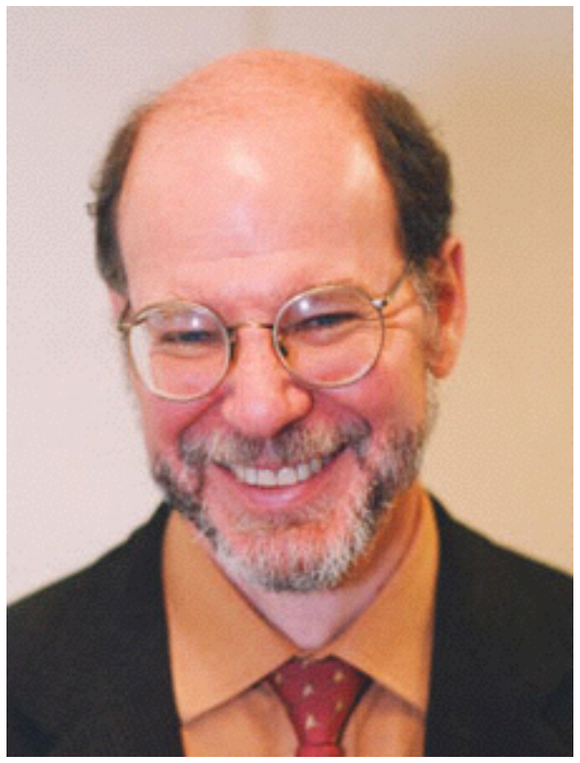
Bio393: Genetic Analysis

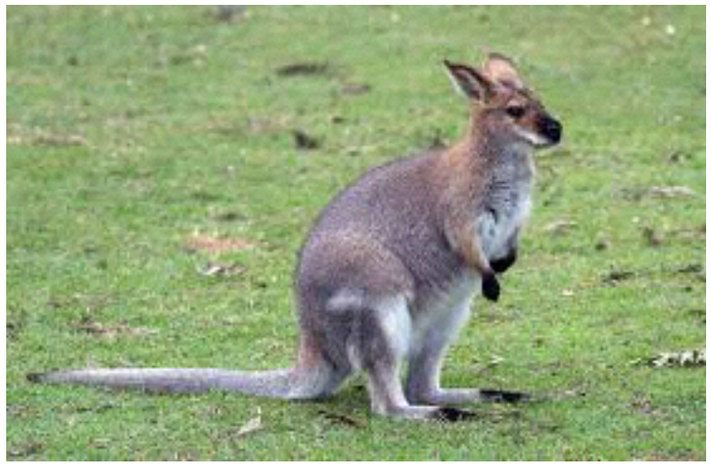
Step-wise genetic analysis



Bob Horvitz

Lecture 8

“Model organisms” are everywhere now



1. Define the problem

?

Let the question choose the organism
(not the other way around)

2. Choose an organism

Organism	Time to 10^6	Space
Bacteriaphage	1 hour	10 nL
Bacteria	15 hours	1 µL
Yeast	1 day	0.1 mL
Worm	10 days	6 cm cube
Fly	6 weeks	0.5 m cube
Mouse	3 years	Half Pancoe
Human	750 years	Evanston + Wilmette

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

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

3. Perform a mutant hunt

To mutagenize or not to mutagenize?

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

3. Perform a mutant hunt

To mutagenize or not 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

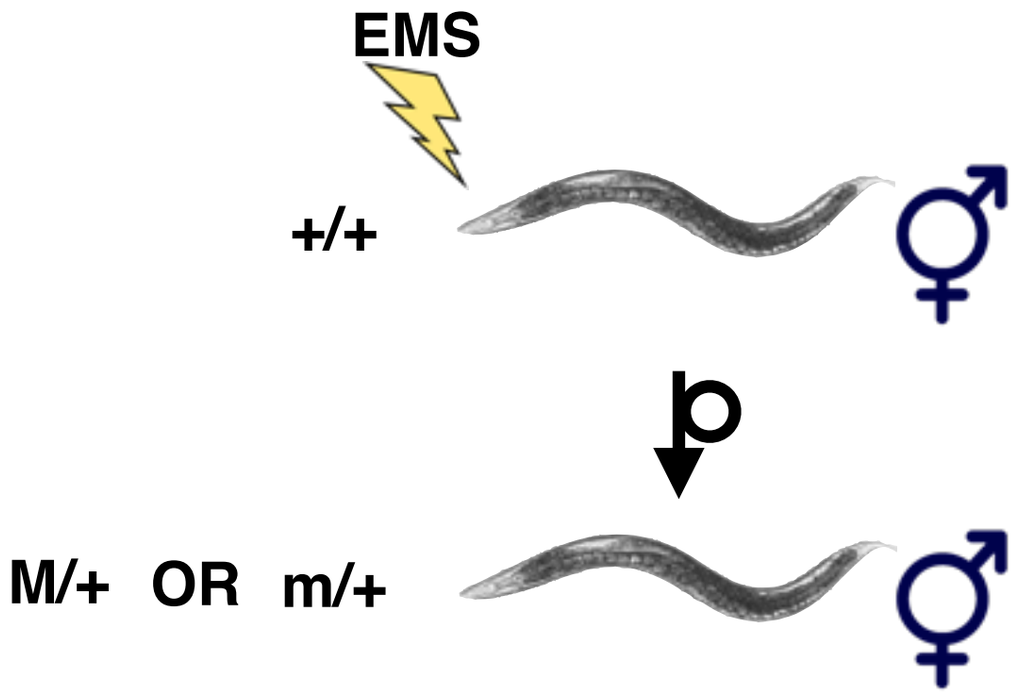
D. melanogaster

~12,000 genes
12 LoF mutations

Screen or selection?

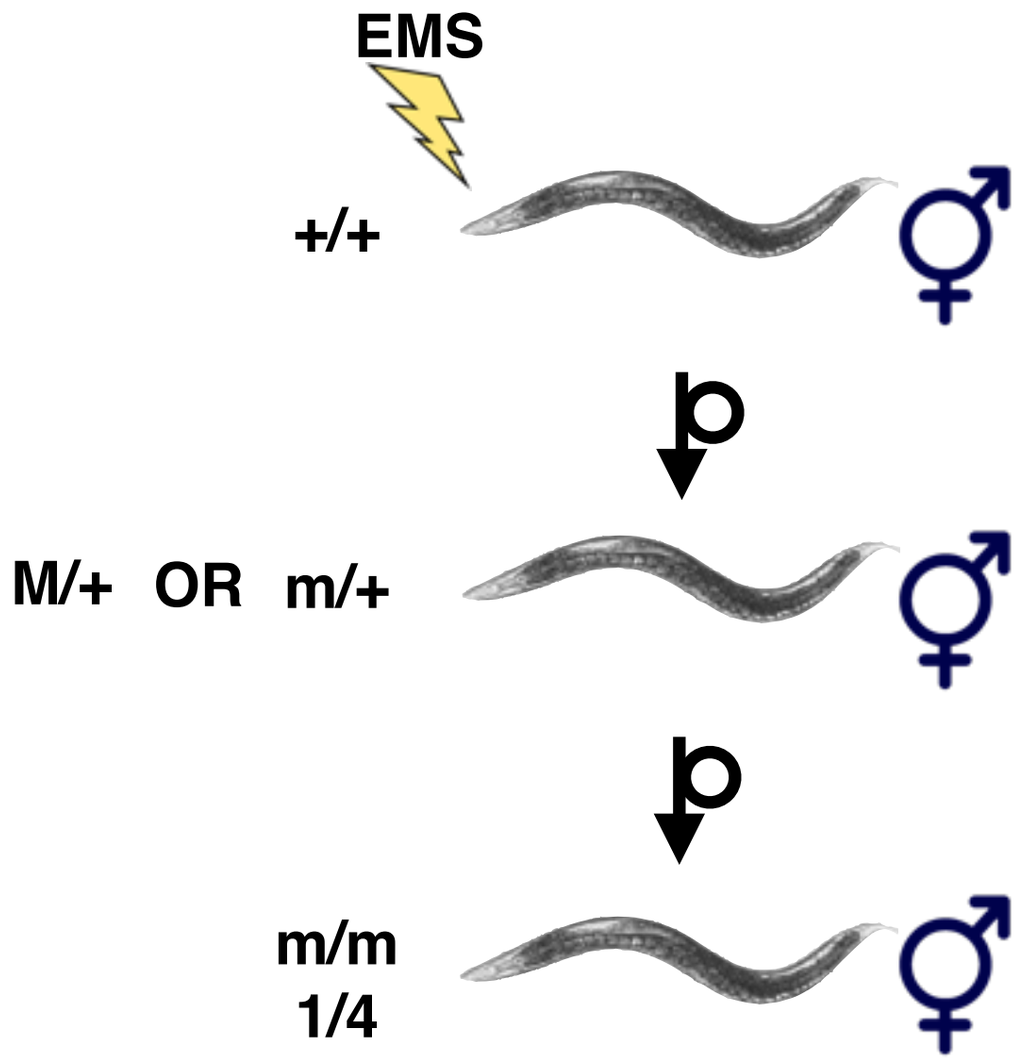
C. elegans screens for dominant or recessive phenotypes

Screen or selection?



