CHROMOSOMAL CONTROL OF FERTILITY IN *DROSOPHILA MELANOGASTER*. I. RESCUE OF *T(Y;A)/bb*$^{l\text{-}158}$ MALE STERILITY BY CHROMOSOME REARRANGEMENT

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ABSTRACT

Many translocations between the *Y* chromosome and a major autosome have no effect on the fertility of *Drosophila melanogaster* males. However, when such translocation-bearing males also carry an *X* chromosome deficient for a large portion of the centric heterochromatin, they are generally sterile. This has been interpreted to be the result of an interaction between the deficiency and the subterminally capped autosome. Using this observation as a starting point, we have developed a selection scheme for radiation-induced translocation resealings that depends on the prediction that fertility in the presence of such a deficient *X* is restored whenever the displaced autosomal tip is brought back in association with an autosomal centromere. The observation and the prediction form the basis for what is referred to as the autosomal continuity model for male fertility.——Such a mutagenesis scheme offers several advantages. (1) It is efficient, producing upward of 1% resealings in some cases. (2) It is simple; since fertility is the basis for the selective screen, many males can be tested in a single vial. (3) It can be used to simultaneously generate both duplications and deficiencies specific for chromosomal material adjacent to the original translocation breakpoints. (4) The target for mutagenesis can be mature sperm.——Analysis of the pattern of male-fertile rearrangements obtained from several translocation lines using this protocol indicates that continuity of the autosomal tips and their centromeres is neither a necessary nor sufficient condition for male fertility in the presence of a bobbed-deficient *X*. Thus, the simple autosomal continuity model is not adequate to explain this complicated mechanism of chromosomal control of fertility and will have to be revised accordingly. Potential future lines of inquiry toward this goal are discussed.

THE genetic control of meiosis and gametogenesis in Drosophila is extremely complex. Evidence exists for numerous individual loci, both autosomal and sex linked (LINDSLEY and TOKUYASU 1980; LINDSLEY and LIFSCHYTZ 1972), which participate in the regulation of gamete production. Some of these act in only one sex, whereas others are nonspecific in that regard. To add a further layer of complexity, there appears to be, at least for spermatogenesis, a supragenic organizational requirement. This has been demonstrated by the discovery in *Drosophila melanogaster* of two apparent distinct classes of chromosomal rearrangements that can cause male sterility in appropriate genetic backgrounds.

The first class comprises *T(X;A)* events (*A* = chromosome *2* or *3*). These translocations often show a type of sterility that is dominant (males with duplications covering the *T(X;A)* breakpoints remain sterile) and generally independent of breakpoint, although the minority of *T(X;A)* cases that are fertile have *X* breakpoints at one of the extreme ends of the *X* chromosome (LINDSLEY 1965). Moreover, such translocations show a common phenotype for the sterility; that is, a failure of proper sperm head elongation during spermatogenesis (SHOUP 1967; LINDSLEY and TOKUYASU 1980). These observations argue against a genic origin for the sterility, as this would require an improbably large number of sites affecting fertility, all capable of mutating to a dominant state and all causing the same defect in gamete production.

LINDLSEY and LIFSCHYTZ (1972) have interpreted these data as evidence for a *cis*-acting control locus that mediates early inactivation of the *X* chromosome during spermatogenesis. In this view, *T(X;A)* events remove part of the *X* from this control, and the consequent disruption of inactivation leads to sterility. Although such asynchronous inactivation of the *X* has not been observed directly in *D. melanogaster*, there is evidence for its occurrence in related Drosophila species (*e.g.*, *D. hydei*, see LINDSLEY and TOKUYASU 1980 for a review).

Further studies involving large deficiencies of the base of the long arm of the *X* chromosome suggest that the putative control region may span the area including the suppressor of forked [*su(f)*] and the bobbed (*bb*) loci and comprise as many as three subsites flanking *bb* (RAHMAN and LINDSLEY 1981). Deletions of part or all of this material often lead to male sterility, especially if they are coupled with *X* duplications in other chromosomes. This mapping is further supported by the observation that fertile *T(X;A)*'s with breaks in the *X* centric heterochromatin are all broken proximal to *bb*, suggesting that, in these cases, the control region is translocated along with the rest of the *X* and can still mediate its inactivation.

