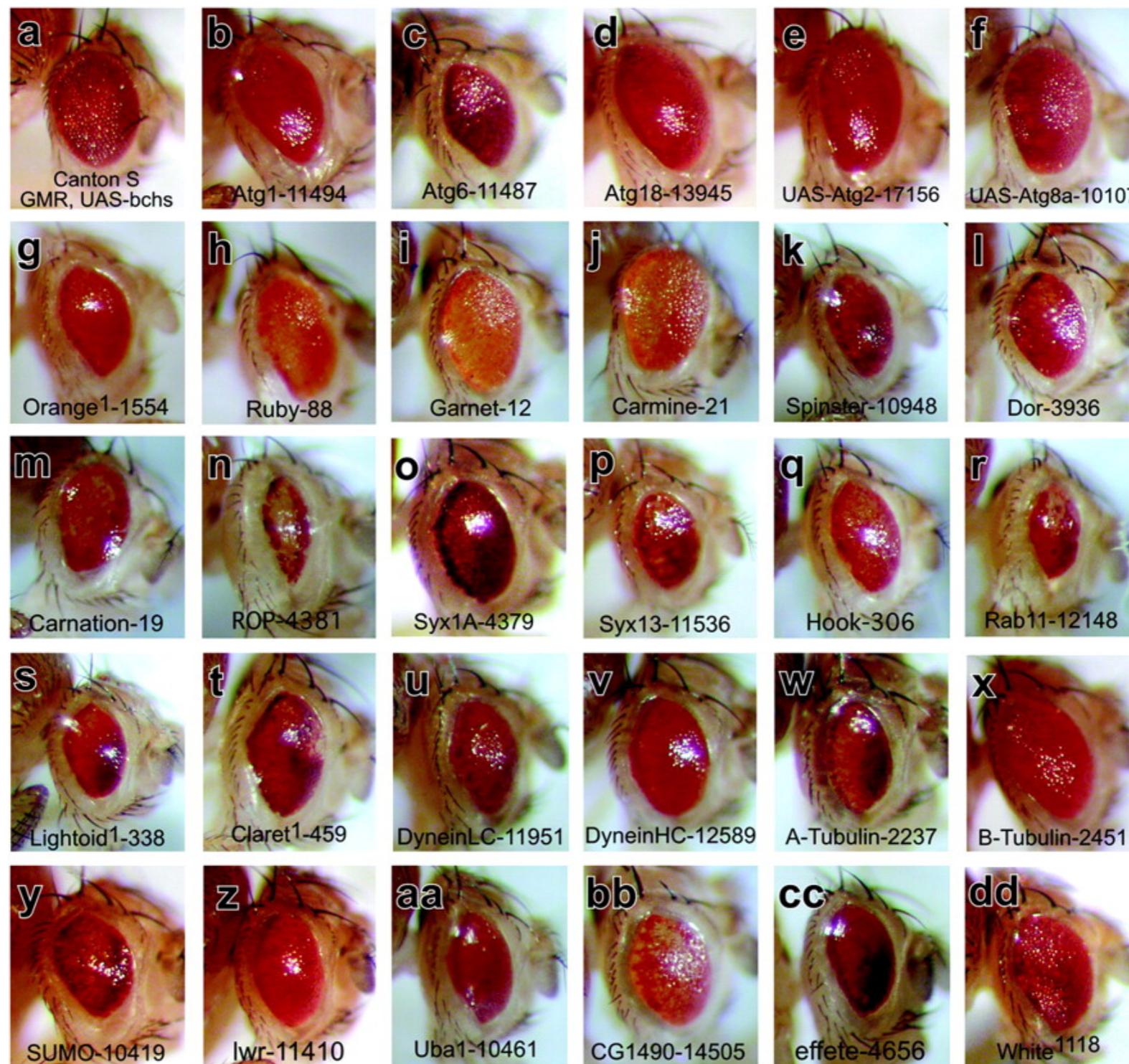
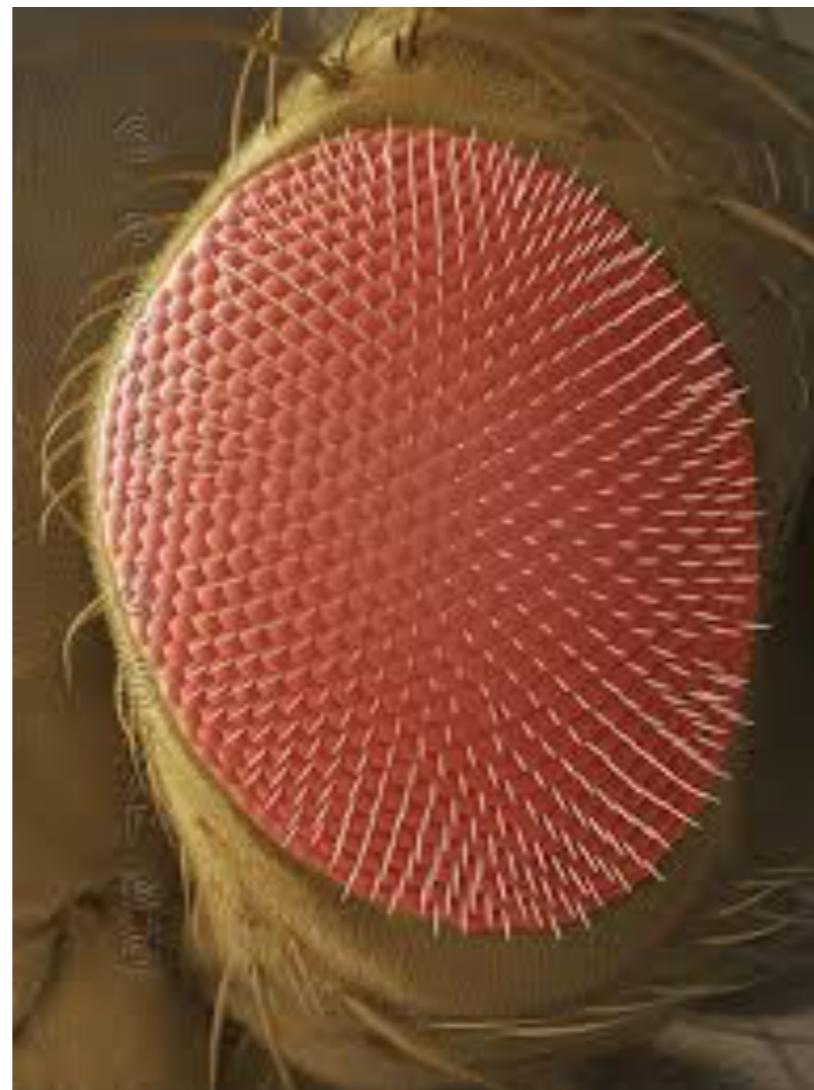


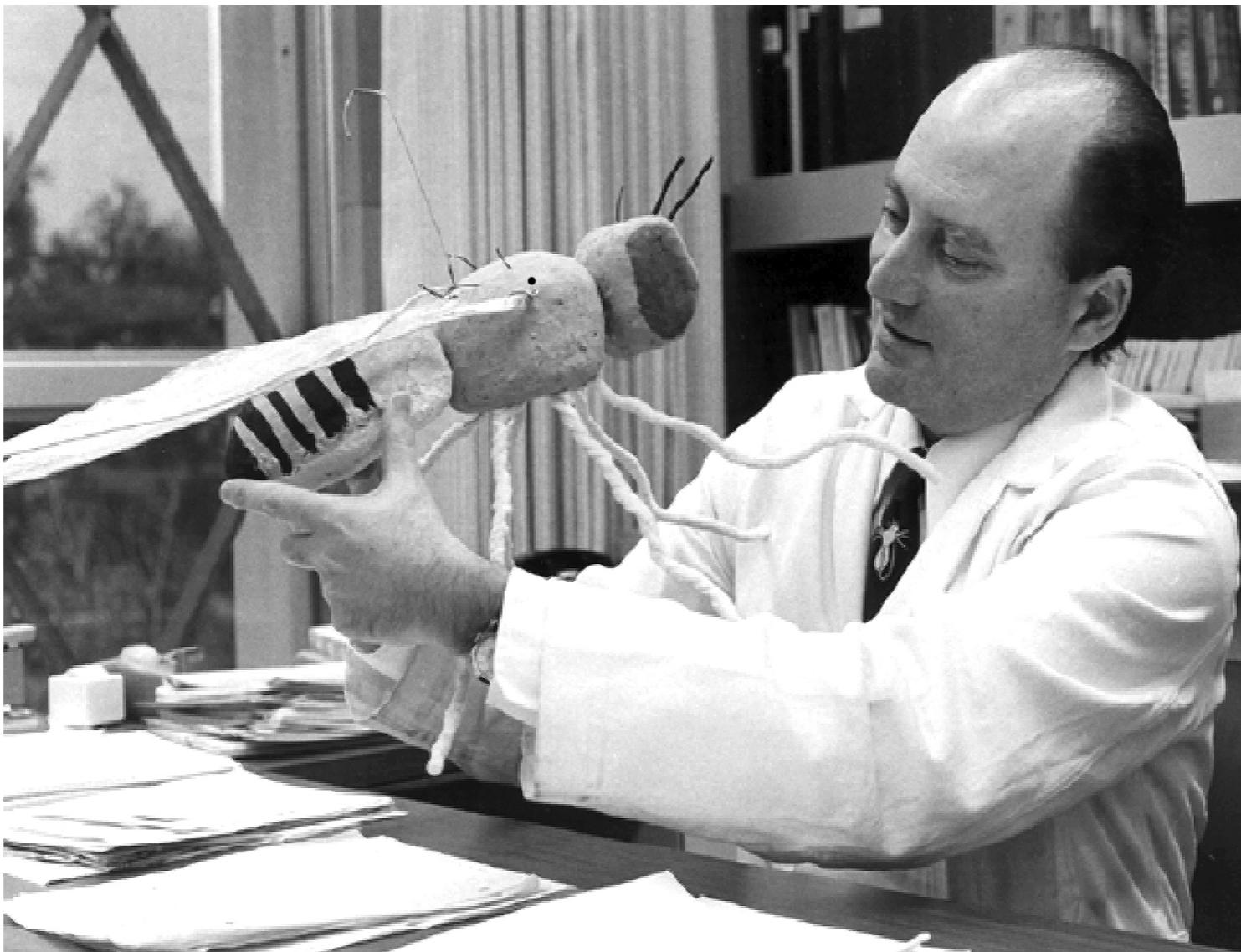
# Bio393: Genetic Analysis

## Developmental genetics II

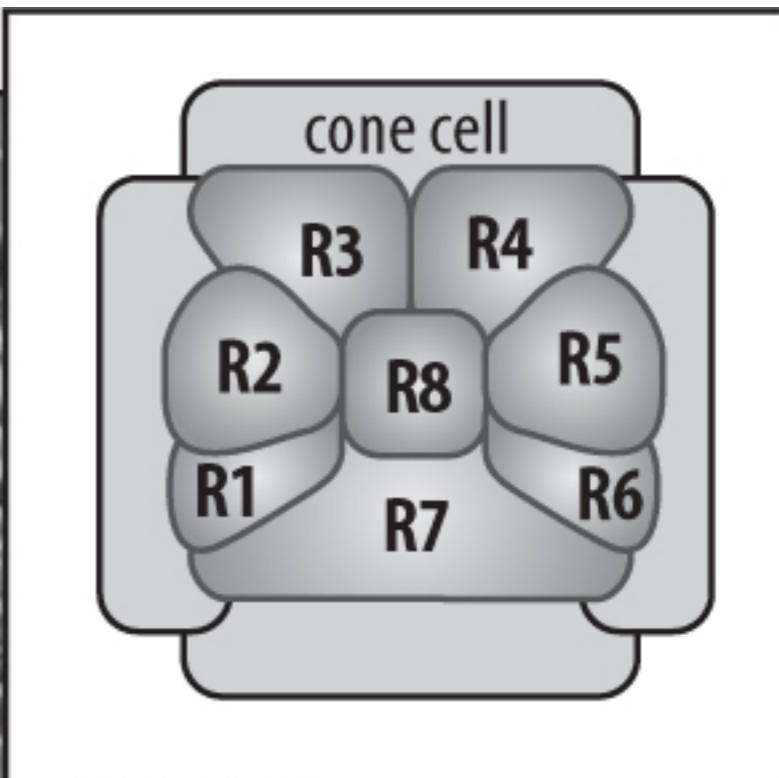
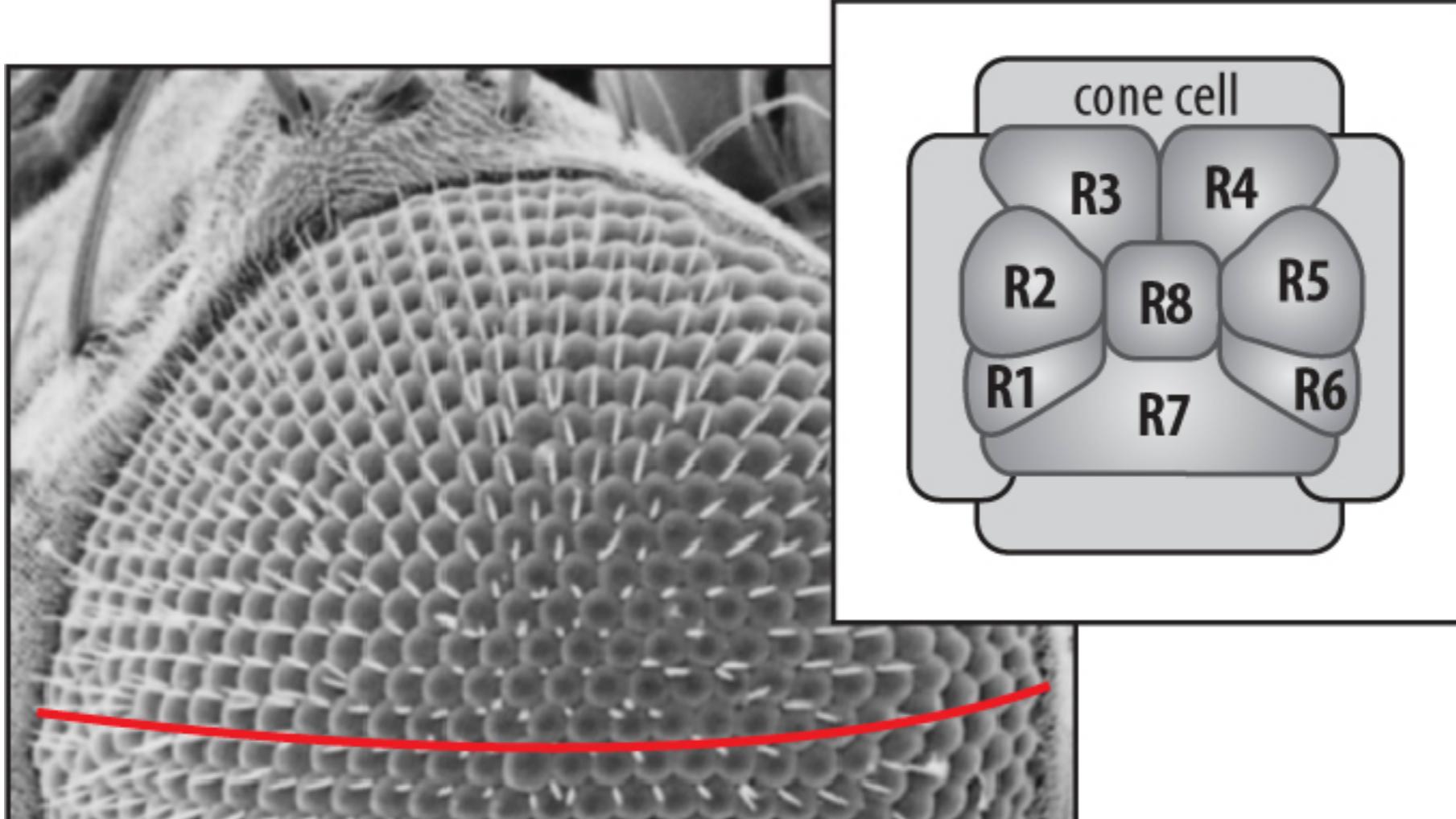
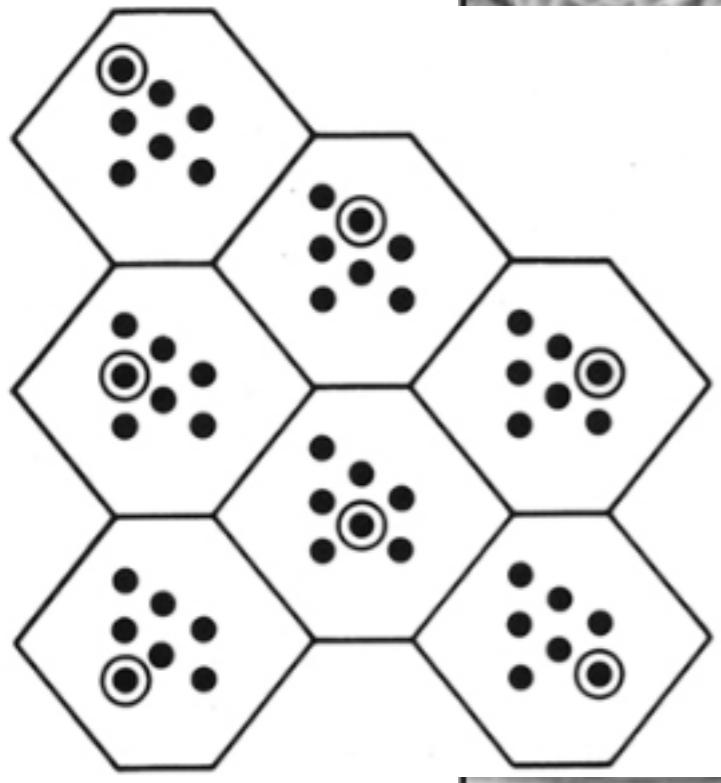