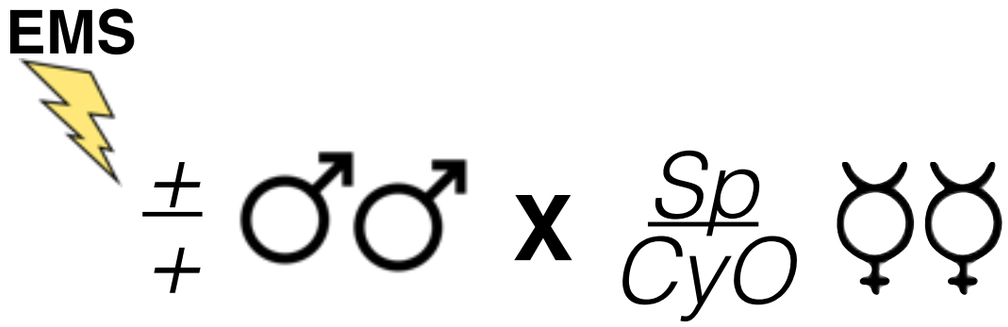
C. elegans screens for dominant or recessive phenotypes

Screen or selection?

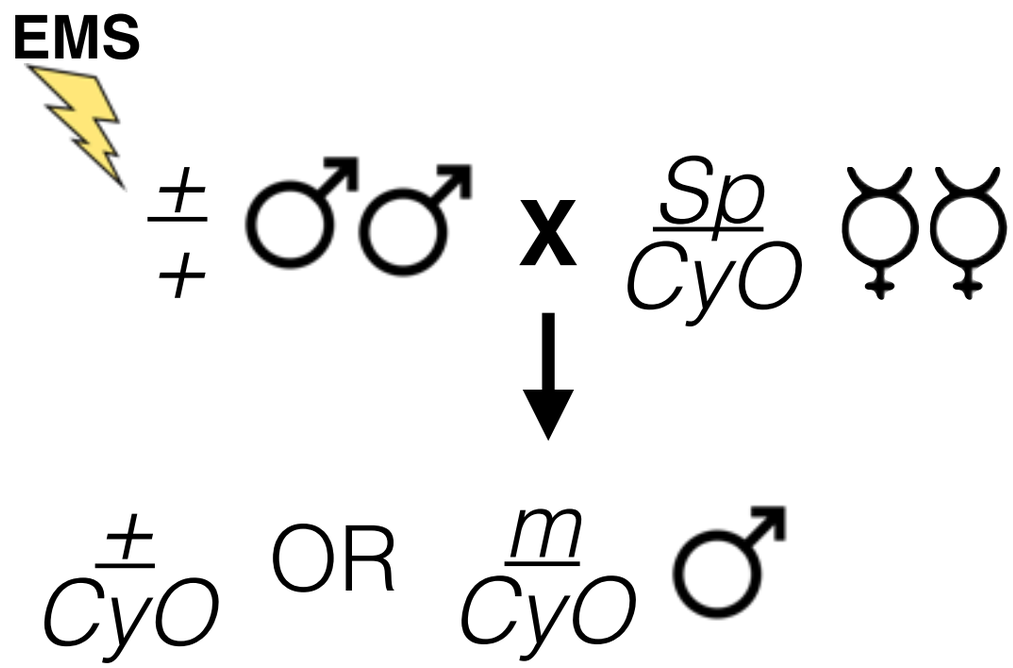


C. elegans screens for dominant or recessive phenotypes

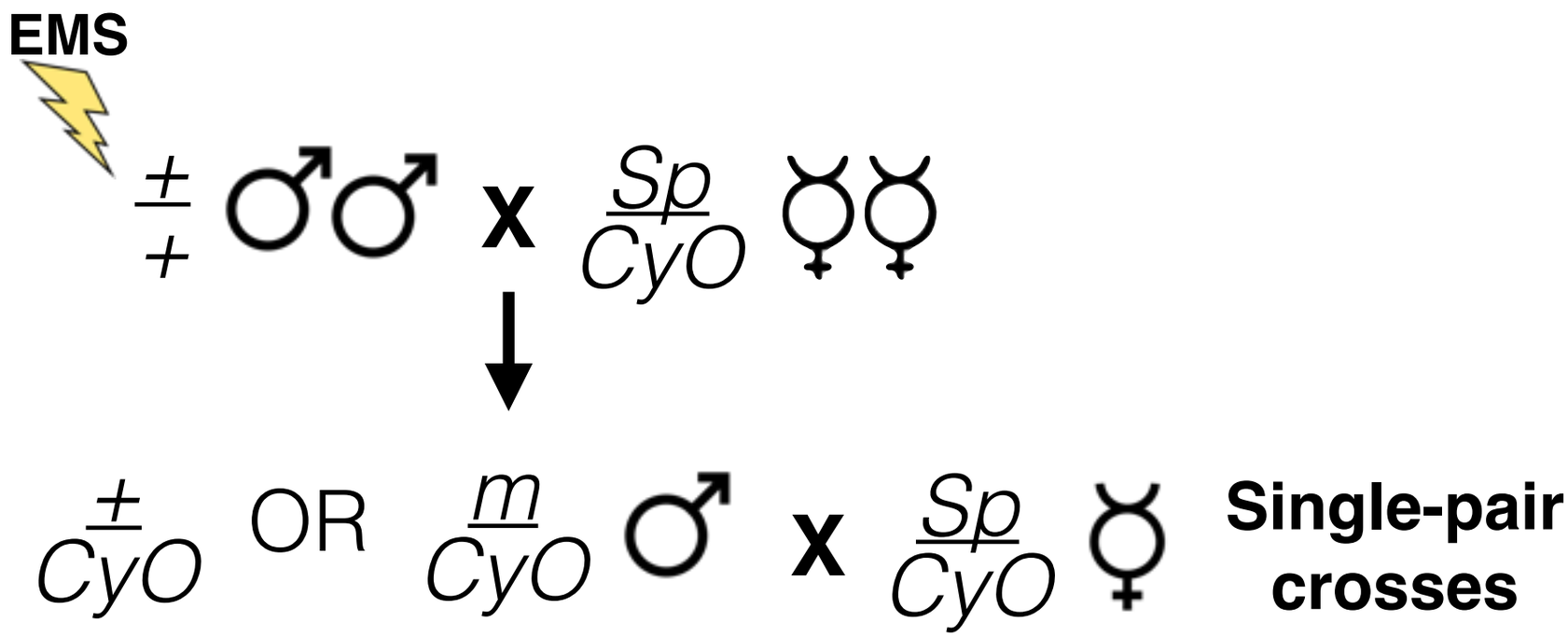
Screen or selection?



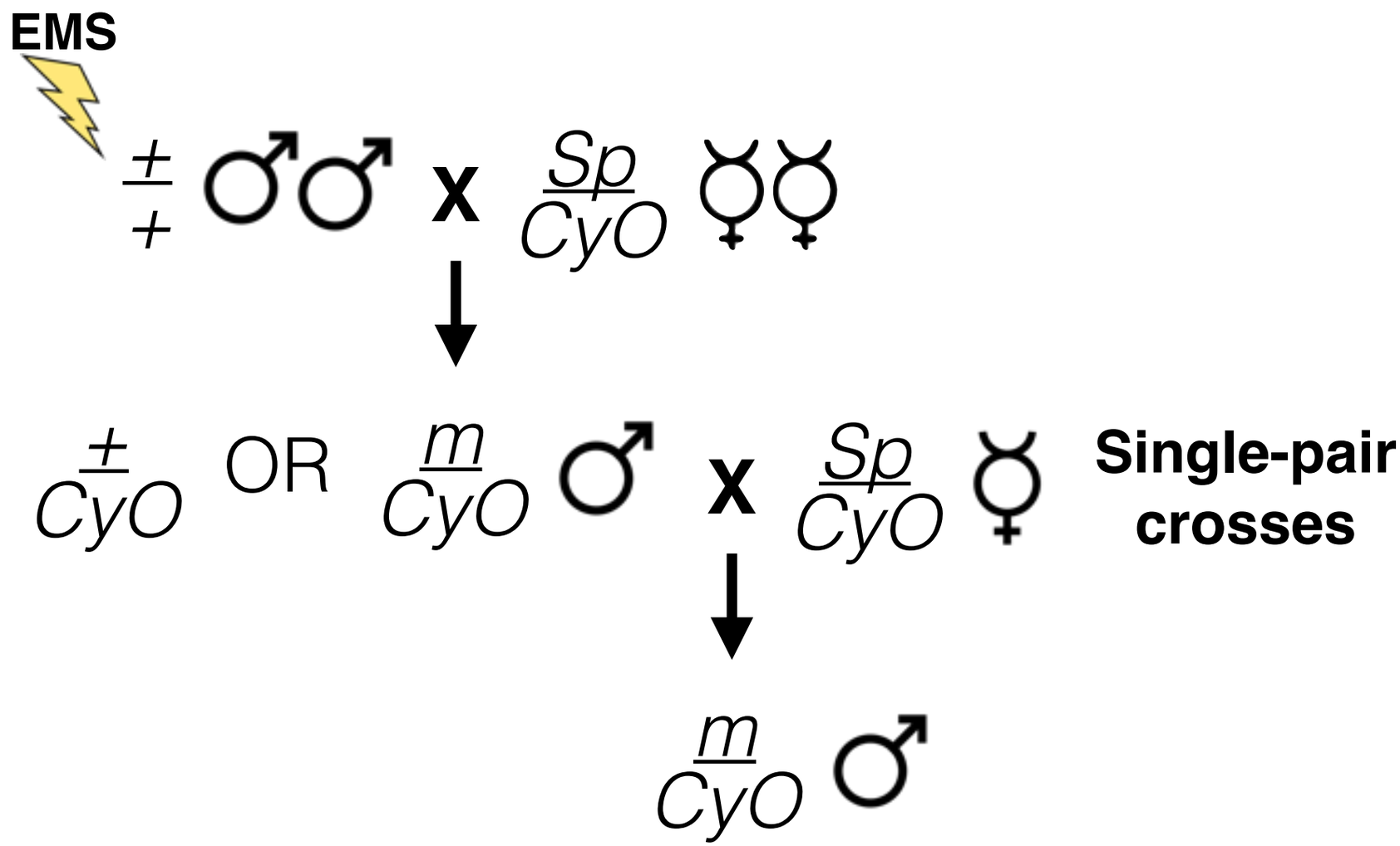
Screen or selection?



