

Prelim review: Fly genetics

MTL

22 April 05

1 *Drosophila* genetics

1.1 Preliminaries

Flies are little people with wings.



Figure 1: Little person with wings.

1.1.1 Chromosomes

Flies have one X and three pairs of autosomes. X and 4 are acrocentric, 2 and 3 have an L arm and an R arm. 4 is mostly heterochromatic and generally does not recombine. Recombination occurs only in females.

1.1.2 Nomenclature

Nomenclature is a mess. The genotype of a fly is written in cytogenetic order, with genes on different chromosomes separated by a semi-colon. For each chromosome, the genotype of one member of the homologous pair is written, followed by a slash, followed by the genotype of the other member. Wildtype alleles are generally left off. If both copies of the chromosome are wildtype, the chromosome is skipped. The male Y chromosome is written as an arrow underneath the X genes. Examples:

$y^1 w^{67c23} \rightarrow$; $dp; e^1/e^s Sb$ Male with yellow and white alleles on X; homozygous dumpy on 2; heterozygous e^1/es at the ebony locus and heterozygous wildtype/Sb on the stubble locus on chromosome 3.

$y^1 w^{67c23}; e^1/Sb$ Female homozygous on y and w loci; wildtype on 2; heterozygous in trans, $e^+ / + Sb$, on 3.

Other abbreviations are as follows (highly simplified...)

Deficiency Df

Duplication Dp

Inversion In

Translocation T

Transposition Tp

1.2 Balancer chromosomes

Balancers are chromosomes with multiple inversions, which causes suppression of recombination. Balancers have a dominant visible marker and are homozygous lethal. Examples of balancers:

FM7c X chromosome balancer with Bar eyes marker

SM6a second chromosome balancer with Curly wings marker

CyO second chromosome balancer with Curly wings marker

TM3 third chromosome balancer with Stubby bristles marker

Let's say you have a recessive lethal mutation m and a linked marker cn . Then a stock of $m\ cn$ over a balancer can be easily maintained without recombination:

$$\begin{array}{c} m\ cn/CyO \times m\ cn/CyO \\ \downarrow \\ CyO / CyO \text{ (dead)} \\ m\ cn / m\ cn \text{ (dead)} \\ m\ cn / CyO \text{ (curly)} \end{array}$$

1.3 Loss of function screens

1.3.1 Insight

You want to identify a loss of function allele for some gene that is possibly lethal. So you induce random mutations in a lot of male flies and hope that at least one of the hits will be inside your gene of interest. From that fly you create a balanced stock, then you attempt to map the mutation to find out what gene it is in.

1.3.2 Procedure

Start with wildtype males and virgin females with a balancer over a marker. You will be screening for mutations that hit that particular chromosome. For example, CyO over glazed eye phenotype,

$$(G0) \quad \text{EMS } \sigma +/+ \times \text{Gla/CyO } \text{♀}$$

Isolate the curly-winged, non-glazed males, which possibly have a lethal mutation on the 2nd chromosome, m^*/CyO , and individually mate with Gla/CyO virgin females:

$$(G1) \quad \sigma m^*/CyO \times \text{Gla/CyO } \text{♀}$$

The result will be both male and female curly flies. You want to keep all the flies without glazed eyes, since this means they're carrying the possibly lethal mutation m^* . Finally, you intercross these flies

$$(G2) \quad \sigma m^*/CyO \times m^*/CyO \text{♀}$$

and look for the absence of straight-winged flies in G3, which would indicate homozygosity (and thus, non-lethality) of m^* . If all the flies are curly-winged, then you have a balanced stock of your mutation.

1.3.3 Saturation

EMS induces random mutations across the entire genome, which roughly follows a Poisson distribution parameterized with the mean number of hits per gene. If you recall the shape of the Poisson distribution, the probability of getting 0 hits in a particular gene is actually sizeable. So you want to increase the odds of getting a hit in each gene by increasing the number of flies you screen, say 3 to 4 fold for chromosome 2, as compared to the number of genes on your chromosome of interest. This will give you around a 95% chance of hitting any particular gene. Beyond that, you asymptotically approach saturation, and you can't expect to do much better than that.

