TECHNIQUES FOR MANIPULATING CHROMOSOMAL REARRANGEMENTS AND THEIR APPLICATION TO *DROSOPHILA MELANOGASTER*. I. PERICENTRIC INVERSIONS

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ABSTRACT

Techniques have been developed for manipulating pericentric inversions in Drosophila that are based on the lethality of grossly aneuploid zygotes and the existence of recombinationally interconvertible genotypes for any heterozygous inversion complex: males of some of these genotypes will produce only aneuploid sperm, which can be used to rescue complementary aneuploid ova and selectively recover recombinational derivatives of inversions. Markers can be recombined into inversions through a sequence of selected single exchanges, and a novel type of duplication can be synthesized from overlapping inversions that has the characteristics of both insertional and tandem duplications; there are also applications to half-tetrad analyses. ——Two cytogenetic screens are developed: (1) the dominant lethality of a large insertional-tandem duplication can be reverted by deletional events that give rise to net deficiencies or duplications, and (2) deficiencies and tandem duplications in proximal regions can be selectively recovered as the results of unequal exchanges within an inversion loop. Recombinants have been recovered between breakpoints separated by distances of as little as fifty bands, arguing against the existence of some small number of sites necessary for the initiation of recombinational pairing. In several instances, hyperploids for four to six numbered divisions were observed to be fertile in both sexes.

IN highly fecund organisms, procedures that selectively eliminate regular classes of offspring provide powerful tools for recovering products of rare genetic events. Good examples of this in Drosophila are provided by the genetic (CHOVNICK 1966) and biochemical (CHOVNICK *et al.* 1970) selective procedures that were used in the recombinational dissection of the rosy locus. This paper describes procedures for the selective recovery of recombinational derivatives of pericentric inversions and the technology that these procedures make possible.

Rearrangement heterozygosity causes local suppression of recombination (STURTEVANT 1931), and this has hitherto been a major obstacle to the recovery of recombinational derivatives of rearrangements. Little can be done to affect the absolute frequency of recombinational events; however, the relative frequency of recovered products of such events can be drastically increased by en-

suring that nonrecombinant chromosomes are incorporated into lethally aneuploid zygotes. As will be seen, this is a feasible approach whose direct applications include the insertion of markers into inversions through a sequence of selected single exchanges and the synthesis of a novel type of duplication that has the properties of both insertional and tandem duplications. Indirect applications include two cytogenetic screens and a system for autosomal half-tetrad analyses.

MATERIALS AND METHODS

All flies used in these experiments were grown at 25° on the medium described by LEWIS (1960). Crosses to select recombinants were usually made in mass matings of 40 to 50 well-aged virgin females to 10 to 20 males; the matings were carried out in vials and the flies transferred to bottles after 1 or 2 days; thereafter, the flies were transferred to bottles containing fresh medium at 3-day intervals. In some crosses, particularly those involving hyperploid males, it was necessary to deviate from this procedure to the extent of mating single males to 30 to 40 females; in these cases, the matings were held in vials for 4 to 6 days before being transferred to bottles.

C(1)M4 (CRAYMER 1974) is present in many of the crosses because of the strong interchromosomal effect that it exerts on recombination; this interchromosomal effect is especially helpful when selecting for recombinants in proximal regions of the autosomes where recombination frequencies are depressed relative to physical distances. This effect has not been carefully quantified, but a small-scale (528 flies) experiment gave an *ast* to *cl* distance of 29 cM in the presence of *C(1)M4*, as compared with the standard distance of 15 cM (LINDSLEY and GRELL 1968); the distance from *th* to p^p was observed to increase from 5 cM to about 20 cM in the presence of *C(1)M4*.

The mutants used as visible markers are described in LINDSLEY and GRELL (1968), with the exception of *Tb* (AUERBACH 1943; CRAYMER 1980) and Ch^V (VALENCIA 1968; CRAYMER 1980).

Rearrangements used in this study include:

In(2LR)ds^{33k} is the *In(2LR)21D;60D* component of *In(2LR)*bw^{V1}.

In(2LR)px^{52g} has breakpoints at 30A and 58E; otherwise as described in LINDSLEY and GRELL (1968).

In(2LR)S^{325} was induced on *Dp(2;2)S, ds* $(S^+$ *ast)(S ast) dp. In(2LR)*$^{325}/ds^W$ shows a ds^-/ds^W phenotype; otherwise as described in LINDSLEY and GRELL (1968).

In(3L)HR15: In(3L)64D2-5; 68C4-9. It is homozygous viable, fertile and phenotypically wild type (ASHBURNER 1972).

In(3L)HR27: In(3L)76C3-4; 80B2-4. Homozygous viable, fertile and shows weak variegation for an eye-color mutant (ASHBURNER 1972).

In(3L)P: In(3L)63B8-11; 72E1-2; otherwise as described in LINDSLEY and GRELL (1968).

In(3LR)A114: In(3LR)80;92A (LEWIS). Originally associated with *T(Y;3)A114* (LINDSLEY *et al.* 1972). Homozygous lethal.

In(3LR)B158: In(3LR)76A;93B (LINDSLEY *et al.* 1972). Originally associated with *T(Y;3) B158*; homozygous lethal.

In(3LR)bxd^{92}*: In(3LR)80;89E* (LEWIS) was X-ray-induced on p^p by N. SHAW, 73e. Homozygote shows extreme *bxd* and *pbx* effects.

In(3LR)bxd^{106}*: In(3LR)72D11-E1;89E2-3.* X-ray-induced on sbd^2 bx^3 by LEWIS, 51k7.

In(3LR)C267: Inversion originally associated with *T(2;3)C267*; described in LINDSLEY and GRELL (1968).

In(3LR)HR33: In(3LR)61A1-2;87B2-4. Homozygote is viable and fertile and shows a *gvl*-like phenotype that often overlaps wild type; the *gvl*-like mutant is recombinationally inseparable from the inversion. Band 61A2-3 does not puff in homozygous larvae (ASHBURNER 1972).

In(3LR)LD3: In(3LR)61F7-62A2;81F5-82A1. X-ray-induced on bx^{34e}, probably in spermatogonia, by L. DEJONGH and detected by transvection by bx^{34e}/Ubx. Homozygous viable, fertile and phenotypically wild type.

In(3LR)LD6: In(3LR)62A10-B1;85A2-3. Origin and phenotype similar to that of *In(3LR)LD3.*

In(3LR)P30: In(3LR)64C7-9;82A2-4. X-ray-induced on bx^{34e} by LEWIS, 50i15. Homozygous viable, fertile and phenotypically wild type.

In(3LR)P41: In(3LR)64A5-7;88D6-8. X-ray-induced on bx^{34e} by LEWIS, 50i15. Homozygous viable, fertile and phenotypically wild type.

In(3LR)P42: In(3LR)70F1-2;81F1. X-ray-induced on bx^{34e} by LEWIS, 50i15. Homozygous viable, fertile and phenotypically wild type.

In(3LR)P91: In(3LR)67C10-D1;81F. Induced with fast neutrons from nuclear detonation by LEWIS, 51h28, on *Ubx* e^4; homozygote not known.

In(3LR)P93: In(3LR)64B10-12;81. Induced with fast neutrons from nuclear detonation by LEWIS, 51h28, on *Ubx* e^4. Homozygous lethal.

In(3LR)sep: In(3LR)65D2-3;85F2-4; puff associated with left end of inversion. Otherwise as described in LINDSLEY and GRELL (1968).

In(3LR)Sta = *In(3LR)Stigmata: In(3LR)79D;94A* (LEWIS) associated with the dominant mutant Stigmata. X-ray-induced on *T(2;3)P10* by LEWIS in 1978. *Sta* is associated with the right end of the inversion; its phenotype is an absence of pigmentation at the corners and mid-anterior edge of the notum, with spread wings, which may be due to muscle attachment site defects; homozygote occasionally survives. RK2A.

In(3LR)Ubx^{16R}*: In(3LR)79D;89E* (LEWIS). X-ray-induced on *Cbx* by T. RAMEY in 1978.

In(3LR)Ubx^{U} = *In(3LR)*Ubx^{Ultra}*: In(3LR)62A2-3;89E1.* X-ray-induced on sbd^2 *ss* bx^{34e} in 1966. Associated with Ubx^U, which has an extreme *Ubx* phenotype with halteres about three times the volume of those of *Df(3R)Ubx/+*; Ubx^U has also been referred to as Ubx^{300}. RK1A.

