Fly Pushing

The Theory and Practice of *Drosophila* Genetics

SECOND EDITION

FREQUENTLY USED MARKERS

heterozygous ♀ hemizygous ♂

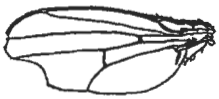
Bar (*B*) *X*
eye narrower than usual, oval shape

forked (*f*) *X*
bristles short with split or bent ends

singed (*sn*) *X*
bristles short, gnarled, and wavy

Curly (*Cy*) *2nd*
wings curled upward instead of flat

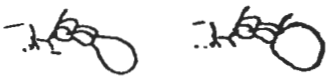
Scutoid (*Sco*) *2nd*
missing bristles, especially
from posterior thorax



Serrate (*Ser*) *3rd*
(also called *Beaded-Serrate*, Bd^{S})
wings notched

Dichaete (*D*) *3rd*
wings extended like jet plane
instead of straight back

Stubble (*Sb*) *3rd*
bristles short and stubby



+/+ *Ubx/+*

Ultrabithorax (*Ubx*) *3rd*
haltere larger and rounder than normal

eyeless-Dominant (ey^{D}) *4th*
very small eyes

Introduction

WITH EACH PASSING YEAR, MORE AND MORE SCIENTISTS are attracted to work with *Drosophila*, lured by the potential for combining genetic and molecular approaches to questions of gene expression, cell biology, development, and neurobiology. They are aware of a large folklore of classical genetic tools lurking somewhere in the fly field—they have seen the results in some of the dazzling findings that have been made—but access to these tools somehow seems to be limited. At times, it may even appear that the wielders of "hard-core" fly genetics preside over a coven with secret rites of initiation. The situation has led many to bemoan the lack of a simple and rational way to gain access to Drosophilid mysteries.

Publication of a compendium of facts and commentaries on *Drosophila* genetics and biology by Michael Ashburner (originally in 1989, with a new edition, Ashburner et al. 2004) has provided fly workers with a comprehensive reference source for all of the folklore (and much more). Still lacking, however, is a bridge to that folklore for the uninitiated.

This book attempts to provide that bridge. It is designed for those graduate students, postdocs, and even laboratory heads wishing to use fly genetics in their work. An elementary knowledge of genetics (e.g., undergraduate level) is assumed, but not much more. The approach used in this book has been worked out over the years in lectures given as part of intensive short-term courses at the Cold Spring Harbor Laboratory, at the University of California at San Francisco, and in my own laboratory. This approach owes a major debt to the pedagogical tradition of the late Larry Sandler as modified and transmitted by his student (my mentor), Jeff Hall.

Although the book does not attempt to be comprehensive, it does refer to more complete treatments of particular topics elsewhere (such as the aforementioned Talmudic commentaries of Ashburner).

Each subject is addressed from a practical standpoint, with a bit of the theoretical foundations (such as they are) to make it intelligible. Illustrations of each technique are taken from the literature and practice problems are provided. Although the working out of problems is helpful in learning the principles, it must be borne in mind that there is no substitute for actually doing genetic experiments and crosses—"fly pushing," as it is affectionately known.

CHAPTER 1

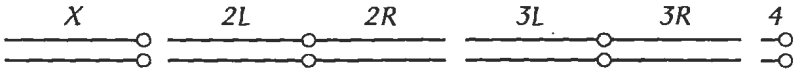
The Basics of Doing a Cross

OVER THE PAST CENTURY, FLY GENETICS HAS BEEN developed to a high art. Some of this is the result of time and accumulated information but much of it is due to the construction of several unique genetic "tools," plus a few intrinsic features of fly biology. The net effect is to make mating schemes more reliable and unambiguous by controlling the randomizing and shuffling effect of recombination in meiosis, and to make the physical location of genes on chromosomes easier to determine. The following sections present some of the basics of fly genetics and looking after fly cultures, as well as a beginner's Rosetta stone of fly nomenclature.

FLY CHROMOSOMES

Flies have four pairs of chromosomes, usually represented as lines and circles for arms and centromeres:

Female



Male

L refers to the left arm and *R* to the right. The *X* and fourth chromosomes

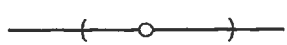
have major left arms and tiny right arms (standardly drawn with no right arm). The size of the *X, 2L, 2R, 3L,* and *3R* are roughly comparable, whereas chromosome *4* is only about one-fifth as large.

Sex determination in *Drosophila* is based on the ratio of *X* chromosomes to autosomal sets. In males, one *X* with two autosomal sets gives a ratio of 0.5, whereas females have a ratio of 1.0. The *Y* chromosome contains few genes and is not required for most aspects of male development, only for proper sperm motility.

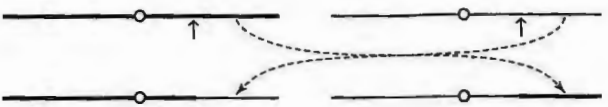
An important feature of fly genetics is the total absence of recombination in males. Whereas recombination is usually lower in the heterogametic sex of a species (the sex with two different sex chromosomes, usually the males), in *Drosophila melanogaster* it is effectively zero. Recombination in females, on the other hand, is alive and well. Its control is achieved by the use of the fly pusher's most distinctive tool: balancer chromosomes. These are chromosomes whose normal gross sequence is so scrambled (the result of multiple breaks and rejoinings induced by radiation) that they are no longer capable of pairing or recombining with their normal homolog during meiotic prophase. In addition, their presence in a fly is easily recognizable by a dominant marker mutation and they contain recessive markers as well. Consequently, their transmission to progeny can be tracked unambiguously. Since they effectively block any recombination with their homologs, the transmission of the homolog to progeny can also be tracked unambiguously. This holds true even if the homolog has no dominant marker, because homologs segregate reliably. Thus, if the progeny did not get the balancer, it must have gotten the homolog. This is the single most important principle in fly mating schemes.

Balancers, which are discussed in more detail later in this chapter, are a special case of the more common kind of chromosome rearrangement known as inversions. Other rearrangements that appear in the course of our discussions are translocations, compound chromosomes, deficiencies, and duplications. The major categories of rearrangement with their representations are listed below.

- Inversions, in which two breakage and repair events have occurred in the same chromosome, resulting in an inverted segment (the breakpoints are symbolized by parentheses):

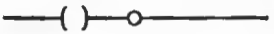


- Translocations, in which breakage events have occurred in two different chromosomes and repaired so that the pieces are swapped:

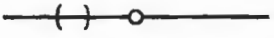


- Compound chromosomes, in which two left or two right arms have become attached to the same centromere (e.g., attached-*X*, attached-*2L*, attached-*4*):

- Deficiencies (also called deletions), in which two breaks have occurred in the same chromosome and the repair event has excluded the excised piece:

