Once you have found a new mutation, you will need to know where it maps. If it has been induced in a scheme that is not directed at a particular region of the genome, you must resort to the tried and true techniques of linkage and recombinational analysis.

Linkage analysis relies on Mendel's principle of segregation of alleles—that the two homologs of a chromosome segregate reliably and inexorably from each other during the first meiotic division. Therefore, if the mutation is on the opposite homolog from a dominant marker, and if there is no recombination between the two homologs, they will segregate from each other in every meiosis and thus in all the progeny. This segregation cannot be confounded by recombination in males because they have none. In females, ambiguities due to recombination can be prevented by using balancer chromosomes.

Chromosome segregation, like all things biological, is not foolproof. Improper segregation does occur, but generally at a rate so low that it is of no consequence for our purposes. It can become significant and troublesome, however, when rearranged chromosomes are involved, such as translocations or multiple inversions. Under certain circumstances, these will not pair and segregate normally. The resulting gametes do not contain a normal genetic complement but may have too much or too little genetic material. This condition is called aneuploidy (as opposed to having the proper genetic complement, called euploidy). Since aneuploidy at the level of whole chromosomes (with the exception of chromosome 4, XXY, and X0) is lethal to embryos, occasional failures of segregation will not even be noticeable in the adult progeny that you score in your analysis.

Recombinational analysis relies on the fact that the frequency of chromosomal exchange between two loci is related to the distance between them. The recombination occurs in females who are heterozygous for your mutation and for other markers on the same chromosome. The basic anal-
ysis has not changed since its first use by Sturtevant to establish the order of genes on the X chromosome of the fly in 1913. In this way, you can establish an approximate position for your gene prior to zeroing in more accurately with deletions, duplications, and breakpoints.

Mapping strategies can also be used to identify genes if there is a convenient assay for the product or for a particular phenotype. Such strategies rely on chromosomal rearrangements systematically to create deletions or duplications of regions of the genome and can serve as an efficient precursor to mutagenesis; i.e., you can often identify the general region containing a gene you want, then screen for mutations there.

SEGREGATION ANALYSIS

Tracking a mutation by its segregation from a dominant marker is fundamental to all mating schemes in the fly. As shown previously in the discussion of mutagenesis schemes, this is what identifies unambiguously which flies are carrying recessive mutations. It requires dominant markers and balancers for the second and third chromosomes. Linkage to the X is easier to see, since recessive mutations are hemizygous in males and, if introduced from a male, are only transmitted to female progeny. The fourth chromosome is usually left out of these schemes. If a mutation does not map to the X, second, or third, linkage to chromosome 4 can be confirmed. There are dominant markers for chromosome 4, such as the dominant alleles of eyeless (eyD) and cubitus interruptus (ciD), but no balancers, since it does not undergoexchange even in females (except in triploids).

Dominant mutations are always the easiest to map, because they can be directly scored in the next generation. One simply mates the mutant flies to a stock carrying dominant markers on one or more of the other chromosomes, selects male progeny heterozygous for all of the dominants, then mates them to a wild-type strain to score segregation in the next generation. When males are used, there is no danger that exchange will cause the mutation to become linked to one of the markers. This could become a problem if the mutation maps far from the marker for its chromosome, because loci that are distant from each other on the same chromosome will appear to assort independently, as if they were unlinked, when exchange is active. An example of a cross to test segregation of a dominant (Dom) is:

$$\frac{Dom^?}{+} \times \frac{In(2LR)O,Cy}{Sco}, \frac{TM6}{Sb}$$

$$\downarrow$$

$$\frac{Sco}{Dom^?} \times +$$

Then score whether the new dominant segregates from Sco or Sb
P-element insertions, either new transformants or enhancer-trap jumps, can be mapped by the same strategy, since these lines are usually marked with the wild-type alleles of rosy (ry') or white (w') and will act as dominant on a mutant background of ry or w (see p. 39).

\[
\frac{w}{Y'} P[w^+] \times \frac{w}{w'} \text{ln}(2LR)O,Cy \quad \text{or} \quad \frac{w}{w'} \text{TM3, Ser} \quad \frac{w}{Sb} \]

\[
\frac{w}{Y'} \text{ln}(2LR)O,Cy \quad \frac{w}{Y'} P[w^+] \quad \text{or} \quad \frac{w}{Y'} \text{TM3, Ser} \quad \frac{w}{w'} \frac{w'}{w'}
\]

Then score whether \(w^+\) segregates from Cy, Ser, or neither.

