This “map” of rudimentary alleles does not imply anything about where the various mutations might lie on a meiotic map.

It is simply a schematic representation of a collection of data from a series of complementation tests designed to determine functional allelism.

The Complementation Map

Extensive analysis was undertaken of the phenotypic expression of crosses involving 31 r alleles, and this enabled the construction of the complementation map illustrated in Fig. 1. It was possible to arrange the mutants in a unique linear sequence involving different or adjacent cistrons. In the construction of this map, mutants which complemented were assigned...

Fig. 1. Complementation map of the r-locus. The number of mutants in each category is indicated in brackets.
Let’s consider three $r$ mutant alleles:

did they complement (Y or N)?

\[ a^{-}/b^{-} : \text{Yes} \]

...and the hybrid fly looked: \textit{wildtype}
The Complementation Map

Extensive analysis was undertaken of the phenotypic expression of crosses involving 31 r-alleles, and this enabled the construction of the complementation map illustrated in Fig. 1. It was possible to arrange the mutants in a unique linear sequence involving different or adjacent cistrons. In the construction of this map, mutants which complemented were assigned

---

**Fig. 1.** Complementation map of the r-locus. The number of mutants in each category is indicated in brackets.

---

did they complement (Y or N)?

No

they **do** overlap on the map

---

**a -/h - : No**
Consider a new \( r \) mutant allele, \( rz^3 \)

Which heteroallelic combination (hybrid) is most likely to have a mutant phenotype?

Which heteroallelic combination is most likely to generate a wildtype allele during meiosis?

\[ rz^3 / i \]

\[ ? \text{ no basis for a determination} \]
This “map” tells us next to nothing about the possible molecular basis for the complex complementation pattern… but so long as we have reason to believe that group $i$ contains at least some point mutants, all the mutants on this “map” are likely to be in the same (thing that we want to call a) gene.

The Complementation Map

Extensive analysis was undertaken of the phenotypic expression of crosses involving 31 $r$-alleles, and this enabled the construction of the complementation map illustrated in Fig. 1. It was possible to arrange the mutants in a unique linear sequence involving different or adjacent cistrons. In the construction of this map, mutants which complemented were assigned

...and $i$ is the most frequent class

Fig. 1. Complementation map of the $r$-locus. The number of mutants in each category is indicated in brackets.
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?
Fig. 20.2 a fruit fly 9h after fertilization

**wildtype (ftz^+)**

**ftz amorph (null mutant) homozygote** (it’s recessive lethal)
something Thomas Hunt Morgan never guessed (among other things): you can get a huge amount of **phenotypic information** out of the **skin of a dead maggot** by 16h after fertilization, even if doomed

denticle belt pattern on the larval cuticle
*(cuticle: “skin,” tho. actually “skeleton”)*
*(denticles: maggot tire treads)*
reveals **where** fly cells think they are in space

Wieschaus and Nüsslein-Volhard, Nobel Prize 1995
**Fig. 20.19**

**maximally informative** mutant phenotypes for understanding metazoan pattern formation

(b) Phenotypes caused by gap gene mutations

- **wildtype**
- **Krüppel**
- **hunchback**
- **knirps**

(*ftz* is in the “**pair rule**” family, not the “**gap**” family of mutant phenotypes)
Mutations (changes in DNA): the lifeblood of genetic analysis

**fact:** it’s easier to mess things up than to make them better

hence most mutations with any functional consequence (**mutant phenotype**) **disrupt** normal (**wildtype**) gene function

Geneticists like to mess DNA up to discover **what genes do what**

so they can tell **biochemists** where to look to learn **how** those genes do it

(...and sometimes geneticists can learn a thing or two about “how” even before the biochemists enter the picture)
**Forward genetics:**

mutant phenotype (functional consequences of disruption) ➔ wildtype molecules to infer wildtype function

**Reverse genetics:**

wildtype molecules (including transcription units) ➔ mutant phenotype (functional consequences of disruption) to infer wildtype function
Mutations: the lifeblood of genetic analysis

In the context of our current goal: infer wildtype function

Not Mendel’s goal: (predict the appearance and breeding behavior of hybrids)

Not Morgan’s goal: (learn how inherited information is transmitted & how it changes)

(neither had any hope of discovering what gene are

(1) What kinds can we make?
(2) How do we make them?
(3) How do we find them?
Mutations: the lifeblood of genetic analysis

In the context of our current goal: to infer wildtype function.

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

Herman Muller (1930s):
In what ways can mutations affect normal gene function?