1.3.4 Mapping

If you have a balanced stock of some mutation, and now you want to know what gene it corresponds to, you have to map it. Meiotic mapping measures map distance based on recombination frequencies – you obviously need to put the mutation over a non-balancer chromosome with a marker of interest (in a female!) in order to observe recombination. Deficiency mapping takes advantage of stocks of flies with deficiencies over various chromosomal segments. Failure to complement over a deficiency suggests the mutation maps to the deficiency region. This is a single-generation screen, but requires you to have the right Df fly (not all possible Dfs exist).

1.3.5 Wrinkles

You can get more than one hit per chromosome, in which case your phenotype could be due to the contributions of more than one gene. You can also get cryptic lethals, which are low-frequency alleles present before mutagenesis but revealed due to population bottle neck induced by the screen; you want to isogenize the stock first to prevent this from happening.

1.4 P elements

Drosophila transposable element that is co-opted to carry a gene of interest, a dominant selectable marker, usually red eyes, and ampicillin resistance. Transformation of a P-element into an embryo results in rare, quasi-random insertion into the genome and the possible expression of the gene encoded in the P-element. This process depends on transposase. You generally start with a white-eyed mutant embryo and see if the P-element can rescue the phenotype of your mutant; this would suggest that your mutant is a lesion in the same gene carried by the P-element.

1.4.1 Insertional mutagenesis

Transposition can be controlled via the source of transposase. An insertion into a gene can result in a recessive phenotype for that gene that is stable as long as there is no transposase around. As an example, start with females homozygous for white eyes and carrying the P-element on the X chromosome, and cross it to males bearing a balanced transposase gene linked to a marker **Sb**:

$$(G0) \quad \text{♀ } w, P\{w^+, YFG\} \times \Delta 2-3 \text{ Sb}/TM6 \text{ ♂}$$

Extract the males, which now carry both the P-element and the transposase gene. Their germlines may exhibit transposition. Cross with white-eyed females:

$$(G1) \quad \sigma \frac{w, P\{w^+, YFG\}}{Y}; \frac{\Delta 2-3}{+} \times w \text{♀}$$

The resulting progeny may include rare red-eyed males, where the red-eye phenotype must be due to transposition of the P-element, since the males are hemizygous for white eyes on the X chromosome. If you choose the ones without the Sb marker, you have a stable P-element insertion:

$$(G2) \quad \sigma \frac{w}{Y}; \frac{P\{w^+, YFG\}}{+}$$

Some flavors of insertional mutagenesis:

Enhancer trap screen Use a P-element with GFP. Insertion downstream an enhancer can reveal (part of) the spatio-temporal expression pattern of the nearby gene

Exon trap Insertion of GFP flanked with splice acceptor/donor within an intron can cause a GFP-protein fusion and spatio-temporal protein localization in the cell, assuming the insertion preserves approximate phenotype

Small deletions The P-element, once inserted, can be induced to jump again with transposase, and in doing so will often bring flanking genomic sequence with it, causing a small local deletion.

Targeted deletions (KOs) P-elements containing a FRT site can be used to induce controlled deletions. P-elements on opposite sides of a gene on homologous chromosomes can be induced to recombine with FLPase, with the result being deletion of everything between the FRT sites.

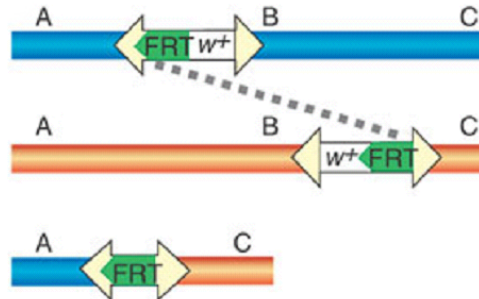


Figure 2: FLP/FRT.

Finding where your P-element inserted can be done by generating sequence tags flanking your P-element, and searching the genome sequence or EST databases.