T(2;3)HR30: T(2;3)34E;70B. Homozygous viable, fertile and phenotypically wild type. (ASHBURNER 1972).

All other rearrangements used in this study are described in LINDSLEY and GRELL (1968).

TECHNIQUES AND RESULTS

To simplify the presentation that follows, a "constellation" of chromosomes will be defined as a set of chromosomes characterized by their cytological structure; that is, two sets of chromosomes with the same gross structure—both might, for example, consist of a pair of chromosomes with sequences 123.456 and ABC.DEF—represent the same constellation regardless of any allelic differences between the two sets. Only constellations involving pericentric inversions and their derivatives will be considered in this paper.

The constellation of chromosomes consisting of an inverted (1234/876.5/9) chromosome and its structurally normal (12345.6789) homologue may give rise to duplicate-deficient (1234/876.54321) and deficient-duplicate (9/5.6789) chromosomes through recombination within the inverted (5.678) region. The constellation that consists of the duplicate-deficient and deficient-duplicate chromosomes can similarly give rise to inverted and structurally normal chromosomes through recombination. Constellations that show this recombinational interconvertibility may be termed "alternative" constellations. Figure 1 diagrams these two constellations and their component chromosomes.

The structurally normal and inverted chromosomes of the *In/+* constellation will be termed "heterosynaptic" since neither chromosome shows internal pair-

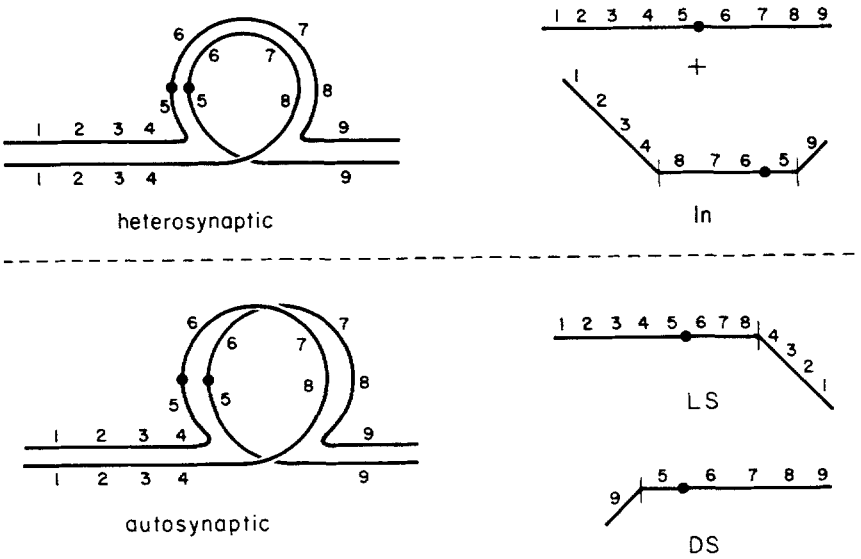

FIGURE 1.—The two attainable chromosome constellations for a simple pericentric inversion heterozygote and their component chromosomes. LS = levosynaptic, DS = dextrosynaptic. Note that the breakpoint relationships (5 adjacent to 9 and 8 adjacent to 4) are preserved from one constellation to the next. Heterosynaptic females can produce LS- and DS-bearing ova as the result of recombination within the inversion loop in addition to the ova bearing nonrecombinant *In* and + chromosomes; autosynaptic females can similarly produce ova bearing any of the four chromosome types. Autosynaptic Drosophila males produce LS-bearing and DS-bearing sperm to the virtual exclusion of other genotypes; crosses of autosynaptic males to either heterosynaptic or autosynaptic females produce predominantly LS/DS offspring (other genotypes being lethal as a consequence of overly extensive aneuploidy), which makes it possible to selectively recover LS and DS chromosomes from such mothers. *In* and + chromosomes can be selectively recovered from crosses of heterosynaptic or autosynaptic mothers to structurally normal males.

ing. The duplicate-deficient and deficient-duplicate chromosomes in the alternative constellation do exhibit internal pairing—1234 with 1234 on one chromosome and 9 with 9 on the other—and thus will be termed "autosynaptic". Within a given constellation, autosynaptic homologues are further classified as being "levosynaptic" (LS) or "dextrosynaptic" (DS); the LS chromosome carries the leftmost (according to the standard sequence) autosynaptic region, while its homologue is arbitrarily labelled as the DS chromosome—in most cases, the DS chromosome will carry the rightmost autosynaptic region, but there are exceptions. In the autosynaptic constellation of Figure 1, the two regions of internal pairing are 1234 and 9; the 1234/876.54321 chromosome is classified as LS since it carries the leftmost (1234) autosynaptic region, and the 9/5.6789 chromosome is classified as DS.

By taking advantage of aneuploid lethality, one can selectively recover the chromosomal components of any particular constellation. An LS/DS male Drosophila, in which there is virtually no exchange, will regularly produce only LS- or DS-bearing sperm, so that maternally derived DS or LS chromosomes are

selectively recovered in surviving offspring of *In*/+ or LS/DS females that have been mated to LS/DS males: the LS/*In*, LS/+, DS/*In*, DS/+, LS/LS and DS/DS offspring die, while the $LS_{maternal}/DS_{paternal}$ and $LS_{paternal}/DS_{maternal}$ offspring survive. Maternally derived *In* or + chromosomes can be selectively recovered from crosses of *In*/+ or LS/DS females to structurally normal males.

Synthesis of autosynaptic stocks

The lack of recombination in Drosophila males, which makes possible the selective recovery of maternally derived LS or DS chromosomes, ensures that an autosynaptic stock is stable—viable individuals in the stock are euploid and must carry the LS/DS constellation since they inherited a paternal LS or DS chromosome—but at the same time makes the initial recovery of autosynaptic males somewhat of a problem. Five basic approaches have been devised for synthesizing autosynaptic males.

(1) *Triploid method*: NOVITSKI (1950) used free-*X* triploids to generate 2*X*:1*A* ova in which the *X* chromosomes were the complementary single exchange products between *In(1)sc*^{8} and *In(1)dl–49*; when such eggs were fertilized by *Y*-bearing sperm, viable diploid females were recovered that carried the *In(1)sc*^{8L}*dl–49*^{R}/*In(1)dl–49*^{L}*sc*^{8R}/*Y* constellation of chromosomes. Males carrying compound autosomes—either $C(A_1L)/C(A_1R)$ (reviewed by HOLM 1976) or $C(A_1)EN$ (NOVITSKI 1976)—produce significant frequencies of nullo–A_1 sperm that can be used to recover viable zygotes from diplo–A_1 ova, thus allowing the simultaneous recovery of complementary autosomal exchange products. $In(A_1)/A_1/A_1$ triploid females will produce some $LS(A_1)/DS(A_1)$ diploid male offspring when mated to compound–A_1 males. By mating single male offspring to both $In(A_1)/A_1$ and structurally normal females carrying an easily scored mutant marker, putative $LS(A_1)/DS(A_1)$ stocks can be identified as cultures lacking the marker. The autosynaptic state is then confirmed by demonstrating that no viable adult offspring are produced by crosses of structurally normal females to males from a putative LS/DS stock. (For some inversions with distal breakpoints, such as *In(2LR)ds*^{33k}, it is possible to obtain viable LS/+ or DS/+ individuals; but for most inversions, LS/+ and DS/+ zygotes die before hatching from the egg, so that crosses of structurally normal females to the LS/DS males appear to be sterile.)

Table 1 presents the results of applications of this method. One would expect that the frequency of autosynaptic males should approach 50% of that of the

TABLE 1

Results of progeny testing "In/+" *male offspring of* In/+/+ *mothers*

Inversion:	Heterosynaptic	Autosynaptic	Sterile
In(2LR)S^{325}	124	4	14
In(2LR)ds^{33k}	107	3	12
In(2LR)bw^{V1}	110	5	
In(3LR)LD3	120	3	10
In(3LR)LD6	57	2	4

phenotypically "*In*/+" males (independent assortment of the inversion breakpoints would make *In*/+ and LS/DS constellations equally frequent among surviving offspring) for inversions as large as those listed, but the maximum frequency of recovered *LS/DS* males is only about 3% of that of the "*In*/+" males. The likely explanation for the low recovery rate is that recombinant chromosomes tend to disjoin, as observed by BRIDGES (1916) and DOBZHANSKY (1933). Nonrandom disjunction (NOVITSKI 1951, 1967), the tendency to recover the shorter chromatid from a heteromorphic dyad at meiosis II, could conceivably result in a reduced recovery frequency, but this can be ruled out as a major cause by the equal recovery of LS*(2)ds*^{33k}/DS*(2)*^{33k} and LS*(2)bw*^{V1}/DS*(2)bw*^{V1} from the same cross: the recombinational event that produces LS*(2)ds*^{33k}/DS*(2)ds*^{33k} does not noticeably alter chromosome lengths, but that producing LS*(2)bw*^{V1}/DS*(2)bw*^{V1} drastically alters chromosome lengths.