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

Muller: spontaneous & radiation-induced

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

Muller: exploited giant polytene chromosomes & invented balancer chromosomes
Muller categorized mutations with respect to change in gene function relative to wildtype

**Loss-of-(wildtype)function (l-o-f) mutant alleles**

- generally recessive \((a^+/a^-):\) one functional copy “suffices”
  - (1) so long as all other genes ok
  - (2) so long as we don’t look too hard

  clear exception: l-o-f mutations in haploinsufficient genes are dominant by definition (Minute mutations in flies; \(Df(M)/+=M/+\))

**lof alleles causing cancer:** identify tumor suppressor genes

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

- generally dominant (often misexpression, wrong time/place): \(\text{Antp}^X/\text{Antp}^+\)

  fly leg where antenna should be

**gof alleles causing cancer:** identify proto-oncogenes
Loss-of-(wildtype)function (l-o-f) mutant alleles

- complete lof: **amorph**(ic) (null)
- partial lof: **hypomorph**(ic) (leaky)

**Important goal of genetic analysis:**
define the null phenotype

**Phenotypic series:**
set of alleles with progressively less function

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

- too much of a good thing: **hypermorph**(ic)
- something new & different: **neomorph**(ic):
  - different in kind (e.g. fusion protein)
  - wrong time/place: **ectopic expression**
- antagonizes (poisons) wildtype: **antimorph**(ic)
  - (dominant negative)
to infer the normal function of a gene:

**LOF alleles** simplest to interpret

**GOF alleles** usually more interesting

but generally **harder to interpret**

.. especially if don’t know amorph (null) phene.

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

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

…and thereby establish allelism between the GOF and LOF alleles

consider the fly gene ovo as a good example:

ovo^{D(ominant)#1} dominant female-specific sterile antimorph

ovo^{e8K} recessive female-specific sterile hypomorph

**normal ovo^{+} function:** establish female identity of fly germ cells?

how do we even know ovo^{D1} and ovo^{e8K} are alleles? (ovo^{D1}/ovo^{e8K}?)
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>ovoD1 / +</code></td>
<td>[tumorous female germline]</td>
</tr>
<tr>
<td><code>Df(ovo) / +</code></td>
<td>[wildtype female germline]</td>
</tr>
<tr>
<td><code>ovo^e8K / +</code></td>
<td>[wildtype female germline]</td>
</tr>
<tr>
<td><code>ovo^e8K / ovo^e8K</code></td>
<td>[tumorous female germline]</td>
</tr>
</tbody>
</table>

**complementation test?**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>ovoD1 / ovo^e8K</code></td>
<td>[wildtype or mutant?]</td>
</tr>
</tbody>
</table>

**cis-trans test?**

...if in same gene, cis phenotype ≠ trans

<table>
<thead>
<tr>
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<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>ovoD1 + / + ovo^e8K</code></td>
<td>[tumorous]</td>
</tr>
<tr>
<td><code>ovoD1 ovo^e8K / ++</code></td>
<td>[less tumorous or wt.]</td>
</tr>
</tbody>
</table>

...if in different genes, cis phenotype ≠ trans

<table>
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<td><code>ovoD1 + / + ovo^e8K</code></td>
<td>[tumorous]</td>
</tr>
<tr>
<td><code>ovoD1 ovo^e8K / ++</code></td>
<td>[tumorous]</td>
</tr>
</tbody>
</table>

**not haplo-insufficient**
…if in same gene, cis phenotype ≠ trans

\[
\begin{align*}
\text{tumorous} & \quad \text{less tumorous or wildtype} \\
\text{ovo}^{D1} + / + \text{ovo}^{e8K} & \\
\text{ovo}^{D1} \text{ ovo}^{e8K} / ++ & \\
\end{align*}
\]

but how construct? Consider a slightly different cis/trans:

\[
\begin{align*}
\text{tumorous} & \\
\text{wildtype} & \\
\text{ovo}^{D1} + / + \text{ovo}^{-\text{null}} & \\
\text{ovo}^{D1} \text{ ovo}^{-\text{null}} / ++ & \\
\end{align*}
\]

what we have: \( \text{ovo}^{D1} + / ++ \) tumorous

\[
\begin{align*}
\text{wildtype} & \\
\text{ovo}^{D1} \text{ ovo}^{-\text{null}} / ++ & \\
\end{align*}
\]

but what if true reversion: \(+ ++ + ?\) (1) look at homozygote

if homozygote is tumorous:

\[
\begin{align*}
\text{tumorous} & \\
\text{ovo}^{D1} \text{ ovo}^{-\text{null}} / + \text{ovo}^{e8K} & \\
\end{align*}
\]

(2) now do complementation test to distinguish the alternatives:

\[
\begin{align*}
\text{wildtype} & \\
++ / + \text{ovo}^{e8K} & \\
\end{align*}
\]
ovo<sup>D1</sup> ovo<sup>-null</sup> / + + vs. + + / + ovo<sup>e8K</sup>

Followed by a complementation test:

ovo<sup>D1</sup> ovo<sup>-null</sup> / + ovo<sup>e8K</sup> vs. + + / + ovo<sup>e8K</sup>

tumorous wildtype

We have to lose the gene function that ovo<sup>e8K</sup> is missing in order to “revert” (suppress) the dominance of ovo<sup>D1</sup>.

..hence we have established that ovo<sup>D1</sup> and ovo<sup>e8K</sup> are functional alleles.