TECHNIQUES FOR MANIPULATING CHROMOSOMAL REARRANGEMENTS AND THEIR APPLICATION TO *DROSOPHILA MELANOGASTER*. II. TRANSLOCATIONS

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ABSTRACT

Translocations have long been valued for their segregational properties. This paper extends the utility of translocations by considering recombinational derivatives of pairs of simple reciprocal translocations. Three major derivative structures are noted. One of these derivatives is suitable for use in half-tetrad experiments. A second should find use in recombining markers with translocation breakpoints. The third is an insertional-tandem duplication: it has a section of one chromosome inserted into a heterologue with a section of the latter chromosome tandemly repeated about the breaks of the insert. All of these structures are contained in "constellations" of chromosomes that regularly segregate aneuploid-1 products (informationally equivalent to nonrecombinant adjacent-1 segregants) for one of the parental translocations but do not segregate euploid products. This is in contrast to the parental T_1/T_2 constellations which segregate euploid products but not aneuploid-1 products. Methods are described for selecting translocation recombinants on the basis of this dichotomy. Several examples of translocation recombinants have been recovered with these techniques, and the recombination frequencies seem to be consistent with those observed for crossovers between inversion breakpoints. Recombinant chromosomes tend to disjoin, but it is observed that the tendency may vary according to the region involved in the recombination, and it is suggested that this difference reflects a difference in chiasmata terminalization times. Special consideration is given to insertional-tandem duplications. Large insertional-tandem duplications are useful in cytogenetic screens. Small insertional-tandem duplications are useful in gene dosage studies and other experiments that require an insert from one chromosome to another. Large duplications can be deleted to form small duplications. To generate a small insert for a specified region, it is only necessary to have one translocation with a breakpoint flanking the region of interest. The second translocation can have a breakpoint quite far from the region: an insertional-tandem duplication containing the region that has one closely flanking breakpoint can be deleted to create a smaller duplication that has two closely flanking breakpoints.

RECOMBINATION is a useful tool for synthesizing new chromosomal aberrations from existing ones. Recombinational derivatives of pericentric inversions (CRAYMER 1981) include a novel type of duplication, a half-tetrad structure and multiple inversion complexes. Analogous structures can be derived from translocations.

Translocations are structural interchanges between nonhomologous chromosomes. Translocation heterozygotes regularly produce both euploid and aneuploid gametes. In crosses of translocation heterozygotes to structurally normal individuals, the aneuploid translocation segregants are incorporated into aneuploid zygotes, which are usually lethal. The euploid segregants are incorporated into viable euploid zygotes so that the translocated chromosomes appear to be linked. This pseudolinkage of nonhomologous chromosomes is often used to eliminate unwanted classes of offspring from mating schemes.

Viable aneuploid genotypes can be created by combining elements from two translocations with similar, but not identical, breakpoints (MULLER 1930). LINDSLEY *et al.* (1972) isolated more than 300 *Y* autosome translocations which were then used to generate a series of synthetic deficiencies spanning approximately 90% of the autosomal genome. Other workers have used these *T(Y;A)*'s to localize a number of structural genes involved in enzymatic pathways (reviewed by O'BRIAN and MACINTYRE 1978).

LINDSLEY *et al.*, (1972) constructed aneuploid derivatives via adjacent-1 segregation in *T(Y;A)*/+ animals. That is, the male parents in their crosses were *T(Y;A)*#1/+, and the female parents were *T(Y;A)*#2/+ (with a compound-*X* present so that the females were *XXY*); the relevant offspring received "half" of the parental *T(Y;A)#1* and half of the maternal *T(Y;A)#2*, along with a normal set of other chromosomes from both parents. Only a fraction of the offspring recovered from a given cross were the derivative aneuploids. Most of the offspring were euploid. Had the crosses been designed so that one parent produced only aneuploid gametes, then only the aneuploid derivatives would have been recovered among offspring of the cross. This approach can be applied to the problem of recovering translocation recombinants.

TECHNIQUES

There are three basic structures for translocation recombinants. One of these can be used as a half-tetrad. The second may be useful for recombining markers with translocations. The third is an insertional-tandem duplication.

Figure 1 diagrams pairs of translocations that will produce each of these three structures. For consistency, each pair of translocations contains the same breaks (2-3; B-C for one translocation and 4-5; D-E for the second) but are differentiated by centromere locations. Figure 2 shows the constellation derived from each pair of translocations as the result of exchange in the CD regions separating breakpoints of these translocations. (I use the term "constellation" to refer to a euploid set of chromosomes with a characteristic structure. Two sets of chromosomes that are structurally identical represent the same constellation regardless of any allelic differences.)

In Figure 2*a*, the novel chromosome (structurally 65/D.C/3456) carries two copies of the 56 region and so might be used as a half-tetrad for this region, much as compound chromosomes (ANDERSON 1925; BALDWIN and CHOVNICK 1967; CHOVNICK *et al.* 1970) or inversion-derived autosynaptic chromosomes (CRAYMER 1981) may be. It may be noted that a further exchange in the 34 region of the figure produces the $T_{11}+T_{12}$ complex, structurally 1.2/BA; 65/