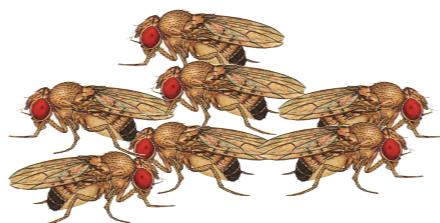
The *Drosophila* eye



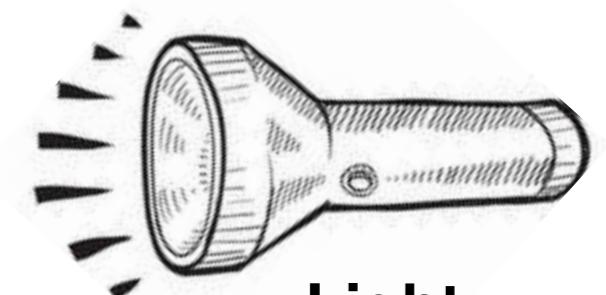
# Seymour Benzer



# A simple behavioral selection

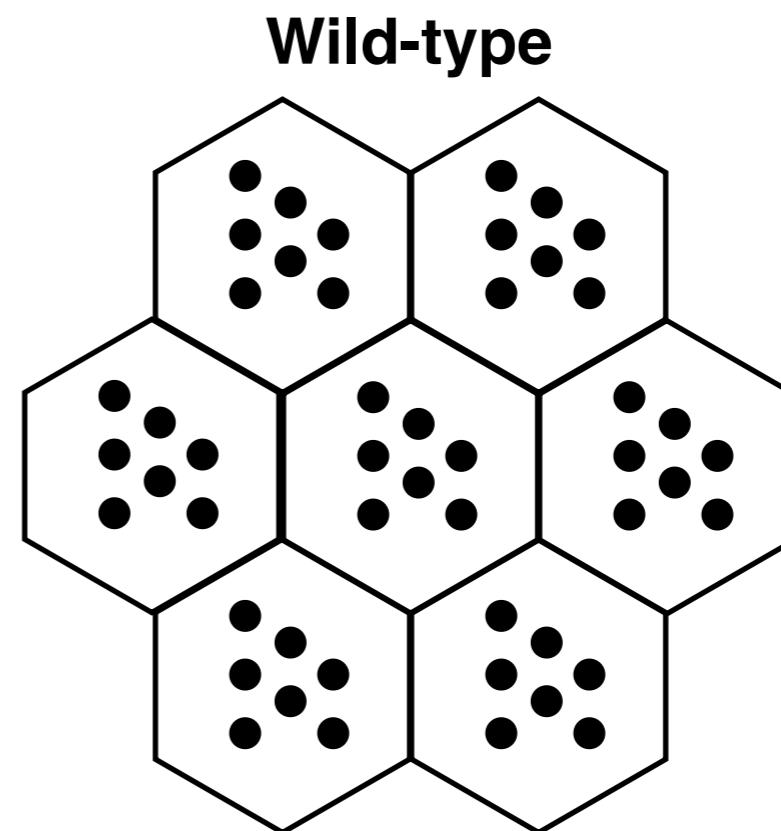
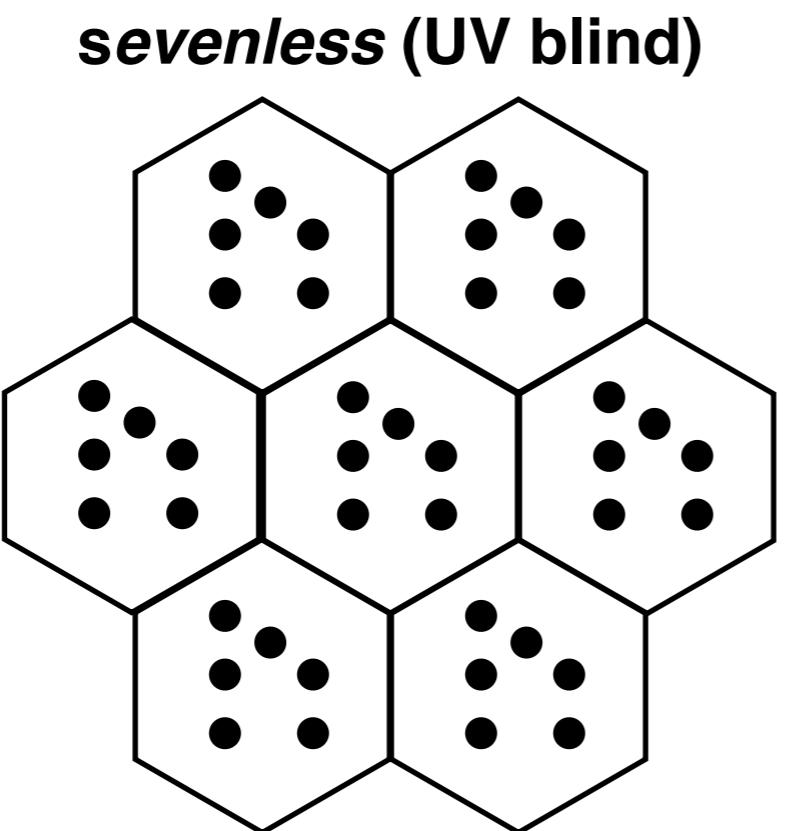
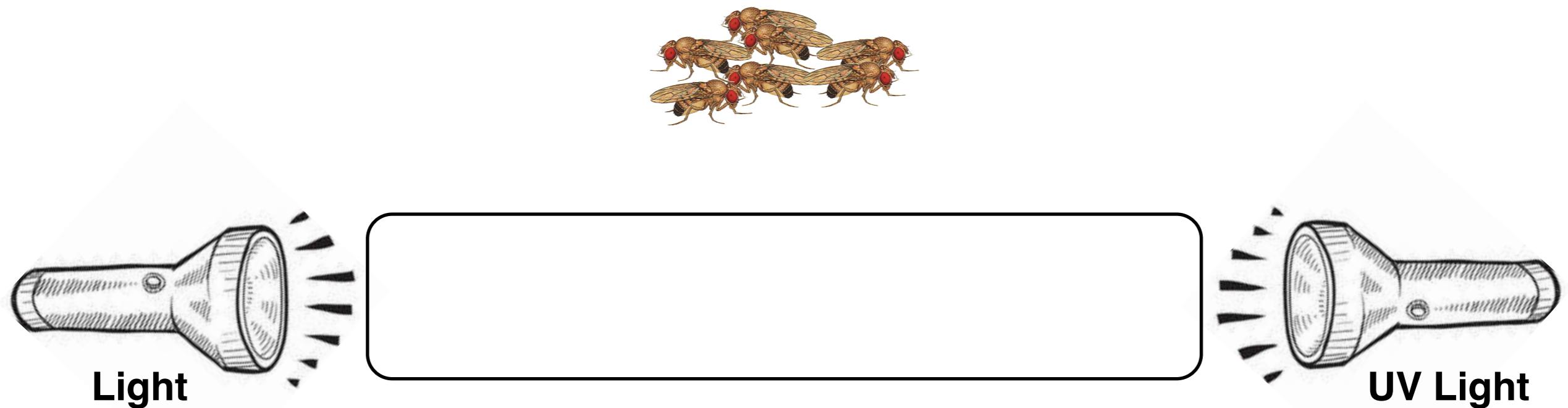


Dark



Light

# A simple behavioral selection

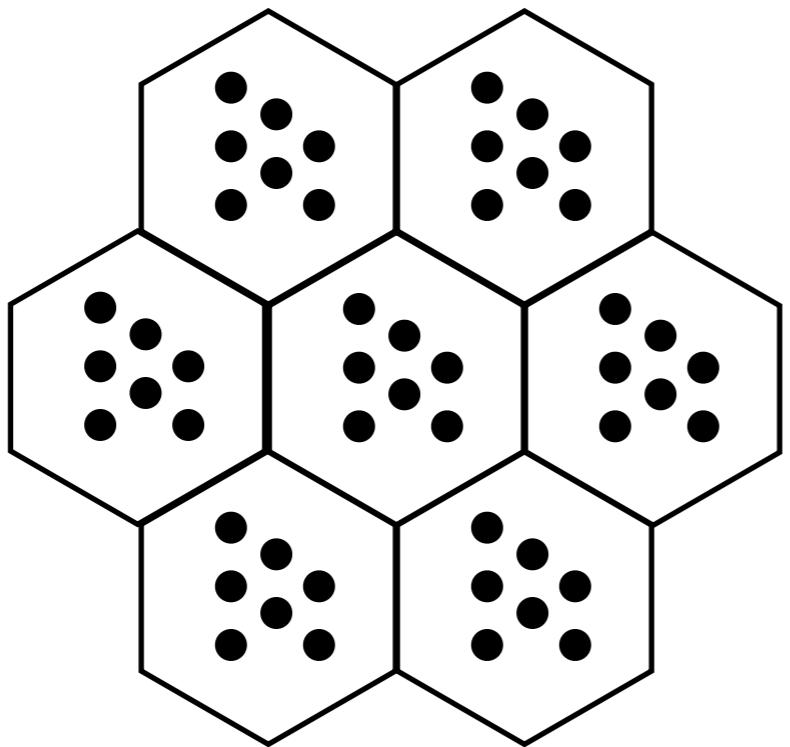




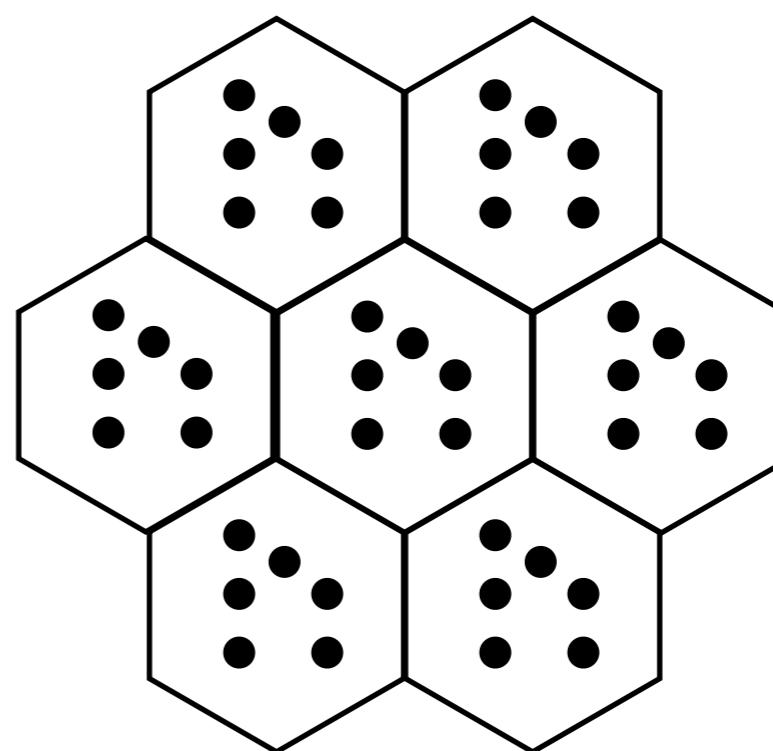
**Gerry Rubin**

# Other UV blind mutants

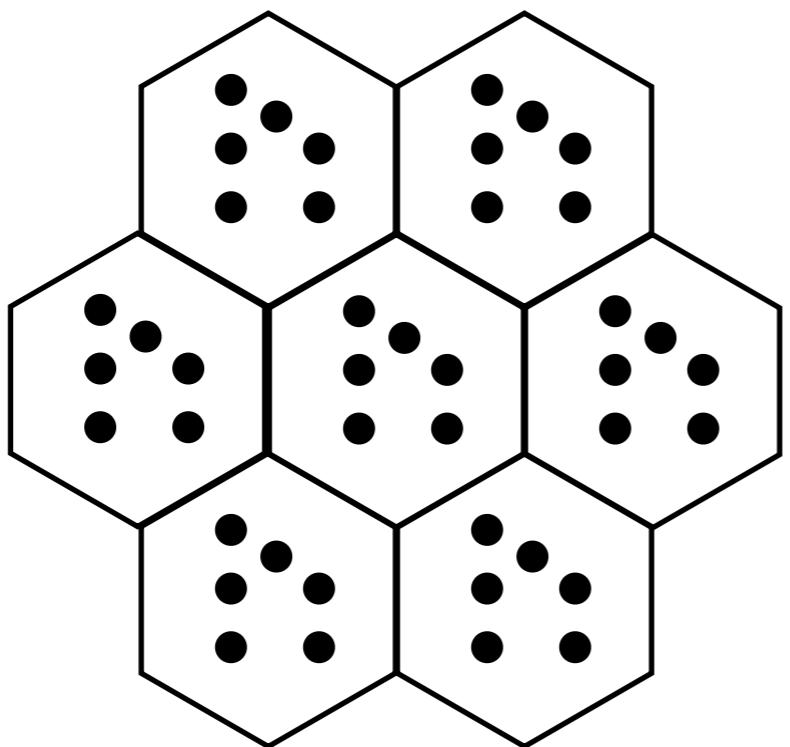
Wild-type



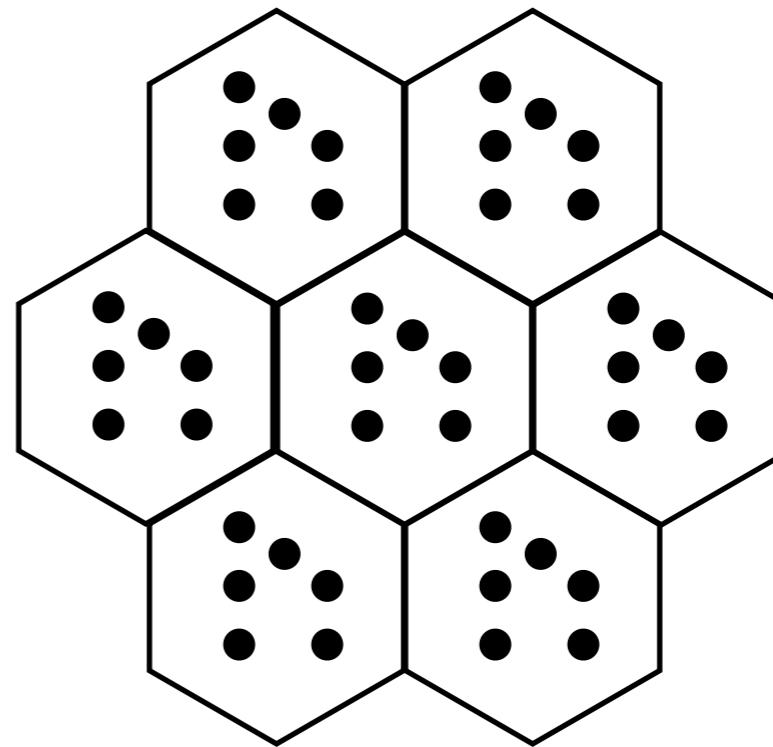
*sevenless* (UV blind)



