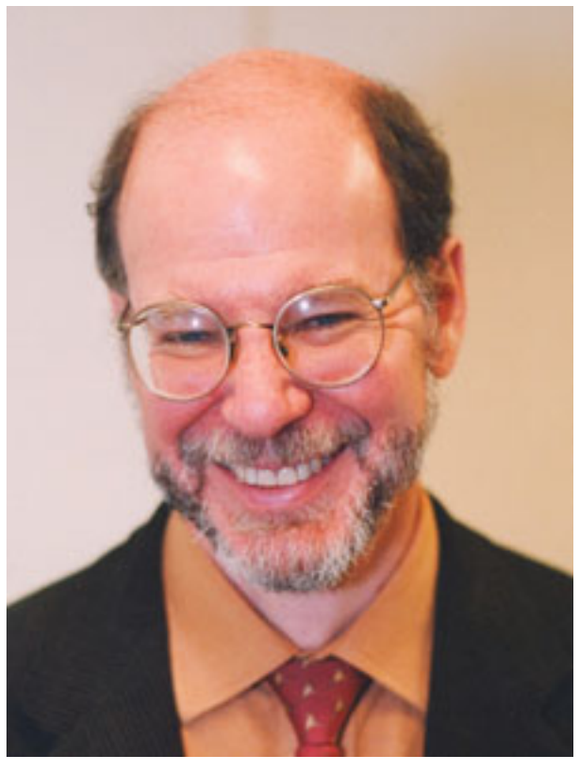
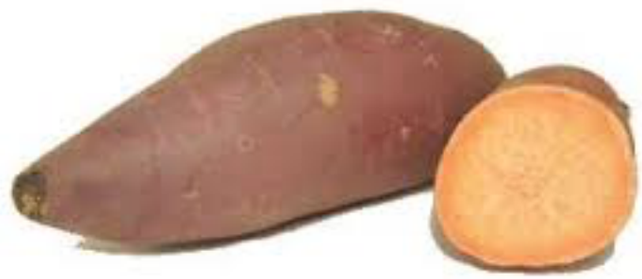
Bio393: Genetic Analysis

Step-wise genetic analysis



Bob Horvitz

“Model organisms” are everywhere now



1. Define the problem

?

Let the question influence the choice of organism
(not the other way around)

2. Choose an organism

Organism	Time to 10^6	Space
Bacteriophage	1 hour	10 nL

10^6 individuals to study 10^{-6} mutation rate

$2^{20} \approx 10^6$ individuals

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Fly	6 weeks	0.5 m cube
Mouse	3 years	Half Pancoe
Human	750 years	Chicago suburbs

10^6 individuals to study 10^{-6} mutation rate

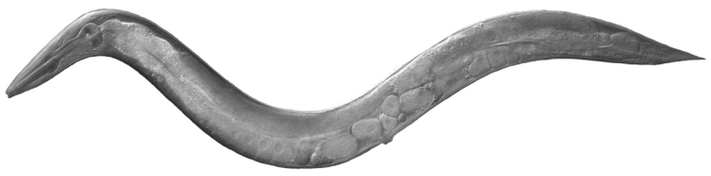
$2^{20} \approx 10^6$ individuals

3. Perform a mutant hunt

To mutagenize?

Yes	**No**	
10^{-3}	10^{-6}	LoF mutation
10^{-5}-10^{-6}	10^{-8}-10^{-9}	Specific mutation

