Reading:
"Transposable genetic elements move from place to place in the genome" pp508-514 (Chp 14)
Also: p548 (insertion sequences in bacteria)

A practice problem set dealing with mutant categories is available on the website. Answers will be posted next Monday.

Lecture schedule:
Wed 3/14: genetic screens
Fri 3/16: sensitized screens
Mon 3/19: genetic mosaics in screens
(hence: no RNAi and one less sex lecture)

Radiation (the first experimental mutagen discovered)

- non-ionizing (lower energy)
  UV light
  photochemical reaction glues adjacent thymines together in cis:
  \[
  \begin{array}{c}
  A \ G \ G \ C \ C \ T \ C \ T \ C \ A \\
  T \ C \ C \ G \ G \ A \ G \ A \ G \ T \\
  \end{array}
  \Rightarrow
  \begin{array}{c}
  A \ G \ G \ C \ C \ T \ C \ T \ C \ A \\
  T \ C \ C \ G \ G \ A \ G \ A \ G \ T \\
  \end{array}
  \]
  DNA repair machinery called out:
  \- light-dependent repair (accurate)
  \- excision repair (error prone)

- ionizing (higher energy)
  X-rays, γ-rays, cosmic rays

Ionizing radiation causes just about every genetic change imaginable.

Categories of transposable genetic elements:
- Retroposons: transposes via an RNA intermediate (actually a virus)
- Transposons: transposes via a DNA intermediate
- Insertion sequences (IS in bacteria, Fig. 15.6)
  generate transposons, some of which are responsible for ultimately producing multiply drug resistant pathological bacteria

Histories of transposable element studies:
- Genetic analysis of unstable genes
  "jumping genes" in corn (1948 Rhoades and McClintock)
  unstable fly mutants in 1920s (crosses between "races")
  unstable bacterial mutants

- Molecular characterisation ultimately related all three
  Led by rediscovery of unstable fly mutants in 70's
  key breakthrough: discovered how to control instability
  ...the phenomenon of "hybrid dysgenesis"
  molecular explanation in early 1980s
  showed generality (and importance) of mobile genetic elements
  ...let to Nobel prize for McClintock 1983

Transposable DNA elements as mutagens
...and as general genetic workhorses

- "all natural" mutagenic agent (like many of the most potent carcinogens)
  responsible for half of all spontaneous mutations in "the fly"
  responsible for generating much of the raw material of evolution
  (eg. chromosome rearrangements, duplications, deletions, etc.)
  TE = 12.5% of fly genome;
  just two of many different in humans (SINEs & LINEs) = 7% of human genome
  they are ubiquitous mobile genetic parasites ("selfish DNA")
  "stripped down" virus -- or real virus in some cases
  can insert into DNA causing damage
  when "tamed", have been used to generate the most powerful tools in modern molecular genetics
  control their mobility

- usually causes double-strand DNA breaks
  (double helix, not sister chromatids)
  cell must repair this damage at any cost to avoid dying after next cell division!!
  ...but what repair template available?
  If none can be found (or found in time), just stick the DNA together blindly.

- Genetically makes single base-pair changes, or at most very small deletions --- in contrast to:
  ionizing radiation causes just about every genetic change imaginable.
  Ionizing radiation causes just about every genetic change imaginable.
Initial evidence for mobile genetic elements in Drosophila:

- Different classes of DNA based on abundance:
  - Single copy
  - Middle repetitive: 80 different types, ~5 kb, x50 = 12.5% total
  - Highly repetitive

- Copia class of middle repetitive DNA cloned and characterized in situ DNA hybridization to polytene chromosomes:
  - 30-50 sites of hybridization,
  - But many differences among wildtype flies
  - Mobile
  - ...but positions seemed relatively stable in individual lab strains
  - ...most don't move often and in most cases, don't know why/when they move

- First to be cloned different classes of DNA based on abundance:
  - Most don't move often and in most cases, don't know why/when they move

- Mobile Fig. 14.24 -- once again, polytene chromosomes were central to the finding

One important copia difference between two fly lines:

- white+ No copia element at band 3C2 (w)
- white+apricot copia element at band 3C2

w¹ was generated by 'PM hybrid dysgenesis'

Sved and Kidwell's discovery of strange incompatibility between wildtype races of flies:

<table>
<thead>
<tr>
<th>Female parent in cross:</th>
<th>Male parent in cross</th>
<th>Strain A</th>
<th>Strain B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Strain A</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Dysgenic</td>
<td>Strain B</td>
<td>Dysgenic: (1) hi mutation rate (2) chrom. rearrgmts. (3) sterility (degenerate gonads)</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Characterisation of any new strain with respect to P or M identity:

- Then Strain X must be M

| Female parent in cross: |
|-------------------------|------------------------|
| M strain maternal for dysgenesis | Strain X |
| Normal | |
| P strain paternal for dysgenesis | Dysgenic |
| Dysgenic | |

Then Strain Y must be P

<table>
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<th>Female parent in cross:</th>
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<tr>
<td>M strain maternal for dysgenesis</td>
</tr>
<tr>
<td>Dysgenic</td>
</tr>
<tr>
<td>P strain paternal for dysgenesis</td>
</tr>
</tbody>
</table>
Origin of \( w^{12} \)

<table>
<thead>
<tr>
<th>Female parent in cross:</th>
<th>Male parent in cross</th>
<th>P strain</th>
<th>dysgenic</th>
<th>produced ( w^{12} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>M strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Odd behavior of \( w^{12} \)

<table>
<thead>
<tr>
<th>Female parent in cross:</th>
<th>Male parent in cross</th>
<th>P strain</th>
<th>dysgenic</th>
<th>( w^{12} ) unstable (reverts to ( w^+ ))</th>
<th>( w^{12} ) stable</th>
</tr>
</thead>
<tbody>
<tr>
<td>M strain</td>
<td>( w^{12} ) stable</td>
<td></td>
<td></td>
<td></td>
<td>( w^{12} ) stable</td>
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<tr>
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<td></td>
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What was wrong with \( w^{12} \)?

It had a P-element transposon in it \([2.9 \text{ kb} \text{ DNA (if intact)}]\)
(generally the P inserts are internally deleted “defective” elements)

It looks as if:
PM hybrid dysgenesis drove a P element into white
partially destroying its function (the element moved!)

What is the difference between P and M strains?

- P strains have intact P elements
- M strains have no P elements

This is the key to the value of P-elements:
“virgin” strains exist that have NO P elements
(not true for any other fruit fly transposons)

P-element transposon has a very typical (simple) transposon structure:

- IR ends needed in cis for mobility (DNA between them is moved)
- Transposase protein can work in trans (on IR ends) to move DNA

Back to the original crosses that define P & M strains:

<table>
<thead>
<tr>
<th>Female parent in cross:</th>
<th>Male parent in cross</th>
<th>M strain</th>
<th>P strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>no P’s</td>
<td>many P’s</td>
</tr>
<tr>
<td>Permissive cytotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M strain</td>
<td>no P’s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal</td>
<td>no P’s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P strain</td>
<td>dysgenic</td>
<td></td>
<td>not mobile</td>
</tr>
<tr>
<td>P strain</td>
<td>many P’s</td>
<td></td>
<td>many P’s</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
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<td>M strain</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P strain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mobile!</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
The P element is a smart parasite:

1. Only makes transposase in germ cells
   - hence only moves and increases in number in germ cells
   thereby avoids collateral somatic damage

2. When invading virgin turf:
   - first moves and increases in number, but once established
   - calms down and avoids further problems for host
   (generates P (repressive) cytotype)

How to get transgenes from *in vitro* to *in vivo*:

- Your favorite gene(s) & \( W^+ \) transposase gene
- \( P \)-element (ends) serve as a "vector" to move DNA of our choice

Need a source of transposase to get it to insert into chromosomes

1. set up a dysgenic cross
2. inject an immobile source of transposase along with the transgene
   (transient source since can’t replicate) (a bit of a nuisance)
3. Use a defective (immobile) integrated P-element as a genetic source

modify so makes transposase in soma as well as germline

Use as a mutagen (make it hop… into genes):

- whatever is useful (e.g. \( W^+ \))
- (a nonautonomous element)

New (random) site of insertion

If so, already well marked and easy to clone!

*Lucky thing* that M strains exist

- Lab strains taken from the wild before 1950’s
- don’t have P elements.
- This DNA parasite invaded *D. melanogaster*
   sometime in the 1950s, then rapidly spread
   nearest P-element relative: in a fly species 50 Myr diverged

Why do M strains exist?

- an example of *horizontal* genetic transmission
  (xfrd in same generation, i.e. gene not introduced from parents (vs. vertical)}