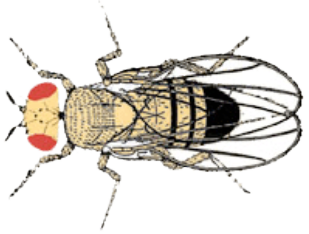
Creating mutant flies

Mutagenesis strategies

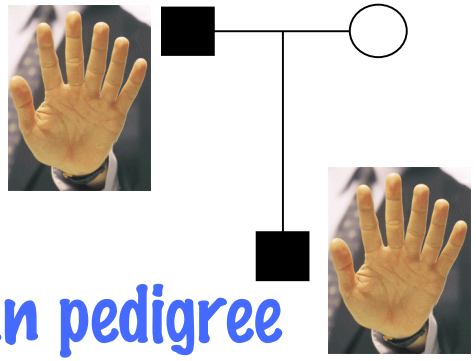
Selecting and screening

Transposon-mediated mutagenesis

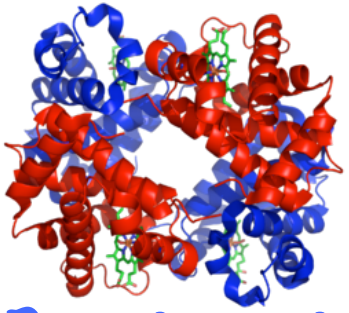
Common theme: linking genotype & phenotype



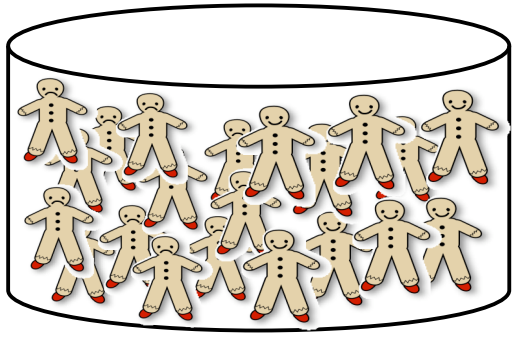
Mutant identified
in a model organism



Human pedigree
segregating a trait



Protein acting in
a biological process

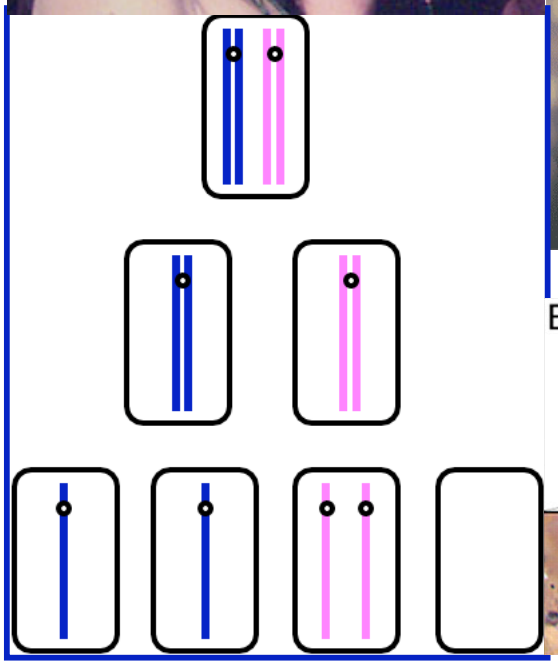


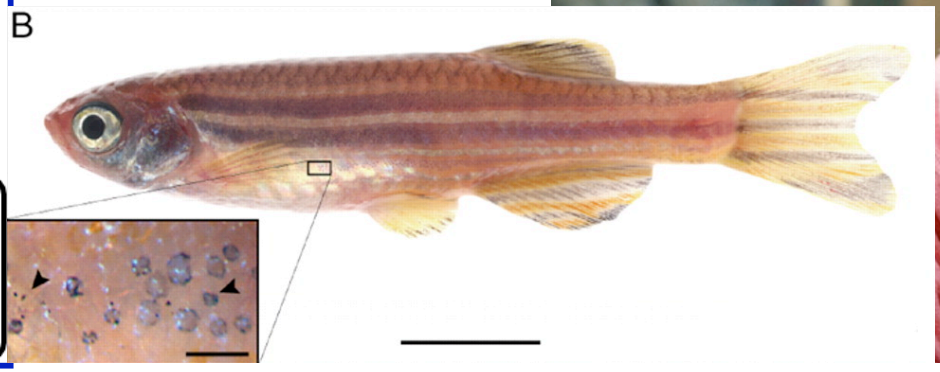
Association study

```
 946  ATT GTC TGT AGC CGA TTG GAG GAG TAC AAC AGC CAT
1009  GGA CCT TTA CGG CGT AAT CCT GGA AAC CAT GAC AAA
1072  GCT GAT GTA GAA TTT TGC CTG AGT TTG ACC CAA TAT
1135  AAT TTC AGC TTT AGA AAT ACA CTG GAA GGA TTT GCT
1198  TCT CAA AGC AGC ATG CAC AAT GCC TTG CAC ATC TAT
1261  GGA TCT GCC AAC GAT CCT ATC TTC CTT CTT CAC CAT
1324  TGG CTC CGA AGG CAC CGT CCT CTT CAA GAA GTT TAT
```

Sequence analysis

Using genetics to study a process: Making mutations





Genetic analysis using mutations

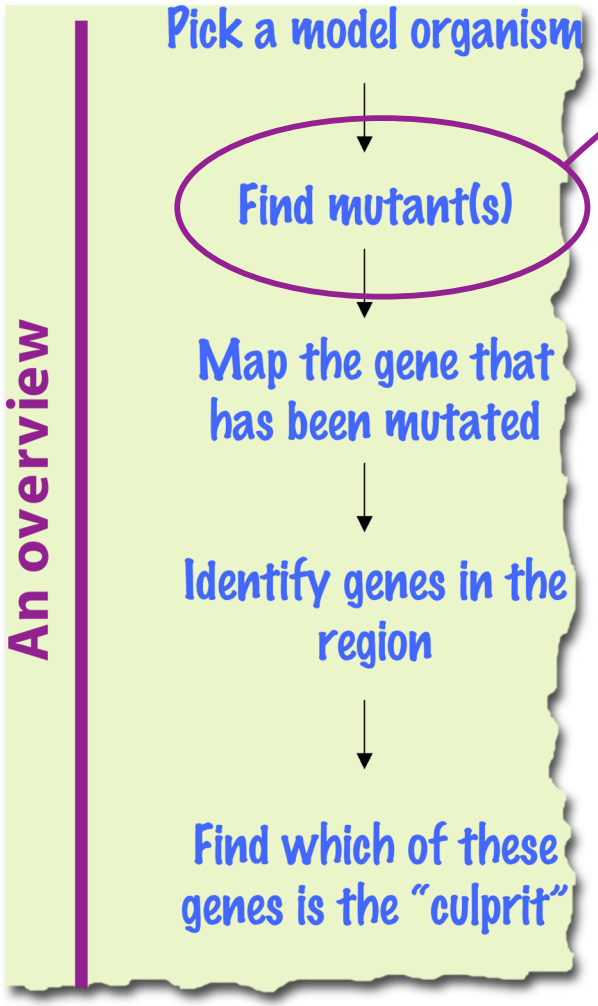
The goal: understanding a biological process or structure

"Forward genetics"

- Start with "unknown" system
- Mutations to identify genes needed
- Mutant phenotypes reveal functions
- Map the genes
- Identify the gene products

"Reverse genetics"…begins with gene or its product, work backward to figure out the process involving the gene

A genetic approach



Find mutant(s) with "interesting" phenotypes

.

.

.

How many genes have we mutated?

Breaking the system… mutagenesis

Start with large population of identical individuals

Use mutagenic process to greatly increase

the number of mutants

Identify interesting mutations affecting the process under study

Identifying interesting mutations—screen vs. selection

Screen — Each member of the population is examined... does it fit the phenotype criteria that have been set up?

Selection — Individuals not meeting the criteria don't survive (or are otherwise eliminated from the population)