C. elegans

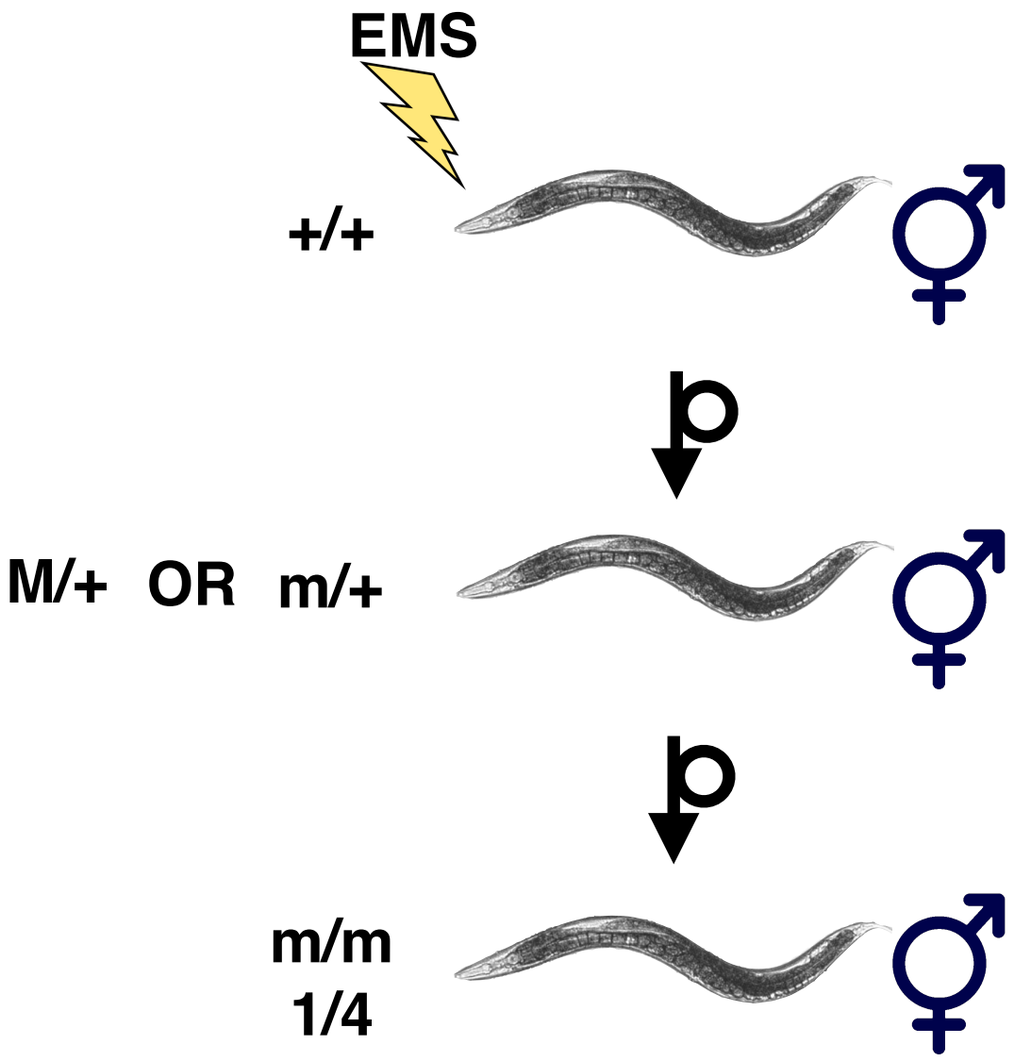


~20,000 genes
20 LoF mutations

D. melanogaster