Recessive mutations are not much more difficult to map, requiring only the generation of males and females heterozygous for both the mutation and the markers. That is, when testing for segregation of a recessive mutation, you must mate heterozygotes to reveal the presence of the mutation. This means that balancer chromosomes must be used as the source of dominant markers in the heterozygous females to prevent exchange.

**Problems with Balancers**

It would be too good to be true if balancers didn’t also present some problems. Although these are not all pertinent to the use of balancers for segregation analysis and meiotic mapping, and will matter more in the next chapter on Synthesizing Specific Genotypes, there is enough reason to mention them now.

One kind of problem, already alluded to, is the probability (albeit low) of a recombination event causing breakdown of a balancer. For this reason, it is wise when keeping and using balanced stocks to check routinely for whether all the flies have the markers they are supposed to have. This simple bit of compulsiveness can save a great deal of time and trouble by allowing the detection of such an event before it takes over the stock. The best way to deal with the discovery of an inappropriate fly is to discard that particular bottle or vial if there are other copies that show correct markers, or to start several new lines from single pairs of males and virgin females carrying the appropriate markers.

A more common problem occurs when a cross requires the generation of flies carrying balancers for two different chromosomes. Females
heterozygous for two different balancers produce far fewer viable offspring than females carrying only one. This is due to the dependence of chromosome segregation at meiosis on exchange between homologs. When no rearrangements are present, the vast majority of X-chromosome and all of the second and third chromosome homologs recombine during female meiosis. When one copy of an inversion chromosome is present, it too still segregates correctly from its homolog, but when two different chromosomes have heterozygous inversions, segregation runs amok. Exchange is prevented and they pair inappropriately. The result is that the wrong chromosomes segregate from each other (e.g., a second chromosome can segregate from a third chromosome or an X from a second chromosome) to produce aneuploid gametes and dead embryos.

Correct pairing and segregation (no balancers)

Correct pairing and segregation (one balancer)

Incorrect pairing and segregation (two balancers)
An attached-X in the same stock with a second or third chromosome balancer will have the same problem of incorrect pairing and segregation. Males, in contrast, do not have such problems. Even in the presence of multiple balancer chromosomes, segregation occurs normally.

A secondary difficulty that surfaces when multiple, heterozygous inversions are present in the same stock is that the likelihood of balancer breakdown increases. This is due to another anomaly, called the interchromosomal effect, in which the heterozygous rearrangements suppress exchange in part of the genome, increasing the likelihood of recombination occurring elsewhere. Thus, the presence of one balancer increases the probability of a rare breakdown event in another balancer. Although not nearly as common a problem as the incorrect segregation described above, this can still be an annoyance. It becomes more than an annoyance when mapping a new mutation meiotically, as it distorts the map distances and accentuates the chances of otherwise rare exchange events.

The remedy for these problems is to anticipate that crosses involving females with two balancers will go poorly and to try to circumvent them in the design of the mating scheme. The easiest way to do this is to transmit the two balancers through males, since they segregate chromosomes perfectly well regardless of the presence of inversions. If there is no alternative, use many more flies than you would normally and keep a sharp eye out for balancer breakdowns in the progeny.

MEIOTIC MAPPING

Once the linkage of a new mutation is known, the most efficient way to find its location on the chromosome, in the absence of cloned pieces or sequence information, is to map it by recombinational analysis. This generally involves a multiply marked chromosome, whose mutations lie at intervals along the chromosome, and a similar chromosome that also carries a dominant marker. The basic strategy is to generate females heterozygous for the chromosome bearing the new mutation and for the marker chromosome. These will recombine during meiosis, and the various classes of recombinant chromosomes, produced in proportion to the distance between markers and mutation, will go into her eggs.