Example 1: Looking for a translator — Russian ↔ English

Screen: read resumés

Selection: advertise in Russian

ЕВРОПЕЙСКАЯ НАУКА
ОБЕСПОКОИЛАСЬ

Example 2: Looking for wingless fly mutants

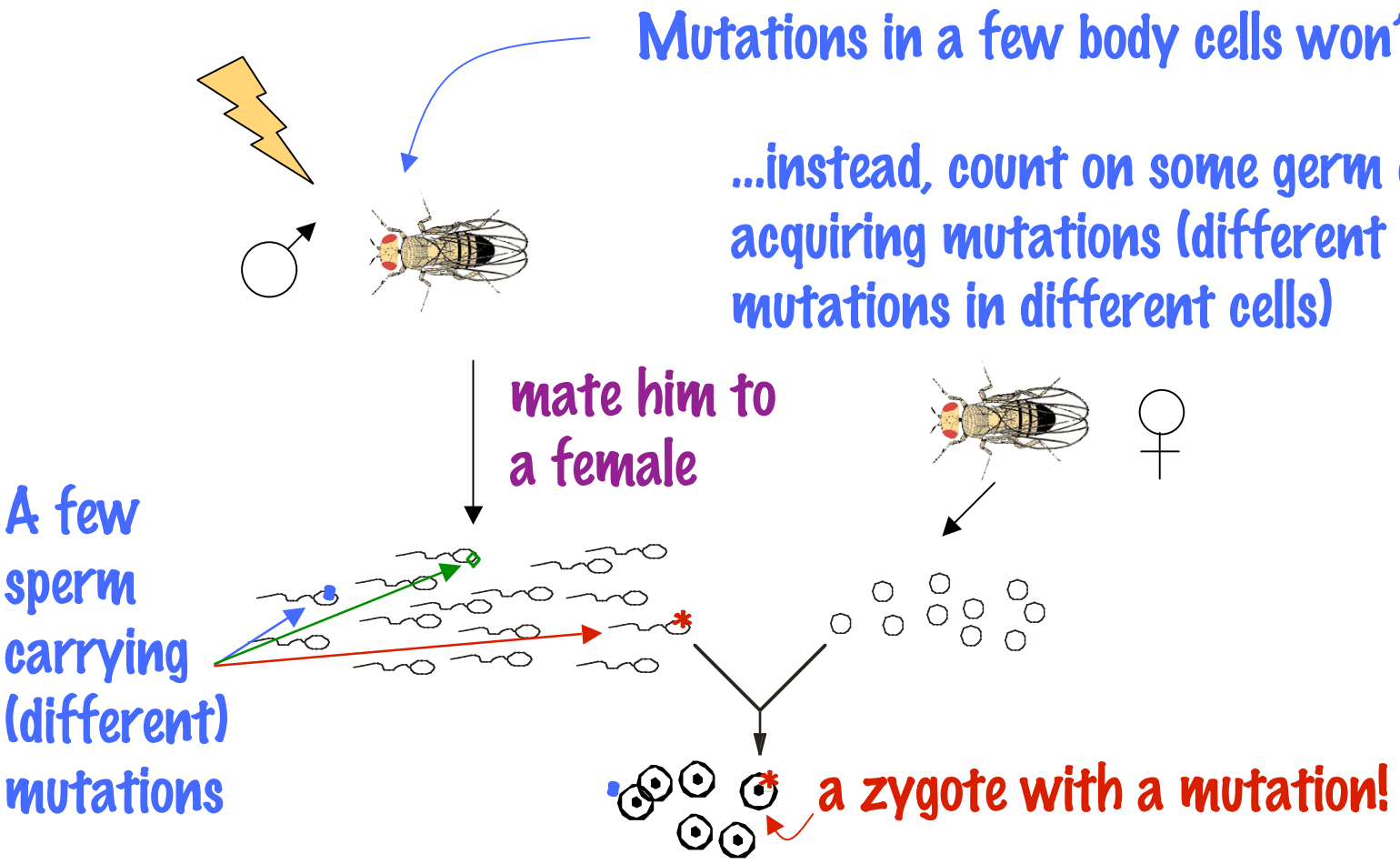
Screen: Look at each fly... wings present?

Selection: Open vial, let flies fly away

Primary selection or screen is often followed by secondary selection or screen

Aside: Mutagenesis strategies

We need mutants where every cell in the body has the same mutation



Overall procedure

We want to…

Control when mutation occurs

↓

Cause mutations during gametogenesis

↓

“Capture” the gametes by fertilization

↓

Identify all progeny with potential mutations

↓

Identify mutants of interest

Generating mutations for genetic analysis

Spontaneous mutations

- too infrequent!

Induced mutations

- chemical mutagenesis
- radiation
- transposon tagging

What are transposons?

Small pieces of DNA that can move from one site in the genome to another

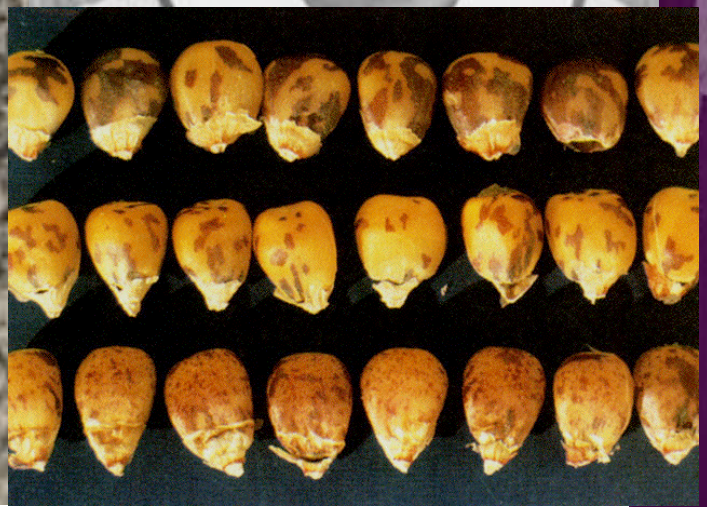
- ALL organisms have them (about 45% of our genome: transposon remnants!)
- Jumping genes, Selfish DNA
- Mechanism for evolutionary change

Discovery of transposons

Barbara McClintock (1902-1992)

1940's: theory of "controlling elements"

Nobel Prize in 1983



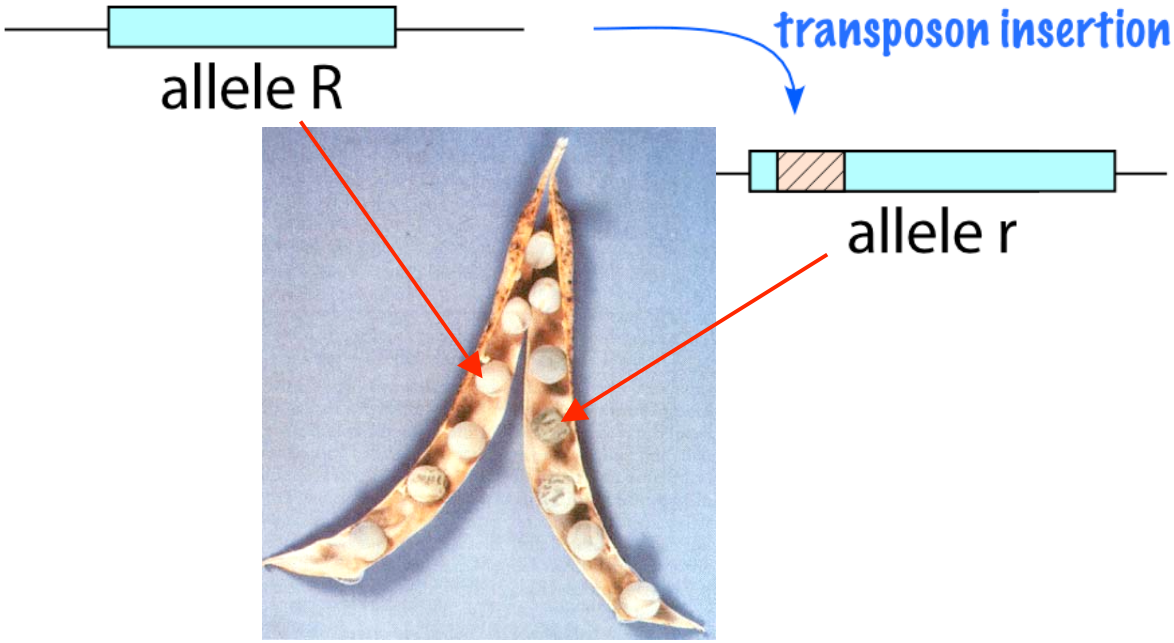
Mutagenesis using transposons

Reminder:

- how a process is broken ⇒ how it works normally

- mutations may break processes

Transposons can cause mutations if they hop into or near genes



Three types of Transposable Genetic Elements

1. DNA-dependent

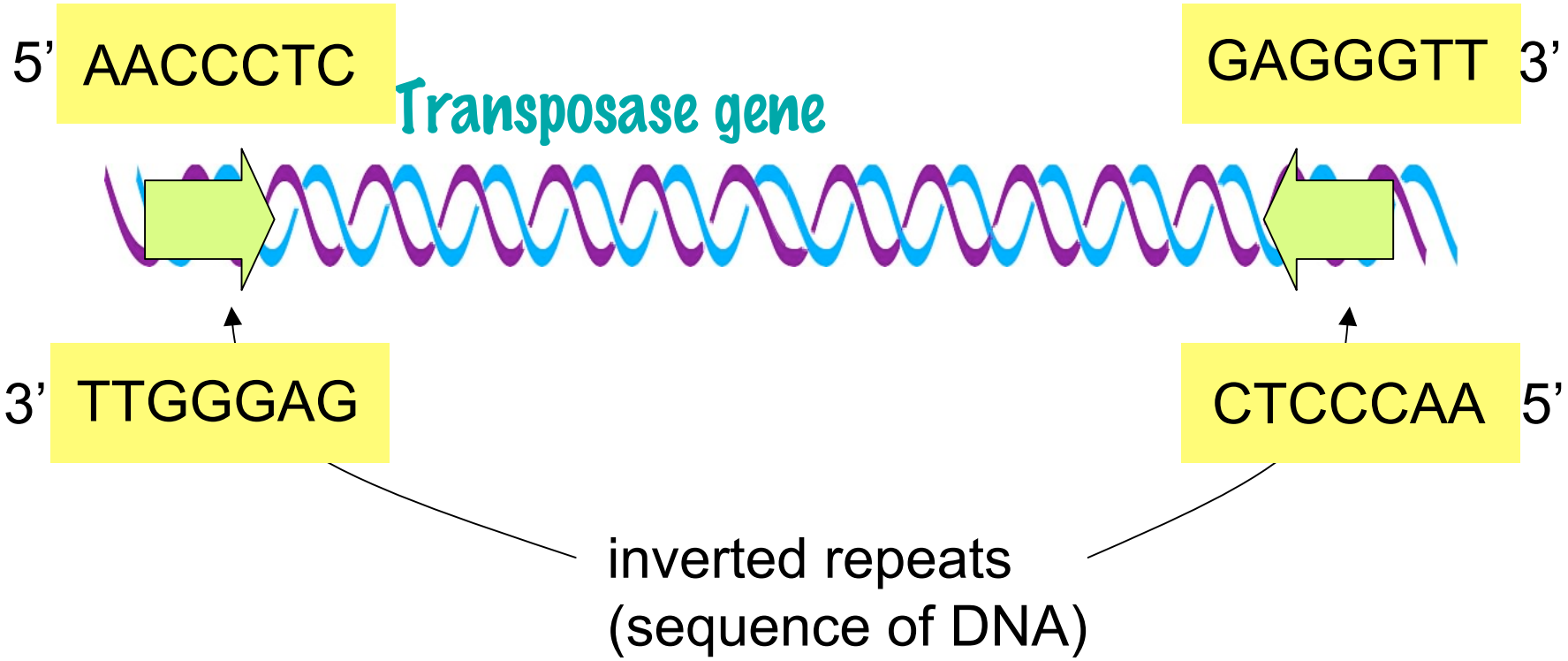
Prokaryotes & Eukaryotes,
DNA intermediates