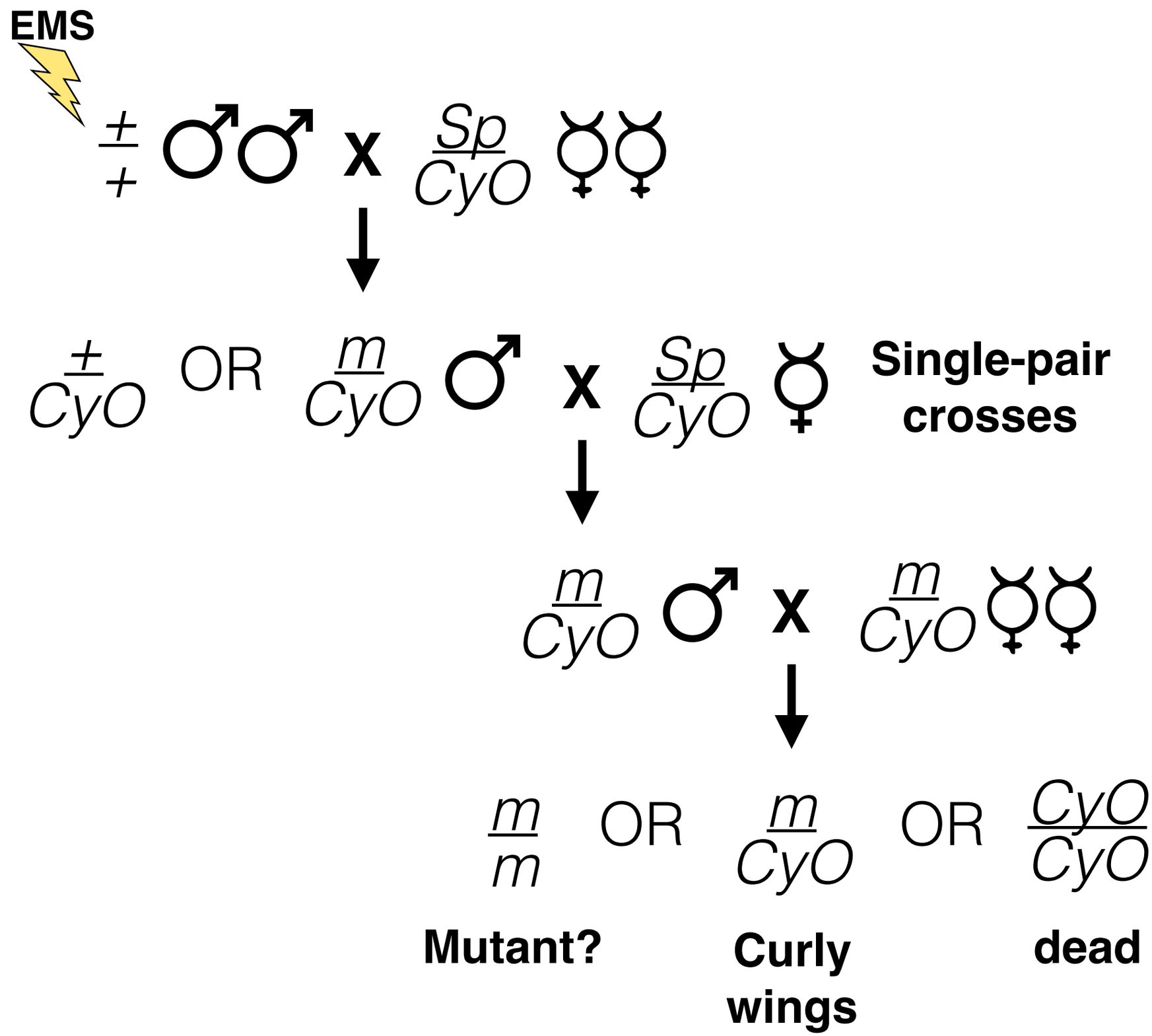
~12,000 genes
12 LoF mutations

Screen or selection?

