2024: Class I: Identify genotype by phenotype
   The First Genetic Screen
   : Balanced chromosome key to fly genome
   : Inversions + human disease
   : (Transgenesis)

Goal of genetic crosses: unambiguously track genotypes across generations

Simplest cross: \( \frac{m}{+} \times \frac{+}{+} \)
\( m = \text{recessive mutant} \)
\( + = \text{wild-type} \)

1) How track recessive mutant \( m \) next generation?
   \( m/+ \times +/- \rightarrow m/+ \) or \( +/- \)

2) What if the mutant is dominant?
   \( M/+ \times +/- \rightarrow M/+ \)

3) What if recessive mutant is in trans to a dominant mutant? (far away vs tightly linked)

4) What if you can do two things:
   a) Dominant mutant in trans to your rec mut
   b) block recombination b/w homologous chromosomes
The First Genetic Screen: Muller, 1927 (1946 Nob.)

1) Known: mutations arise spontaneously, in nature, but very slowly; mutations follow Mendel's Laws
   - Goal: Generate mutations on demand ⇒ accelerated gene research.
   - Observations: Many substances reported to be mutagens - lead, alcohol, X-rays - but no definite evidence.
   - Question/Hypothesis: Are X-rays mutagenic? X-rays are mutagens?

3) Assay: Male lethality

   **Experiment:**
   - Cross: leth-ar, female X - chrom
   - Cross: 
     - $\frac{+m}{+m} \times \frac{CIB}{+m} \rightarrow$ lethal free
     - $\frac{+m}{+m} \times \frac{CIB}{+m} \rightarrow$ lethal free
     - $\frac{+m}{+m} \times \frac{+m}{+m} \rightarrow$ lethal free
   - Progeny: $\frac{+m}{+m}$, $\frac{CIB}{+m}$, $\frac{CIB}{+m}$, $\frac{+m}{CIB}$
   - 7000 single female crosses
   - Contains dominant marker: Bar

   - 100% suppression of ability to obtain recombinant progeny.

   **Conclusion:**
   - About 1 in 1000 $^+m$ chroms acquire a lethal mutation $b/w G0 \rightarrow E_1$
   - Why do this? What does it tell you?

   - 6340: $\frac{+m}{+m}$, $\frac{CIB}{+m}$, $\frac{CIB}{+m}$, $\frac{+m}{CIB}$ - Male = present
   - 6: $\frac{+m}{+m}$, $\frac{CIB}{+m}$, $\frac{CIB}{+m}$, $\frac{+m}{CIB}$ - Male = absent
   - 1 in 1000
Repeat screen with X-rays

X-rays

\[ \frac{t}{7} x CIB/+m \]

Adult male: inherited lethal free X chrom. from mother

\[ \text{F}_1 \left( \frac{t}{7} x +P \right) \times 2000 \text{ single female matings} \]

\[ \text{F}_1 \text{ genotypes:} \frac{+m}{7} \frac{+m}{7} \frac{CIB}{7} \frac{+P}{7} \]

Phenotypes: 

<table>
<thead>
<tr>
<th>Gender</th>
<th>Phenotype</th>
<th>Count</th>
</tr>
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<tbody>
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<td>250</td>
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<tr>
<td>f</td>
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<tr>
<td>φ</td>
<td>φ</td>
<td>1350</td>
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</tbody>
</table>

15% of crosses contained a newly induced lethal mutation in \( +P \) (\( \text{F}_1 \)): 150-fold increase.

Conclude: 1) X-rays are mutagenic

2) X-ray induced mutations follow Mendel's laws

3) Created \(~100\) visible mutations in one month
   - 200 known mutations before screen
   - mutagens = substrates for Muller's Mop

Common Types of mutagens:

1) X-rays: chrom. aberrations + point mutations

2) Chemicals: ENU/EMS \( \rightarrow \) G:G \( \rightarrow \) A:T

3) Transposons \( \rightarrow \) \( \Delta \) \( \rightarrow \) \( \Delta \)
Why does CIB block the ability to obtain recombinant progeny?

