Steps in forward genetics:

decide what to study
generate informative mutant alleles
recover informative mutant alleles
study informative mutant allele (do molecular biology)
write paper
reap rewards
Mutations affecting segment number and polarity in *Drosophila*

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In systematic searches for embryonic lethal mutants of *Drosophila* melanogaster we have identified 15 loci which when mutated alter the segmental pattern of the larva. These loci probably represent the majority of such genes in *Drosophila*. The phenotypes of the mutant embryos indicate that the process of segmentation involves at least three levels of spatial organization: the entire egg as developmental unit, a repeat unit with the length of two segments, and the individual segment.

The construction of complex form from similar repeating units is a basic feature of spatial organisation in all higher animals. Very little is known for any organism about the genes involved in this process. In *Drosophila*, the metameric nature of the pattern is most obvious in the thoracic and abdominal segments of the larval epidermis and we are attempting to identify all loci required for the establishment of this pattern. The identification of these genes and the description of their phenotypes should lead to a better understanding of the general mechanisms responsible for the formation of metameric patterns.

In *Drosophila*, the anlagen for the individual segments arise as equally sized subdivisions of the blastoderm, each segment represented by a transverse strip of about three or four cell diameters. A cell lineage restriction between neighbouring segments is established at or soon after this stage. Two basic types of mutation have been described which change the segmental pattern of the *Drosophila* larva. Maternal effect mutations transform abdominal segments into mesothoracic segments. However, the homeotic loci do not affect the total number, size or polarity of the segments, nor do they point to any other step which might intervene between the maternal gradient and the final pattern of segments.

We have undertaken a systematic search for mutations that affect the segmental pattern depending on the zygotic genome. We describe here mutations at 15 loci which show one of three novel types of pattern alteration: pattern duplication in each segment (segment polarity mutants; six loci), pattern deletion in alternating segments (pair-rule mutants; six loci) and deletion of a group of adjacent segments (gap mutants; three loci) (Table 1, Fig. 1).

The segmental pattern of the normal *Drosophila* larva

Figure 2 shows the cuticular pattern of a normal *Drosophila* larva shortly after hatching. The larval body is comprised of...
mutant phenotypes informative for understanding pattern formation in metazoans
Fig. 1 Semi-schematic drawings indicating the regions deleted from the normal pattern in mutant larvae. Dotted regions indicate denticle bands, dotted lines the segmental boundaries. The regions missing in mutant larvae are indicated by the hatched bars. The transverse lines connect corresponding regions in mutant and normal larvae. Planes of polarity reversal in runt and Krüppel are indicated by the arrows. The two segment polarity loci patch and gooseberry are represented at the left. For indication of the polarity of the patterns, see Fig. 3. The patterns of fused and c" (not shown) look similar to the gooseberry pattern, whereas in hedgehog and wingless the deleted regions are somewhat larger, cutting into the denticle bands at either side. Four pair-rule mutants are shown in the centre. The interpretation of their phenotypes is based on the study of weak as well as strong alleles, combinations with Ubx (see text) and, in the case of runt, on gynandromorphs (unpublished). They probably represent the extreme mutant condition at the respective loci. The phenotypes of all known barrel and engrailed alleles (not shown) are somewhat variable and further studies are needed to deduce the typical phenotype. At the right, the two gap loci Krüppel and knirps are shown. Both patterns represent the amorphic phenotype as observed in embryos homozygous for deficiencies of the respective loci. The only known hunchback allele (not shown) deletes the meso- and metathorax.
They regulate…

**Drosophila** homeotic gene clusters

**Mammalian** Hox gene clusters

**Mouse embryo**

Fig. 20.22 p741

Fig. 20.26 p744
Two general categories of mutant allele recovery strategies:

(1) genetic screens ("brute force" screens)

make mutations randomly, then
you sift through chromosomes (often one at a time)
looking for mutant alleles of interest/use

(2) genetic selections

make mutations randomly, then
let nature eliminate all undesired mutant alleles
so you are only left with the good stuff

2 easier than 1, but often not possible & potentially more biased
By the way:
an important but under-appreciated step in genetic analysis:

- generate mutant allele
- recover mutant allele
- study mutant allele
- **maintain mutant allele**
- write paper
- reap rewards

Homework problem:
How much **food** (corn meal, molasses, yeast) has T.H. Morgan’s original **white** mutant line **consumed** since 1910?

Strategies & tools that help us **recover** mutant alleles can also help us **maintain** them.
Maintaining mutant stocks (lines) in model genetic systems:

**To freeze or not to freeze**

| most microbes (spores are nice) | mouse (embryos) |
| arabidopsis ("the weed") & corn | fish |
| seeds |
| worm | fly |
Basic facts to consider in designing screens and selections:

(1) Most **LOF** mutant alleles are **recessive** (all else being equal)  
    (**LOF** mutations are the **most frequent** class)

(2) Most **null** alleles of genes with an obvious LOF phenotype are **lethal**, or at least **sterile**.