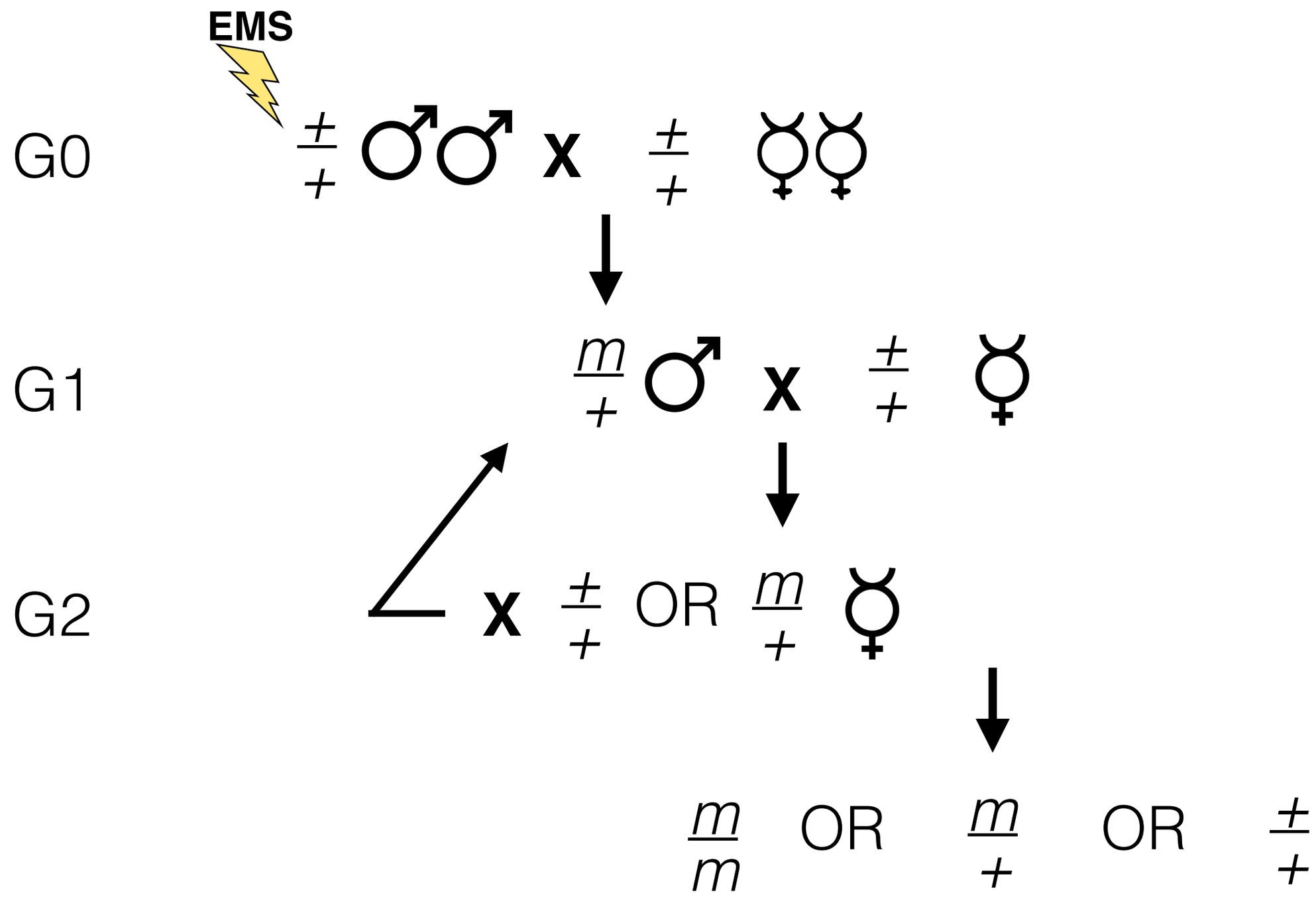


C. elegans screens for dominant or recessive phenotypes

Screen or selection?



Screen or selection?



Mouse screens for dominant or recessive phenotypes

Remember hemizygous screens too

4. Screen until saturation?

Use Poisson sampling and common sense

Change mutagens

Saturation of the investigator's patience

Why might we miss genes?

Numbers are too small
Pleiotropy (sterility or lethality)
Redundancy

The most common phenotypes are sterile or dead!

