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Induction of P element transposition using $\Delta 2-3$ transposase to determine whether the number of inserts affects transposition rate in *Drosophila melanogaster*.

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Introduction

In *Drosophila* many different transposons are known, most of them present at variable sites throughout the genome. Transposon movement is a useful tool to geneticists for mutagenesis, for the introduction of genes by using the element as a vector, and to identify endogenous genes by enhancer trapping, most recently extended to gain of function screens (*e.g.*, EP lines). Because of this, there is a strong interest to optimize the movement of elements in this genetic system. One of these mobile genetic sequences is the P element transposon, with its movement controlled by the presence of transposase.

The objective of this study is to establish whether the presence of more than one marked P element is a factor that affects transposition rate. Transposition is a method of transferring genes as transposons to random locations in the genome. There are few known factors that influence transposition rate. One factor is the location of the insert; the same marked element may more readily serve as a source, or template, from one location but infrequently from another location in the genome. Also, size may be an issue, as larger elements are more difficult to transform initially and may reestablish less frequently.

In this study, one P element was marked with y^+ and another P element was marked with w^+ . Both elements are present on the same X chromosome. These marked elements were introduced into the fly by transformation and the movement of these elements was induced by crossing in the $\Delta 2-3$ transposase element located on the third chromosome. “Dysgenic” males with the X linked elements and the $\Delta 2-3$ transposase were crossed to yw females (Figure 1). Relocation of either the w^+ marker or the y^+ marker from the X chromosome in a male to an autosome in a son is one classic way of recognizing a transposition event. It is often used for enhancer trapping and EP studies. Any marked element on the X chromosome in males will ordinarily pass to their female progeny but none of their male progeny. Those rare sons that inherit their father’s marked element inevitably have it located at a new location on one of the autosomes. The insert is stable if the sons do not receive the copy of the third chromosome bearing the $\Delta 2-3$ insert; therefore, this third chromosome is also marked using Ki , so it may be selected against. The transposition event is frequently pre-meiotic and results in a cluster of identical exceptional sons. Typically dysgenic males are cultured singly with the yw female, and only one exceptional son from each cross is used for subsequent crosses in order to avoid wasting effort through analyzing identical inserts. Under these conditions, the transposition rate for comparison with other experiments is the fraction of dysgenic males that yield at least one exceptional son.

It is assumed often that the transposition for several elements will be additive, but tangible data is absent. Therefore we want to determine if the transposition of one element interferes or interacts with

another in a multiple element genotype. In order to examine this question we compared transposition rates from the same fathers for two differently marked elements on the X chromosome. We also looked at the independence of w+ and y+ transposition as another measure of interactions in the process.

Source of Marked Transposable Elements

One element is the y+ marked UAS EP insert # 4, which was found to transpose from X to A in about 50% of the dysgenic fathers in a previous study (Crisp and Merriam, 1997, DIS 80: 90-92). The second element is the w+ marked Gal 4 enhancer trap A9, based on pGaw B and furnished by Ethan Bier. It transposes at a lower rate with approximately 20% of the dysgenic males yielding a w+ exception.

Experimental Design

Females (strain 4A9) homozygous for both elements on the X chromosome with a background genotype of yw were crossed to males having the third chromosome with the kinked marker (Ki) and Δ2-3 transposase. Their sons, the “dysgenic males,” were cultured individually with two to three yw females. Resulting progeny were scored for regular classes and for exceptional y+w sons and yw+ sons (Figure 1).

