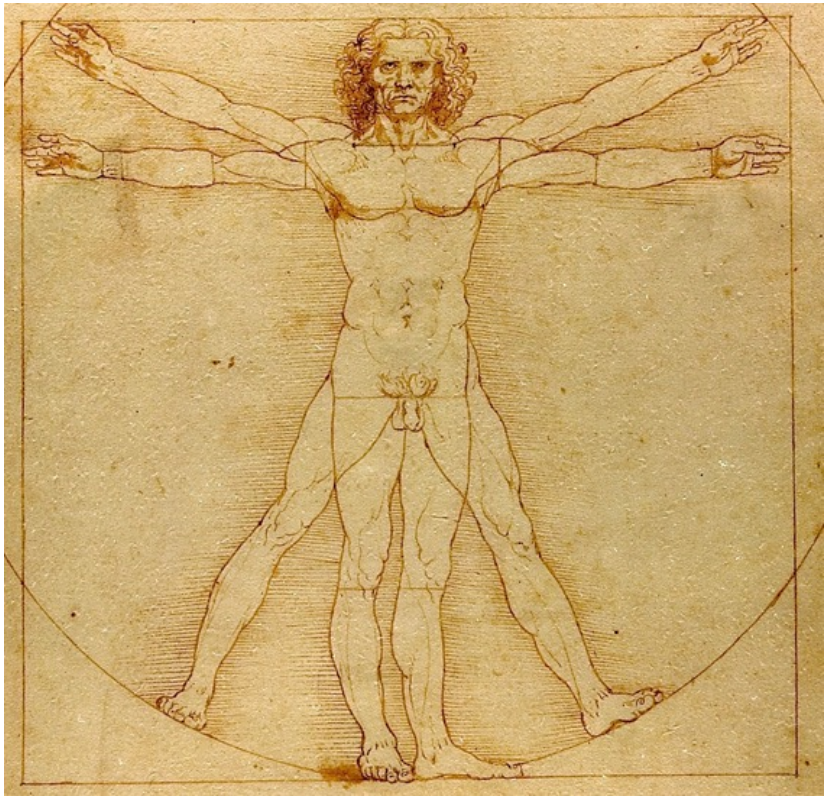
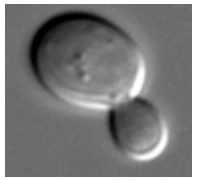
Drosophila toolbox

Peter Gallant
Biochemistry & Molecular Biology



Relevance

0	90	800	1’100
			
3’500	3’300	180	12

Evolutionary Divergence (mio years)

Genome Size (MB)

Experimental accessability

Human diseases genes in *Drosophila*

Disease	Human gene symbol	Fly gene symbol
Cancer		
Tuberous sclerosis	*TSC1*, *TSC2*	*tsc1*[§], *tsc2*[§]
Endometrial carcinoma	*PTEN*	*Pten*[§]
No known disease mutations in homologue		
Renal cancer lines		
No known disease mutations in homologue		
Bladder and colorectal cancer		
No known disease mutations in homologues		
B-cell leukaemia		
Melanoma		
Retinoblastoma		
Hepatocellular carcinoma		
Ectodermal dysplasia		

Disease	Human gene symbol	Fly gene symbol
Dysmorphology		
Synpolydactyly	*HOXD13*[‡]	*Abd-B*[§]
Single bone in zeugopod	*HOXD3–HOXD13* (heterozygous deletion)	*Abd-B*[§]
Hand-foot-genital syndrome	*HOXA13* or heterozygous *HOXA11–13* deletion	*Abd-B*[§]
Aniridia		
Townes-Brocks syndrome		
Saethre-Chotzen syndrome		
Pfieffer syndrome		
Apert syndrome		
Crouzon syndrome		
Saethre-Chotzen syndrome-like		
Alagille syndrome		
Spondylocostal dysostosis		
Primary congenital glaucoma		
Cardiac disease		
Congenital heart disease		
Holt–Oram syndrome		
DiGeorge syndrome		
Venous malformations		

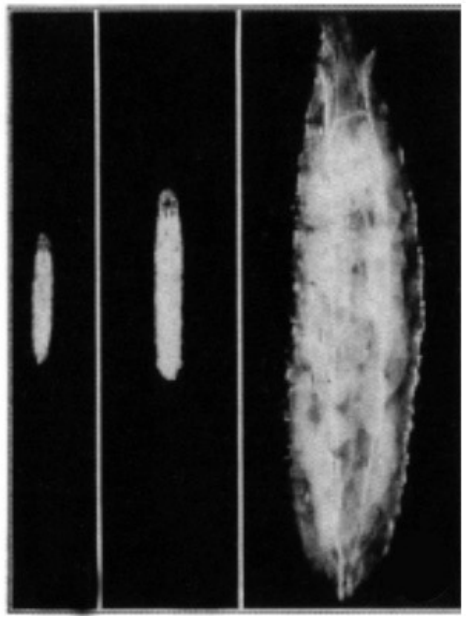
Disease	Human gene symbol	Fly gene symbol
Neurological		
Spinocerabellar ataxia	*SCA1* (also known as *ATXN1*) *SCA2* (also known as *ATXN2*) *SCA6* (also known as *CACNA1A*) *SCA14* (also known as *PRKCG*) *SCA17* (also known as *TBP*)	*CG4547* *CG5166* *cac*[§], *Ca-α1D*[§] *inaC*[§], *Pkc53E* *Tbp*[§]
Huntington disease	*HD*	*huntingtin*[§]
Spinal and bulbar muscular atrophy 3	*AR*	*ERR*, *svp*[§]
Parkinson disease	*PARK2* *PARK5* (also known as *UCHL1*) *PARK7* *NR4A2* *MAPT* *PINK1* *SNCA*	*park*[§] *Uch* *dj-iβ*, *CG6646* *Hr38*[§] *tau*[§] *CG4523*[§] None known
Alzheimer disease	*PSEN1*, *PSEN2* *APP*	*Psn*[§] *Appl*[§]
Fragile X syndrome	*FMR1*	*Fmr1*[§]
Angelman syndrome	*UBE3A*	*dube3A*[§]

Bier (2005), *Nat Rev Gen 6*, 9.

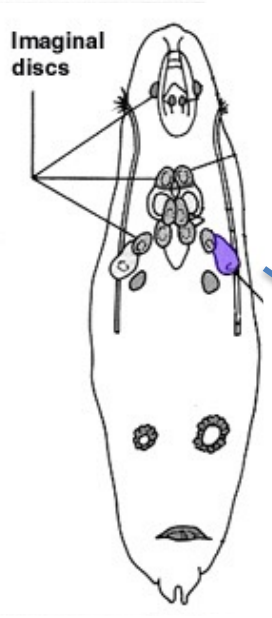
Drosophila toolbox

- *Drosophila* biology
- *Drosophila* genetics
- Methods:
  - Transgenesis
  - CRISPR-based mutagenesis/misexpression
  - Binary overexpression
  - Mitotic clones / Mosaicism

Drosophila development

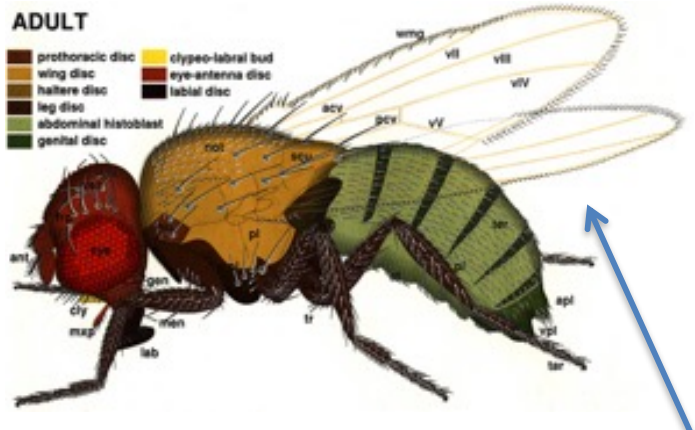


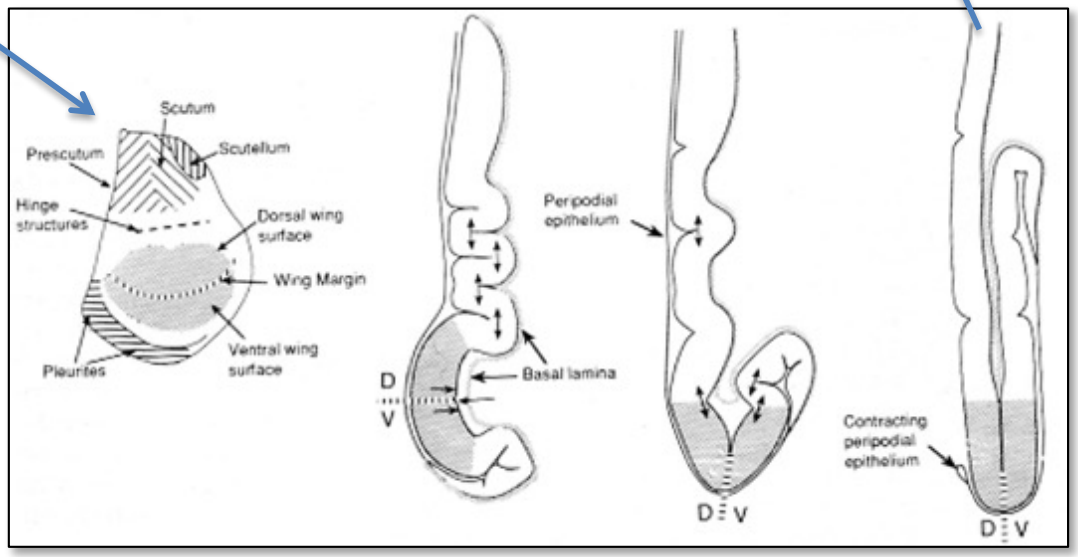
L1 L2 L3



P

Bodenstein (1950), in: Demerec (ed.),
The Biology of Drosophila.

