Office hours:
  Monday  5-6pm (door closes @ 5:15p)
  Friday 4-5 pm

11 Koshland Hall
What does a geneticist mean by “gene”? …revealed by how a geneticist tests for “allelism”

Alleles: DIFFERENT (alternative) forms of the same gene

Given:

- mutant a (lozenge-shaped eyes) vs. wildtype
- mutant b (lozenge-shaped eyes) vs. wildtype

Are mutant A and mutant B alleles?

**YES:** A&B are alternative forms of the same gene

\[ lozenge^a \text{ and } lozenge^b \]

**NO:** A&B are alternative forms of different genes

\[ lozenge^a \text{ and } lozengelike^b \]
First must ask:
(1) are each of these mutant phenotypes **likely** to be **caused** by **single-gene differences** from the wildtype?
(2) do the mutants map to the **same general region** of the genome?

(sequencing mutant A and mutant B genomes would not necessarily answer either question!)

Given:
- **mutant a** vs. **wildtype**
- **mutant b** vs. **wildtype**

Before we ask:
Are **mutant a** and **mutant b** alleles?
are each of these mutant phenotypes likely to be caused by a single-gene differences from the wildtype? 

(2) do the mutants map to the same general region of the genome?

**low resolution segregation tests:**

(1) \[ \frac{a}{a} \times +/+ \rightarrow \frac{a}{+} \rightarrow \frac{a}{a} \& \frac{a}{+} \& +/+ \text{ only} \]

(2) \[ m_{1^-}m_{2^-}, a^+ \times m_{1^+}m_{2^+}, a^{lz} \rightarrow \text{ trihybrid} \rightarrow \]

least frequent gamete classes: \[ m_{1^-}m_{2^-}a^{lz} \]

\[ \& m_{1^+}m_{2^+}a^+ \]

**3-factor cross rule:** least frequent class two outside parental, inside nonparental: hence order: \[ m_{1} \ a \ m_{2} \]
Are **mutant a** and **mutant b** alleles (i.e. genetic alternatives)?

Do we go with Mendel or (Lewis)/Benzer?

- high-resolution segregation test for recombination
- breeding behavior of hybrids
  
  **mutant a**
  
  **mutant b**

<table>
<thead>
<tr>
<th>a</th>
<th>+</th>
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</thead>
<tbody>
<tr>
<td>+</td>
<td>b</td>
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**OR**

<table>
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Are a & b alternative units of segregation during meiosis?

i.e. are they **segregational alleles**?

- complementation test for function
- phenotype of hybrids

<table>
<thead>
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Are a & b alternative cis-acting units of function during development?

i.e. are they **functional alleles**?
Are mutant a and mutant b alleles (i.e. genetic alternatives)?

High-resolution segregation test for recombination and mutant behavior of hybrids.

Complementation test for function phenotype of hybrids.

Must first establish: that a & b are recessive?

\[
\begin{align*}
\text{mutant a} + \text{mutant b} \\
\begin{array}{c}
a + \\
+ b
\end{array} = ?
\end{align*}
\]

Gametes

\[
\begin{align*}
\text{a +} = & \text{a} \\
\text{b +} = & \text{b} \\
\text{+ +} = & \text{w.t.} \\
\text{a b} = & \text{?}
\end{align*}
\]

Not alleles

\[
\begin{align*}
\text{a +} = \text{a} \\
\text{b +} = \text{b} \\
\text{+ +} = \text{w.t.} \\
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Alleles

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\text{b +} = \text{b} \\
\text{+ +} = \text{w.t.} \\
\text{a b} = \text{?}
\end{align*}
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Wildtype

\[
\begin{align*}
\text{mutant a} + \text{wildtype} & = \text{w.t.phene.} \\
\text{mutant b} + \text{wildtype} & = \text{w.t.phene.}
\end{align*}
\]

Phenotype

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Mutant

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+ b
\end{align*}
\]

Wildtype

\[
\begin{align*}
\text{mutant a} + \text{mutant b} \\
\begin{array}{c}
alleles
\end{array}
\]

Alleles

(hence one functional copy sufficient)
Are mutant a and mutant b alleles (i.e. genetic alternatives)?

