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Directed Synthesis of a Segmental Chromosomal Transposition: An Approach to the Study of Chromosomes Lethal to the Gametophyte Generation of Maize

James A. Birchler and Daniel M. Levin

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

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ABSTRACT

Because of the haploid nature of the gametophyte generation of plants, most mutations that are lethal or detrimental to the gametophytes cannot be recovered. Our laboratory is currently developing several techniques to overcome this situation. In this paper, a procedure is described to generate directed segmental chromosomal transpositions. The method involves recovery of recombinants between reciprocal translocation overlaps such that one region of the genome is inserted into a nonhomologous chromosome in a predetermined and directed manner. This duplicated segment then could serve to cover deficiencies or mutations, lethal to the gametophytes, in the region from whence it originated. The manipulation of segmental chromosomal transpositions for analyzing mutants lethal or detrimental to the gametophyte generation is discussed. The procedure to generate transpositions, the translocations between normal A and supernumerary B chromosomes that generate deficiencies in the male gametes, the *r-X1* chromosome that generates deficiencies in the female gametes and other techniques available in maize form a system to analyze gametophyte lethal mutations.

THE life cycle of plants consists of a sporophyte and a gametophyte generation. The endproduct of the sporophyte generation is a chromosomally reduced cell that results from meiosis. This cell differentiates into the gametophytes, which in higher plants are spatially separated and serve to form an egg cell in the megagametophyte or sperm cells in the microgametophyte. Therefore, in diploid species the gametophytes are monoploid.

Evidence from isozyme comparisons indicates that the gametophytes synthesize their own gene products. Heterozygotes for genes encoding multimeric enzymes inevitably show only homomultimeric isozymes when their pollen is examined. There is also substantial overlap of the genes expressed between the sporophytic and gametophytic generations (SCHWARTZ 1971; TANKSLEY, ZAMIR and RICK 1981; SARIGORLA *et al.* 1987; PEDERSON, SIMONSEN and LOESCHCKE 1987). Moreover, from an independent approach of comparing the messenger RNA complexity in pollen and shoots of maize, it was found that 24,000 diverse sequences were expressed in the mature pollen compared to 31,000 in shoots. Cross hybridization studies suggest that the pollen sequences shared with the sporophyte could be as high as 90% (WILLING, BASHE and MASCARENHAS 1988).

The extent to which gametophytic gene expression is vital was addressed in a study by C. M. RICK and G. S. KHUSH (discussed in ZAMIR 1983), who irradiated mature pollen of tomato and used it in pollinations. The hybrids were examined cytologically and used in genetic crosses. It was found that even small deficiencies, excepting those involving centric heterochromatin, would not transmit through the gametophyte generation. A similar situation exists in maize, where there are few examples of deficiencies that can survive (CARLSON 1977). In addition, inspection of M1 ears and pollen of maize that result from ethyl methanesulfonate treatment of kernels shows high abortion (*e.g.*, BIRCHLER and SCHWARTZ 1979). Thus, there are many mutants that can be induced but that are eliminated in the gametophytes.

Taken together, these observations indicate that there is considerable overlap of gene expression between the two generations, the gametophytes express their own products and many genes are vital; therefore mutations in a substantial fraction of genes (perhaps a majority) in plants can not currently be recovered. Mutations in genes whose products are vital to metabolism, cell division, gametophytic development, gametophytically expressed determinants of sporophytic development, etc. will be eliminated at this stage of the life cycle. Indeed, mutations that merely slow pollen tube growth could not be recovered in standard mutageneses that involve self pollination, because they cannot successfully compete against normal pollen in achieving fertilization. Our laboratory is developing a variety of techniques for

the recovery and analysis of deficiencies and gametophytically lethal mutants. The experiments outlined in this paper detail one of these approaches. A procedure for the directed synthesis of a segmental chromosomal transposition is described.

The principle of this technique is to select a region of interest and recombine that segment, via overlapping reciprocal translocations in segmental monosomics created by B-A translocations, into another chromosome. With this segment now duplicated on another centromere, chromosomes with deficiencies or mutants lethal to the gametophytes within the segment from whence the duplicated segment came are not selected against in mutageneses because a functional allele is still present in the nucleus. Because the duplication will independently assort in meiosis, mutant gametophytes can be produced for study. Further, mutant sporophytes could also be produced because methods exist in maize to eliminate certain chromosomes late in either mega or microgametophyte development that would remove the duplication from the gamete. Procedures also exist to create chimeric sporophytes in a heterozygote once the mutant chromosome has been delivered in an uncovered state to one of the two gametes.