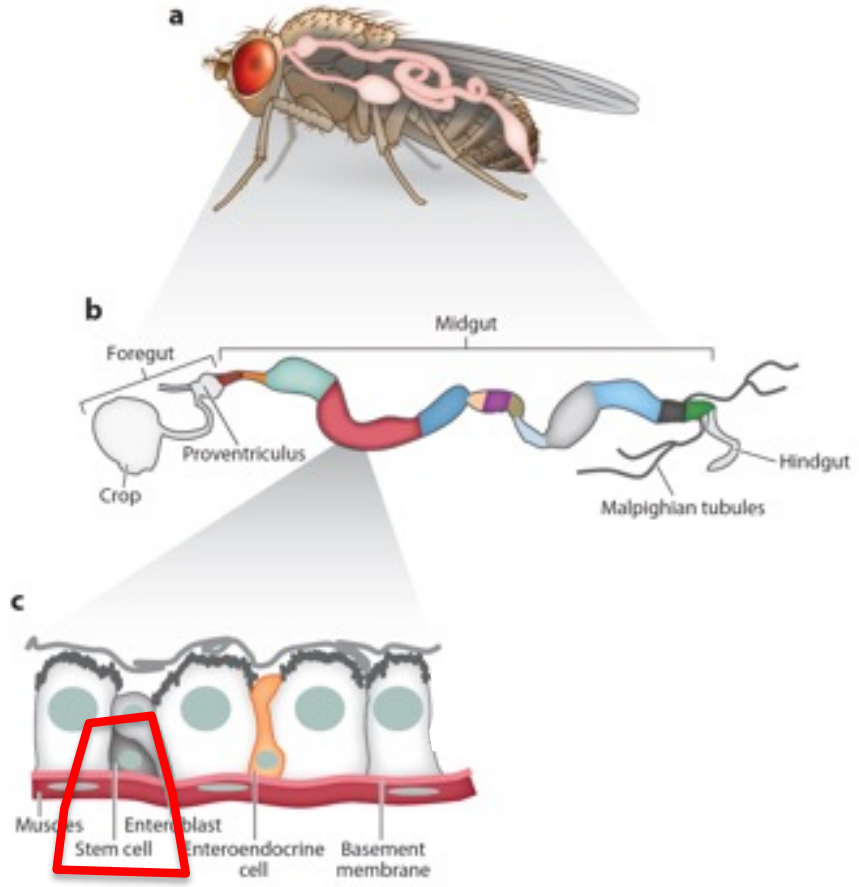




Fristrom & Fristrom (1993); Hartenstein (1993)

Adult *Drosophila*

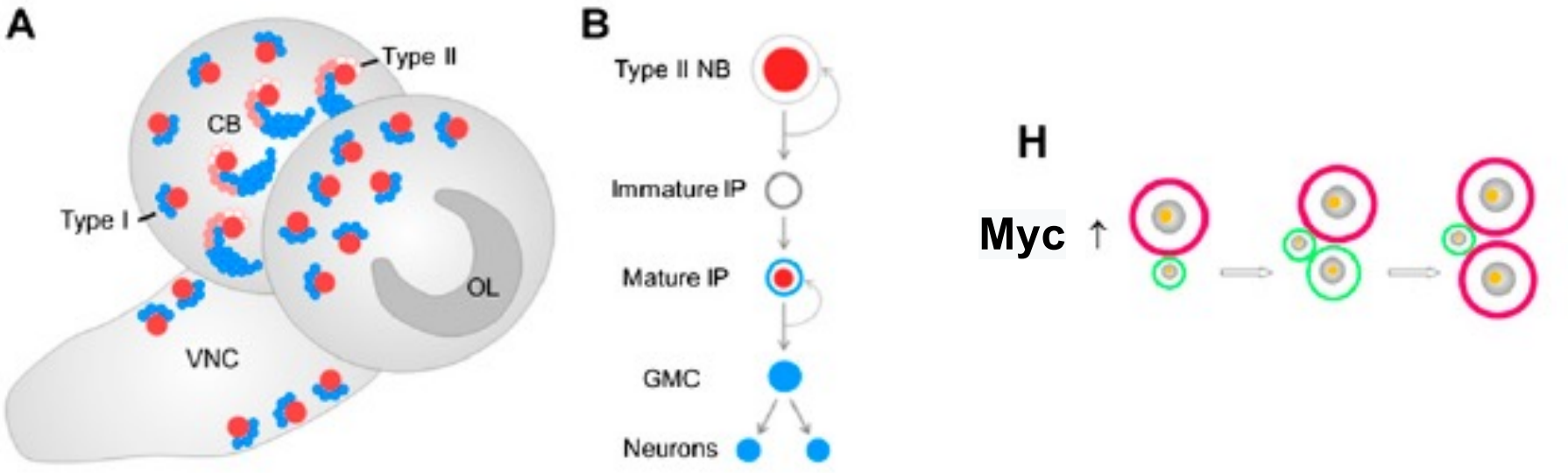
- midgut as model for “colon cancer”



Lemaitre & Miguel-Aliaga (2013), *Ann Rev Genet 47*, 377

Adult *Drosophila*

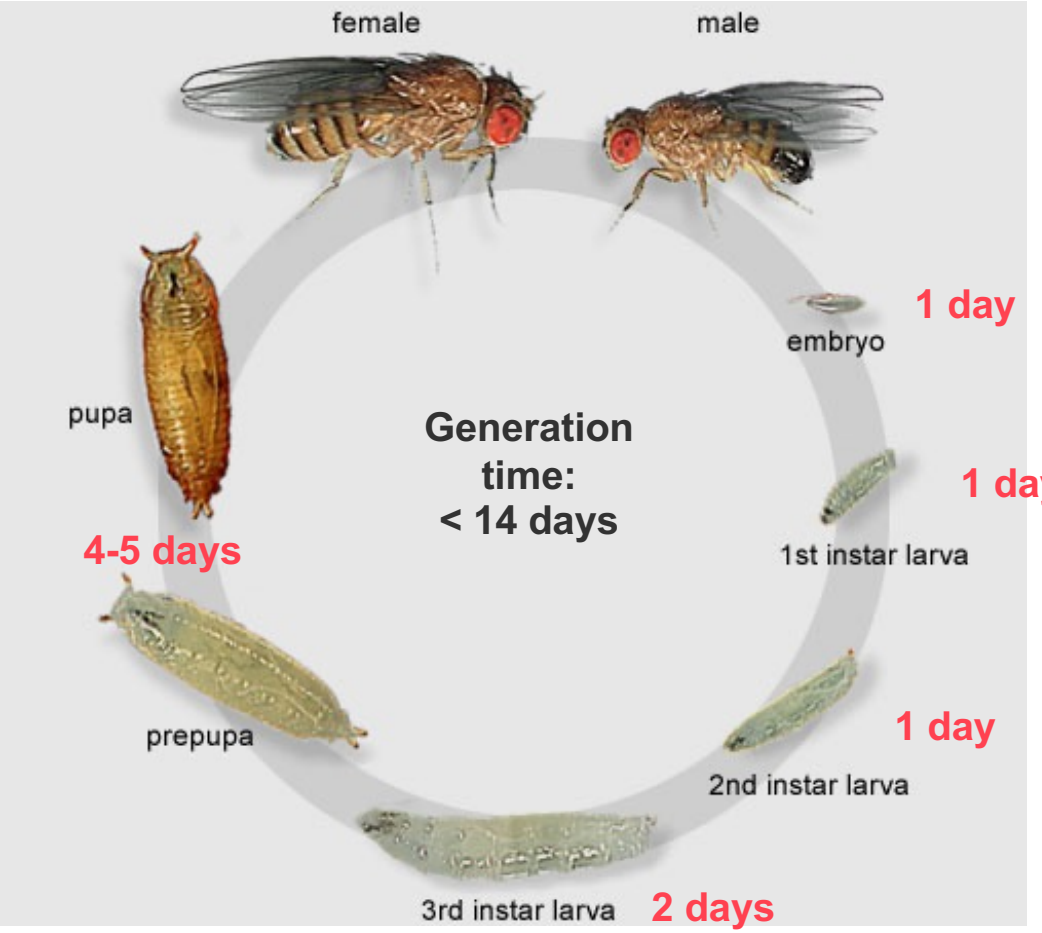
- midgut as model for “colon cancer”
- nervous system stem cells as model for “brain tumors”



adapted from Song & Lu (2011)