This is the most generally applicable of the various methods: it can theoretically be applied to generate any attainable autosynaptic constellation. However, the low recovery rate makes this the least efficient method, so that it is best applied only when no other approach is feasible.

(2) *Inchworm method*: Consider the overlapping inversions *InA*, 123/76.54/89, and *InB*, 1234/876.5/9. If an LS-*A*/DS-*A* stock exists, then *InB*/+ females can be crossed to LS-*A*/DS-*A* males to recover LS-*B*/DS-*A* offspring; LS-*B*/DS-*B* animals can then be recovered by crossing *InB*/+ females to the LS-*B*/DS-*A* males. Similarly, one can generate an LS-*A*/DS-*A* stock if one has an LS-*B*/DS-*B* stock. Multiple repetitions of this process can be used to generate autosynaptic constellations for a sequence of inversions.

The LS-*B*/DS-*A* genotype is hyperploid for regions 4 and 8; for the above scheme to work, such hyperploids must be viable and fertile. Table 2 lists several sequences of inversions for which this method has been successfully employed; it is clear from the table that Drosophila is quite tolerant of hyperploidy. The single-step intermediate hyperploid genotypes are typically duplicated for four to six numbered divisions; despite showing a variety of phenotypic effects, all of the hyperploids are viable and fertile in both sexes. The only six-division or smaller hyperploid that I have observed to cause sterility is the 87E to 93C duplication derived from *Tp(3)MKRS*; this duplication is sterile in both sexes. The hyperploid phenotypes may include a squat-bodied appearance, lengthened or shortened and broadened or narrowed wings, shortened and fused or missing tarsi, abnormal pigmentation, various bristle abnormalities, and reduced and roughened eyes; in the case of LS*(3)C267*/DS*(3)P21*, hyperploid for 72E to 74F and 63C to 65A and 88A to 88D, the eyes also have a slightly glazed appearance. In matings of single hyperploid males to 30 or more females, the males are often observed to survive for a week or more.

Since this method is selective—only the LS-*B*/DS-*A* offspring survive from the cross of *InB*/+ females to LS-*A*/DS-*A* males and only the LS-*B*/DS-*B* and LS-*B*/DS-*A* offspring survive from the cross of *InB*/+ females to LS-*B*/DS-*A* males, with the LS-*B*/DS-*A* genotype usually showing reduced viability—this

TABLE 2

Inversion series to which inchworm method of generating autosynaptic constellations has been applied

Inversions:	Breakpoints
(A) *In(3LR)LD3*	61F;82A
1. *In(3LR)P93*	64C-E;81F
2. *In(3LR)P91*	67C-D;81F
3. *In(3LR)P42*	70F;81F
(B) *In(3LR)HR33*	61A;87B
1. *In(3LR)P41*	64A;88D
2. *In(3LR)C190*	69F;89D
3. *In(3LR)bxd*^{106}	72D;89E
4. *In(3LR)P21**	65A;87F-88A
5. *In(3LR)C267*	74F;88D
(C) *In(3LR)Sta*	79D;94A
1. *In(3LR)B158*	76A;93B
(D) *In(3LR)bxd*^{92}	80;89E
1. *In(3LR)A114*	80;92A
(E) *In(2LR)C251*	36F;57B
1. *In(2LR)bw*^{V32g}	40;59D
(F) *C(2L)P4; C(2R)P4*	
1. *In(2LR)Sco*$^{R+9}$	35B;41
2. *In(2LR)Sco*$^{R+1}$	35B;44

* *In(3LR)P21* was induced on *In(3L)P*; new order is 61 to 63C/72E to 65A/87F to 72E/63C to 65A/88A to 100.

Inversions labelled A to F were used to initiate the inching process for which autosynaptic stocks had been derived by other means; under each initiating inversion is a list of the inversions in the series, the Arabic numeral denoting the number of steps taken to derive an autosynaptic stock of the numbered inversion: for example, an LS*(3)P93*/DS*(3)P93* was derived through a LS*(3)P93*/DS*(3)LD3* intermediate and is thus one step removed from LS*(3)LD3*/DS*(3)LD3*, while the LS*(3)P93*/DS*(3)P93* stock was derived through a LS*(3)P91*/DS*(3)P93* intermediate and is thus two steps removed from LS*(3)LD3*/DS*(3)LD3*.

is a powerful method for generating autosynaptic constellations in those cases where it is applicable.

(3) *Breakaway method*: The inchworm method can rarely be applied when one has a pair of included inversions—hypoploidy is much less well tolerated by the organism than is hyperploidy—but it is possible to use a maternally generated duplication to rescue a hypoploid LS-*B*/DS-*A* intermediate genotype. Figure 2a illustrates the synthesis of an autosynaptic constellation through such an intermediate step. Figure 2b shows an elegant extension of this idea: by beginning with an inversion that is "wrapped" around a pair of free autosomal arms, it is possible to recover the LS and DS chromosomes that can be generated from that inversion. By crossing females that are heterozygous for the inversion-plus-free-arm complex to males that are autosynaptic for an inversion with very proximal and very distal breaks—such males are essentially free-arm over a complementary chromosome—a maternally derived LS or DS chromo-

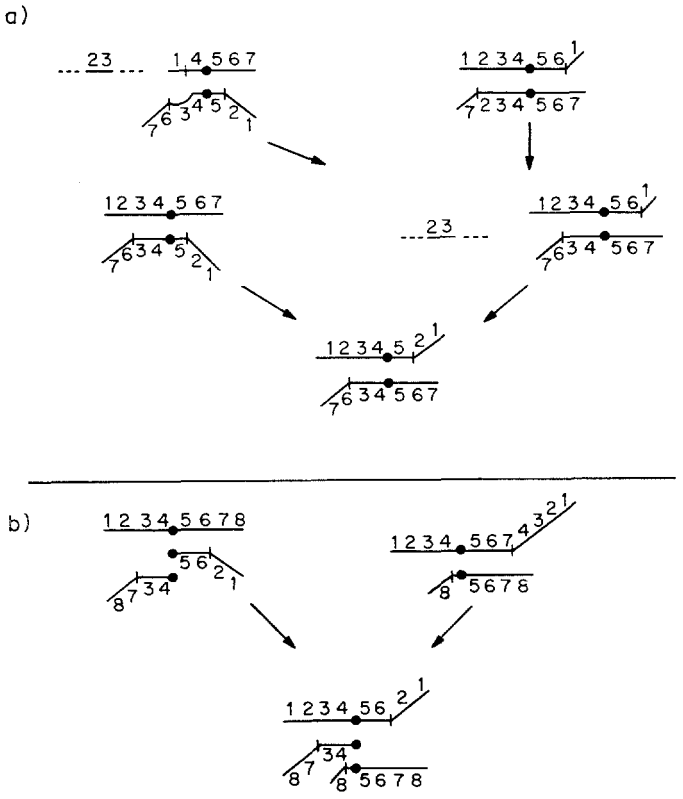

FIGURE 2.—The breakaway method of generating autosynaptic inversion complexes. (a) shows the use of a translocation that inserts the 23 region elsewhere in the genome to recover an autosynaptic constellation involving the 2/3; 5/6 inversion and a structurally normal chromosome, given an autosynaptic stock of the 1/2; 6/7 inversion. (b) shows the recovery of an LS chromosome from a +/*In*-plus-free-arms heterozygous female by crossing her to an autosynaptic male whose DS chromosome is essentially a free arm; a similar cross using autosynaptic males whose LS chromosome is essentially a free arm can be used to recover the DS chromosome from the inversion wrapped around the free arms.

some is derived in a gamete complementary to the free arm; other offspring genotypes are lethally aneuploid. The cross shown in Figure 2b recovers an LS chromosome; the DS chromosome is recovered by a similar cross involving males that are autosynaptic for a 1/.432/5678 inversion.