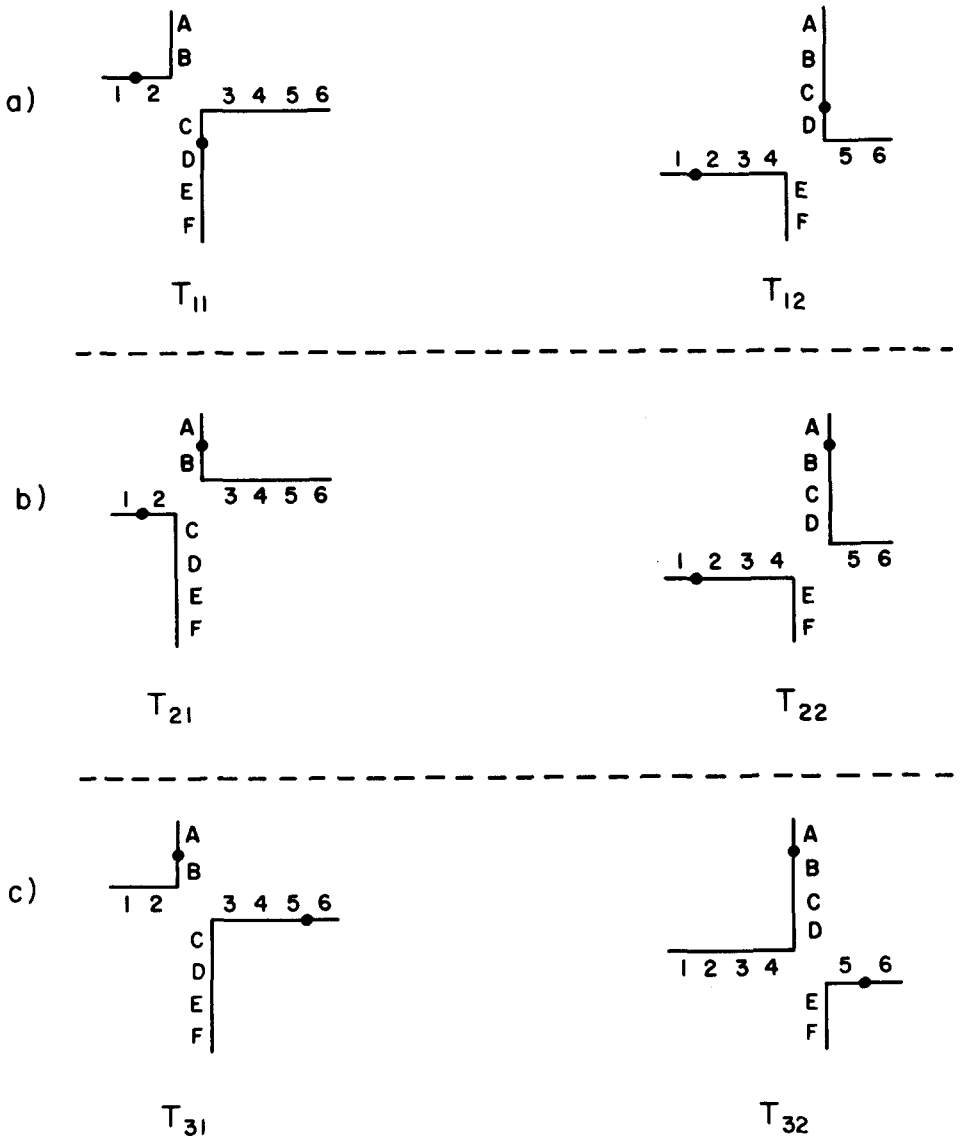

FIGURE 1.—Representative pairs of translocations for deriving each of the three classes of translocation recombinants. T_{11}, T_{21} and T_{31} have 2-3; B-C breaks, whereas T_{12}, T_{22} and T_{32} have 4-5; D-E breaks. Except for T_{11}, each translocation is drawn with the L^PN^D chromosome above the N^PL^D chromosome. [L denotes the lettered chromosome, and N the numbered chromosome. Proximal (P) and distal (D) relate the new chromosome order to the original with respect to centromere location. 1.2/BA, for example, is the $N^PL^DT_{11}$ chromosome since 2 is proximal to the 2/B breakpoint, and BA is distal since it is normally distal to the C/B point where the translocation break occurred.] The translocations with identical breaks are mainly differentiated by centromere locations: T_{21} differs from T_{11} and T_{31} in having 2/C; B/3 breakpoints instead of 2/B; 3/C breakpoints, but T_{12}, T_{22} and T_{32} all have 4/E; D/5 breakpoints. An alternative version of the T_{21}-T_{22} pair of translocations has centromeres separating 3 from 4 and C from D. Exchanges in the CD regions separating breakpoints of each pair of translocations produces the constellations diagrammed in Figure 2.