Working with *Drosophila*

- Short generation time
- Easy maintenance
- **Genetics**

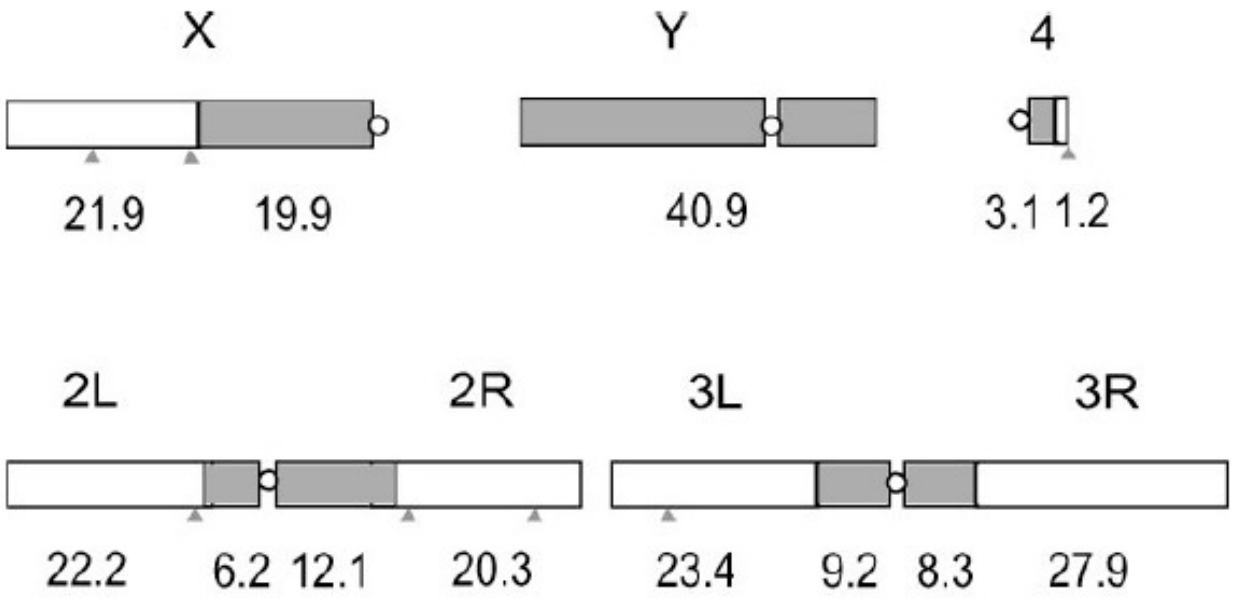


Weigmann et al (2003), *Trends Genet 19*, 310

Drosophila toolbox

- *Drosophila* biology
- ***Drosophila* genetics**
- Methods

Basic genetics: the genome

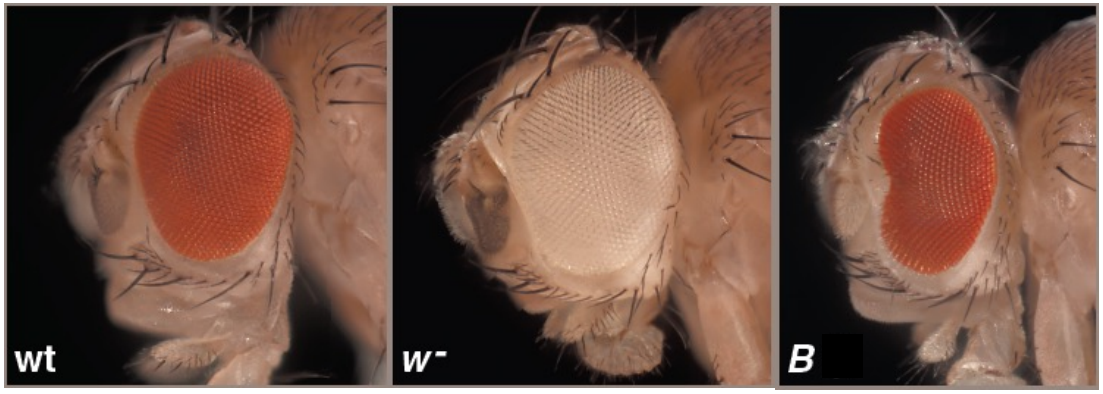


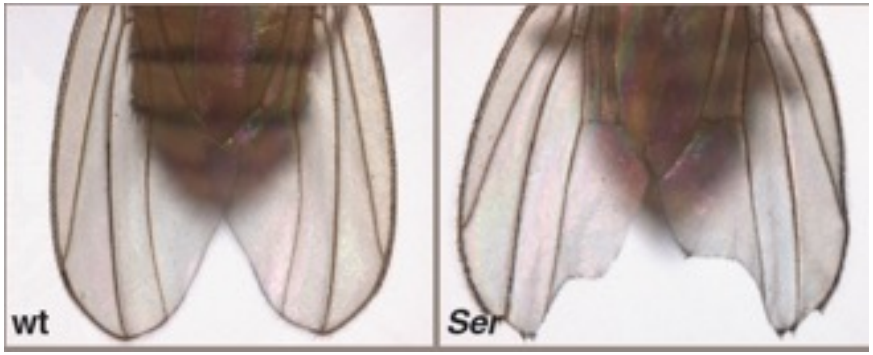
Females: X/X; 2/2; 3/3; 4/4
Males: X/Y; 2/2; 3/3; 4/4

ca. 18‘000 genes

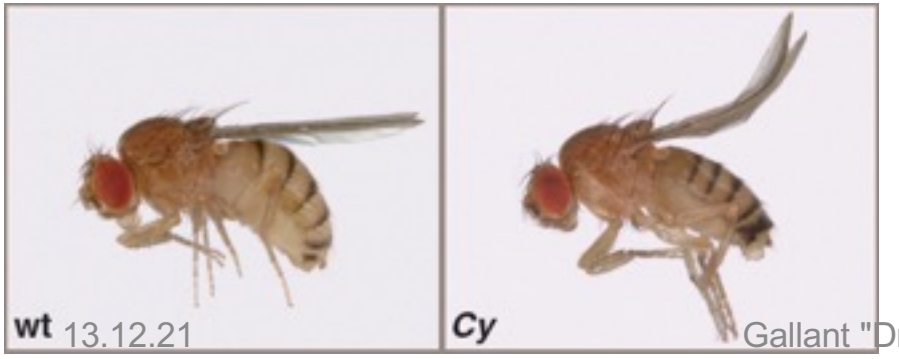
Basic genetics: the genes

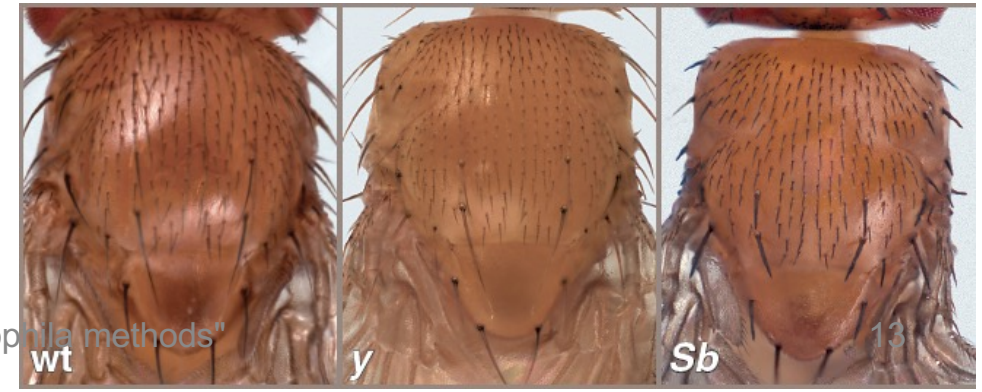
- Genes named after first *mutant* phenotype; examples:
  - *white*: lacks all eye pigment
  - *apterous* and *wingless*: lack wings
  - *eyeless*: lacks eyes
- Markers:
  - Dominant or recessive mutations with a visible phenotype



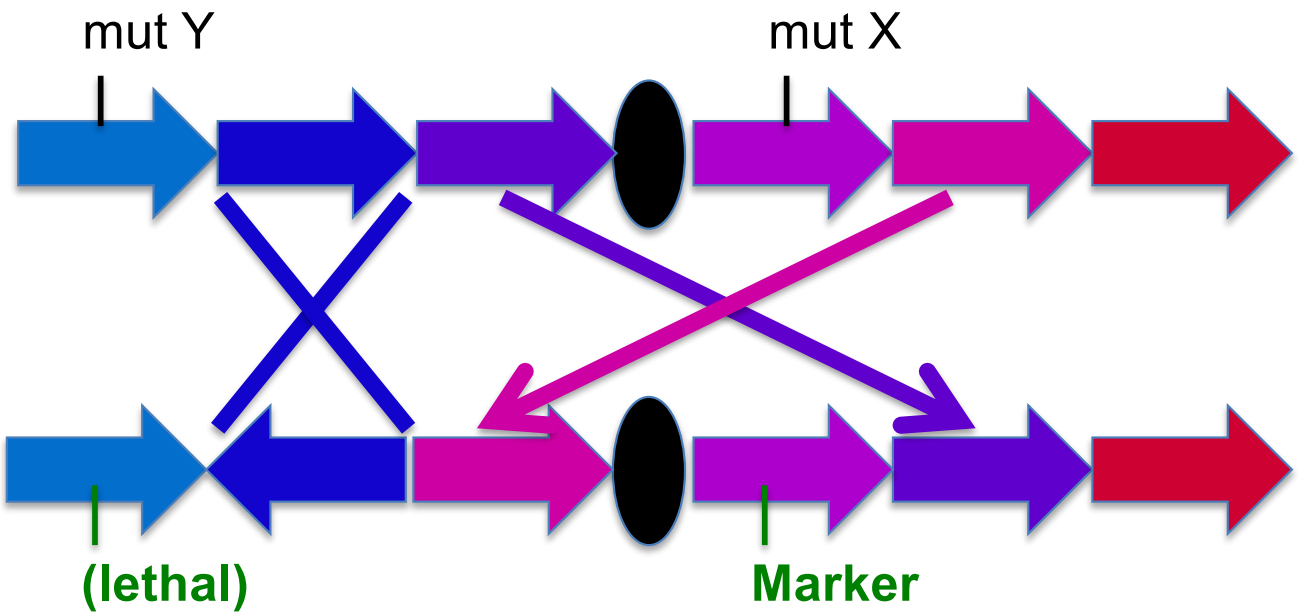


Childress et al. (2005), *genesis 43*, cover.





Basic genetics: balancer chromosomes

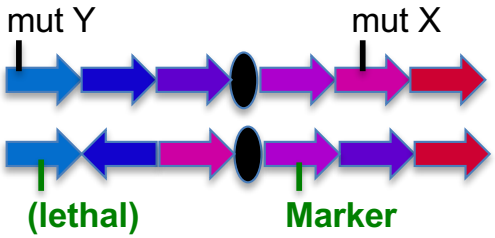


"wt" chromosome

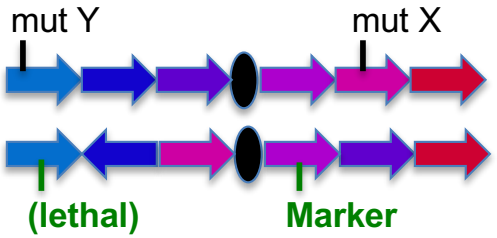
Balancer chromosome

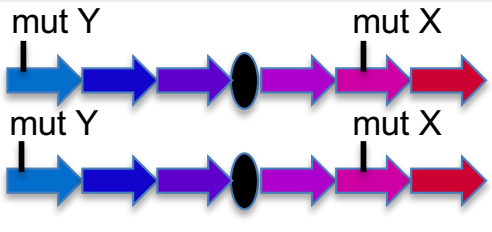
- Multiple translocations and inversions
  → prevent meiotic recombination
- Marker mutations

Basic genetics: balancer chromosomes

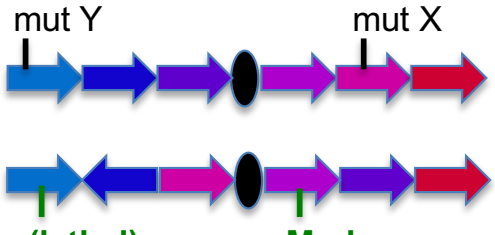


X

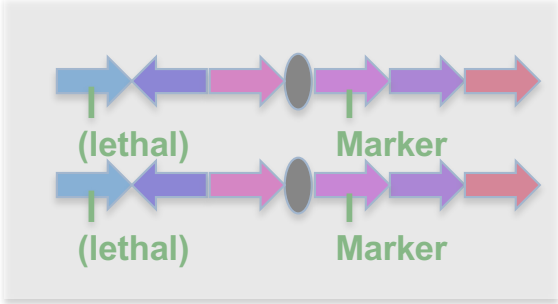




$$\frac{Y\ X}{Y\ X}$$



$$\frac{Y\ X}{+\ +}\quad\frac{}{(\text{lethal})\ \text{Marker}}$$



+

$$\frac{+\ +}{+\ +}\quad\frac{(\text{lethal})\ \text{Marker}}{(\text{lethal})\ \text{Marker}}$$

Drosophila toolbox

- *Drosophila* biology
- *Drosophila* genetics
- **Methods**

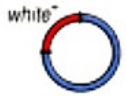
Methods

- Analysis:
  - Microscopy
  - Electrophysiology
  - Behavioural studies
  - Physiology
  - ...
- Genetics:
  - **Transgenesis**

Transgenesis in mice

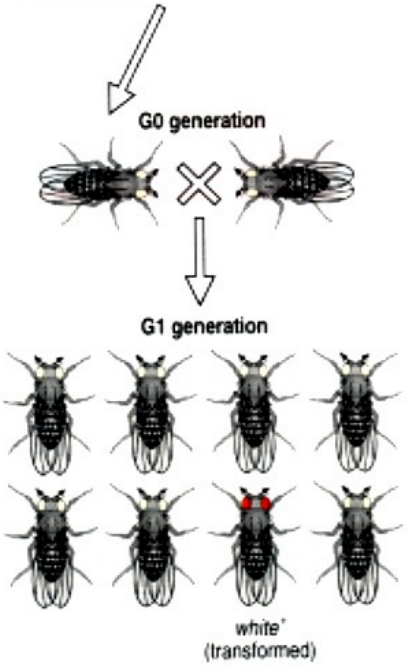
- ES cells cultured in vitro
- ... transfect plasmid with desired payload
- ... select for transformants, e.g. via neomycin-resistance
- ... inject transformed cells into blastocyst & implant in foster mother
- ... identify mosaic adult mice (coat color) & mate with partners