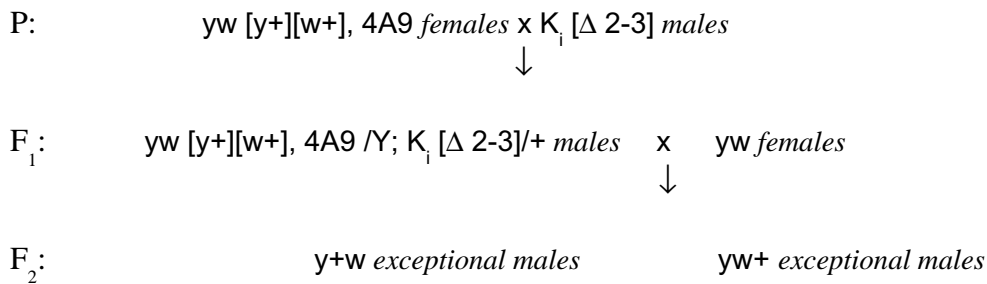


Figure 1. Experimental layout to generate two types of exceptional males.

Two series of identical crosses were made at different times. The results are comparable except for the difference that the second series only scored exceptions that were non-Kinked (half of the exceptions are Kinked and half are non-Kinked since the dysgenic father are heterozygous for the Kinked containing chromosome). In practice, screens to generate new transposition-locations routinely discard the exceptional males that also received the Δ2-3 chromosome from the dysgenic father (in order to have only stable transpositions). For this reason, the transposition frequency that is most relevant is the fraction of the fathers producing a non-Kinked (non Δ2-3) exceptional son(s). Because of clustering of exceptional sons, most dysgenic males that yield any exceptional sons will have at least one that is non-Kinked. Hence the transposition frequency measured this way for the second series is not much lower than the measure counting any exceptional male.

Results

Series I

Out of 312 fertile crosses in series one, there was a higher frequency of fathers yielding $y+w$ exceptional sons than those yielding $yw+$ exceptional sons, as expected (Figure 2). 178 dysgenic males, out of 312 crossed in series I yielded at least one $y+w$ son and 59 yielded at least one $yw+$ son. Several $y+w+$ sons were found which turned out to be sterile. Their origin is attributed to nondisjunction and they are not discussed further.

Series II

Out of 1669 fertile crosses in series two, there was again a higher frequency of $y+w$ exceptions than $yw+$ exception (Figure 3). 728 dysgenic males yielded at least one $y+w$ son, 343 dysgenic males yielded at least one $yw+$ son.

Analysis of Results

What is observable from both series of crosses is that the frequency of transposition for either element appears to be unchanged by the presence of the other element. That is about 50% of the dysgenic fathers yield one or more $y+$ sons and about 20% of the dysgenic father yielded one or more $w+$ sons. The results are approximately additive in that the frequency of dysgenic males yielding a $y+$ son and $w+$ son is higher than 50%. Does this mean that transposition at either element is independent of the other? What determines if a dysgenic male will give at least some exceptional sons? If, for example, transposase or transposition is somewhat limited and not completely additive, then dysgenic males could appear to yield either $y+$ sons or $w+$ sons with fewer yielding both classes; this will appear as non independence of $y+$ and $w+$. Alternatively, if some males are physiologically better able to promote transposition, they should be more likely to yield both $y+$ sons and $w+$ sons. This also will appear as non-independence.

The percentages of dysgenic males defined by the kinds of exceptions they yield, a $y+$ only exception or a $w+$ only exception, or both $y+$ and $w+$ exceptions, or neither exception, are presented in Figure 4 and Table 1.

Table 1 compares the numbers of dysgenic males for each category with the numbers expected from independence of occurrence of $y+$ exceptions and $w+$ exceptions. The expected numbers are calculated from a two by two contingency table, with the $y+$ transposition positive and $y+$ transposition negative frequencies forming one axis and $w+$

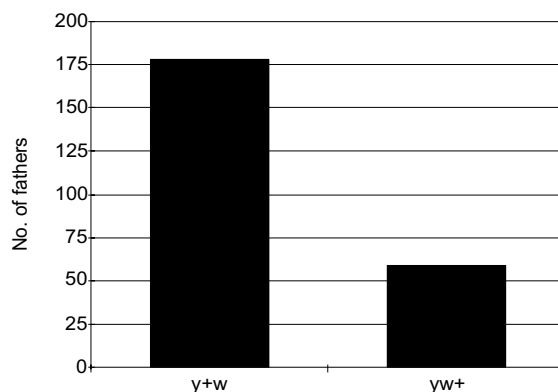


Figure 2. Numbers of dysgenic males generating an exceptional son, by marker, in series I.

