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A Translocation X;Y System for Detecting Meiotic Nondisjunction and Chromosome Breakage in Males of *Drosophila Melanogaster*

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A nondisjunction and chromosome breakage screening system devised by Craymer and modified in our laboratory, involves an X;Y translocation with the short arm of the Y (Y^S), marked with the wild type allele of yellow, attached to the distal end of an X (break point 11D) carrying the recessive marker *y*; and the long arm of the Y chromosome (Y^L), marked with the dominant locus Bar of Stone (B^S), attached to the proximal end of the X. A female tester strain carrying normal chromosomes homozygous for the yellow allele is employed in the mating scheme. Following normal disjunction in the male, all zygotes, which in this case receive aneuploid paternal sex-chromosomes and a normal euploid maternal complement, will die as a result of genetic imbalance. Thus all survivors from this cross can be classified as exceptions arising from: (1) nondisjunction in the female; (2) gross deletion of the paternal X;Y chromosome; (3) complete loss of the paternal X;Y chromosome; or (4) primary meiotic nondisjunction in the male. Results indicate the sensitivity of this scheme for the detection of events induced by x-rays and various chemicals. Positive results have been obtained with the known mutagens EMS and x-radiation.

Introduction

In recent years it has been established that a substantial proportion of birth defects and spontaneous abortions in human populations are due to meiotic nondisjunction (*1*). Because of this enormous impact as both a health and economic burden, efficient screening methods for detection of environmental agents capable of inducing segregational anomalies are a vital part of a responsible testing battery. This is particularly important because of the possibility of agents capable of inducing nondisjunction but not inducing other genetic endpoints. These agents would be overlooked in traditional screening regimens.

Drosophila melanogaster is an efficient, economic, and widely used organism for detection of mutagenicity (*2*). It is useful for sampling a variety of germ cell stages and has a high rate of relevance to man (*3, 4*). It is, therefore, a likely organism in which to study induction of segregational anomalies.

The system described here is a single generation test in *Drosophila melanogaster* in which only exceptional progeny survive. It is rapid and involves minimal labor. Furthermore, the products recovered reflect several types of damage, thus facilitating the comparison of the relative importance of multiple effects caused by a given environmental agent. Specifically, primary meiotic nondisjunction, chromosome loss, gross deletions, exchange between X and Y heterochromatin, and the reduction of progeny recovered as a result of the exposure, can be monitored simultaneously.

The System

The nondisjunction and chromosome breakage screening system described here was devised by Dr. Loring Craymer (*5*) and improved in our laboratory. It involves an X;Y translocation with the short arm of the Y chromosome (Y^S), marked with the wild type allele of yellow, attached to the distal end of the X (breakpoint 11D) carrying the recessive marker yellow; and the long arm of the Y chromosome (Y^L)

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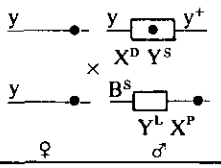
Table 1. Progeny recovered from cross of translocation X;Y male with a normal X-bearing female.



	Class 1		Class 2			
	1A	1B	2A	2B	Class 3	Class 4
Event	Nondisjunction in ♀ – noninduced (Internal control)	Nondisjunction in ♀ or exchange between X and Y heterochromatin	Gross deletion of X^P, leaving B^S marker	Gross deletion of X^D, leaving y^+ marker	Chromosome loss of either translocated X chromosome in ♂	Nondisjunction of translocation X;Y chromosome in ♂
Phenotype	y^+ ♀	yB^S ♀	yB^S ♂	y^+ ♂	y ♂ (XO)	y^+B^S ♀ (X,X;Y)
Chromosome constitution	y y^+ y y	B^S y y or y B^S X^D Y^S X^P Y^L y	B^S y	y^+ y	y	y y^+ B^S y

marked with the dominant locus Bar of Stone (B^S), attached to the proximal segment of the X.

A female tester strain carrying normal X-chromosomes homozygous for the yellow allele is employed in the mating scheme. This tester strain is also homozygous for the recessive autosomal marker poliert (pol) to exclude nonvirginity of the parental female as a possible origin of F_1 products. In addition, the female tester strain has a marked Y chromosome so XXY parental females can be recognized and discarded.

Following normal disjunction in the parental male and female, all zygotes receive aneuploid paternal sex chromosomes and a normal euploid maternal complement and consequently die prior to eclosion as a result of genetic imbalance. Six phenotypic types of exceptional progeny are routinely recovered. These result from four major classes of event (Table 1) and are described as follows: (class 1) nondisjunction in the female; (class 2) gross deletion of paternal X;Y chromosomes; (class 3) complete loss of the paternal X;Y chromosome; and (class 4) primary nondisjunction in the male.