Random Transgenesis in flies



+ Transposase enzyme

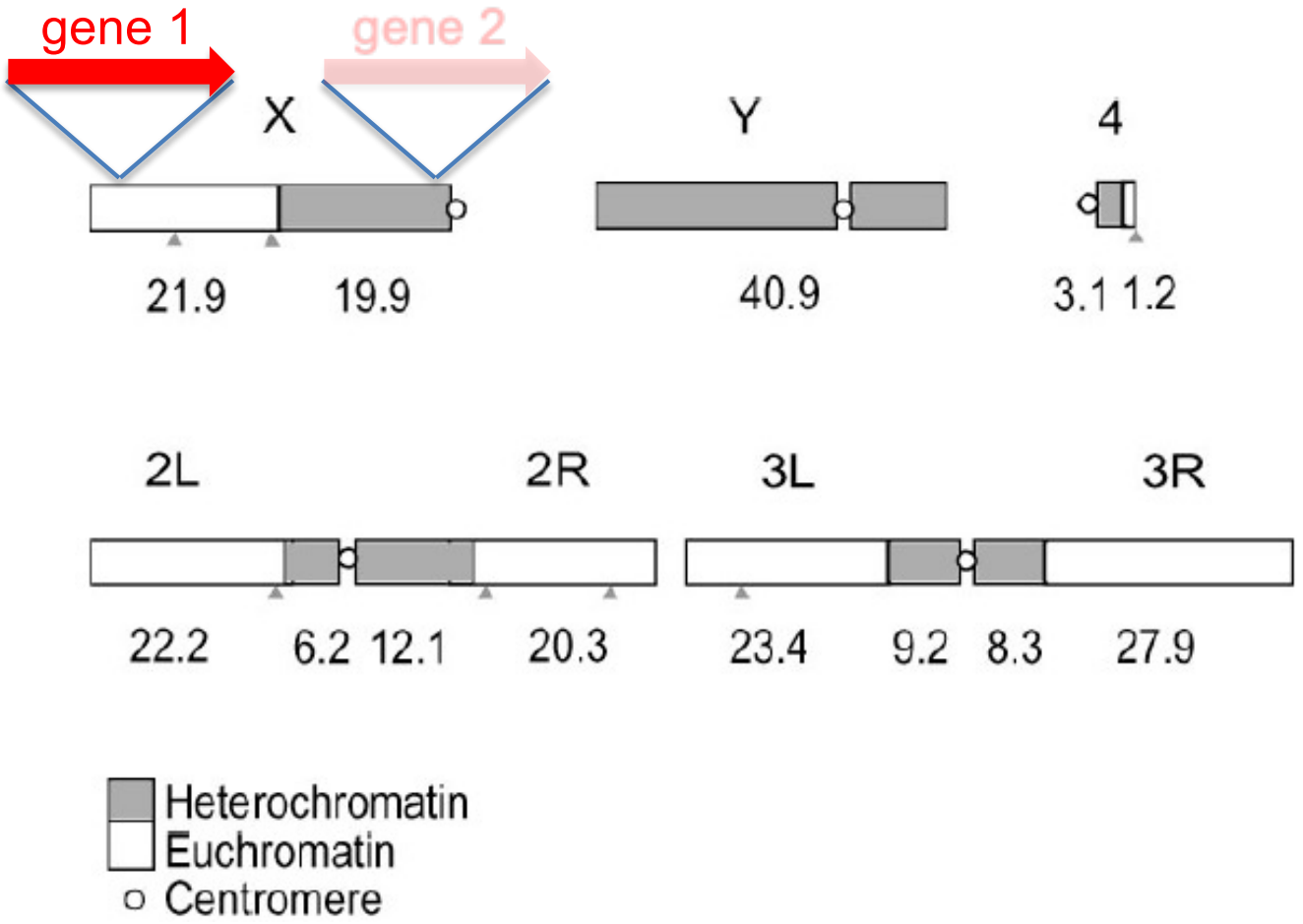
Plasmid with appropriate sequences:
- Marker (e.g. ***white*** gene)
- any additional “pay load”



Embryo:
- Mutant for the marker (e.g. ***white***$^{-}$)
- Transgene integrates in ≤1 germ cell / embryo

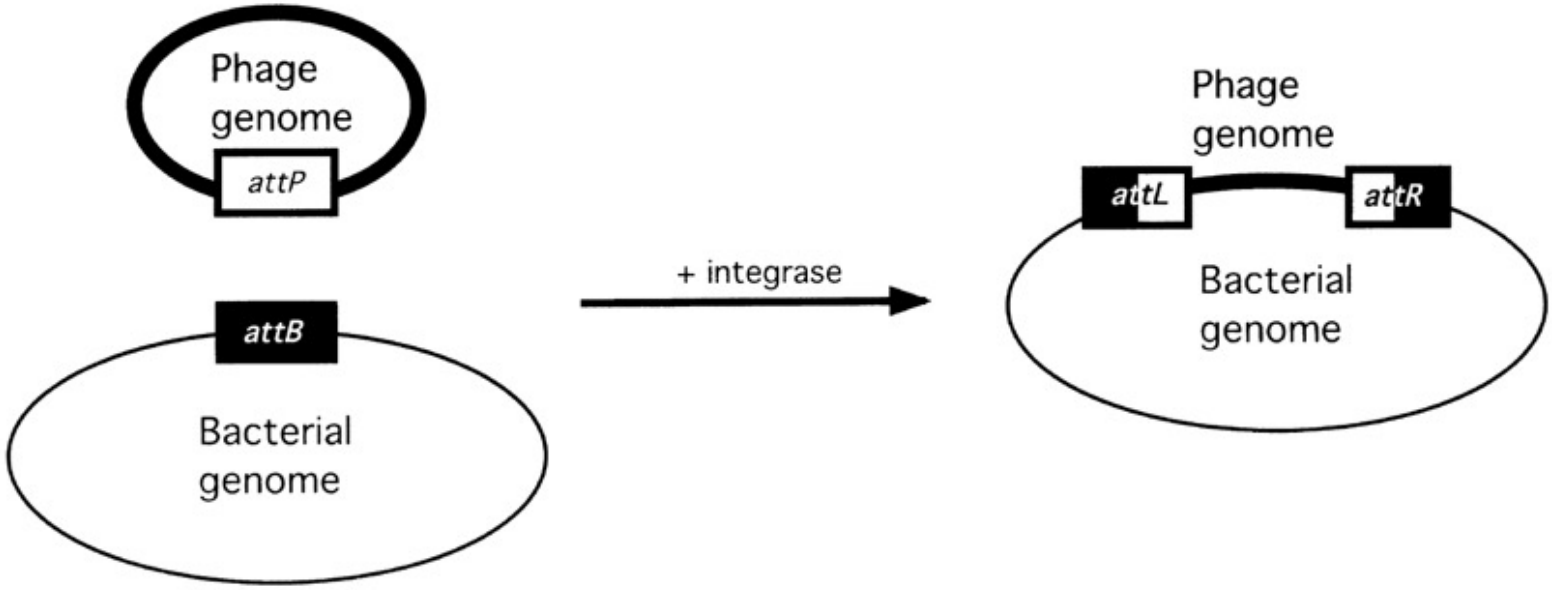
G1 generation:
- Derived from the germ cells of G0
- Only few animals carry the transgene
  → recognized e.g. by ***white+*** eyes

Figure after Wolpert (1998)


gene 1
gene 2
X
Y
4
21.9
19.9
40.9
3.1
1.2
2L
2R
3L
3R
22.2
6.2
12.1
20.3
23.4
9.2
8.3
27.9
Heterochromatin
Euchromatin
Centromere

Targeted Transgenesis

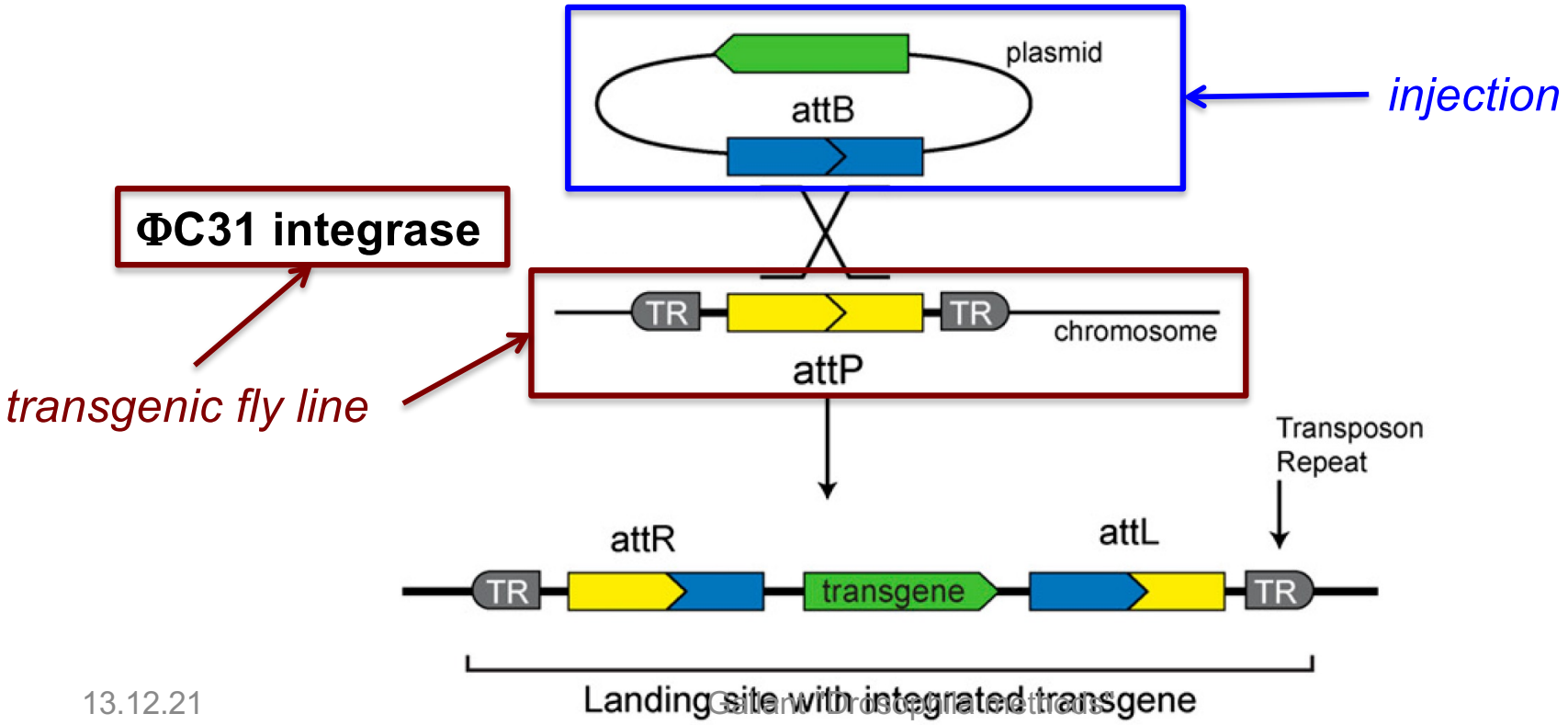
- Site-directed integration: **b**acteria- and **p**hage ΦC31-derived "**att**achment sites" (**attB** & **attP**) and phage-derived **integrase**
- Resulting hybrid sites "**attL**" & "**attR**"