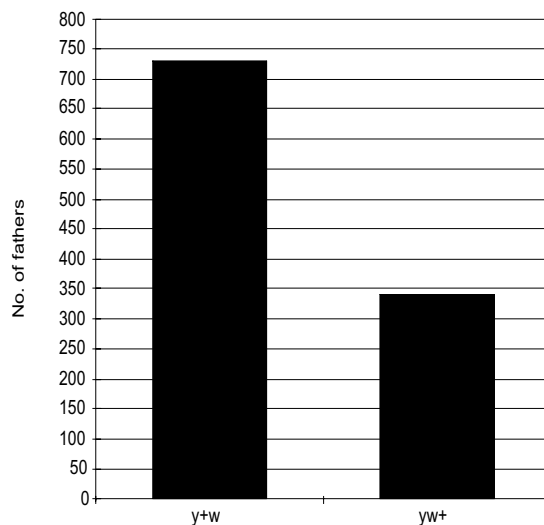


Figure 3. Numbers of dysgenic males that generated an exceptional son, by marker in series II.

transposition positive and w+ transposition negative frequencies forming the other axis.

Although the expected numbers compared to counted data in contingency tests may be evaluated by Chi-squared with one degree of freedom, the comparisons made here are too variable to be to be appropriate for Chi-squared analysis. Instead, any significance stems from both cross series giving similar results. More of the dysgenic fathers yield both y+ exceptional sons and w+ exceptional sons than expected assuming independence. While the differences are not large, they support the notion that there is an underlying area of variability between dysgenic males making some more potent transposers,

although perhaps not greatly so. The results suggest a degree of “hyperadditivity.” To this extent, the strategy of combining multiple elements in the same dysgenic male to take advantage of transposition opportunities seems appropriate. An extension to this question would be to measure a combination of P element transposition, *hobo* element transposition or mariner element transposition

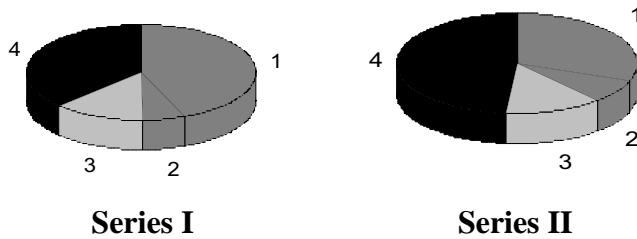


Figure 4. Pie diagram of the proportion of dysgenic males yielding only y+ exception(s) (1), only w+ exception(s) (2), both y+ exceptions and w+ exceptions (3), or not yielding any exceptions, (4).

in the same fly to ask if it is a property of the element or is a property of the fly and its culture condition.

A common use of generating transposed inserts at new locations in the genome is for enhancer trapping and EP studies. The y+ marked element is an EP type driver (Crisp and Merriam, 1997, DIS 80: 90-92), and the y+w exceptions from series I were further tested by crossing one exceptional male to two to three virgin females bearing the actin promoter Gal 4 drivers marked with w+ and balanced over CyO. In this test, the “experimental” progeny receiving both the w+ marked Gal 4 driver and the y+ marked UAS responder (to express the adjacent endogenous gene) are clearly distinct from their siblings. We found 28 of the 178 lines in series I were found to be completely lethal for the y+w+ experimental progeny. The importance of this figure is its implication that approximately one third of genes are capable of harming fly development when misexpressed. This subject will be addressed in another report.

Table 1. Observed and calculated proportions of the dysgenic males classified according to their exceptional progeny.

