Mutations (changes in DNA): the lifeblood of genetic analysis

(1) **What kinds can we make?** (categories)

(2) How do we make them? (mutagenesis)

(3) How do we find them? (mutant screens & selections)

(4) Why bother?
Muller categorized mutations with respect to change in gene function relative to wildtype.

- **Loss-of-(wildtype)function (l-o-f)** mutant alleles
- **“Gain”-of-(over wildtype)function (g-o-f)** mutant alleles
to infer the **normal function of a gene:**

**LOF alleles** simplest to interpret

**GOF alleles** …to understand what (if anything) they are telling us about the wildtype gene function one needs to **know the null mutant phenotype.**

Can use **GOF alleles** to generate **LOF alleles** relatively easily:

“revert” (suppress) the dominance of a GOF allele ➔ LOF allele

Illustrated by:

ovo$^{D1}$ antimorph
ono$^{e8K}$ hypomorph

How would we know if these female-sterile mutant alleles are in the same gene?
To “revert” the dominance of the antimorph $ovo^{D1}$, we had to lose the gene function *in cis to $ovo^{D1}$* that $ovo^{e8K}$ is missing

.. hence we have established that $ovo^{D1}$ and $ovo^{e8K}$ are functional alleles
Same for Antp$^{32a5}$ and Ns$^1$, two GOF mutant alleles that cause the antenna to develop as a leg instead.

revert their dominance
(one gets a recessive lethal in each case)
and see if the resulting recessive mutant chromosomes fail to complement.
Muller did not just define the five basic ways the functioning of a gene can be changed by mutation (without, he noted, changing its ability to faithfully replicate).

He gave us operational tests to determine to which class a given mutant allele might belong.

**Loss-of-(wildtype)function (l-o-f) mutant alleles**
- complete lof: amorph(ic) (null)
- partial lof: hypomorph(ic) (leaky)

**“Gain”-of-(over wildtype)function (g-o-f) mutant alleles**
- too much of a good thing: hypermorph(ic)
- something new & different: neomorph(ic)
- antagonizes (poisons) wildtype: antimorph(ic)
Muller’s tests: how does the phenotype change when you:

1. hold the number of **mutant** alleles constant and change the number of **wildtype** alleles.

2. hold the number of **wildtype** alleles constant and change the number of **mutant** alleles.

**increased dose of mutant, phenotype more wildtype**

The **white** gene started it all, and has kept it all going to this day.

<table>
<thead>
<tr>
<th><strong>wa/wa</strong> (darker, more “wildtype”)</th>
<th><strong>wa/Df(w)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>2 copies <strong>wa</strong> allele</td>
<td>1 copy <strong>wa</strong> allele</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>wa/Y</strong> (lighter, less “wildtype”)</th>
<th><strong>wa/Y; Dp(wa)/+</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 copy <strong>wa</strong> allele</td>
<td>2 copies <strong>wa</strong> allele</td>
</tr>
</tbody>
</table>
See how the phenotype changes when you:

(1) hold the number of mutant alleles constant and change the number of wildtype alleles. **increased dose of wildtype, phenotype more wildtype**

(2) hold the number of wildtype alleles constant and change the number of mutant alleles. **increased dose of mutant, phenotype more wildtype**

\[
w^a/w^+ \text{ (darker, more “wildtype”) } > w^a/Df(w)\\
1 \text{ copy } w^+ \text{ allele } \quad 0 \text{ copies } w^+ \text{ allele}
\]

\[
w^a/Y \text{ (lighter, less “wildtype”) } < w^a/Y; Dp(w^+)/+\\
0 \text{ copies } w^+ \text{ allele } \quad 1 \text{ copy } w^+ \text{ allele}
\]
Loss-of-(wildtype)function (l-o-f) mutant alleles

- Complete lof: **amorph**(ic) (null)

partial lof: **hypomorph**(ic) (leaky) 

“Gain”-of-(over wildtype)function (g-o-f) mutant alleles

- Too much of a good thing: **hypermorph**(ic)
- Something new & different: **neomorph**(ic)
- Antagonizes (poisons) wildtype: **antimorph**(ic)
**Partial lof:**  hypomorph(ic)