The task is then to measure the proportion of different recombinant chromosomes and thus determine the position of the new mutation relative to the markers. To obtain a preliminary map interval, it is easiest to cross the heterozygous female to another version of the marker chromosome—one that contains a dominant marker as well. Then, males carrying recom-
binant chromosomes can be recognized and picked up as heterozygotes for the dominant marker. Whether a male has a particular recombinant interval can easily be scored by how many of the recessive markers are now homozygous. These males are then mated individually to the original stock carrying the lethal mutation to assess the presence of the lethal on the recombinant chromosome.

A simple mapping scheme for a new, recessive lethal (ɛ) on the third chromosome uses a multiply marked chromosome known affectionately as "ruca" which carries roughoid (ru) 3-0.0, hairy (h) 3-26.5, thread (th) 3-43.2, scarlet (st) 3-44.0, curled (cu) 3-50.0, striped (sr) 3-62.0, ebony (e) 3-70.7, and claret (ca) 3-100.7, and its cognate chromosome "ruPrica" which also has the dominant marker Prickly (Pr) 3-90.0:

\[
\frac{TM6, Ubx}{ɛ} \times \frac{ru \ ca \ cu}{ru \ cu \ ca} \quad \text{P}
\]

Essential to collect females ♀♀ ru cu ca ɛ ♂♂ ruPrica ♂♂ F1

Select males heterozygous for ɛ and markers.
Select males heterozygous for ruPrica and with cross-overs in each interval, recognizable by homozygosity of appropriate markers, and cross individually to original lethal-bearing chromosome.

ru (ɛ?) + ruPrica
ru h (ɛ?) + ruPrica
ru h st (ɛ?) + ruPrica
ru h th (ɛ?) + ruPrica
ru h th st (ɛ?) + ruPrica

ru h th st cu (ɛ?) + ruPrica
ru h th st cu sr (ɛ?) + ruPrica
ru h th st cu sr e (ɛ?) + ruPrica
ru h th st cu sr e ca (ɛ?) + ruPrica

♀♂ ♂♂ × TM6, Ubx

♂♂ TM6, Ubx ♂♂ ♂♂ F2

Score progeny for absence of Ubx and Pr, indicative of the presence of the lethal on the recombinant chromosome.
(These hypothetical recombinant chromosomes are drawn to reflect single cross-over events. In reality, many would have double cross-overs [e.g., ru h + + sr e ca] but could still serve your purposes.)

This analysis defines the chromosomal interval in which the new mutation lies and can be accomplished with a few flies of each genotype. Finer grain localization can then be carried out either by deletion mapping with available deficiencies of the region or by collecting many recombinants between the two markers in an interval and scoring the actual percentage of cross-overs separating the lethal from one versus the other.

Map positions, as reported in Lindsley and Zimm (1992), are denoted by the chromosome followed by a normalized value for the meiotic map position, e.g., (2-36.8). The normalization is an attempt to order the genes on the chromosome in an approximate fashion by adding map values from left tip to right tip of the chromosome. Thus, it is possible for a locus to have a map position of greater than 50 map units, even though no one could ever measure a value greater than 50 in a single mapping experiment. These numbers, and the gene order derived from them, are to be taken and used with caution. Direct mapping between specific loci is the only way to obtain reliable results. Meiotic map positions are standardly measured using heterozygous females that have been raised at 25°C. This is because recombination frequency is sensitive to temperature (Ashburner 1989).

Some of the value of traditional meiotic map positions has diminished as the molecular mapping of the fly genome has progressed with its correlation of loci with sequences. The persisting value of meiotic mapping lies in the narrowing down of a new mutation’s position from that of the whole chromosome to a rough interval and in the demonstration that a phenotype is due to a single gene. This can be readily accomplished by using a few markers and obtaining a relative map. Once the interval is known, a finer localization can be obtained with deletions or with marked P-element insertions mapped to the region.