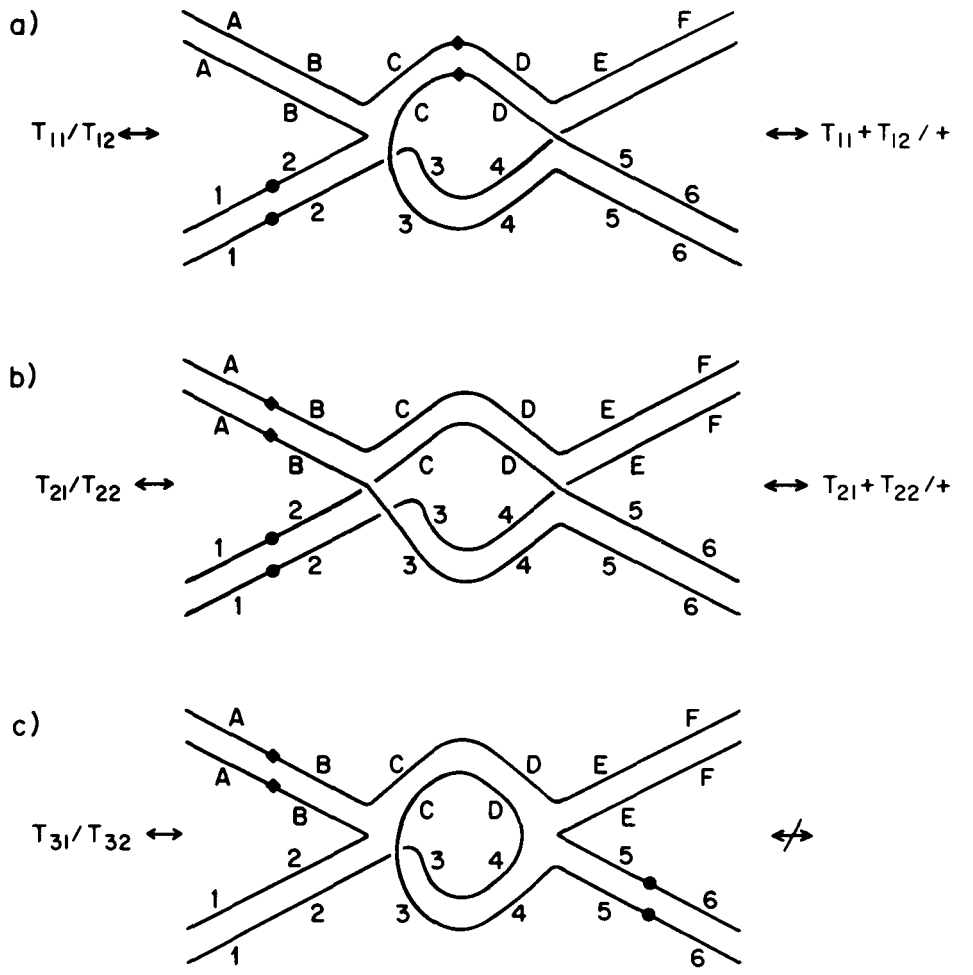

FIGURE 2.—The typical constellations containing novel chromosomes. The novel chromosomes in each constellation result from CD exchange between the corresponding pair of translocations shown in Figure 1. a) consists of $+_L$, $N^PL^DT_{11}$, $N^PL^DT_{12}$ and $L^PN^DT_{11}+L^PN^DT_{12}$ (65/D.C/3456). b) consists of $+_L$, $L^PN^DT_{21}$, $N^PL^DT_{22}$ and $N^PL^DT_{21}+L^PN^DT_{22}$ (1.2/CD/56). c) consists of $+_L$, $L^PN^DT_{31}$, $N^PL^DT_{32}$ and $Dp(L,N;N)T^P_{32}T^D_{32}$ (1234/DC/345.6). The $Dp(L,N;N)$ notation indicates that material from both L and N is duplicated onto an N chromosome. The $T^P_{31}T^D_{32}$ notation indicates that the proximal breakpoint of the duplication is derived from T_{31} and the distal breakpoint from T_{32}. The 65/D.C/3456 chromosome in a) can provide a half-tetrad for the 56 region. The b) constellation may be used to recombine markers onto either T_{21} or T_{22}. The 1234/DC/345.6 chromosome in c) is an insertional-tandem duplication. Not shown is a constellation that differs from b) only in locating the centromeres as C·D and 3·4 rather than A·B and 1·2. This constellation has segregational properties similar to those of the a) constellation: it segregates aneuploid-1 products for each of the component translocations. CD exchange in any of the diagrammed constellations will reconstitute the parental translocations for the constellation. This is indicated by the double-headed arrows in the figure, although it must be noted that the T_{i1}/T_{i2} and diagrammed constellations are interconvertible only in the sense that exchange in one constellation generates the components of the other. Further exchange in the 34 region of a) produces $T_{11}+T_{12}$, structurally FE/43/C.D/56; 1.2/BA, and 34 exchange in b) produces $T_{21}+T_{22}$, structurally A.B/34/EF; 1.2/CD/56. 34 exchange in c) does not produce a double-translocation complex; instead, an exchange in the $Dp(L,N;N)T^P_{31}T^D_{32}$ produces an acentric ring and a $+_N$ chromosome.

D.C/34/EF. This has the segregational properties of T_{11}, differing only in the presence of the 5/D; 4/E "inversion" of the $L^PN^DT_{11}$.

The structure in Figure 2*b* is of interest as an intermediate between T_{21}/T_{22} and $T_{21}+T_{22}/+$, but it may also be used for recovering marked versions of T_{21} or T_{22}. Consider an L chromosomal mutant m_1, located between C and D. By taking advantage of the segregational patterns of the constellation shown in Figure 2*b*, the $+_L$ chromosome can be replaced with an L chromosome carrying m_1. A crossover in region C of the Figure 2*b* constellation (with m_1) then leads to T_{22}, m_1 [A.B/3456; 1.2/Cm_1DEF]. A crossover in region D would similarly produce T_{21}, m_1.

The novel chromosome in Figure 2*c* is of particular interest. It is an insertional-tandem duplication, insertionally duplicated for CD and tandemly duplicated for 34. Insertional-tandem duplications (CRAYMER 1981) are useful in cytogenetic screens and as substitutes for insertional duplications; when necessary, an insertional-tandem duplication can be deleted to form a smaller insertional-tandem duplication, going from 1234/DC/345.6 to 123/C/345.6 or to 1234/D/45.6, while leaving one of the breakpoints (C/3 or 4.D) of the original duplication intact.

Recombination is locally suppressed by heterozygous rearrangement breakpoints. Consequently, recombination in a region separating translocation breakpoints is rare unless the region is large. To recover translocational recombinants, it is necessary to design crosses that selectively incorporate recombinant-bearing gametes into viable zygotes and nonrecombinant-bearing gametes into lethal zygotes.

For each structure diagrammed in Figure 2, one regular segregation pattern produces aneuploid-1 products for one of the component translocations. (ZIMMERING (1956) used the term "aneuploid-1" to refer to translocation segregants with either the $+_L$; N^PL^D or L^PN^D; $+_N$ structures that regularly arise from adjacent-1 segregation or alternate segregation with a crossover between centromere and translocation breakpont from a $T/+$ animal. I extend the usage to also include rearranged versions of either structure: $L^PN^DT_{11}+L^PN^DT_{12}$; $N^PL^DT_{11}$, for example, is aneuploid-1 for T_{12}.) This can be seen from examination of Figure 2 but is laid out more explicitly in Table 1. 2a segregates $+_L$; $N^PL^DT_{11}$ (and necessarily, a complementary product) and also $+_L$; $N^PL^DT_{12}$. 2b segregates $+_L$; $N^PL^DT_{22}$. 2c segregates $+_L$; $N^PL^DT_{32}$. None of the precursive double-translocation (T_{i1}/T_{i2} or $T_{i1}+T_{i2}/+$, where i may be either 1, 2, or 3) heterozygotes segregate equivalent products. To select for translocation recombinants, it is necessary to devise crosses in which only the aneuploid-1 products can be incorporated into viable, euploid zygotes. This means that the male parents in selective crosses must segregate T_{i2}-aneuploid-1 (T_{11}-aneuploid-1 is also feasible in the case of T_{11}/T_{12} females) products but not segregate euploid products or $L^PN^DT_{i1}$; $N^PL^DT_{i2}$ or $L^PN^DT_{i2}$; $N^PL^DT_{i1}$.

Adjacent-1 segregation in $T/+$ heterozygotes produces gametes that could be used in recovering translocation recombinants. However, $T/+$ heterozygotes also produce euploid gametes from alternate segregation, and these euploid gametes would complement the nonrecombinant translocation segregants. Figure 3 shows a modified "$T/+$" genotype, which does not produce euploid

TABLE 1

Regular segregation patterns from the constellations shown in Figure 2 and from the T_{i1}/T_{i2} from which they were derived

Constellation	Segregant	*Dp*	*Df*	Complementary segregant
2a	$+_L$; $N^PL^DT_{11}$	AB	3456	$L^PN^DT_{11} + L^PN^DT_{12}$; $N^PL^DT_{12}$
	$+_L$; $N^PL^DT_{12}$	EF	56	$L^PN^DT_{11} + L^PN^DT_{12}$; $N^PL^DT_{11}$
T_{11}/T_{12}	T_{11}			T_{12}
	$L^PN^DT_{11}$; $N^PL^DT_{12}$	34, EF	AB	$L^PN^DT_{12}$; $N^PL^DT_{11}$
2b	$+_L$; $N^PL^DT_{22}$	EF	56	$L^PN^DT_{22}$; $N^PL^DT_{21} + L^PN^DT_{22}$
	$+_L$; $N^PL^DT_{21} + L^PN^DT_{22}$	CD	34	$L^PN^DT_{21}$; $N^PL^DT_{22}$
T_{21}/T_{22}	T_{21}			T_{22}
	$L^PN^DT_{22}$; $N^PL^DT_{21}$	CD	34	$L^PN^DT_{21}$; $N^PL^DT_{22}$
2c	$+_L$; $N^PL^DT_{32}$	EF	1234	$L^PN^DT_{31}$; $Dp(L, N; N)T^P_{32}T^D_{31}$
	$+_L$; $Dp(L, N; N)T^P_{32}T^D_{31}$	CD, 34		$L^PN^DT_{31}$; $N^PL^DT_{32}$
T_{31}/T_{32}	T_{31}			T_{32}
	$L^PN^DT_{32}$; $N^PL^DT_{31}$	CD, 34		$L^PN^DT_{31}$; $N^PL^DT_{32}$

Entries in the "segregant" column indicate a particular segregation pattern; the "complementary segregant" in the same row is also produced by that segregation pattern, but the *Dp* and *Df* entries refer to the segregant entry.

segregants in the absence of recombination. The *LS+T/DS* constellation shown may be recovered from crosses of *T/In* females to *LS/DS* males and maintained by repeated backcrosses of *LS+T/DS* males to either *LS/DS* or *T/+* females, provided that adequate markers are present to identify the *LS+T/DS* animals. (*LS+T/DS* stocks are usually not stable: recombination in region 5 of Figure 3 in females followed by adjacent-1 segregation in both sexes will reconstitute the *T/In* constellation.)