2. Retroviruses

3. Retrotransposons

Eukaryotes only,
RNA intermediates

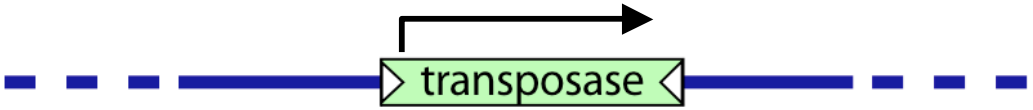
DNA-dependent transposons (no RNA intermediate)



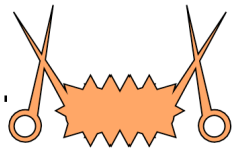
Transposase protein will recognize the IRs, cut out the transposon and find another place in the genome for insertion

How does transposition work?

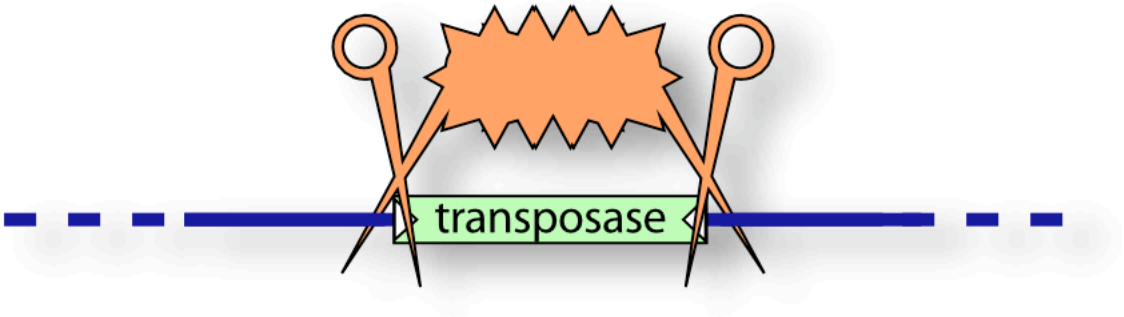
1. Transposase gene is transcribed



2. Transposase protein is made…

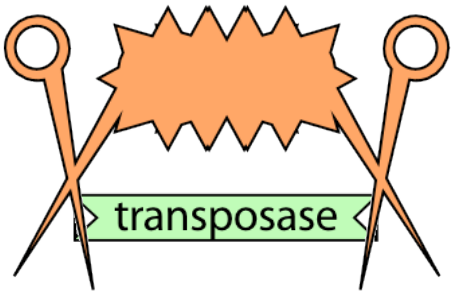


3. Transposase recognizes and cuts at similar inverted repeats (wherever they may be in the genome)



How does transposition work? (cont'd)

4. Cut-out transposon can be degraded…



5. …or inserted at a new location

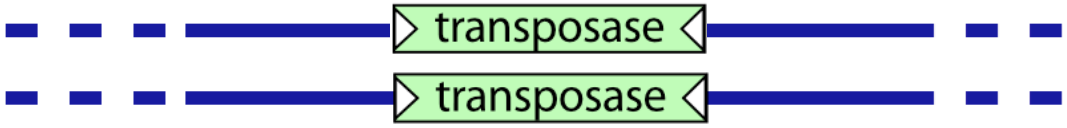
transposition!

what about the original location?

How does transposition work? (cont'd)

At original location…

- cut site is repaired using a template (sister chromatid or homologous chromosome)
- if the template has the transposon, repaired DNA will have the transposon also



- if the template does not have the transposon, it's lost from the original site

Elements needed for transposition

1. Functional transposase gene

   okay if no repeats flanking the transposase gene

2. A pair of intact inverted repeats (in proper spacing)

   anywhere in the genome

   - need not enclose a functional transposase gene

Mutagenesis using transposons—the big picture

We want to make mutations by making a transposon jump into or near genes

- we want control of when and in whom the jump happens
- once the transposon has jumped, we want to prevent it from jumping again (Why?)

Bigger picture reality check:

What is our overall goal?

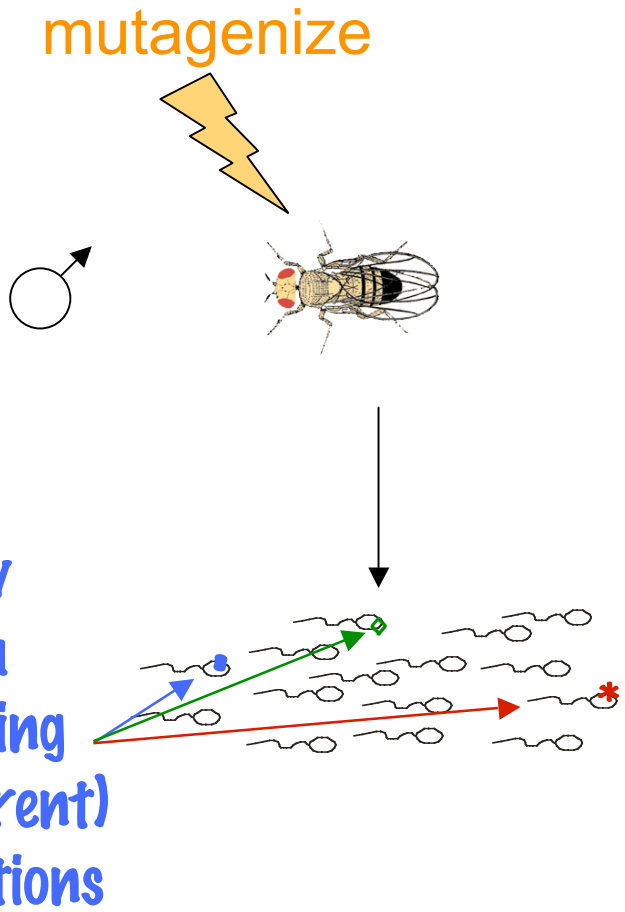
To understand how some process works...

...by "breaking the system" (i.e., making mutations)

e.g., How do flies develop wings?

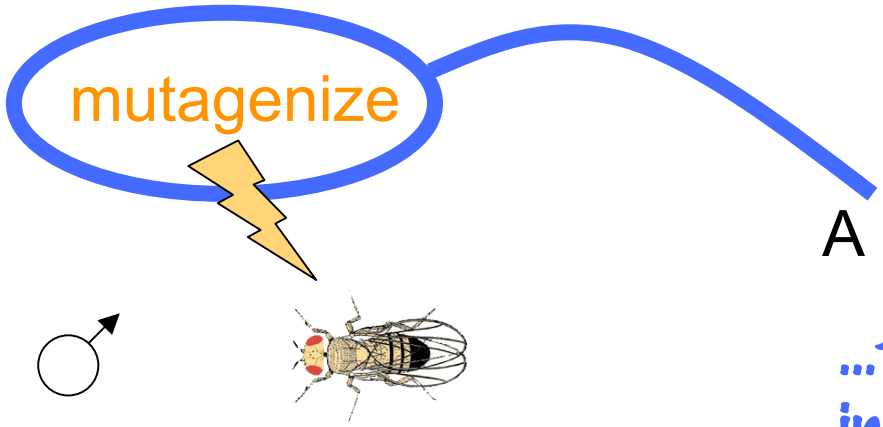
What do we need?

We need mutants where every cell in the body has the same mutation



Count on some germ cells acquiring mutations (different mutations in different cells)

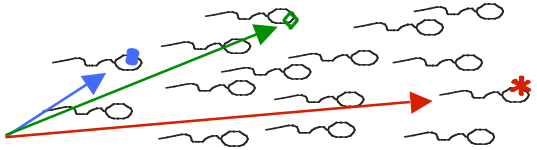
How are we going to make mutations?