An important issue to bear in mind is that the markers should not interfere with the phenotype of the new mutation. If the new mutation causes an embryonic pattern defect that can be scored in an individual offspring, there will be little danger of interference from markers. If, on the other hand, scoring requires a population of flies of like genotype (e.g., for a behavior mutant), there could be significant trouble due to marker phenotypes that blur the distinction between behavioral mutant and wild type. In such a case, using a hypothetical example on chromosome 2, one might set up crosses that test for the presence of two benign markers [straw, stv, 2-55.1 for bristle color and brown, bw, 2-104.5 for eye color] and generate the population of flies to be tested for the new mutation (n) in parallel.
Virgin females
in which crossing-over can occur

\[
\frac{\text{stw; bw}}{\text{stw; bw}} \times \frac{\ln(2LrO,Cy)}{n}
\]

Collect males heterozygous for either \(\ln(2LrO,Cy)\) or \(Sco\), who will carry potentially recombinant chromosomes, and mate them individually in vials to a mixture of two types of females

\[
\frac{\text{stw; bw}}{\text{stw; bw}} \times \frac{\ln(2LrO,Cy \text{ or } Sco)}{n}
\]

\(\text{}\)

\(\text{Single } \sigma\)

\(\text{}\)

After a suitable mating period (5-7 days), place the two types of females into separate vials and score progeny

What markers are present?

Is \(n\) present?

The relative proportion of lines that have \(\text{stw with } n, \text{ bw with } n, \text{ both with } n, \text{ and neither with } n\), will tell which marker \(n\) is closer to and whether it is between them or outside of them. (The closer \(n\) is to a marker locus, the less often will recombination occur between them and the more often will \(n\) segregate with the allele \([\text{marker}^+\text{ in this case}]\) to which it was originally linked.)

PROBLEM 7 Assume you have just isolated a new, viable learning mutation on an X chromosome already carrying vermilion (\(v\)) at 1-33.0. Design a scheme for mapping the new mutation relative to \(v\) and to yellow (\(y\)) at 1-0.0 and forked (\(f\)) at 1-56.7.

\[y \quad v \quad f\]

Relative positions of \(y\), \(v\), and \(f\)
DELETION MAPPING

Once the approximate location of a mutation is known from meiotic mapping, a more accurate placement can often be assigned by testing various chromosome deletions for their ability to uncover a recessive mutant phenotype. When a mutation is caused by an insertion element, concordance of the mutant phenotype with the site of insertion can be tested most rapidly by deletion mapping. The smaller the deletion, the more accurate the localization down to the limiting case of a chromosomal breakpoint.

This kind of mapping is indistinguishable from a complementation test in which one is testing for the failure of complementation between a deletion and a mutation (see Chapter 2). After testing a variety of deletions for a given region, the gene can be localized to the shortest interval between the available breakpoints.

Most deletions have been induced by radiation. Some result from chemical mutagenesis (e.g., EMS), and an increasing number are being created by imprecise P-element excisions. These latter excision-generated deletions are often (but by no means always) small, generally not affecting more than one locus or its immediate neighbor. They are listed in Lindsley and Zimm (1992) and in the updated computerized FlyBase (see Appendix).

For those regions of the genome that cannot be conveniently tested with existing deletions, it is possible to synthesize a deletion using chromosome rearrangements specifically designed for the purpose (see section below on Synthesis of Deletions and Duplications). Alternatively, it is also possible to generate new deletions by the technique of imprecise P-element excision (see section on Inducing Deletions in Chapter 4).

DUPPLICATION MAPPING

Precise localization of a locus can make use of any kind of chromosome aberration to bracket the gene between known breakpoints. Duplications serve this purpose when a recessive phenotype can be covered (i.e., rescued) by the presence of a duplication carrying the wild-type locus. Duplications are often the reciprocal products of the radiation events that produce deletions: A chunk of chromosome is chopped out and re-inserted elsewhere.