5. Establish a strain

True-breeding stocks

Balancers, balanced stocks

Freeze organisms

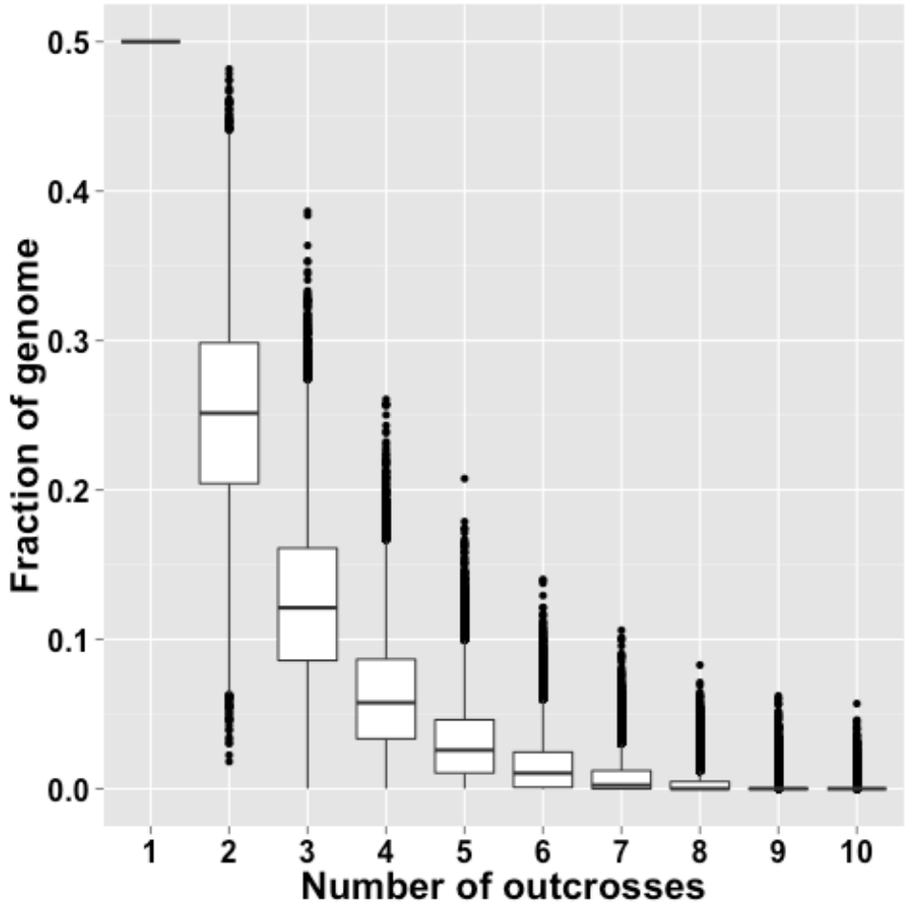
The most common phenotypes are sterile or dead!

6. Backcross and/or outcross

Mutagenesis adds hundreds of mutations randomly throughout the genome.

Backcross = cross to parent used in the screen/selection

Outcross = cross to a wild-type strain



7. Test for dominance

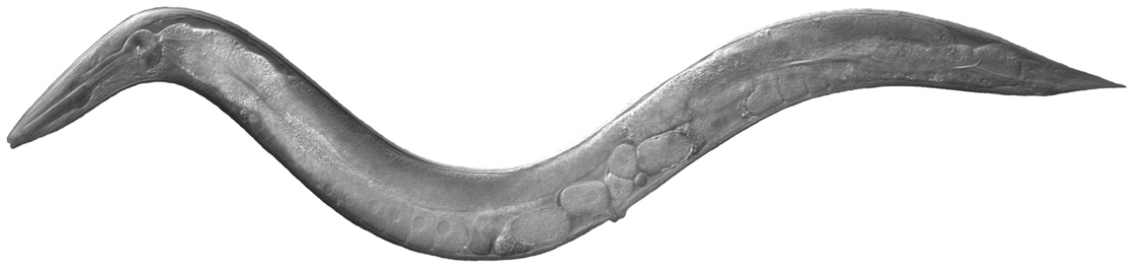
8. Single-gene phenotype?

9. Mapping and complementation

What have we discovered so far?

10. Characterize the phenotype

Look at the wild-type and mutant organisms *in detail*



Let's say you screened for mutants that failed to lay eggs

What could be mutated?

No embryos
No vulva
No vulval muscles
No neurons
Or malfunction of any vulva, muscle, or neuron

Pleiotropy

A single mutation causes many different mutant traits

Mutation in gene X

Mutant with…

long hairs — gene X ⊣ short hair

disrupted sleep patterns — gene X → normal sleep

slow growth — gene X → normal growth

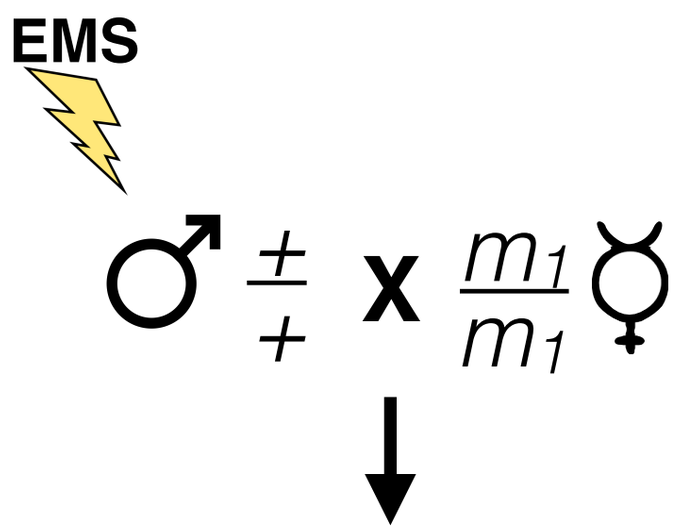
enhanced metabolism of high fat diet — gene X ⊣ fat metabolism