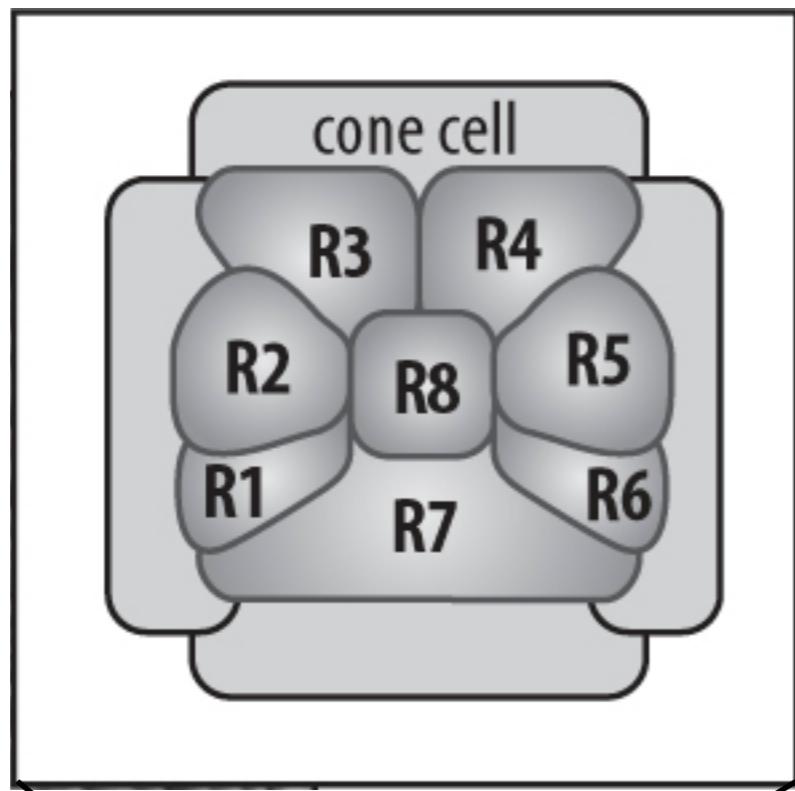
*bride of sevenless* (UV blind)



*seven-in-absentia* (UV blind)



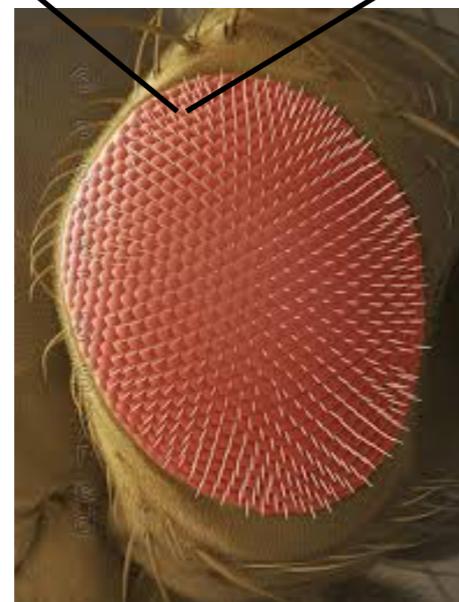
# Development of an ommatidium



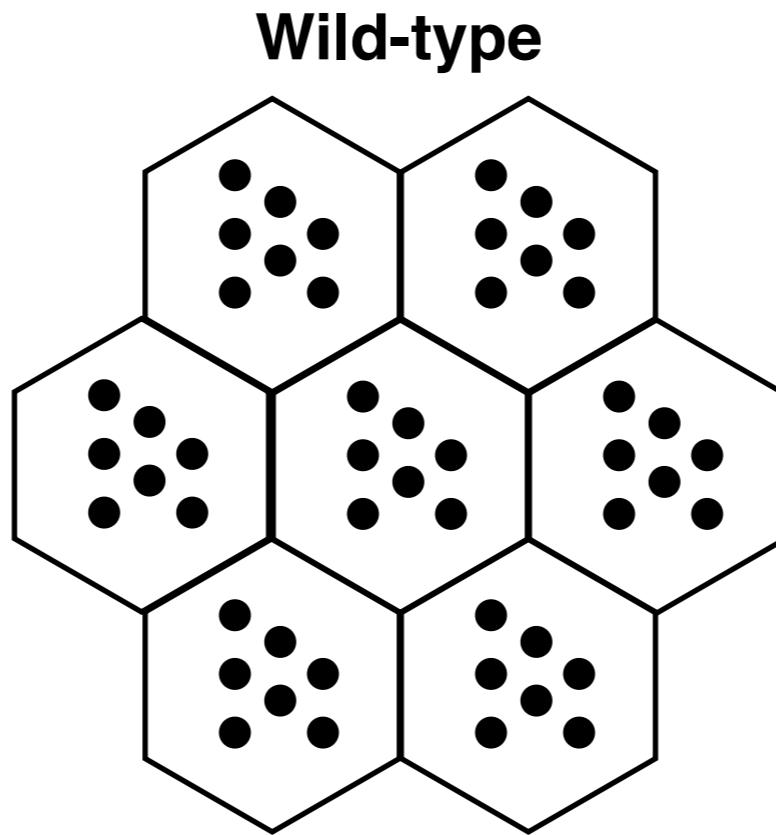
R8 born first

R1-R6 born after

R7 last to be born



# Is the function of *sev*, *sina*, or *boss* required in the R7 cell?



Function in R7 is considered cell autonomous

Function in any cell besides R7 is considered cell non-autonomous

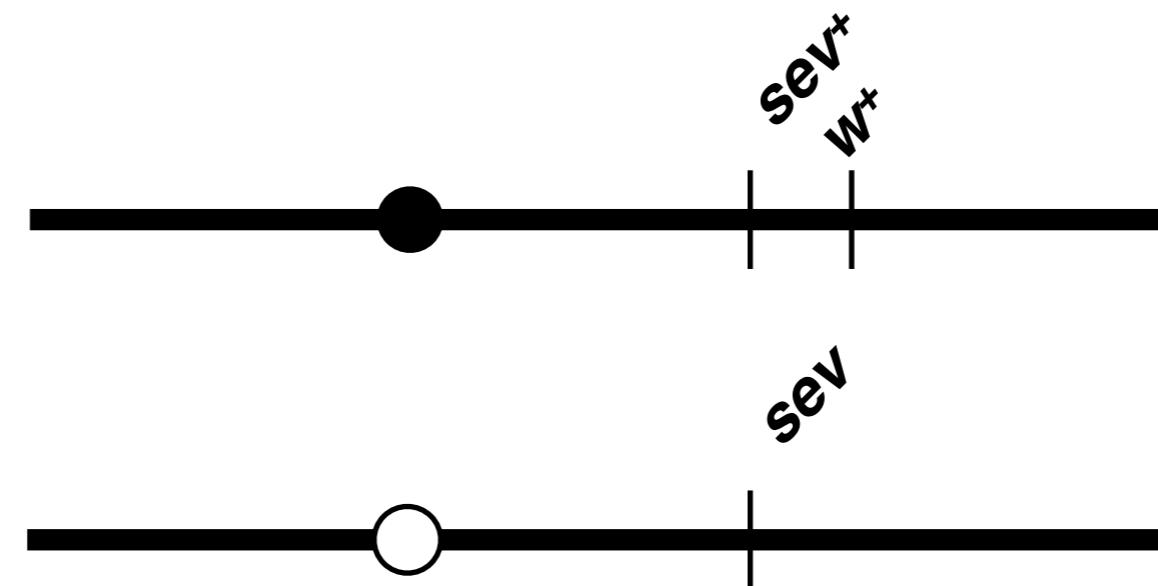
# Are genes required in ommatidia for cell viability?

We want to make flies that lack the *sev* gene in certain cells

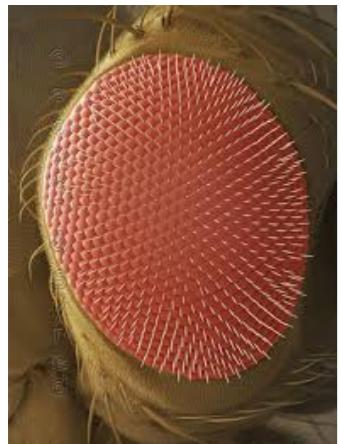


1.  $\frac{\underline{sev}}{+} ; \frac{w}{W}$

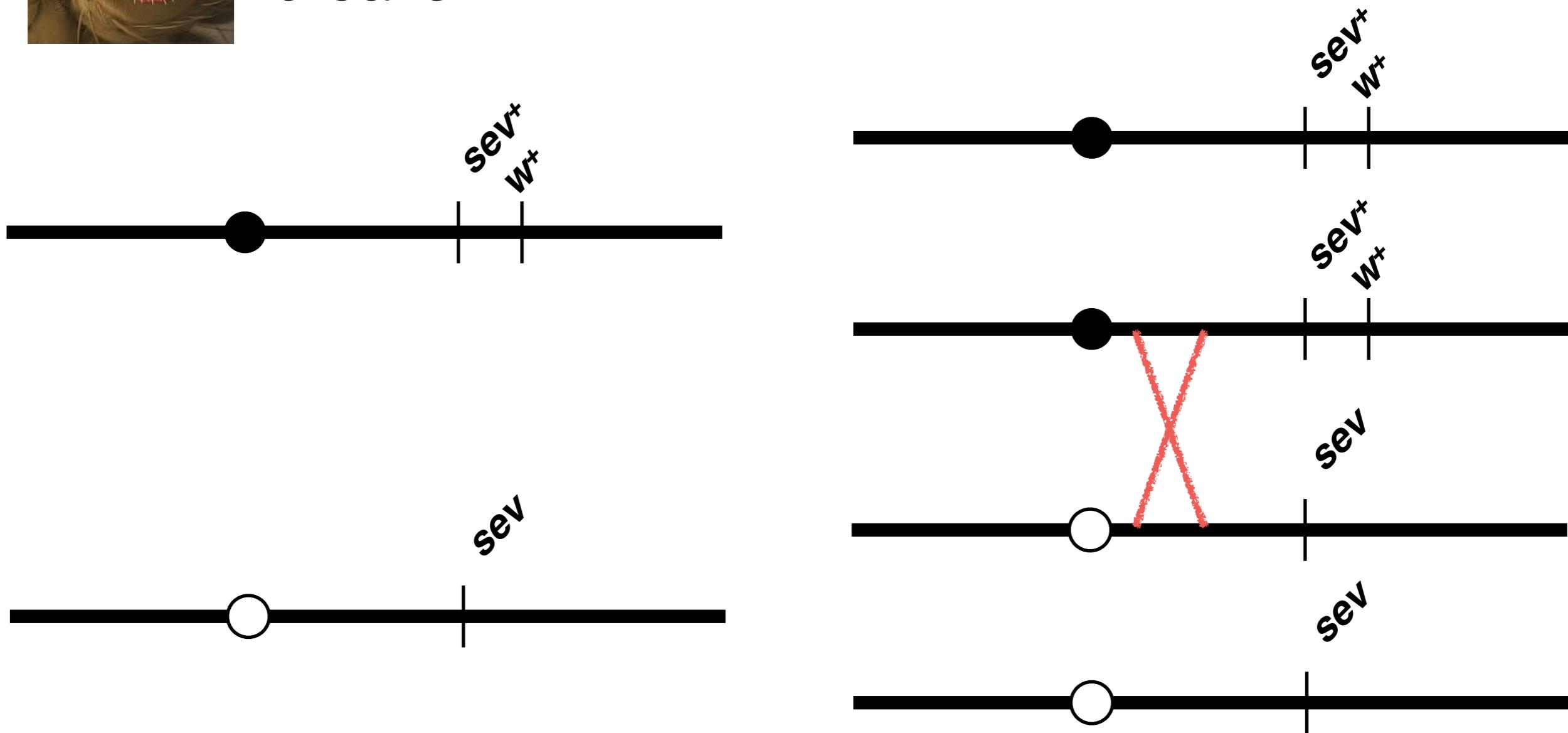
2. Use a P element with  $w^+$  distal  
to the wild-type *sev* gene



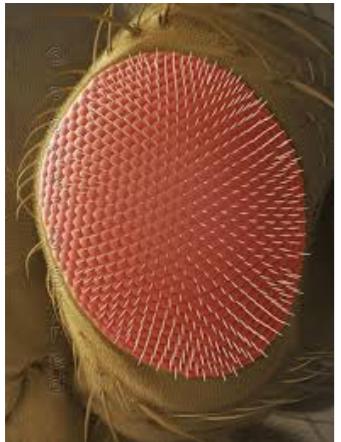
# Are genes required in ommatidia for cell viability?