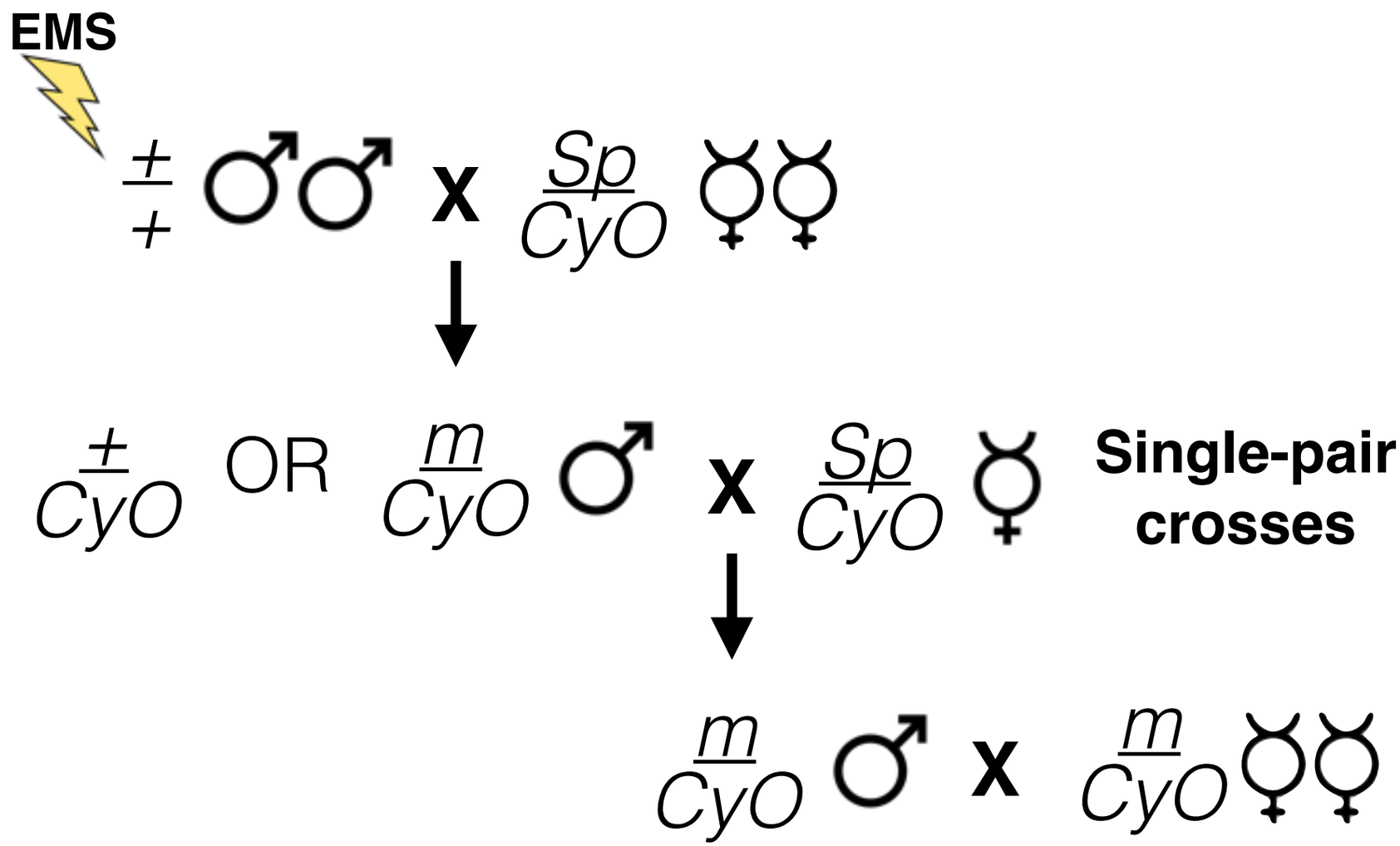
Screen or selection?



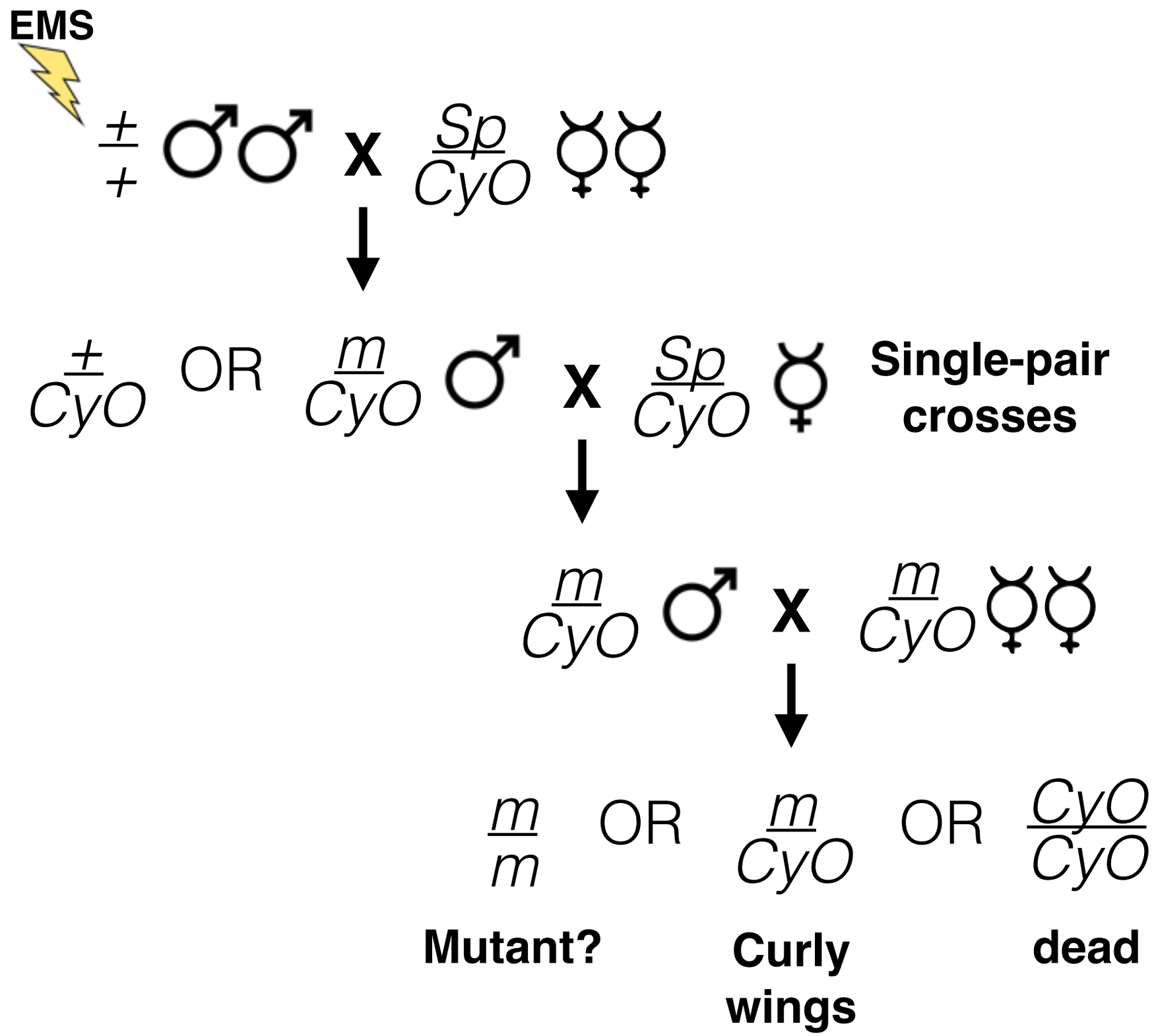
Screen or selection?



Screen or selection?



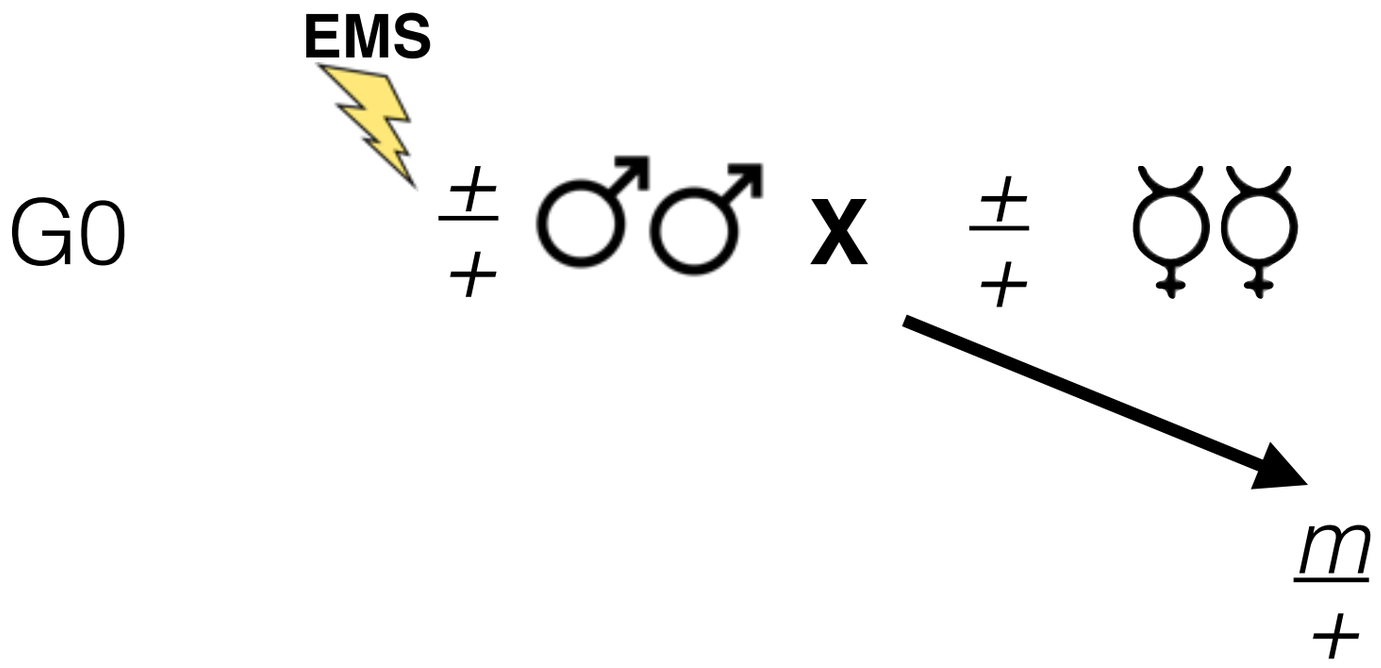
Screen or selection?