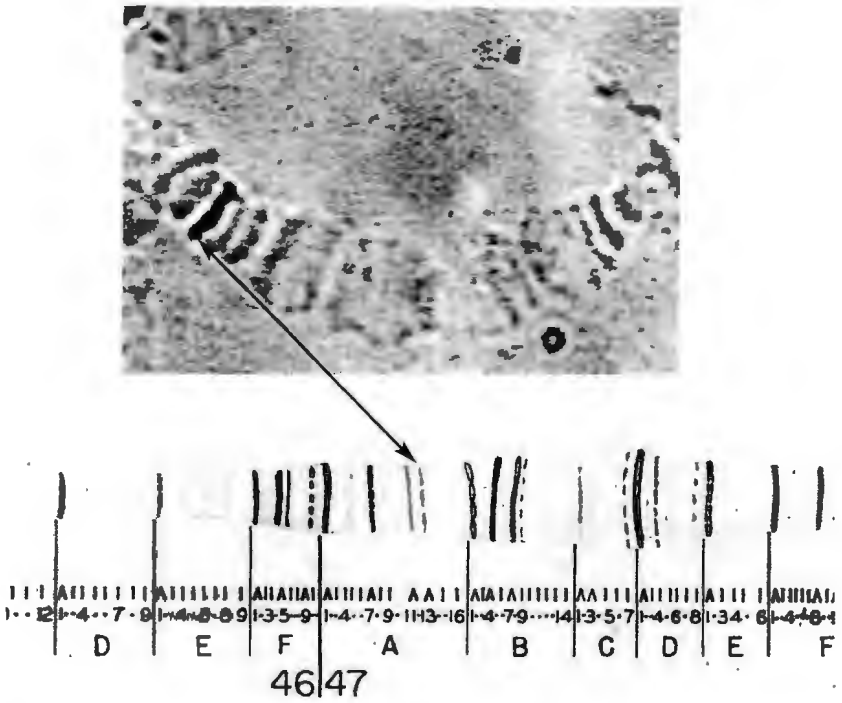


- Duplications, in which an excised piece is inserted into another chromosome:



Another important intrinsic feature of the fly is the presence of polytene chromosomes in the salivary glands. These have distinctive, high-resolution banding patterns. Early on, they made it possible to correlate map positions of genes with physical features of the chromosome and determine the breakpoint locations of chromosome rearrangements. In the molecular era, they have facilitated the mapping of cloned DNA sequences to physical locations.

Each of the major chromosome arms is divided into 20 numbered segments: 1–20 for *X*, 21–40 for *2L*, 41–60 for *2R*, 61–80 for *3L*, and 81–100 for *3R*. Chromosome *4* is divided into regions 101–104. Each numbered region is then divided into lettered regions (A,B,C,D,E) and each lettered region into numbered bands. (The number of lettered regions and numbered bands for each numbered region is not constant along the chromosome, but depends on local topography.) Centromeric regions are 20 for the *X*, 40–41 for the second, 80–81 for the third, and 104 for the fourth



In situ hybridization to larval polytene chromosome. (*Top*) Enzymatically stained DNA hybridized to band 47A11-14 region (courtesy of P. Tolias). (*Bottom*) Bridges' original drawing of the same chromosome region (Bridges 1916).

chromosome. The corresponding telomeric regions are 1 (*X*), 21 (*2L*), 60 (*2R*), 61 (*3L*), 100 (*3R*), and 101 (*4*).

RECOGNIZING MARKERS

Marker mutations are the key to deciphering genotypes. Sometimes they are used to mark the chromosomes that you are specifically trying to follow, but more often they mark the chromosome that you are trying to lose. In any event, a vast array of mutations affecting eye color, eye shape, wing shape, wing vein morphology, bristle color, bristle shape, and cuticle pigmentation—to name the major categories—serve to tag the various chromosome arms.

Descriptions of mutant phenotypes can be found in Lindsley and Zimm (1992), for those preferring the printed page, and on FlyBase for the cybernetically inclined (FlyBase Consortium 2003; see the Appendix). One simply becomes accustomed to recognizing these descriptions. The important points to bear in mind about markers concern their consistency of expression and their interactions with each other.

Consistency of expression is reflected in the likelihood that a fly of mutant genotype will show a mutant phenotype (penetrance) and if so, the extent of the range of those phenotypes (expressivity). The "rank" assigned to mutations in Lindsley and Zimm embodies these parameters, with the highest (RK1) showing the greatest consistency. When you select positively for a given marker, you can tolerate a certain amount of inconsistency. The worst that will happen is that you will miss a few flies. Much more dangerous, however, is selecting against a particular marker (i.e., saving flies based on the absence of the marker). In these cases, it is crucial to be able to rely on the marker's consistency of expression—or at least to be aware of any possible inconsistency. The RK rating helps you to discern the ones that are problematic until you get a feel for it.

Interactions between markers become important as soon as you find yourself in the situation of using two that affect the same trait. For instance, if you are using two different mutations affecting bristle shape, it is crucial to be familiar with the double mutant to determine whether it is distinguishable from each single mutant. Rarely can this information be found in Lindsley and Zimm or on the FlyBase (except for certain eye-color mutations). Instead, one often has to proceed empirically.

NOMENCLATURE

Fly nomenclature is a paper tiger.

From the uncollected sayings of Mao Zedong.

The shorthand of *Drosophila* genetics can be reduced to a few simple rules, illustrated in the examples that follow (see also FlyBase Consortium 2003).

1. $\frac{f;\ cn\ bw;\ TM2}{tra}$

This example demonstrates several points:

- The genotype of a chromosome is indicated only if there is a mutation or some other kind of variant on it, and the chromosomes are always listed in the order *X/Y, 2, 3, and 4*. In this example, *f; cn bw; TM2/tra* refer to the *X, 2,* and *3* chromosomes, respectively. If the pertinent mutations are on the *X* and *3* only, then you would skip any designation for chromosome *2*, e.g., *f; TM2/tra*.
- Fly genotypes (e.g., *f*) are always italicized, as are mutant and gene names. This book follows the traditional convention.
- Mutant names are abbreviated with three or fewer letters (although this rule has been abandoned in recent years because of the amount of mutants to name [e.g., *norpA* and *disco*]; *f* denotes *forked,* affecting bristle morphology, *cn* is for *cinnabar* and *bw* for *brown* (both affecting eye color such that together they produce a white eye), *TM2* indicates the balancer chromosome "third multiple 2," and *tra* denotes *transformer*, a gene required for sex determination.
- Lowercase abbreviations indicate recessive phenotypes and uppercase indicate dominants, with locus names taken from enzyme or protein names (e.g., *Adh* for the structural gene for *Alcohol dehydrogenase*) or, as in this case, a particular chromosome rearrangement (the balancer chromosome *TM2*).
- Semicolons separate the genotype symbols for different chromosomes. In the example above, genotypes of the *X, 2,* and *3* chromosomes are indicated.
- Commas follow the name of a rearrangement and indicate mutations on that chromosome (e.g., the full name of *TM2* is *TM2, Ubx^{130}* because it carries a mutant allele of *Ultrabithorax* known as number 130).
- A chromosomal genotype written on a single line indicates that the stock is homozygous for that genotype; heterozygosity is denoted by a two-line genotype (as in *TM2/tra* above), each line corresponding to one of the homologs present (in publications, the genotype would be denoted as *TM2/tra*, all on one line).
- Anything that is not shown is presumed to be wild type. Thus, *f* means that the *X* chromosome carries a mutant allele of *forked*; all other *X*-chromosome loci are presumed to be wild type. Similar-

ly, when heterozygosity is indicated, only the mutant loci (or rearrangements) are shown for each chromosome.