What about the cis/trans test?

Where is the cis control?

complementation test for function phenotype of hybrids

\[
\begin{align*}
\text{mutant a} & \quad \text{wildtype} = \text{w.t.phene.} \\
\text{mutant b} & \quad \text{wildtype} = \text{w.t.phene.}
\end{align*}
\]

\[
\begin{array}{c}
\text{a} & \text{b} \\
\text{in trans} & \text{mutant a} & \text{mutant b} \\
\text{a} & \text{+} & \text{b} & \text{ OR } & \text{a} & \text{+} & \text{b} & ?
\end{array}
\]

\[
\begin{align*}
\text{a & b in cis} & \quad \text{wildtype phenotype (complement)} \\
\text{a & b not alleles}
\end{align*}
\]

\[
\begin{align*}
\text{a & b in cis} & \quad \text{mutant phenotype (don’t complement)} \\
\text{alleles}
\end{align*}
\]
Are mutant a and mutant b alleles (i.e. genetic alternatives)?

--- are we being misled by the apparent failure of these recessive mutants to complement in trans?

complementation test for function phenotype of hybrids

mutant a + mutant b

When do we need the cis control

...could mutants be recessive individually and in different cis-acting units of genetic function (i.e. not be functional alleles), but interact in combination to appear dominant together)

If so, the cis control will also be mutant!
Are mutant a and mutant b alleles (i.e. genetic alternatives)?

**CIS control**

[mutant a] [mutant b]  +  +

**TRANS**

[mutant a] [mutant b]  +  +

Wildtype phenotype
...we can believe it!

Mutant phenotype
Don’t appear to complement
*but can we believe it?*

CIS phenotype ≠ TRANS phenotype, hence functional alleles

*BUT* if mutant phenotype
...we’ve been misled!!!

cis vs. trans matters!

cis vs. trans doesn’t matter!

CIS phenotype = TRANS phenotype, hence NOT functional alleles
Are **mutant a** and **mutant b** alleles (i.e. genetic alternatives)?

**CIS/TRANS test**

- **same recessive mutants in CIS**
  - mutant a  mutant b
  - +      +

- **recessive mutants in TRANS**
  - mutant a
  - mutant b
  - -      -

**wildtype**

Phenotype

... we can trust the trans result

But nobody bothers with the cis control for **recessive** mutants when doing complementation tests despite what all the textbooks say (not even Benzer did it, as we will see)

It is **too hard** in most cases, and in most cases **unnecessary**.

...then why am I wasting your time with this?
Are mutant a and mutant b alleles (i.e. genetic alternatives)?

The true cis/trans test will allow us to determine allelism even if one or both of the mutants are not recessive!

CIS phenotype $\neq$ TRANS phenotype, hence functional alleles

CIS phenotype = TRANS phenotype, hence NOT functional alleles

…and all that matters is whether the cis vs. trans phenotypes are the same or different, not whether either one is wildtype.

Remember: the “complementation test” per se is limited to recessive mutants. Most mutants are recessive, but some of the most useful & interesting are not.
Are mutant a and mutant b alleles (i.e. genetic alternatives)?

complementation test for function

alleles as alternative cis-acting units of function

high-resolution segregation test for recombination:

alleles as alternative units of segregation (recombination)

\[ \text{mutant a} \]
\[ \text{mutant b} \]

\[ \text{a} \text{ & } \text{b} \] may appear to be allelic by the functional test (failure to complement)

results may conflict

\[ \text{mutant a} \]
\[ \text{mutant b} \]

...yet NOT allelic by the functional test (nonparental alleles recovered by meiotic recombination)

Mendel said that genes are the units of segregation, which led to the “beads on a string” model of genes & chromosomes:

genetic map (linear like chromosome)
Are mutant a and mutant b alleles (i.e. genetic alternatives)?

complementation test for function
alleles as alternative cis-acting units of function

Intuitively, a better operational definition of alternative forms of genes

conflict favored

high-resolution segregation test for recombination:
alleles as alternative units of segregation (recombination)

The basis for making genetic maps

Genetic map (linear like chromosome)

If one can get recombination between functional alleles (alternative forms of a gene), then how do the genetic maps one can therefore construct within single genes compare to the genetic maps that can be (had been) constructed between genes?