Since a test of rescue by a duplication requires introducing three components, the two mutant alleles and the duplication, it is desirable to use duplications in which the insertion has gone into a different chromosome. An illustration of such a test is shown below for a second-chromosome
lethal (ɛ) mutation with a duplication of the segment containing the wild-type engrailed locus inserted into the third chromosome:

\[
\sigma\sigma \quad \text{In}(2LR)O, Cy, \quad \text{Dp}(2;3) \quad \text{en}^+ \quad \text{Sb} \quad \frac{\text{ɛ}}{\text{ɛ}} \quad \frac{\text{ɛ}}{\text{ɛ}} \quad \text{In}(2LR)O, Cy \quad \varpi \varpi
\]

See if there are any \( \text{ɛ}\) \( \text{Dp}(2;3) \) \( \text{bw}^+ \) flies in the progeny \( \text{ɛ}^+ \) \( \text{ɛ}^+ \)

If there are, this indicates that \( \text{Dp}(2;3) \) \( \text{en}^+ \) covers the lethality of \( \text{ɛ} \). This localizes the mutation inside the breakpoints of the duplication. The approach is not foolproof, however, if a second lethal had by chance been induced on the same chromosome. A duplication capable of rescuing one lethal would not rescue the other, producing a false negative. To circumvent this problem, one can use a different, independently isolated allele of the gene if it exists, since it would never have the same two random lethals induced. (See Chapter 4 for techniques to synthesize strains.)

**SYNTHESIS OF DELETIONS AND DUPLICATIONS**

Not all regions of the genome are represented by existing deletions and duplications. Those that are can be obtained as a kit from the Drosophila Stock Center at Bloomington, Indiana (see Appendix). If you are stuck with solving the problem yourself, strains were constructed by Lindsley et al. (1972) to permit synthesis of duplications almost at will and, to a lesser extent, deletions. These strains are translocations between either the second or third chromosome and a \( Y \) chromosome marked with \( y^+ \) (the wild-type allele of yellow body color) and \( B^S \) (an allele of the dominant eye mutation Bar of Stone) known as \( T(Y;A)Ls \). Each translocation has the general structure:

```
Bal

\( y^+ \) \( B^S \)
```

in which there is a reciprocal translocation between marked \( Y \) and autosome such that each piece of the broken autosome has a different \( Y \)-linked marker and a centromere. (Chromosome fragments have no future if
they don't have centromeres.) The entire autosome is present, but it is now broken into two pieces. These are balanced by an appropriate autosomal balancer chromosome (Bal). By choosing two T(Y/A)s with relatively nearby breakpoints and with opposite configurations of y* and B⁸:

```
           Bal
          /     \
         /       \
        /         \
   y*        y*  B⁸
```

one class of progeny is produced that gets the short end of the translocations, producing a deletion; another class gets the long ends, producing a duplication; and all the others are euploid (normal amount of genetic material).

```
       Deletion
       /     \   
      /       \  
     /         \ 
   y*        y*  B⁸

       Duplication
       /     \   
      /       \  
     /         \ 
   y*        B⁸  y*
```

The deletion is uniquely marked with two doses of y* but not B⁸, whereas the duplication is unique with two doses of B⁸ and none of y*. The aneuploid (deleted or duplicated) progeny are a small fraction of the total progeny at best, and reduced viability associated with aneuploidy can decrease the recovery even further. An analogous set of translocations between the X and the same marked Y were generated by Stewart and Merrim (1973) and are known as T(X;Y)s.

The initial study for which these rearrangements were made surveyed the "gross structure" of the Drosophila genome (Lindsley et al. 1972) and defined several haplo-lethal loci (lethal when present as a heterozygous deletion), one of which is also triplo-lethal (lethal when duplicated). In general, they found that heterozygous deletions larger than one numbered region and duplications larger than four or five numbered regions were lethal. These are only averages, however, and there are examples of very
large duplications (e.g., the entire left arm of chromosome 2) and deletions (e.g., 37B-408) that are viable.