Percentage	Series I	Observed Value	Expected Value
1 43.9	y+ (w) only exceptional males	137	144
2 5.8	(y) w+ only exceptional males	18	25
3 13.1	y+ (w) and (y)w+ exceptional males	41	34
4 37.2	No exceptions	116	109

Percentage	Series II	Observed values	Expected Value
1 30.6	y+ (w) only exceptional males	512	578
2 7.6	(y) w+ only exceptional males	127	193
3 12.9	y+ (w) and (y)w+ exceptional males	216	150
4 48.6	No exceptions	812	746

The y+ exceptions from series II were used in a screen for minute mutation suppressors, although none were found. A partial list of phenotypes observed in crosses to bristle Gal 4 drivers is included in Table 2.

Crosses with y+w exceptions were scored for abnormalities such as short/inhibited bristle growth, possible minute suppressors, semi or total lethality, and wing deformities. Occasionally flies had a very dark body color due possibly to an extra y+ marker, seen in HM 537, HM 776, and HM 853. Often the macrochaete was more affected than the microchaete, indicating that the larger bristles possibly have a lower tolerance and are more vulnerable for change than others. As for the other phenotypes obtained, it was interesting to note that HM 165 was able to have one bristle on a specific locale on the fly body. Also another line (HM 421) had bristles that were all present but did not grow beyond the thorax shell. This reinforces the idea that larger or longer bristles are more vulnerable for change. Rarely it was observed during scoring that the gray flies had yellow wings and yellow bristles while still retaining the gray body color. This rare event was attributed to the position effect seen in HM160.

Similarly, crosses with w+ exceptions from both series were scored for minute suppression that restore normal bristles size. A similar screen was used to select the B11-98 gal 4 driver (Merriam, in preparation). Each w+ exception was tested for Gal 4 production in bristles by means of crosses to flies with a minute mutation (66D) together with the UAS and cDNA of its gene product (rpL14). Eight additional lines marked with w+ exhibited suppression for the minute phenotype by restoring the bristle to normal size and a darker color. They will be described in another report.

The goal in this experiment was to determine if the presence of one or more P inserts influences the transposition rate of either insert and how it influences the rate. From our data as well, it can be concluded that the presence of an insert can be powerful in inducing phenotypes.

Table 2. Progeny from y+ w fathers tested with MB in series II. Various developmental defects ranging from bristle deformities to lethality consistent for all y+ w+ progeny. MB is a balanced stock containing M (3)66D and the novel gal 4 driver B11-98 (Merriam, in preparation).

y+ w line originated from one dysgenic male	Scored phenotype for the y+ w+ class
HM 12	Very minute bristles
HM 17	Short bristles
HM 30	Extremely short bristles
HM 36	Very minute bristles
HM 54	Short bristles
HM 56	Extremely short bristles
HM 122	Bristles absent
HM 160	Yellow wings, yellow bristles
HM 165	1 small bristle
HM 210	Bristles absent
HM 274	Lethal
HM 298	Very short bristles
HM 304	Curly wings
HM 385	Bristles absent
HM 388	Bristles absent
HM 400	Lethal
HM 415	Bristles absent
HM 421	Bristle growth stops at shell line
HM 431	Lethal
HM 501	Lethal
HM 505	Bristles absent
HM 515	Winkled wings
HM 530	Very minute bristles
HM 537	Very dark body color (extra y+)
HM 617	1 fly (semi-lethal)
HM 689	Very minute bristles, curly wings
HM 696	Lethal
HM 745	Very short bristles
HM 776	Very dark body color (extra y+)
HM 781	Very thin bristles
HM 786	Wrinkled wings
HM 801	Short bristles
HM 804	1 fly (semi-lethal)
HM 805	Bristle growth stops at shell line
HM 813	Lethal
HM 840	Lethal
HM 853	Very dark body color (extra y+)
HM 857	Wrinkled wings