The second version of the breakaway method cannot be applied unless the free-arm-plus-inversion complex exists or can be synthesized. This limits its utility for pre-existing inversions, but it is possible to screen for new pericentric inversions as translocations between free arms and to then apply the method. Such a screen has not yet been carried out, but autosynaptic constellations of *In(2LR)px*^{52g} and *In(3LR)C190* have been generated by this version of the method. This method is obviously applicable to inversions with noncentric breaks; the observed occasional breakdown of free arms/balancer stocks indi-

cate that it should be applicable to any inversion. In cases where one breakpoint is in the centric region, one would not try to recover LS and DS chromosomes independently; instead, a stock of the final genotype shown in Figure 2b would be maintained until it "broke down" into the LS/DS form.

Free chromosome–*2* arms were derived by crossing *In(2LR)bw*^{V32g}/+/+ triploid females to LS*(2)*S^{325}/DS*(2)*S^{325} males; the diploid offspring had an LS*(2)*S^{325}/DS*(2)bw*^{V32g}/+ genotype with the desired LS*(2)*S^{325} and DS*(2)bw*^{V32g} free arms. Free chromosome *3* arms were obtained by crossing *T(2;3)*S^{M}/+/+ triploid females to LS*(3)LD3*/DS*(3)LD3* males to recover the *LS(3)LD3/2*^{D}*3*^{P}S^{M}/+ offspring. Free autosomal arms have also been synthesized by methods involving compound chromosomes (GRELL 1976). Once the free-arm combinations had been generated, free arms derived from *T(Y;A)*'s (LINDSLEY *et al.* 1972) were substituted to produce free-arm genotypes that were less hyperploid.

(4) *Product-mimic method*: Rearrangement segregants can sometimes be produced that mimic desired LS or DS gametes; the simplest example of this is provided by the single steps in the inchworm method. Consider an inversion with order 1234/76.5/89 and the translocations T_1, ABCD.E/321; HGF/45.6789, and T_2, ABCD.EF/789; 12345.6/GH. Some of the sperm produced by T_1/T_2 males will carry the ABCD.EF/789 and HGF/45.6789 chromosomes; when these fertilize an LS-bearing (ABCD.EFGH; 1234/76.54321) egg, a viable and fertile male is produced that is hyperploid for F, 4 and 7. Such males can be backcrossed to *In*/+ females and a LS/DS stock established from the offspring.

The monumental study of LINDSLEY *et al.* (1972) produced a large number of *Y*-autosome translocations that can be used in applications of this method. LS*(3)Sta*/DS*(3)Sta* was synthesized using *T(Y;3)B27* (94E) and the LS*(3)LD3/2*^{D}*3*^{P}S^{M} free-arm combination to obtain a *3*^{P}*Y*^{D}*B27*/LS*(3)LD3*/DS*(3)Sta* intermediate. Autosynaptic constellations for *In(2LR)C251* and *In(3LR)C269* were constructed in a slightly less direct manner, using *T(Y;2)H174* (37D) and *T(Y;3)J162*, respectively. *C(1)M4*, y^2; *In*/+ females were crossed to *T(Y;A)* males to recover *C(1)M4*, y^2/A^DY^P; +/DS females, and these females were then crossed to attached-*XY* males to recover $\overline{XY}/A^DY^P$; +/DS males. These males were then crossed to *C(1)M4*, y^2; *In*/+ females to recover $\overline{XY}/Y$; LS/DS males, which were then used to derive an autosynaptic stock.

(5) *Pseudo-translocation method*: It is possible to construct a pericentric inversion genotype that has the segregational properties of a translocation heterozygote; this is shown in Figure 3. To construct this genotype, an inversion (12/5.43/6) has been recombined with a homozygous viable translocation (123/C.DEF; AB/4.56) and the *T* + *In*/*T* genotype generated: regular segregation patterns in this genotype produce *T*, *T* + *In*, *LS* + *T*, and *DS* + *T* gametes, with the latter two being the gametic types desired for synthesizing a LS/DS stock.

Generating autosynaptic constellations from single inversions by this method requires the recovery of the *T* + *In* complex from a double crossover in a *T*/*In*

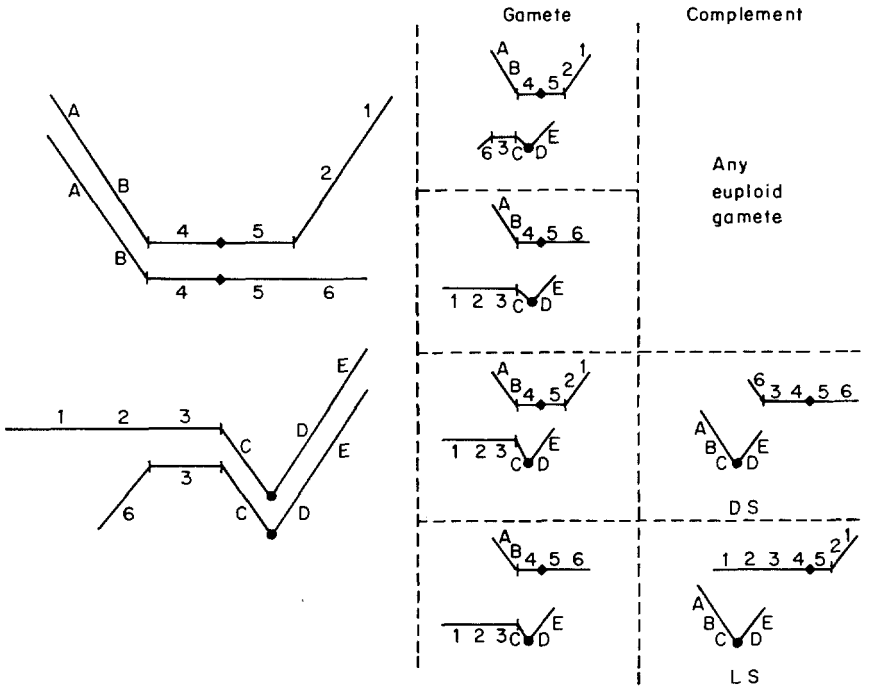

FIGURE 3.—The pseudo-translocation method for recovering autosynaptic constellations. The $T + In/T$ genotype, shown at the left, which is homozygous for a translocation with B/C; 3/4 breaks and heterozygous for an inversion with 2/3; 5/6 breaks (an inversion that can be "wrapped" around the translocation), segregates 4 gametic types. Two of the gametic types are euploid, but the remaining two are complementary to gametes bearing LS or DS chromosomes, so that the LS and DS chromosomes generated by an $In/+$ mother can be recovered by mating her to the $T + In/T$ males.

female; such a crossover was recovered between *T(2;3)HR30* and *In(3LR)HR33* and used in the construction of an LS*(3)HR33*/DS*(3)HR33* stock, but screening for the double recombinant is a tedious process and feasible only for large inversions. The utility of the method is in dealing with multiple inversion complexes. By crossing T/In females to LS/DS males, both the LS + T and DS + T complexes can be recovered; LS + T/DS + T and $T + In/T$ are identical genotypes, differing only notationally, so that the $T + In$ complex is easily isolated. $T + In$ complexes for two separate inversions can be generated, and the $T + InA/T + InB$ genotype used to generate $T + InA^{L}B^{R}$ and $T + InB^{L}A^{R}$ sperm for recovering $InB^{L}A^{R}$ and $InA^{L}B^{R}$ recombinants between the two inversions.

Notation: The notation used here for autosynaptic chromosomes was chosen to conform as closely as possible to the standard notation of LINDSLEY and GRELL (1968). The LS and DS labels are used only when no other alternative presents itself: specifically, the LS or DS notation is not applied to any chromosome which can be properly described as a duplication (*Dp*; no associated deficiency) or as a recombinant between two inversions ($InB^{L}C^{R}$; the recom-

binant with the left breakpoint of some *InB* and the right breakpoint of some *InC*). Marker notation for autosynaptic chromosomes is similar to that applied to compound chromosomes. Markers distal to the defining breakpoint are listed first, followed by a dash, and then by a list of markers present on the remainder of the chromosome. When a dash does not appear in a label, all markers listed are present in the region of the chromosome that is not distal to the defining breakpoint. For example, if the inversion of Figure 1 is labelled *InA* and a second inversion, *InB*, is introduced with sequence 1/6.5432/789 and m_1 m_2 is the standard order of two mutants in the 12345.678 region, then LS-*A*, *InB*, m_1 m_2 denotes a chromosome with sequence 1/6.5432/78/4321, which carries m_1 and m_2 in the 1/6.5432/78 region of the chromosome; DS-*A*, m_3 m_4—m_2 m_3 shows a chromosome with m_3 m_4 present in the region 9 distal to the 5/9 breakpoint and m_2 m_3 present on the 5.6789 region of the chromosome, the standard mutant order being m_2 m_3 m_4.