Most normally fertile *T(Y;A)* rearrangements can also lead to sterility if present in a male carrying certain *X*-proximal deficiencies. This second type of chromosomal control of fertility was first observed by BESMERTNAIA (1934), rediscovered by LINDSLEY *et al.* (1979) and extended by my laboratory (LYTTLE 1981). LINDSLEY and his co-workers made several experimental observations that led them to postulate that it was the interaction between the *X* deficiency and a subterminally capped autosome that led to sterility. That is, since (1) almost all *T(Y;A)* rearrangements that are fertile with *bb* deficiencies have very distal autosomal breakpoints, and since (2) replacing the A^Y (= autosome capped with *Y* tip) portion of sterile translocations with a normal autosome restores fertility, whereas substituting a normal *Y* for the Y^A portion does not, the implication is that fertility in *bb*-deficient males requires some very distal locus or region in each major autosome arm to remain contiguous with an autosomal centromere. This interpretation has obvious common elements with the LINDSLEY and LIFSCHYTZ model for *T(X;A)* sterility described earlier; but note that here we have a purely operational explanation with no suggestion as to the biological mechanisms involved. This will hereafter be referred to as the autosomal continuity model.

This form of chromosomal control of fertility is interesting both because the elucidation of the rules governing its occurrence may shed considerable light on the normal process of spermatogenesis and because it suggests a potential selection system for site-specific insertional translocations of autosomal material into the *Y* chromosome and vice versa. That is, if continuity of autosomal tips with their centromeres is a requirement for male fertility in the presence of a *bb* deficiency, then it is possible that mutation events induced in sperm that reseal translocations (*i.e.*, restore the arms to their original centromeres) may also restore fertility to otherwise sterile *T(Y;A)/bb* offspring. If this resealing process were to involve breaks offset from those of the original *T(Y;A)*, then there would be the potential for inserting either adjacent autosomal or *Y* material into the opposite chromosome. This paper provides a description of such a selection procedure and its efficiency in producing the desired resealings. In addition, the types of rearrangements obtained and their implications for the autosomal continuity model outlined earlier are considered.

MATERIALS AND METHODS

D. melanogaster *stocks*

y $bb^{l\text{-}158}$*/FM7/*y^{+}*y* is used as the source of the *bb*-deficient *X* chromosome since $bb^{l\text{-}158}$, which is a recessive lethal in females, removes 82% of the centric heterochromatin (YAMAMOTO and MIKLOS 1978) and shows the strongest sterility effects in *T(Y;A)* males (D. L. LINDSLEY, personal communication). *FM7* is a multiply inverted chromosome carrying w^{a}, *B* and *sc* (see LINDSLEY and GRELL 1968 for a description of all mutant alleles).

cn bw is a standard second chromosome carrying the eye color mutants cinnabar (*cn*) and brown (*bw*).

Table 1 lists the *T(Y;A)* translocations that were used as targets for the mutagenesis procedure. Autosomal breakpoints according to the standard cytological map of BRIDGES (1935) are included, along with the *Y* arm involved when known (*L* = long, *S* = short). All *T(Y;A)* lines are fertile in males carrying a normal *X* but sterile with $bb^{l\text{-}158}$. None of the translocations were marked with visible mutants, and each line was maintained by repeated backcrossing to *cn bw* females.

Mutagenesis protocol

T(Y;2)/+; *cn bw* males, 2–5 days old, were irradiated with 4500 r of γ-rays (approximately 5500 r/min) from a Co^{60} source. These were then mated to *y* bb^{l}*/FM7* virgin females in half-pint bottles of standard cornmeal-molasses food, with approximately 15 pairs/bottle. After 7 days the males were discarded to promote sampling of sperm that were primarily postmeiotic at the time of irradiation, whereas the females were brooded into new bottles and discarded after an additional 7 days. Non-*FM7* male offspring were collected from each bottle over an 18-day period and immediately mated with *cn bw* females in 6-dram shell vials, with 25 pairs each. Test vials not showing larvae within 10 days were discarded as were vials producing <5 offspring (usually females), the latter being attributed to the "leaky" fertility of an unrearranged male. Vials producing five or more progeny were considered to qualify as fertile, and males hatching from such vials were assumed to be sons of a single F_1 male who was himself the product of an induced resealing. These, in turn, were backcrossed repeatedly to *cn bw* females in order to maintain each recovered line. The type and chromosomal extent of the rearrangement events obtained were determined both by direct observation of salivary gland polytene chromosomes and by analysis of the segregation of genetic markers in the backcross generations. For example, resealing events that resulted in the insertion of only a small piece of a second into a *Y* chromosome often produced viable aneuploid progeny (*i.e.*, phenotypically wild-type females or *cn bw* males) from the backcross, whereas these were absent in the stock crosses of the parent translocation line.

