1) How to identify genotype by phenotype.
   a) The 1st Genetic Screen
   b) Balanced chromosomes: the keys to fly genetics
   c) C. V. S.: Inversions/Hemophilia
   d) Transgenesis

Goal of genetic crosses: Unambiguously follow genotype through generations. How

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pheno</th>
</tr>
</thead>
<tbody>
<tr>
<td>m/+</td>
<td>+</td>
</tr>
<tr>
<td>m/+</td>
<td>+</td>
</tr>
<tr>
<td>+/+</td>
<td>+</td>
</tr>
<tr>
<td>+/+</td>
<td>+</td>
</tr>
</tbody>
</table>

Questions:

1) If an organism is heterozygous for a recessive mutant, how do you follow it in the next generation?

2) What if it's a dominant mutant?

3) What if the recessive mutant is in trans to a dominant one - distantly linked? - tightly linked?

4) What if you could block recombination between homologs and put a dominant mutant in trans to your recessive mutant of interest?
The First Genetic Screen: 1927 Muller (1946 Nobel)

**Known:** Mutants arise spontaneously in nature at very slow rate. Mutants follow Mendel's Laws.

**Problems:** Low number of gene mutants limits research on genes.

**Goal:** Generate mutants on demand.

**Observations:** Reports of many substances inducing mutants, e.g., lead, alcohol, x-rays, but no definitive evidence.

**Question/Hypothesis:** Are x-rays mutagenic? / X-rays are mutagenic?

**Assay:** Male lethality

\[ C_{IB} \]

\[ \frac{C_{IB}}{m} \]

\[ \frac{C_{IB}}{p} \]

\[ \frac{C_{IB}}{T} \]

\[ \frac{C_{IB}}{w} \]

\[ \frac{C_{IB}}{f} \]

\[ \frac{C_{IB}}{\phi} \]

\[ \frac{C_{IB}}{\psi} \]

\[ \frac{C_{IB}}{\Omega} \]

\[ \frac{C_{IB}}{\Phi} \]

\[ C_{IB} = \text{magic chromosome} \]

1) Lethal in \( \phi \): \( C_{IB} \rightarrow \phi \)

2) Contains Dom. mutat. A - Bar

3) Completely suppresses the product of recomb. progeny.

\[ \text{Conclusion:} \ 6/6346 \text{ crosses (0.1%) of } \frac{C_{IB}}{p} \text{ chromosomes contained a newly induced lethal mutant.} \]
Experiment: Repeat 2 X-ray treatment

1. $\frac{m}{4}$ lethal fly?
2. $\frac{m}{4}$ Bar $\phi$?
3. $\frac{m}{4}$ No $\alpha^2$

Conclude:
1. X-rays are mutagenic.
2. New mutations obey Mendel's laws.
3. Make 1000 visible mutants in a month (vs. muts, time of screen).
   * Catalyzed research on genes/gene mutants.
   * Did this simply by using phenotype to follow genotype.

Common Types:
1. X-rays - chrom. breaks + point mutation of Mutagens
2. Chemicals: EMS/ENU - Gic - \( \alpha^4 \)
   - high frequency can hit all genes
3. Transposons: \( \rightarrow \)
4. TALENS, CRISPR = targeted mutat of a specific gene.
Why does C1B block the ability to obtain recombinant progeny?

1) It contains a large inversion.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3 4 5 6</td>
<td>7 8</td>
</tr>
</tbody>
</table>

- A) paracentric
- B) pericentric

2) Why do inversions suppress the ability to obtain recombinant progeny?

\[
\begin{align*}
(3 4 5 6) & \quad \rightarrow \quad 1 2 \quad (5 6) \\
1 2 (3 4 5 6) & \quad \rightarrow \quad 1 2 \quad (4 5 6)
\end{align*}
\]

Can visualize pairing b/w chromosomes in 2 ways:

- \[
\begin{align*}
1 2 3 4 5 6 7 8 \\
8 7 (3 4 5 6) 2 1
\end{align*}
\]
- \[
\begin{align*}
1 2 3 4 5 6 7 8 \\
1 2 3 (4 5 6) 7 8
\end{align*}
\]

1) What happens if a single crossover w/in inverted region?
2) """" double crossover ""

Take home: 1) Recombinants can occur b/w inverted & wild type.
2) Products of single crossover are inviable.
1. **4 chromosomes**

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>X(II)</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