Groth & Calos (2004), JMB 335, 667

Targeted Transgenesis

- High efficiency (10->50%)
- e.g. integration of complex constructs (with FRT, LoxP, attB,...)
- e.g. first targeted insertion of attP in specific locus, then efficient integration
- Sequence-specificity: (almost) no untargeted integration



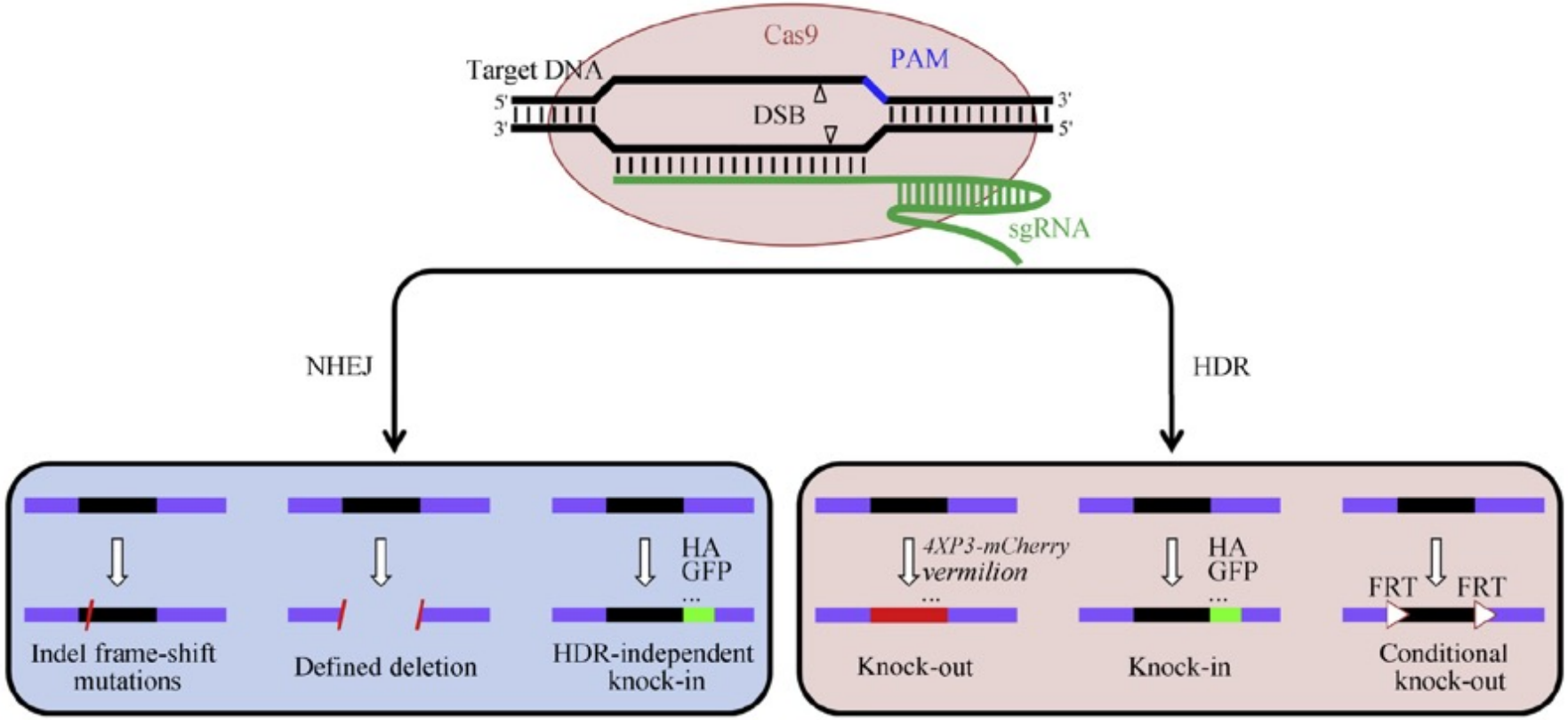
Knapp et al. (2015), Genetics 199, 919 (based on Bischof et al. 2004)

Methods

- Analysis:
  - Microscopy
  - Electrophysiology
  - Behavioural studies
  - Physiology
  - ...
- Genetics:
  - Transgenesis:
    - random insertion
    - ϕC31-mediated targeted insertion
  - **CRISPR-based mutagenesis/misexpression**

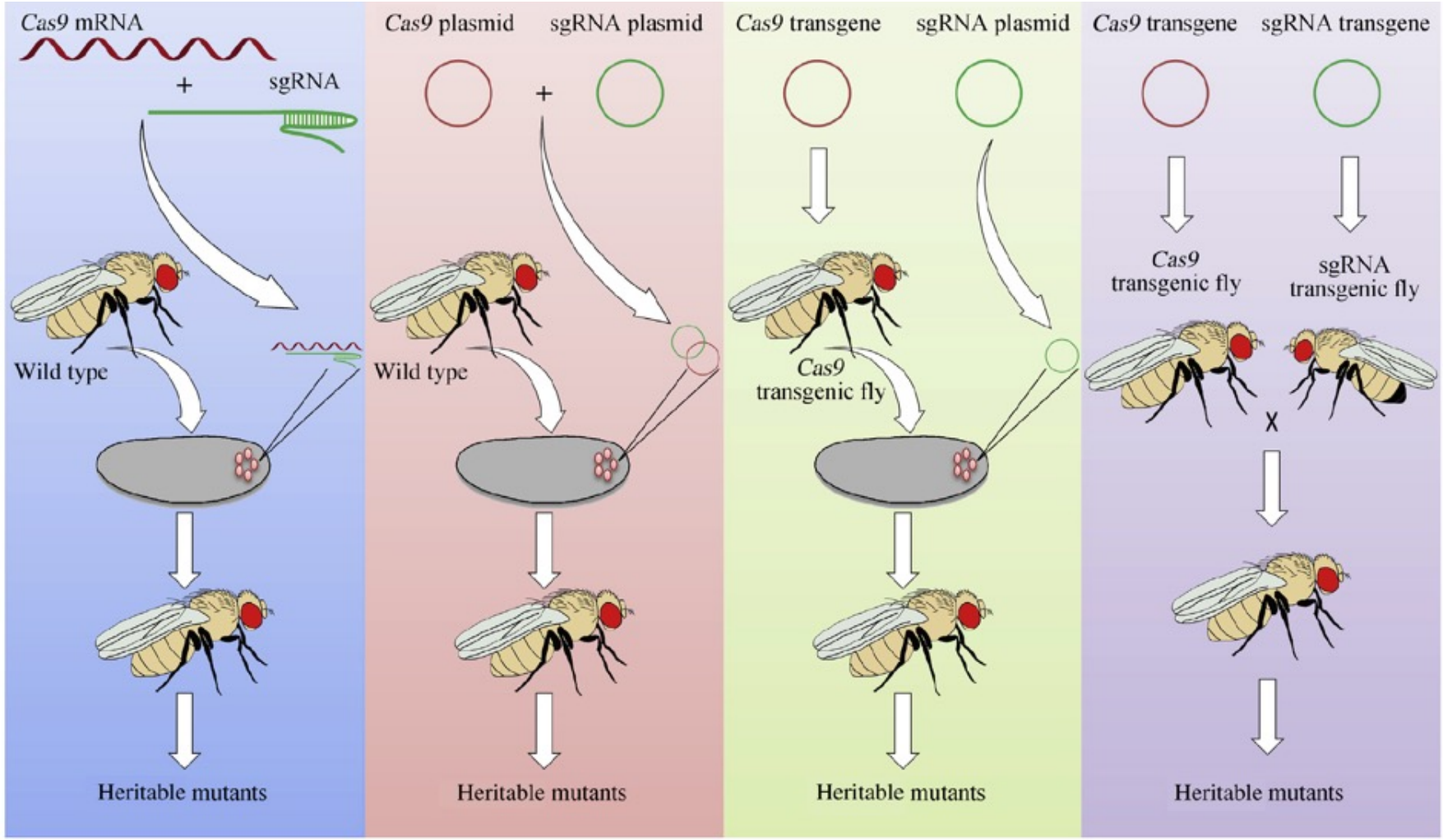
CRISPR

- Based on the bacterial CRISPR/Cas9-based defense system:
  - Enzyme Cas9
  - Target sequence specific sgRNA