The Classes

Class 1A. y^+ females are produced following the fertilization of a disomic egg resulting from nondisjunction of the X's in the untreated female parent and a sperm bearing the y^+ marked translocation chromosome. These hyperploid females are sterile. This phenotypic type serves as an internal control class, for females of this type are derived solely from events occurring in the untreated female parent. Thus, a reduction in y^+ females in the treated series compared to the control series is indicative of zygotic killing or sperm disfunction resulting from the exposure.

Class 1B. yB^S females are produced following the fertilization of a disomic egg resulting from nondisjunction of the X's in the untreated female parent and a sperm bearing the B^S marked translocation chromosome. These hyperploid yB^S females are sterile. yB^S females are also produced from a sperm that resulted from a recombination event in the male between X and Y heterochromatin to restore a complex X (marked with B^S) fertilizing a normal single X-bearing egg from the female. These females are fertile when mated with normal X-bearing males.

Class 2A. yB^S males result from a sperm which suffered gross deletion of X^P but retained the B^S marker, fertilizing a normal X-bearing egg. These males are functionally XO and are sterile.

Class 2B. y^+ males result from a sperm which suffered gross deletion of X^D but retained the y^+ marker, fertilizing a normal X-bearing egg. These males are functionally XO and are sterile.

Class 3. y males result from a nullo-sex chromo-

some sperm fertilizing a normal X-bearing egg. The nullo-sex chromosome sperm could result from first or second division nondisjunction or from breakage and subsequent loss of either of the translocation chromosomes. These males are sterile due to the absence of the Y chromosome.

Class 4. y^+B^S females are produced when a sperm resulting from a first division nondisjunction in the male fertilizes a normal X-bearing egg. These females are fertile when mated back to the translocation X;Y stock.

Methods

Exposures

Exposures in the experiments described were performed as follows.

In the x-ray low exposure, 1-day-old males were irradiated by using a Picker Vanguard x-ray machine operating at 18 mA and 280 kVp with 1 mm Al and ½ mm Cu filters; 360 males were exposed per series.

In the x-ray high exposure, 2-day-old males were irradiated using a Picker Vanguard x-ray machine operating at 18 mA and 280 kVp with 1 mm Al and ½ mm Cu filters; 240 males were exposed per series.

In the EMS exposure, 1 to 2-day-old males were fed for 2 days on concentrations of 0.05% and 0.5% EMS in a 1.0% glucose solution; 335 males were exposed per series.

In the exposure to run-off from a smelter, 1-day-old males were fed for 2 days on 0.2% run-off in a 1.0% glucose solution; 272 males were exposed per series.

General Procedure

Matings are done en masse, typically with 10 treated males and 40 untreated 3 to 5-day-old virgin females per ½ pint milk bottle. Males are brooded every 2 or 3 days to test a variety of germ cell stages. Females are brooded at least once to ensure maximum recovery of sperm.

Scoring is done one to three times from the tenth day to the seventeenth day post-mating. The number of progeny recovered from each class is recorded. Matings to confirm the chromosome constitution of recovered progeny can be performed.

The estimated cost of testing one exposure rate of a single agent by this procedure is $600-1000.

It is crucial that control and treated series be handled identically with regard to culture conditions, number of males in the series, age of males, number of males per bottle, age of females, number of females per bottle, time of brooding, and time of scoring.

Results

Tables 2-5 show representative screening data from experiments with the translocation X;Y system. Results are shown for low and high x-ray exposures, an EMS adult feeding exposure, and an adult feeding exposure to aqueous run-off from a smelting operation. For each experiment the number of eggs laid can be roughly estimated from the number of y^+ females present in the control. This has been done based on the assumption that the frequency of spontaneous nondisjunction in the untreated females is approximately 1/3000 (*6*).

The appearance of yB^S males and y^+ males in the controls is consistent with a Poisson distribution each experiment. Therefore, the significance of numbers in treated series for these phenotypic classes can be statistically validated according to confidence limits set for this distribution (*7*).

The yB^S female class has been omitted here because of the ambiguity in determining the origin of flies of this phenotype.

Low X-Ray Exposure

Approximately 369,000 male germ cells are rep-

Table 2. Effects of low x-ray exposure on the induction of chromosome aberrations in germ cells in adult *Drosophila melanogaster* males.

	Exposure, R	Number of F_1 offspring from *postmeiotic* stages	Number of F_1 offspring from *meiotic and* premeiotic stages
Class 1 y^+ ♀ / $\frac{y}{y}$ $\frac{y^+}{}$	Control	123	41
	100	113	46
	200	124	39
Class 2A yB^S ♂ / $\frac{B^S}{y}$	Control	9	2
	100	40	19
	200	77	47
Class 2B y^+ ♂ / $\frac{y^+}{y}$	Control	43	6
	100	98	29
	200	59	38
Class 3 y ♂ / $\frac{y}{}$	Control	652	173
	100	695	183
	200	746	209
Class 4 y^+B^S ♀ / $\frac{y}{B^S}$ $\frac{y^+}{}$ / $\frac{}{y}$	Control	120	52
	100	126	50
	200	128	49

Table 3. Effects of high x-ray exposure on the induction of chromosome aberrations in germ cells in adult *Drosophila melanogaster* males.