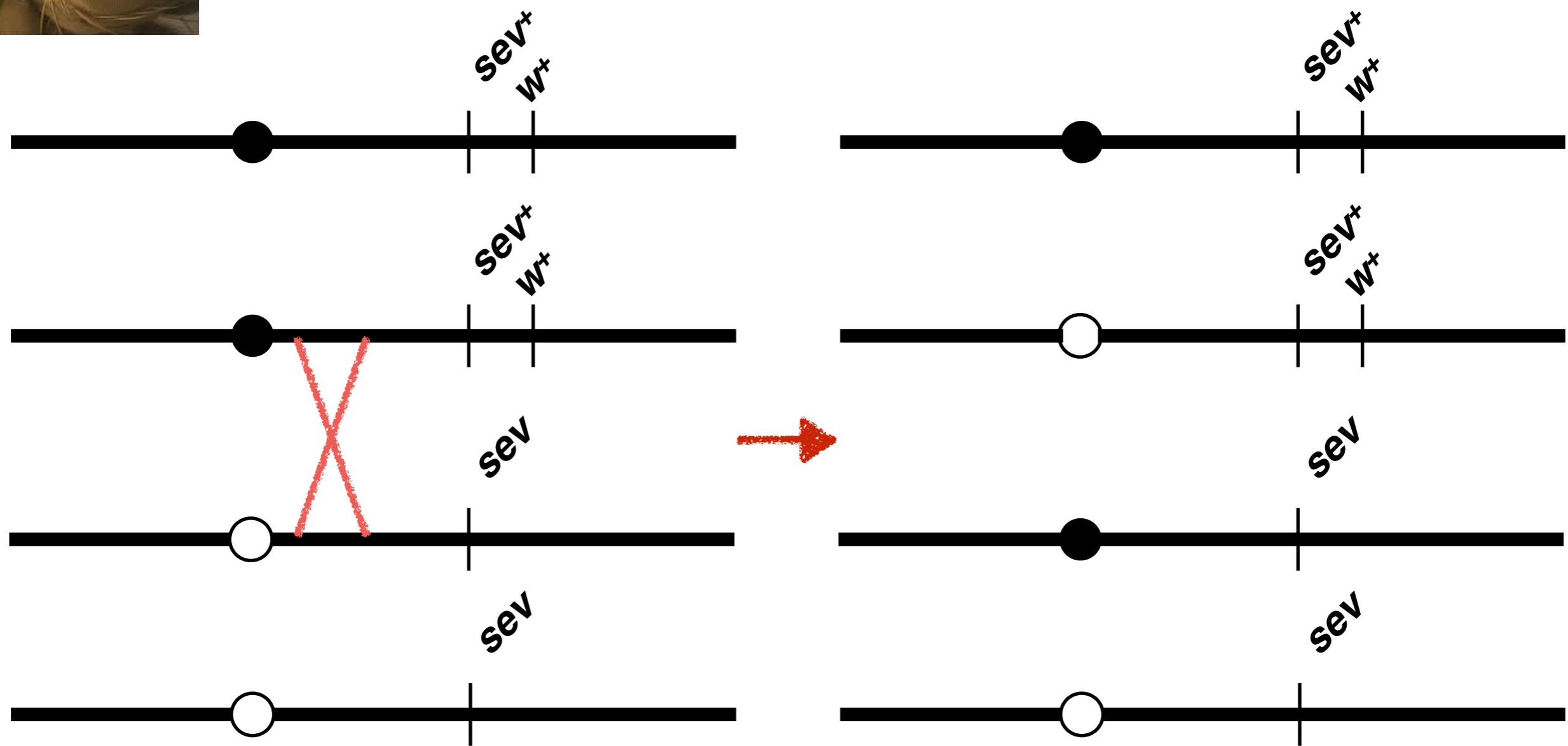
During mitosis for the cells that make up the eye, recombination can occur to repair double-strand breaks.



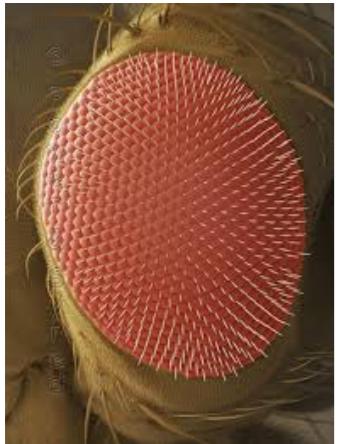
# Are genes required in ommatidia for cell viability?



During mitosis for the cells that make up the eye, recombination can occur to repair double-strand breaks.

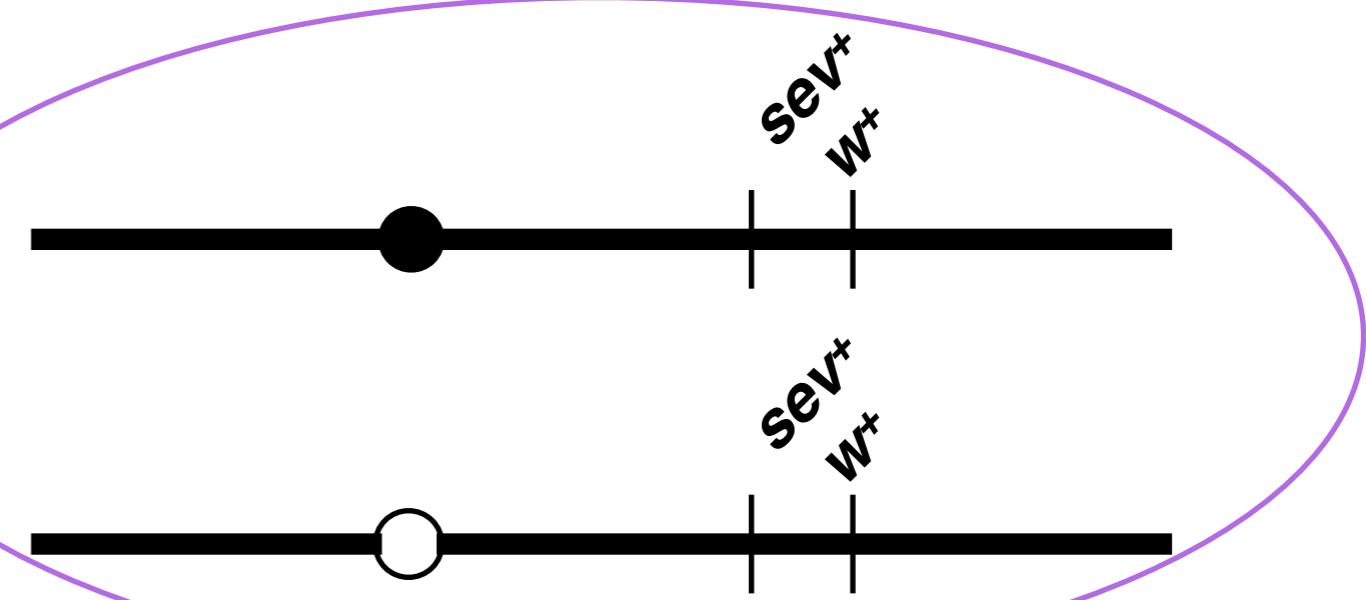


# Are genes required in ommatidia for cell viability?



During mitosis for the cells that make up the eye, recombination can occur to repair double-strand breaks.

*sev* WT and pigment



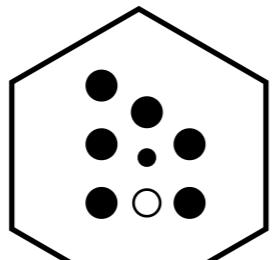
*sev*

*sev*

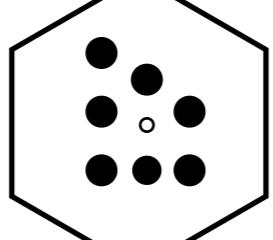
*sev* mutant  
and no pigment

**Twin spots!**

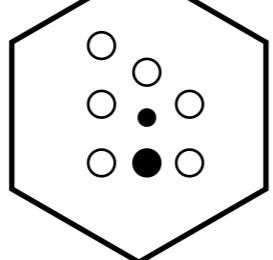
# ***sev* acts cell autonomously to regulate the R7 fate**



R7 cell *white* and *sev* mutant

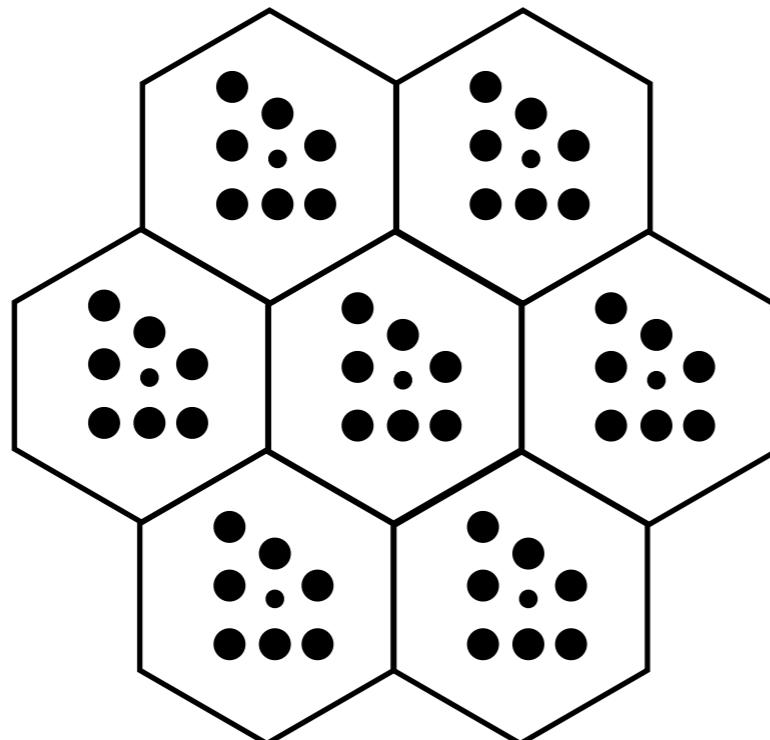


R8 cell *white* and *sev* mutant

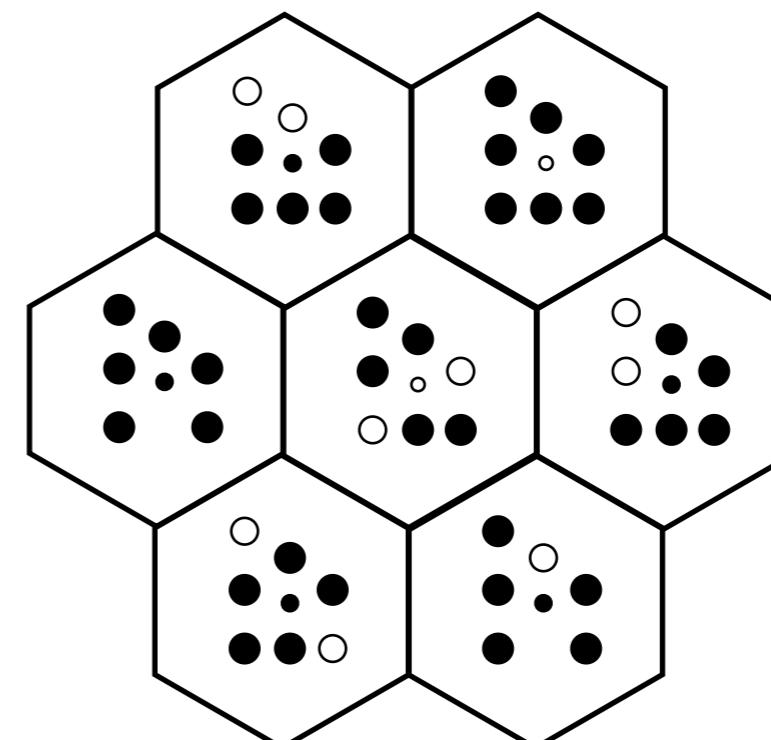


R1-R6 cells *white* and *sev* mutant

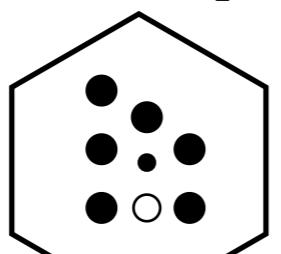
Wild-type



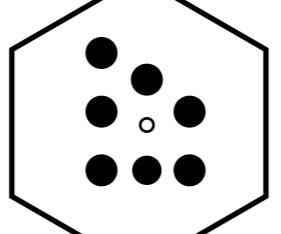
*sev* mutant



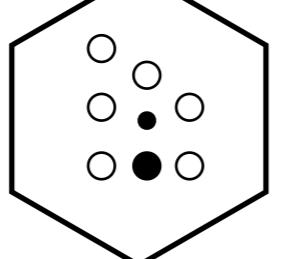
# ***boss* and *sev* are required in different R cells for R7 fate**