11. Define the nature of the mutant allele(s): gene dosage

1. Dominant or recessive?
2. Frequency of mutant?
3. Where is the mutant allele in allelic series?
4. Look at deficiency heterozygotes for haploinsufficiency
5. Antagonism by wild-type copies of gene

What if you only have one mutant?

12. Perform non-complementation screens



$\frac{m_1}{+}$ OR $\frac{m_1}{m_2}$

Common (wild-type)

Rare (mutant)

OR $\frac{m_1 \quad +}{+ \quad M}$

Rare (dominant)

13. Define the null phenotype

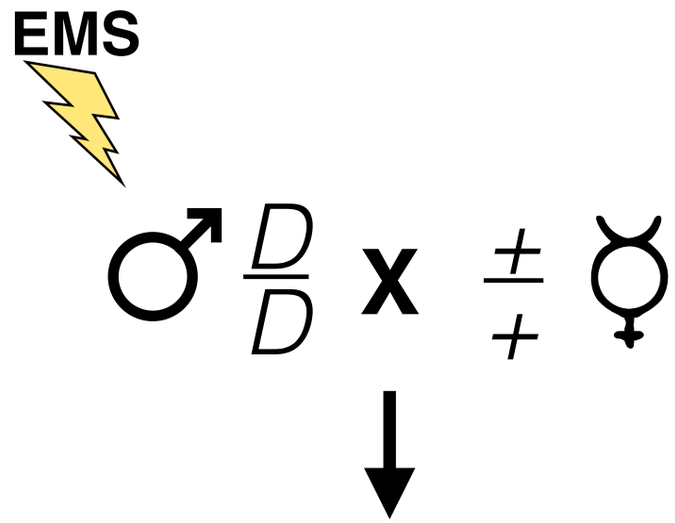
What happens with a complete loss of gene function?

Dosage studies, non-complementation screens, and characterization of the mutant phenotype tell you about the null phenotype

You need a null to characterize gene function. Why?

What if you have a mutant with a dominant gain-of-function phenotype?

Cis-dominant suppressor screen



Common (mutant) | Rare (wild-type)

What could *D** be?

Cis-dominant suppressor screen

♂ $\frac{D}{D}$ x $\frac{\pm}{+}$ ♀

$\frac{D}{+}$ OR $\frac{D^*}{+}$

Common (mutant) | Rare (wild-type)