Maize is particularly well suited for such an analysis. First of all, there are nearly 900 reciprocal translocations available as starting material. These translocations provide the potential to create such duplications for basically all segments of the genome, save immediate regions around the centromeres and the terminal regions, both of which can be covered by other techniques. Second, translocations can be followed easily by inspection for semisterility on the ears, or in the pollen. Third, a nearly complete set of B-A translocations are available for use in generating whole arm deficiencies, which is critical for achieving recombination in the translocation overlaps, and lastly, genetic tools are available for elimination of the duplications.

MATERIALS AND METHODS

Maize stocks: Translocations *1–3 (5267)* and *1–3 (5242)* were originally obtained from the Maize Genetics Cooperative, University of Illinois. A segmental tetrasomic involving the regions between the breakpoints on *1L* and *3L* was constructed (BIRCHLER *et al.* 1981). Translocation *1–3 (5267)* is linked to an alcohol dehydrogenase *Adh-F* allele and *1–3 (5242)* to an *Adh-S*. The *Adh* locus resides between the *1L* breakpoints of these two translocations (BIRCHLER 1980, 1981). The segmental tetrasomic line, therefore, is *Adh-F/F/S/S*.

The B-A translocation, *TB-1La*, was obtained from the Maize Coop. Originally, it carried *Adh-F*, which was replaced by crossing over with *Adh-C* (BIRCHLER 1979).

Root tip analysis: Root tip chromosome counts were performed by the method of KINDIGER and BECKETT (1983) as modified by BIRCHLER, CHALFOUN and LEVIN (1990).

Alcohol dehydrogenase electrophoresis: Electrophoresis and staining of ADH isozymes was by the method of BIRCHLER and HART (1987). The alleles of alcohol dehydrogenase and further information are described in FREELING and BIRCHLER (1981).

RESULTS

The rationale for the experiments described here is to create a transposed duplication of a certain chromosomal segment. This will place a region of one chromosome into another in a directed, preconceived manner. Once such a transposition is produced, it could then be used to recover and maintain chromosomal deficiencies and mutations that are lethal to the gametophyte generation in that region of the genome. The example described outlines the method by which such transpositions can be produced.

Transpositions can be created by recombination in overlaps of reciprocal translocations, in which the overlap does not delete any part of the genome. When two reciprocal translocations involving the same two chromosome arms are crossed together, duplicated or deficient spores are produced during meiosis. By judicious choice of breakpoints, spores can be produced that are duplicated for both regions between the breakpoints in each of the two chromosomes. For this to be the case, the relative orientation of breakpoints requires one translocation to have a proximal break in chromosome "Y" and a distal breakpoint in chromosome "Z." The second translocation would have a distal break in chromosome Y and a proximal break in Z. This orientation will generate four classes of spores: (1) those balanced for the first translocation, (2) those balanced for the second translocation, (3) those duplicate for all regions between the breakpoints of the two translocations, and (4) those deficient for these regions (GOPINATH and BURNHAM 1956). The last class usually aborts (see Figure 1).

One approach to creating transpositions might be to select the duplicated progeny from such translocation heterozygotes and eventually generate a segmental tetrasomic, as has previously been done (BIRCHLER, ALLEMAN and FREELING 1981). The desired recombinant could be selected in the progeny of the tetrasomic. However, after study of this stock, it was found that recombination was not detected in the translocation overlap, presumably because chromosome pairing is greatly preferred between the homologous chromosomes.

Consequently, a procedure was sought that would result in a pairing configuration in which only the region of overlap was available for pairing and recombination. To achieve this situation, a heterozygote for the translocations, *T1–3 (5267)* and *T1–3 (5242)*, marked by *Adh-F* and *Adh-S*, respectively, was crossed by the B-A translocation *TB-1La*, marked by *Adh-C*. As noted above, the translocation heterozygote will generate one class of gamete that is duplicated for the