(3) Most “**developmentally interesting**” genes are **essential** for viability or fertility

Hence:
screen/selection schemes must provide for the recovery of recessive **lethals** and **steriles**
The “diploid advantage” for recessive lethal studies:

**Diploid:**

\[
\text{lethal} / + \quad \text{alive (fertile)}
\]

(\(+\) holds the fort)

**Haploid:**

\[
\text{lethal} \quad \text{dead (sterile)}
\]

(let naked and exposed)

Often for microbes rely on **conditional** lethals in generating mutations:
Haploid:

<table>
<thead>
<tr>
<th>lethal</th>
<th>dead (sterile)</th>
</tr>
</thead>
</table>

(let naked and exposed)

rely on **conditional** lethals in generating mutations:

**condition A**

- growth
- all grow
  - (mutagenize wildtype)

**condition B**

- no growth
  - only mutants of interest don’t grow

Two key tricks for microbes:

p212: Fig. 7.5 Replica Plating

& p558: Fig. 15.15

augmented by:

p547: Fig. 15.5 (Penicillin) enrichment

**genetic selection**
condition A  |  condition B
---|---
growth vs. no growth
all grow (mutagenized) only mutants of interest don’t grow

Two key tricks for microbes:
p212: Fig. 7.5 & p558: Fig. 15.15

Replica Plating

genetic screen
condition A

growth
all grow (mutagenized)

condition B

no growth

only mutants of interest don’t grow

two key tricks for microbes:
p212: Fig. 7.5
& p558: Fig. 15.15

replica plating

augmented by:
p547: Fig. 15.5 (Penicillin) enrichment

replica plate on (diluting out the penicillin)

Mutagenized culture in rich medium contains auxotrophic and prototrophic cells.

Centrifuge

Resuspend in medium minus one nutrient plus penicillin

Transfer to rich medium

Auxotrophs

Only prototrophs grow. Locate auxotrophic colonies on original plate.

Prototrophs grow and 99% are killed by penicillin. Auxotrophs cannot grow and are not killed. Culture is enriched for auxotrophs.
The "diploid advantage" for recessive lethal studies:

**Diploid:**

- **lethal** / + alive (fertile)
  (+ holds the fort)

**Haploid:**

- lethal dead (sterile)
  (let naked and exposed)

The "diploid handicap" for recessive lethal studies:

+ masks lethal effects of lethal immediately obvious
The problem with diploids in hunting for new recessive mutations:

Female: ++/+ × Male: ++/+ 

PARENTS: ++/+ a+/b+/

Form zygotes:

F1 PROGENY: ++/b−, ++/a−
The problem with diploids in hunting for new recessive mutations:

mutagenize (altv.: dysgenic)

<table>
<thead>
<tr>
<th>Female</th>
<th>X</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td></td>
<td>+/+</td>
</tr>
<tr>
<td>+/+</td>
<td></td>
<td>+/a−</td>
</tr>
<tr>
<td>+/b−</td>
<td></td>
<td>+/+</td>
</tr>
</tbody>
</table>

F1 PROGENY

+/-     +/+     +/+     +/+     +/−

given: we are interested in (finding) the $a^{-}/a^{-}$ phenotype

How do we know who (if anyone) is carrying $a^{-}$?

…the individual who can produce $a^{-}/a^{-}$ offspring.
The problem with diploids in hunting for new recessive mutations: mutagenize (altv.: dysgenic)

Female

Male

+/-

+/-

+/-

+/-

X

F1 PROGENY

How do we know who (if anyone) is carrying \( a^- \)?

To whom do we mate to find out?

If we can “self” this individual, we are effectively mating to +/a- for sure

of course, we had to self everyone: No \( a^-/a^- \)

YES! & we know in the F2

...the individual who can produce \( a^-/a^- \) offspring.
The problem with diploids in hunting for new recessive mutations:

- Mutagenize (altv.: dysgenic)

Female +/+  x  Male +/+  

F1:
- +/+ (homozygous wild type)
- +/b⁻ (heterozygous for recessive mutation)
- +/a⁻ (heterozygous for recessive mutation)

To whom do we mate to find out -- if we can't self?
- +/+
- +/+ (homozygous wild type)
- +/a⁻ (heterozygous for recessive mutation)

.... we still don't know in the F2!

