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P-M hybrid dysgenesis using geographically separate P strains of *Drosophila melanogaster*

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The similarity or otherwise of the mutation spectra generated by different P strains in P-M hybrid dysgenesis is of considerable theoretical and practical importance. We report here that the mutations generated on the X-chromosome by different P strains, Π2 and Loua 83, are indistinguishable both in quantity and in quality.

INTRODUCTION

P-M hybrid dysgenesis is a syndrome of abnormalities observed in the F1 of crosses between males of strains carrying a transposable genetic element, the P-factor (P-strains); and females from strains which lack this element (M-strains). The abnormalities include sterility at high temperatures, male recombination and an increased mutation rate (Bregliano and Kidwell, 1983). The mutations observed usually result from the insertion of P-factors (or their smaller, deleted products P-elements) into or near structural genes (Engels, 1983). Some genes are particularly mutable in hybrid dysgenesis, notably the sex-linked *singed* (*sn*) locus (Green, 1977). Different P-strains have P-factors situated at different chromosomal locations (Engels, 1983). If the positions of new P-factor sites generated by transposition depend upon the positions of donor P-factor sequences then positional variation between strains would be expected to result in different mutation spectra. This is potentially important, as insertional mutagenesis with P-elements is now important in cloning *Drosophila* genes by isolating mutated genes through homology to their inserted sequences (*e.g.*, Searles *et al.*, 1982). However, some genes appear not to be susceptible to P sequence insertions. If this result was a consequence of the P-strain used in the dysgenic cross, cloning of such genes might yet be possible using an exotic P-strain as a P sequence donor.

MATERIAL AND METHODS

Here we test the hypothesis that different P-strains create different mutation spectra on the X chromosome. The strains used were Π2 (a strong P-strain from Wisconsin (Engels and Preston, 1979)), Loua 83 (a strong P-strain, as measured by the high temperature induction of gonadal dysgenesis, collected recently in Central Africa (Anxolabéhère *et al.*, 1984)), Canton-S (a long-established laboratory M-strain and C(1) DX, *yf*:CS (an attached -X *yellow forked* stock with the Canton S genetic background). The following crosses were carried out, each at 22°C.

CANTON S♀ × P-Strain♂
↓
C(1)DX *yf*♀ × F1♂
↓
F2♂s

(Examined for visible mutations)

The F2♂s thus would carry visible mutants if P elements had been inserted into or near major genes in the X chromosomes of the germ cells of F1♂s. Loua 83 and Π2 were used as paternal P-strains in separate experiments, and the F2s examined over a 4 week period, during which two generations (referred to below as F2 and F3) emerged.

RESULTS

Visible mutations were detected at only two loci; *singed* (with 31 mutations) and *scalloped* (*sd*)

Table 1

Strain	Generation	Wild type ♂s	*sn* ♂s	*sd* ♂s
LOUA83	F2	3339	7	1
	F3	2431	1	0
Π2	F2	6829	16	2
	F3	4841	7	0

(with three mutations). (table 1) χ^2-tests were carried out to compare the two P-strains for their mutation rates at *singed*. The results were:

F2: Loua 83 v Π2 $\chi^2 = 0.060$ Not significant
F3: Loua 83 v Π2 $\chi^2 = 1.589$ Not significant
F2+F3 Loua 83 v Π2 $\chi^2 = 0.750$ Not significant
Loua 83 F2 v F3 $\chi^2 = 2.699$ Not significant
Π2F2 v F3 $\chi^2 = 1.128$ Not significant
Loua 83+Π2 F2 v F3 $\chi^2 = 3.309$ Not significant.

The overall rate of mutation from wild-type to *singed* was about 1 in 600 chromosomes. This is lower, (though not significantly) than the rates observed in a similar study by Simmons *et al.* (1984) using both Π2 and the Q strain v_6 as P-element donors. In their experiment the initial cross was to an attached -X strain, so the mutations subsequently observed were in the P-strain X chromosome and not that of the M strain. This explains their finding of many *beadex* mutations when using Π2, due to chromosome breaks arising at the P-element adjacent to *Beadex* in the Π2 X-chromosome. There is evidence in our data for the clustering of mutations indicating that the mutations occur premeiotically and are shared by groups of flies from the same parents. The *sn*♂s in the F2 Π2 data include six which consist of two sets of three brothers, and two of the Loua 83 *sn*♂s in the F2 could also have been brothers. Thus there may have been, in the data set, only 26 independent *sn* mutations, not 31.

Many of the *sn* mutations generated in this study have been maintained for up to 15 generations, either as males in a stock with attached -X females (as some of these mutations appear to be female-sterile) or as strains in which both males and females possess the mutation. The mutations frequently prove to be unstable in these stocks, reverting either to wild-type or to a weaker singed phenotype (table 2). The revertants are dominant over the *singed* alleles from which they are derived. Differences between stocks in their reversion rates may be due to differences between the *sn* mutations in their intrinsic reversion rates and also possibly to differences between stocks in cytotype.

These data, albeit limited, appear to indicate that the mutation spectrum of X-chromosomal insertion mutations in P-M hybrid dysgenesis is insensitive to the P strain used. Mutationally refractory loci are hence likely to remain so whichever cross is attempted.

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Table 2

Mutation (name indicates P-strain of origin)	*sn* Chromosomes examined	Wild-type	*sn* weak	Reversion rate %
LOUA sn^1	4198	8	1	0.09-*0.21*-0.35
LOUA sn^2	3830	0	0	*0.00*-0.08
LOUA sn^3	439	0	0	*0.00*-0.68
LOUA sn^4	866	5	3	0.44-*0.92*-1.91
LOUA sn^5	3882	0	1	0.00-*0.03*-0.16
LOUA sn^6	359	0	0	*0.00*-0.83
Π2 sn^1	3092	14	2	0.26-*0.51*-0.76
Π2 sn^2	4662	1	0	0.00-*0.02*-0.13
Π2 sn^3	5958	10	77	1.14-*1.44*-1.74

The numbers preceding and following the reversion rate estimates (which are based upon the sums of wild-type and sn^w) are 95 per cent confidence limits based upon the binomial standard error of a proportion or upon the limits of the expectation of this distribution (Fisher and Yates 1963). They are unrealistically close to the rate estimate as they assume falsely that every fly showing a revertant phenotype represents an independent reversion event.

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