Figure 1 illustrates the mutagenic protocol and the spectrum of potential results expected for

TABLE 1

Translocation lines used in the fertility selection scheme

Translocation line	Breakpoints: autosomal (*Y*)	Tested males	Fertile rearrangements	% Fertile (95% confidence interval)
T(Y;2)B10, $B^{S}Yy^{+}$; SD Roma	36D2-3 (*L*)	3100	22	0.710 (0.44–1.07)
T(Y;2)T22, SD(NH)-2	36E (*L*)	250	3	1.20 (0.25–3.50)
T(Y;2)L141, $y^{+}Y$; +	56F (*S*)	3825	15	0.392 (0.22–0.65)
T(Y;2)B13 $B^{S}Yy^{+}$; SD Roma	57A (*S*)	300	1	0.330 (0.00–1.20)
T(Y;2)A2 $B^{S}Yy^{+}$; SD(NH)-2	41A-C (*L*)	1335	10	0.749 (0.36–1.38)
T(Y;2)C5; $B^{S}Yy^{+}$; SD(NH)-2	38B1-2 (?)	1349	3	0.222 (0.05–0.65)

For all lines except *B13*, at least some of the fertile rearrangements have been resealings. The 95% confidence intervals are based on a Poisson distribution for rearrangements, and mutually overlap in the range 0.440–0.647% fertile males.

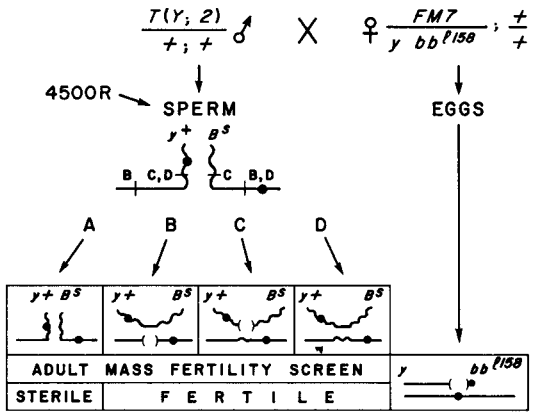

FIGURE 1.—A schematic representation of the fertility selection scheme for the generation of translocation resealings. F_1 males that arise from irradiated sperm are mass tested for fertility. + represents a standard *X* chromosome or second or third autosome. In the lower part of the figure, the egg and sperm chromosome constitutions are shown schematically; wavy lines represent *Y* material, and straight lines represent the *X* and autosome. Parentheses indicate material that has been deleted and moved. Unaltered sperm lead to sterile males (A), whereas males carrying a resealed translocation are expected to be fertile. Resealings can involve insertions of autosomal material into a *Y* (see breakpoints labeled B, and corresponding product in the F_1), insertions of *Y* material into an autosome (C) or both (D). See text for further details.

male offspring arising from an arbitrary *T(Y;A)* line. Suppose that there is no resealing in an irradiated sperm that subsequently fertilizes an egg carrying a bobbed-deficient *X*, such as in path A. Then the autosomal continuity model predicts that the continued displacement of the autosomal tip from its centromere (Figure 1A) should ensure the sterility of the resulting F_1 male. On the other hand, fertile resealings could be any one of three simple types, distinguished by whether a block of autosomal material (B), *Y* material (C) or both (D) is inserted into the complementary chromosome. (The possibility of having resealing breaks at precisely the sites involved in the original translocation is not excluded but is uninteresting.) The distinction among these three types of resealings is not trivial, since it might be possible that an insertion of *Y* material into a resealed

autosome would not qualify as an event that restores continuity of an autosomal tip with its centromere. In this case, we would not expect it to be fertile with $bb^{l\text{-}158}$.

The recovery of sterile aneuploid males carrying only the Y^2 portion of a fertile resealing (*e.g.*, the *cn bw* males described earlier) was taken as evidence that the resealing involved the insertion into the autosome of *Y* material containing fertility factors, as might be the case in path C or D of Figure 1. Resealing events of type D allow a further determination as to the origin of the fertility factors, *i.e.*, proximal or distal to the original *Y* chromosome break. This follows from an examination of the diagrammed breakpoints in the figure. It is clear that type D resealings with autosomal breaks in the 2^Y portion of the original *T*(Y;2) constrain inserted fertility factors to be of proximal origin, whereas resealings with Y^2 autosomal breaks (not shown here) could only insert distal fertility factors.

RESULTS AND DISCUSSION

The application of the mutagenic protocol yielded fertile rearrangements from all the *T(Y;2)* lines listed in Table 1 and described earlier; although, as will be seen, not all rearrangements were of the simple kind illustrated by Figure 1. The efficiency of the resealing process is quite high, approaching 1% fertile males recovered from some lines (see Table 1). This may be an underestimate of the true recovery rate, since some of the discarded vials exhibited the "leaky" fertility attributed to unrearranged males. Consequently, these were not counted as resealings, although some may well have been. Moreover, some potentially fertile males may have failed to mate for unrelated reasons.