	Exposure, R	Number of F_1 offspring from postmeiotic stages[a]	Number of F_1 *offspring* from meiotic and premeiotic stages[a]
Class 1 y^+♀ / y — y^+ / y / y	Control	110	25
	1500	61	5
	3000	26	5
Class 2A yB^S ♂ / B^S / y	Control	13	4
	1500	502 (904)	24 (120)
	3000	549 (2322)	17 (85)
Class 2B y^+ ♂ / y^+ / y	Control	43	7
	1500	77 (139)	33 (165)
	3000	80 (338)	18 (90)
Class 3 y ♂ / y	Control	504	63
	1500	675 (1215)	62 (310)
	3000	555 (2348)	39 (195)
Class 4 y^+B^S ♀ / y — y^+ / B^S / y	Control	102	16
	1500	46 (83)	3 (15)
	3000	16 (68)	3 (15)

[a]Numbers in parentheses are corrected for F_1 viability decrease resulting from exposure.

Table 4. Effects of EMS feeding exposure on the induction of chromosome aberrations in germ cells in adult *Drosophila melanogaster* males.

	Exposure, %	Number of F_1 offspring from postmeiotic stages[a]	Number of F_1 offspring from meiotic and premeiotic stages[a]
Class 1 y^+♀ / y — y^+ / y / y	Control	104	16
	0.05	71	6
	0.5	31	5
Class 2A yB^S ♂ / B^S / y	Control	5	2
	0.05	15 (22)	0 (0)
	0.5	93 (312)	20 (64)
Class 2B y^+ ♂ / y^+ / y	Control	48	4
	0.05	45 (66)	6 (21)
	0.5	37 (124)	10 (32)
Class 3 y ♂ / y	Control	211	49
	0.05	190 (277)	38 (101)
	0.5	299 (1002)	37 (118)
Class 4 y^+B^S♀ / y — y^+ / B^S / y	Control	41	12
	0.05	42 (60)	11 (29)
	0.5	17 (57)	4 (13)

[a]Numbers in parentheses are corrected for F_1 viability decrease resulting from exposure.

resented from the postmeiotic stages and approximately 123,000 male germ cells are represented from the meiotic and premeiotic stages.

Comparison of the number of y^+ females recovered in the exposed and control series (Table 2) indicates that neither 100 R nor 200 R had an observable effect on the viability of F_1 offspring. Both exposures do produce a significant ($p < 0.05$) increase in gross deletional events (yB^S males and y^+ males) in all germ cell stages studied. A slight increase in XO males is observed in progeny derived from all germ cell stages. No exposure related increase in nondisjunctional classes is observed.

High X-Ray Exposure

Approximately 330,000 male germ cells are represented from the postmeiotic stages and approximately 75,000 male germ cells are represented from the meiotic and premeiotic stages.

Following 1500 R and 3000 R exposures (Table 3), large exposure-dependent decreases in numbers of recovered y^+ females are observed. These decreases are presumed to be a result of zygotic killing or sperm disfunction and must cause similar magnitudes of viability reductions in the other progeny classes. A crude correction for this decrease can be made by multiplying each data point in the exposure series by the appropriate ratio of the number of control y^+ females to the number of exposure y^+ females.

Even without a correction for this overall decrease in viable F_1, 1500 R and 3000 R produce enormous increases over the control with regard to gross deletional events in postmeiotic germ cell stages in the B^S (X^DY^P) translocated chromosome. When the correction for F_1 viability decreases is made, large increases in gross deletions (yB^S males and y^+ males) and chromosome losses (y males) are also observed following either exposure. The correction factors for the meiotic and postmeiotic stages contain a high degree of unreliability because of the tiny sample

size of recovered y^+ females in the exposed groups. This is the most likely explanation for the anomalous dose-response relationship observed in classes 2 and 3 (yB^S males, y^+ males, and y males) when the numbers are corrected for the F_1 viability decrease.

No induced increase in y^+B^S females derived from postmeiotic germ cell stages would be expected, and when the numbers are corrected for overall decrease of viable F_1, no difference is noted between the exposed and control series. This corroborates the validity of the crude correction factor for the postmeiotic stages and confirms the generality of the F_1 viability decrease. Finally, no increase with exposure is noted for the nondisjunctional class (y^+B^S females) from meiotic and premeiotic germ cell stages.