2. Polytenic numbering system
   - X(II): 1A1 - 20F
   - 2: 2A1 - 40F 4B1 - 60F
   - 3: 6A1 - 80F 8A1 - 100F
   - 4: 101A1 -

3. Genes 13-14k

4. Size: 160-170 Megabases, ~120MB euchromatin, 40-50MB heterochrom

---

**Other types of chromosomal abnormalities:**

1. **Deficiency:** D (3R) XYZ

2. **Duplication:** Dp (2R:2R) XYZ

3. **Transposition:**

4. **Translocation:**

   A) Philadelphia chromosome - T-locus b/w chrom 9 + 22: BCR-ABL fusion

   B) Burkitt's Lymphoma
   - Ig enhancer
   - Myc

---

---
**Keys to Fly Genetics**

1. **Mendel's Laws**
   1. No recombination in males
   2. Recombination in males

3. **Balancer chromosomes**
   A) Key features of Balancer chromosomes
   1. Multiply inverted: suppress ability to get viable recomb. progeny across entire chrom
   
   E.g.: TM6 Tb: 61Rx18962-TSc1941-100F3/92D1-89C8161A2-63B87262-TSc1941-92E1/100F3
   
   2. Balancer chrom. carry @ least one dominant marker
   a) Can track balancer in crosses by dom. marker
   b) Can unambly track the unmarked homologous chrom. by the absence of the balancer.
   - e.g.: \( \frac{3}{4} x \frac{1}{4} \) vs \( \frac{3}{4} \) \( \frac{1}{4} \)

3. Carry @ least one lethal mutation: \( \frac{Balancer}{Balancer} = 0 \)
   
   a) Except for X(1) chromosome
   - \( Q \) viable, fertile
   - \( Q \) semi-viable, sterile.

4. Carry multiple recessive mutations

B) **Common Balancer Chromosomes**
   1. FM7 Bar y w sn
   2. CyO Cc en bw
   3. TM3 Sb e TM6 Tb e

C) Utility of Balancer chromosomes: \( \frac{1}{TM3}; \) see e.g. Muller screen

Nomenclature: \( yw; \) on bw sn
\( \frac{ywj}{FM7}; \) en bw sn
\( \frac{j}{TM3}; \) en bw sn
\( \frac{yw}{FB1}; \) en bw sn
\( \frac{j}{TM3}; \) en bw sn
CNVs - How do inversions arise?

Hemophilia A - About 40% of severe hemophilia A cases arise from similar molecular event at Factor VIII gene
- occurs in different ethnic backgrounds

1) Factor VIII Gene
X-linked

Segmental duplications: Copy Number Variation
- ~ 9.5 kb intronless gene
- ~ over 99.9% identical

The following event occurs at a low frequency during male meiosis (all ethnic backgrounds)

Homologous but non-allelic recomb

1) Event occurs almost exclusively in male, not female, meiosis
   - why?

2) Occurs de novo at a low freq. in all ethnic backgrounds
   - why?

3) CNVs/Segmental Duplications
   A) 5-15% of human genome
   B) substrates for homologous, but non-allelic recom

C) What are the consequences of recombinatn b/w direct/inverted repeats on the same molecule.
Transgenesis:

Insert exogenous DNA into the germ line genome.

1. Requirements for germ line transgenesis:
   A. DNA must insert into the germ line DNA.
   B. You must know it got in (ideally track it).
   C. DNA must stay in the genome (be stable).

2. If you can get exogenous DNA into germ line then what can you do with it.

Drosophila = model organism in which transgenesis has been leveraged to the greatest extent.

1. Key aspects of transgenesis.
2. Power of transgenic tools:
   → Think about how to transfer these tools to other systems.
P elements: DNA Transposon (P. elegans, Minos...)

1. P element = DNA transposon: jumps as a DNA intermediate

2. Two types of P elements
   - Autonomous P element: can jump on its own
     - exons
     - all 4 exons
     - encode
     - transposase
     - LTRs required in cis for I-position
   - Non-autonomous P element: can jump but not by self
     - internally deleted
     - non-fetal transposase

How can you use P elements for transgenesis?

- Add DNA into
- Know it got in
- Keep it in track

1. Germline transposition: Two component system (LTR/Trage)
2. Helper plaque (wings clipped)
3. Destroy LTRs

- w-/+ w-
- P elements: germline
- Cells: germline
- w-/+ w-
- Assess progeny

* Can jump, can track