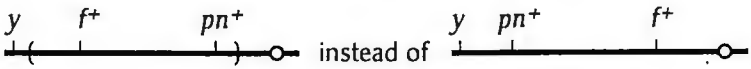
2. $\frac{C(1)RM,\ y^2}{Y}$; $\frac{In(2LR)O,\ Cy}{Sco}$; $\frac{ci^D}{ey^D}$

- *C(1)RM* denotes a compound chromosome. *C(1)* shows that it is a compound of the first chromosome, and *RM* refers to the fact that it is reversed metacentric, i.e., the centromere is in the middle (metacentric) and in the linear order of the chromosome. One arm is reversed relative to the other (i.e., both are attached at the same end); a common shorthand for *C(1)* is $\widehat{XX}$, attached-*X*.
- This particular attached-*X* is homozygous for the y^2 allele (the second one found) for the gene affecting cuticle color yellow; it has black bristles and a yellow cuticle, which differs from (the first) yellow by the fact that its bristles are yellow as is its cuticle.
- Since the attached-*X* contains both homologs of the *X* on one centromere, they do not segregate from each other. Usually these stocks are kept such that both males and females carry a *Y*; this is to ensure that all of the males will be fertile. (The presence of a *Y* chromosome has nothing to do with sex determination and has no effect on females with two *X*s, but it is essential for sperm motility. Since the *Y* segregates from the *X* in males, the only way their sons can receive a *Y* is if they obtain it from their mothers, a result of its segregation from the attached-*X*. This occurs reliably by having a *Y* present in all flies of the stock.)
- The genotype of the second chromosome in this stock is heterozygosity for two different chromosomes: One is a balancer known as *In(2LR)O,Cy* (sometimes referred to colloquially and variously as "*Curly-O*," *CyO*, "*Curly-Oster*," or "*Curly of Oster*," named after Irwin Oster, who produced it), which carries the dominant mutation *Cy* causing wing curling, and the other is a chromosome with the dominant mutation *Scutoid* (*Sco*, subsequently renamed noc^{Sco} as an allele of *no ocelli*), which eliminates certain thoracic bristles; the *In(2LR)* refers to the multiple inversions (*In*) involving the left (*L*) and right (*R*) arms of the second (*2*) chromosome.
- The final chromosome genotype refers to the fourth chromosome, heterozygous for two dominant alleles of genes for which most other alleles are recessive—*eyeless* and *cubitus interruptus*. This

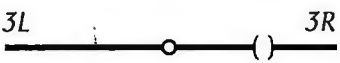
represents an exception to the convention of uppercase letters for dominant mutations, instead using a superscript *D* to indicate dominance of these alleles. Since both of these alleles are recessive lethal, all flies will be heterozygous for both (this apparent exception to the naming convention follows the rule that loci originally defined by recessive mutations, such as *ey*, will continue to be designated by lowercase letters even after dominant alleles are found).

- Chromosome rearrangements are designated by an abbreviated symbol followed by the chromosome affected (*1, 2, 3,* or *4*) and the name of the rearrangement. For example,

In(1)sc^{4} inversion on the *X* chromosome called "*scute 4*," producing a mutant phenotype because of a break in the *scute* locus (breakpoints are symbolized by parentheses):



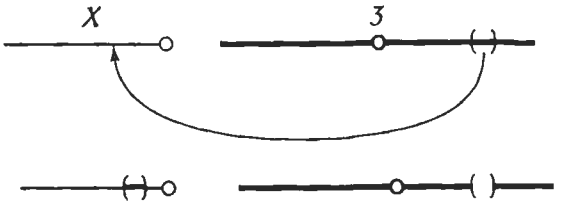
Df(3R)P14 a deletion (deficiency) of part of the right arm of the third chromosome (*3R*), whose name, *P14*, stands for Pasadena 14:



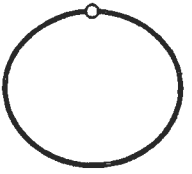
T(1;4)B^{5} translocation between the first and fourth chromosomes, in which there is a break in each and a reciprocal rejoining, producing a severe *Bar* eye phenotype called *Bar of Stone* (*B*^{5}):

Tp(3;1)ry^{35} transposition (*Tp*) of a piece of the third chromosome into the *X*, one of whose breakpoints produces a mutant phenotype in the eye-color gene *rosy*; sometimes designated as translocation *T(3;1)ry*^{35}; transpositions can also refer to the movement of a segment from one place to anoth-

er in the same chromosome. When this *X* is present in a strain whose third chromosomes are both normal, it becomes a "duplication," designated by *Dp(3;1)ry*^{35}:

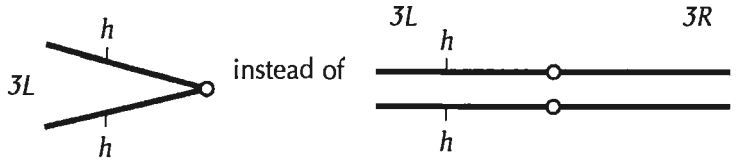


R(1)w^{vC} ring *X*, an *X* chromosome with no free ends. This particular one has abnormal expression (variegation, hence the "*v*") of the *white*^{1} gene affecting eye color (the "*C*" refers to Catcheside, its originator):



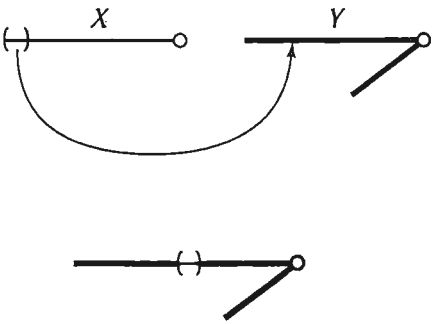
C(3L)RM, h compound chromosome consisting of two left arms of the third chromosome, also known as "attached-*3L*," homozygous for the original allele of the mutation *hairy* (*h*), producing extra hairs on the thorax and head. "*RM*" stands for reversed metacentric, jargon meaning that the two left arms of chromosome *3* are attached to the same centromere instead of each one being attached to a separate centromere with a right arm. "Metacentric" refers to the fact that the centromere is in the middle and "reversed" refers to the order of genes from one *3L* to the next. This reverses when you pass through the centromere, such that the normal *3L*-chromosome tips are still at the tips. This contrasts with a "tandem metacentric," in which the gene order would repeat identically

when passing through the centromere, and where the tip of one arm is now at the centromere:



F(2L) "free" left arm of the second chromosome, in which only a single left arm (*2L*) is on one centromere:

y+Y duplication on the *Y* chromosome of the chromosomal segment carrying a wild-type allele of *yellow*, also designated as *Tp(1;Y) y+*:



Note: On above figures, *Y* signifies the chromosome, *y* the *yellow* locus.

A new kind of gene name has emerged after sequencing the fly genome: "*CG*" (see FlyBase Consortium 2003). These genes, not previously identified, are those predicted from the genome sequence. Each one is designated by a number preceded by the letters CG (referring to Celera Genome), e.g., *CG5170*. CG names are temporary and will eventually give way to more descriptive gene names as functions are assigned to this vast set of many thousands of genes.

EVOLUTION OF GENE NAMES

Since genes are generally pleiotropic, independently isolated mutations with different phenotypes and different names often eventually turn out to be alleles of the same locus. When this occurs, the name that prevails for the locus is identified first, and the other name becomes a superscripted allele designation. For example, the olfactory mutation *smellblind* (*sbl*) turned out to be an allele of the sodium channel gene *paralytic* (*para*). The final name thus becomes $para^{sbl}$. Several of the dominant markers used routinely in fly crosses, and whose names permeate the fly literature, have suffered the same fate. In this book, the traditional names are used, but are noted if recently subsumed into another locus name.