Applications

Insertion of markers into pericentric inversions: In crosses of *In*/+ females to LS/DS males, offspring are selectively recovered that carry a maternally derived LS or DS chromosome along with a complementary paternal homologue: the aneuploid genotypes (LS/LS, LS/+, LS/*In*, +/DS, *In*/DS, and DS/DS) are lethal in most cases, so that only the LS/DS progeny survive to adulthood. Crosses of LS/DS females to structurally normal males selectively recover recombinant *In* and + chromosomes. Through the sequential selection of single-exchange products, it is possible to generate a double-crossover chromosome; this sequential exchange procedure is convenient for the insertion or removal of markers from an inversion.

Assume that we wish to insert a mutant marker, m_2, located between 7 and 8 on a standard-sequence chromosome (12345.67$m_2$89) into the inversion of Figure 1 (1234/876.5/9); also assume that the numbered regions are of equivalent recombinational lengths. DS recombinants produced by an *In*/m_2 female will predominantly carry m_2 because recombination in the 5.67 region is more frequent than recombination in the 8 region. Thus, we can cross *In*/m_2 females to LS, m_1/DS, m_3—m_3 males to recover LS, m_1/DS, $m_2^?$ offspring, where m_1 is a marker located between 4 and 5 (LS, m_1 = 1234$m_1$5.678/4321), which effectively marks the left inversion breakpoint, and m_3 is a recessive marker in the 9 region, which is used to identify the paternal DS chromosome; LS, m_1/DS, $m_2^?$ females can be crossed to m_1 m_2 males, and the *In*, m_2 chromosome should be present in all m_1^+ m_2 offspring. Alternatively, we can cross *In*/m_2 females to LS/DS, m_3—m_3 males to recover LS/DS, $m_2^?$ males; these males can then be crossed to *In*/m_1 m_2 females and LS, m_1 m_2/DS, m_2 offspring recovered as being phenotypically m_2; by then crossing LS, m_1 m_2/DS, m_2 females to m_1 m_2 males, the *In*, m_2 chromosome can be recovered in the m_1^+ m_2 offspring.

The sequential exchange technique can be extended to the "nesting" of included inversions, shown diagrammatically in Figure 4. Table 3 presents data obtained in the course of nesting several pairs of inversions. The data in Table

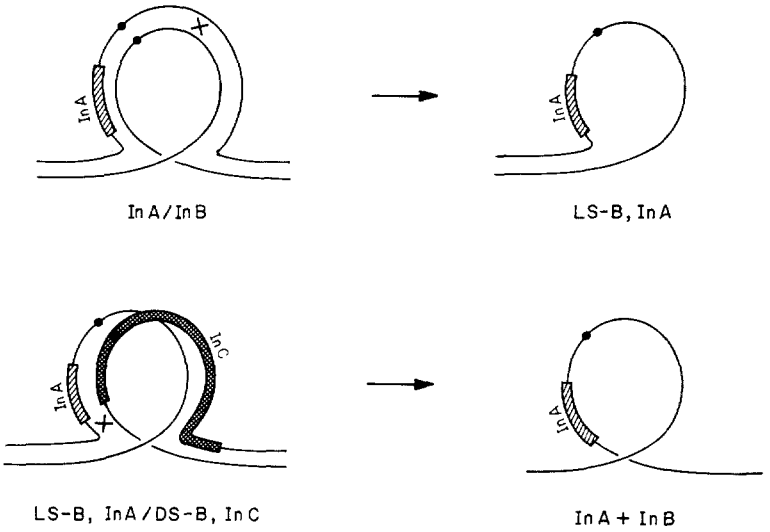

FIGURE 4.—The "nesting" of included inversions. In this example, a paracentric inversion *InA* (indicated by the hatched region; for presentational clarity, neither *InA* nor *InC* are shown as they would be expected to pair) is to be recombined into a pericentric inversion (indicated by the inversion loop). Within *InB*, the region to the left of *InA* (according to the standard chromosome order; as it is drawn, this region is below *InA*) is much smaller than the region to the right of *InA*, so that maternally derived LS-*B* chromosomes from crosses of *InA/InB* females to LS-*B*/DS-*B* males will predominantly carry *InA*; this is shown at the top of the figure. To suppress or zygotically eliminate the products of recombination to the right of *InA*, *InC* is introduced on a DS-*B* chroomsome; the *InA* + *InB* chromosome can then be recovered among offspring of crosses of the LS-*B*, *InA/DS-B*, *InC* females to structurally normal males.

3 show not only that recombinants can be selected for between quite close breakpoints—the 64A-64D region of experiment 5 and the 61A-62B region of experiment 6 are about 50 bands and the 86B-87B region of experiment 6 is about 70 bands in length, according to BRIDGES' revised map (LINDSLEY and GRELL 1968)—but also the recombination frequencies are probably a direct function of physical distances. If there are specific sites for the initiation of recombinational pairing, then they occur at a level too fine to be detected in these experiments. The three regions covered in experiments 5 and 6 are of similar lengths and show similar recombination frequencies; the 62B-64D region of experiment 1 and the 64A-65E region of experiment 2 also have corresponding lengths (110 to 120 bands) and recombination frequencies.

The data of experiments 3 and 4 suggest that the added interchromosomal effect of *CyO*/+ (*Pu*[2] is not visibly associated with a rearrangement and is not known to affect recombination) affects recombination in the 80B-81F region. Several clusters of offspring appeared as the result of male recombination events in these two experiments, including one cluster of more than 20 adults: these data do not appear in the table. Maternal recombinants did not detectably occur in clusters, so that the maternal 80B-81F exchanges were probably meiotic events.

TABLE 3

Selected recombinants between nearby breakpoints

Cross:	Region	Bottles	In or LS	+ or DS	3N/2X3A	r/b	Limits
(1) *C(1)M4, y²;* $\frac{\text{LS}(3)LD6,\ In(3L)HR15}{\text{DS}(3)LD6,\ In(3LR)C190}$ × *su(ve) ru ve h th*	62B-64D	120 × 2 = 240	16	12	1/0	0.12	0.077 to 0.17
	69F-85A, 85A-89D		—	347		1.4	1.3 to 1.6
(2) *C(1)M4, y²;* $\frac{In(3LR)P41,\ bx^{34e}}{TM3\ Sb\ Ser}$ × LS*(3)P41*, R/DS*(3)P41*	64A-65F	20 × 2 = 29	2	3	0/0	0.17	0.056 to 0.40
(3) *C(1)M4, y²;* $\frac{Pu^2}{+}$; $\frac{In(3LR)P42}{In(3L)HR27}$ × LS*(3)P42*/DS*(3)P42*	70F-76C	20 × 2 = 40	35	29	0/0	1.6	1.2 to 2.1
	80B-81F		9	18		0.68	0.44 to 1.0
(4) *C(1)M4, y²;* $\frac{CyO}{+}$; $\frac{In(3LR)P42}{In(3L)HR27}$ × LS*(3)P42*/DS*(3)P42*	70F-76C	20 × 2 = 40	37	36	14/0	1.8	1.4 to 2.3
	80B-81F		24	16		1.0	0.7 to 1.4

TABLE 3--Continued

Cross:	Region	Bottles	In or LS	+ or DS	3N/2X3A	r/b	Limits
(5) $C(1)M4, y^2; \frac{LS(3)P41, In(3L)HR15}{DS(3)P41, TM3\ Sb\ Ser} \times p^p\ Mcp$	64A-64D	$240 \times 4 = 840$	3	13	140/52	0.019	0.011 to 0.031
(6) $C(1)M4, y^2; \frac{LS(3)HR33, In(3LR)LD6}{DS(3)HR33, In(3R)Hu} \times cv\text{-}c\ sbd^2$	61A-62B	$240 \times 5 = 1020$	12	12	163/113	0.024	0.015 to 0.035
	86B-87B		22	13		0.034	0.024 to 0.048
(7) $\frac{LS(3)P41, In(3LR)P30, p^p\ cu}{DS(3)P41, bx^{34e}—In(3R)Ubx^{125}} \times st\ in\ ri\ p^p$	64A-64C	$60 \times 3 = 180$	0	1	7/1	0.006	0.0014 to 0.031