--- WHAT IS THE NATURE OF GENETIC FINE STRUCTURE?

...and what is the basic (minimal) unit of recombination (i.e. what are true segregational alleles)?
That two distinct kinds of substances—the d’Hérelle substances and the genes—should both possess this most remarkable property of heritable variation or "mutability," each working by a totally different mechanism, is quite conceivable, considering the complexity of protoplasm, yet it would seem a curious coincidence indeed. It would open up the possibility of two totally different kinds of life, working by different mechanisms. On the other hand, if these d’Hérelle bodies were really genes, fundamentally like our chromosome genes, they would give us an utterly new angle from which to attack the gene problem. They are filterable, to some extent isolable, can be handled in test-tubes, and their properties, as shown by their effects on the bacteria, can then be studied after treatment. It would be very rash to call these bodies genes, and yet at present we must confess that there is no distinction known between the genes and them.
Phage kill bacteria for a living (and replicate in the process). Geneticists count “centers of killing” (plaques).
T4 plaques on a “lawn” of *E. coli* cells
Presence indicated as plaques

add: 100 phage in 0.1 ml media
to: \(10^7\) cells in 0.1 ml media
let set 20 min
add: 2.5 ml molten agar
pour on a plate
grow overnight at 37 C

\[
\text{moi is } 100:10^7 = 1:100,000\ldots.
\]

…..the chance of a single cell being infected
by 2 phage is \(10^{-10}\)

“multiplicity of infection”
Must have a **phenotypic difference** between pure-breeding lines to do genetics.
Phage phenotypes (differences): based on **growth**

- Plaque **morphology**
  - large/small (growth rate)
  - clear/turbid
  - smooth edged/rough edged

- Growth **conditions**
  - bacterial host range
  - temperature range (T4: 25-42 C)
By early 1950s, Alfred Hershy had isolated a number of phage T4 mutants and constructed a (circular) genetic map based on

(1) complementation (to group the mutants into genes)

and

(2) recombination to organize those genes on a map.

…and along came physicist Seymour Benzer
The young S. Benzer: (1950s) **phage T4** as his genetic workhorse

- reconciled the segregational and functional definitions of the gene
- set up the experimental system used in the 2nd most elegant genetics paper in history

An older Benzer: (1967-present) **fruit fly (D.melanogaster)** as his genetic workhorse

- founded many of the most interesting areas of modern behavioral genetics (clocks & learning, etc.)
- set up the experimental system most effective for studying fly development: the **compound eye**
FINE STRUCTURE OF A GENETIC REGION IN BACTERIOPHAGE

By Seymour Benzer

This paper describes a functionally related region in the genetic material of a bacteriophage that is finely subdivisible by mutation and by genetic recombination. The group of mutants resembles similar cases which have been observed in many organisms, usually designated as "pseudo-alleles." (See reviews by Lewis and Pontecorvo.) Such cases are of special interest for their bearing on the structure and function of genetic determinants.

The phenomenon of genetic recombination provides a powerful tool for separating mutations and discerning their positions along a chromosome. When it comes to very closely neighboring mutations, a difficulty arises, since the closer two mutations lie to one another, the smaller is the probability that recombination between them will occur. Therefore, failure to observe recombinant types among a finite number of progeny ordinarily does not justify the conclusion that the two mutations are inseparable but can only place an upper limit on the linkage distance between them. A high degree of resolution requires the examination of very many progeny. This can best be achieved if there is available a selective feature for the detection of small proportions of recombinants.