A transposon

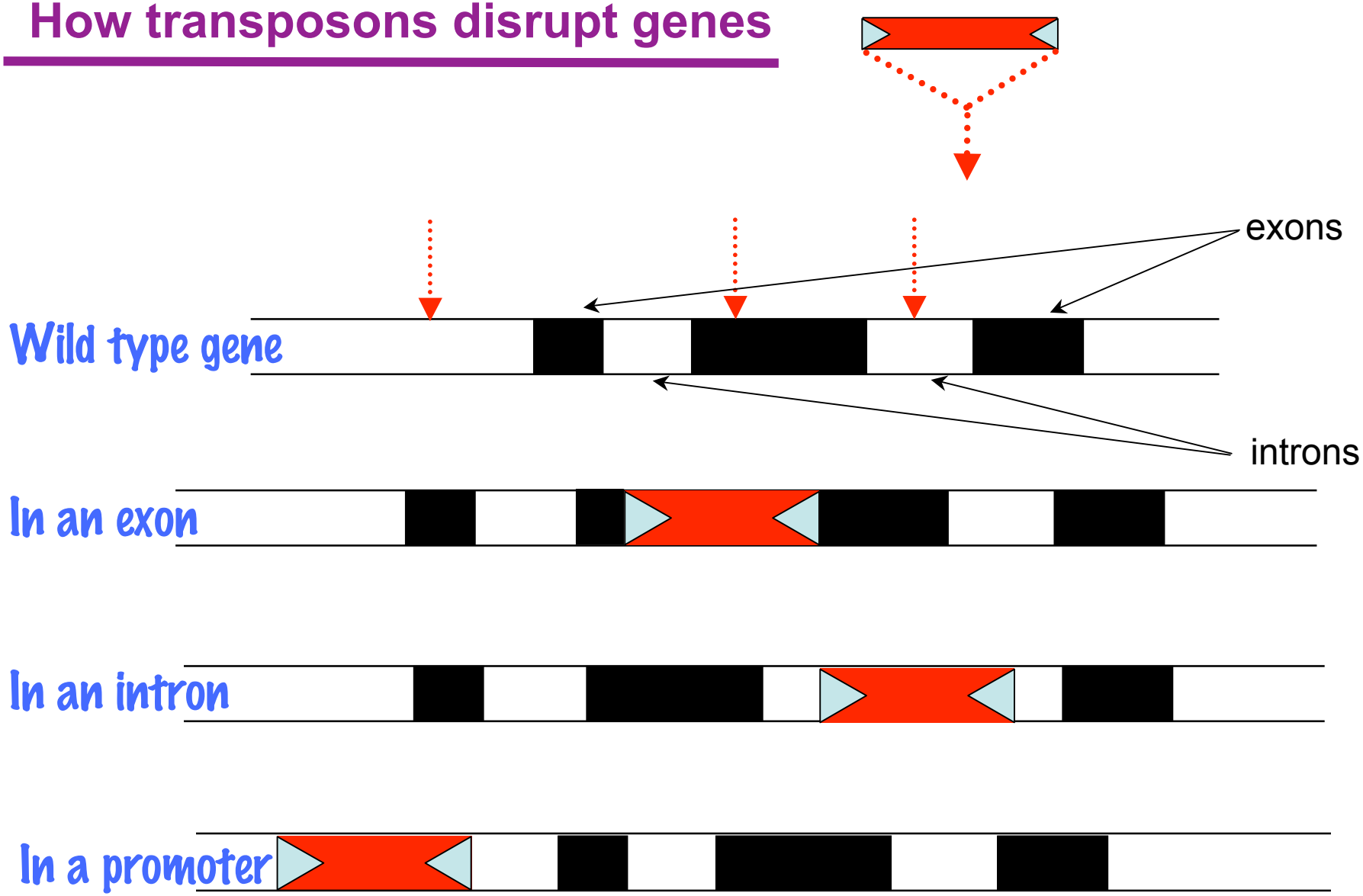
...to "jump" to random locations in genome

A few sperm carrying (different) mutations

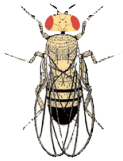


Why would that cause mutations?

How transposons disrupt genes

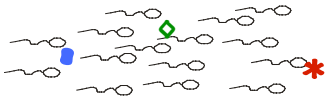


Mutagenesis using transposons



transposon will jump in cells of his gonads (during spermatogenesis)

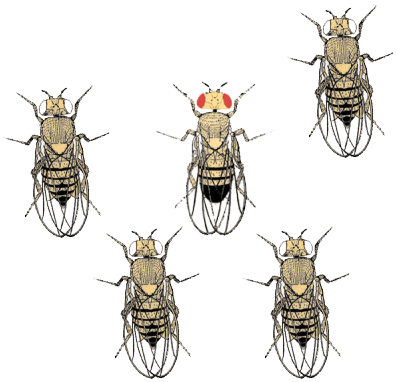
mate him to females



» Most of his sperm... no jump

» a few sperm... successful jumps

» even fewer... jumps into or near genes that affect our biological process of interest



and prevent further jumps!

identify progeny made by sperm with jumped transposon

How to control the jump?

Elements needed for transposition:

1. Functional transposase gene

   okay if no repeats flanking the transposase gene

   ...but then it cannot itself hop

2. A pair of intact inverted repeats

   anywhere in the genome

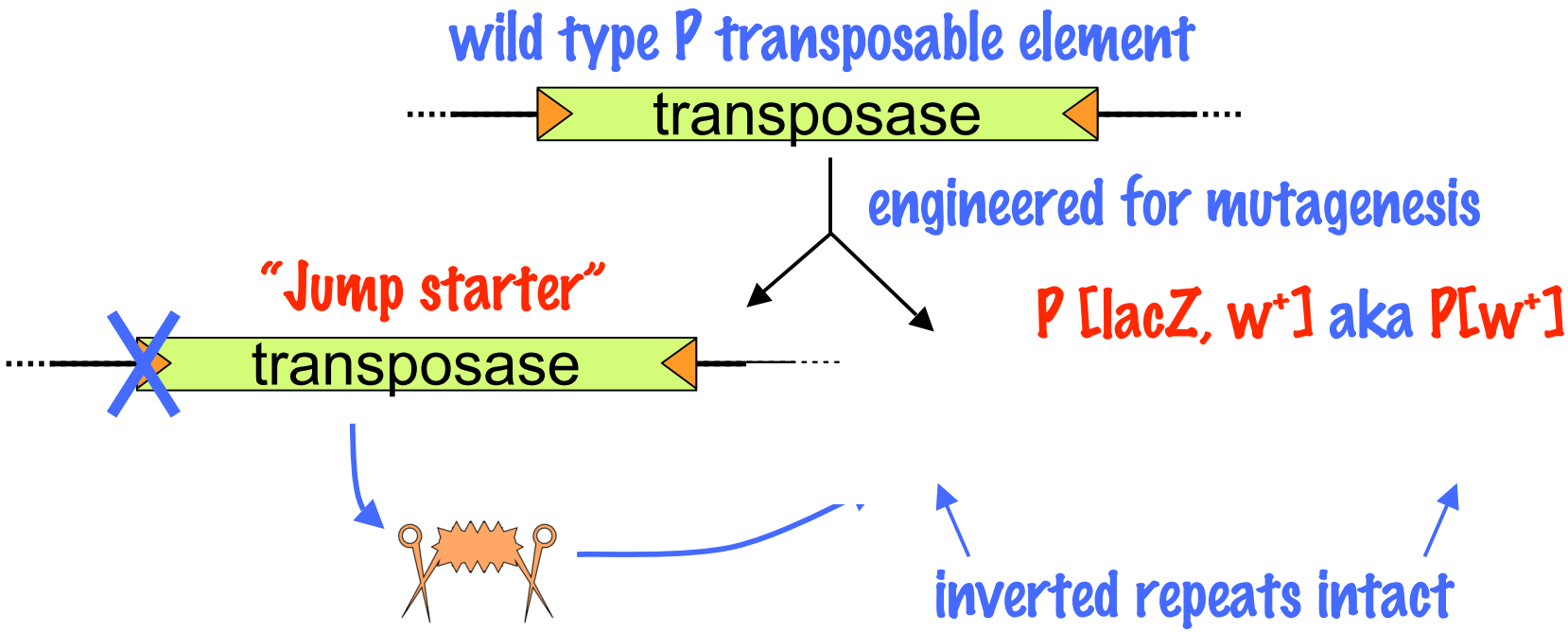
   - need not enclose a functional transposase gene

     ...but then it depends on transposase from elsewhere

Controlling the jump — P elements in Drosophila

Separating the two components of transposons → control over when transposition occurs

Drosophila P element system:



What's w^+??