Entries to the left of the equals sign in the column labelled "Bottles" give the number of bottles set up for the cross times the number of broods in the cross; numbers to the right are the total numbers of bottles used in the experiments. Some consolidation of parents took place in the last brood of experiments 2, 5, and 6 (experiment 5 had three broods of 240 bottles each and one of 120), so that there is no exact equivalence between the numbers on the two sides of the equals sign in these experiments. Recovered recombinants are listed in the *In* or LS and + or DS columns. Triploid exceptions are listed in the *3N/2X3A* column: there was, for example, one *3N* female found in experiment 1 and no *2X3A* intersex. The r/b column gives recombinants per bottle; the Limits column gives 95% confidence limits as calculated from STEVENS (1942). In experiment 1, the 69F-85A, 85A-89D "region" describes a 3-strand double-exchange event. Also in experiment 1, the LS*(3)LD6* chromosome carried *th* and the DS*(3)LD6* chromosome *h*; in experiment 2, the DS*(3)P41* chromosome carried bx^{34e}—*In(3R)Ubx*^{125}; in experiments 3 and 4, the *In(3LR)P42* chromosome carried *cp in ri*, the LS*(3)P42* chromosome carried *th st cp in ri*, and the DS*(3)P42* chromosome carried *cp in ri* Ch^V *Ki*; in experiment 6, the DS*(3)HR33* chromosome also carried sbd^2—*ru* bx^3 *bxd pbx*.

The data of experiment 7 appear in Table 3 since they argue that exchange in an interval as short as the 64A-64C region does not require the interchromosomal effect of *C(1)M4* for its occurrence. This argument is weakened by the fact that the recombinant recovered was not the definitive *In(3LR)P41+P30* chromosome, but recombinants over larger regions—85A to 87B from *In(3LR)HR33* and *In(3LR)LD6*, and 87B to 89E in the synthesis of *Dp(3;3)S1* (to be discussed later)—have been selected in the absence of heterologous rearrangements.

Estimates of recombination frequencies do not appear in Table 3 largely because of the difficulty of assigning error bounds. Various lines of evidence suggest that about 1000 *C(1)M4/Y* and *+/Y* zygotes are produced per bottle in *C(1)M4* crosses with 40 to 50 female parents, regardless of the rate of survival to adulthood, but this might vary by as much as a factor of 2 since egg-laying rates are dependent on culture conditions and parental age and background genotype. Survival rates in selective crosses also depend on culture conditions, but dead larvae and pupae were much less commonly observed in the selective experiments than were survivors, so that survival may not be a significant factor.

Several exceptional progeny types appear as noise in selective experiments. Triploids and triploid intersexes tend to occur in large scale experiments; usually, the distribution of markers and the lack of clustering indicate that the observed triploid exceptions result from a failure of meiosis I in either sex, but the high incidences observed in experiments 5 and 6 of Table 3 were associated with clustering. In crosses involving autosynaptic males, the major source of noise tends to be recombination in the male; the incidence varies from cross to cross and apparent frequencies of resultant survivors range from less than 1 in 10,000 to about 1 in 1,000 zygotes. Very rarely are survivors observed to result from simultaneous maternal and paternal nondisjunction.

Synthesis of insertional-tandem duplications

It is possible to select for exchange products that involve overlapping inversions, with the interesting consequences diagrammed in Figure 5. From a mating of *InA/InB* females to LS-*A*/DS-*A* males, an LS-*A*, *InB* chromosome can be recovered as the result of exchange in the 78 region, which happens to be internal to *InA* and external to *InB*. From a mating of LS-*A*, *InB*/DS-*A* females to $InA^{L}B^{R}/InB^{L}A^{R}$ males, or through some application of the product mimic or pseudo-translocation method of recovering autosynaptic chromosomes, it is possible to recover a novel duplication. This duplication will be denoted $DpB^{P}A^{D}$: the inversion supplying the proximal (P) breakpoint is noted first in the duplication label and then the inversion supplying the distal (D) breakpoint. One other duplication is derivable from the *A* and *B* inversions: this is $DpA^{P}B^{D}$ (12345.678/432/789), which is derived after selecting for an exchange in the 234 region of *InA/InB* heterozygotes in matings to LS-*B*/DS-*B* males. $DpB^{P}A^{D}$ is informationally equivalent to $InA^{L}B^{R}$, but differs in structure. As is seen in Figure 5, $DpB^{P}A^{D}$ is insertionally duplicated for the 78 region and tandemly

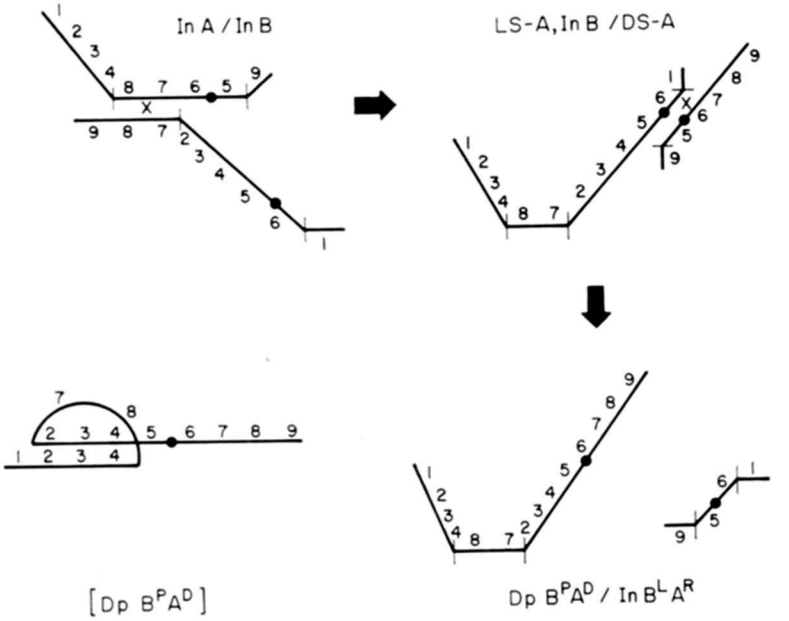

FIGURE 5.—The synthesis of an insertional-tandem duplication from overlapping pericentric inversions. *InA/InB* (*InA* has sequence 1234/876.5/9; *InB* has sequence 1/6.5432/789) females are crossed to LS-*A*/DS-*A* males so that an LS-*A*, *InB* chromosome may be recovered, and LS-*A*, *InB*/DS-*A* females are later crossed to $InA^{L}B^{R}/InB^{L}A^{R}$ males or to their product mimic or pseudo-translocational equivalent in order to recover $DpB^{P}A^{D}$ (notation defined in text), which is insertionally duplicated for 78 and tandemly duplicated for 234.

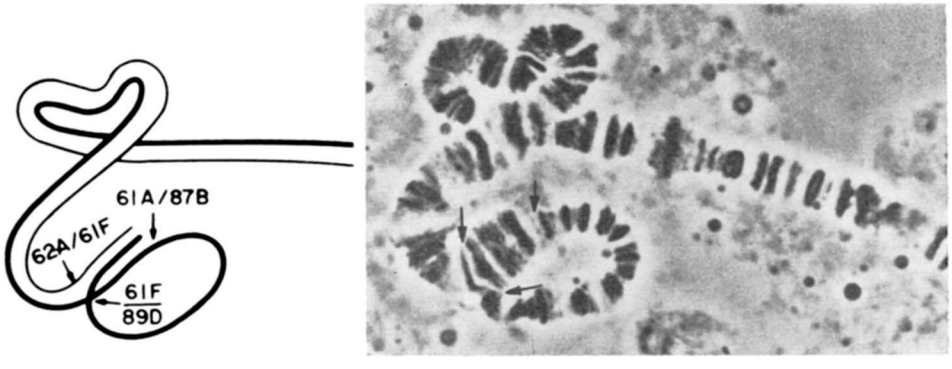

FIGURE 6.—*Dp(3;3)S1/+* = *Dp(3;3)HR33^P Ubx^UD/+*. *Dp(3;3)S1* has order 61A to 62A1.2/89D to 87B/61A to 100, and is tandemly duplicated for 61A to 62A1.2 and insertionally duplicated for 87B to 89D. The 61A to 62A regions of the duplicated chromosome are paired with that of the normal homologue in the photograph, while the 87B to 89D insert is unpaired; this is shown diagrammatically at the left. Since the 61A breakpoint of *In(3LR)HR33* is effectively terminal, the chromosome arm appears to end in a ring-like structure.

duplicated for the 234 region. Figure 6 shows a photomicrograph of a polytene preparation taken from a heterozygote for such an insertional-tandem duplication.