It should be noted that we use the term "resealing" to specifically describe a secondary two-break event that simultaneously rejoins the two parts of an autosome and *Y* chromosome separated by the earlier breaks of a *T(Y;A)* rearrangement. In its final form, a resealing of the type seen in Figure 1, path B, will, consequently, have the appearance of a very specific type of four-break rearrangements, *i.e.*, one in which a pair of nonoverlapping inversions sharing a common breakpoint are inserted into the *Y*. Figure 2 shows the polytene chromosomes from two salivary gland nuclei of *X/Dp(2;Y) B10-3*, y^+YB^S; *cn bw/ cn bw* (see Figure 3 for breakpoints), illustrating the typical conformation of such inserted autosomal material. Similar results obtain for the other types of resealings.

Two of the *T(Y;2)* lines (*B10* and *L141*) have produced enough resealings to warrant their use in some qualitative comparisons as to the type of rearrangements obtained and their implication for the autosomal continuity model. Figure 3 and Table 2 give the recovered fertile rearrangements for *T(Y;2) B10* (and also include the few resealings obtained from *T(Y;2) T22*, as these give qualitatively similar results). Most recovered rearrangements were the expected resealings, with only three of the 25 involving more complicated breaks. We will return to these later. Several points are illustrated by these results. First, resealings that involve insertions of *Y* material into the autosome can still rescue fertility (*cf.*, *T22-1*, *T22-3*, and *T22-5* in Figure 3, all examples of Figure 1D resealings). In addition, the type and frequency of *Y* insertions obtained vary with the site of the translocation *Y* break. Thus, *T22*, which gave only type D resealings, must be broken in the middle of Y^L. This can be argued in the

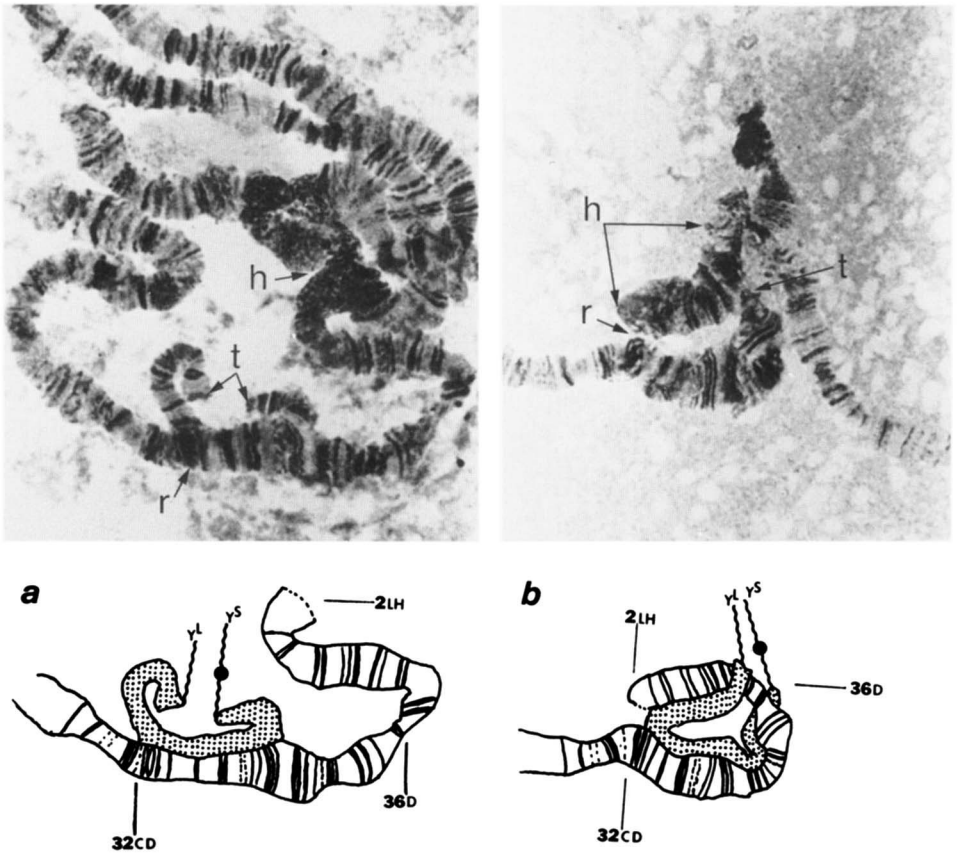

FIGURE 2.—The topology of resealings. The polytene salivary gland chromosomes of two nuclei from *Dp(2;Y)B10-3* (derived from *T(Y;2) B10-3*, see Table 2) are shown, along with a schematic of their pairing conformation, demonstrating the fact that resealings of this type (path B in Figure 1) essentially are the equivalent of inserting a pair of nonoverlapping inversions that share a common breakpoint, into a *Y* chromosome. When fully paired, such duplications give the doughnut conformation shown in b. h = centromeric or Y heterochromatin. r = breakpoint(s) involved in resealing event. t = original translocation breakpoint.