R7 cell *white* and *sev* mutant

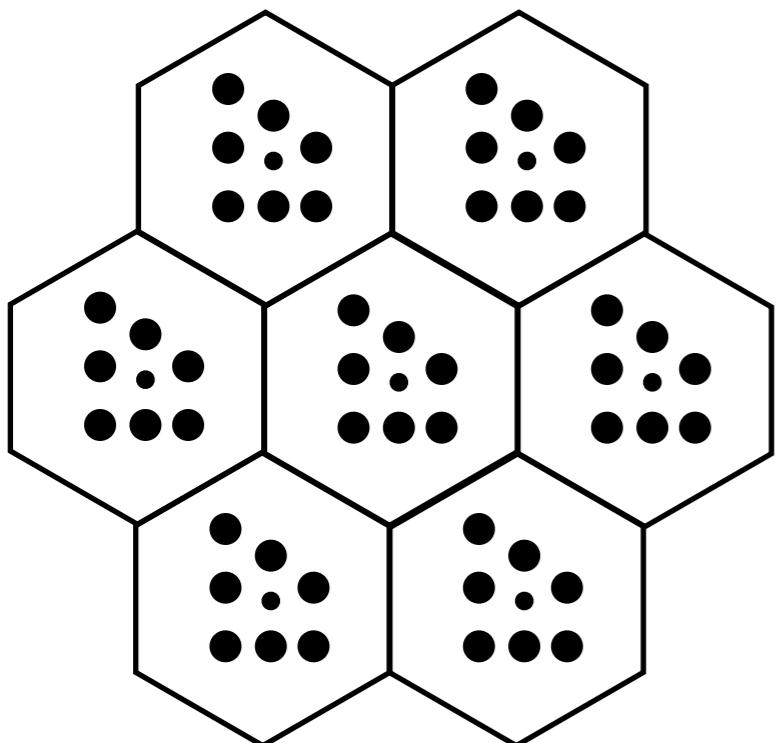


R8 cell *white* and *sev* mutant

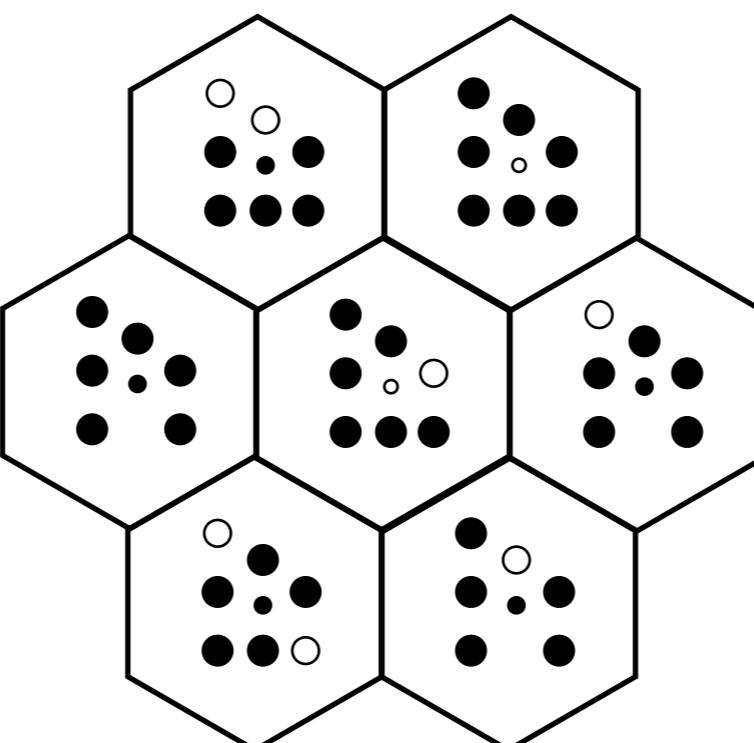


R1-R6 cells *white* and *sev* mutant

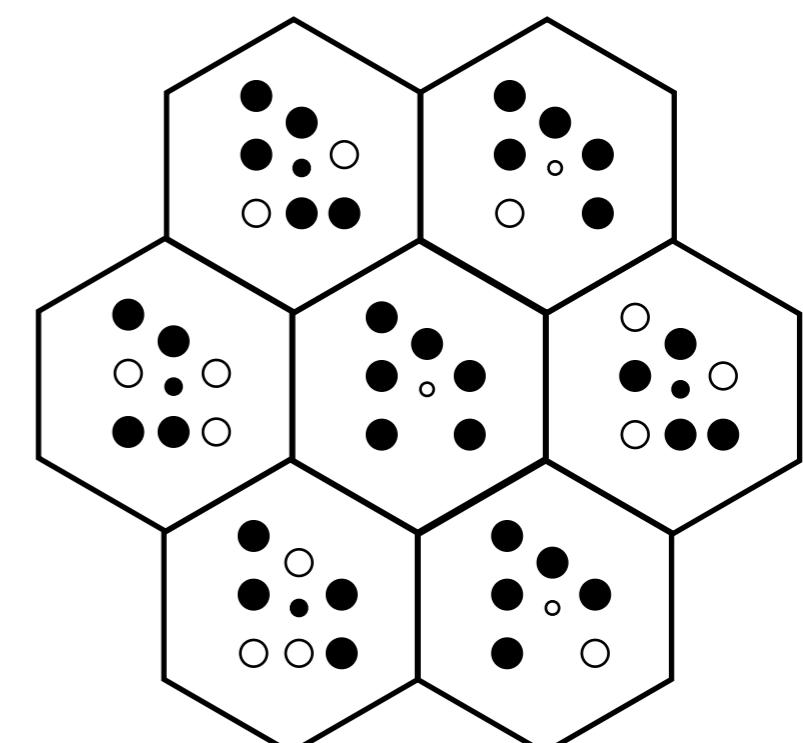
Wild-type



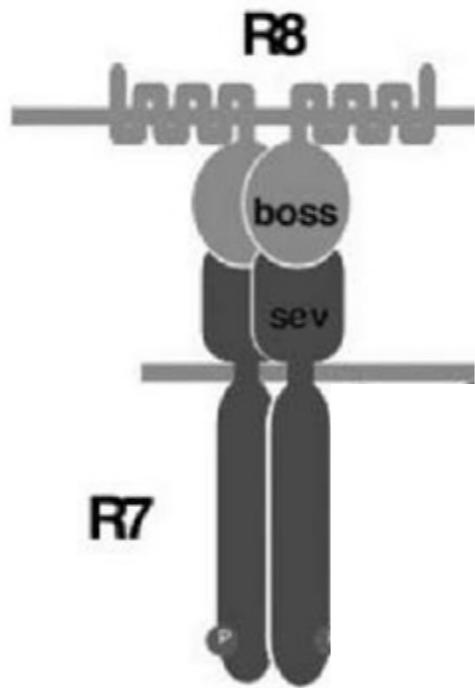
*sev* mutant



*boss* mutant

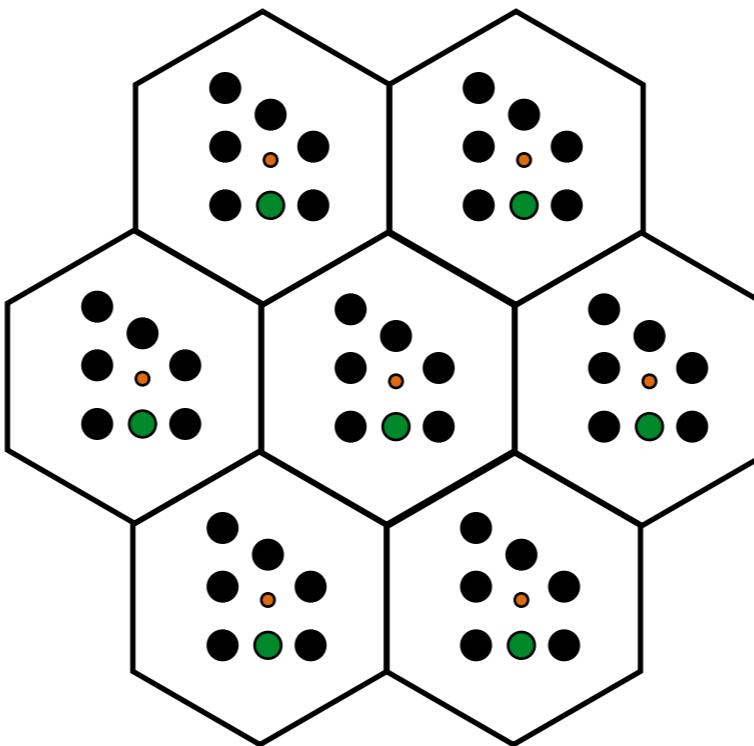


*boss* encodes  
a membrane-bound protein



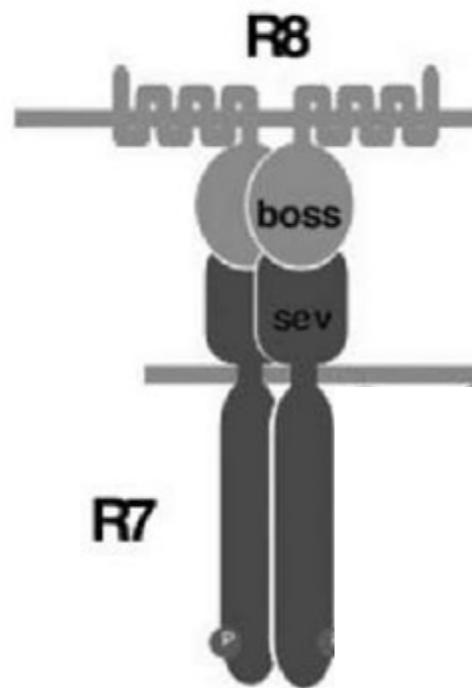
*sev* encodes  
a membrane receptor tyrosine kinase

# Expression of *sev* and *boss*



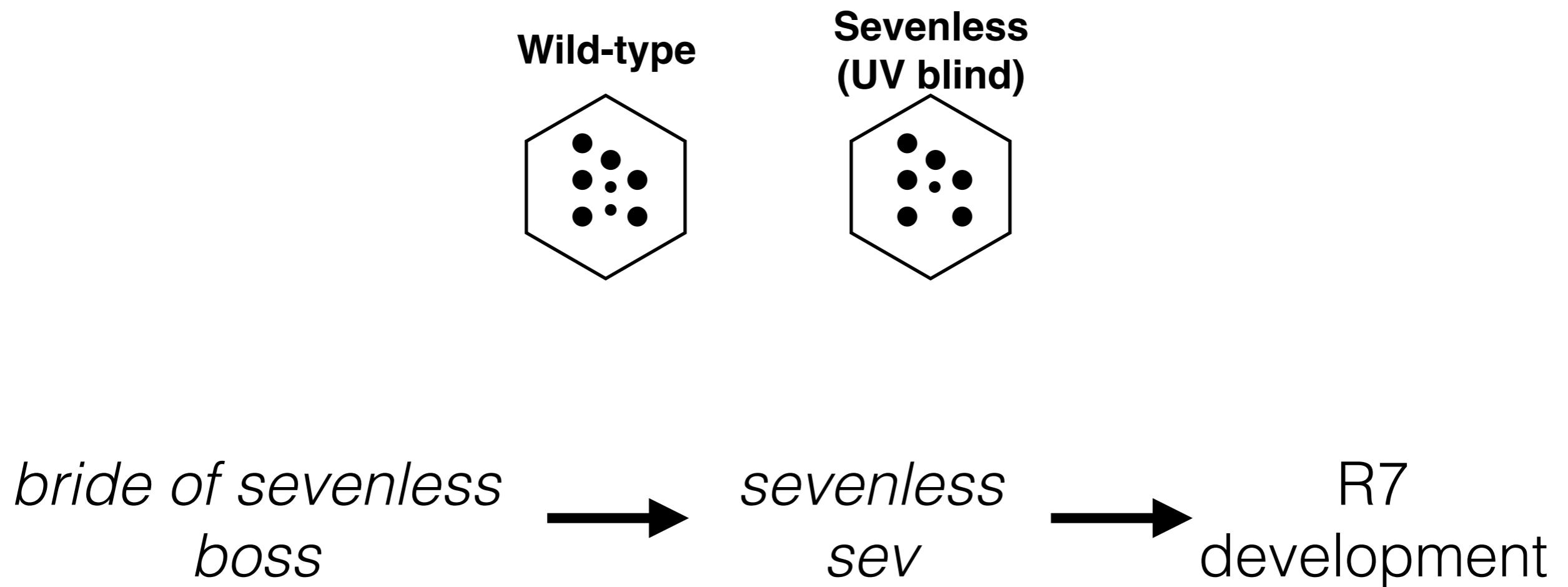
boss expressed in R8

sev expressed in R7



boss internalized into R7

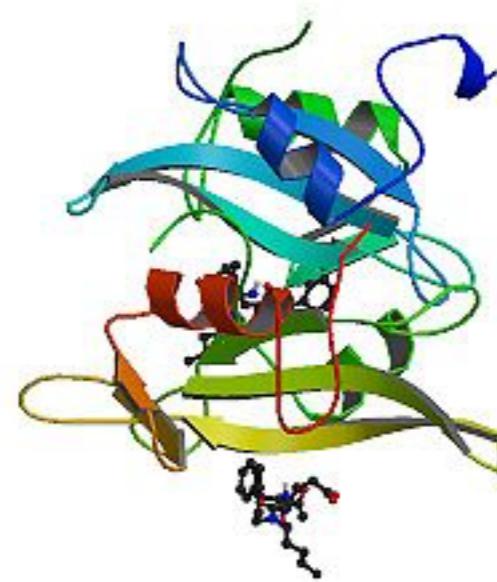
# UV blind mutants led to elements of signaling pathway



# Virus gene mutant from chicken to *Drosophila*



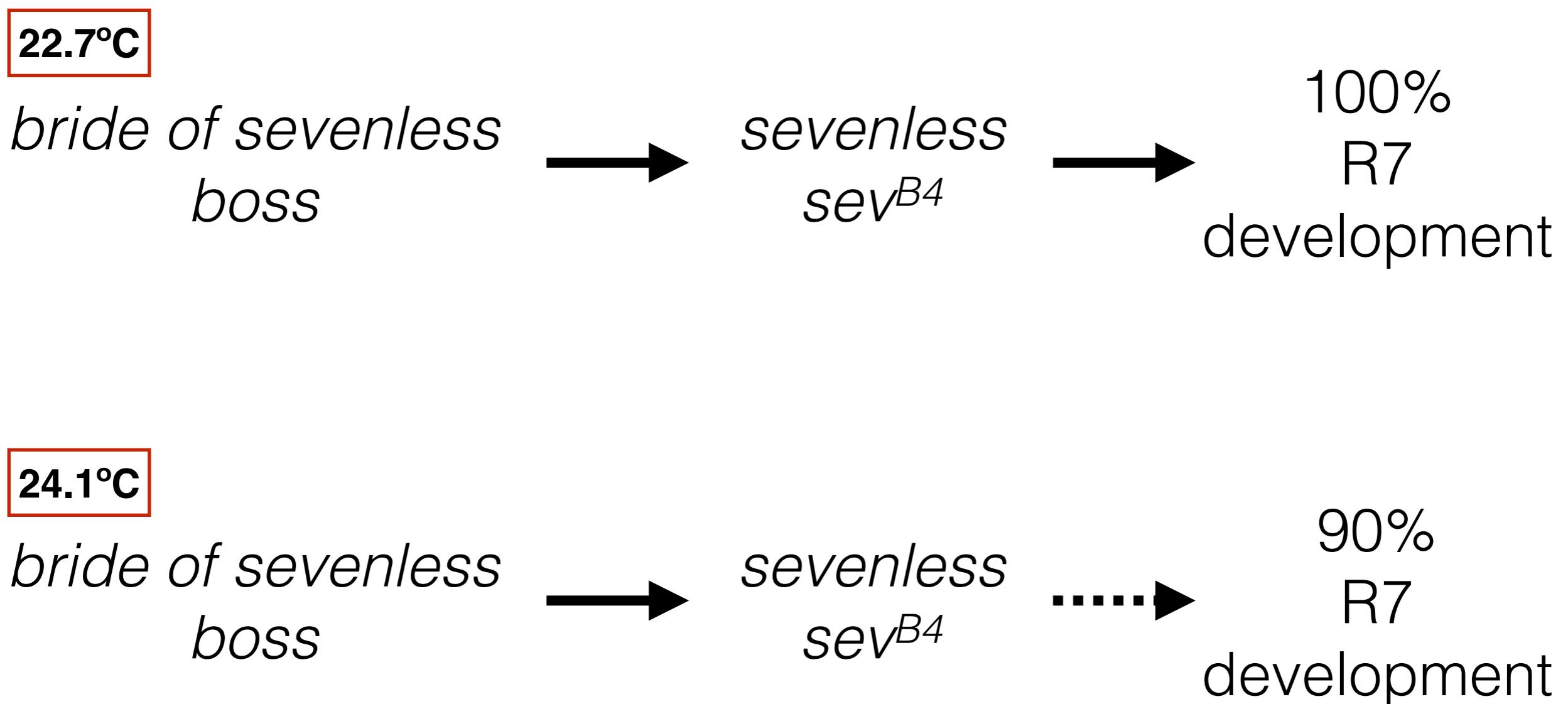
Peyton Rous discovered the first oncogenic virus in chicken