In the paleolithic age prior to cloning, these stocks were successfully used to identify structural loci for several enzymes of neurobiological interest by "dosage effects." The strategy relied on the fact that duplications and deletions of these loci were not dosage compensated. That is, flies with a duplicated enzyme locus would have roughly 1.5 times as much enzyme as a normal, euploid fly. Those that were heterozygous for a deletion would have roughly 0.5 as much as euploids. (In reality, the values are quite variable and average 1.3 for duplications and 0.6 for deletions.) Crosses of 30 T(Y^2A)s produce a series of contiguous duplications for chromosomes 2 and 3. Crosses of two appropriately chosen T(X;Y)s are sufficient to produce duplications for each half of the X. The fourth chromosome can be tested in one cross between a stock carrying an attached-4, C(4)RM, and a stock homozygous for a fourth-chromosome marker (e.g., eyeless, ey) produces progeny that are either triplo-4 or haplo-4.

\[
\begin{array}{c}
C(4)RM \\
\times \\
\uparrow \\
\downarrow \\
\text{ey} \\
\text{ey} \\
\text{Tripl}-4 & \text{Haplo-4} \\
M^+ ey^+ & M ey
\end{array}
\]

Half of the progeny will have three copies of chromosome 4 and half will have one copy. Those with three copies will look wild-type, those with one copy will look eyeless (ey), due to hemizygosity for the mutation, and Minute (M), a phenotype characterized by thin bristles and retarded development, due to hemizygosity for the Minute locus on chromosome 4 (see Chapter 4 for discussion of Minutes).

In this fashion, loci for acetylcholinesterase (Hall and Kankel 1976), dopa decarboxylase (Hodgetts 1975), cAMP phosphodiesterase (Kiger and Golany 1977), and choline acetyltransferase (Greenspan 1980) were initially found. It was because of this cytological localization of cAMP phosphodiesterase that the molecular identity of the learning mutant dunce was solved, based on the initial realization that they mapped to the same place (Byers et al. 1981; story recounted in Greenspan 1990).

When using these stocks, it is best to carry out a series of pilot crosses (a vial or two) to make certain they are behaving as they should. This is...
much easier than making chromosome squashes from each. Y-autosomal breakpoints are difficult to score. The markers indicating duplication and deletion progeny are one indicator. If the two strains in the cross have breakpoints that are nowhere near each other, no duplication or deletion progeny will be produced. If they are several numbered regions apart, duplications should survive but not deletions, and so on. A more detailed characterization involves scoring the proportion of duplication progeny that are male versus female (Lindsley et al. 1972; Ashburner 1989).

To figure out which markers should indicate duplication and which deletion, one need only consult the list of these stocks to see which arm of the Y chromosome is broken in the translocation, either the short arm (Y5) or the long arm (Y4). In the original Y chromosome, B5 was on Y4 and y+ on Y5:

The attrition of these stocks over the years has been significant, but many still remain. Some have lost the B5 marker, but this is not always a problem if one of the two strains retains it.

**PROBLEM 7** Determine what classes of progeny you could distinguish if you mated two T(Y;A)5s, both of which have breakpoints in Y5. Determine what classes of progeny you could distinguish if you mated two T(Y;A)4s, one of which has a breakpoint in Y5 and one of which has a breakpoint in Y4 but has lost its B5 marker.

The strength of this technique for adult flies is obvious. For embryos and larvae, on the other hand, B5 is completely unrecognizable and y+ is scorable only in third-instar larval mouth parts and setae (tiny hairs). It is thus not possible to know which progeny are duplicated or deleted for the segment at these pre-adult stages. This precludes most kinds of analysis except for those in which one needs only to see a distinctive phenotype in some fraction of the progeny, as in the identification of new loci.
Identification of New Embryonic Genes by Deletion Mapping

Most deletions are homozygous lethal. This severely limits their utility for identifying new loci based on phenotype unless the phenotype can be scored at the lethal stage. For embryonic development, this is quite feasible, and a variety of new loci have been identified starting from a morphological defect in 25% of the progeny of a strain heterozygous for a deletion, or in the aneuploid progeny of a T(X;Y). Even when multiple genes are deleted, as is virtually always the case, the lethal phenotype is predominantly caused by a single, early-acting gene. This is ultimately sorted out by isolating individual mutations in the deleted region (see Chapter 2) and seeing which, if any, recapitulate the phenotype of the homozygous deletion. One is limited only by the ability to screen for morphological aberrations.