Constellations of the 2a [or alternative version of 2b (see legend to Figure 2)] type are also useful sources of aneuploid-1 segregants. If a stock exists that produces T_{11}-aneuploid-1 but not euploid segregants, then the 2a constellation can be recovered from T_{11}/T_{12}. The 2a constellation produces both T_{11}-aneuploid-1 and T_{12}-aneuploid-1 segregants but does not produce euploid segregants: for any two-break translocation, T_{1s}, a 2a-like constellation can be derived either from T_{11}/T_{1s} or from T_{1s}/T_{12}. Furthermore, if either T_{11} or T_{12} carries a 34 region rearrangement that locally suppresses recombination, then a stable stock of the 2a constellation can be constructed by replacing the $+_L$ chromosome with a balancer chromosome that suppresses recombination in the C·D region.

Recombination, disjunction and recovery frequencies

Table 1 does not list all of the segregants that result from crossovers. Crossovers in the 34 region of the T_{i1}/T_{i2} (*i* being 1, 2 and 3) heterozygotes also produce novel chromosomes and segregational products. Furthermore, crossovers occur at the four-strand stage of meiosis so that each produces an oocyte with both an intact parental constellation and a crossover constellation of chromatids. Segregants can be produced from the crossover oocyte that can be produced by neither the parental nor the daughter constellation. For example,

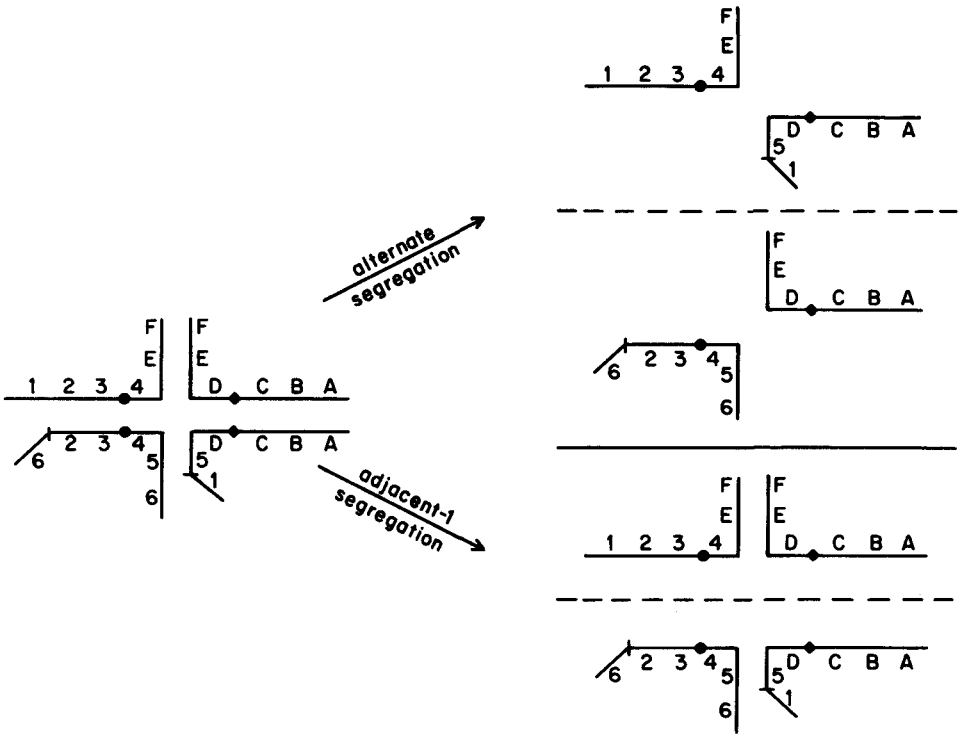

FIGURE 3.—An inversion/translocation-derived constellation and the segregational patterns that make it useful for selecting aneuploid-1 translocation derivatives. The parental inversion has structure 1/54.32/6, whereas the translocation is ABC.D/56; 123.4/EF in structure. An exchange in region 5 generates the constellation diagrammed. Alternate segregation leads to ABC.DEF; 6/234.56 and ABC.D/5/1; 123.4/EF gametes. The former is simply a DS-bearing (6/23.456) gamete, whereas the latter complex may be described as *LS+T* (the *LS* and *DS* notation is described by CRAYMER 1981). Either is recoverable from crosses of *In/T* females to *LS/DS* (*LS* = 123.45/1) males since euploid offspring (*LS/DS* or *LS+T/DS*) result. Neither should be recoverable over $+_L$; $+_N$: $+_L/+_L$; $+_N/DS$ is hypoloid for region 1 and hyperploid for 6, whereas $LS+T/+_N$; $+_L$ is hypoploid for 6 and hyperploid for 1. Adjacent-1 segregation in the diagrammed constellation leads to aneuploid-1 gametes.

an $N^P L^D T_{22}$ chromosome could be obtained from the T_{21}/T_{22} constellation of the crossover oocyte and combined with a $+_L$ from the 2b constellation. The $N^P L^D T_{22}$; $+_L$ segregant thus produced differs from the segregants produced by either the T_{21}/T_{22} or the 2b constellation. Figure 4 shows the tetrads that result from either 34 or CD recombination in T_{i1}/T_{i2} heterozygotes. Also shown are the predicted planes of segregation.

Table 2 lists the aneuploid-1 segregants from crossover meiocytes and their frequencies among regular segregants (for the cases discussed, regular segregation patterns are those in which chromosomes with homologous centromeres disjoin). d_{CD} and d_{34} appear in the calculated frequencies to take into account the tendency for crossover chromosomes to disjoin (DOBZHANSKY 1933; BROWN 1940; PIPKIN 1940).