following way. First, note in figure 3 that resealings *T22-3* and *T22-5* involve autosomal breaks in the Y^2 portion of *T22*, and, therefore, the inserted fertility factors in these lines must originate in Y^L, distal to the original *Y* breakpoint. Conversely, *T22-1* has a 2^Y autosomal break and inserts proximal *Y* fertility factors into the resealed second chromosome. It must, therefore, be the case that fertility factors flank the original Y^L breakpoint of *T22*, perhaps explaining the high incidence of resealings that move them. On the other hand, the original Y^L breakpoint of *B10* is inferred to be distal to all such loci. This conclusion follows from the observation that some *B10* rearrangements involve *Y* breaks distal to the B^S marker, leading to its insertion into the autosome at the site of the original translocation breakpoint (*cf.*, *B10-1*, *B10-2*, *B10-5*, *B10-8* and *B10-28* in Table 2, Figure 3). In these cases all of the Y^L material distal

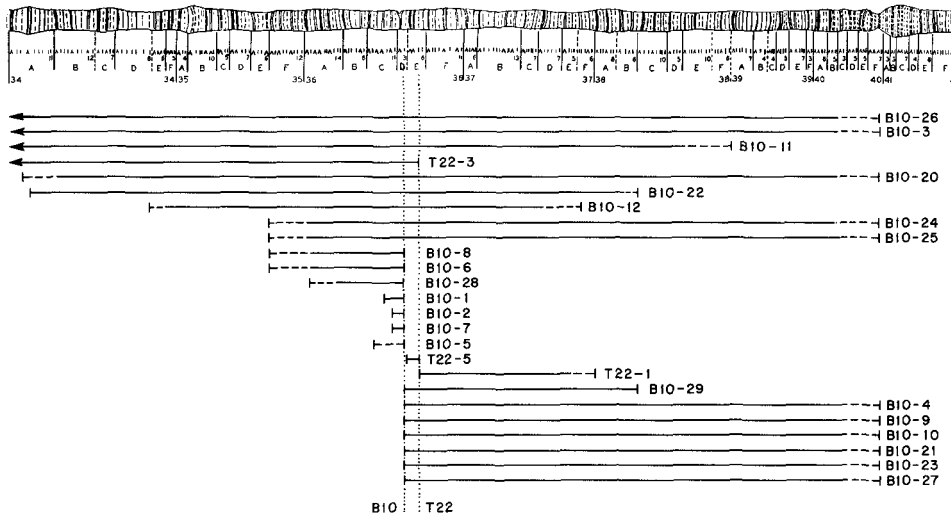

FIGURE 3.—A cytological mapping of the resealings from *B10* and *T22* (see Table 2). The labeled horizontal lines show the extent of the autosomal material inserted into the *Y* chromosome for the appropriate resealing line. Dashed lines indicate uncertainty as to the exact terminus of an insertion, whereas an arrow indicates that the insertion continues off the figure. Vertical dotted lines mark the original *T(Y;2)* autosomal breakpoints.

to the original *T(Y;2)* break must be inserted as well, yet none of these yielded insertions of fertility factors; thus, the original Y^L break must *a fortiori* be distal to all fertility loci.

So far zero of 22 fertile rearrangements recovered from *B10* insert fertility factors, in contrast to the results with *T22*. Whether this bias implies a deficiency of type C and D resealings (see Figure 1) or simply reflects a large distance between the Y^L breakpoint of *B10* and the nearest proximal fertility factor is as yet unknown. Nevertheless, it is clear that the position of the *Y* breakpoint in the initial translocation has an important influence on the pattern of resealings obtained.

The results from the fertility rescue protocol using *L141* and *B13* are presented in Figure 4 and Table 3. The recovered events are qualitatively quite different from those described for *B10* and *T22*. Here, the great majority of fertile rearrangements, although still two break events, involve the exchange of the originally translocated *2R* tip with a *2L*, *3R* or *3L* arm (*cf.*, Figure 4). However, a few true resealings were also obtained.