Xu et al. (2015), JGG 42, 141

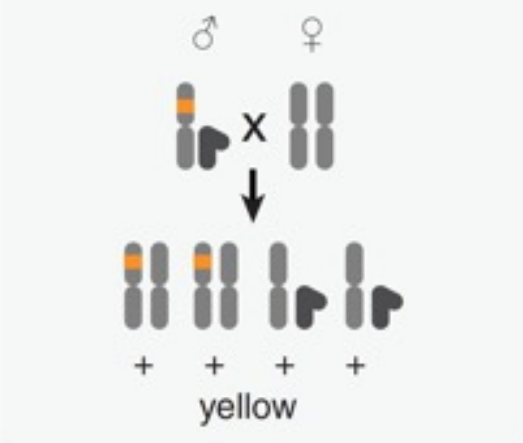
CRISPR



Xu et al. (2015), JGG 42, 141

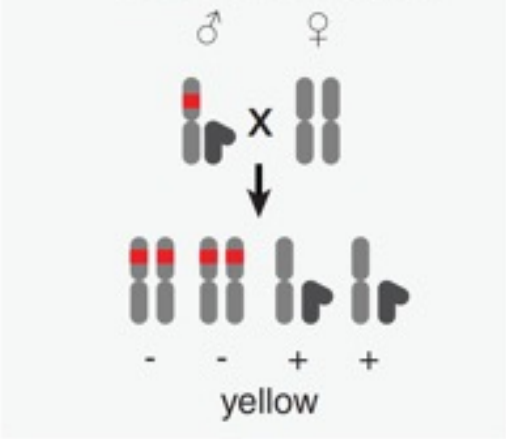
CRISPR: Gene Drive

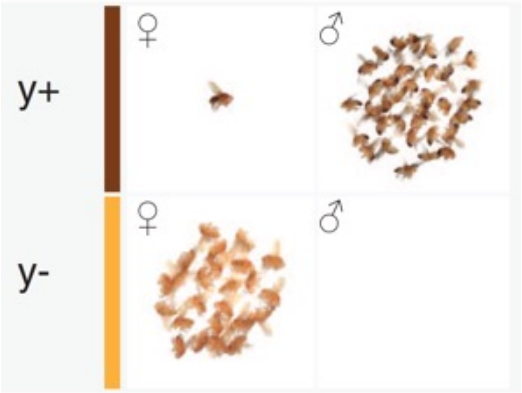
Mendelian inheritance

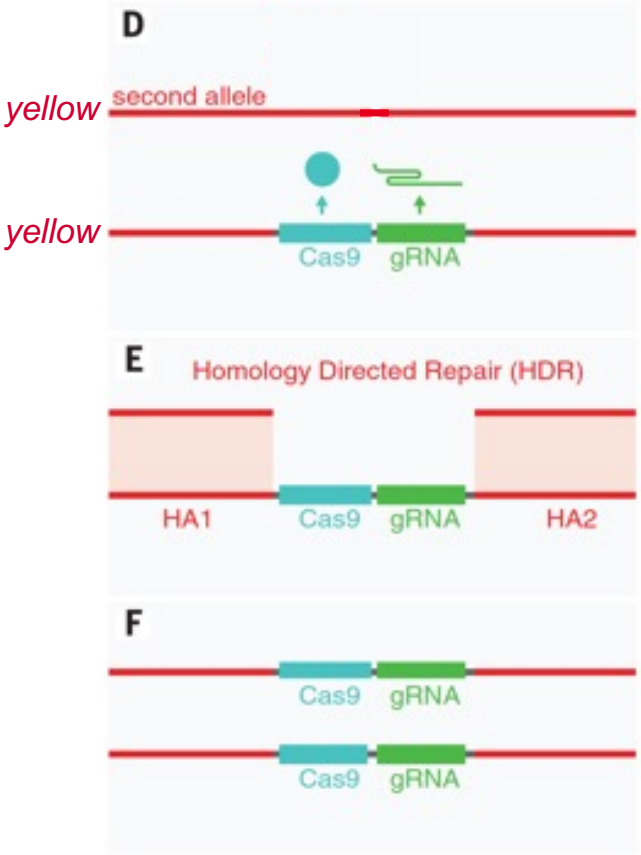


y - *y* +

Gene drive







Gantz & Bier (2015), Science 348, 442

CRISPR: Gene Drive

Mendelian inheritance

+/- * +/+ → +/- (50%), +/+ (50%)
+/- * +/- → +/+ (25%), +/- (50%), -/- (25%)

Gene drive

+/- (→ -/-) * +/+ → -/- (100%)

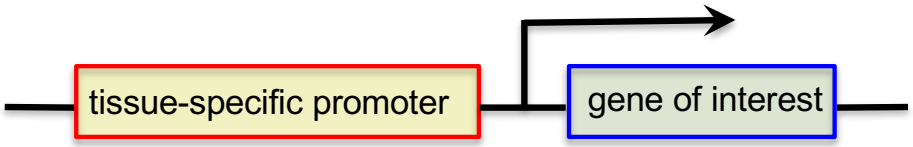
Gantz & Bier (2015), Science 348, 442

Methods

- Analysis:
  - Microscopy
  - Electrophysiology
  - Behavioural studies
  - Physiology
  - ...
- Genetics:
  - Transgenes
  - CRISPR-based mutagenesis/misexpression
  - **Binary overexpression**

Overexpression

Traditional



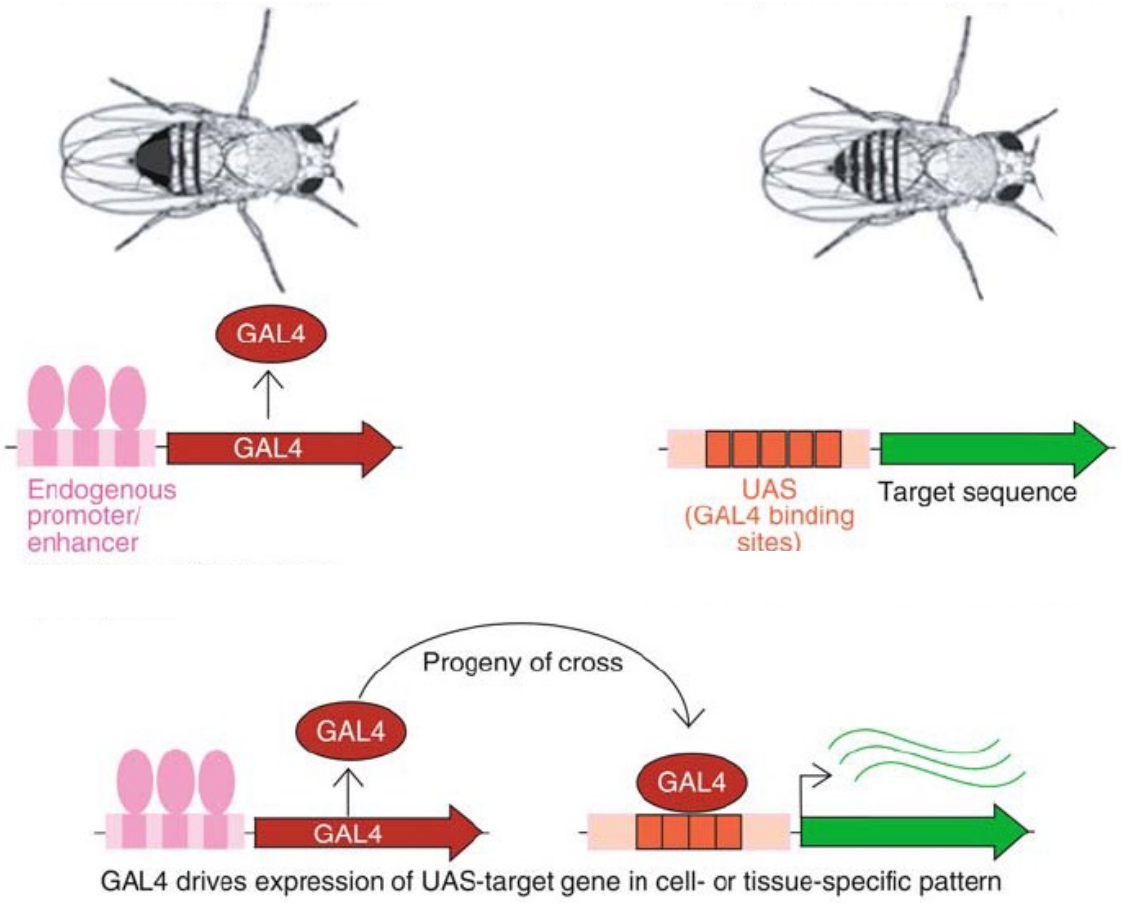
Drosophila

Transcriptional activator from
Saccharomyces cerevisiae

- overexpression of cDNA
- expression of dsRNA → RNAi

Elliott & Brand (2008), in: *Methods in Molecular Biology: Drosophila: Methods & Protocols* (C.Dahmann, ed.).

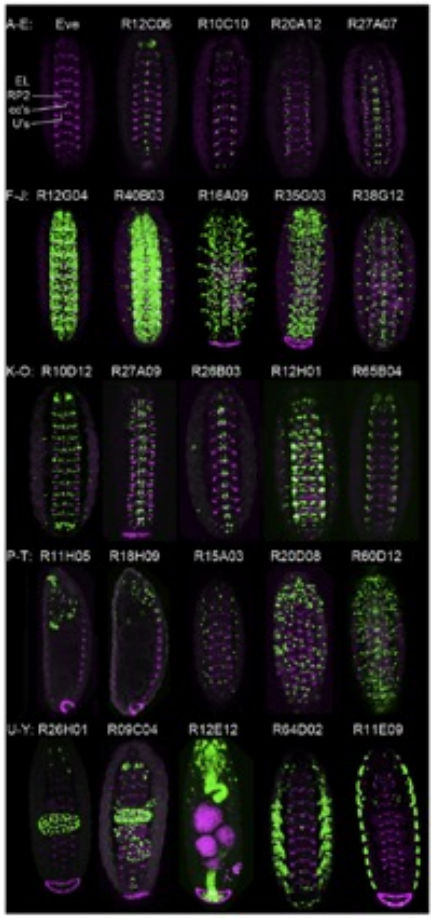
Overexpression



Elliott & Brand (2008)

Overexpression

embryonic CNS



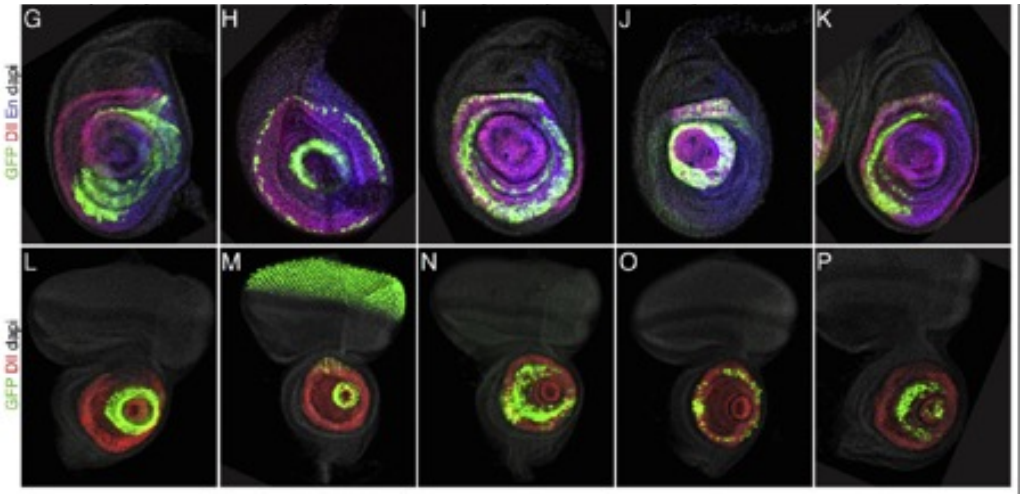
Manning et al. (2012), *Cell Reports 2*, 1002

x-GAL4 x UAS-GFP

- 6'000 transgenic GAL4 lines with different patterns
- many 1'000s in other collections