Screen or selection?

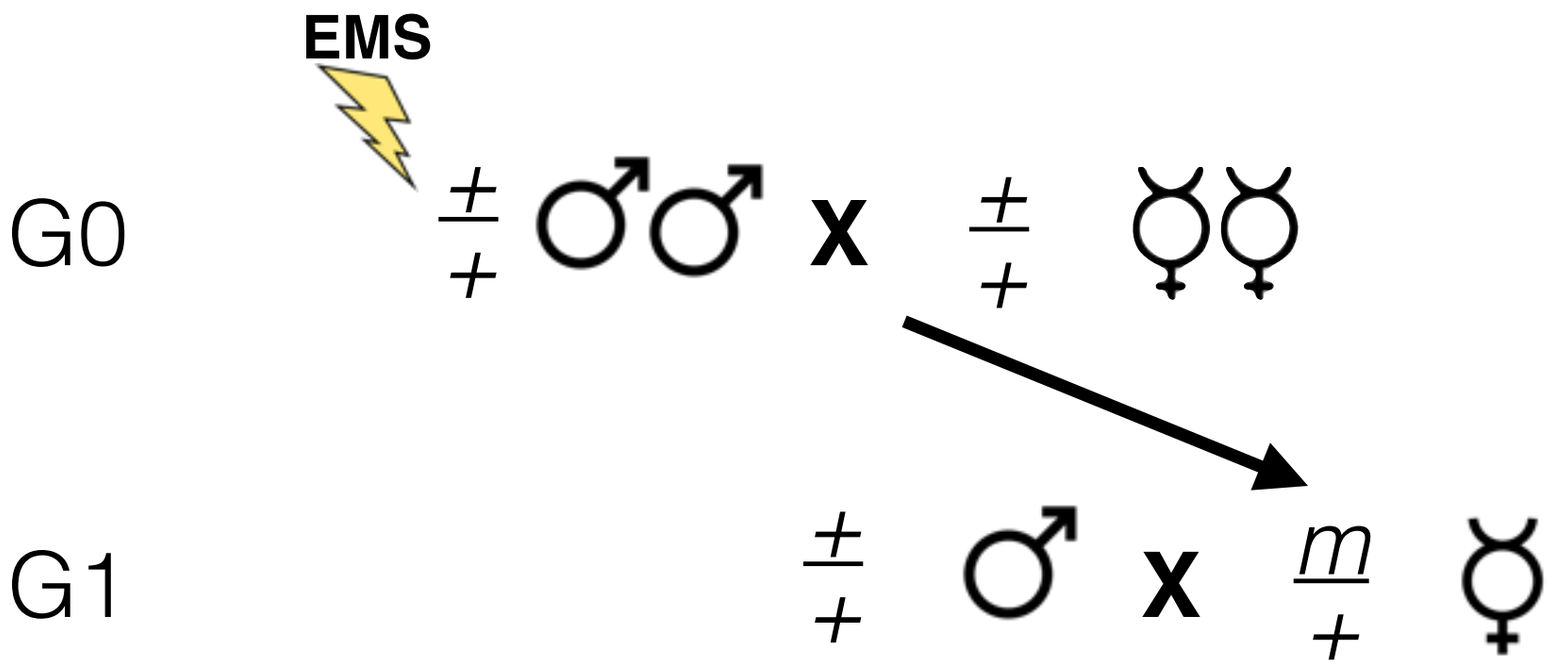
Mouse screens for dominant or recessive phenotypes

Screen or selection?



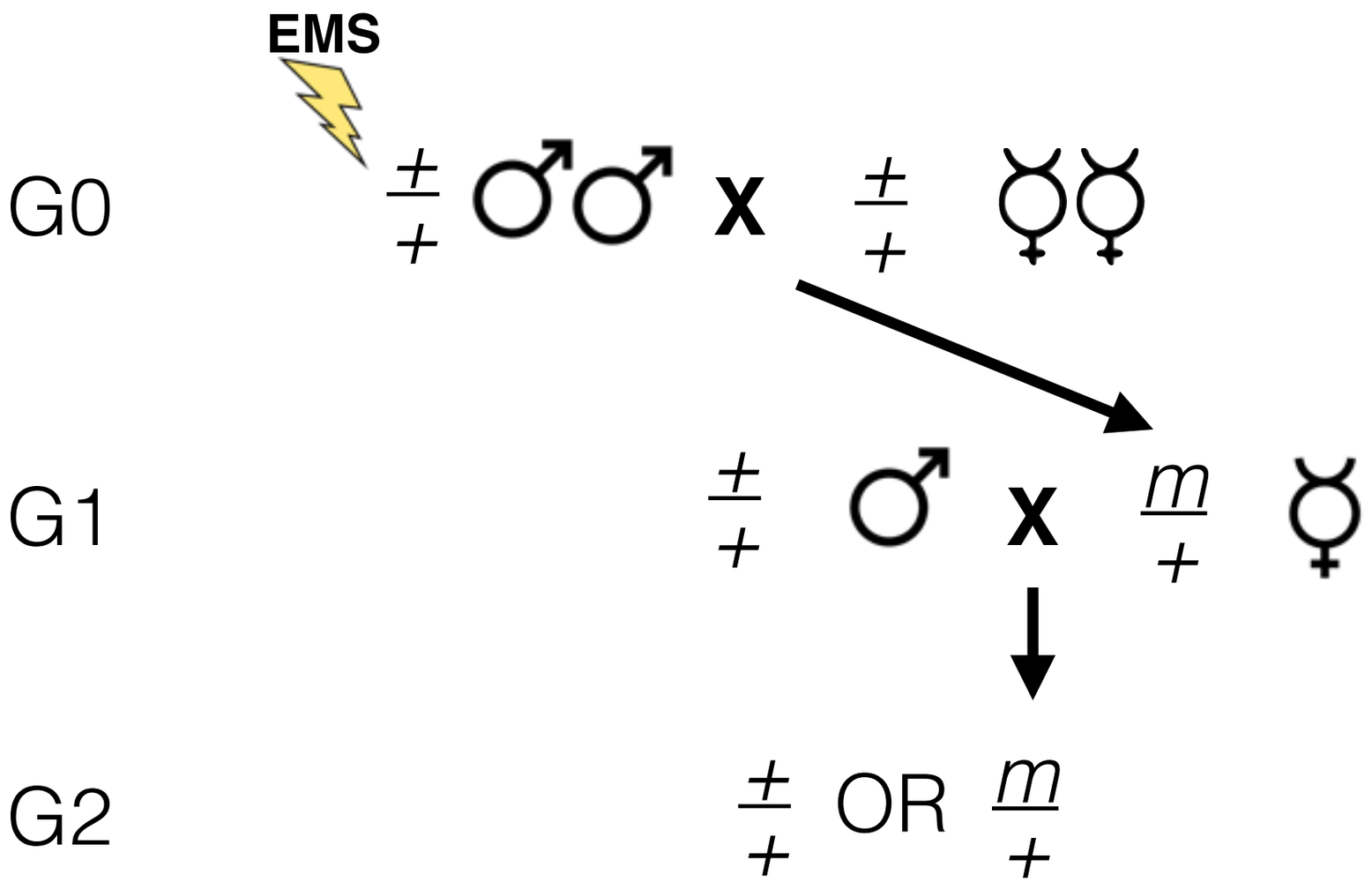
Mouse screens for dominant or recessive phenotypes

Screen or selection?