These results would seem to invalidate the autosomal continuity model for $T(Y;a)/bb^l$ sterility and its rescue by resealing. That is, since we know that *T(Y;3)* lines are also sterile with $bb^{l\text{-}158}$ (LINDSLEY *et al.* 1979; D. L. LINDSLEY, personal communication), we would expect a rearrangement that simply exchanges a *Y*-translocated *2R* tip for, say, a *3L* tip, to remain sterile with $bb^{l\text{-}158}$. Here we have evidence that at least some of these exchanges can restore fertility.

Since these lines are essentially *T(Y;2;3)* rearrangements, we might ask whether having two autosomal tips displaced in general negates the $T(Y;A)bb^l$

TABLE 2

Fertile rearrangements from T(Y;2) B10 *and* T22

Rearrangement line	Breakpoints	Autosomal material inserted in *Y*?/*Dp* fertile?	*Y* material inserted in 2?	Resealing type
B10-1	*T(Y;2;3)*[a] 36C10-D1; Y^L; 62A	+[b]/+	+[c]	D
B10-2[d]	*T(Y;2)*[a] 36D1-2; Y^L	+/+	+[c]	D
B10-3[d]	*T(Y;2)* 32BC; 2LH	+/+	−	B
B10-4	*T(Y;2)*[f] Y^L; 2LH	+/+	?	B or D
B10-5	*T(Y;2;4)*[a] 36C5-D2; Y^L; 101F	+[f]/+	+[c]	D
B10-6	*T(Y;2)*[a] 35F; Y^L	+/+	?	B or D
B10-7	*T(Y;2)*[a] 36D1-2; Y^L	+/+	?	B or D
B10-8	*T(Y;2)*[a] 35F; Y^L	+/+	+[c]	D
B10-9	*T(Y;2)*[f] Y^L; 2LH	+/+	?	B or D
B10-10	*T(Y;2)*[f] Y^L; 2LH	+/+	?	B or D
B10-11	*T(Y;2)* 33A; 38EF	+/+	−	B
B10-12	*T(Y;2)* 34DB-E3; 37D3-E1	+/+	−	B
B10-20	*T(Y;2)* 34A; 2LH	+/+	−	B
B10-21	*T(Y;2)*[f] Y^L; 2LH	+/+	?	B or D
B10-22	*T(Y;2)* 34A4-6; 38B	+/+	−	B
B10-23	*T(Y;2;3)*[f] Y^L; 2LH; 92A; 95EF	+[g]/+	?	B or D
B10-24	*T(Y;2)* 35F; 2LH	+/+	−	B
B10-25	*T(Y;2)* 35F; 2LH	+/+	−	B
B10-26	*T(Y;2)* 30B6-8; 2LH	+/?	−	B
B10-27	*T(Y;2)*[f] Y^L; 2LH	+/?	?	B or D
B10-28	*T(Y;2)*[a] 36AB; Y^L; 62A +*In(3L)* 66A; 70EF	+[h]/+	+[c]	D
B10-29	*T(Y;2)*[f] Y^L; 38B6	+/+	?	B or D
T22-1	*T(Y;2)*[f] Y^L; 37E	+/−	+[i]	D
T22-3	*T(Y;2)*[a] 33A; Y^L	+/−	+[i]	D
T22-5	*T(Y;2)*[f] 36E?; Y^L	?/−	+[b]	C or D

See Figure 1 for resealing type. See Figure 3 for a schematic representation. Note that all rearrangements are resealings, although three involve secondary translocations as well.

[a] Broken in translocated portion of *Y*.

[b] New order on *Y*: $y^+Y^S.Y^L$|36D1-2|61A1-61F8.

[c] B^S inserted into resealed autosome.

[d] Df lost.

[f] Broken in untranslocated portion of *Y*.

[f] New order on *Y*: $y^+YS.Y^L$|36CD|101F-102F8.

[g] 92A-95EF inserted into resealed autosome.

[h] New order of *Y*; $y^+Y^S.Y^L$|36A-36D|61A1-61F8.

[i] Fertility factor(s) inserted into resealed autosome.

sterility. To examine this, we have tested several independently derived *T(Y;2;3)* and *T(Y;2)+T(2;3)* lines. Only two of ten such lines were fertile, both *T(Y;2;3)*'s, with distal *3R* from *95CD* and *97F*, respectively, capping an unknown *Y* arm as a result. Thus, there is no evidence that *T(Y;2;3)* lines *per se* are any more likely to be fertile with bb^1 than either *T*(Y;2) or *T(Y;3)* rearrangements.