→ a single UAS-transgene can be expressed in **many temporal & spatial patterns.**

larval imaginal discs



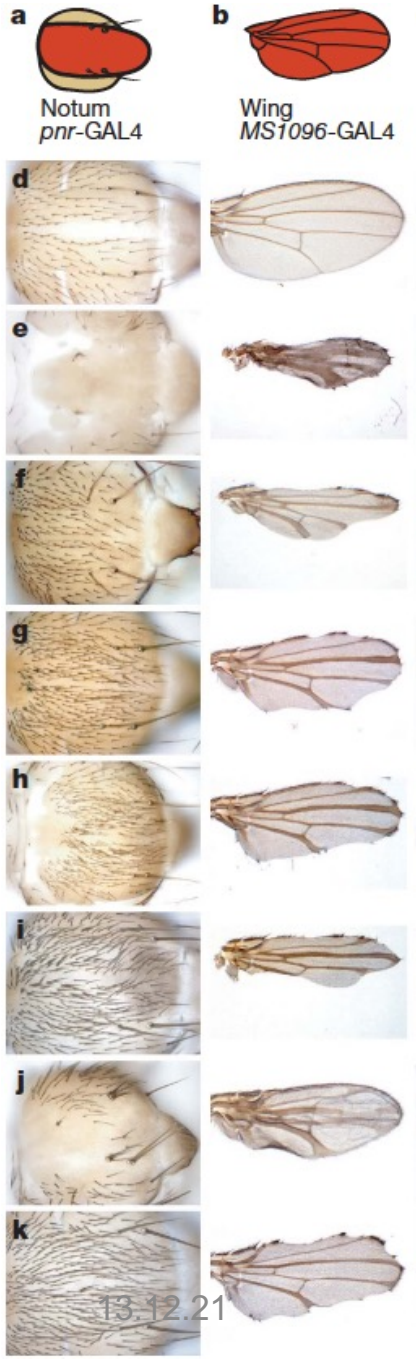
Jory et al. (2012), *Cell Reports 2*, 1014

Genetic screen

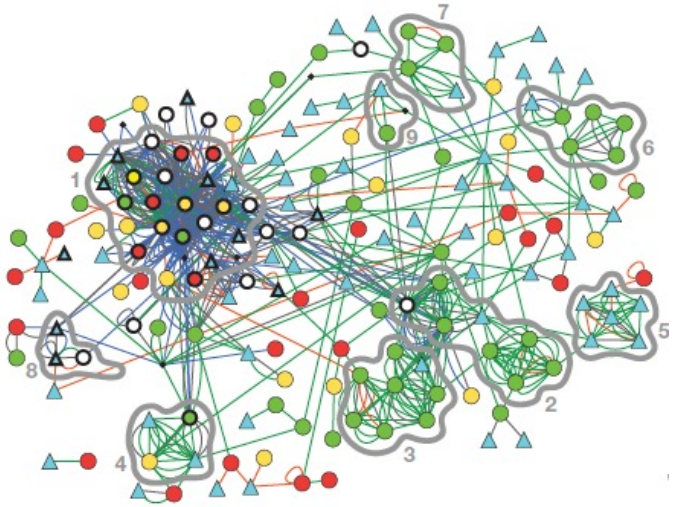
Starting question:
how is a particular process controlled (e.g. „organ growth“)?
which genes control this process?

small head normal head

Approach:
knock-down / knock-out one gene after another & analyze
the effects on this particular process



Overexpression



UAS-dsRNA collection

- >30'000 different lines
- Targets every gene (≥ 1x)
  → **genetic screen**

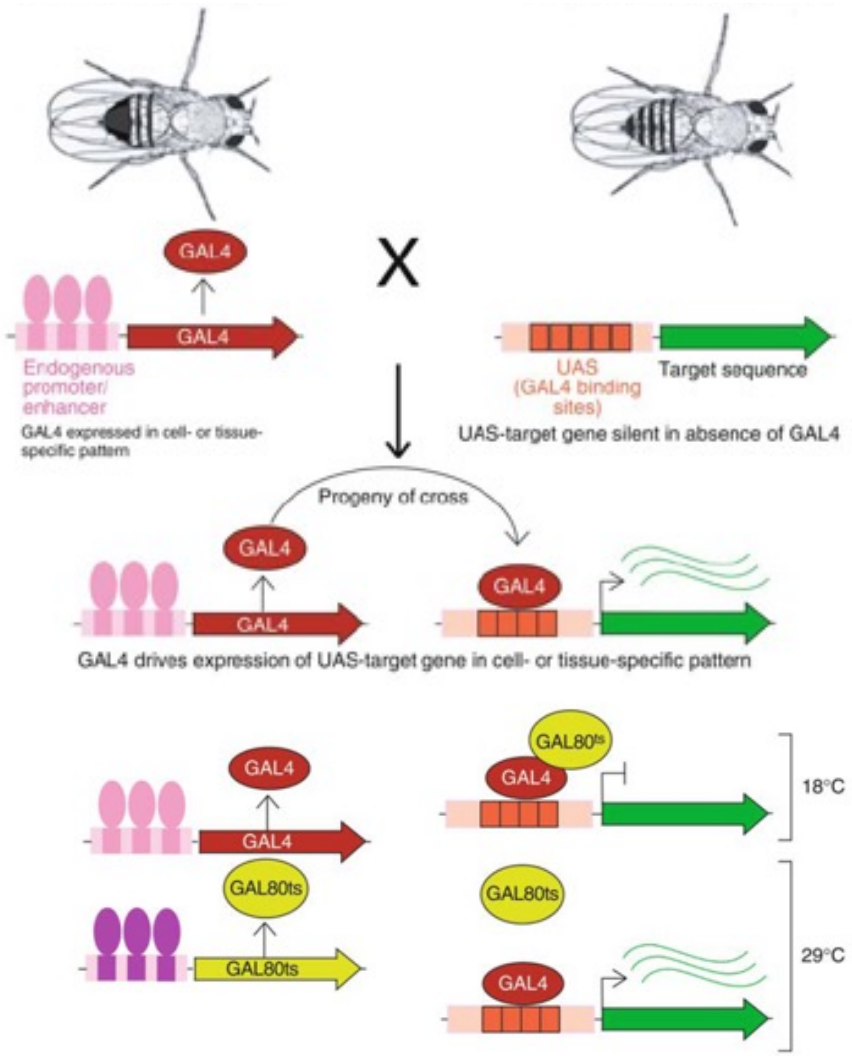
UAS-miRNA collection

UAS-TF collection:

- Overexpression of all transcription factors

Mummery-Widmer et al. *(2009), Nature* 458, 987.

Overexpression



→ temporal & spatial control

Elliott & Brand (2008)

Overexpression

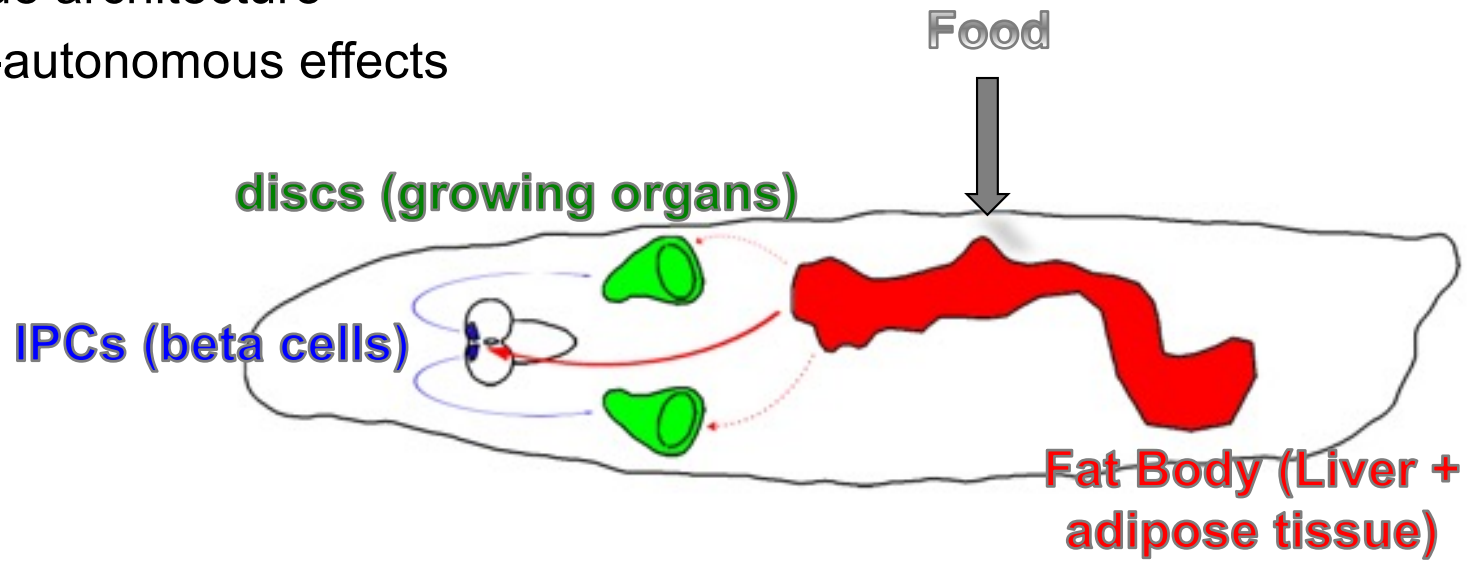
- GAL80 –l GAL4 → UAS
- QS –l QF → QUAS

[Potter et al. (2010), *Cell 141*, 536]

Logic Gate	A-GAL4 B-QF	Additional Transgenes Required
A OR B		UAS-R, QUAS-R
B NOT A		UAS-QS, QUAS-R
A NOT B		QUAS-GAL80, UAS-R
A AND B		1) UAS-FLP, QUAS><stop>R 2) QUAS-FLP, UAS><stop>R
NOT A		UAS-QS, QUAS-R, (B= tubP)
NOT B		QUAS-GAL80, UAS-R, (A= tubP)
A → B		tubP>QF> , UAS-FLP, QUAS-R
B → A		tubP>GAL4>, QUAS-FLP, UAS-R
A XOR B		UAS-QS, QUAS-GAL80, UAS-R, QUAS-R
A NOR B		tubP>R> , UAS-FLP, QUAS-FLP
A NAND B		tubP>R>QF, QUAS-FLP, UAS-QS, QUAS-R