The virus expressed v-src (a tyrosine kinase) to control cell cycle

Mutagenesis of v-src led to temperature-sensitive alleles

# A sensitized enhancer screen for the *sevenless* pathway



# Strains used for the sensitized screen

$sev^{d2}; TM3\ ry / CxD$

$sev^{d2}$  = complete loss of  $sev$

$TM3$  = third chromosome balancer

$ry$  = allele conferring recessive rosy eye phenotype

$CxD$  = third chromosome balancer

$P[ry^+ sev^{B4}]$

$sev^{B4}$  = temperature-sensitive  $sev$  hypomorph

# Screen for dominant enhancers of sensitized phenotype

$sev^{d2}; TM3\ Sb\ ry\ P[ry^+ sev^{B4}]/\text{CxD}$

**22.7°C**

*bride of sevenless  
boss*



*sevenless  
sev<sup>B4</sup>*



100%  
R7  
development

**24.1°C**

*bride of sevenless  
boss*



*sevenless  
sev<sup>B4</sup>*



90%  
R7  
development

**22.7°C**

*bride of sevenless  
boss*



*sevenless  
sev<sup>B4</sup>*

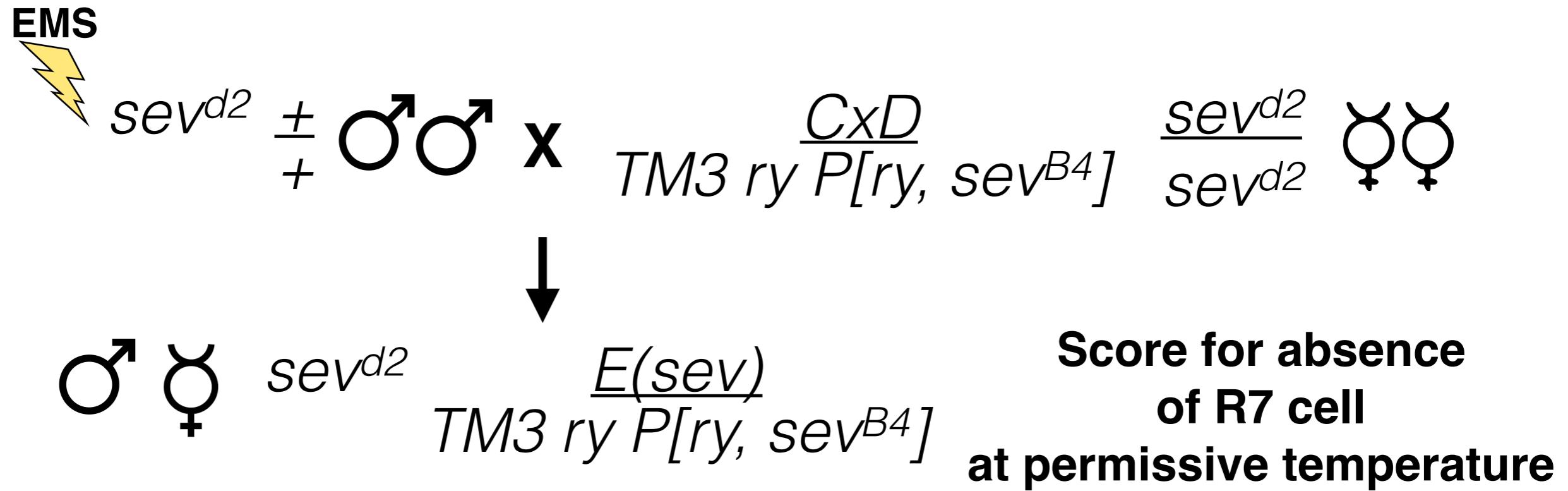


$E(sev)$



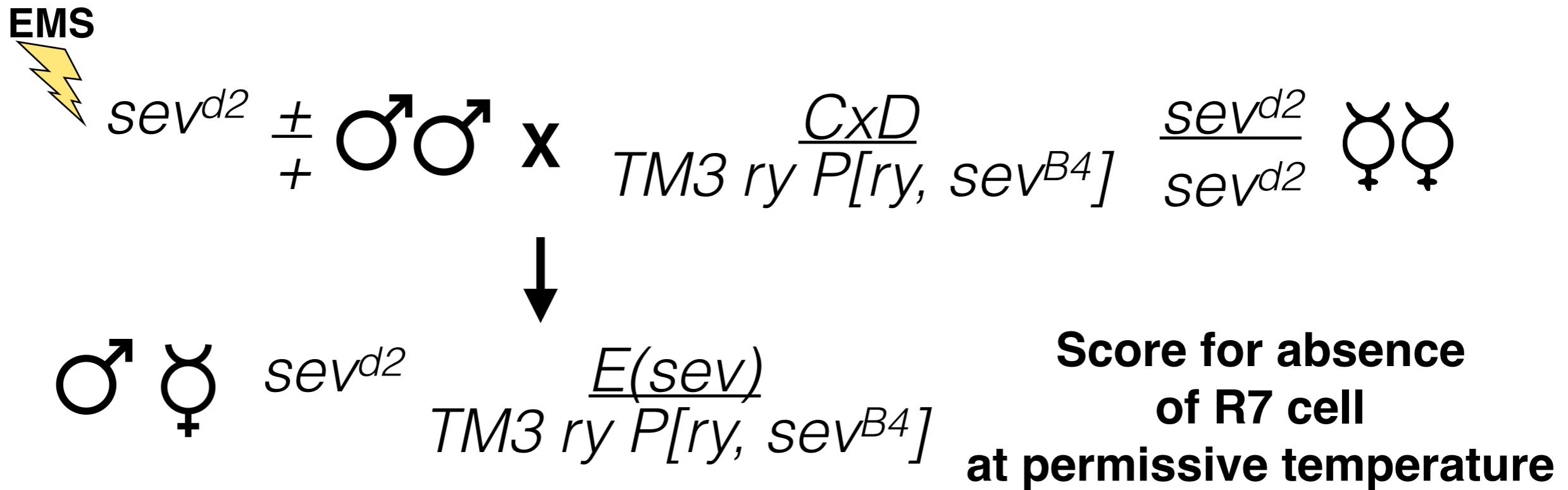
~10%  
R7  
development

# Screen for dominant enhancers of sensitized mutant R7 phenotype



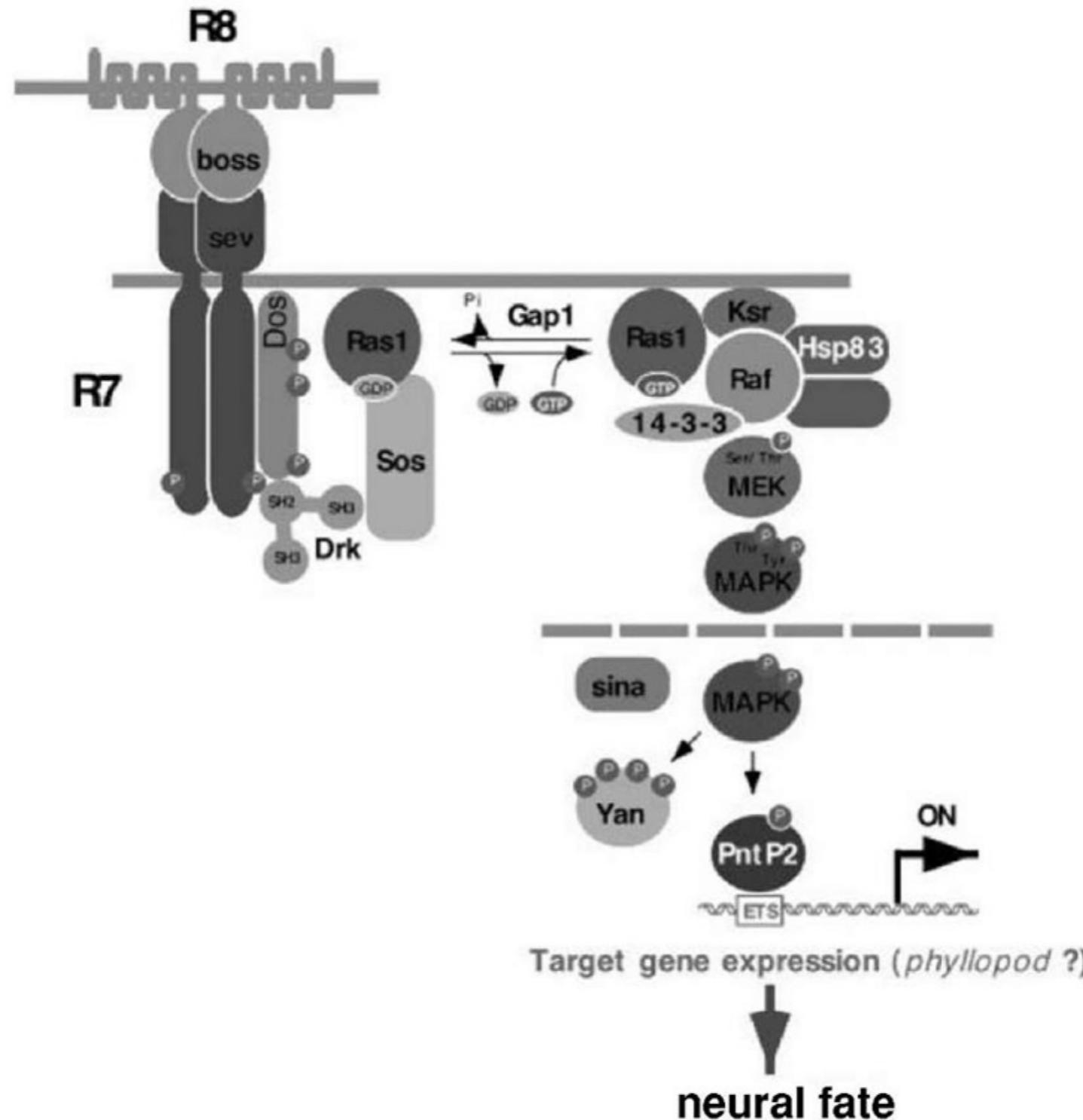
Assumptions: (1) Mutations in downstream genes required for viability and R7 fate  
(2) Most genes are not haploinsufficient

# Screen for dominant enhancers of sensitized mutant R7 phenotype



Screened 30,000 flies using pseudopupil technique  
Got 20  $E(sev)$  in seven complementation groups

# R7 fate is determined through a Ras pathway



What cells require *E(sev)* for function?

Mitotic recombination

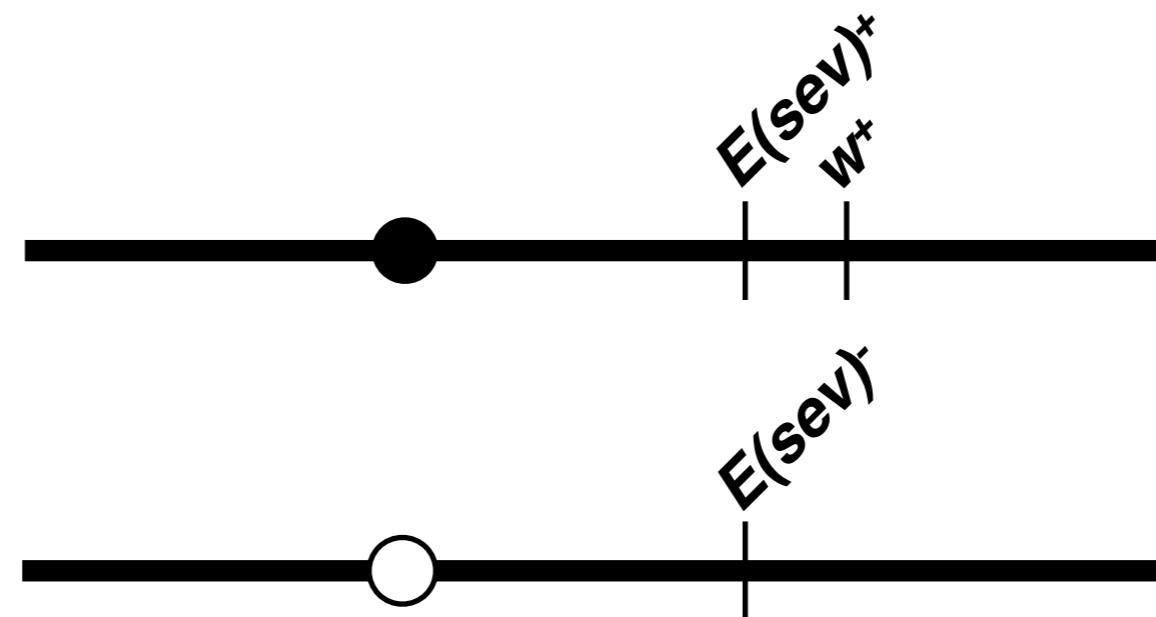
Lecture 10

# Are genes required in ommatidia for cell viability?

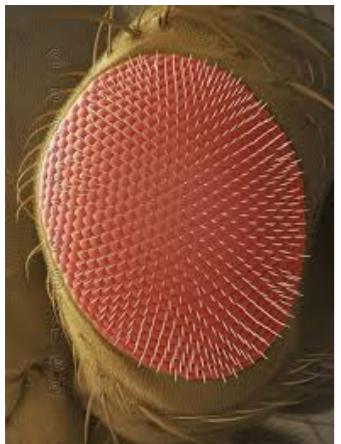
We want to make flies that lack the *E(sev)* gene in certain cells