All but one of the rearrangements that are fertile with $bb^{1\text{-}158}$ involve distal

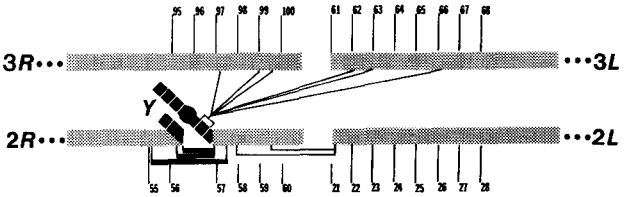

FIGURE 4.—A schematic representation of the fertile rearrangements obtained from *L141* and *B13* (see Table 3). The tips of the major autosomal arms for *T(Y;2) L141* are juxtaposed, with the standard cytological divisions (after BRIDGES) provided as a reference. Thin lines mark the points at which tips are exchanged in secondary translocation events; thick lines indicate resealings of the same type as Figure 3. Note that *JL-6* is not included.

TABLE 3

Fertile rearrangements obtained from T(Y;2) L141

Rearrangement line	Breakpoints	*Dp*'s fertile?	New order	Resealing type
JL-1	*T(Y;2;3)*[a] 30B; 99F; Y^S	+	60F-56F|30B-56F|Y^Sy^+; 61A-99F|30B-21A; Y^LY^S|99F-100F	n.a.
JL-2	*T(Y;2;3)*[a] 62B; Y^S	+	21A-56F|Y^Sy^+; 60F-56F|62B-100F; Y^LY^S|62B-61A	n.a.
JL-3	*T(Y;2)*[a] 56C; Y^S (Resealing)	+	21A-56C|56F-60F; Y^LY^S|56C-56F|Y^Sy^+	B or D
JL-4	*T(Y;2)*[ab] Y^S; Y^S (Resealing) +*In(2R)* 42A; 57F	+	21A-42A|56F-56F|Y^S heterochromatin|56F-42A|56F-60F; $Y^LY^Sy^+$ (deficient for Y^S material)	C
JL-5	*T(Y;2;3)*[a] 66B; Y^S	+	21A-56F|Y^Sy^+; 60F-65F|66B-100F; Y^LY^S|66B-60A	n.a.
JL-6	*T(Y;2;3)*[a] 60A; 87F; Y^L	+	21A-56F|Y^Sy^+; 61A-87F|Y^L; 60A-60F|Y^LY^S 65F-60A|87F-100F	n.a.
JL-8	*T(Y;2;3)*[a] 97B; Y^S	+	21A-56F|Y^Sy^+; 61A-97B|56F-60F; Y^LY^S|97B-100F	n.a.
JL-9	*T(Y;2;3)*[a] 63A; Y^S	+	21A-56F|Y^Sy^+; 60F-56F|63A-100F; Y^LY^S|63A-61A	n.a.
JL-10	*T(Y;2;3)*[a] 99F; Y^S	+	21A-56F|Y^Sy^+; 61A-99|56F-60F; Y^LY^S|99F-100F	n.a.
JL-11	*T(Y;2)* 21A; 57F	+	60F-57F|21A-56F|Y^Sy^+; Y^LY^S 56F-57F|21A	n.a.
JL-12	*T(Y;2)* 21A; 59D	+	60F-59D|21A-56F|Y^Sy^+; Y^LY^S 56F-59D|21A	n.a.
JL-15	*T(Y;2)* 55A; 57C (Resealing)	+	21A-55A|57C-60F; Y^LY^S|55A-57C|Y^Sy^+	B
B13-1	*T(Y;2)*[a] 99CD; Y^S	+	21A-57A|Y^Sy^+; 61A-70F|97CD-70F|57A-60F; Y^LY^S|99CD-100F	n.a.

See Figure 4 for a schematic representation. Note that most are secondary translocations rather than resealings. n.a. = not applicable.

[a] Broken in untranslocated portion of *Y*.

[b] Broken in translocated portion of *Y*.

breaks in *2L*, *3R* or *3L*. Thus, in order to restate the model of autosomal continuity to accommodate these new results, we must suppose that it is not the terminal portion of the autosome itself, but a somewhat more proximal region that must remain continuous with an autosomal centromere in order to maintain fertility. With this revision, the results obtained with the rearrangements of Table 4 (with one exception) would be compatible with the hypothesis that the required region was located at least as proximal as *66B*, *97B*, and *21A* for *3L*, *3R* and *2L*, respectively. However, this would imply that plain T(*Y;3*)'s with breaks distal to *97B* and *66B* would be fertile with bb^{1}, and this is apparently not generally the case (D. L. LINDSLEY, unpublished results).