X^w

LOF mutation → white eyes

Notation:
w^+ is wild type
w is mutant

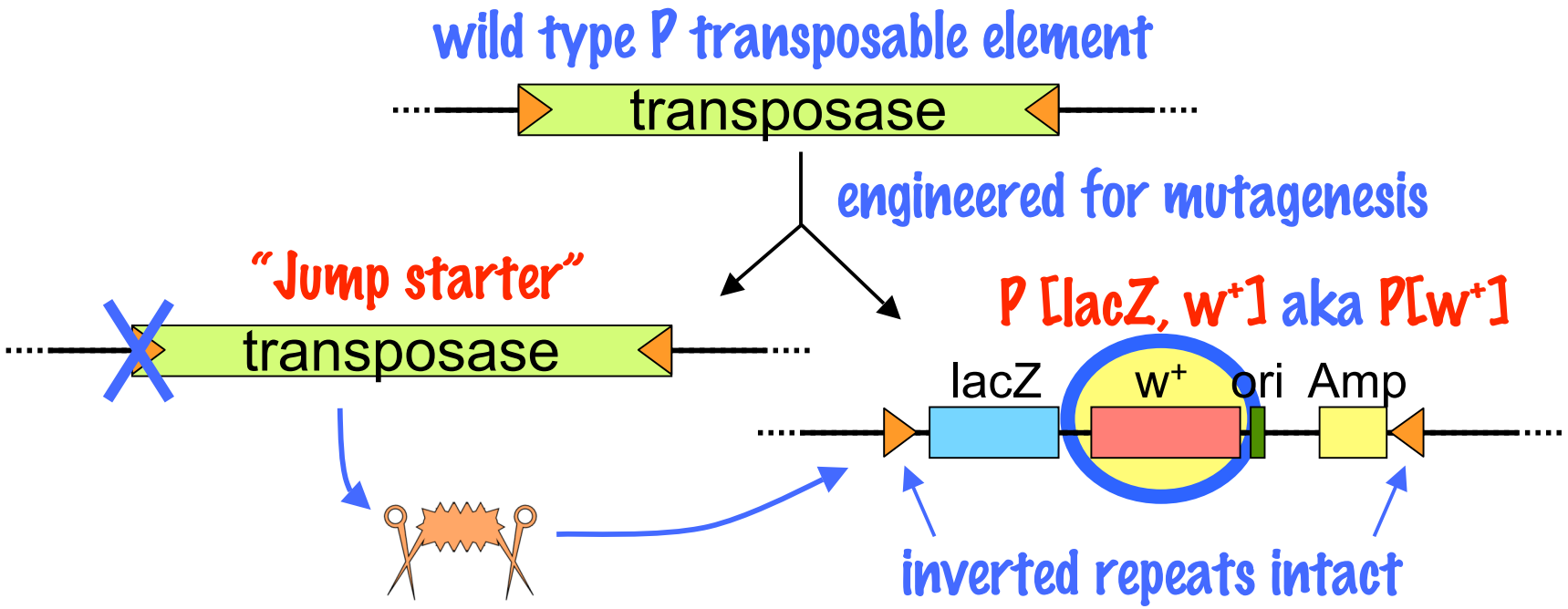
X^{w+}

wild type allele → red eyes

Controlling the jump — P elements in Drosophila

Separating the two components of transposons → control over when transposition occurs

Drosophila P element system:

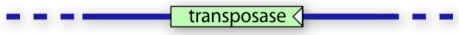


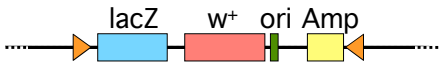
How to control the jump?

To have control over the jump… split up the two components!

one line of flies… Jumpstarter (transposase, no inverted repeats)

separate line of flies… P[lacZ w+] (inverted repeats, no transposase)



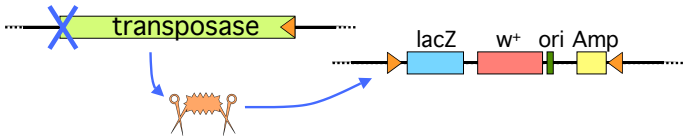


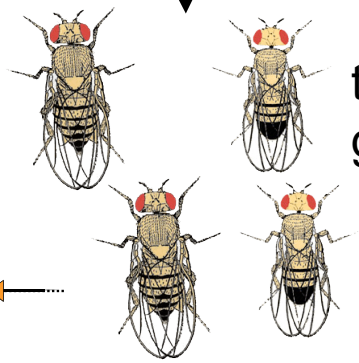
Mate

CROSS 1

when we want transposition…

progeny whose cells have both components





transposon will jump in cells of his gonads (during spermatogenesis)

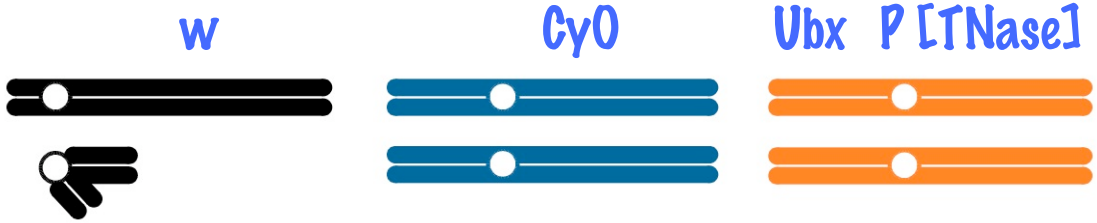
CROSS 1: to bring the transposase into the same flies as the transposon that will create the mutations

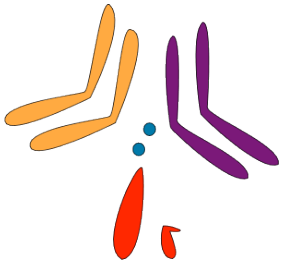
How to control the jump?

To have control over the jump… split up the two components!

one line of flies...
Jumpstarter
(transposase, no
inverted repeats)

depiction of genotype





sex chr (I)

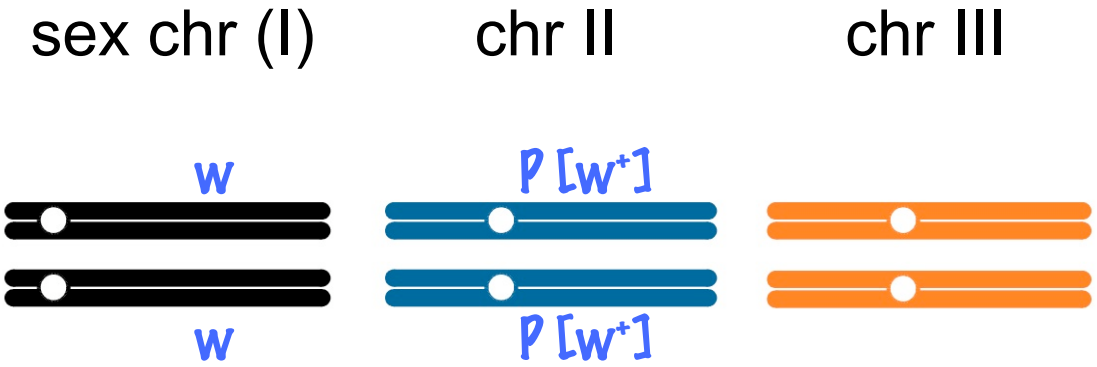
II

III

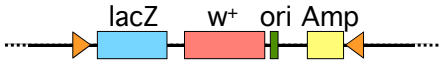
Drosophilamelanogaster

Male Female

Harnessing transposons

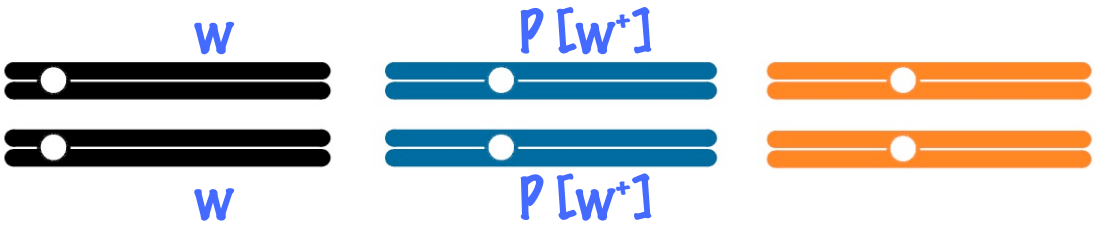


separate line of flies... P[lacZ w+] (inverted repeats, no transposase)