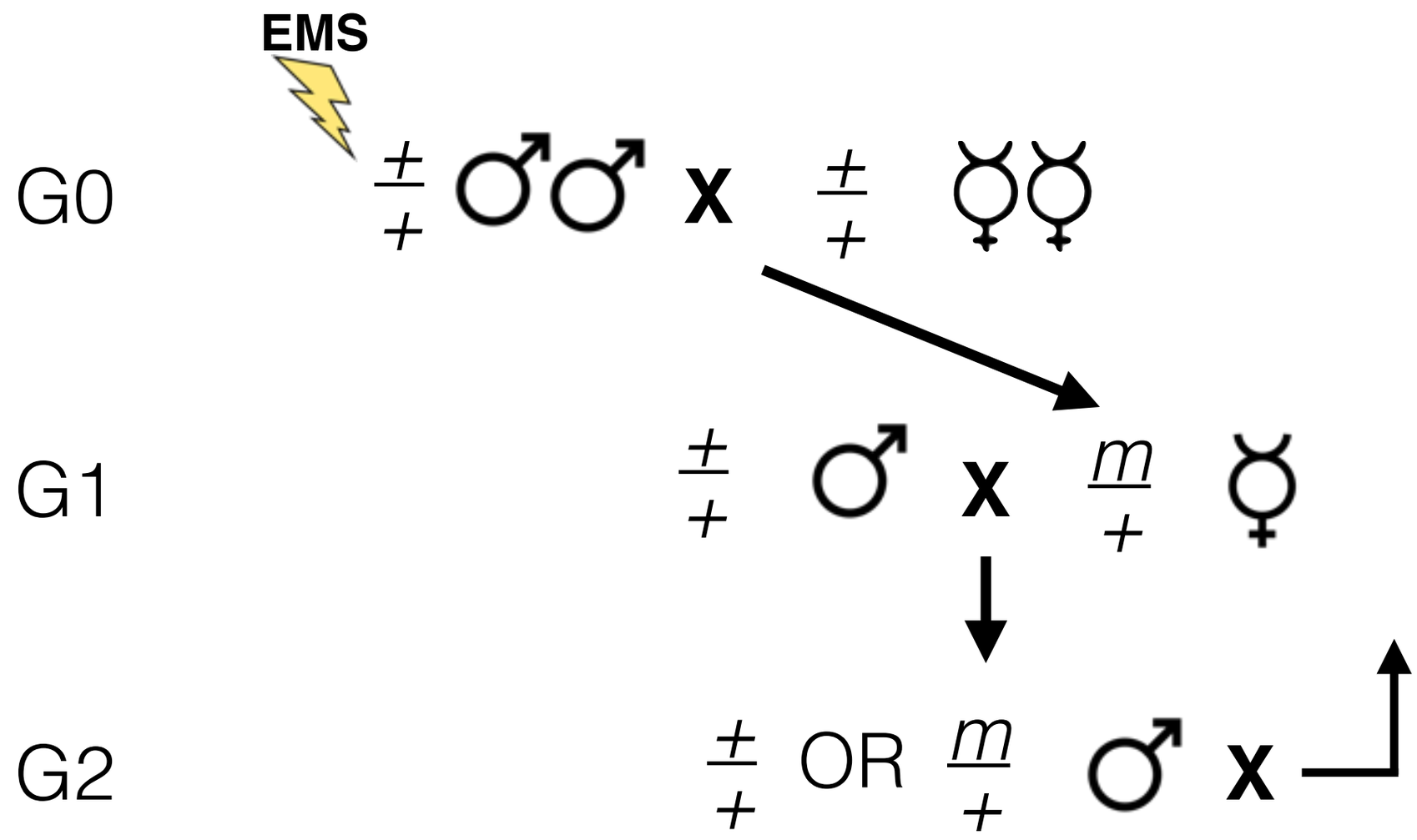
Mouse screens for dominant or recessive phenotypes

Screen or selection?



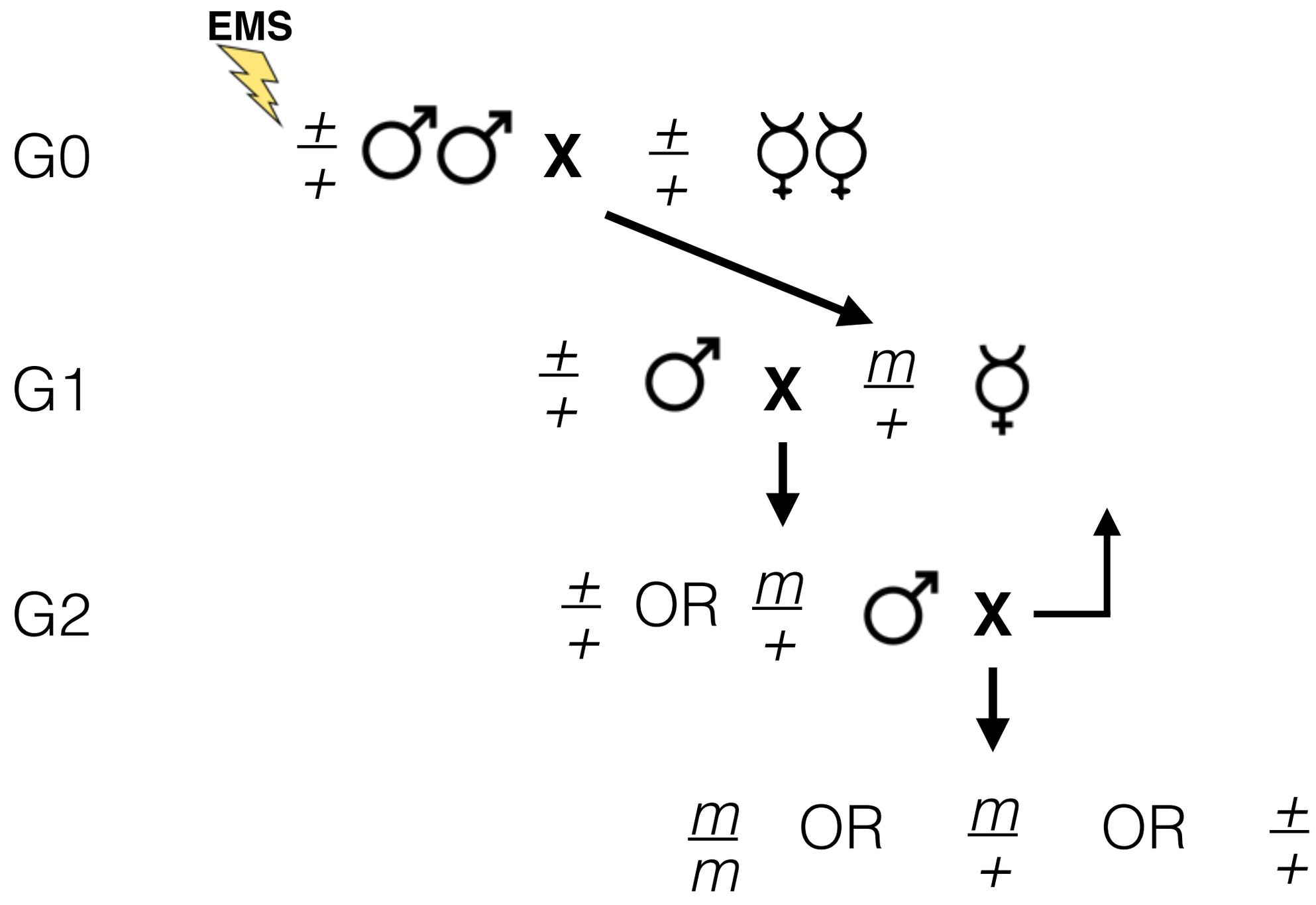
Mouse screens for dominant or recessive phenotypes

Screen or selection?



Mouse screens for dominant or recessive phenotypes

Screen or selection?



Mouse screens for dominant or recessive phenotypes

Remember hemizygous screens too

4. Screen until saturation?

4. Screen until saturation?

Use Poisson sampling and common sense

4. Screen until saturation?

Use Poisson sampling and common sense

4. Screen until saturation?

Use Poisson sampling and common sense

Saturation of the investigators patience

4. Screen until saturation?

Use Poisson sampling and common sense

Saturation of the investigators patience

4. Screen until saturation?

Use Poisson sampling and common sense

Saturation of the investigators patience

Change mutagens

4. Screen until saturation?

Use Poisson sampling and common sense

Saturation of the investigators patience

Change mutagens

Why might we miss genes?

4. Screen until saturation?

Use Poisson sampling and common sense

Saturation of the investigators patience

Change mutagens

Why might we miss genes?

Numbers are too small

4. Screen until saturation?

Use Poisson sampling and common sense

Saturation of the investigators patience

Change mutagens

Why might we miss genes?

Numbers are too small
Pleiotropy

4. Screen until saturation?

Use Poisson sampling and common sense

Saturation of the investigators patience

Change mutagens

Why might we miss genes?

Numbers are too small
Pleiotropy
Redundancy

5. Establish a strain

5. Establish a strain

True-breeding stocks

5. Establish a strain

True-breeding stocks

5. Establish a strain

True-breeding stocks

Balancers, balanced stocks

5. Establish a strain

True-breeding stocks

Balancers, balanced stocks

5. Establish a strain

True-breeding stocks

Balancers, balanced stocks

Freeze organisms

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.

6. Backcross and/or outcross

Mutagenesis adds hundreds of mutations randomly throughout the genome.

Backcross = cross to parent used in the screen/selection

6. Backcross and/or outcross

Mutagenesis adds hundreds of mutations randomly throughout the genome.