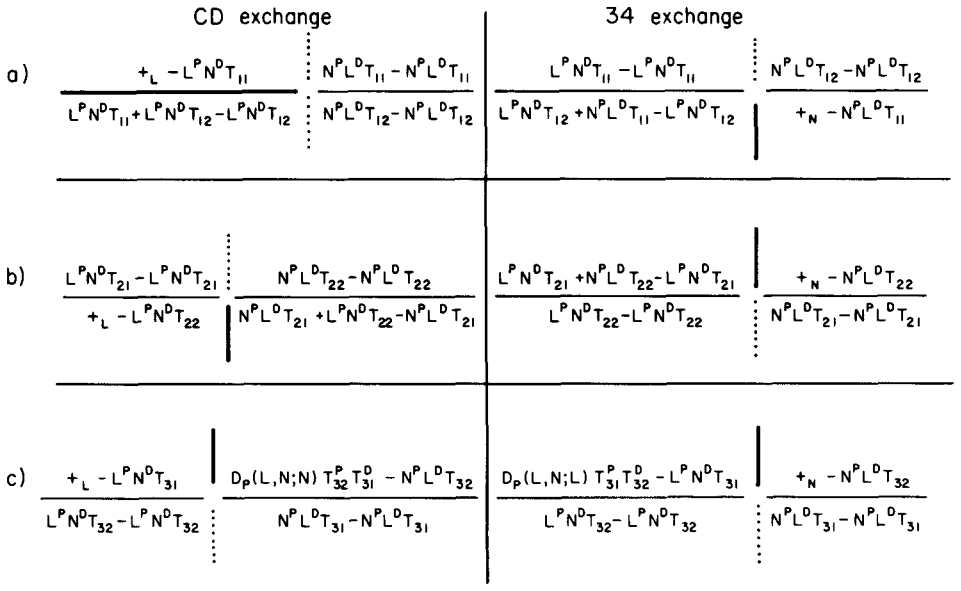

FIGURE 4.—Tetrads resulting from single exchange in either the CD or 34 regions of the translocation pairs presented in Figure 1. Lines separating chromosomes indicate disjunctional tendencies. Sister chromatids are separated by dashes to indicate that they disjoin in meiosis II. Heavy lines separate recombinant chromosome pairs; recombinant chromosomes have a strong tendency to disjoin (DOBZHANSKY 1933; BROWN 1940; PIPKIN 1940). Lighter lines separate chromosomes with homologous centromeres since these usually disjoin, and this tendency may be reinforced by recombination between the homologues. Dotted lines separate chromosomes that are not directly driven to disjoin. In all but the CD recombinant of T_{11}/T_{12}, the preferred segregation pattern is the "alternate" one; in this exceptional case, the L^P chromosomes disjoin and the N^P chromosomes disjoin, but L^P chromosomes assort independently of N^P chromosomes.

Examination of Table 2 reveals that whenever it is possible to generate an aneuploid-1 segregant carrying a novel chromosome it is also possible to generate an equivalent aneuploid-1 segregant that does not contain the novel chromosome. Where the T_{11}-aneuploid-1 segregant $L^PN^DT_{11}$ + $L^PN^DT_{12}$; $N^PL^DT_{12}$ can be generated from T_{11}/T_{12}, it is also possible to generate $L^PN^DT_{11}$; $+_N$ (equivalent in content but not in structure). All other aneuploid-1-like segregants containing novel chromosomes listed in the table are accompanied by equivalent aneuploid-1 segregants that do not contain novel chromosomes. Thus, paternal markers in a translocation-crossover-selection cross do not unambiguously identify offspring carrying novel chromosomes. To resolve the ambiguity, it is necessary to genetically test for the presence of novel chromosomes. Cytological anlaysis of putative novel chromosome-bearing karyotypes is also helpful.

Selective crosses and tests for novel constellations

T_{11}/T_{12}: It can be seen from Table 1 that the $L^PN^DT_{11}$ plus $L^PN^DT_{12}$ chromosome is recoverable in crosses that select from either T_{11}-aneuploid-1 or T_{12}-aneuploid-1 segregants (that is, in crosses of T_{11}/T_{12} females to males that

TABLE 2

Aneuploid segregants produced after exchange in a T_{i1}/T_{i2} *mother*

Maternal genotype	Gamete$_L$	Frequency	Gamete$_N$
T_{11}/T_{12}	$+_L$; $N^PL^DT_{11}$	$r_{CD}/4$	$L^PN^DT_{11} + L^PN^DT_{12}$; $N^PL^DT_{12}$
		$(1 - d_{34})r_{34}/4$	$L^PN^DT_{11}$; $+_N$
	$+_L$; $N^PL^DT_{12}$	$r_{CD}/4$	$L^PN^DT_{21} + L^PN^DT_{22}$; $N^PL^DT_{21}$
	$L^PN^DT_{11} + N^PL^DT_{12}$	$d_{34}r_{34}/2$	$L^PN^DT_{12}$; $+_N$
T_{21}/T_{22}	$+_L$; $N^PL^DT_{22}$	$d_{CD}r_{CD}/2$	$L^PN^DT_{21}$; $N^PL^DT_{21} + L^PN^DT_{22}$
	$L^PN^DT_{21} + N^PL^DT_{21}$; $N^PL^DT_{21}$	$d_{34}r_{34}/2$	$L^PN^DT_{22}$; $+_N$
	$+_L$; $N^PL^DT_{21}$	$(1 - d_{CD})r_{CD}/4$	
		$(1 - d_{34})r_{34}/4$	$L^PN^DT_{21}$; $+_N$
T_{31}/T_{32}	$+_L$; $L^PN^DT_{32}$	$d_{CD}r_{CD}/2$	$L^PN^DT_{31}$; $Dp(L, N; N)T^P_{31}T^D_{32}$
		$(1 - d_{34})r_{34}/4$	$L^PN^DT_{32}$; $+_N$
	$+_L$; $N^PL^DT_{31}$	$(1 - d_{CD})r_{CD}/4$	
	$Dp(L, N; N)T^P_{32}T^D_{31}$; $N^PL^DT_{32}$	$d_{34}r_{34}/2$	$L^PN^DT_{31}$; $+_N$

The gamete$_L$ column lists $+_L$-containing and equivalent (in terms of content) aneuploid-1 gametes. The gamete$_N$ column includes the complementary aneuploid-1 segregants. The frequency calculations only take into account cases where chromosomes with homologous centromeres disjoin. r_{CD} and r_{34} are recombination frequencies; d_{CD} and d_{34} are measures of disjunctional tendencies for recombinant chromosomes.

show either T_{11}-aneuploid-1 or T_{12}-aneuploid segregation patterns). From Table 2, the T_{11}-aneuploid-1 crossover selection will confound $L^PN^DT_{11}$+$L^PN^DT_{12}$; $N^PL^DT_{12}$ with $L^PN^DT_{11}$; $+_N$ insofar as carrier phenotype is concerned. T_{12}-aneuploid-1 crossover selections confound $L^PN^DT_{11}$+$L^PN^DT_{12}$; $N^PL^DT_{12}$ with $L^PN^DT_{12}$; $+_N$.

In most practical situations, the difference in recovery frequencies will be small and it will not matter which aneuploid-1 selection is used. For half-tetrad analyses, the 34 region will be centric heterochromatin and will rarely undergo recombination. When the 2a constellation is to be used for T_{11}-aneuploid-1 selections, a T_{12} will usually be chosen that carries further rearrangements that suppress recombination in the 34 region.

The $L^PN^DT_{11}$+$L^PN^DT_{12}/+_L$; $N^PL^DT_{11}/N^PL^DT_{12}$ constellation has T_{11}-aneuploid-1 and T_{12}-aneuploid-1 segregation patterns, whereas the confounding $T_{11}/+$ (or $T_{12}/+$) constellation shows both an orthoploid and T_{11}-aneuploid-1 segregation patterns. The two constellations may be distinguished by individually crossing males to $T_{12}/(Bal_L; Bal_N)$ females. For the $L^PN^DT_{11}$+$L^PN^DT_{12}$-bearing males, offspring will carry either Bal_L or Bal_N, but not both or neither. $T_{11}/+$ males will have offspring that carry either both balancers or neither.