WHAT MAKES FLIES SO GREAT? BALANCER CHROMOSOMES

Balancer chromosomes are what set fly genetics apart from genetics in all other organisms. Most recessive mutations are invisible in heterozygous condition. The ability to carry out crosses such that invisible genotypes can be scored in progeny with virtually 100% reliability has given fly genetics a degree of ease and power unmatched in other diploids.

H.J. Muller invented the idea of balancers, as he did much of the rest of what has become hard-core genetic analysis in the fly (Alfred Sturtevant was responsible for most of the rest), when he first identified the chromosome *C1B* as a suppressor of exchange on the *X* and used it to isolate new *X*-linked lethal mutations (Muller 1918). Since then, the principle that multiply inverted chromosomes are highly unlikely to undergo exchange with their normal homologs has been elaborated. When these chromosomes are also carriers of marker mutations, they become powerful tools in segregation analysis and the predictable synthesis of defined genotypes. Since the markers are often recessive lethals themselves, the chromosomes provide a means for constructing true-breeding stocks for defined lethal mutations—"balanced lethal" stocks in which only those adults doubly heterozygous for the balancer and for the lethal-bearing homolog survive.

Many balancers exist for the *X*, *2*, and *3* chromosomes. They are not necessary for chromosome *4* because there is no exchange on that chromosome (although balancing of recessive lethals with a dominant marker that is also a recessive lethal is still needed). The most effective balancers are those that suppress exchange all along the chromosome. Those that fail to do so usually have a large enough portion in normal order to per-

mit occasional synapsis with a homolog and consequent double crossovers within the short intervals that succeed in pairing. This can result in "breakdown" of the balancer—replacement of portions by a normal sequence, with transfer of some markers to the normal homolog. These are clearly situations to be avoided because they confound the usefulness of balancers.

Since the *X* chromosome must exist in hemizygous condition in males, most *X*-chromosome balancers do not contain recessive lethals. Instead, some *X*-chromosome balancers carry recessive female sterile mutations to prevent them from "taking over" the stock (i.e., becoming the only *X* chromosomes present, which will occur if the other chromosome carries mutations that are unhealthier than those on the balancer).

Balancers are usually named with a letter for their chromosome (*F* for first, which is the *X*; *S* for second; and *T* for third), with an *M* for multiply inverted and with a number and sometimes a lowercase letter to identify its place in a series. The name is sometimes followed by the genetic symbol for the principal markers carried by that balancer. The most efficient balancers are as follows:

X chromosome

- *FM7a* (real name *In(1)FM7, y^{31d} sc^{8}*), which carries the dominant marker *Bar* (*B*), as well as recessive alleles of *yellow* (y^{31d}), *scute* (sc^{8}), *white-apricot* (w^{a}), and *vermilion* (v^{Of}).
- *FM7b*, which carries y^{31d}, sc^{8}, w^{a}, and a recessive female-sterile allele of *lozenge* (lz^{sp}).
- *FM7c*, which carries y^{31d}, sc^{8}, w^{a}, and a recessive female-sterile allele of *singed* (sn^{X2}) in addition to alleles of *vermilion* (v^{Of}) and *garnet* (g^{4}).

Second chromosome

- *SM6* (real name *In(2LR)SM6, al^{2} Cy dp^{lv1} cn^{2} sp^{2}*), which carries the dominant marker *Curly* (*Cy*) as well as various recessives *dumpy* (*dp*), *cinnabar* (*cn*), and *speck* (*sp*).
- *In(2LR)O, Cy dp^{lv1} pr cn^{2}* (sometimes referred to as *CyO* or "*Curly of Oster*"), and it carries *Curly* (*Cy*) and the recessives *dumpy* (*dp*), *purple* (*pr*), and *cinnabar* (*cn*).

Third chromosome

- *TM3* (real name *In(3LR)TM3, y^{+} ri p^{p} sep bx^{34e} e*), which carries the wild-type allele of *yellow* (y^{+}) and the recessives *radius incompletus* (*ri*), *pink peach* (p^{p}), *sepia* (*sep*), *bithorax* (bx^{34e}), and *ebony* (*e*). More useful are those versions carrying the dominant marker *Serrate* (*Ser*) sometimes accompanied by *Stubble* (*Sb*).

- *TM6* (real name *In(3LR)TM6, Hn^{P} ss^{P88} bx^{34e} Ubx^{P15} e*), which carries the dominants *Henna* (*Hn^{P}*, not very easy to score) and the more reliable *Ultrabithorax* (*Ubx^{P15}*) plus the recessives *spineless* (*ss^{P88}*), *bithorax* (*bx^{34e}*), and *ebony* (*e*).
- *TM6B* (real name *In(3LR)TM6, Hu e*), which carries a dominant allele of *Antennapedia* (*Hu*), often with the additional dominants *Dichaete* (*D^{3}*) or *Tubby* (*Tb*, a good marker for larval and pupal stages as well), and the recessive ebony (*e*).
- *TM8* (real name *In(3LR)TM8, l(3R)DTS th st Sb e*), which carries a dominant temperature-sensitive lethal (*l(3R)DTS*), the dominant *Stubble* (*Sb*), and the recessives *thread* (*th*), *scarlet* (*st*), and *ebony* (*e*). *TM9* is a further derivative of this.
- *T(2;3) CyO; TM9*, which is a double balancer for chromosomes *2* and *3*, the result of a radiation-induced reciprocal translocation between *In(2LR)O,Cy* and *TM9*. Since it is a reciprocal translocation, in which part of *In(2LR)O,Cy* is now linked to part of *TM9* and the remainders are also linked together, the only progeny that you will recover are those that have all the pieces present in the same gamete for it to be euploid (i.e., to have a complete haploid genome). This helps to preselect progeny if you only want those that have inherited balancers for both chromosomes.

A great many variants of these balancers have been derived that have useful transgenes inserted into them. A few of these are discussed in this book; all are listed at http://flystocks.bio.indiana.edu (the Bloomington Stock Center's list).

DECIPHERING MATING SCHEMES

After nomenclature, the next most obfuscated realm of fly lore is the mating scheme. Mating schemes are shorthand for the genotypes that you need to collect and mate to get the progeny you want. What is confusing is that the schemes do not show all of the possible progeny from a cross, only the genotype of the unique desired class. These flies will be unique in phenotype as well as genotype if the author of the scheme has planned correctly. The unwritten assumptions are that homologous chromosomes pair and segregate from each other in the first meiotic division, all possible combinations of haploid segregants will be produced with equal frequency in the male's sperm and female's eggs, and likewise all possible combinations of diploid genotypes will be produced with equal frequency when eggs are fertilized. Whether they all survive is a separate matter.