Insertional duplications have a variety of uses (gene dosage studies, genetic fine-structure mapping of lethal loci and as somatic-cell markers), but are frequently not available for a specific region and are difficult to recover from mutagenesis experiments. Insertional-tandem duplications can be used for the same purposes and their synthesis is limited primarily by the availability of rearrangements with breaks in the region of interest. This is less of a limitation than it appears since an insertional-tandem duplication with sequence 1234/87/2345.6789 can be deleted to produce insertional-tandem duplications with representative sequences 1234/8/45.6789 and 12/7/2345.6789; only one breakpoint (4/8 or 7/2) needs to be specified by the original rearrangements.

Cytogenetic screens

Overly extensive hyperploidy results in zygotic lethality, so that one can select for deletional derivatives of large insertional-tandem duplications; in crosses of structurally normal females to mutagenized duplication-bearing males, only offspring carrying deletional derivatives of the duplication will survive to adulthood. These deletional derivatives include tandem duplications and deficiencies, in addition to the smaller insertional-tandem duplications and other products.

Figure 7 illustrates the events that produce tandem duplications and deficiencies. After introducing mutant markers into the parental duplication as shown in the figure, it is possible to identify surviving progeny that carry deficiencies or tandem duplications in the subregion bounded by the two loci. In crosses of m_1 m_2 females to mutagenized males carrying the marked duplication, phenotypically m_1 m_2 progeny will carry either a tandem duplication or a deficiency in the m_1 to m_2 interval. The tandem duplications will not include either locus, but the deficiencies may.

In the first step of the duplication synthesis shown in Figure 5, it is possible to recover a LS-*A* chromosome (instead of the LS-*A*, *InB* chromosome) from a three-strand double-exchange event in which one exchange takes place in the 234 region and the second in the 5.6 region. This can be a problem in synthesizing a large insertional-tandem duplication for a cytogenetic screen—optimal duplications have small inserted regions and large tandemly duplicated regions—since the double-exchange event may be more frequent than the single exchange that produces the LS-*A*, *InB* chromosome, but is easily guarded against: a recessive marker in region 1 of *InA* will be homozygosed by the double exchange but not by the single exchange.

One should avoid screening for deficiencies in the neighborhood of a breakpoint of the mutagenized duplication, since the presence of undetected secondary rearrangements or mutations near the breakpoint could lead to ambiguities in the analysis of deletional derivatives. This is an especially serious problem in dealing with centromeric regions where secondary rearrangements are cyto-

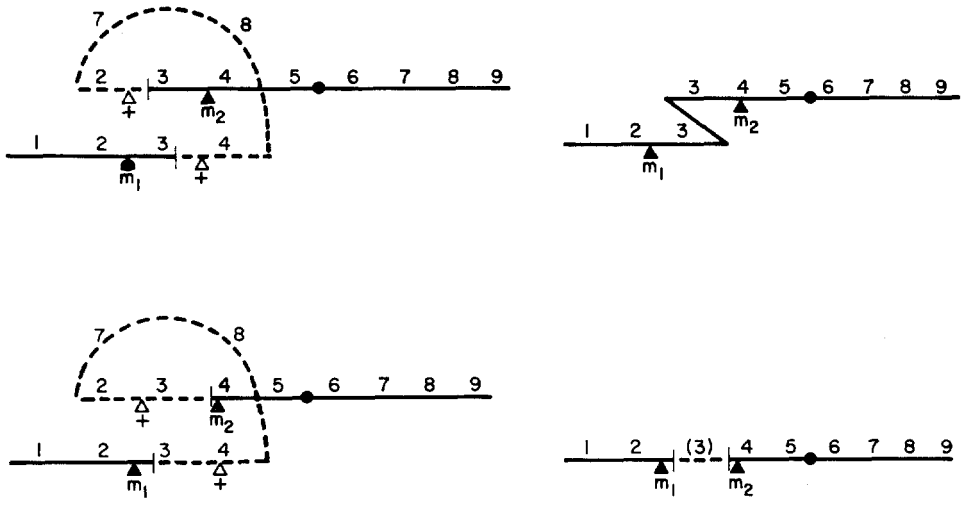

FIGURE 7.—The recovery of tandem duplications and deficiencies as deletions of an insertional-tandem duplication. The dashed regions in the structures at the left are deleted to give rise to the structures at the right: the deletional event shown at the upper left produces the tandem duplication at the upper right; the event shown at the lower left produces the deficiency at the lower right. When the insertional-tandem duplication is marked as shown ($m_1\ m^+_2$—$m^+_1\ m_2$), phenotypically $m_1\ m_2$ offspring of $m_1\ m_2$ females and mutagenized males carrying the insertional-tandem duplication will carry either a tandem duplication for material between, but not including either locus, m_1 and m_2, or a deficiency that may include either locus.

logically undetectable. As an alternative to the duplication-based screen, one can select for induced exchange events in a heterozygote for an inversion that (ideally) has breaks five to six numbered divisions to either side of the centromere. In the absence of heterologous rearrangements, little spontaneous exchange occurs within the inversion; the majority of surviving offspring from a cross of mutagenized autosynaptic females to structurally normal males will be the results of induced exchanges. Many of these exchange events will be unequal and give rise to deficiencies and tandem duplications. This method differs only slightly from the compound-autosome detachment method of BALDWIN and SUZUKI (1971), but has the advantage that secondary rearrangements can be excluded by substituting material in the centric region of the autosynaptic inversion complex before it is used for screening. M. CROSBY (personal communication) recovered a tandem duplication (77A-B to 80B-C) for Pc^+ among 50 survivors from a cross of irradiated LS*(3)P42, th st cp in ri*/DS*(3)P42* females to *st in ri* p^p males; *Pc* deficiencies have been recovered by T. RAMEY (unpublished) in a similar screen.

Half-tetrad applications

Compound chromosomes (ANDERSON 1925; BALDWIN and CHOVNICK 1967; LEWIS 1967; CHOVNICK *et al.* 1970) are useful for recombinational analyses.

The attachment of two homologous regions to a common centromere leads to the simultaneous recovery of two of the four copies of that region present at the four-strand stage of meiosis. Using a compound-*X* chromosome, ANDERSON (1925) was able to show that recombination occurred at the four-strand stage of meiosis in Drosophila; CHOVNICK *et al.* (1970) used compound-third chromosomes to investigate the relationship between gene conversion and reciprocal recombination at the *ry* locus.

Autosynaptic chromosomes also attach homologous regions to a common centromere and can be used in half-tetrad analyses. Figure 8 shows a set of chromosomes that were used in a small-scale experiment to test the feasibility