An alternative approach is to establish stocks of the recovered chromosomes and later cross males from each stock to structurally normal females. The crosses with $L^PN^DT_{11}$+$L^PN^DT_{12}$-bearing males will produce no viable progeny, whereas crosses with $T_{11}/+$ males will produce viable progeny.

T_{21}/T_{22}: A T_{22}-aneuploid-1 selection is required to recover the $N^PL^DT_{21}+L^PN^DT_{22}$ chromosome from T_{22}/T_{21} mothers. The $L^PN^DT_{21}$; $N^PL^DT_{21}$ + $L^PN^DT_{22}$ ova will be confounded with $L^PN^DT_{22}$; $+_N$ ova. The novel chromosome-bearing ova will be a fraction $d_{CD}r_{CD}/(d_{CD}r_{CD} + d_{34}r_{34})$ of those showing the appropriate phenotype. If r_{CD} is much less than r_{34}, an excessive number of tests may be needed to find an offspring carrying the $N^PL^DT_{21}+L^PN^DT_{22}$ chromosome. In that case, it is better to first select for the $L^PN^DT_{21}+N^PL^DT_{22}$ novel chromosome and then select for the $N^PL^DT_{21}+L^PN^DT_{22}$ chromosome. The second selection is carried out by crossing $L^PN^DT_{21}+N^PL^DT_{22}/L^PN^DT_{22}$; $N^PL^DT_{21}/Bal_N$ females to structurally normal males to recover $T_{22}+T_{21}$ ($L^PN^DT_{21}+N^PL^DT_{22}$; $N^PL^DT_{21}+L^PN^DT_{22}$) among the offspring. ($Bal_N$ suppresses recombination in the 34 region so that only products of CD region recombination are recovered.)

$L^PN^DT_{21}/+_L$; $N^PL^DT_{21}+L^PN^DT_{22}$ can be distinguished from $T_{22}/+$ by crossing males to structurally normal females after stocks have been established. The $L^PN^DT_{21}/+_L$; $N^PL^DT_{21}+L^PN^DT_{22}$ constellation does not have a euploid segregation pattern, so that the crosses involving males with this constellation will produce no viable offspring.

T_{31}/T_{32}: $Dp(L,N;N)T^P_{31}T^D_{32}$ is recovered from T_{31}/T_{32} females via T_{32}-aneuploid-1 selections. The $Dp(L,N;N)T^P_{31}T^D_{32}$; $L^PN^DT_{31}$ ova are confounded with $L^PN^DT_{32}$; $+_N$ ova, and a fraction $2d_{CD}r_{CD}/[2d_{CD}r_{CD} + (1 - d_{34})r_{34}]$ of the offspring with markers denoting a paternal "$+_L$; $L^PN^DT_{32}$" will carry the duplication.

The corresponding $Dp(L,N;L)T^P_{32}T^D_{31}$ is recovered from T_{31}/T_{32} females via T_{31}-aneuploid-1 selections. It may be noted that the translocation used to select for a duplication is the one with the proximal break in the chromosome where the duplication is to be inserted.

The $L^PN^DT_{31}/+_L$; $Dp(L,N;N)T^P_{31}T^D_{32}/N^PL^DT_{32}$ males produce no euploid sperm but do product T_{32}-aneuploid-1-like sperm. Such males can be distinguished from their $T_{32}/+$ sibs by crossing to $T_{32}/+$ females, or to structurally normal females after a stock has been established, much as the 2a or 2b constellations are tested for. An additional genetic test is possible for the insertional-tandem-duplication-bearing constellation. $N^PL^DT_{31}/Bal_L$; $Dp(L,N;N)T^P_{31}T^D_{32}/N^PL^DT_{32}$ females will produce no viable Bal^+_L offspring when crossed to structurally normal males. This behavior is characteristic only of insertional-tandem constellations.

Diploid exceptions from 3:1 segregations

Occasionally, there will be some survivors from a translocation-recombinant selective cross as the result of 3:1 segregation. For example, 2a males may produce $N^PL^DT_{12}$ sperm, and T_{1s}/T_{12} females may produce $T_{1s}/L^PN^DT_{12}$ ova. In a cross of T_{1s}/T_{12} females to 2a males, there will thus result some T_{1s}/T_{12} offspring from 3:1 segregation in both sexes (or 3:1 segregation in one sex and loss of a chromosome during meiosis in the other).

The 3:1 exceptions are readily identified if the $L^PN^DT_{11}+L^PN^DT_{12}$ and $+_L$ chromosomes of the male parent each carry dominant markers. The expected progeny will carry one or the other of the marked chromosomes. The 3:1 exceptions will carry both or neither.

TABLE 3

Example syntheses of some of the constellations diagrammed in Figures 2 and 3

No.	Mother	Father	Relevant offspring genotype
1	$\frac{T(2;\,3)P8}{In(3LR)B158}$	$\frac{LS(3)B158}{DS(3)B158}$	$\frac{LS(3)B158,\ T(2;\,3)P8}{+;\ DS(3)B158}$
2	$\frac{T(2;\,3)P8}{SM5;\ TM1}$	$\frac{LS(3)B158,\ T(2;\,3)P8}{+;\ DS(3)B158}$	$\frac{LS(3)B158,\ T(2;\,3)P8}{SM5;\ DS(3)B158}$
3	$\frac{T(2;\,3)P8}{T(2;\,3)S^{L}}$	$\frac{LS(3)B158,\ T(2;\,3)P8}{SM5;\ DS(3)B158}$	$\frac{3^{D}2^{P}S^{L}+2^{P}3^{D}P8}{SM5};\ \frac{3^{P}2^{D}S^{L}}{3^{P}2^{D}P8}$
4	$\frac{T(2;\,3)S^{L}}{T(2;\,3)P71}$	$\frac{3^{D}2^{P}S^{L}+2^{P}3^{D}P8}{SM5};\ \frac{3^{P}2^{D}S^{L}}{3^{P}2^{D}P8}$	$\frac{3^{D}2^{P}S^{L}+2^{P}3^{D}P71}{SM5};\ \frac{3^{P}2^{D}S^{L}}{3^{P}2^{D}P71}$
5	$\frac{T(2;\,3)S^{L}}{T(2;\,3)C287}$	$\frac{3^{D}2^{P}S^{L}+2^{P}3^{D}P71}{SM5};\ \frac{3^{P}2^{D}S^{L}}{3^{P}2^{D}P71}$	$\frac{3^{D}2^{P}S^{L}+2^{P}3^{D}C287}{SM5};\ \frac{3^{P}2^{D}S^{L}}{3^{P}2^{D}C287}$
A	$\frac{T(2;\,3)P8}{T(2;\,3)205}$	$\frac{LS(3)B158,\ T(2;\,3)P8}{SM5;\ DS(3)B158}$	$\frac{2^{P}3^{D}205}{SM5};\ \frac{Dp(2,\,3;\,3)205^{P}P8^{D}}{3^{P}2^{D}P8}$
B	$\frac{T(2;\,3)C287}{T(2;\,3)P71}$	$\frac{3^{D}2^{P}S^{L}+2^{P}3^{D}P71}{SM5};\ \frac{3^{P}2^{D}S^{L}+3^{P}2^{D}P71}{3^{P}2^{D}P71}$	$\frac{2^{P}3^{D}C287}{SM5};\ \frac{Dp(2,\,3;\,3)C287^{P}P71^{D}}{3^{P}2^{D}P71}$
C_0	$\frac{T(2;\,3)C287}{T(2;\,3)P71}$	$\frac{3^{D}2^{P}S^{L}+2^{P}3^{D}C287}{SM5};\ \frac{3^{P}2^{D}S^{L}}{3^{P}2^{D}C287}$	$\frac{3^{D}2^{P}S^{L}+2^{P}3^{D}C287}{Dp(2,\,3;\,2)P71^{P}C287^{D}};\ \frac{3^{P}2^{D}S^{L}}{3^{P}2^{D}C287}$
C_1	$\frac{T(2;\,3)C287}{bw^{V1};\ TM6B;\ D^{3}}$	$\frac{3^{D}2^{P}S^{L}+2^{P}3^{D}C287}{Dp(2,\,3;\,2)P71^{P}C287^{D}};\ \frac{3^{P}2^{D}S^{L}}{3^{P}2^{D}C287}$	$\frac{2^{P}3^{D}C287}{Dp(2,\,3;\,2)P71^{P}C287^{D}};\ \frac{TM6B,\ D^{3}}{3^{P}2^{D}C287}$