JL-6 offers the most striking example of the problems with the autosome continuity model. Not only is this rearrangement fertile despite having a majority of *3R* added to the *Y*, but all of the *2R* tip originally translocated also remains. The first tip apparently confers sterility with $bb^{1\text{-}158}$, whereas the addition of a second tip actually restores fertility. Note that in this rearrangement, as in several others of Table 3, the 2^{Y} portion of the translocation is unaltered; yet, as described before, it would be to this portion that LINDSLEY *et al.* (1979) would assign the source of the sterility interaction. There seems to be no reasonable way to accommodate all of these observations with the simple model of autosomal continuity that has been suggested.

Although the rearrangements derived from *L141* and *B13* seem to render the autosomal continuity model untenable, and, in fact, defy the superimposition of any simple pattern on the breaks necessary to restore fertility, a reexamination of Table 2 reveals the converse: an example of an extraordinary repetition of fertile rearrangement pattern. Only three lines listed (*B10-1*, *B10-5* and *B10-28*) involve T(*Y;2;3*) or T(*Y;2;4*) events; yet, these constitute three of the five cases of B^{S} insertion into a resealed chromosome *2*. Moreover, two of these were T(*Y;2;3*)'s with breaks at *62A*, and all three involve resealing breaks in *36B-D*, very near the original translocation site of *B10*. Whether this represents coincidence or an important clue to the pattern of rearrangements required for the restoration of fertility remains a question for future study.

Aside from the implications for model building, we must also consider why the distribution of rearrangement types differs so drastically between Tables 2 and 3. Is it the fact that *B13* and *L141* are themselves near an autosomal tip that predisposes the fertile rearrangements obtained from these lines to be secondary translocations rather than resealings? Contrarily, is it the fact that *B13* and *L141* involve a Y^{S} (rather than Y^{L}, as in *B10* and *T22*) break that leads to this asymmetry? As yet, these questions cannot be answered, but they will certainly be important in assessing the general applicability of the selection protocol for the generation of *Dp/Df* resealings.

Our incomplete understanding of the mechanism of fertility control involved does not negate the value of the selection procedure outlined for producing a high proportion of specific classes of rearrangements (particularly resealings) for specific autosomal regions. The protocol has several advantages. (1) It is simple: mutagenesis can be of mature sperm rather than oocytes (the normal target for resealing experiments); (2) it can be highly efficient (Table 1); (3)

when resealings occur, both the *Dp* and *Df* portions of the resulting insertional translocation are recovered (unlike the situation in standard resealing methods, or in simple induction of deficiencies); and (4) it can be used to insert dominant markers into particular autosomal locations. B^S insertions have accompanied several of the resealings. We expect that y^+ could be similarly inserted, but because only three resealings involved an original Y^S break, we have insufficient evidence on this point. However, the resealing protocol could conceivably be used to insert these dominant markers, or any others similarly placed on a *y* chromosome, into any desired autosomal site. One obvious use would be the linking of cell autonomous markers to important autosomal loci for use in fate mapping studies.

As a first step in characterizing this phenomenon, this analysis inevitably raises more questions about the chromosomal control of fertility than it answers. Considerable further study is required before we can begin to characterize the nature of the fertility mechanism that is altered when these chromosomal rearrangements are combined with proximal *X* deficiencies. Several of the observations that have been discussed can be summarized in the following points, which any revised model of control must satisfy:

1. Maintenance of autosomal tip-centromere continuity is neither a necessary (*cf.*, Table 3) or sufficient [some *T(Y;2)* insertional translocations that leave autosomal tips and centromeres joined are sterile, *c.f.*, LINDSLEY *et al.* 1979] condition for fertility in the presence of *bb* deficiencies.

2. However, most *T(Y;A)* and *T(2;4)* lines tested are sterile with a bb^l, except those that involve autosomal breaks distal to all cytologically visible bands (LINDSLEY *et al.* 1979).

3. Many secondary rearrangements that restore fertility of otherwise sterile *T*(Y;2) lines are *T(Y;2;3)*'s, yet unselected *T(Y;2;3)* lines are themselves usually sterile with $bb^{l\text{-}158}$. In general, however, fertile *T(Y;2;3)*'s move only very distal portions of an autosome to the *Y*.

4. Replacing the Y^2 portion of a translocation with a complete *Y*, so as not to change the 2^Y chromosome, does not restore fertility with bb^l; however, simply involving the Y^2 piece in secondary rearrangements often does (again, see Table 3).

It is apparent that, whatever the ultimate resolution of these complexities, we are dealing with a rich new level of an intricate control structure for spermatogenesis in Drosophila and perhaps other animal species as well.

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