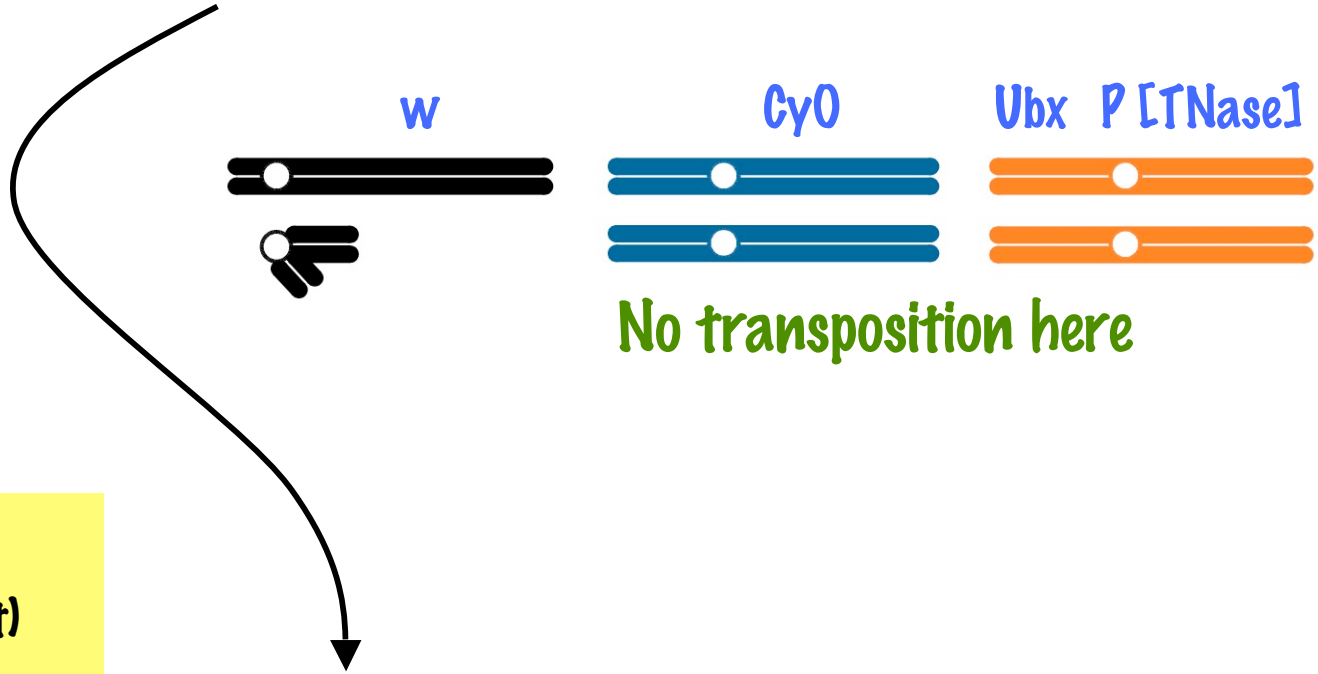
Harnessing transposons

sex chr (I) chr II chr III



No transposition here

X = CROSS 1



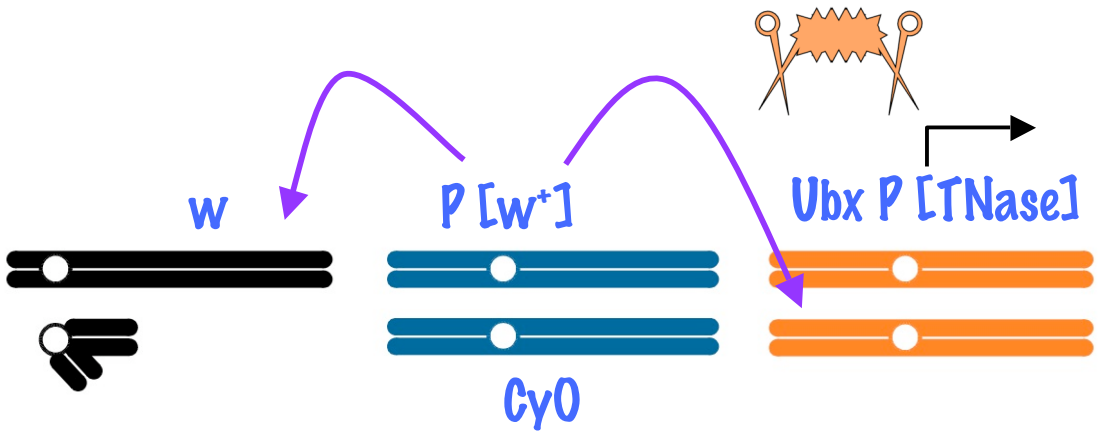
No transposition here

P [TNase] = Jump starter

CyO = curly wing (dominant)

Ubx = ultrabithorax (dominant)

Harnessing transposons (cont'd)



Transposition during gametogenesis…

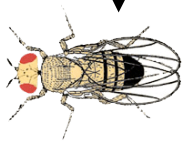
Why pick males?

- no recombination!

Jump-starter

P[w+]

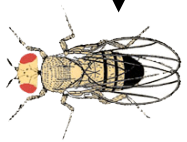
mate



Which flies did we pick?

← Fly in which mutagenic event will happen… what phenotype?

mate



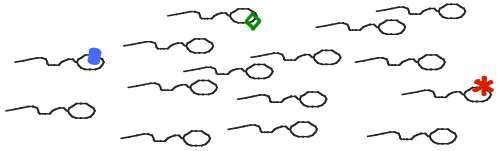
Which flies did we pick?

Red-eyes... must have P[w$^+$]

Enlarged halteres... must have P[ITNase]

Male... no recombination

Curly wings... thinking ahead (need to identify successful hops!)

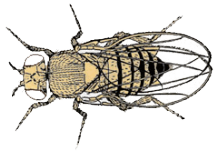


Are the sperm any good to us by themselves?

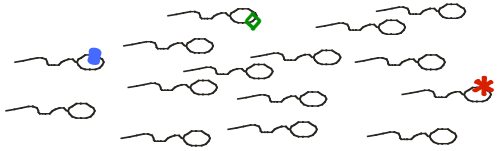
… and which sperm are we interested in?

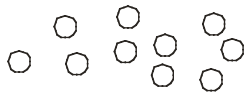
male progeny from cross 1

mate him to
a female



??





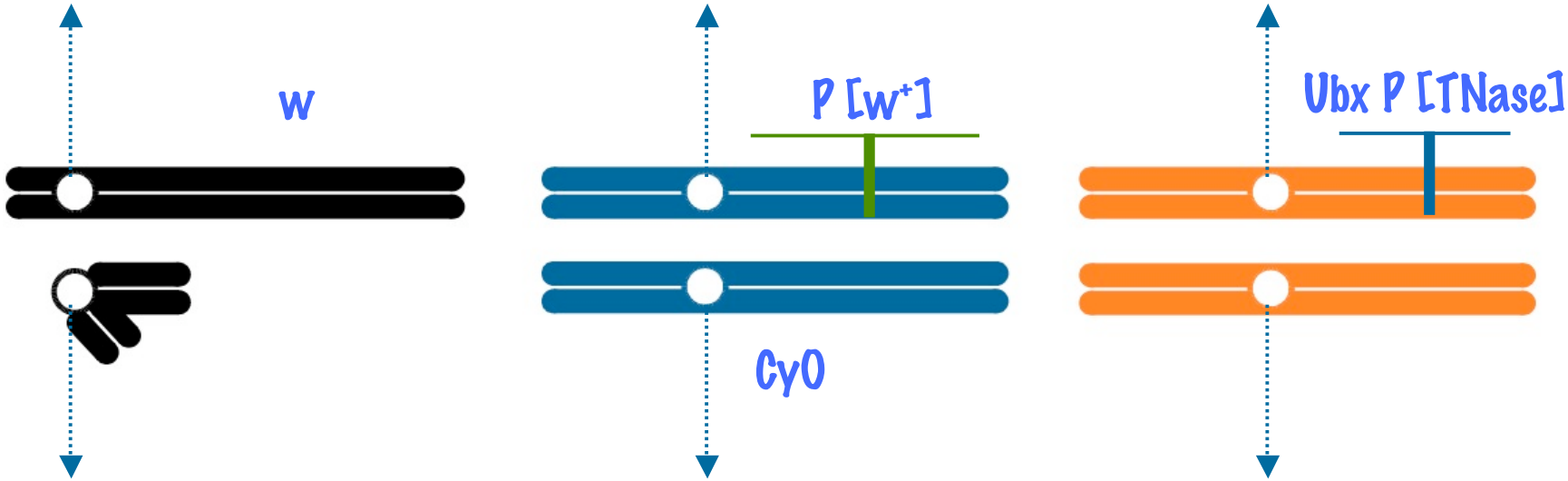
a zygote with a mutation!

sperm with...

What genes?

How to detect "hops"?

How do we detect hops?



Mate the males to white-eyed females…

X ♀ $\frac{w}{w}$

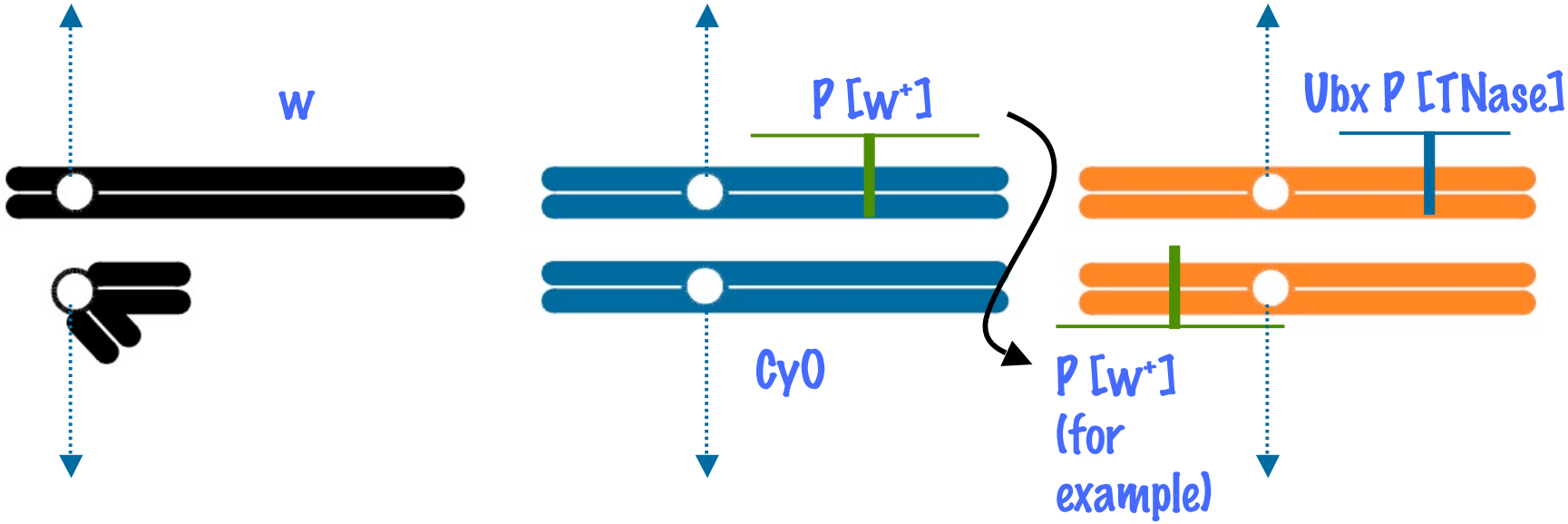
If NO hops:

CROSS 2

w^+ and CyO segregate away from each other…

…all curly-wing progeny have white eyes

How do we detect hops?

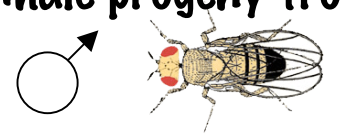


Mate the males to white-eyed females…

If curly wing progeny flies have red eyes…

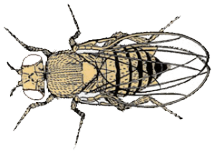
there must have been a hop!

male progeny from cross 1

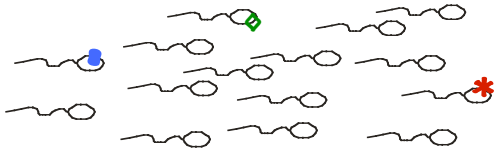


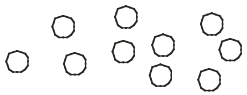
mate him to
a female

white eyes



??





sperm with...