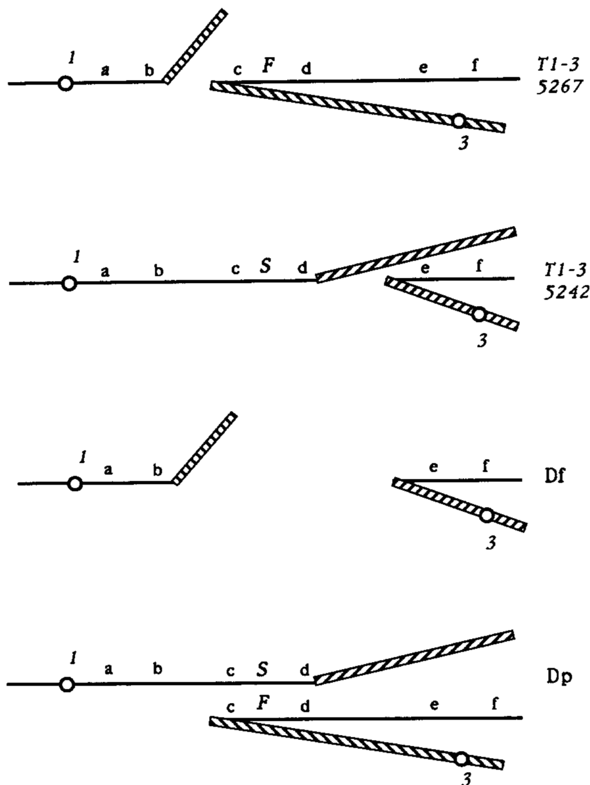

FIGURE 1.—Diagram of meiotic segregation from a double translocation heterozygote. The translocations used in the present study are labelled *T1-3 5267* and *T1-3 5242*. In *T1-3 5267* the breakpoint is proximal to the *Adh* locus (*F* allele) in the long arm of chromosome *1*. *T1-3 5242* is broken distally to *Adh* (*S* allele). The relative proximal-distal breaks in chromosome *3* are reversed. The four sections of the diagram depict the types of meiotic products that result from heterozygotes of the two translocations. Each translocation is recovered as well as products deficient (Df) or duplicate (Dp) for all regions between the translocation breakpoints. The deficiency spores can not support gametophytic development and abort. The duplicated gametes are present in one-third of the progeny when the double translocation heterozygote is crossed as a female and are marked by *Adh S/F*. The centromere of each chromosome is labeled. The long arm of chromosome *1* is divided into section labeled a–f to illustrate the deficient and duplicate regions produced.

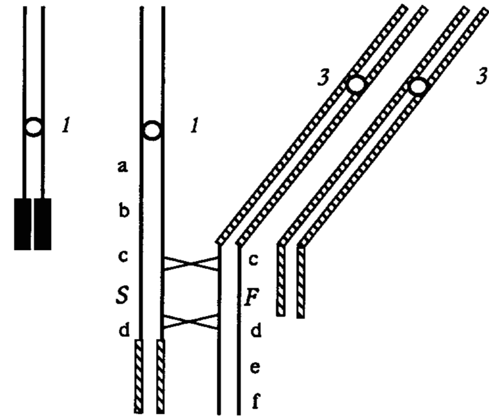

FIGURE 2.—Diagrammatic representation of a monosomic for *1L* with the translocation overlap. The solid line represents chromosome *1*. The filled block represents the terminal segment of the B chromosome. The hatched line represents chromosome *3*. Circles represent centromeres, which are labeled respectively. *S* and *F* indicate the *Adh* alleles present. The top cross illustrates a crossover proximal to *Adh* while the bottom, a crossover distal to *Adh*. The long arm of chromosome *1* is divided into sections labeled a–f to illustrate the duplicated segment surrounding *Adh*.

1L and *3L* regions between the breakpoints. The B-A translocation used is broken in the long arm of chromosome *1* at a position proximal to the duplication generated by the translocation overlap. Because of the property of the B chromosome centromere to nondisjoin at the second microspore division, which immediately precedes sperm differentiation in maize, male gametes produced from B-A translocations are duplicated or deficient for the A chromosomal region translocated to the B chromosome. In the case under consideration here, *TB-1La* is marked by *Adh-C*. Therefore, the deficient gametes will be missing this allozyme and if they fertilize the egg cells that are duplicated, an ADH pattern of only *F S* will result. The zygotes that result from fertilizations by male gametes that are balanced for the B-A translocation or that are duplicated will all have *Adh-C* present. Those zygotes that result when deficient male gametes fertilize the nonduplicated egg cells will have only F or S present. As a result, all pertinent classes of zygotes can be distinguished by ADH allozyme typing.

A schematic of the desired class is shown in Figure 2. The *1*-B chromosome does not contribute the distal portion of *1L* to the genotype, thus the regions of overlap from the two *1–3* reciprocal translocations are the only homologous regions of *1L* present. A normal chromosome *3* is contributed from the *TB-1La* parent. This configuration leaves only the region of overlap to pair and recombine, having removed the obstacle of pairing of homologues that occurs in the segmental tetrasomic.