Our assumption is that they will be produced initially (with the caveat that some abnormal chromosomes deviate from normal expectations). The shorthand indicates the genotypes of the relevant pairs of homologs for the cross at hand. A typical, simple scheme is shown below.

$$\text{♂♂}\quad \frac{nd}{Y} \times \frac{FM7a}{FM7a};\ \frac{In(2LR)O,Cy}{Sco}\quad \text{♀♀}$$

Males ↓ Virgin females

$$\frac{FM7a}{nd};\ \frac{In(2LR)O,Cy}{+}$$

This represents a cross between *notchoid* (*nd*) males (they must be males because they have one *X* and one *Y*, and the fact that more than one male is symbolized by ♂♂) and females (virgins, of course, symbolized by ♀♀) homozygous for the balancer chromosome *FM7a* on the *X* and heterozygous for the balancer *In(2LR)O,Cy* and the dominant marker *Scutoid* (*Sco*) on the second chromosome. Since the male's genotype does not show anything about his second or third chromosomes, he is assumed to be free of genetic variations and mutations (+). (In the Methods section of a paper, these balancers would be designated by their formal names: *In(1)FM7a* and *In(2LR)O,Cy*. In laboratory shorthand, they would usually be written as above. As mentioned, some forms of *FM7* carry a recessive, female-sterile mutation; *FM7a* does not and so would work in this cross.)

This cross produces many different classes of progeny. Only one of them is shown: females heterozygous on the *X* for *FM7a* and *nd*, and heterozygous on the second chromosome for *In(2LR)O,Cy*. Meanwhile, elsewhere in the bottle you will also find

$$\underset{\text{♂}}{\frac{FM7a}{Y};\ \frac{In(2LR)O,Cy}{+}}\ \text{and}\ \underset{\text{♂}}{\frac{FM7a}{Y};\ \frac{Sco}{+}}\ \text{and}\ \underset{\text{♀}}{\frac{FM7a}{nd};\ \frac{Sco}{+}}$$

Each is phenotypically, as well as genotypically, unique. The dominant marker on the *FM7a* chromosome, *Bar* (*B*) eye, makes any progeny carrying *FM7a* unambiguously recognizable. Similarly, on the second chromosome, the dominant marker on *In(2LR)O,Cy*, the *Curly* (*Cy*) wing mutation, and the dominant marker mutation, *Scutoid* (*Sco*), which eliminates

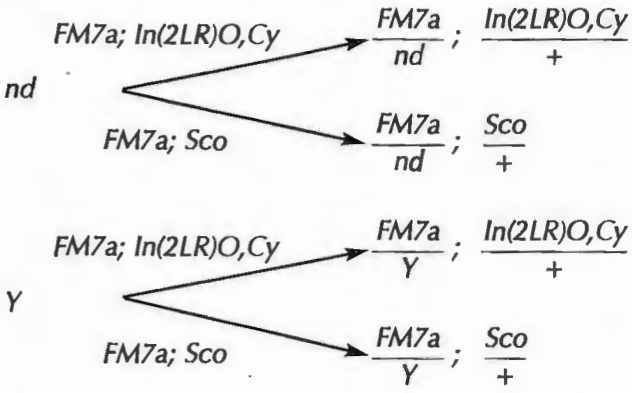
posterior thoracic bristles, make any fly carrying these mutations recognizable in any combination, since the mutations do not mask each other. The *nd* mutation is effectively invisible. It has no morphological effect when heterozygous (in females). (*Bar* is particularly well suited as a dominant marker for the *X* chromosome because it is viable in homozygous as well as heterozygous condition, and the two are distinguishable—*B/B* females are more severely affected than *B/+*. Males have no homozygosity for the *X*, only hemizygosity [*B/Y* in males is as severe as *B/B* in females], so *Bar*'s viability is very helpful in allowing survival of males carrying *FM7a*. In contrast, the dominant markers on autosomal balancers never need to survive in hemizygotes because hemizygosity for chromosome *2* or *3* is lethal.)

The unwritten assumption in these schemes is that meiosis causes each pair of homologs to segregate from each other so that only one of each pair is transmitted to any given progeny (Mendel's law of segregation). This occurs independently for each pair of homologs (Mendel's law of independent assortment). The parental males in the cross are hemizygous for their sex chromosomes, *nd/Y*, and consequently display the mutant *nd* phenotype. Since the *X* and *Y* pair and segregate at meiosis, they give rise to gametes of two possible genotypes: *nd* or *Y*. Even though they carry balancers and marker mutations for two different chromosomes, the parental females are only heterozygous on the second chromosome and thus give rise to only two possible gametes: *FM7a; In(2LR)O,Cy* or *FM7a; Sco*. These four gametic genotypes can combine in four possible combinations, as shown above. Heterozygosity on any other chromosomes increases the possible types of gametes and the corresponding classes of progeny. If in doubt about the classes of progeny that might arise from a cross, you can always set up a Punnett square (below) to ensure that you are imagining all possible combinations.

Female gametes

Male gametes	*FM7a ; In(2LR)O,Cy*	*FM7a ; Sco*
nd	$\frac{FM7a}{nd}$; $\frac{In(2LR)O,Cy}{+}$	$\frac{FM7a}{nd}$; $\frac{Sco}{+}$
Y	$\frac{FM7a}{Y}$; $\frac{In(2LR)O,Cy}{+}$	$\frac{FM7a}{Y}$; $\frac{Sco}{+}$

Even better than the Punnett square is the "algebraic" or branching approach, which is better suited to multiply mutant genotypes:



It is important to ensure that the genotypic class you want is phenotypically unique and thus recognizable among all the other progeny. This is the key to fly genetics.

BASIC FLY HUSBANDRY

Fortunately for many of us in the fly field, working with *Drosophila* does not take much technical skill—care, yes, but not manual skill. Much of the power of fly genetics comes from the ability to perform crosses in which each possible genotype in the progeny is recognized easily and unambiguously. To achieve this, be certain that the only progeny you obtain are from the intended cross, rather than from stray flies or unwanted pregnancies (i.e., nonvirgin females). Often, the class of progeny you are trying to generate will be a small fraction of the total and not particulary healthy. This means that it is equally important to start the cross with enough flies to recover enough progeny to continue the experiment.

The easiest way to grow flies is at room temperature. This also protects your stocks from incubator failures—by far the major cause of catastrophic loss of flies. The healthiest way to keep fly stocks, on the other hand, is at 25°C, 60% relative humidity. At this temperature, you get the fastest generation time (approximately 9–10 days from egg to emerged adult) and the best viability. (The generation time increases by roughly 2 days if the mold inhibitor Tegosept is present. See Ashburner et al. 2004.) Stocks can also be maintained routinely at temperatures as high as 29°C and as low as 18°C with correspondingly faster or slower generation times (see Ashburner et al. 2004) but with poorer yield.

For stocks that are true breeding (i.e., all adult progeny are the same genotype), the only time limitation on the life of the culture is that of health—older cultures produce less healthy flies and serve as a breeding ground for mold and mites, the scourge of all stock collections. A good rule of thumb for these cultures is to transfer them every 2 weeks and keep them a maximum of 18 days at 25°C. Cultures that are produced from a specific cross have an additional constraint: Eighteen days after the start of the culture, you will start to obtain second-generation progeny whose genotype will probably be a complete mystery, since you will not have known their parents.