CyO

P[w+]

X or Y

...but not Ubx

a zygote with a mutation!

CROSS 1: to bring the transposase into the same flies as the transposon that will create the mutations

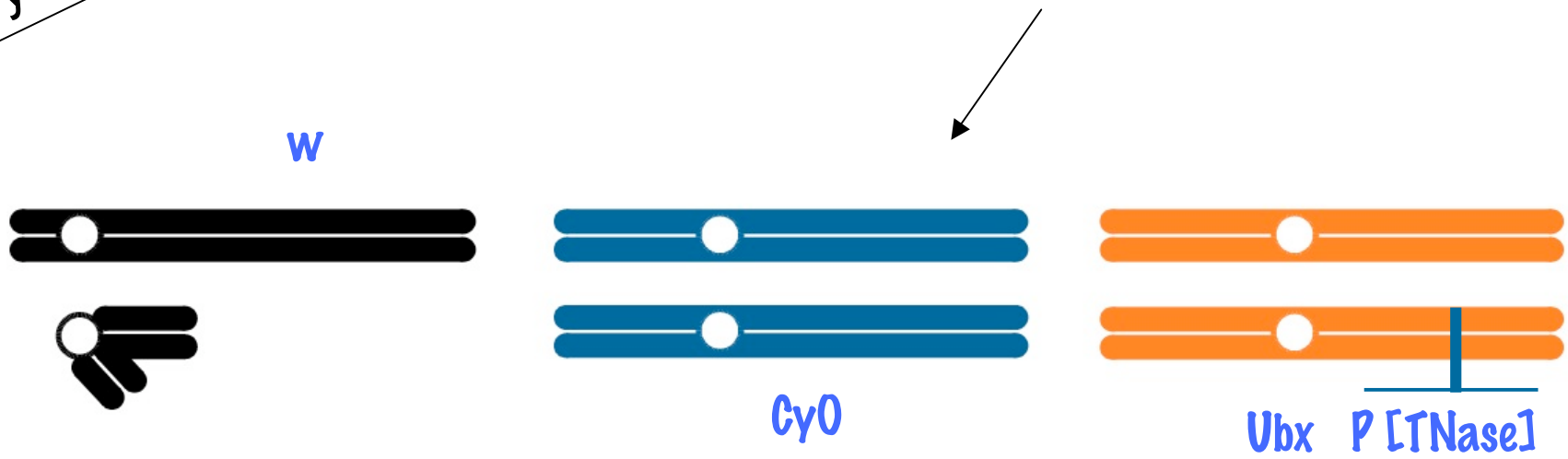
CROSS 2: to identify flies in which a mutation due to hopping of the transposon has occurred

If you are confused about how we can identify jumps…

tear off the top half of the next page, do the exercise that's on it.

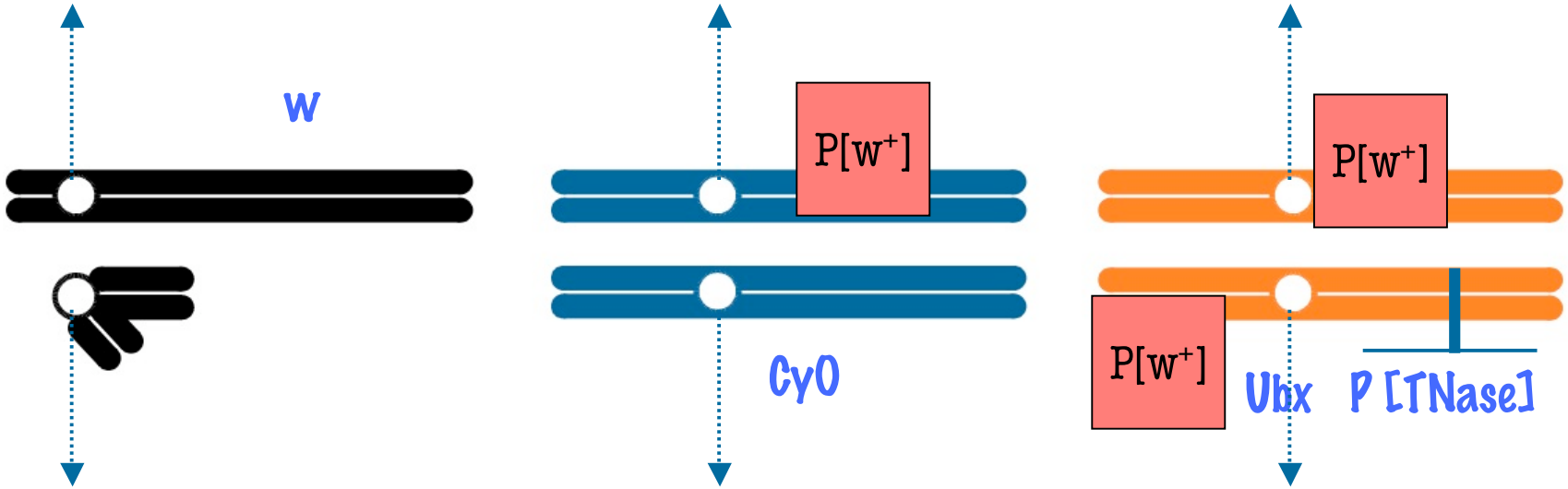
Try this at home

1. Put a Postit note (=P[w+]) on this homologue



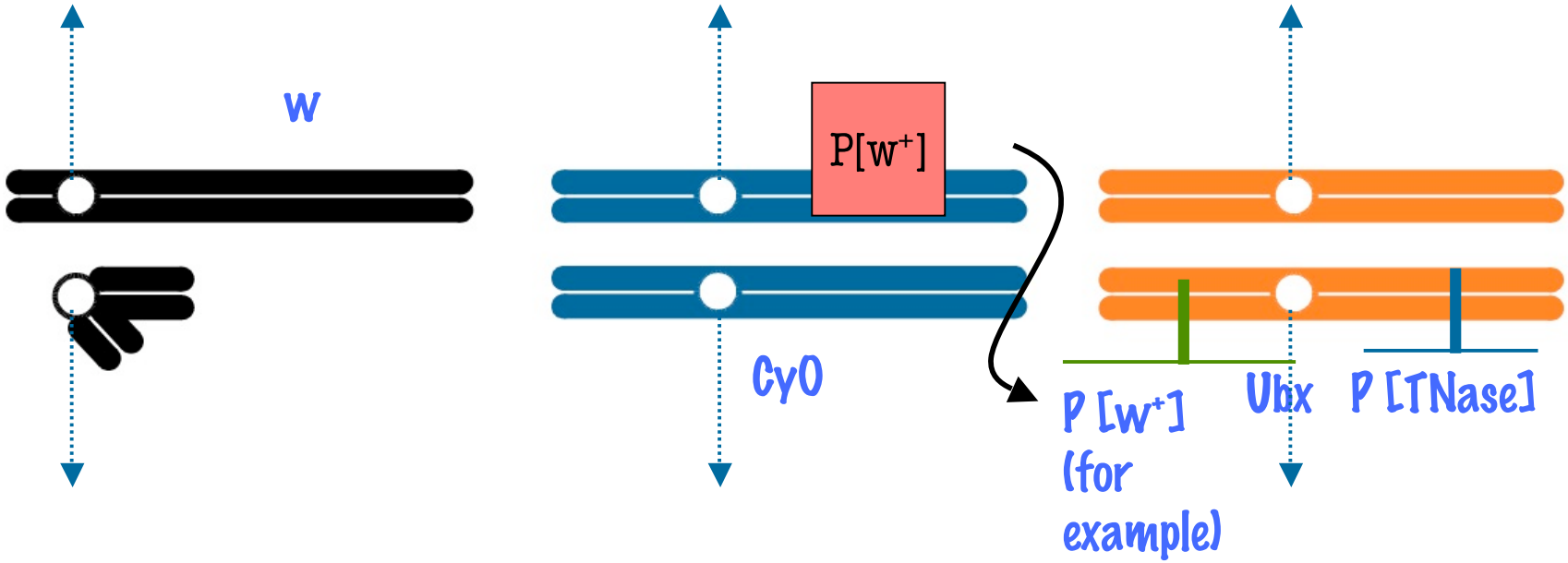
2. Do Anaphase I (cut the paper between the homologues) WITHOUT allowing P[w+] to jump
3. Is there a marker with which w+ will never co-segregate?
4. Now repeat 2 & 3 after allowing the transposon to jump (e.g., to chromosome III)... see a difference?

1. Now allow P[w+] to jump anywhere in the genome



2. Do Anaphase I

3. What segregation pattern would identify a definite "hop"?

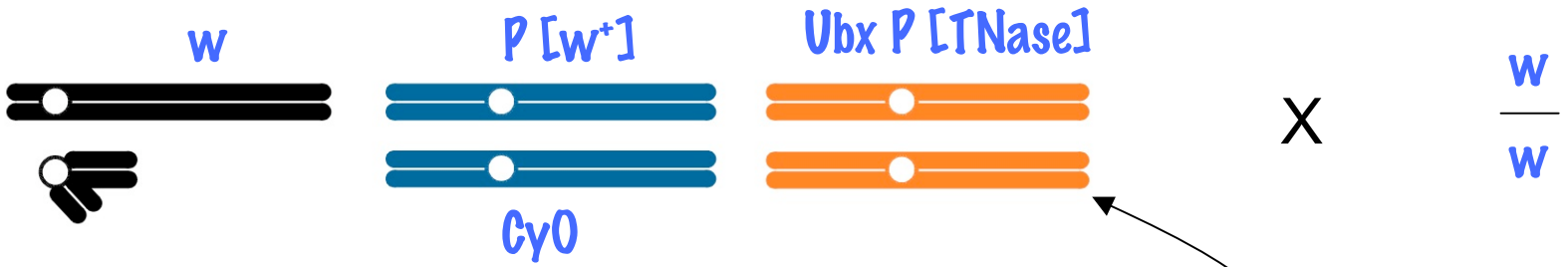


Why was it important to pick males?