In practice, kernels from a cross of the translocation heterozygote by *TB-1La* were analyzed for *Adh* alleles from a sliver of the scutellum. Those kernels exhibiting an *Adh-F/S* genotype were saved and planted. These individuals represent the cases of monosomy for the region of *1L* involved in *TB-1La* and that receive a duplication from the heterozygous translocation parent. Twenty such individuals were crossed as females by *Adh-W* males. The plants were short in stature relative to parental stocks and exhibited high levels of ovule abortion, as expected. The ovule abortion results because the segregation of the A-B chromosome produces gametes that are deficient for most of *1L*. In the other classes of gametes, both chromosomes involved in the overlap are required to prevent significant deficiency, which occurs in one half of the spores. The theoretical level of ovule abortion is therefore 75%.

In the absence of recombination in the overlap, the only type of viable gamete produced from the geno-

type depicted in figure two is one possessing the overlap itself. When fertilized by normal pollen the resulting individuals will produce ears that exhibit 50% ovule abortion. The viable gametes are present in approximately 50% overlap versus 50% normal chromosome *1* (BIRCHLER 1980). Both classes are transmitted through the female side but the normal chromosome is recovered more frequently on the male side as is often the case in competition with duplicated pollen.

Recombination within the overlap has different consequences for marker distribution depending on the site of the event (see Figure 2). The two chromosomes generated are a normal chromosome *1* and an insertion of the *1L* region between the breakpoints of the original translocations into a duplication of the *3L* region between the breakpoints. If the recombination occurs proximal to *Adh*, the normal chromosome *1* will carry *Adh-F* and the transposition, *Adh-S*. If the crossover occurs distal to *Adh*, then the normal chromosome *1* will be marked by *S* and the transposition by *F*.

The possible assortment of chromosomes *1* and *3* at anaphase I in a meiocyte with a chiasma in the overlap region is given in Figure 3. The two types on the left will all result in spores that are deficient for *1L* at the completion of meiosis II. The type at the top right will produce a viable spore if the chromatid that is a normal chromosome *1* assorts with the chromatid that has the *1L* → *3L* transposition. Only one quarter of the spores that result from this type are viable, all the remaining ones will be deficient and result in abortion. The last type shown in the lower right will also produce viable spores that carry a normal chromosome *1* and a normal chromosome *3*. Of the four possible combinations of chromatids that could result at the end of meiosis II, half are viable.

Therefore, from a cell in which a crossover has occurred in the overlap, only 3/16 of the possible combinations of chromatids will produce a viable spore at the conclusion of meiosis. Among the survivors, the expected frequency of transposition plus chromosome *1 versus* normal chromosomes *1* and *3* is 1:2.

The viable products from a crossover, when fertilized by normal pollen, will not produce ovule or pollen abortion in the subsequent generation because no deficiencies are generated. This is in contrast to the situation involving noncrossers. The descendents of recombinants can easily be recognized because the ears will have normal fertility as characterized by an orderly array of kernels while the descendants of noncrossovers give partially filled ears. See Figure 4.

As a consequence, the selfed progeny of the cross of the segmental monosomics by *Adh-W* were screened for recombinants by inspection of the ears. From a total of 160, four did not exhibit semisterility. Kernels from these ears were analyzed for ADH allozymes. The data are in Table 1. Two ears gave kernels indicative of selfing of *S/W* plants. One was a self of *F/W*. The last was an S/W self with independent assortment of *Adh-F*.