The single-minded (sim) locus was identified in this fashion, starting from a screen of embryos from heterozygous stocks of autosomal deletions. Dead embryos were stained and examined in whole mounts. Aberrations of the ventral nerve cord were found in homozygotes for a 14-band deletion Df(3R) ry619 (Thomas et al. 1988). EMS mutagenesis and a screen for lethal mutations uncovered by this deficiency confirmed that this phenotype was due to a single gene defect.

More dramatically, one of the earliest-acting zygotic genes was identified through deletion mapping as being predominantly responsible for the phenotype produced in an embryo having no X chromosome (nullo-X). Nullo-X embryos have long been known to arrest development very early with no cellularization at blastoderm stage (Poulson 1940). This was always assumed to be a "syndrome" due to the loss of 1/5 of the genome. Wieschaus and Sweeton (1988) created large deletions of the X to define the region responsible for this phenotype. They mated T(X;Y) males to attached-X females (each with its own intact Y) and cleverly scored which portion of the X an embryo received by the presence or absence of the folded-gastrulation (fog) phenotype. fog- embryos fail to form a posterior midgut and so are easily recognized.
Those embryos receiving the Y from their mothers and a fragment of the \( T(X;Y) \) from their fathers will have large deletions of the X, distinguishable by the presence or absence of the posterior invagination induced by \( fag^* \) activity. After obtaining a rough localization by this method, these investigators zeroed in on the locus by means of careful deletion mapping of the embryonic phenotype. This revealed, contrary to previous expectation, that a single, two-band region was responsible for the nullo-X phenotype of failed cellularization. The nullo locus was ultimately identified as the culprit (Rose and Wieschaus 1992).

Taking this approach to the autosomes, Merrill et al. (1988) utilized a different set of chromosome rearrangements to produce complete or partial deletions of autosomal arms. They used compound autosome stocks in which the two left arms are attached to a common centromere and the two right arms are attached to a common centromere. In most stocks of this sort, each set of compound arms is also homozygous for a recessive marker for ease of determining if the stock is uncontaminated (see p. 11).

When males and females of this stock are mated, some will lack 2L and others will lack 2R. (Segregation of these compound autosomes in males occurs randomly, whereas in females the 2L will segregate regularly from the 2R. Thus, males will produce gametes containing random mixtures of the various chromosome rearrangements, whereas females will produce gametes that contain one or the other.) A similar stock exists for the third chromosome. To monitor the frequency of the various classes of segregants in these stocks, they were crossed to embryonic cuticle pattern mutants located on each arm.

From this analysis, Merrill et al. (1988) were able to determine which chromosome arms carried genes that acted prior to gastrulation, similar to the nullo-X locus previously identified. By combining various \( T(X;Y) \)'s with the C(2L;2R) stock, they were able to create smaller deletions of the appropriate arms.
A small fraction of the progeny embryos from this cross will be totally deleted for the distal left arm:

\[ y^+ \]

This analysis identified seven new loci with developmental defects preceding gastrulation, two of which affected cellularization.

Compound chromosomes of this sort are produced by radiation. To introduce recessive markers onto their arms, which is essential for knowing what you have, one produces triploid females in which the one normal set of chromosomes carries the desired recessive mutations, \( m_1, m_2, m_3, m_4, \) and \( m_5 \) (e.g., Hardy 1975):

\[
\begin{align*}
X & & 2L & & 2R & & \quad 3L & & 3R \\
\quad m_1 & & \quad m_2 & & m_3 & & \quad m_4 & & m_5
\end{align*}
\]

**Mapping by in situ hybridization**

A technique that has become as standard as linkage analysis for mapping insertion sites of P-elements is in situ hybridization. It represents the fastest and most direct way to localize a new insertion site. However, a mutant phenotype must be independently mapped to the site of a new insertion, either by excision, deletion, or recombination analysis. Techniques for in situ hybridization are described in Ashburner (1989).

Cytological positions obtained from in situ hybridization (or from analysis of chromosome breakpoints) are expressed as "band numbers" in reference to the chromosome bands visible in preparations of larval salivary gland chromosomes (see p. 6).

The correspondence between meiotic map positions and cytological band positions is not a simple one, varying with position along the chromosome. Specific correlations can be found (or guessed at) in the computerized listing of FlyBase.