EMS Exposure

Approximately 312,000 male germ cells are represented from the postmeiotic stages and approximately 48,000 male germ cells are represented from the meiotic and premeiotic stages.

Following exposures to 0.05% and 0.5% EMS (Table 4), large exposure dependent reduction in y^+ females from exposed series compared to those from control series are observed. Correcting for this reduction, increases are noted for both gross deletional classes (yB^S males and y^+ males) and for the chromosome loss class (y males) in all germ cell stages studied. The nondisjunctional class (y^+B^S females) shows no increase over the control for either exposure.

Exposure to Aqueous Run-off from Smelter

Approximately 207,000 male germ cells are represented from the postmeiotic stages and approximately 30,000 male germ cells are represented from the meiotic and premeiotic stages.

Feeding exposure to aqueous run-off from a smelting operation had no apparent effect on any of the phenotypic classes (Table 5). The same concentration (0.2%) fed to adult males showed no increase over the control in a sex-linked recessive lethal test.

Table 5. Test for chromosome aberrations induced in germ cells in adult *Drosophila melanogaster* males by feeding exposure to aqueous run-off (0.2%) from a smelting operation.

	Exposure	Number of F_1 offspring from postmeiotic stages	Number of F_1 offspring from meiotic and premeiotic stages
Class 1 y^+ ♀ / y — y^+ / y / y	Control	69	10
	Aqueous runoff	68	13
Class 2A yB^S ♂ / B^S / y	Control	7	4
	Aqueous *runoff*	4	3
Class 2B y^+ ♂ / y^+ / y	Control	38	16
	Aqueous runoff	28	8
Class 3 y ♂ / y	Control	232	48
	Aqueous runoff	243	74
Class 4 y^+B^S ♀ / y — y^+ / B^S / y	Control	50	21
	Aqueous runoff	53	17

Discussion

For both mutagens studied, class 2A events (leading to yB^S males) differ from class 2B events (leading to y^+ males) with regard to sensitivity to induction. This is probably due to topological differences in the translocated chromosomes. That is, the deletion of X^P (in the B^S marked translocated chromosome) is probably favored by the presence of heterochromatic regions adjacent to B^S and near the X centromere.

X-Ray Data

The system described is exquisitely sensitive to detecting x-ray-induced gross deletional events. For this class of events, the system is capable of monitoring significant increases at exposures as low as 100 R and increasing in an exposure related fashion through exposures of 3000 R. Detection of sex-linked recessive lethal induction following an exposure of 100 R would require testing roughly 20,000 germ cells and involve a large expenditure of time and labor. Induced chromosome loss is readily detected at a 1500 R exposure for all germ cell stages and is suspected at doses as low as 200 R for all stages. Very little or no induced nondisjunction is expected at exposures below 1000 R (*6, 8*) and it is, therefore, not surprising that none was obtained. An increase in primary meiotic nondisjunction is expected following 1500 R and 3000 R exposures (*6, 8*),

but none was obtained in these experiments. This was probably due to the low rate of recovery of meiotic and premeiotic germ cells from the treated male.

EMS Data

Although EMS is not generally considered an efficient chromosome breaking agent (*9*), Bishop and Lee (*10*) have reported that EMS is effective in producing breakage in X and Y heterochromatin in *Drosophila melanogaster*. This is consistent with the results presented here for the induction of gross deletions (yB^S males and y^+ males). These events presumably result from breakage and subsequent loss in the heterochromatic regions associated with the X and Y chromosomes. X chromosome loss was also induced by EMS. This result confirms that obtained by Mittler (*11*). No evidence for the induction of primary nondisjunction was obtained.

The lack of conclusive primary meiotic nondisjunction data is a result of low recovery of progeny in the later broods. The cause of this reduced recovery is twofold. First, these stages are the most susceptible to cell killing caused by the exposures, and consequently, a low recovery of progeny from these germ cell stages following exposure to an effective mutagen is expected. Second, males from this translocation X;Y stock appear to suffer early sterility, leading to a marked decrease in the number of sperm recovered from later broods in both control and exposed series. To overcome this problem, future work will focus on larval treatment, and it is presumed that sensitivity for the detection of induced nondisjunction will increase to the level observed for induced deletional events.

Summary

The translocation X;Y system described here has the capacity to detect multiple effects of a mutagen in a single experiment. It has been shown to be a very powerful method for detecting both gross deletions and chromosome losses in *Drosophila melanogaster* males. The system has the advantage of combining the rapid screening capacities of prokaryotic systems with the relevance to humans of eukaryotic systems. Furthermore, it has the capacity to be used as a screening test for meiotic nondisjunction, an important and neglected genetic endpoint in environmental mutagen screening regimens currently in use.

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