1.   $\underline{E(sev)}^- ; \underline{w}$   
+  $w$

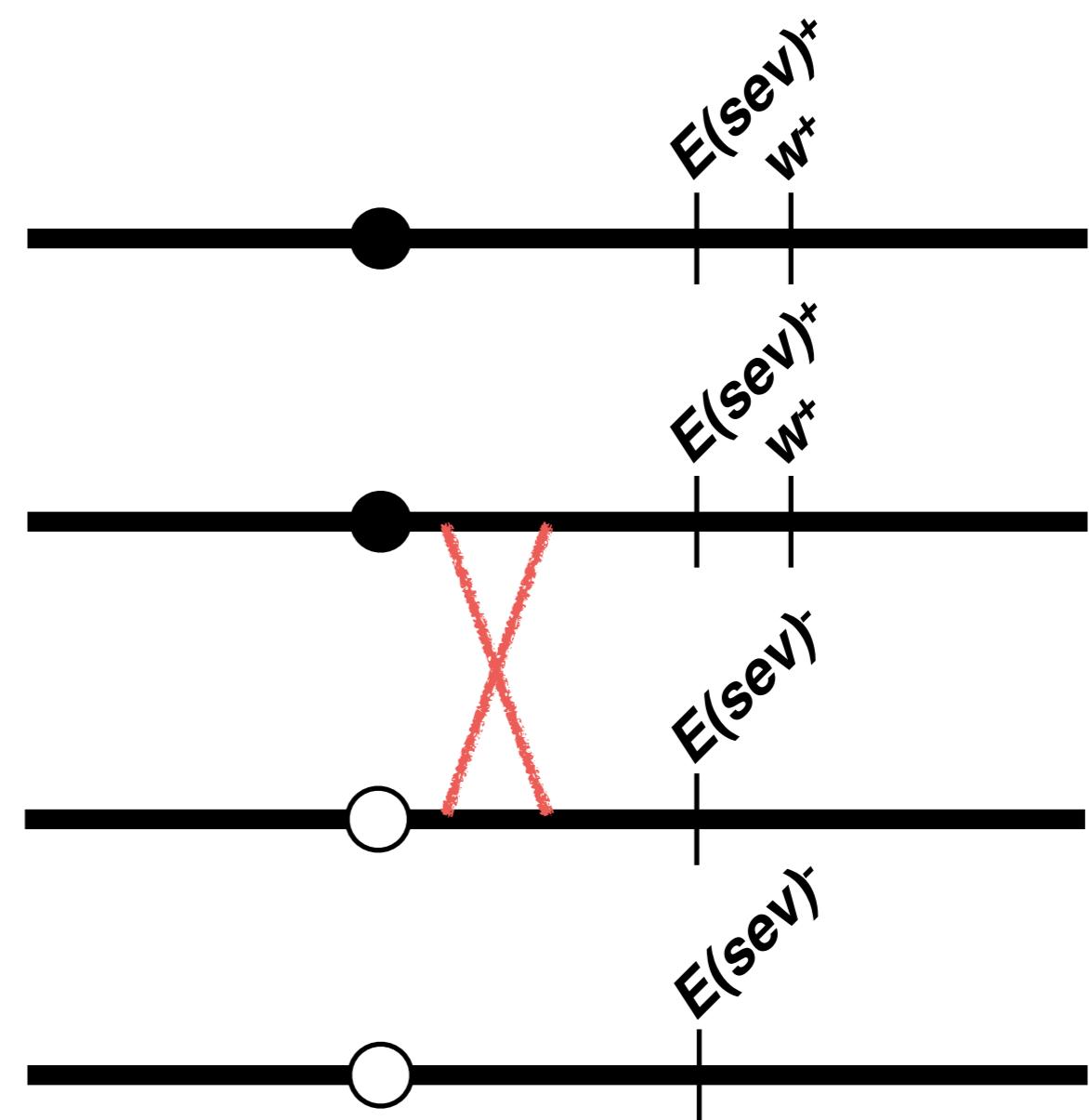
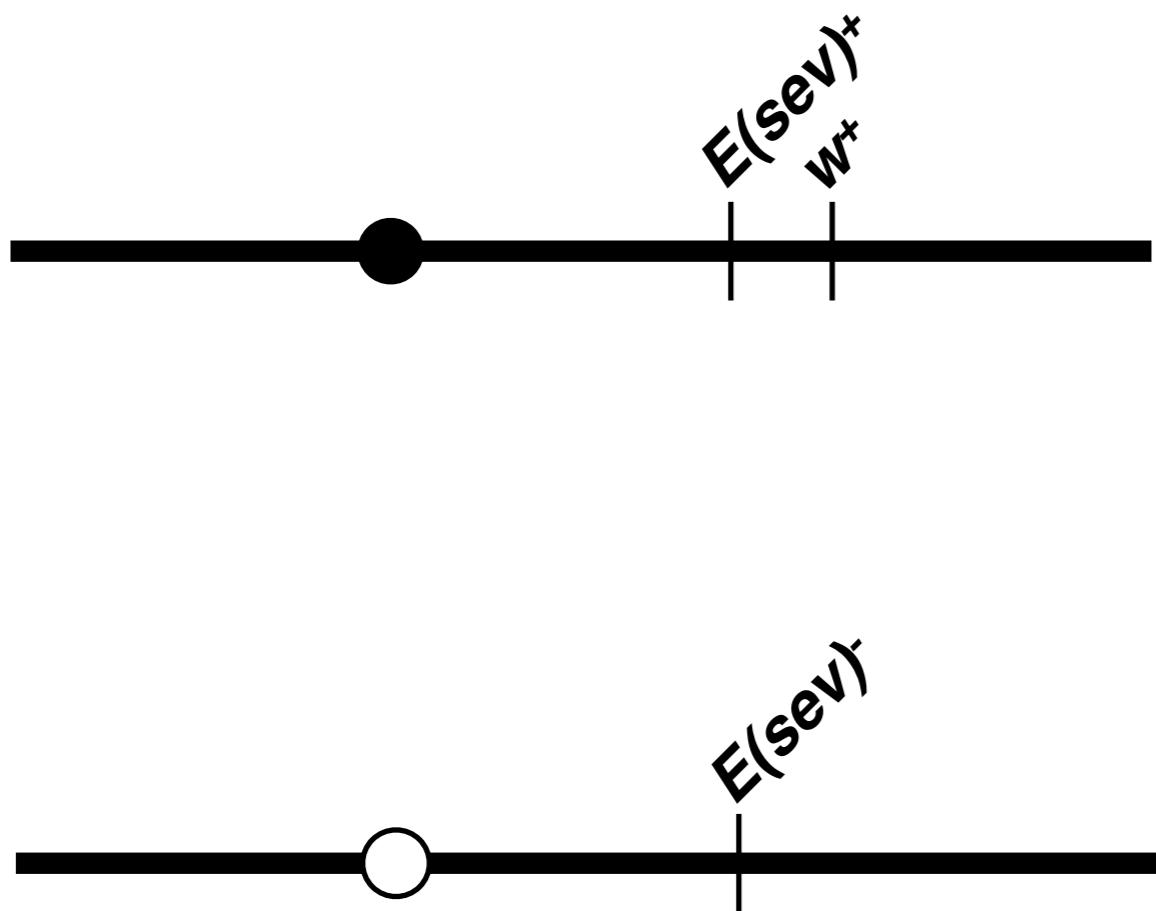
2. Use a P element with  $w^+$  distal  
to the wild-type *E(sev)* gene



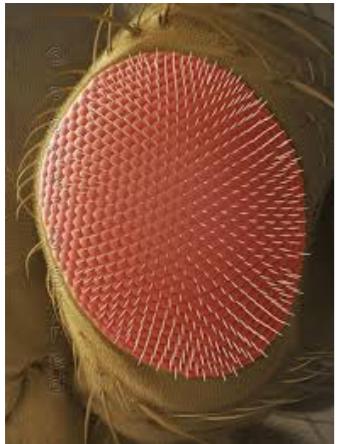
# Are genes required in ommatidia for cell viability?



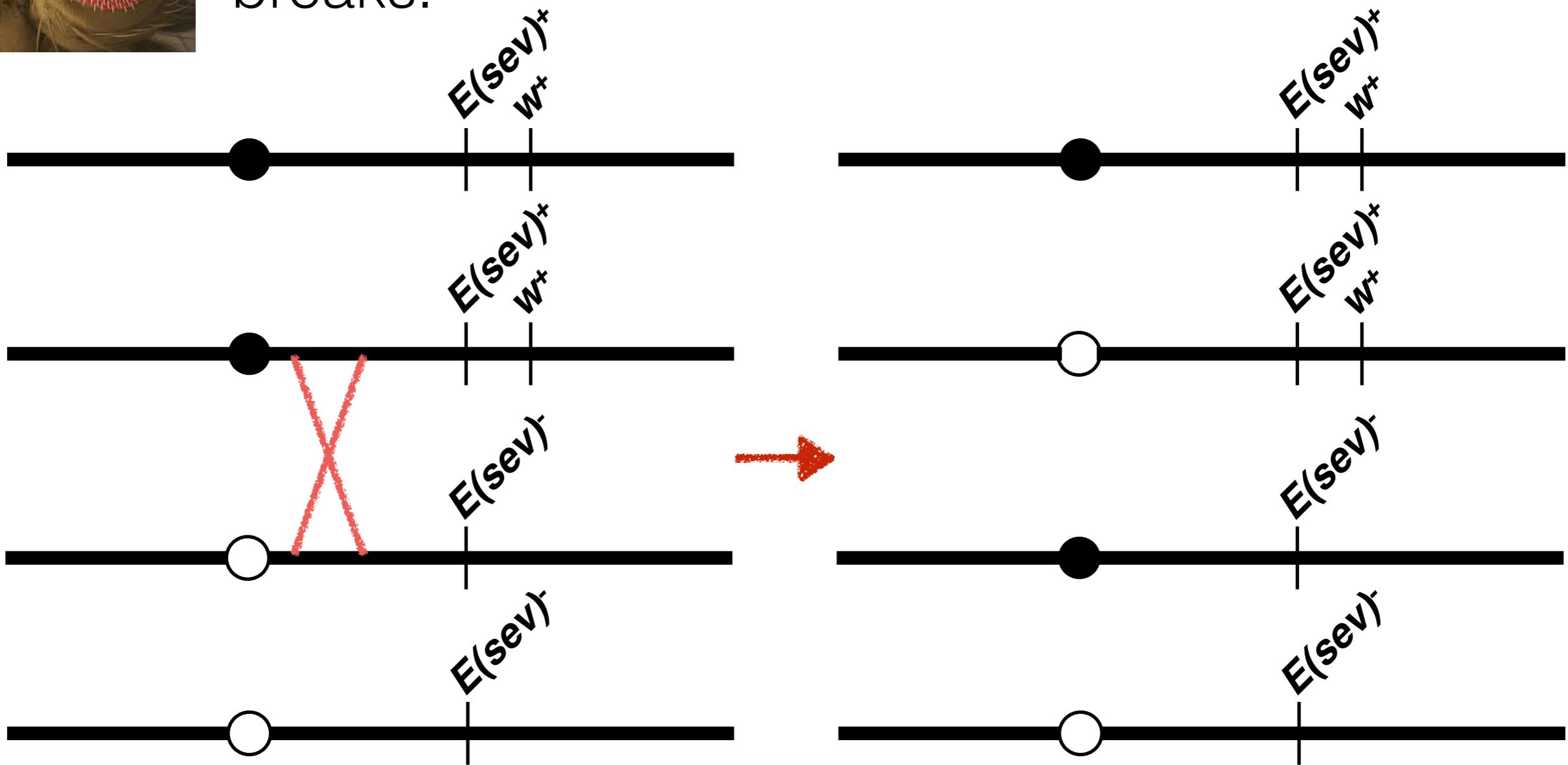
During mitosis for the cells that make up the eye, recombination can occur to repair double-strand breaks.



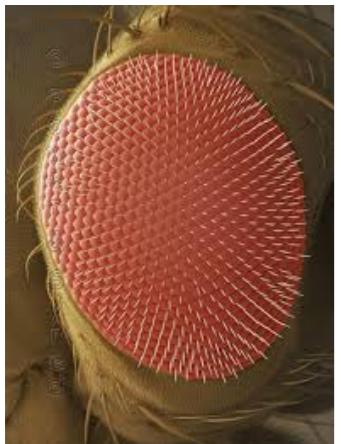
# Are genes required in ommatidia for cell viability?



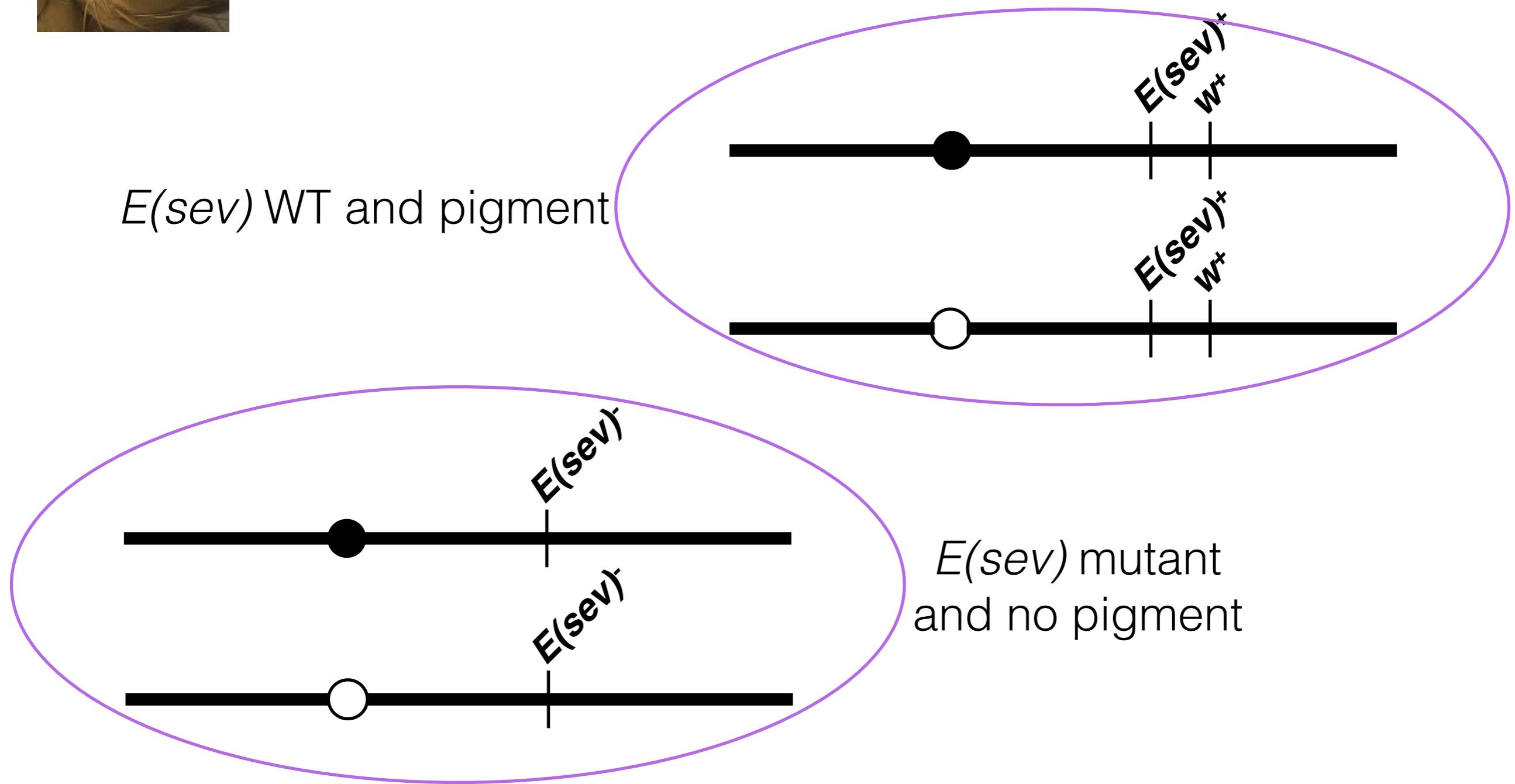
During mitosis for the cells that make up the eye, recombination can occur to repair double-strand breaks.



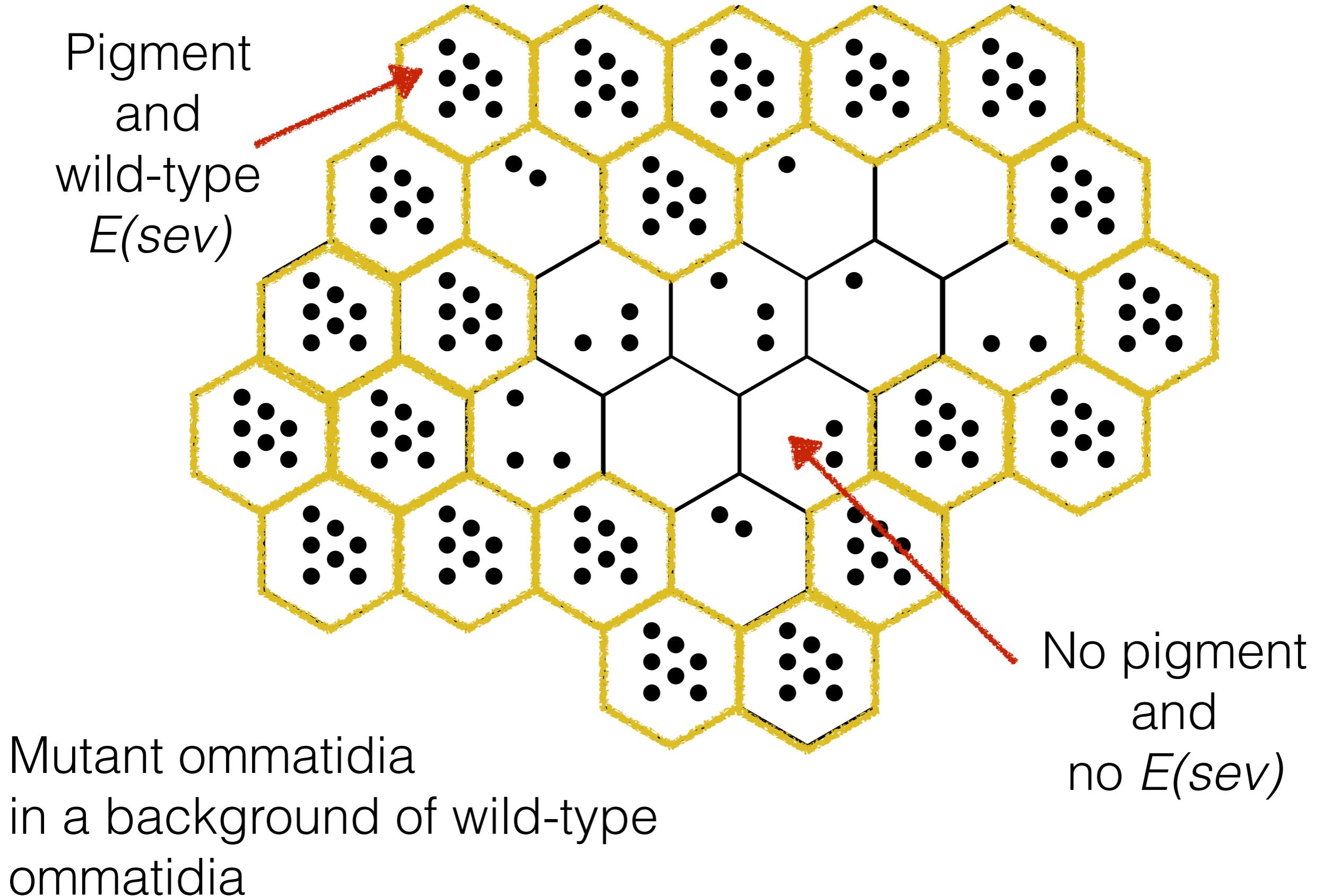
# Are genes required in ommatidia for cell viability?