1) B/c it has an inversion: order relative to w:
   \[ 1 \underline{2(3\ 4\ 5\ 6)} \rightarrow 1 \underline{2(3\ 4\ 5\ 6)} \]

2) Types of Inversions: paracentric \( \rightarrow \) pericentric \( \rightarrow \)

3) Why do inversions suppress the ability to obtain recombinant progeny? \( \rightarrow \) block recombin\( \text{a} \)?
   \[ 1 \underline{2\ 3\ 4\ 5\ 6\ 7} \rightarrow 1 \underline{2\ 6\ 5\ 4\ 3\ 7} \]

Can visualize pairing b/w \( w^T \) + inverted chrom:

\[ 1 \underline{2\ 3\ 4\ 5\ 6\ 7} \rightarrow \begin{array}{c}
1 \underline{2(6\ 5\ 4\ 3)\ 7} \\
\end{array} \]

1) What happens if a single x-over in inverted region?
2) "" "" double "" ""?

Take home: x-overs that occur within inverted region, but products of single x-overs are inviable.
Drosophila Genetics

1) 4 chromosomes: X(1) → 2L 2R 3L 3R 4 (det)

A) Polytene numbering system
   - X: 1A1 → 20F
   - 2: 21A1 → 40F 41A1 → 60F
   - 3: 61A1 → 80F 81A1 → 100F
   - 4: 101A1

B) Genes: ~16k → 13k protein coding

C) Size: ~160 Mb - about 170 euchromatin

2) Other types of chromosomal aberrations

1) Deficiency: Df(3L) X Y Z

2) Duplicat: Dp(2L:2L) A B C

3) Transposition:

4) Translocation: A B C → A B C

Recurrent
A) Philadelphia Chrom: Chrom 9 + 22: Bcr + Abl
B) Burkitt's Lymphoma: Chrom 8 + 14

Enhancer

Myc
Keys to Fly Genetics:
1) Mendel's Laws
2) No recomb in males
3) Balancer chromosomes: effectively suppress the ability to obtain recombinant progeny even entire chrom. How?
   a) Multiply inverted
      e.g., TM6-Tb: 61A1-89C2-75C1-94A1-100F1-92D1-89D4-61A2-63B8-72E2-75C1
      C-mere
   b) Carry @ least one dom. mutat.
      1. Can unambiguously balancer = dom. marker
      2. "" unmarked homologous chrom by absence of the marker.
   c) Carry @ least one recessive lethal mutat
      1. b balancer/balancer = dead
      2. Except" = X-chrom: 0 viable, fertile & semi-sterile
   d) Carry multiple recessive visible mutat
      \[ \frac{m^1}{+} \times + + \text{ versus } \frac{m^1}{m^2} \times + + \text{ Balancer} \]
      m/cyo x m/cyo \rightarrow \text{ m/cyo; } 2x \text{ m/cyo; CyO/cyo } \phi
   e) Common Balancers:
      1) F17 Bar y w sn
      2) CyO Cy en bw
      3) TM3 6b c
   f) Nomenclature y w; en bw sp; e vs F17
        - draw out
How do inversions arise in nature? (Genome ~ 10 kb)

Hemophilia A: About 40% of severe hemophilia A cases arise from a nearly identical molecular event @ factor VIII gene

Factor VIII gene: X-linked gene; required for blood clot

Locus:

9.5 kb intronless gene: 2% 99.9% seq identity
- homologous on sequence, but non-allelic

The following mutates arised de novo @ a low frequency in all ethnic backgrounds
- preferentially occurs during meiosis in o

Non-allelic, but hom. recombinant but non-allelic recombinant
- CNVs = 5-10% of human genome
- substrates for homologs
- color, blindness...

What happens/is the product of recomb. b/w direct repeats on same DNA molecule?
Transgenesis: Insert exogenous DNA \(\rightarrow\) genome site

Three Needs:
1. DNA needs to get in the genome
2. You need to know it got in
3. It needs to stay in

A Case study: P elements = DNA Transposon

1. Autonomously P element: can jump on its own
   - A) LTRs required in cis
   - B) 4 OREs = Transposase
     \[\text{required in trans} \quad \text{Exons} \quad \text{LTR}\]
     \[\text{2907 bp}\]

2. Non-autonomous P element: can jump, but needs...
   - A) Intact LTRs
   - B) Internally deleted
     \[\text{non-functional} \quad \text{transposase} \quad \text{LTR} \quad \text{LTR} \quad \text{required in cis}\]

How can you use P elements \(\rightarrow\) DNA \(\rightarrow\) gene

What part is needed to jump?

- LTRs
- \(\text{Are they separable?}\)