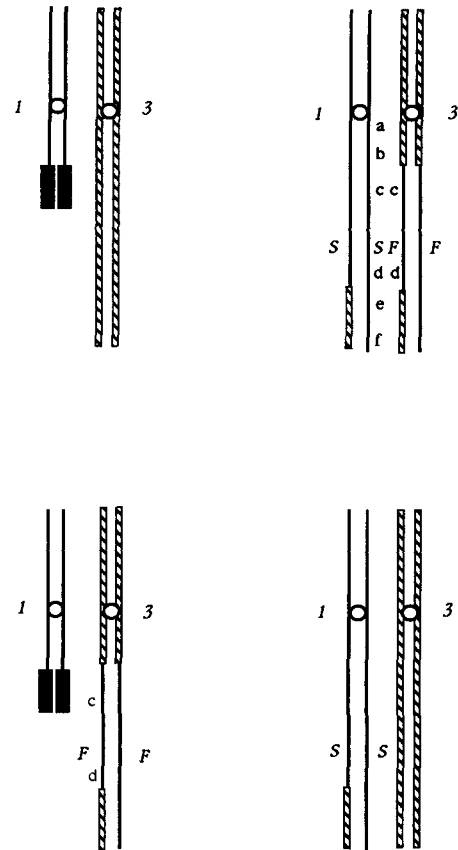

FIGURE 3.—Diagrammatic representation of the classes of meiotic assortment at anaphase I that result from a meiocyte with a crossover in the overlap region. Top left, *1*-B chromosome and normal chromosome *1*. No viable spores can be produced. Bottom left, *1*-B chromosome and *1* chromosome centromere with a crossover chromatid. No viable spores can be produced. Top right, chromosomes with *1* and *3* centromeres, the former carrying a crossover chromatid that is a normal sequence *1* and the latter carrying a crossover chromatid with an insertion of a segment of *1* into *3*. The assortment at anaphase II will produce a viable spore if the normal *1* and the insertion proceed to the same pole. Bottom right, chromosome *3* and chromosome *1* centromere with a crossover chromatid that is a normal chromosome *1*. Viable spores will be produced when the normal chromosome *1* is included in the meiotic endproduct with the normal *3*. In the upper right, the long arm of chromosome *1* is divided into sections labeled a–f to illustrate the region of *1L* inserted into *3L*.

The first three ears are interpreted as resulting from cases of recombination in the overlap with recovery of the normal chromosomes *1* and *3*—the situation expected more frequently. The last is interpreted as a potential transposition marked by *Adh-F* and recovered with a normal chromosome *1* marked by *Adh-S*.

Before proceeding to a further description of the

FIGURE 4.—Ovule sterility in recombinant and nonrecombinant ears. Right, nonrecombinant exhibiting semisterility. Left, recombinant shows return to normal fertility.

TABLE 1

ADH genotypes from ears with normal ovule fertility

Ear	ADH genotype							
	S/S	*S/W*	*W/W*	*W/F*	*F/F*	*S/S + F*	*W/S + F*	*W/W + F*
A	2	10	8					
B	4	8	8					
C			5	10	5			
D	3	17	14			14	18	4

The number of individuals with the corresponding *Adh* genotype are listed for each of the four ears showing normal ovule fertility. *S*, *F* and *W* refer to the ADH allozymes used in the study.

transposition, it should be noted that potentially recombination could occur in the overlap region of *3L* to insert this segment into *1L*. This would also create an F_1 plant that bears a fully fertile ear; however, the ADH isozymes S and F would be linked. This is diagrammed in Figure 5. No such case was recovered, nor was it expected at high frequency, because the *3L* overlap is much shorter in length than the *1L* segment and a normal chromosome *3* is present that serves as a pairing competitor.

The recovery of four recombinant products and

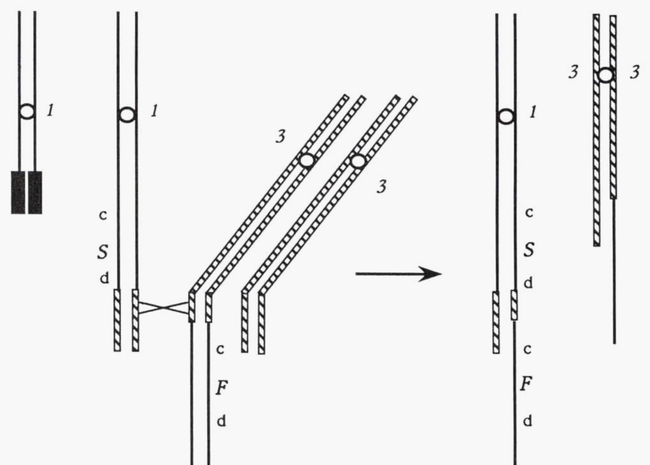

FIGURE 5.—Diagrammatic representation of the products if a recombination were to occur in the *3L* overlap. At left is the *1L* monosomic translocation overlap redrawn to show pairing in the *3L* overlap region. At right, the potential recombinant chromosomes are shown. The assortment of the duplicated, *Adh F/S* chromatid with a normal chromosome *3* would produce a viable spore. The duplicated region around *Adh* is labeled c,d as in previous figures to illustrate the order on the recombinant chromosome.

TABLE 2

***Adh* genotypes in the progeny of the transposition recombinant**

Ear	*Adh* genotypes						Parental genotypes
	W/W	*W/S*	*S/S*	*W/W* + *F*	*W/S* + *F*	*S/S* + *F*	
1	15						*W/W*
2	15						*W/W*
3		4	5				*W/S*
4	2	4	3				*W/S*
5	7	7	2				*W/S*
6	4	7	8				*W/S*
7	4	4	7				*W/S*
8	3	9	2				*W/S*
9			20				*S/S*
10			13				*S/S*
11	9			9			*W/W/F*
12	6			10			*W/W/F*
13	7	9	14	6	10	13	*W/S/F*
14	13	22	7	14	21	8	*W/S/F*
15	18	33	16	16	16	13	*W/S/F*
16	8	13	7	14	25	7	*W/S/F*
17	1	2	1		2	3	*W/S/F*
18			6			9	*S/S/F*
Totals from ears segregating	62	79	51	69	74	53	
Adh-F (11–18)		192			196		

Individuals from the recombinant transposition ear were grown and self polinated. *Adh* allozyme analysis for 18 of the resulting ears is given above. The number of individuals with the corresponding *Adh* genotype are listed for each ear. At the far right is the deduced genotype of the parent.