Breakpoints of the rearrangements listed in the table are:

In(3LR)B158 76D; 94A
T(2; 3)P8 48C; 84D
T(2; 3)S^{L} 21D; 81F; 88D [T(2; 3)21D; 88D plus In(3R)81; 88D]
T(2; 3)P71 60E; 81F
T(2; 3)C287 56D; 89F.

TM6B is a new complex balancer for the third chromosome; other mutants and balancer chromosomes are described by LINDSLEY and GRELL (1968). Cross 1 generates an example of the constellation diagrammed in Figure 3; cross 2 replaces the wild-type second chromosome of the constellation recovered in cross 1 with a complex balancer chromosome, *SM5*. Crosses 3, 4 and 5 generate samples of the constellation shown in Figure 2*a*. The A, B and C_0 crosses generate insertional-tandem duplication. The C_1 cross replaces the $2^{P}3^{D}S^{L}+2^{P}3^{D}C287$; $3^{P}2^{D}S^{L}$ complex with $2^{P}3^{D}C287$; *TM6B*, D^{3}. All crosses were carried out according to the protocol given in the preceding paper (CRAYMER 1981).

APPLICATIONS

Practical examples of the constellations shown in Figure 2, *a* and *c* and Figure 3 have been constructed. Table 3 lists these examples and the crosses from which each was derived.

The first two crosses listed in Table 3 were used to derive and identify an example of the Figure 2 constellation. Putative *LS(3)B158, T(2;3)P8/DS(3)B158* offspring from cross 1 were identified on the basis of markers carried by the paternal *DS(3)B158* chromosome. By then mating the "*LS(3)B158, T(2;3)P8/DS(3)158*" males individually to *T(2;3)P8/[SM5; TM1]* females (cross 2), presence of the translocation was verified by the production of *SM5*, *TM1*$^{+}$ and *SM5*$^{+}$, *TM1* progeny (result of adjacent-1 segregation in both sexes). The *LS(3)B158+T(2;3)P8/SM5; DS(3)B158* stock has proven to be stable. There is little recombination between the 84D break of *T(2;3)P8* and the 94A break of *In(3LR)B158*, and the tendency for recombinant chromosomes to disjoin is

sufficiently strong as to prevent the formation of a 2^P2^DP8; *In(3LR)B158* ova and subsequently a *T/In* "breakdown" of the *LS+T/DS* constellation. Several *LS+T/DS* constellations have been constructed from other inversions and translocations; the distance between breakpoints was considerably greater in these cases, and the resulting constellations were not stable.

Crosses 3, 4 and 5 were used to generate examples of the 1a constellation. The 81; 88 inversion associated with $T(2;3)S^L$ effectively prevented recombination between the third chromosomal breaks of $T(2;3)S^L$ and either *T(2;3)P8*, *T(2;3)P71* or *T(2;3)C287* so that only the listed constellation and its complement were recovered from each cross. (The region between third chromosomal breakpoints corresponds to region 5 of Figure 1*a*). *SM5* prevents recombination in the two-centric region separating translocation breakpoints, so that stocks of these constellations are stable.

Crosses A, B and C_0 were used to recover insertional-tandem duplications. Four bottles each of the A and B crosses were set up. Two phenotypically Cy S^+ animals were recovered from each cross. These animals received an *SM5* second chromosome and a 3^P2^D chromosome from the translocation used for the selection [*T(2;3)P8* for A and *T(2;3)P71* for B]. On testing, all proved to have received an insertional-tandem chromosome from the mother and to have the genotypes shown in Table 3. Eight bottles of the C_0 cross were set up, and 18 Cy^+ S male progeny were recovered and tested. Sixteen of these proved to have the genotype expected. A larger scale experiment (20 bottles) produced 136 S^+ Cy, 171 S Cy^+, 3 S^+ Cy^+ and no S Cy offspring.

Recombination between the 81F break of *T(2;3)P71* and the 89F break of *T(2;3)C287* is much more frequent than is exchange between the 56F and 60E breaks. One would expect, therefore, a relatively high frequency of the *T(2;3)P71/SM5* "nondisjunctional" progeny from the B cross. No such nondisjunctional progeny were recovered. By the same token, the $3^D2^PS^L$ + $2^P3^DC287/SM5$; $3^P2^DS^L/3^P2^DC287$ constellation should be relatively rare in offspring of the C_0 cross. Yet two of the 18 animals tested seem to have been of this genotype. Clearly, the recombination-disjunction relationships differ for the two regions.

A model for predicting recombination-disjunction correlations is desirable. Unfortunately, current understanding in this area is still rather primitive. The argument that seems most likely to bear on this case is that chiasma should terminalize early for the products of crossing over in the 56–60 region, but chiasma should terminalize late for 81–89 region recombination. Pairing problems at the 56/89 break will drive chiasma terminalization in the first case: the chiasma will be forced in a distal direction, and terminalization should occur early because there is no opposing force. For the latter case, chiasma terminalization is opposed by a force resulting from the pairing problems at the 56/89 break; there is no driving force in favor of terminalization at an early stage. These relations are shown in Figure 5. In neither case is chiasma terminalization driven by the disjunction of homologous centromeres as is true in most other cases.