Backcross = cross to parent used in the screen/selection

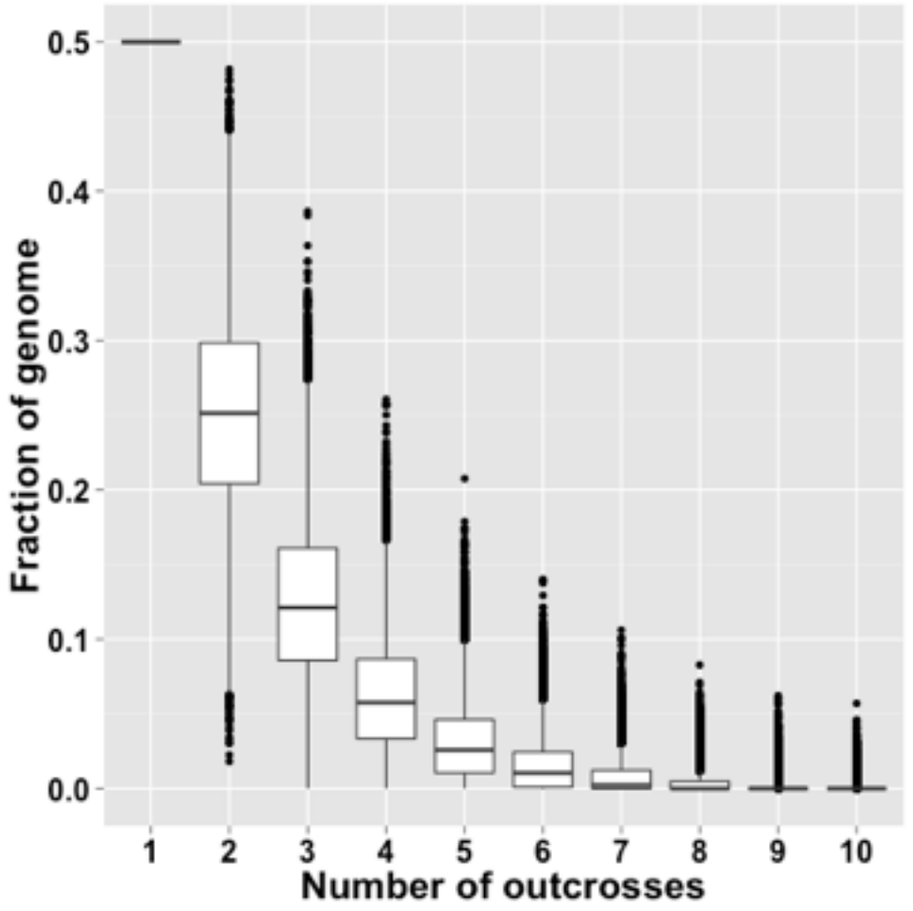
Outcross = cross to a wild-type strain

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

7. Test for dominance

8. Single-gene phenotype?

7. Test for dominance

8. Single-gene phenotype?

9. Mapping and complementation

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*

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?

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

11. Define the nature of the mutant allele(s)

11. Define the nature of the mutant allele(s)

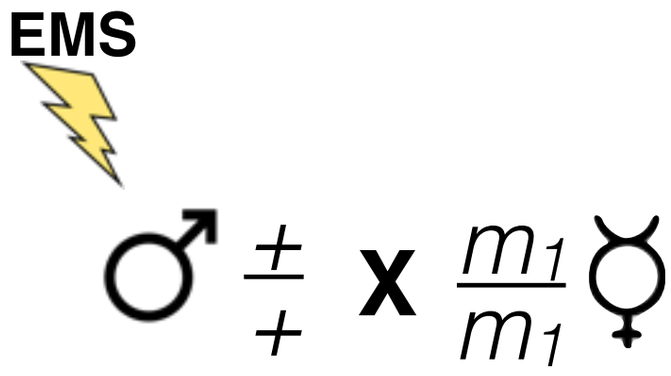
Loss of function more likely than gain of function

11. Define the nature of the mutant allele(s)

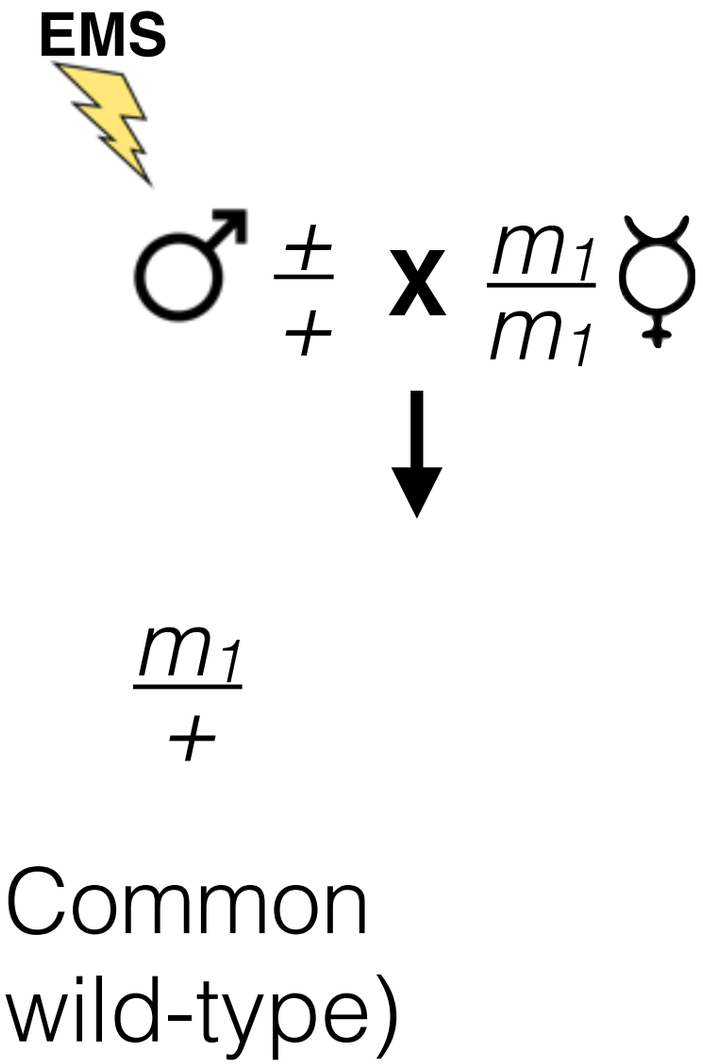
Loss of function more likely than gain of function

What if you only have one mutant?

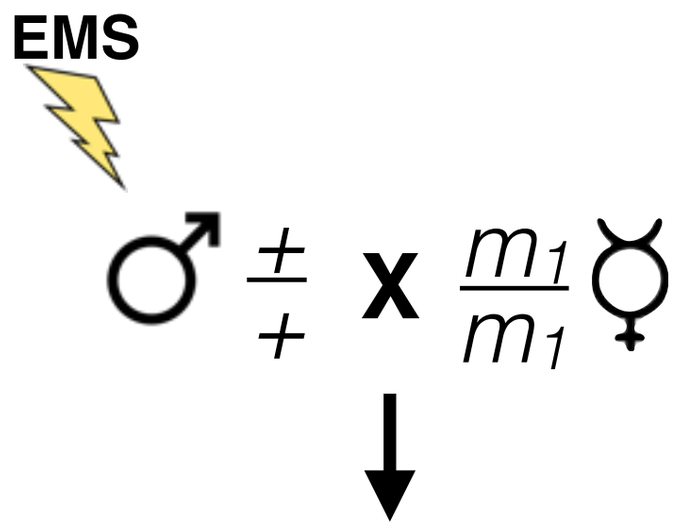
12. Perform non-complementation screens



12. Perform non-complementation screens



12. Perform non-complementation screens



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

Common (wild-type)

Rare (mutant)

13. Gene dosage

We know the location of the gene

Use deficiencies and duplications to study dosage

13. Gene dosage

We know the location of the gene

Use deficiencies and duplications to study dosage

1. Is the phenotype dominant or recessive?

13. Gene dosage

We know the location of the gene

Use deficiencies and duplications to study dosage

1. Is the phenotype dominant or recessive?
2. Recessive: hypomorph or null

13. Gene dosage

We know the location of the gene

Use deficiencies and duplications to study dosage

1. Is the phenotype dominant or recessive?
2. Recessive: hypomorph or null
3. Dominant: hypermorph, neomorph, antimorph

14. Define the null phenotype

What happens with a complete loss of gene function?

14. 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

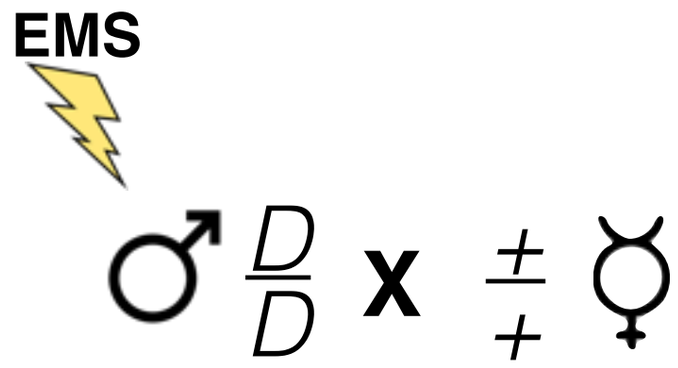
14. Define the null phenotype

What happens with a complete loss of gene function?

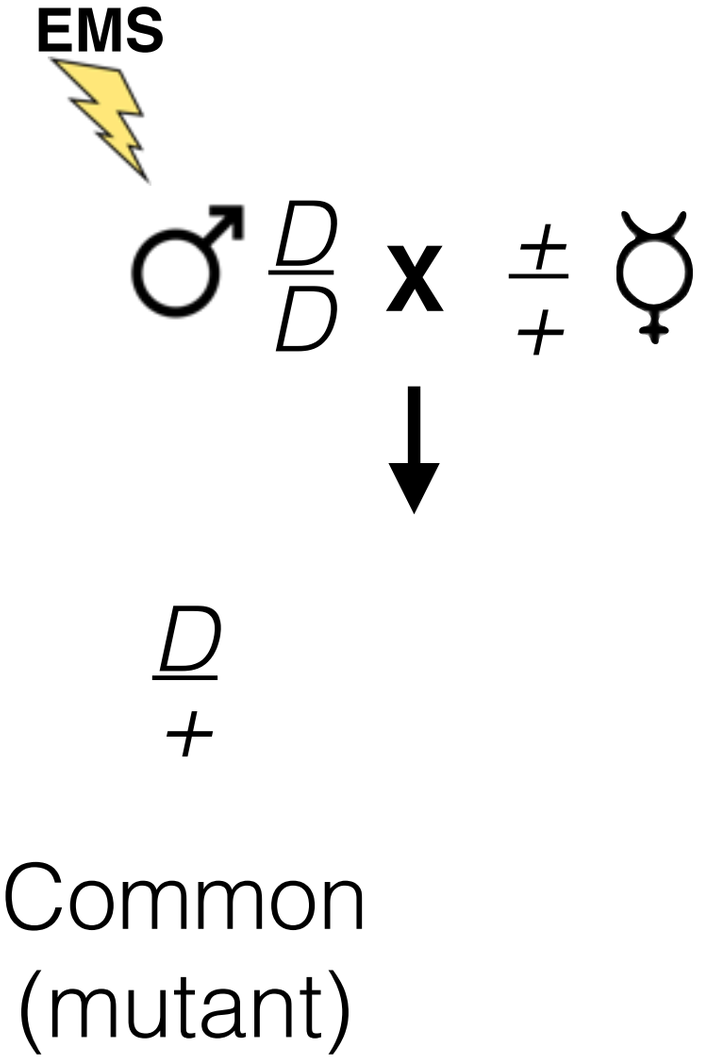
Dosage studies, non-complementation screens, and characterization of the mutant phenotype tell you about the null phenotype