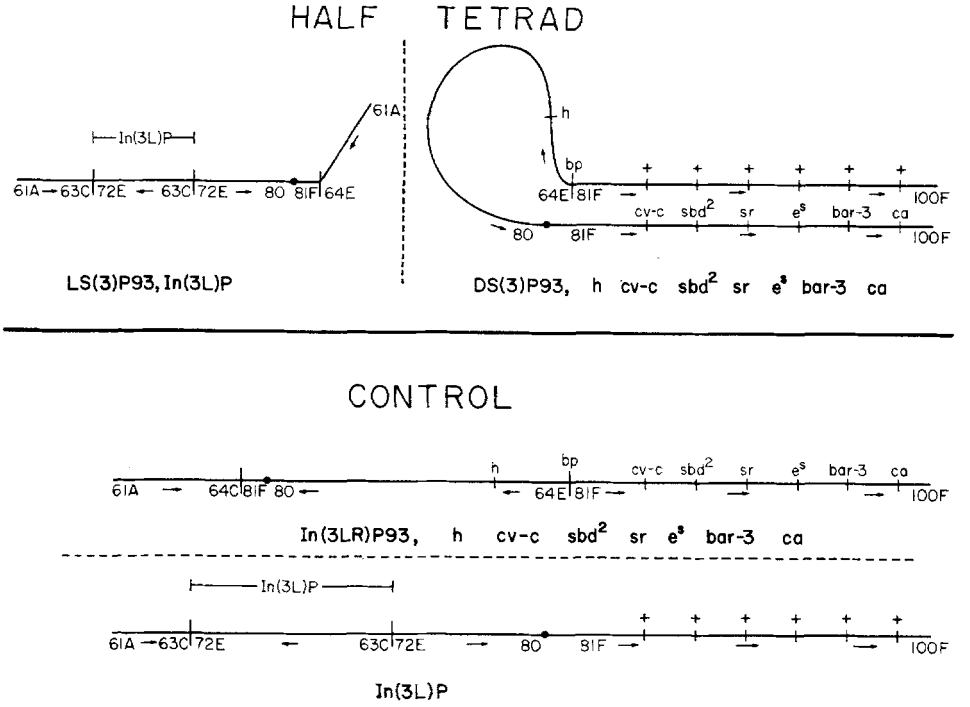

FIGURE 8.—Maternal chromosomes in the half-tetrad and corresponding control experiments. In the half-tetrad experiment, LS*(3)P93*, *In(3L)P*/DS*(3)P93*, *h cv-c sbd² sr e⁵ bar-3 ca* females were mated to LS*(3)P93*/DS*(3)P93*, *Ki* males, and *Ki*⁺ (carrying a maternal DS chromosome) offspring were scored for homozygosis of markers. Control females were mated to *ru h red cv-c sbd² sr e⁵ bar-3 tx ca*/*TM6*, *h* males, and all non-*TM6* offspring were scored. *In(3L)P* was present in both experiments to suppress recombination within the *(3LR)P93* inversion loop and thus to avoid possible recovery problems resulting from nonrandom disjunction (NOVITSKI 1951). In the control experiment, *In(3L)P* also served to link *h* inseparably with *In(3LR)P93*. The maternal DS*(3)P93* chromosome in the half-tetrad experiment also carried *red* in *cis* with the other markers; this marker was ignored when scoring offspring since *red* and *cv-c* are only 0.5 cM apart, but might have further reduced viability of the LS*(3)P93*/DS*(3)P93*, (*red*) *cv-c sbd² sr e⁵ bar-3 ca*—*h* (*red*) *cv-c sbd² sr e⁵ bar-3 ca* class of offspring.

of using autosynaptic chromosomes as half-tetrads; Table 4 gives the results of this experiment.

Markers are easily removed from an autosynaptic half-tetrad. From the observed fertility of crosses of LS*(3)P93*/DS*(3)P93* females to structurally normal males, the frequency of recombination within the inversion loop is on the order of 10%, so that it is a simple matter to recover the inversion-plus-marker or uninverted marked chromosome from the half-tetrad. In the half-tetrad cross, it is possible to limit the recovered offspring to those carrying a maternal DS*(3)P93* chromosome: if the LS*(3)P93*, *In(3L)P* maternal chromosome and the paternal DS*(3)P93* chromosome carry a common lethal in the 64E to 81F region, then the offspring that carry these chromosomes die. Another refinement is possible for the fine-structure analysis of a lethal locus, say *l(3R)A*, so that DS*(3)P93* could be used to provide the half-tetrad. The maternal LS*(3)P93*, *In(3L)P* chromosome could carry a duplication for the *l(3R)A* locus and the maternal DS*(3)P93* carry $l(3R)A^1$—$l(3R)A^2$ so that in crosses of the LS(3)*P93*, *In(3L)P*, *Dp*, *l*(3)*c*/DS(3)*P93*, $l(3R)A^1$—$l(3R)A^2$ females to LS*(3)P93*/DS*(3)P93*, *1(3)c* males [*l(3)c* is some lethal in the 64E to 81F region] the surviving offspring will carry a paternal LS*(3)P93* chromosome and a maternal DS*(3)P93* chromosome that carries a reconstituted $l(3)A^+$ allele.

DISCUSSION

The classic obstacles to the recombinational manipulation of pericentric inversions have been chromosome interference and the local suppression of recombination by heterozygous rearrangement breakpoints (STURTEVANT 1931) or by the centromere (BEADLE 1932, 1933). The approach developed here makes it possible to circumvent interference in most cases by dealing only with single-exchange products, while the capability of selectively recovering recom-

TABLE 4

Control and half-tetrad data

Interval	Standard distance	Control ($N = 507$)		Half-tetrad ($N = 428$)	
		Number of recombinants	% recombination	Number of recombinants	% recombination
bp to *cv-c*	6.6 cM	43	8	14	7
cv-c to sbd^2	4.1	15	3	7	3
sbd^2 to *sr*	3.8	23	5	5	2
sr to e^s	8.7	32	6	18	9
e^s to *bar-3*	8.4	29	6	7	3
bar-3 to *ca*	20.6	99	20	46	21
bp to *ca*	53.2	241	46	97	45

Standard distances are calculated from the map locations given in LINDSLEY and GRELL (1968); bp (see Figure 8) was assigned a location of 47.5, which is consistent with the centromere location. Recombination % for the half-tetrad experiment were calculated as twice the number of observed recombinants times 100 divided by 428. In the control experiment, the presence of *h* effectively marked the inversion breakpoint (bp); in the half-tetrad experiment, the breakpoint was inseparable from the maternal DS*(3)P93* chromosome.

binants makes it feasible to run experiments on a scale that is sufficient to overcome the more general problem of recombination suppression. The latter point is convincingly demonstrated by the inversion "nesting" experiments of Table 3 in which recombinants were obtained between breakpoints separated by distances of as little as 50 bands and at zygotic frequencies that are on the order of 10^{-5}.

Probably the most useful application of this technology is the generation of insertional-tandem duplications. Insertional duplications of a convenient size rarely exist for a specified region and are neither easily induced nor easily screened for, but insertional-tandem duplications can be synthesized with almost any desired inserted region. (Insertional-tandem duplications with inserts from heterologous chromosomes can be synthesized from translocations; details of these manipulations will be published elsewhere). Large insertional-tandem duplications, duplications of a size sufficient to cause lethality when heterozygous with a euploid homologue, can be used to screen for deficiencies and tandem duplications in a specified autosomal region (the region that is tandemly duplicated in the insertional-tandem duplication). Reversion of the dominant lethality should provide a powerful screen: if K is the appropriate kinetic constant for two-break events, d is the length of the region involved and f is the average maximum recoverable deficiency length in the region, then straightforward calculation gives an induction rate of Kfd for deficiencies and $Kd^2/2$ for tandem duplications. Thus $2f/d$ is the expected ratio of recoverable deficiencies to induced tandem duplications.

From known deficiencies, an f value of 1 numbered division appears reasonable; the tandemly duplicated portion of the insertional-tandem duplication might commonly be on the order of 10 numbered divisions, which would give a ratio of 1 deficiency to 5 tandem duplications among surviving progeny. The actual recovery ratio would probably be greater than this since large duplications will suffer from reduced viability. However, this may not be a relevant consideration since the parental duplication can be marked to allow attention to be restricted to a particular subregion with a much smaller d value. Virgin collection can be automated so that the size of such a screen is limited by physical facilities (available bottles, maximum amount of culture medium which can be made at a time and incubator space) and not by available manpower.

The efficiency of an experiment designed to recover recessive lethal or sterile mutants often suffers from the lack of an appropriately marked balancer chromosome that effectively suppresses recombination. The technique of recombining markers with an inversion through a sequence of selected single exchanges should make it possible to construct complex combinations of inversions and markers for use as balancer chromosomes. One step in this direction has been to recombine the *SM1* and *CyO* inversions to produce a new balancer for chromosome *2*, but examination of Table 3 makes it clear that much more extensive manipulation is feasible.

Some basic results concerning chromosomal phenomena have appeared in applications of this technology. The available selective power has made it pos-

sible to select for recombinants between breakpoints separated by distances of as little as 50 bands; no attempt has been made to recover recombinants over shorter distances. No region has been found in which it is not possible to recover recombinants between breakpoints. Although few regions have been tested as yet, it seems safe to conclude that there is not some small number of specific sites that are necessary for the local initiation of recombinational pairing; the resolution of the experiments is not sufficient to rule out the possibility that such specific sites may exist at the single band level. Meiotic recombination was also observed in a region of centric heterochromatin bounded by inversion breakpoints.

In the course of applying the inchworm method of generating autosynaptic inversion constellations, it was necessary to deal with several cases of hyperploidy for four to six numbered divisions of the polytene map. In all but one case, these hyperploids proved to be fertile in both sexes; the exceptional case was sterile in both sexes. LINDSLEY *et al.* (1972) observed occasional survival of hyperploids for nine to ten numbered divisions, and FITZ-EARLE and HOLM (1978) observed survival to the late pupal stage of individuals that were hyperploid for all of the left arm of chromosome *2*. Extensive hyperploidy appears to be quite well tolerated in Drosophila.

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