The translocation manipulations described here provide a powerful analogue to the inversion techniques described in the previous paper (CRAYMER 1981).

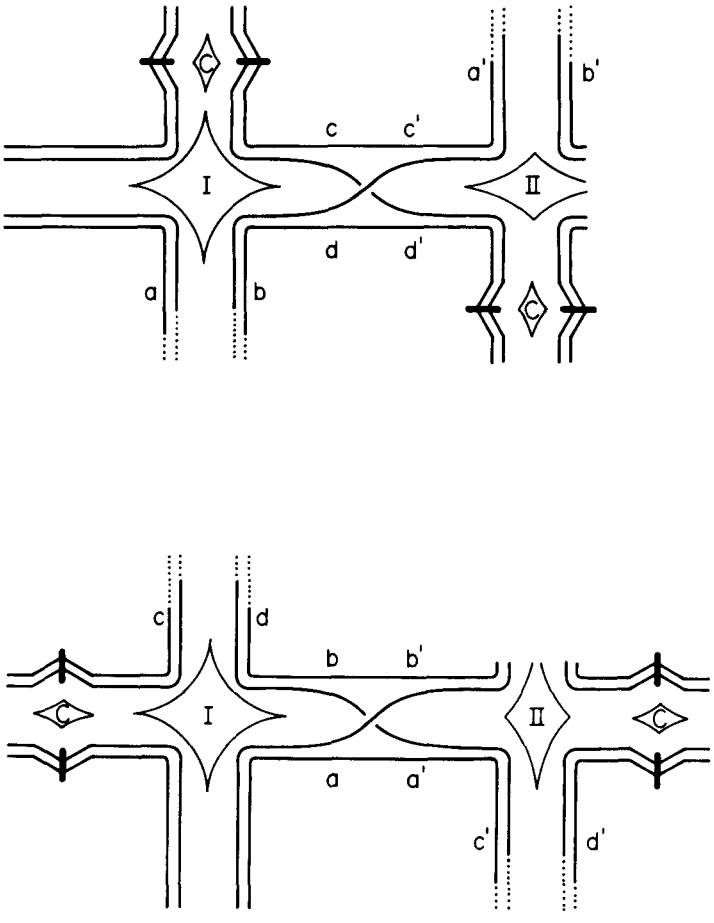

FIGURE 5.—Suggested terminalization tendencies for chiasmata between translocation breakpoints. The upper and lower figures differ only in the location of chiasmata: the dotted lines are artificial separations of translocated chromosomes, and the two figures would appear similar if the a-a′, b-b′, c-c′ and d-d′ connections were drawn in. The chromosomes are drawn as heavy lines, whereas the lighter lines outline "empty" space between homologous chromosomes. The "C" areas represent the separation of homologous centromeres, whereas "I" and "II" represent the gaps at a translocation breakpoint. At each breakpoint, pairing in the horizontal segments should stress the synapsed regions of the vertical segments, whereas pairing in the vertical segments should stress the synapsed regions of the horizontal regions; in each case, the greatest stress is exerted on the regions nearest the breakpoint. Improved pairing along one axis helps to "unzip" the paired segments on the other axis. At breakpoint I, all arms of the translocated figure extent sufficiently far that pairing tendencies are about equal for each of the arms of the figure extending from the breakpoint. At breakpoint II, the short arm is rarely paired; as a result, the flanking arms pair more easily, and the opposed arm is pulled apart. At each breakpoint, the tendencies toward asynapsis should determine terminalization tendencies: the forces exerted on the chiasma by homologous centromeres proceeding to opposite meiotic poles should be weak until relatively late in anaphase I. In the upper figure, the asynaptic tendencies of I and II that influence chiasma terminalization should balance, and the chiasma would not be expected to terminalize until late in anaphase I. In the lower figure, I has a much greater influence on terminalization than does II so chiasmata have a significant chance of terminalizing at an earlier stage of meiosis. In a), the tendency for terminalization is met by an opposing force, and one would expect terminalization to occur at anaphase I. In b), there is no opposing force, and terminalization should occur early.

Translocation-based half-tetrad schemes using constellations of the 2a type should be useful for dealing with *X* chromosomal loci. Two simple *X* autosome translocations would be chosen whose *X* chromosomal breakpoints were in proximal heterochromatin. The autosomal breakpoints would be widely separated so that the half-tetrad is easily constructed and easily dismantled. Markers can be readily recombined onto the translocations before the half-tetrad is constructed. An example of a half-tetrad experiment using an inversion-derived analogue of the 2a structure is described by CRAYMER (1981). Construction of appropriately marked *C(1)RM*'s is not as straightforward, and it is rather tedious to detach a *C(1)RM* to isolate a recombinant in a form amenable to further analysis.

Insertional-tandem duplications are useful for cytogenetic analyses and gene dosage studies. Large insertional-tandem duplications act as dominant lethals unless a complementary deficiency is present. Males carrying a large insertional-tandem duplication can be mutagenized and mated to structurally normal females. Surviving offspring will then be the result of events that delete all or part of the parental duplication. The net result of such an event may be a deficiency or tandem duplication for part of the region tandemly duplicated in the parental duplication may be a smaller insertional-tandem duplication than the original or may be a more complicated derivative. The parental insertional-tandem duplication can be marked in a manner that helps to restrict attention to specific subregions of the chromosome (CRAYMER 1981).

Small insertional-tandem duplications should be quite useful in gene dosage or other experiments in which it is desirable to have the wild-type allele of a specified locus inserted into a heterologous chromosome. Such duplications have the advantage of being synthesized in a straightforward manner, in contrast to "standard" insertional duplications that are difficult to recover and troublesome to screen for. The desired insertional-tandem duplication can be created in a two-step process. The first step is to synthesize a large insertional-tandem duplication that inserts the locus of interest into an appropriate region; it is necessary that the locus be near one breakpoint of the duplication. The second step is to delete most of the large duplication to leave a chromosome with a small insert including the desired locus. *Dp(2,3;3)205^{P}P8^{D}*, which has sequence 61A to 80.81 to 89D/41 to 48C/84D to 100F, was built for the purpose of generating a small insertion of *en*$^{+}$ (in 48A) into chromosome *3*. A deletion with one break in 84D-85 and the second in 47 would produce a chromosome with sequence 61A to 80.81 to 84-85/47 to 48C/84D to 100F; this would be a chromosome with the desired characteristics. Similarly, *Dp(2,3;3)P71^{P}C287^{D}*, with sequence 21A to 40.41 to 60E/81F to 89F/56D to 60F, could be deleted to produce a small insert of 89E (which contains the bithorax complex) into chromosome *2*. Neither of the large insertional-tandem duplications have been deleted to form the smaller inserts. However, a small-scale (20 male survivors tested) experiment involving a large, inversion-derived, insertional-tandem duplication produced several moderate sized (three to four numbered divisions) insertional-tandem derivatives and one chromosome that carried a small insert but was deleted for material at the point of insertion. It

seems likely that small insertional-tandem derivatives could be recovered without undue effort.

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