True-breeding stocks can be transferred without anesthetization. This technique constitutes one of the only manual skills in fly pushing: Tap the flies down to the bottom of the old vial (gently, so they do not all get stuck), quickly remove the vial's plug (usually cotton, rayon, or foam rubber), and place an open fresh vial down on top of it, holding the two vial mouths together tightly. Flip them over and tap the flies down into the new vial (gently, so you do not transfer the old food as well). Then quickly plug the new vial. For the first few weeks, many stray flies will probably escape. To keep the number of stray flies in the laboratory to a minimum, use some kind of fly trap. Low-tech versions consist of a clean culture bottle with some vinegar in the bottom and a dash of detergent, topped with an open funnel to impede the flies' escape before they fall into the liquid. High-tech fly traps are the commercial "bug lights" familiar to suburban backyards. Screen doors have sometimes been used (S. Hawley, pers. comm.), but may violate fire regulations.

Since fly stocks can only be maintained by live culturing (they do not readily survive freezing) it is worthwhile to keep duplicates of each stock. This also ensures cultures available for virgin collection whenever you want. For stocks kept in vials, which is true for most of what you keep, this can be done either by keeping two copies on the same schedule or staggering by a half generation. Labeling stocks is equally important, most easily accomplished by using a movable ring tag (a cardboard disc attached to the vial by a rubber band) with the full genotype of the stock indicated on the vial. The date of initiation of each culture (when flies are first put on that food) should also be written on the vial or bottle so that you can easily tell their age.

The number of flies needed to start a culture varies with the viability of the genotype, and a golden mean exists for each. A little empiricism goes a long way when it comes to figuring out how many flies to put in a bottle. In general, the more mutants present (especially dominants), the

poorer the viability. Chromosome rearrangements (e.g., balancers, compound chromosomes, etc.) reduce the fertility of stocks: Too few flies cause the culture not to "take," or it becomes overwhelmed by mold or bacteria. Too many flies results in the culture becoming so soggy from the cumulative waste products that when you dump flies from it, the food will come out as well, leaving a mess on your anesthetizer and fly desk. In addition, soggy cultures cause the flies' wings to stick to their bodies, making it difficult or impossible to score the wing phenotype of many useful genetic markers. If a culture looks like it is becoming too soggy, it is possible to rescue it by stuffing a small piece of paper towel into the food. To prevent the paper from growing mold, it too may need to be treated with mold inhibitor.

Wild-type stocks or those with a single marker mutation may require only 10–15 females for small bottles (see below), 25–35 females for a large bottle, and 4–8 for a vial. Fewer males are required, since one male can make himself known to many females. Stocks with multiple markers, dominants, or rearrangements may require two or three times as many flies. The best way to compensate for not knowing exactly how many flies to start with is to watch the culture as it develops. When the food starts to look churned up (especially near the surface), enough larvae have emerged and it is time to dump out the parents (empty them from the bottle before the first progeny appear). This can take roughly 4 days, depending on the genotypes. When doing a cross, it is also important to dump the parents to eliminate the danger of mistaking progeny for their parents. This is another good reason for writing the date on the culture at the beginning.

CODDLING DIFFICULT STRAINS

Also known as cosseting (in the U.K.), this refers to the gentle art of keeping sickly stocks, rescuing those that have deteriorated to very few flies, or carrying out crosses with a single male and female pair. The basic principles of sound fly husbandry apply: Use a fresh food vial garnished with fresh yeast paste ("Baker's Dried Active Yeast" dried to the consistency of peanut butter), keep the culture in ideal conditions (25°C, 60% humidity), and say an occasional prayer.

If you managed to start out with both males and females in the vial (which does not always happen with a deteriorating stock) and you find that progeny are appearing, it is best to collect them as they emerge and place them in a fresh vial. (They will not live long if left in the original cul-

ture vial. In this fashion, you can start a new culture.) If you have obtained enough flies for the next culture to proceed more quickly (i.e., larval churning of the food after several days), you may transfer the parents to a fresh vial midway and thus increase the number of progeny you ultimately obtain.

COLLECTING FLIES FOR CROSSES

To carry out crosses cleanly, you must start with virgin females. At 25°C, female flies will not mate within the first 8 hours of emergence as adults. This means that virgins are most easily obtained by collecting flies twice a day—once in the morning and again 7–8 hours later. Morning is when the majority of flies emerge from the pupa case (if your incubator is on a light:dark cycle); this is one of their most predominant circadian rhythms (also the origin of the name *Drosophila*, as you may have learned in high school). Although you cannot assume that all of the females present in the culture in the morning are virgins, many of them will be newly emerged and thus recognizable by their pale pigmentation and a dark spot in their translucent abdomens. (This dark spot is the fly's version of meconium, leftovers in the intestine from their last meal as larvae.) If you have been careful about clearing all the adults from the bottle in the morning (including those that have stuck to the side or the food), then any females present 7–8 hours later will also be virgins, even if they no longer look newly emerged.

A more efficient method for maximizing the number of virgins present in the morning is to place the cultures at 18°C overnight, after your collection at the end of the day. Development is slowed down sufficiently at this temperature so that it is roughly 98% probable that newly emerged females will not mate for 16 hours. Thus, your morning collections can be assumed to contain all virgins (if the bottles have been properly cleared at the last collection) and you can simply alternate the cultures to between 25°C during the day and 18°C at night. The 2% error will not matter if nonvirginity is distinguishable among progeny of the cross (see below). If nonvirginity is indistinguishable, then the more conservative approach is better.

For many crosses, it is possible to control for nonvirginity by the use of a "virginity marker"—any recessive marker mutation that is homozygous in the stock from which the females come and which will not be homozygous in the intended cross. If progeny that carry this marker appear, you know that their parents are not virgins and they can be tossed.

When you are in the middle of a mating scheme in which you do not start from homozygous strains, it is still often possible to compensate for nonvirginity by anticipating the possible genotypes and marker combinations that you would see if there were some nonvirgins in the cross. If you can arrange the scheme so that these will be different from the markers that distinguish the progeny you want, you will be safe.

Males for a cross may be collected at almost any time; the only consideration is that they must be at least 3 days old and less than 10 days old, since they will not mate efficiently if they are too young or too old. This is because of the maturation process that males experience after eclosion, which makes them more likely to court and mate females. In this sense, females are more mature than males when they emerge as adults. (So what else is new?) Although the sex ratio is indeed 1:1, it is not necessarily so on each day's collection. Female flies develop faster than males, so there will be more of them in the earlier days of the culture than later.

It is often most convenient to store the flies you have collected before mating them so that you can start the cross all at once—perhaps because it takes several days to collect enough flies, the food has not yet been made, or you may prefer to start all of your crosses on Fridays. (The virtue of starting crosses on Fridays is that the first progeny emerge 10 days later, on Monday. Thus, you can collect progeny for 5 days, start a new cross the next Friday, and in this fashion free up the occasional weekend day. But do not tell your advisor where you heard this.) Fresh food vials (i.e., those that have not had flies in them) are the best place to store flies, 20–30 flies to a vial. If they are to be kept this way for more than a few days, you will need to change the vials to keep the flies healthy. In uncrowded, fresh vials flies can live for 40–60 days, but their fertility gradually decreases to near zero by this time.

During this storage period, it is also possible to ensure that you have virgins by making certain that no larvae begin to churn up the food. Toward the end of the storage period, you can also ensure that the culture will go quickly when you transfer the flies to bottles by "preincubating" males and females a day or so in advance in vials. In this way, many of the females will have mated by the time you put them in bottles. Like any second-order reaction, mating proceeds more quickly in the crowded conditions of a vial, in which collisions between males and females are more likely.