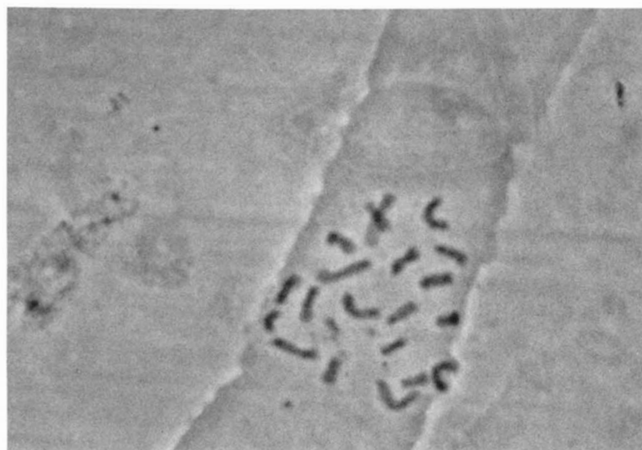

FIGURE 6.—Root tip chromosome squash of an *Adh W/S/F* seedling.

156 nonrecombinants allows a determination of the frequency of meiocytes that had a crossover. As noted above, in the case of nonexchange cells, only one quarter will give a viable spore. In the case of crossovers, only 3/16 give a viable spore. Therefore, the calculation of percentage of meiocytes with a crossover in the overlap is done by multiplying the nonrecombinant frequency by four and the recombinant frequency by 5.33. Then, the calculated recombinant frequency is divided by the sum of the two. In the example considered here, 3.26% of the meiocytes have a crossover in the overlap region. The percent recombination, projected in this way, is therefore 1.63. This value is undoubtedly lower than the recombination frequency for this segment, which is comprised of 18% of the long arm, the whole of which normally has over 100 map units. However, given the complex pairing of the configuration, it is expected that the recombination frequency might be reduced.

A potential alternative explanation for the recombinant would be that nondisjunction occurred during meiosis I or II to give a *1*-B, *1–3, 3–1* or *1–3, 3–1, 3* gamete. The hypothetical trisomic condition that results might be expected to alleviate the ear sterility to some degree and to produce assortment of three *Adh* alleles upon self pollination. To distinguish this possibility from that of recombination to produce a transposition, root tip chromosome counts were made. If trisomy were responsible for the observed *Adh* segregation then individuals with zymograms typical of three alleles would have 21 chromosomes whereas the presence of a transposition would only have 20. Accordingly, two seedlings with *Adh S/S/F* genotypes, one with *W/W/F,* three with *W/S/F* and one with *S/W* were examined for chromosome number. In all cases the chromosome number was found to be 20.

For further characterization, kernels from the transposition ear were grown and the resulting plants self pollinated. These were typed for ADH (Table 2). Examination of the data shows that *Adh-S* and *W* behave as segregating alleles and independently of *Adh-F*. Overall, the ears in which *Adh-F* was present, it occurred in slightly greater than 50% of the prog-

TABLE 3

Test of male transmission of the transposition

Plant genotype and #	*Adh* genotypes of progeny						% F transmission
	W/W	*W/S*	*S/S*	*W/W + F*	*W/S + F*	*S/S + F*	
S/S/F 90117-1 self			21			28	57
W/S × *S/S/F* 90120 × 90117-1		22	18		4	5	18
S/S/F 90117-7 self			23			25	52
W/S × *S/S/F* 90120 × 90117-7		10	11		17	12	58

Individuals were self pollinated and outcrossed as males to an *Adh W/S* heterozygote. The number of individuals with the corresponding *Adh* genotype are listed for each ear.

eny. This suggests that duplicated pollen marked by *F* does not compete well in competition with normal. When the translocation overlap involving this same duplicated region was tested against normal, the male transmission was reduced (Birchler, Alleman and Freeling 1981). If competition were nearly equal, an expected frequency of *F* would be 75%. No attempt was made to discriminate cases of one or two copies of *F*. Root tip chromosome counts were performed on each of the electrophoretic classes on an ear segregating for *W, S* and *F* (*W/W, W/S, S/S, W/W/F, W/S/F* and *S/S/F*). In every case the chromosome number was found to be 20 (Figure 6).

