to induce gonadal dysgenesis and *singed-weak* mutability in his progeny. For each line tested, I crossed up to ten males in this fashion, so that for each line I scored up to 250 females for gonadal dysgenesis and up to 1500 females for sn^w mutability.

To test the Cross A* potential of the lines I crossed ten virgin females from a line individually to three Harwich males at 28° to test for gonadal dysgenesis. Progeny from this cross were aged and dissected in a manner identical to that for the Cross A sterility tests. At the same time, ten more virgins were individually crossed to three sn^w; π_2 males at 25° to initiate the *singed-weak* mutability test. Five virgin female progeny from each of these matings were crossed to three w^c sn^3 males, and up to 150 female progeny were scored for mutations to sn^e. Thus for each line I scored up to 250 females for sterility and up to 1500 females for mutability. Further details of the crossing scheme used in these experiments may be found in Simmons (1985).

3. Results

Fig. 1 shows the mean levels of gonadal dysgenesis plotted against mean levels of *singed-weak* mutability for the twelve lines. Each point represents the mean sterilities or mutabilities of up to ten crosses for each line, where in each cross I scored 25 females for sterility and up to 150 females for mutability. Table 1 shows the results of within-line correlation tests, as measured by Kendall's τ, a non-parametric measure of correlation (Siegel, 1956). Although only two of the 12 lines show a positive correlation between the two

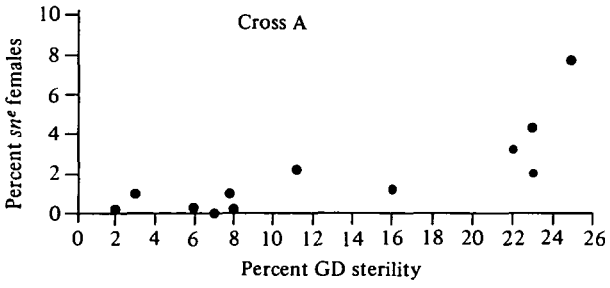

Fig. 1. Mean gonadal dysgenesis vs. mean *singed-weak* mutability produced in Cross A tests of 12 *X* chromosome lines.

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Table 1. *Results of simultaneous tests of gonadal dysgenesis potential and* singed-weak *mutability potential for* 12 *X chromosome lines*

Line	Sterility (gonadal dysgenesis) mean (S.D.)	Mutability (*singed-extreme*) mean (S.D.)	τ	P
W103X	0·08 (0·11)	0·00 (0·01)	0·2335	NS
W104X	0·25 (0·28)	0·08 (0·05)	0·0899	NS
W137X	0·16 (0·18)	0·01 (0·01)	−0·1826	NS
W143X	0·22 (0·20)	0·03 (0·01)	0·0501	NS
W148X	0·03 (0·07)	0·01 (0·01)	−0·4202	*
W155X	0·06 (0·09)	0·00 (0·00)	—	—
W159X	0·11 (0·12)	0·02 (0·03)	0·5543	*
W162X	0·02 (0·05)	0·00 (0·00)	—	—
W164X	0·07 (0·08)	0·00 (0·00)	—	—
W168X	0·08 (0·10)	0·01 (0·01)	0·0514	NS
W169X	0·23 (0·19)	0·02 (0·01)	0·5976	*
W170X	0·23 (0·26)	0·04 (0·04)	0·4001	NS

Gonadal dysgenesis is given as the proportion of ovaries that are missing in a sample from the daughters of the tested males. Mutability is given as the proportion of *singed-extreme* grand-daughters of the tested males. τ is Kendall's non-parametric measure of correlation (Siegel, 1956). P is the statistical significance of the correlation: NS = $P > 0{\cdot}05$, * = $P < 0{\cdot}05$.