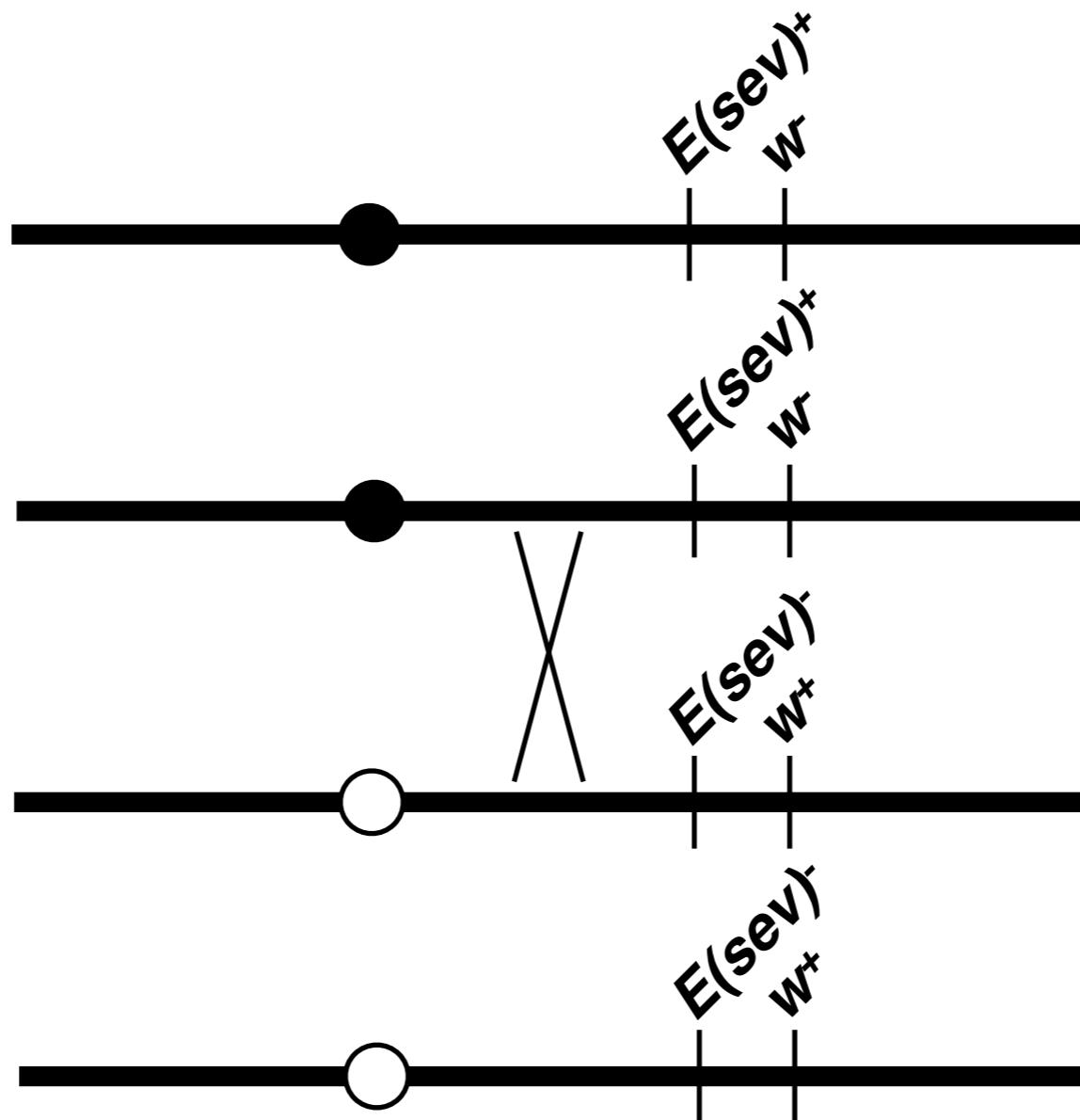
During mitosis for the cells that make up the eye, recombination can occur to repair double-strand breaks.



# Are genes required in ommatidia for cell viability?

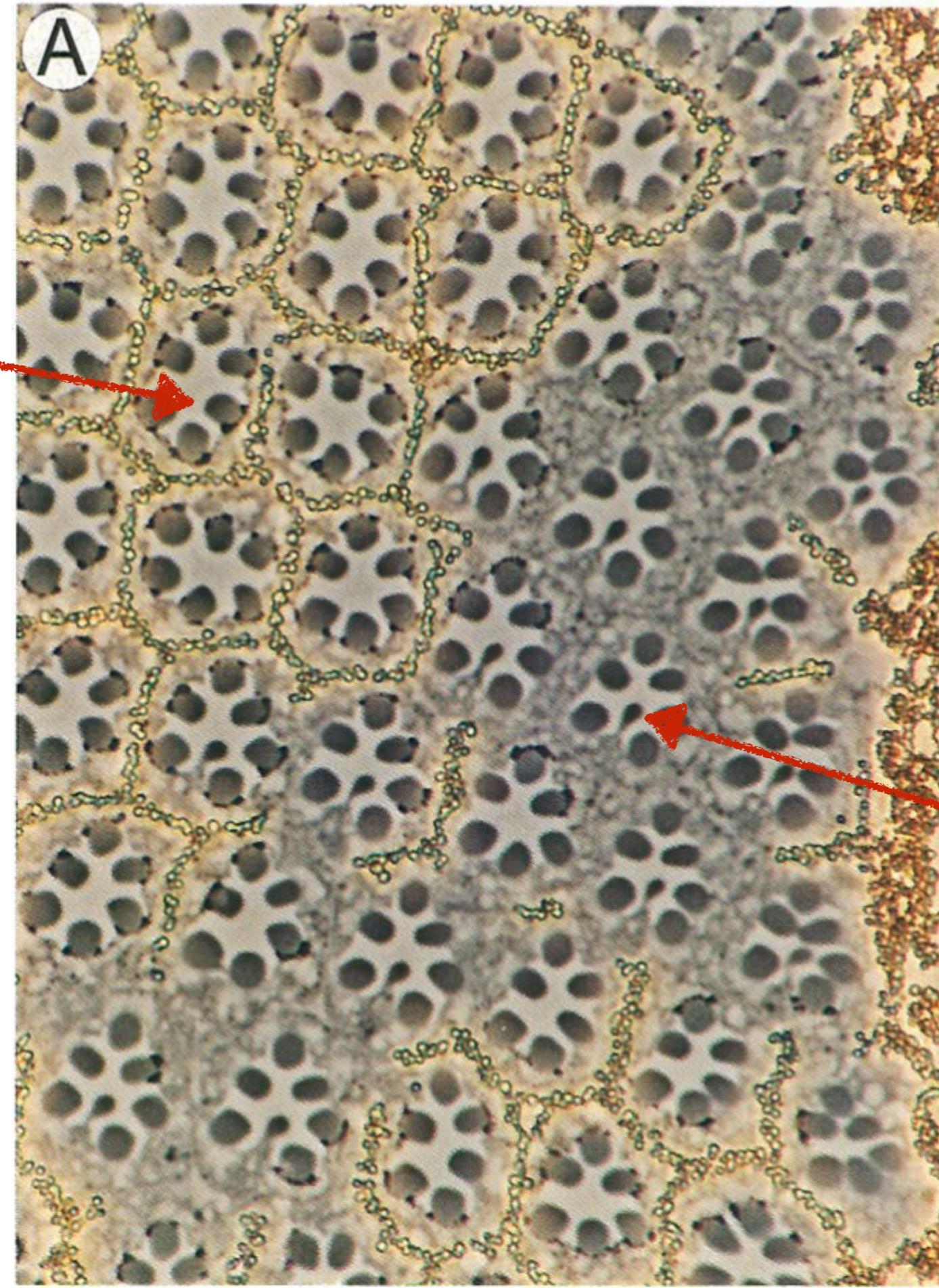


# Are genes required in the R7 cell for controlling R7 cell fate?



Wild-type  
ommatidia  
in a  
background  
of mutant  
ommatidia

Pigment  
and  
*E(sev)*  
mutant



No pigment  
and  
wild-type  
*E(sev)*

# **Methods to ascertain cell autonomy**

1. Expression of gene product in specific cell or tissue and look for rescue of a mutant phenotype
2. Lineage loss of unstable DNA that expresses the gene product and rescues a mutant phenotype (*C. elegans*)
3. Mitotic recombination to create clones of cells that express the gene product and rescue the mutant phenotype (*Drosophila*)

# Screen for dominant enhancers of sensitized phenotype led to the Ras pathway controlling R7 fate

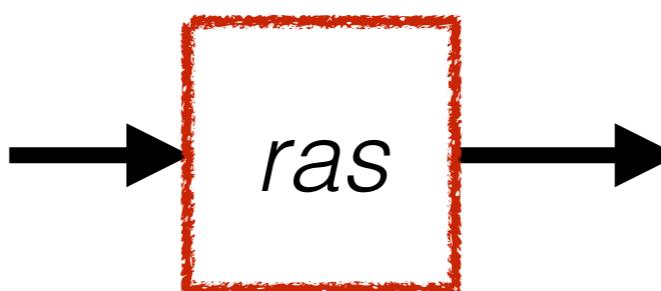
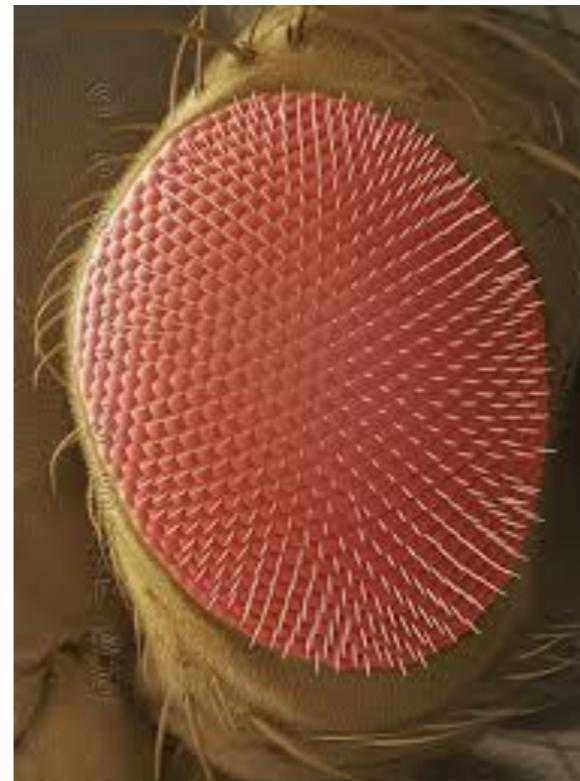
*bride of sevenless*  
*boss*



*sevenless*  
*sev*

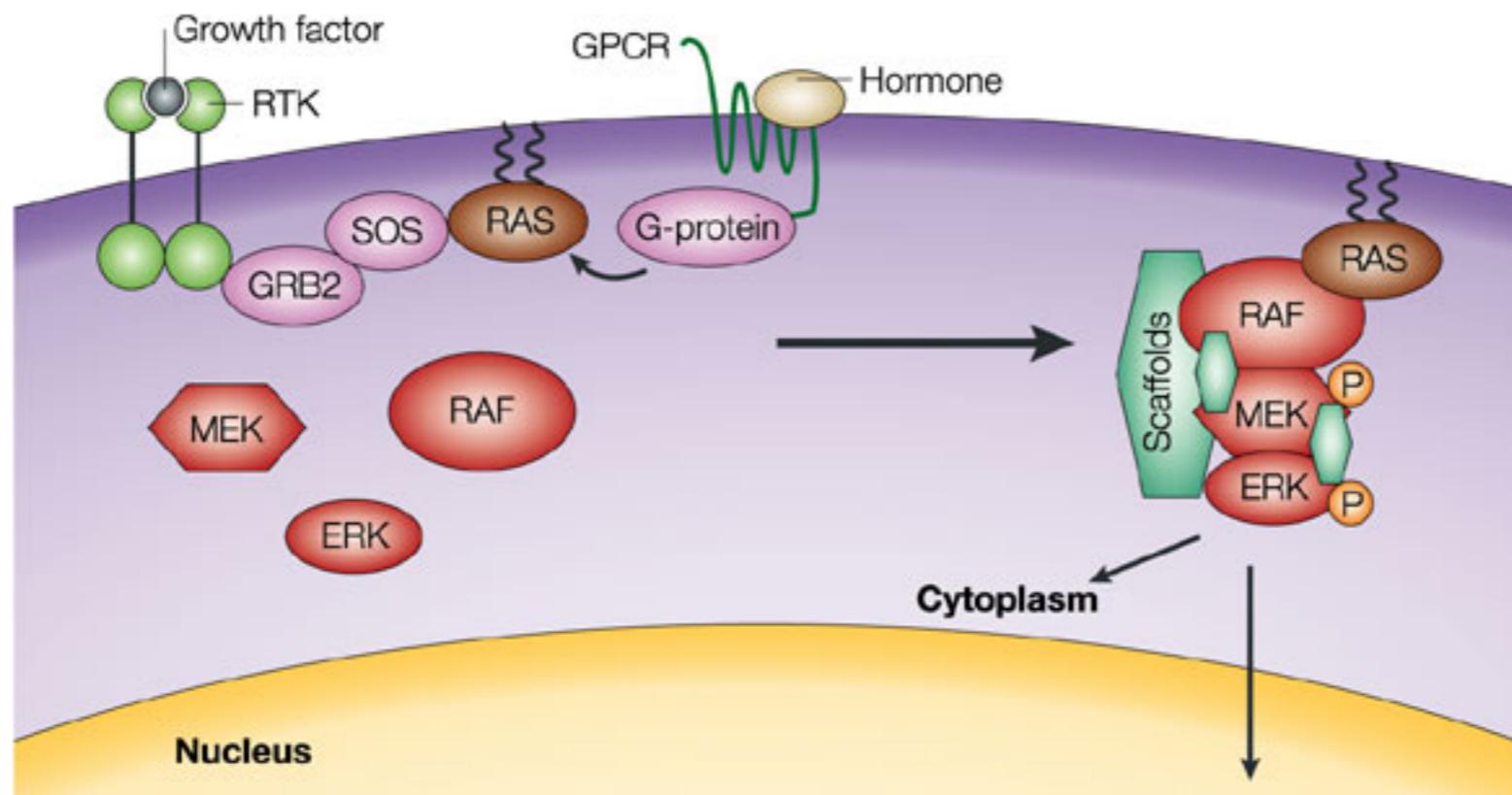


*son of sevenless*  
*sos*



R7  
development

# Two decades of research in *Drosophila* and *C. elegans* led to these pathways



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