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

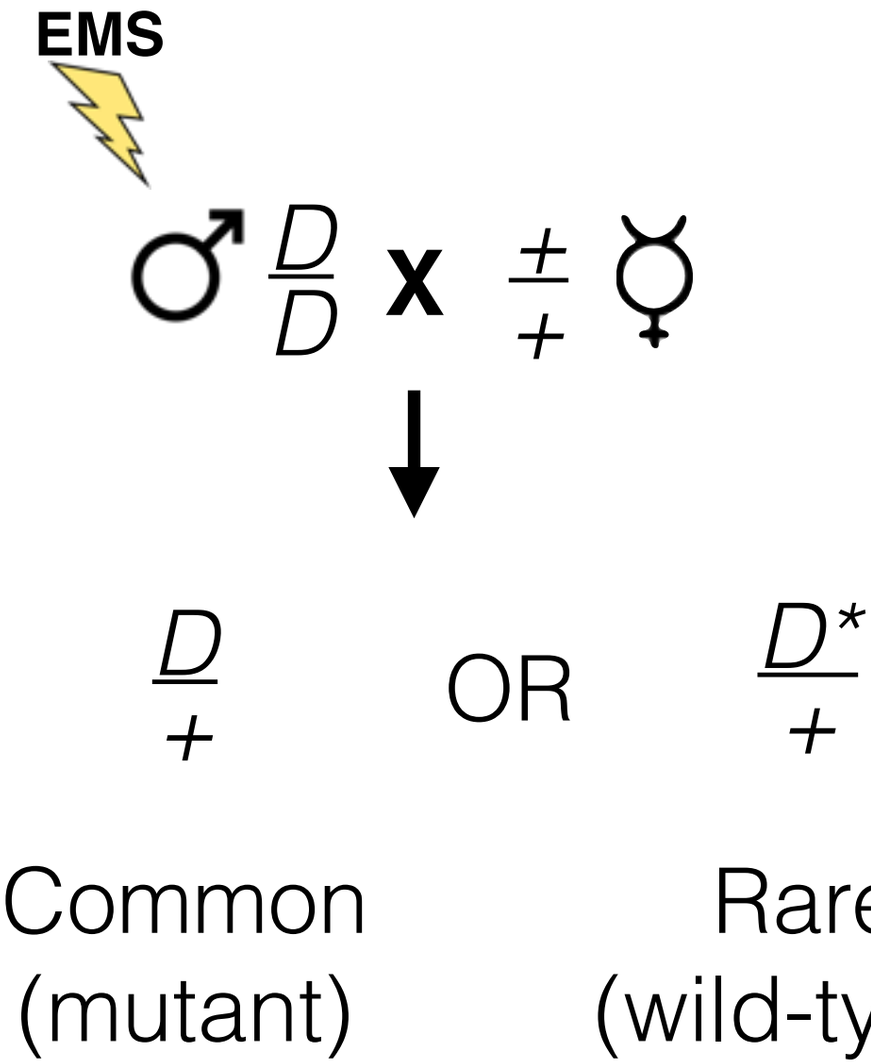
Cis-dominant suppressor screen



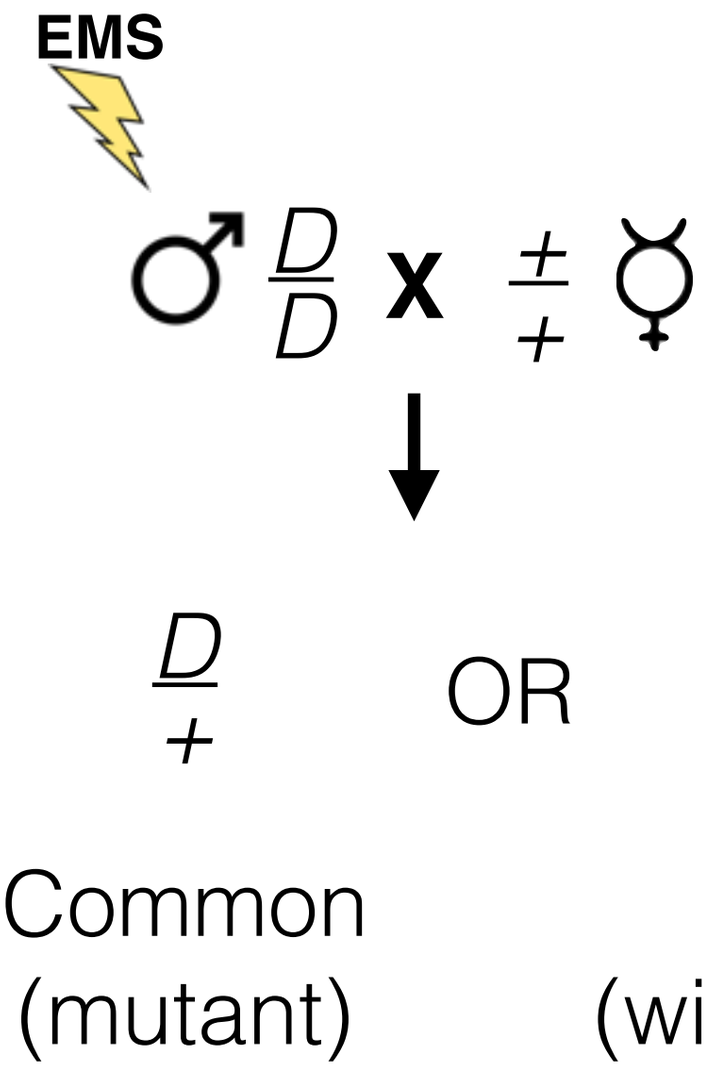
Cis-dominant suppressor screen



Cis-dominant suppressor screen

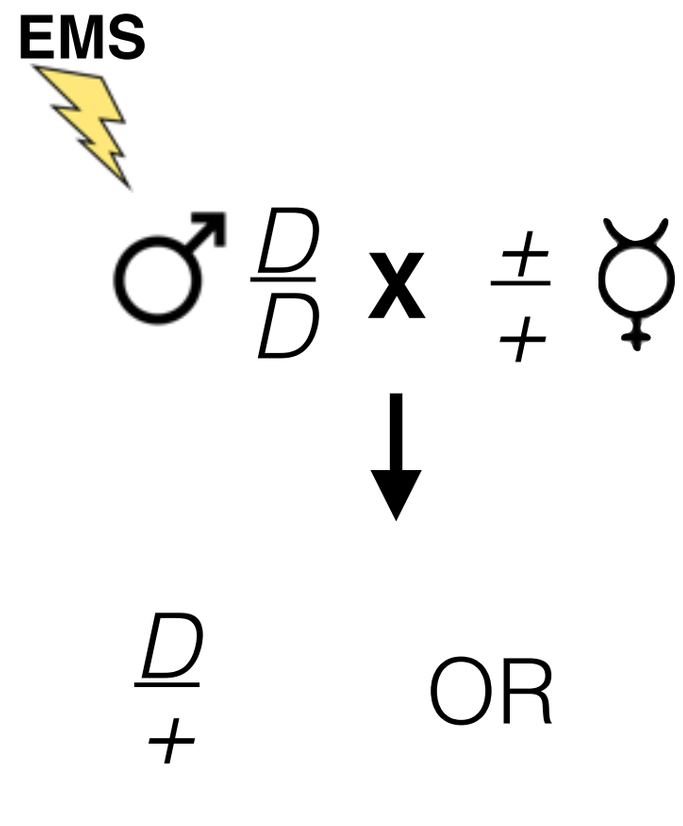


Cis-dominant suppressor screen



What could *D** be?

Cis-dominant suppressor screen



Common (mutant)

Rare (wild-type)

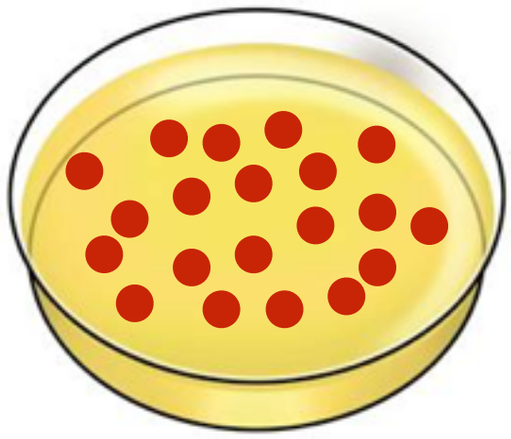
What could *D** be?