Meanwhile:
- +/+ (homozygous wild type)
- +/a⁻ (heterozygous for recessive mutation)
- +/+ (homozygous wild type)

(continued on next page)
The problem with diploids in hunting for new recessive mutations:

mutagenize (altv.: dysgenic)

Female

\(+/+\)

Male

\(+/+

\(-- \text{if we can't self?}\)

\(+/+\)

\(+/b^-\)

\(+/a^-\)

F1

\(+/+\)

\(+/a^-\)

\(+/+\)

\(+a^-\)

F2

\(+/+\)

\(+/a^-\)

\(+/+\)

\(+a^-\)

\(+/+\)

Mate inter se

at best

\(+/+\)

\(a^-/a^-\)

Mate inter se

..but at least now we have potential mates with a-

..but at least now we still don't know in the F2!

(must keep populations separate!)
The problem with diploids in hunting for new recessive mutations:

**mutagenize** (altv.: dysgenic)

Female

\[ +/+ \]

Male

\[ +/+ \]

--- if we can’t self?

**F1**

\[ +/a^- \]

\[ +/+ \]

\[ +/b^- \]

\[ +/+ \]

\[ x \]

**F2**

\[ +/+ \]

\[ +/+ \]

\[ +/+ \]

\[ +/a^- \]

\[ +/+ \]

\[ a^-/a^- \]

if we cross them, \( a^-/a^- \) will come:

only \[ +/+ \]

**Mate inter se**

can we do better than mating *inter se*?
It would help if we could keep track of chromosomes:

Female

+/

X

mutagenize (altv.: dysgenic)

Male

+/

+/

+/

+/

+/

F1

+/

F2

+/

+/

+/

+/

+/

+/

+/

+/

+/

+/

+/

+/

we can do better than mating inter se

if we cross them, a^-/a^- will come:

a^-/a^-
our friend, Herman Muller had the answer (early ‘30s):

(1) used them to determine **mutation frequency**: …how often a **new** recessive lethal **arose** on a given fly chromosome

(2) used them to “**maintain**” deleterious recessive alleles of interest

**Balancer chromosomes:**

(a) a chromosome you can **distinguish** from the others.

**dominant marker** mutant alleles (**Bar**, **Curly**, **Stubble**)

(b) a chromosome that will **not recombine** with others

**crossover suppressors** (multiple inversions)

(c) a chromosome that will **not “become” homozygous**

(i.e. that would either be **lethal** or **sterile** if homozygous)

recessive lethal or sterile alleles
Fig. 14.17

Balancer chromosome

\[ m_1 \]

Normal chromosome with mutations of interest

Key

\[
\begin{align*}
\text{[ ]} & \quad \text{Breakpoints of pericentric inversions} \\
\text{( )} & \quad \text{Breakpoints of paracentric inversions}
\end{align*}
\]
Balancer chromosomes:

(1) use them to follow chromosomes in order to create new genotypes (such as in mutant screens)

(2) use them to “maintain” deleterious recessive alleles of interest

3 essential features:

(a) dominant marker mutant alleles

(b) crossover suppressors

(c) recessive lethal or sterile alleles
Balancer chromosomes:

(2) used them to “maintain” deleterious recessive alleles of interest

recall problem without balancer:

\[
+/- \times +/+ \rightarrow +/+ \\
let/+ \times +/+ \rightarrow +/+ \& let/+ \\
let/+ \times let/+ \rightarrow +/+ \& let/+ \& let/let
\]

with balancer: let/Bal

\[
let-A^同类 let-B^同类 Dom^同类 /Bal, let-A^同类 Dom let-B^- Inv
\]

\[
let-A^-/Bal \times let-A^-/Bal \rightarrow let-A^-/let-A^- \text{ lethal} \\
let-A^-/Bal \rightarrow \text{ O.K.} \\
Bal/Bal \rightarrow \text{ lethal}
\]

Balanced lethal condition
Balancer chromosomes:

(2) used them to “maintain” deleterious recessive alleles of interest

What about an X-linked recessive lethal?

Balanced condition

...unlabeled, labeled, or labeled X + unlabeled, labeled, or labeled Y

female
let-A^-/Bal

X

male

let-A^-/Y or Bal /Y

recessive
female-specific sterile

 lethal

let-A^-/Bal

O.K. female

Bal/Bal

sterile female

let-A^-/Y

lethal male

Bal /Y

O.K. male
Balancer chromosomes:
(1) use them to follow chromosomes in mutant screens

Consider the brute-force screen that led to the last fly Nobel Prize N-V & W:

**Aim:** find genes that allow cells to know where they are so that those cells can know what they should be expected of mutant phenotype for “pattern formation” genes:

(1) embryonic recessive lethal

(2) altered **dentical belt** pattern (exoskeleton) in **dead** embryos (dying fly embryos can still differentiate a lot)

Ant. Post. polarity>>> wildtype

Post. “bicaudal”