Nowadays, most people use CO_2 to anesthetize flies for virgin collection and for examining markers. Various gadgets have been devised for

delivering the CO_2 through diffusers on a flat surface so that a low level of gas can be maintained while you sort through the flies. (The porous polyethylene that is used to plug the bottoms of chromatography columns works well for this purpose.) Usually, a CO_2 hose is inserted into the bottle or vial to anesthetize the flies (while keeping the vial inverted to prevent the flies from sticking on the food) or else they are dumped into an anesthetizer—a porous vessel that exposes the flies to the gas.

The traditional way of anesthetizing flies is with ether. Ether anesthetizers consist of an enclosed container (often an empty fly bottle) with a cotton wad in the bottom into which ether is poured and a porous tube that holds the flies. Care is always needed to avoid overanesthetizing the flies—something that is much less likely with CO_2. Once the flies are anethestized, it is necessary to sort before they reawaken, which could happen quickly during summer heat.

The principal ill effects of such anesthetization are behavioral. Both CO_2 and ether impair neural activity for some time afterwards. For this reason, wait a minimum of 24 hours (sometimes longer) after anesthetization before conducting any behavioral test.

CHAPTER 2

Isolating New Variants

THE REASON THAT WE WORK WITH FLIES IS NOT BECAUSE they are cute, although Ed Lewis has said that you have to love your organism. We work with them primarily because of the potential for getting and analyzing mutations. Much of today's enormous edifice of molecular manipulation in *Drosophila* is predicated on this central fact.

Most forms of mutagenesis do not permit targeting the event to a predetermined gene—as in the so-called "knock-out" technique with mice (a name as descriptive of the effect on one's thinking as on the gene in question). Instead, they approximate a random process from which lesions in the desired gene are generally obtained by genetic testing of individual mutation-bearing flies for complementation or homozygosity. Few selection schemes of the sort that are used with microorganisms are applicable to flies.

Mutagenesis will never become obsolete, if only because random chance always produces more interesting lesions than those that we can think up. But the day is approaching when every gene in the fly will be mutated (i.e., "knocked out") as part of a genome-wide effort of the Berkeley Drosophila Genome Project and Exelixis (Spradling et al. 1995, 1999; http://www. fruitfly.org/p_disrupt/index.html; Thibault et al. 2004). (At that moment, we may hear little cries coming from our fly cultures that will sound something like, "We give up!")

Principles

Mutagens are not completely random in their action, but they approximate it closely enough for the purposes of designing mutant screens. Generally, you treat male flies with a mutagen, or perform a cross to produce

males with mobilized transposable elements, and then "clone" individual treated chromosomes in the F1 generation. This is necessary because after a mutagenesis treatment each sperm is unique. Thus, unless you are able to recognize and recover a newly induced mutation in the next generation, you will be out of luck. Many of the mutations you will want are either hard to recognize or recover. For instance, it is hard to get a lethal mutation out of a dead fly.

Males are most often used because (1) mature sperm are quite sensitive to mutagens, (2) the flies are still capable of performing their conjugal duties after the treatment, and (3) one male will mate with many females to propagate the treated chromosomes. Treatment of females creates problems because of the deleterious effects of mutagens on the oocyte itself and the tendency of the oocyte to soak up mutagen, decreasing its effective concentration in the nucleus. However, females may be the only choice because the mutagenic event requires the presence of both homologs and must therefore occur in a cell whose chromosomes have not yet completed the first meiotic segregation. The induction of compound chromosomes, whose homologous arms are attached to the same centromere, is an example.

Most individual F1 flies carry a mutagenized set of chromosomes in all of their germ cells. However, since chemical mutagens often act on a single strand of the DNA double helix, F1 progeny will potentially be mosaic for the lesion as a result of the semiconservative replication of that chromosome. That is, alteration of the sequence on one strand of the DNA by the mutagen will be transmitted to one of the two daughter cells at the first mitosis. As a result, a new mutation will be present in some cells of the animal. This could produce a mutant phenotype without being transmissible if it is present in the tissues crucial to the phenotype, but not in the germ cells. However, the chances are low that the germ cells in one F1 individual will be mosaic. This means that the screen should be designed so that F1 flies will be testcrossed in such a way as to reveal the presence of new mutations in one class of progeny, while allowing recovery of the mutation-bearing chromosome in sibling progeny (see below).

It is generally assumed that with sufficient ethylmethanesulfonate (EMS) mutagenesis, "saturation" can be obtained for any phenotype or chromosomal segment. Although this is true in principle, the claim must be qualified by the realization that mutagens all show some bias with respect to sequence or chromatin structure and in addition, saturation is only as good as your ability to recognize the phenotype of a mutated gene.

MUTAGENS

The kind of mutagen to use depends on the kind of mutation you want and the amount of time you want to spend looking for it. Chemical mutagens are most suitable for obtaining point mutations (or small, intragenic deletions) at a reasonable rate. Thus, they are best for obtaining an allelic series, conditional (e.g., temperature-sensitive) mutations, and screens aimed at obtaining predefined phenotypes (e.g., learning mutants). Radiation is most suitable for producing rearrangements, i.e., translocations, duplications, deletions, and inversions. Insertional mutagenesis gives the best leverage for rapid molecular cloning of the mutated gene. Its derivative, enhancer trapping, allows you to identify genes on the basis of enhancer-driven expression patterns. A detailed account of various mutagens, their properties, and uses can be found in the Ashburner et al. (2004) commentaries. The following account is abbreviated.

Ethylmethanesulfonate

EMS is the most commonly used chemical mutagen. It is an alkylating agent that produces a high proportion of point mutations, although it also produces small deletions and, occasionally, other rearrangements as well (Pastink et al. 1991). The dose required depends on the kind of screen: a lower dose when searching for a new mutation and a higher dose when isolating new alleles of existing mutations. This is because the chromosome will be made homozygous in the former case, whereas it will be tested in *trans* with another chromosome in the latter. The rationale is that when you are making treated chromosomes homozygous to look for a particular phenotype, you do not want to complicate the situation by having more than one homozygous mutation present—especially a recessive lethal mutation.

For a low dose, males are fed a solution of 0.025-mM EMS in 1% sucrose overnight (see Ashburner et al. 2004). This results in an average of one lethal hit per chromosome arm, or a rate of one hit per 1000 chromosome arms per locus. (That is, if you test progeny from a mutagenized male, you will find an average of one new lethal mutation on each individual's *X*. If you are screening for mutations at a particular locus, you will find it in approximately one out of 1000 chromosomes you screen. This corresponds to the fact that there are roughly 1000 lethally mutable genes on each of the major chromosome arms [*X, 2L, 2R, 3L,* and *3R*].) A twofold higher dose may be used for screens in which treated chro-

mosomes are scored when heterozygous (as in a screen for new mutations uncovered by a deletion).

The treated flies are mated to females for 4–5 days, after which time the males are removed. This is done to maximize the proportion of uniquely mutant chromosomes, since the mutagen can affect gonial stem cells as well as mature sperm. When gonial cells are affected, multiple sperm with the same mutation will be produced. Since the stem cells are less sensitive, the overall frequency of individual hits will be lower. To avoid this, the treated males are discarded before stem cell daughters have had time to become mature sperm, thus ensuring that mutations will be independently induced. The virtue of having independently induced mutations is that you do not waste your time analyzing the same allele more than once and, if you are attempting saturation mutagenesis, the only way to estimate when you have attained saturation is by the frequency of repeated, independent mutations of the same locus.