Revertant, extragenic suppressor, intragenic suppressor, or null mutant

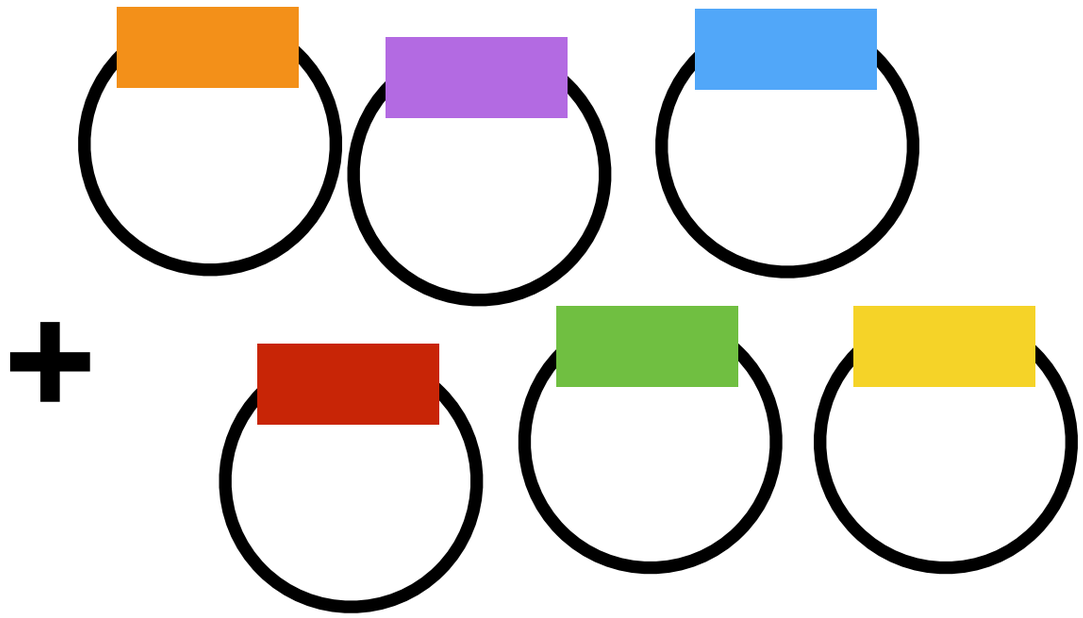
15. Clone the gene

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

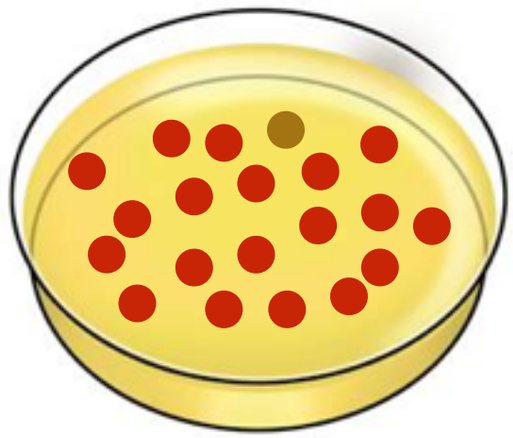
Cloning by complementation in bacteria and yeast



ade2 mutants

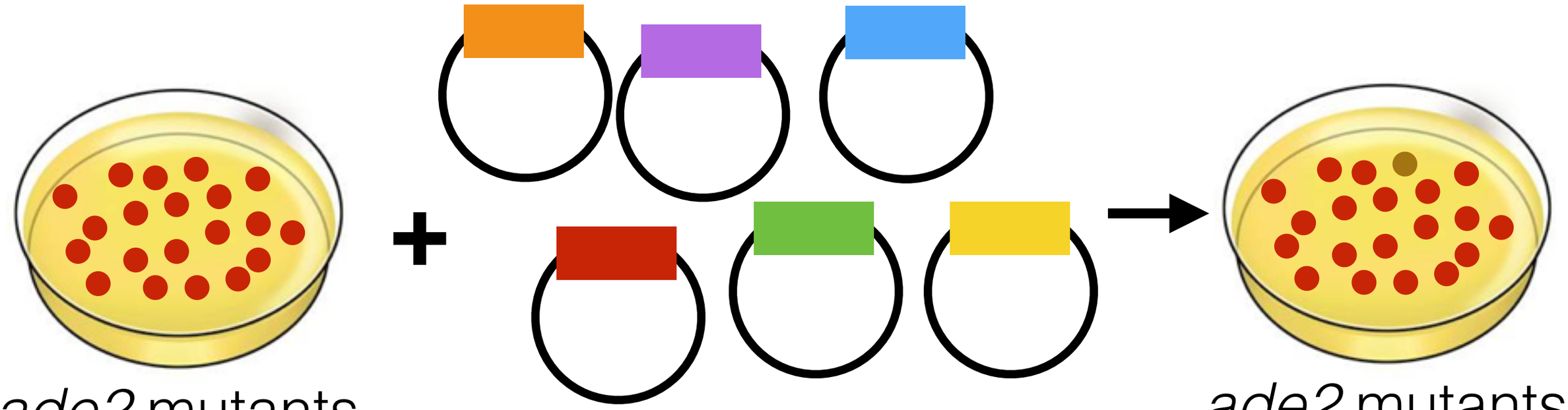


Plasmids each containing a different gene



ade2 mutants + plasmids

Cloning by complementation in bacteria and yeast



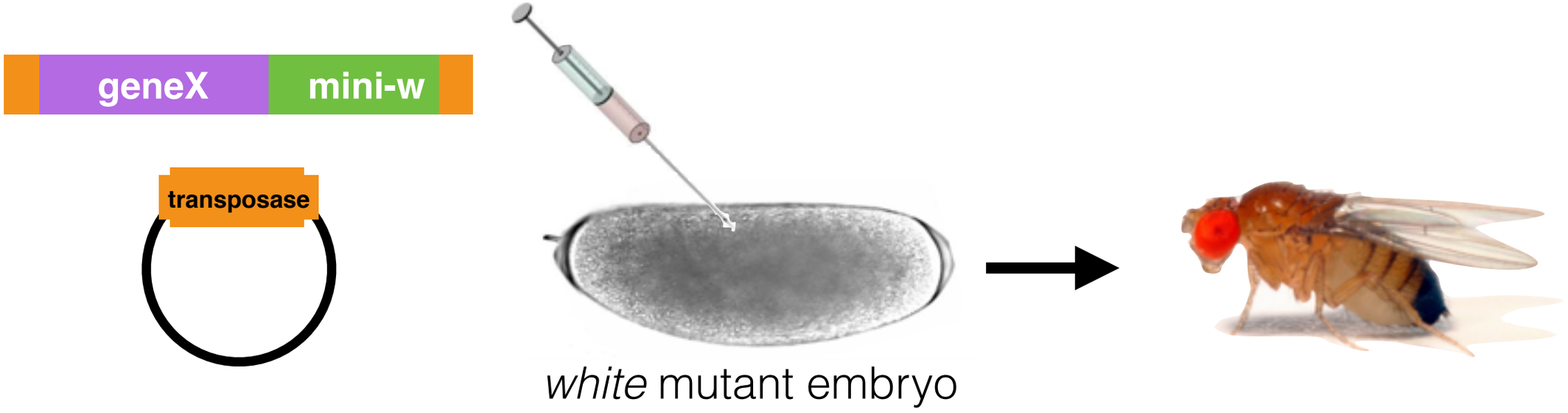
ade2 mutants

Plasmids each containing a different gene

ade2 mutants + plasmids

Caveat: overexpression bypass suppressors

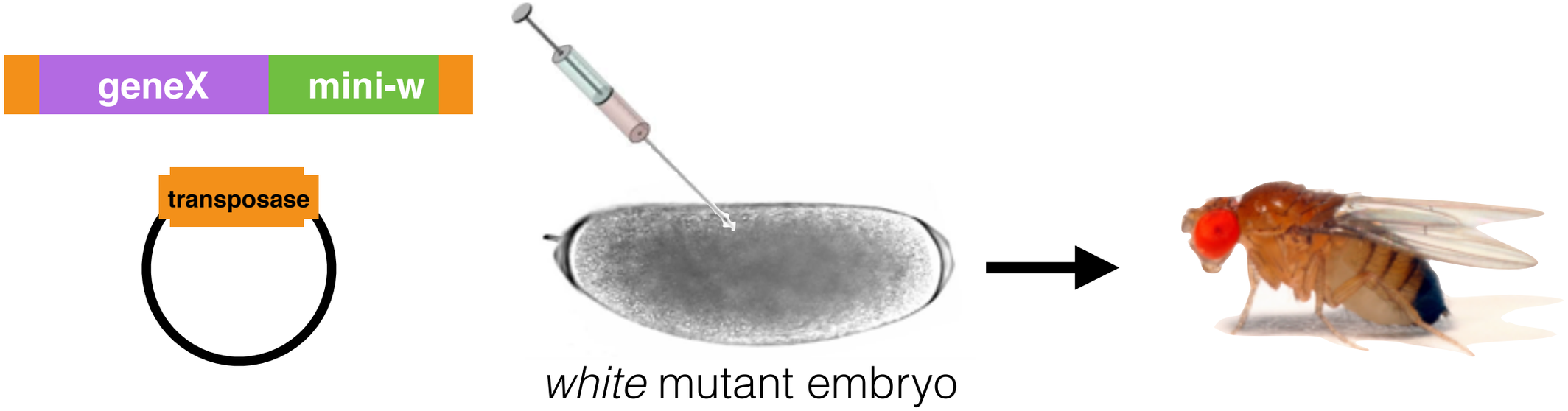
Cloning by complementation in worms and flies



Transgenesis and rescue

Cloning by complementation in worms and flies

Caveat: overexpression bypass suppressors and not stable



Transgenesis and rescue

Cloning by complementation in worms and flies

Caveat: overexpression bypass suppressors and not stable

Caveat: overexpression bypass suppressor and variable expression

Transgenesis and rescue