Increased dose of **mutant:**  phenotype more wildtype

Increased dose of **wildtype:**  phenotype more wildtype

But isn’t in RATHER curious then that:

\[ w^a/w^a = \text{(same color as)} \ w^a/Y \]

\[ \varnothing \]

**Muller** thought so, and realized that he had discovered

**X-chromosome dosage compensation**

XX=XY  (more about that later)
Truth be known, geneticists take shortcuts (c.f. cis/trans test)

The characterization of a mutant allele as a amorphic (null) vs. hypomorphic is generally made based only on a comparison of the homozygous mutant to the hemizygous mutant (and with the knowledge that the mutant is recessive): \( \text{white}^x/\text{white}^x \) vs. \( \text{white}^x/\text{Df}(w) \)

...potential pitfalls, but a good place to start
“Gain”-of-(over wildtype)function (g-o-f) mutant alleles

too much of a good thing:  \textbf{hypermorph(ic)}
  - Increased dose of \textbf{mutant}: phenotype more mutant
  - Increased dose of \textbf{wildtype}: phenotype more mutant

something new & different:  \textbf{neomorph(ic)}:
  - Increased dose of \textbf{mutant}: No change or more mutant in phenotype
  - Increased dose of \textbf{wildtype}: No change in phenotype

our friend from the X-files, \textit{Antp}

antagonizes (poisons)\textit{wildtype}:  \textbf{antimorph(ic)}  \textit{ovo}^D_1
  - Increased dose of \textbf{mutant}: phenotype more mutant (if possible)
  - Increased dose of \textbf{wildtype}: phenotype more wildtype
Mutations (changes in DNA):

Categorized with respect to:

1. change in gene function relative to wildtype

   A) single base pair (point mutation)
      - transition (Pur→Pur or Pyr→Pyr)
      - transversion (Pur→Pyr)
      - frameshift (reading frame changed)
      - missense (amino acid difference)
      - nonsense (translation terminates)

   B) more than a single base pair
      - chromosomal (enough to see cytologically)
      - pseudopoint (cytologically invisible, and small enough to make it hard to distinguish genetically from a point)
        - e.g. transposon insertions

2. molecular nature (Chp 7 and a bit of Chp 8)
Mutations (changes in DNA):

Categorized with respect to:
(1) change in gene function relative to wildtype
(2) molecular nature

(3) Effect on organism
   a) Lethal (generally developmental) (most common)
   b) sterile (sterility is the genetic equivalent of death)
   c) homeotic (replacement by an inappropriate structure)
   d) visible (“morphological”)
      most useful as “genetic markers” if:
      easily distinguishable (high expressivity)
      reliably distinguishable (high penetrance)
      fully viable and fertile

   e) conditional [key for microbial (haploid) genetics
      but useful for all genetics]

in many cases, molecular markers are supplanting (RFLP, PCR, Microarrays)
categories of conditional mutations:

1. auxotrophic (new nutritional requirement for growth relative to wildtype [prototroph])

2. host range (infectivity/virulence) parasite “nutrition”

3. sex-limited/specific (including dominant lethals & steriles)

4. genetically suppressible:

   ...variant allele at a different gene counteracts

   $a^{mut}/a^{mut}; sup^+/sup^+$ = mutant phenotype

   $a^{mut}/a^{mut}; sup^{Mut}/sup^+$ = wildtype phenotype

   $a^{mut}/a^{mut}; sup^{mut}/sup^{mut} = wildtype$ phenotype

   (in haploids: $a^{mut}; sup^{mut} = wildtype$ phenotype)

A mutation that makes a bad situation better is more likely to be informative than one that makes a bad situation worse

  (...and of course is likely to occur less frequently!)
categories of **conditional mutations:**

1. auxotrophic (new nutritional requirement for growth relative to wildtype [prototroph])
2. host range (infectivity/virulence) parasite “nutrition”
3. sex-limited/specific (including dominant lethals & steriles)
4. genetically suppressable
5. temperature-sensitive (ts)