Why was Curly important?

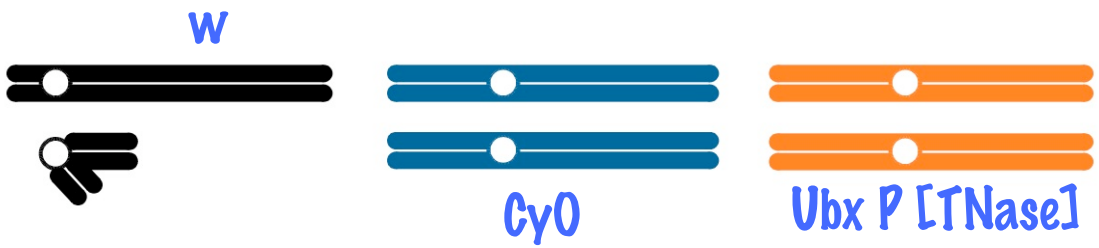
Will the homologue pairs necessarily line up in this orientation?

Homework — do before this week's quiz section



- What are the possible progeny if there was NO hop?
- What are the possible progeny if there was a hop to chromosome III?

A system of notation…



$$\frac{X^{w}}{Y} \; ; \; \frac{+}{CyO} \; ; \; \frac{+}{Ubx\ P[TNase]}$$

homologue pairs

separate (non-homologous) chromosomes

Something to think about...

Try to articulate the strategy for finding hops in general terms (i.e., without specifying Curly, etc.).

Where did the P-element land when it hopped?

In the quiz section:

You will pick a mutant with a hop…

which chromosome had the P element hopped into?

Why is this important?

…we'll revisit this question

Back to our QS cross: "Big picture" reality check

Why would one mess around with this transposon, anyway?

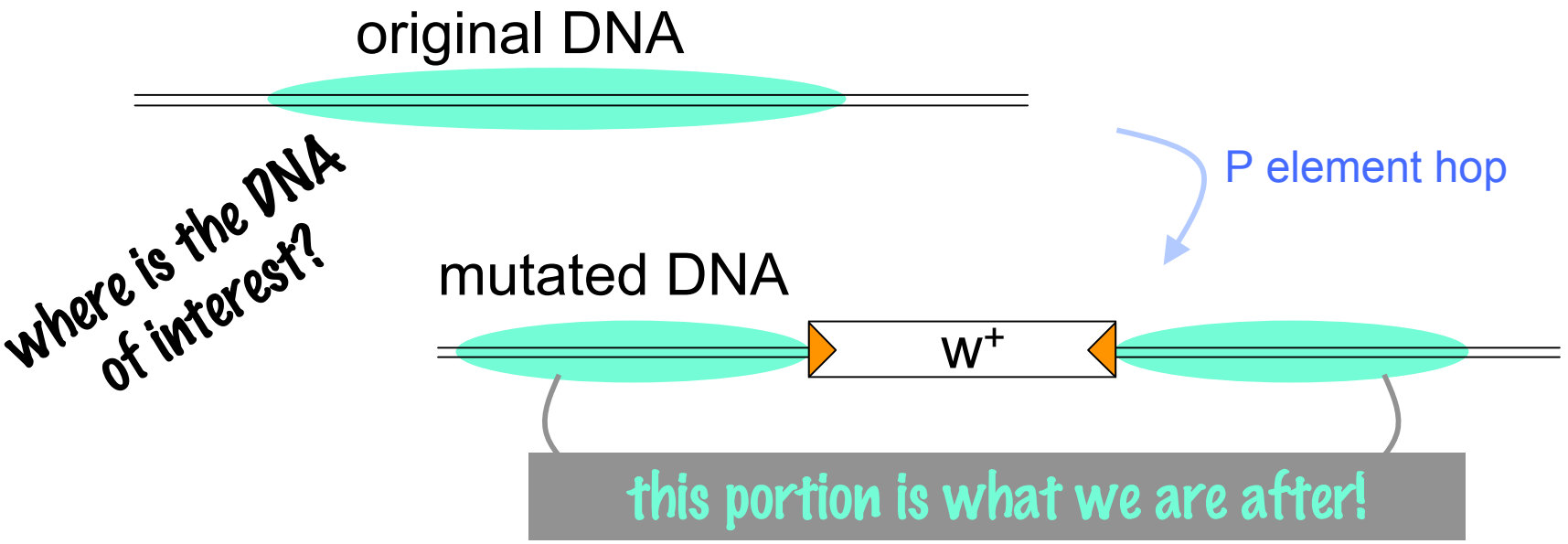
In the cross (= CROSS 3) you set up this week... what progeny phenotype would you want to pick for further analysis? Why?

white-eyed female x red-eye, curly-wing male

...because those flies have a hopped transposon

...and therefore may have an interesting mutation where the transposon landed

“Big picture” reality check (cont’d)



- Curly, TNase, Ubx, etc. let us deliver the mutagenic “blow” and identify when the mutagenic event has happened
- P[w+] causes the mutation; the w+ portion lets us track where the mutation is (which fly has it)

CROSS 1: to bring the transposase into the same flies as the transposon that will create the mutations

CROSS 2: to identify flies in which a mutation due to hopping of the transposon has occurred

CROSS 3: to identify the chromosome that the mutation is on

and to make a stock strain carrying the mutation

Where did the P-element land when it hopped?

In the quiz section:

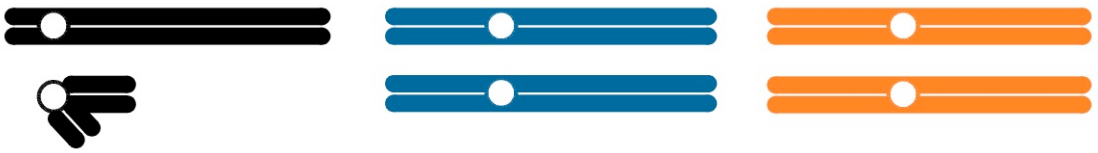
You will pick a mutant with a hop…

which chromosome had the P element hopped into?

Suppose your mutant had a hop into chromosome III

Mate these again to white-eyed females… CROSS 3

$$\frac{X^w}{X^w};\frac{+}{+};\frac{+}{+} \quad x$$



pick red-eye progeny (male or female)

Genotype?

_____ ____ _____

…and suppose they don't show any abnormal phenotype!

Where's the phenotype?

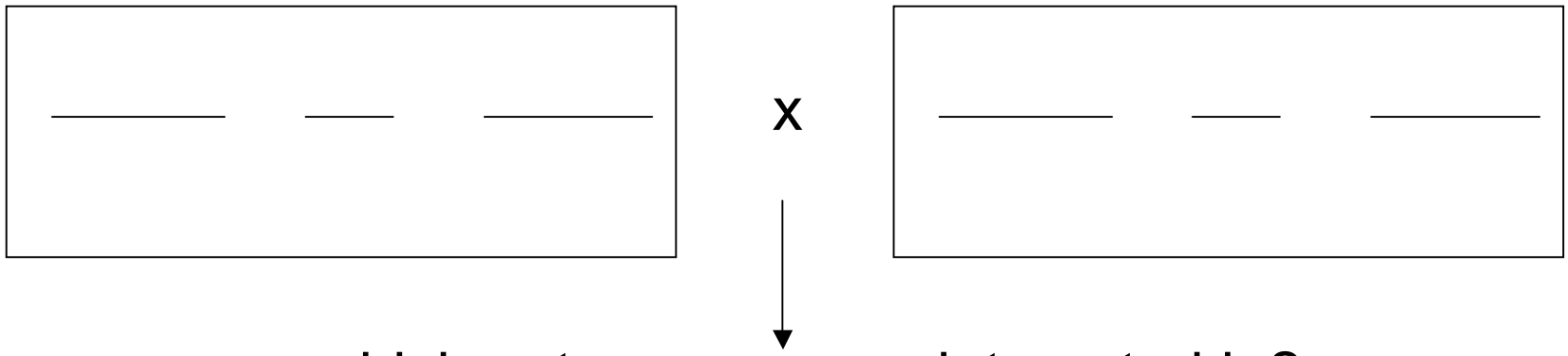
Genotype—same as previous slide

_____ ____ _____

We *know* it has a "hopped" transposon…

so why might it not show any abnormal phenotype?

Looking for a phenotype



Looking for a phenotype (cont'd)

The problem:

How do we identify homozygotes? What would they look like?

Making the mutant homozygous

The problem:

How do we identify homozygotes? What would they look like?

Can we say for sure that

$$\frac{X^w}{X^w \text{ or } Y} ; \frac{+}{+} ; \frac{+}{P[w^+]}$$

will look any different from

$$\frac{X^w}{X^w \text{ or } Y} ; \frac{+}{+} ; \frac{P[w^+]}{P[w^+]}$$

Making the mutant homozygous

Drosophila geneticists have established strains with special chromosomes that allow the construction of homozygous mutants

You need to know the chromosome that the mutation is on in order to use the correct strain