Revertant $\frac{D^*}{+} \rightarrow \frac{\pm}{+}$

Null mutant $\frac{D^*}{+} \rightarrow \frac{\Delta\, D}{+}$

Intragenic supp. $\frac{D^*}{+} \rightarrow \frac{sup\ D}{+}$

Dominant extragenic suppressor

$\frac{D^*}{+} \rightarrow \frac{D}{+}\ ;\ \frac{Sup}{+}$

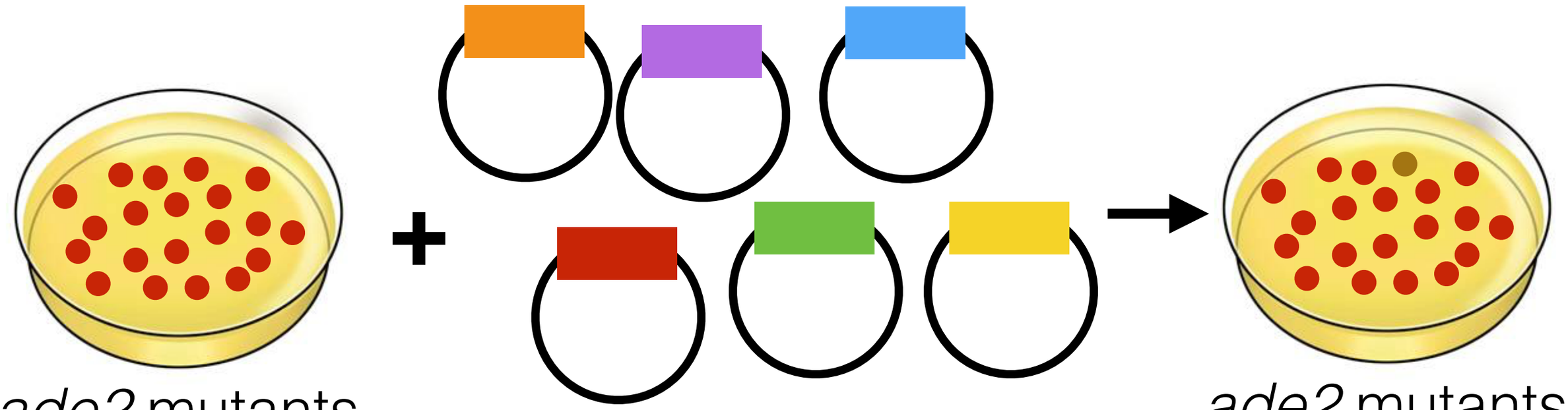
How can we tell what we got?