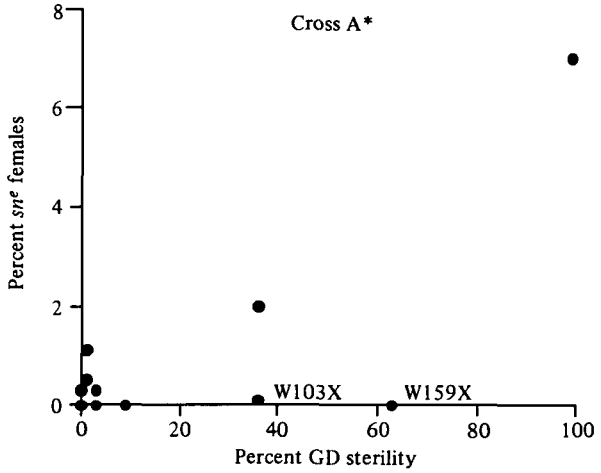

Fig. 2. Mean gonadal dysgenesis vs. mean *singed-weak* mutability produced in Cross A* tests of 11 *X* chromosome lines.

traits, the correlation over all lines tested was significantly positive ($\tau = 0{\cdot}3035$, $P < 0{\cdot}001$, $n = 102$). Fig. 2 shows the relationship between sterility and mutability for the pooled Cross A* results for 11 of the 12 lines. Kendall's τ was significantly positive ($\tau = 0{\cdot}4211$, $P < 0{\cdot}05$, $n = 11$). Unlike the Cross A results, however, there were two lines that produced rather high levels of gonadal dysgenesis (lines W103X and W159X) but virtually no sn^w mutability. These two lines have intermediate levels of Cross A* sterility that are unexplained by the current models of cytotype production (Simmons, 1986).

4. Discussion

The Cross A results reported here agree with Engels' result and with the observations of Kocur *et al.* (1986) although the correlation is weaker than that found by Engels. The lack of consistently positive within-line correlations allows us to reject a simplistic model in which both sterility and mutability are part of the same process; the two phenomena are related in a more complicated way. In fact, the significant correlations observed within some lines may be due to variation and linkage disequilibrium that have accumulated in the lines as they have evolved in the laboratory (Simmons, 1985).

The results of Cross A*, however, show two lines that clearly violate the correlation between sterility and mutability. The two lines with anomalous behaviour (W103X and W159X) are those that have previously been shown to have stable intermediate levels of Cross A* sterility (Simmons, 1986). These lines do not fit Engels' (1979*a*) model of cytotype in which intermediate levels of Cross A* sterility represents lines in transition between *M* and *P* cytotype. Simmons' intermediate lines resemble the pseudo-*M* strains recently reported by Kidwell (1985), but unlike Kidwell's lines, lines W103X and W159X produce some gonadal dysgenesis in Cross A.

In the present experiments lines W103X and W159X continue to display intermediate levels of sterility in Cross A*, similar to those reported previously. As shown in fig. 2, however, these lines display little or no sn^w mutability in Cross A*. By the sn^w test, these lines are strictly *P* cytotype (Engels, 1981) but by the gonadal dysgenesis test they are intermediate. These results argue that despite the overall correlation between gonadal dysgenesis and

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sn^w mutability there are conditions under which they can be separated.

In addition to the Cross A* results here, Green (1984) has observed high-mutator chromosomes (as assayed by specific visible mutation frequency) that produce little or no gonadal dysgenesis. Engels (1984) and Kocur *et al.* (1986) have also observed inbred lines that produce 'too much' gonadal dysgenesis given their levels of *singed-weak* mutability. It is also interesting to note that line W103X, which produces gonadal dysgenesis in both Cross A and Cross A* but produces little or no sn^w mutability in either cross, does produce mutations at the *singed* and *raspberry* loci in a Cross A specific visible screen at a rate of $1{\cdot}1 \times 10^{-3}$ (G. M. Simmons, unpublished data). It is apparent that the sn^w assay does not accurately reflect all aspects of hybrid dysgenesis-induced mutability in this line. There are currently no data on the correlation between *singed-weak* mutability and production of specific visible mutations in hybrid dysgenesis.

Although there does exist an overall correlation between gonadal dysgenesis and mutability of the *singed-weak* allele, the data presented here suggest that the relationship is complicated. It is thus premature to assume that the observed correlation between these phenomena is due to a simple common mechanism.

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