Kernels from an ear bearing *F* were planted. Individuals were selfed and outcrossed to an *Adh S/W* heterozygote to determine the frequency of *F* male transmission. Two sets of successful selfs and outcrosses were analyzed (Table 3). In both self's the frequency of *F* was slightly above 50%, but the two outcrosses differed with regard to the percent transmission through the male, being 18% in one case and 58% in the other. These data together with those noted above suggest that the frequency of male transmission is usually reduced but is greatly influenced by pollination conditions.

DISCUSSION

As noted above, there is considerable evidence that a significant fraction of mutations are lethal or detrimental to the haploid gametophytes. Such mutations cannot be recovered in conventional mutageneses. It is likely that these vital genes would encode steps in many cellular, developmental and metabolic processes. In order to apply genetic dissection to certain phenomena or to establish the functionality of genes cloned via the gene product, procedures that would permit the recovery of gametophyte lethal chromosomes are needed. These techniques optimally would provide a means to cover the mutant or deficiency during the gametophyte generation but would also establish a means to eliminate the covering duplication during the production of gametes or in the sporophyte so that the mutant effect could be studied.

Our laboratory is developing genetic tools to address this situation. The major focus is on two techniques. The first, described in this paper, involves a method to generate transpositions of preselected cytological length and chromosomal location. The second involves the construction of a comprehensive set of compound B-A translocations involving *TB-10L18* and eighteen other chromosome arms. *TB-10L18* is unique in that it is broken in the short arm of the supernumerary B chromosome and exhibits autonomous nondisjunction at the second microspore division. This property will be conferred to the other eighteen arms in the compound translocations. These constructs will be described in a separate publication.

There presently exists a fairly comprehensive set of B-A translocations in maize that covers 18 chromosome arms. The potential exists that these could be used to recover gametophytically lethal chromosomes in tertiary trisomics (*i.e.,* A A B-A genotypes). The procedures being developed have significant advantages over such an approach in several ways.

The B-A chromosomes when used as a tertiary trisomic could cover deficiencies and mutations only through the megagametophyte, because as a general rule, the cytological length is too great for transmission through the pollen. Moreover, the B-A chromosomes do not undergo nondisjunction at the second microspore division in the absence of the terminal euchromatin of the B chromosome in the same nucleus. Consequently, even in cases when the extra chromosome could be transmitted, it would not be eliminated to uncover the mutation in the male gamete. The production of interstitial duplications gives a more specific coverage that is transmissible through both the male and female gametophyte and that could be recombined onto the respective B-A translocation so that it could be eliminated in the male gametes.

Also, the generation of smaller duplications that are transmissible through both parents provides the potential to construct a homozygous stock of the lethal chromosome and the duplication.

The procedure outlined above describes the construction of a directed transposition of one region of the genome into a preselected target. Because one segment is borne on a different chromosome than normal, it will allow the recovery and analysis of deficiencies and mutants lethal to the gametophyte generation that occur in the duplicated region of the normal chromosome. Given that there are nearly 900 reciprocal translocations available in maize (LONGLEY 1961), the procedure could be extended to involve most of the genome. The potential exists to generate very fine dissection using the available translocations, yet the genome could be reasonably covered with far fewer.

The example described above utilized allozyme markers to follow the construction. The procedure could be modified, however, to use restriction fragment length polymorphisms (RFLP) for which an extensive map is available in maize (HELENTJARIS, WEBER and WRIGHT 1988). An RFLP difference could be identified between the parental pair of translocations and also the respective B-A translocation involving the chromosome arm in question. Maize is the most polymorphic for restriction sites of any organism examined to date, making this approach feasible (BURR *et al.* 1988). The two translocations could be crossed together and the F_1 heterozygote crossed by the proper TB-A. Leaf samples could be taken for DNA isolation and Southern analysis to identify those individuals that are duplicate for the region between the breakpoints and monosomic for the chromosome arm. These individuals could be crossed by normal pollen and the progeny self pollinated. Alternatively, these monosomics could be used as males onto normal to reduce the number of plants needed. The individuals in the progeny that were products of recombination in the overlap will have normal ovule fertility while the nonrecombinants will have 50% abortion. This allows the potential to screen many individuals quite rapidly to select the endproduct. Pollen fertility could also be used as a criterion for selection of recombinants at the time of flowering followed by self pollination of the selected individuals only. RFLP analysis need only be performed on the selected ears to distinguish those recombinants that are returned to normal sequence from those that form transpositions.

The procedure described above can not cover the centromeric or telomeric regions of the genome. However, other means can be used. For centromeric regions, one could create a B-A-B chromosome from opposite arm B-A translocations as CARLSON (1983) has demonstrated. The two TB-A's are crossed together and recombinants in the centromeric regions in common will create a small chromosome with the respective centromere and capped on both ends by a terminal piece of the B chromosome. These chromosomes could also be used to cover gametophytic lethals. Terminal regions could be covered by generation of compound B-A translocations with the autonomously nondisjoining *TB-10L18*, a technique that will be described elsewhere.