14. Clone the gene

Identify a DNA sequence that contains your gene of interest

1. Clone by complementation
2. Clone by phenocopy
3. Clone by sequencing

Cloning by complementation in bacteria and yeast



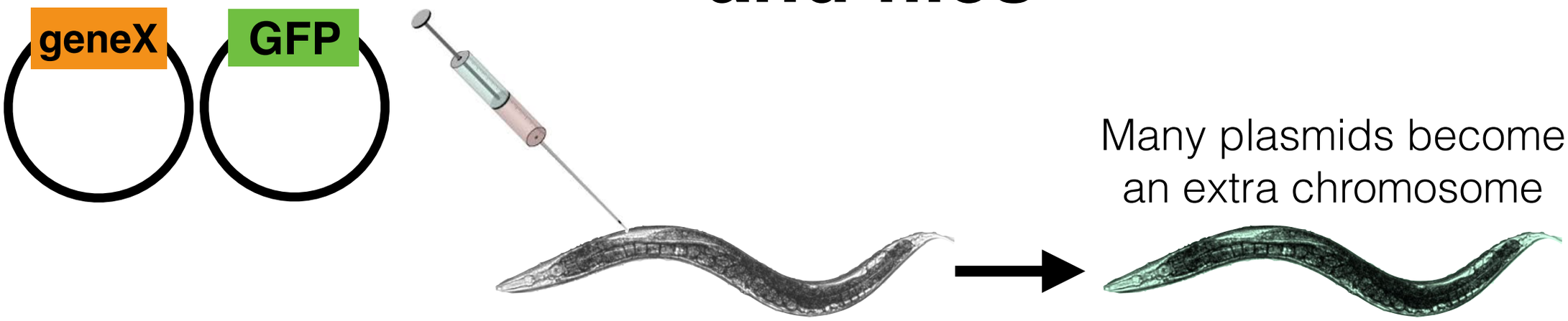
ade2 mutants

Plasmids each containing a different gene

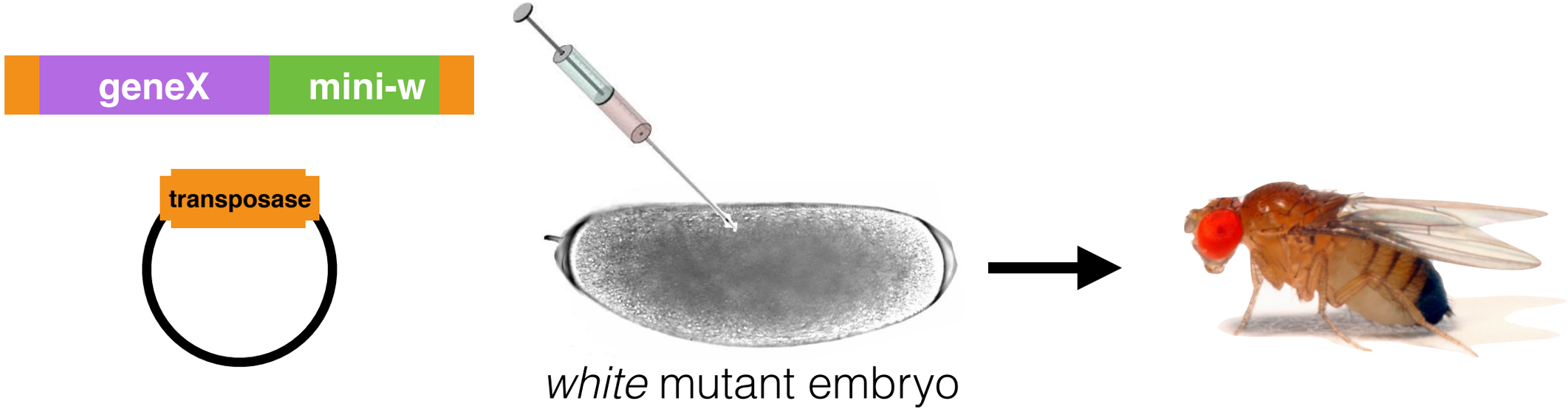
ade2 mutants + plasmids

Caveat: overexpression bypass suppressors

Cloning by complementation in worms and flies



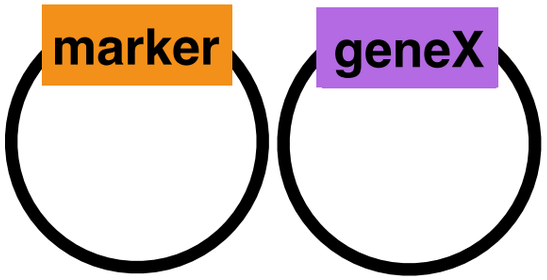
Caveat: overexpression bypass suppressors and not stable

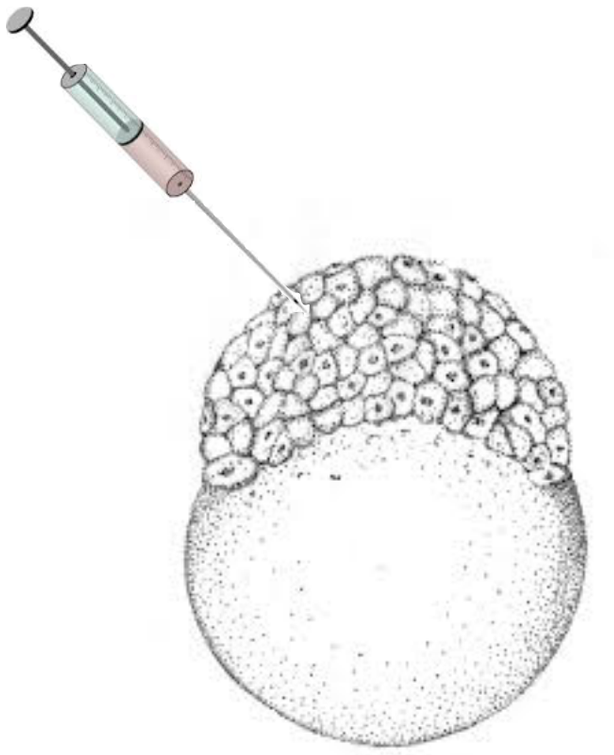


Caveat: overexpression bypass suppressor and variable expression

Transgenesis and rescue

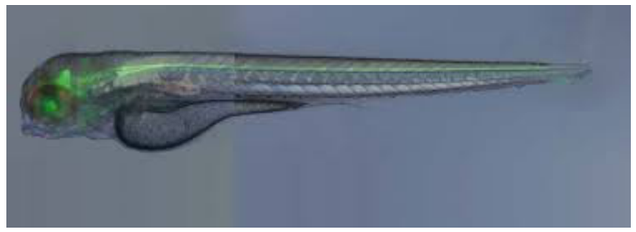
Cloning by complementation in fish and mice





mutant blastoderm

Look for marker expression



Caveat: overexpression bypass suppressors and variable expression