As an alkylating agent, EMS acts on one strand of the double helix. This means that F1 progeny will be mosaic, at least to some extent, for the new mutation. In practice, this only matters if you are trying to score a mutant phenotype in the F1 since then you are counting on the fact that the new mutation will be present in the germ cells as well as in the somatic tissues you have scored and that a somatic phenotype can be detected even when all cells might not be mutant.

It is best to treat males that are 3–5 days old, the age at which they mate most readily. But be careful since many will not survive the overnight exposure to mutagen, not all will be fertile after the treatment, and many of the F1 progeny males will be sterile. It is thus a good rule of thumb to mutagenize a large number of males—equal to half the number of F1 individuals that you plan to testcross (see below). Equally wise is to run a pilot to assess the mutagen sensitivity of the flies that you actually plan to use. Another virtue of a pilot run is that it tells you whether your crosses will produce the classes of progeny you expect. Many ideas look great on paper but fail utterly when it comes to real flies.

Ethylnitrosourea

We assume, for operational purposes, that chemical mutagens such as EMS are completely random in their site of action. This is not true, in fact, and nowhere has it been revealed more clearly than in the different spectrum of mutations obtained with another alkylating agent, ethylnitrosourea (ENU). With ENU, the mutability of genes is different, as are the kinds of

mutations obtained, although the overall frequency is comparable. The frequency of rearrangements also appears to be reduced, as does the frequency of mosaicism in the F1 progeny (Pastink et al. 1988). In all other respects, the same principles and practices apply as with EMS. A feeding dose of 7.5 mM produces lethals (standard jargon for "lethal mutations") on approximately 40% of the treated chromosome arms.

ENU is even more hazardous for mammals than EMS; it causes an extraordinarily high frequency of brain tumors in mice. Many fly workers do not consider its benefits worth the added risk and the extreme precautions it requires.

Radiation

Radiation in the form of X rays was the first mutagen ever to be used, and it remains an essential tool for geneticists. The most conveniently available sources of radiation are X-ray machines and cobalt or cesium sources (for gamma rays). All are capable of inducing chromosome breaks, which are then sometimes repaired to produce translocations, deletions, transpositions, and inversions (Pastink et al. 1987). The breakpoints that comprise these rearrangements also cause mutant phenotypes if they fall within a gene or if, by juxtaposing certain chromosome regions, they produce a phenomenon known as the "position effect" (see Ashburner et al. 2004). Radiation can also cause point mutations.

Mature sperm is the most susceptible target for irradiation. The possible range of rearrangements produced by irradiation of mature sperm is limited by the fact that each sperm has only a haploid set of chromosomes. Thus, events involving pairs of homologs (including the *X* and *Y*) cannot occur.

The frequency of these events is considerably lower than for chemical mutagenesis. A dose of 4000r to males yields a frequency of roughly 5% lethal hits (i.e., lethal mutations) per chromosome arm, as opposed to 60% with the standard dose of EMS. This dose is calibrated as the highest dose that does not cause excessive sterility. Since the breakage event is double stranded, there are no problems with mosaicism in the progeny. Otherwise, the flies are handled similarly as with chemical mutagens.

Insertional Mutagenesis

Disruption of genes by the insertion of transposable elements is not the easiest form of mutagenesis, but the advantages conferred by having an

insertion to "tag" a gene or an enhancer trap to reveal an expression pattern often outweigh the inconveniences. The principle is that of mobilizing a mobile element to transpose and reintegrate at a new site in the genome; this is also known as hybrid dysgenesis. P elements are the most commonly used transposons, with *hobo* and *PiggyBac* as subsequent additions. (The above-mentioned inconveniences are steadily decreasing with the Berkeley Drosophila Genome Project and Exelixis' comprehensive production of insertions throughout the fly genome [Spradling et al. 1995, 1999; http://www.fruitfly.org/p_disrupt/index.html; Thibault et al. 2004]. In time, one will be able to order insertional lesions for any and all genes.)

P-element mutagenesis may be carried out with either intact transposons, starting with strains carrying many such elements in their genome, or with various kinds of inactive elements capable of being transposed, but which lack the activity itself. Inactive elements must be activated by the addition of the transposase activity separately, usually in the form of a chromosome carrying a stably integrated transposase gene.

The advantage of the inactive elements is that the induction of jumps can be controlled by simply adding and removing the transposase activity through appropriate crosses (see below). With this technique, the equivalent of a mutagenized male is the progeny from a cross that brings together the transposable element and the transposase activity in the same fly. Each sperm in these males is treated as if unique, just as if the fly had been fed a mutagen. In subsequent generations, there is no mosaicism to be resolved, just the separation of transposase activity from transposable elements to prevent further jumps. (P-element mutagenesis also works in females, but unlike chemical mutagenesis, it does not cause random damage to the egg.)

The frequency of jumps is a combination of (1) the frequency of excisions, which seems to depend in a somewhat unpredictable way on the size and sequences present in the transposon as well as the site of insertion, and (2) the frequency of insertions, which depends on the chromatin structure present at a given locus (Liao et al. 2000). Some loci are hot spots for insertions, such as the *singed* (*sn*) locus, at which insertions occur at a rate of roughly 1/100. Other loci seem to be completely refractory to insertions, such as *Alcohol dehydrogenase* (*Adh*). The overall average is roughly 1/2000. Part of the rationale for developing alternatives to P elements, the *hobo* and *PiggyBac* systems, was to exploit a different set of insertion biases (Smith et al. 1993; Horn et al. 2003; Thibault et al. 2004). These other systems work on the same principle: A disabled transposable element carries an exogenous gene and a source of transposase activity. The genetic crosses for manipulating them (outlined below) are correspondingly similar.

GENETIC SCHEMES FOR DETECTING MUTANTS

Tests for new mutations vary with the situation but usually fall into two categories: those that are concerned with a particular locus or chromosome region and those that are focused on a particular phenotype regardless of where the genes map. The first kind involves testing the treated chromosome in heterozygous condition—usually in *trans* with a known mutation or deletion-bearing chromosome. The second kind requires that the treated chromosomes be made homozygous.

New Alleles at an Autosomal Locus

A frequent source of insight into a gene's action comes from the analysis of an allelic series. The more complex the process in which a gene is involved, the more important such analyses become. The basic strategy is the classic complementation test, whereby the newly mutagenized chromosome is made heterozygous with a known allele of the locus in question. Crucial to this strategy is the ability to distinguish the chromosome bearing the new allele from the old. The following shows a simple scheme for isolating new alleles of a recessive lethal, third-chromosome mutation (*naked cuticle, nkd*).

Mutagenize wild-type males and mass mate to virgins carrying a balancer *TM6* marked with the dominant mutation *Ubx*, heterozygous with a different dominant marker *Sb*. Actually, one rarely uses wild-type males, instead using males homozygous for some gratuitous marker such as the cuticle color mutation *ebony* (*e*).

♂♂ $\frac{e}{e}$ x $\frac{TM6,\ Ubx}{Sb}$ Balancer dominant ♀♀

produces

$\frac{e}{TM6,\ Ubx}$ and $\frac{e}{Sb}$ F1 progeny, both ♂♂ and ♀♀

Of the two types of F1 progeny produced, one is *Ubx* but not *Sb*