A potential protocol to select gametophytically lethal deficiencies and mutations covered by the duplication follows; however, the particular region or biological problem addressed may require or permit modifications to suit the particular situation. A stock homozygous for the duplication could be used as a female parent in crosses by mutagenized pollen. The progeny would be heterozygous for the duplication. If no mutation is induced, the resulting plant will exhibit no ovule or pollen abortion. If, however, a gametophytically lethal mutation is generated outside the limits of the duplication, semisterility (50% abortion) will be exhibited by the F_1 individual. In the event that a mutation or deficiency is induced in the region covered by the transposition, the resulting F_1 individual will exhibit approximately 25% ovule and pollen sterility. This is due to the segregation of the lethal on one chromosome and the independent assortment of the duplication on another that rescues the lethality when present. This system will select those cases in which the only lethal in the genome is the one covered—all others will be eliminated. In practice, an F_2 screen may prove to be most effective. In this case, the F_1 individuals would be crossed by a chromosomally normal individual (perhaps carrying genetic markers on the chromosome under consideration). In this step, all extraneous gametophytic lethals will be eliminated. Samples of the F_2 progeny could then be screened for those exhibiting 25% abortion. Because the combination, if present, is expected in one-third of the progeny, a sample size of eleven individuals would give a 99% probability of inclusion of at least one representative (HANSON 1959).

Once mutants or deficiencies lethal to the gametophytes are recovered, it would, of course, be useful to have the capability to rid the genotype of the duplication in order that the mutant effect could be studied. Fortunately, methods exist in maize that eliminate chromosomes from the gametes by nondisjunction at a late stage of megagametophyte or microgametophyte development such that the viability of the gametophyte is not affected.

In the megagametophyte, the *r-X1* deficiency on chromosome 10 causes nondisjunction at the second mitotic division, resulting in gametes that are deficient or duplicate for individual chromosomes (ZHAO and

WEBER 1988; LIN and COE 1986). Therefore, the *r-X1* chromosome could be combined with the lethal chromosome plus the duplication. Then, in the subsequent generation the duplication could be lost to uncover the lethal chromosome. By using the appropriate plant markers, the loss of the duplication carrying chromosome can be recognized. The lethal chromosome should be able to be transmitted through the megagametophyte, even with the loss of the duplication at the second division because all monosomics have been recovered from *r-X1* plants. This suggests that megagametophytic vital gene expression is basically carried out by the time of the second of the three major divisions.

In the microgametophyte, chromosomal segments can be eliminated from the genotype if they are translocated to a B chromosome centromere. This centromere nondisjoins at the second mitosis in the development of the microgametophyte. Because this division produces the maize sperm, gametes are produced that are duplicate or deficient for the B centromere linked sequences (ROMAN 1947; BECKETT 1978). In the situation under consideration here, the duplication could be recombined onto the respective B-A translocation in the stock used to cover the gametophytic lethal. Then when used as a male parent, the B centromere would nondisjoin resulting in a fraction of gametes that expose the lethal mutant or deficiency. Because the microgametophyte functions are specified by the tube nucleus, which is independent of the sperm, such a condition is not selected against.

Both of the above procedures would deliver the mutant to the zygote in a heterozygous condition that would still require a mosaic analysis to examine the mutant in the sporophyte. In this case, a genotype could be created such that the mutant is present on one homologue and a chromosome breaking state of *Dissociation* (*Ds*) is present in the other homologue with *Activator* (*Ac*) represented in the genome. This system has been developed by M. G. NEUFFER (1989). *Dissociation* is a nonautonomous transposable element that responds to the autonomous *Activator* element. The particular form of *Ds,* recovered in proximal regions of most chromosome arms in this system, will fragment the chromosome at the site of insertion whenever *Ac* is present in the same genotype. In the plant, *Ds* transposes late in development, so mosaic individuals are created, because *Ds* was proximal to the mutation.

The particular system outlined here is of necessity regional in its application. For some studies such as deficiency mapping of a certain chromosomal segment, this would be desirable. However, for genetic dissection of vital functions, screening the entire genome is needed. Approaches to address this need are under investigation.

The system described in this paper could be developed because of the extensive genetic tools already available in maize. The wealth of reciprocal translocations, B-A translocations, the *r-X1* system and the *Dissociation* mosaic system form a foundation on which an analysis of gametophytically lethal mutations can be performed.

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