Example: *in vivo* genetic screen

- RNAi in vivo:
  - Tissue architecture
  - Non-autonomous effects



fb-GAL4 UAS-RFP UAS-RenLuc

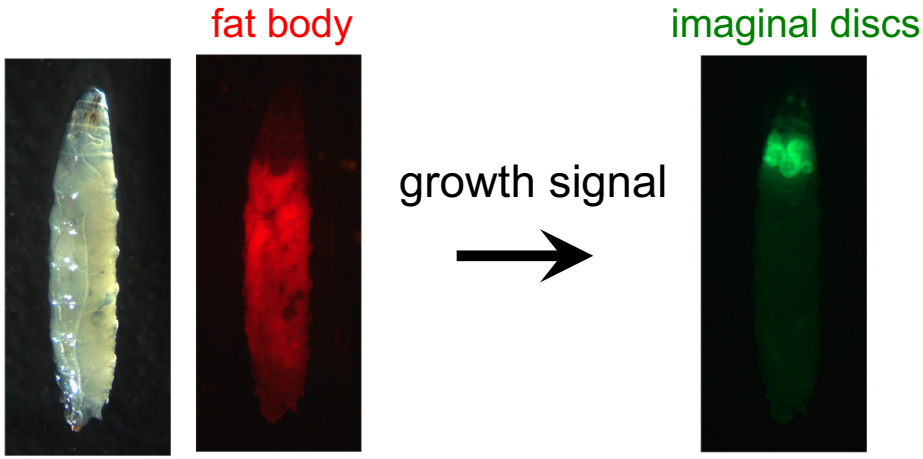
id-QF QUAS-GFP QUAS-FireLuc

x 1'600 different UAS-dsRNA lines

Carreno et al., submitted

Example: *in vivo* genetic screen

- RNAi in vivo:
  - Tissue architecture
  - Non-autonomous effects



fb-GAL4 UAS-RFP UAS-RenLuc

id-QF QUAS-GFP QUAS-FireLuc

x 1'600 different UAS-dsRNA lines

Carreno et al., submitted

Considerations: RNAi screens

- Conceptual limitations of RNAi:
  - Off-target effects
  - Efficiency is variable and rarely 100%
    - Bad: RNAi is not as efficient as a Knockout
    - Good: RNAi is not as efficient as a Knockout
  - Identification of novel gene classes

Methods

- Analysis:
  - Microscopy
  - Electrophysiology
  - Behavioural studies
  - Physiology
  - ...
- Genetics:
  - Transgenes
  - CRISPR-based mutagenesis/misexpression
  - Binary overexpression
  - **Mitotic clones (mosaicism)**

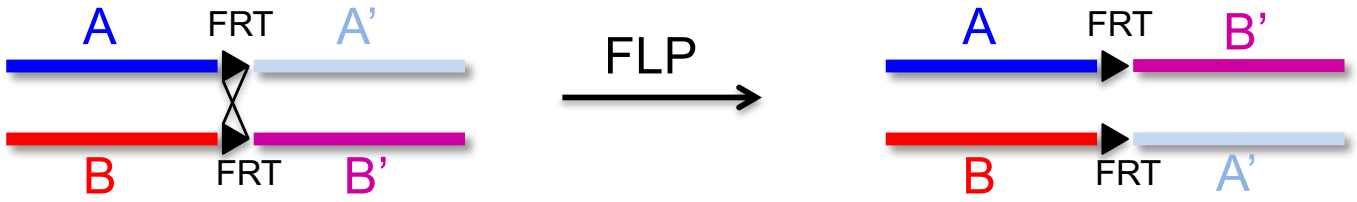
Mitotic clones (mosaicism)

- Mosaicism:
  - Organism is composed of genetically different cells

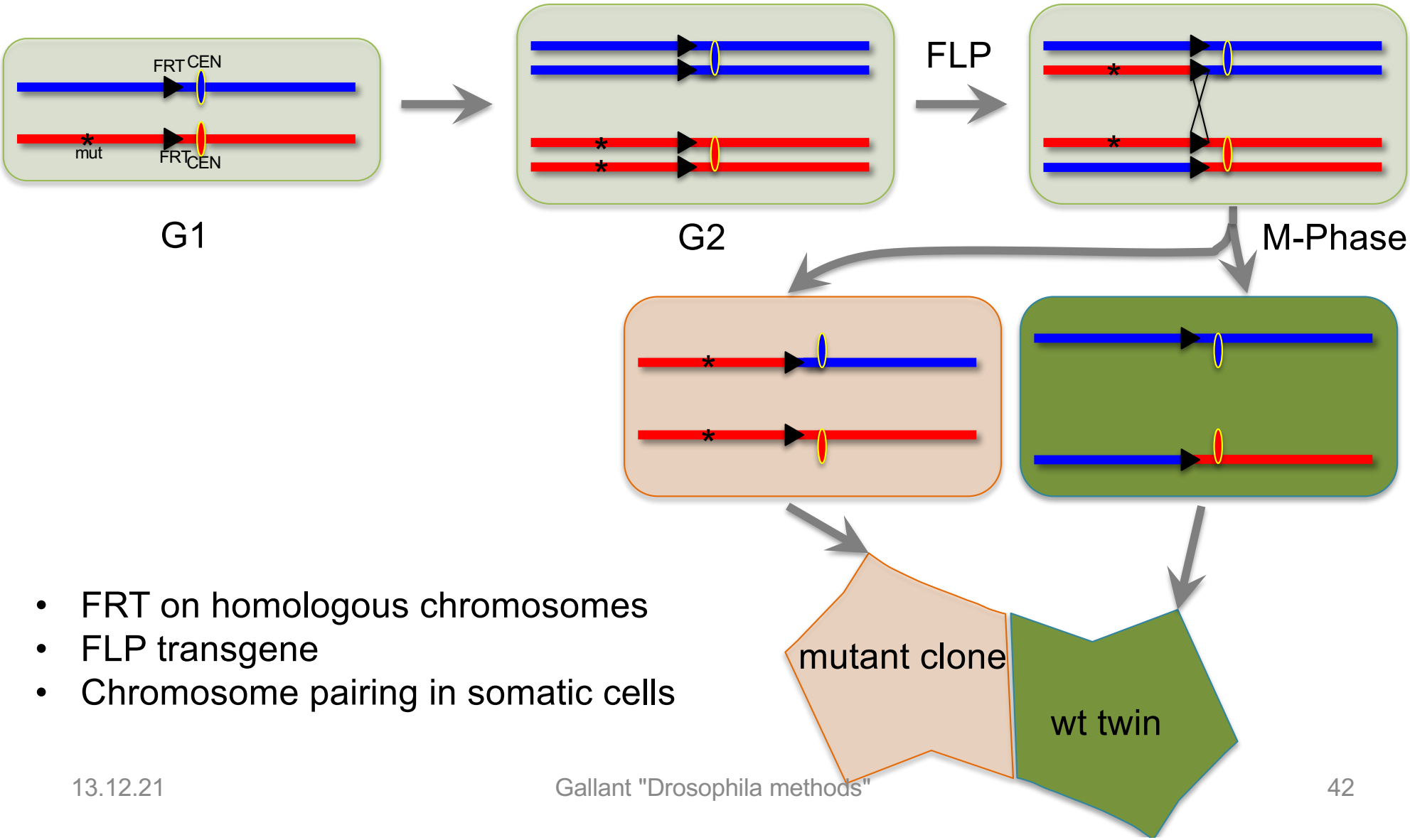
- Applications:
  - Investigation of organismal-lethal genes
  - Cell lineage analysis
  - Examination of cell-autonomy
  - Interactions of different cells
  - …

Mitotic clones

- FRT:
  - ≈70 bp DNA sequence
  - “FLP recombinase target”
- FLP recombinase:
  - Enzyme from *Saccharomyces cerevisiae*
  - Recognizes 2 FRT sequences and catalyses exchange (analogously: ***Cre*** recombinase and ***LoxP*** sites)

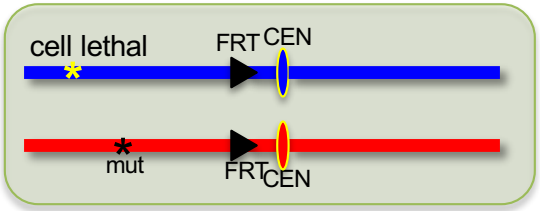


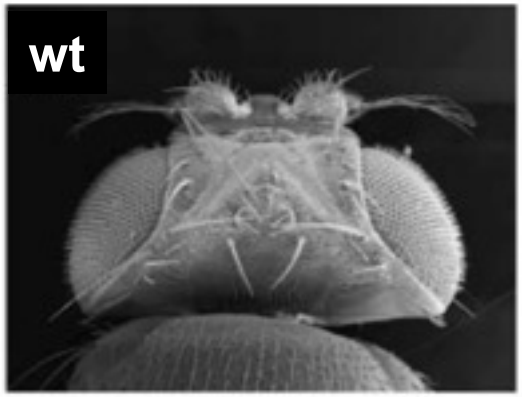
Mitotic clones

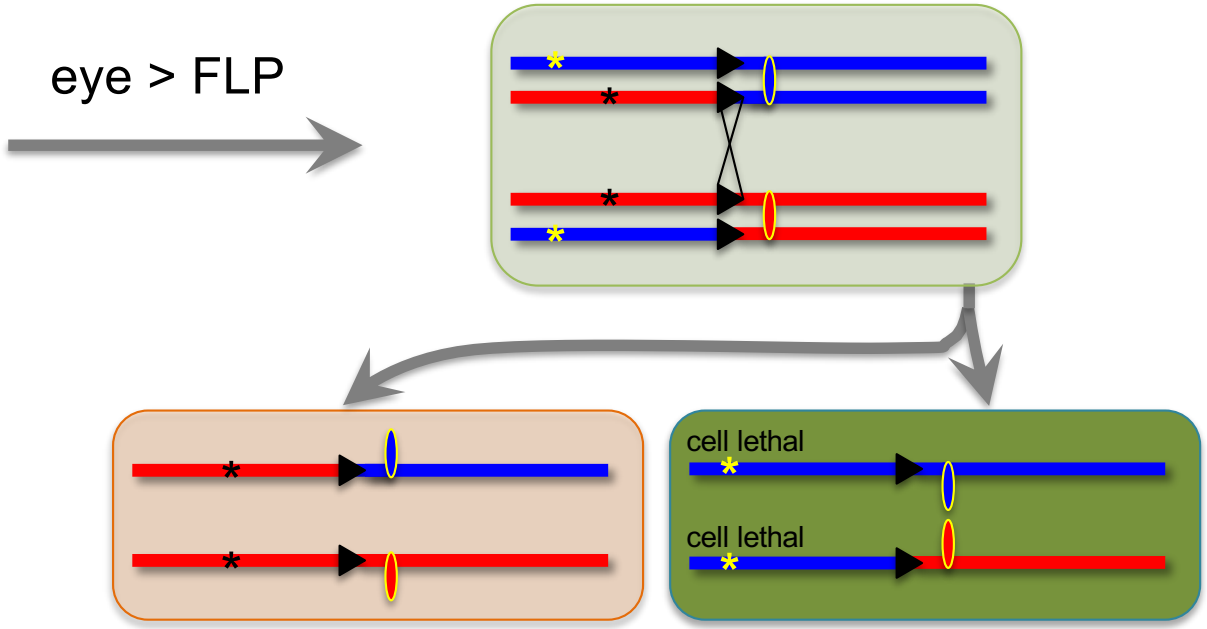


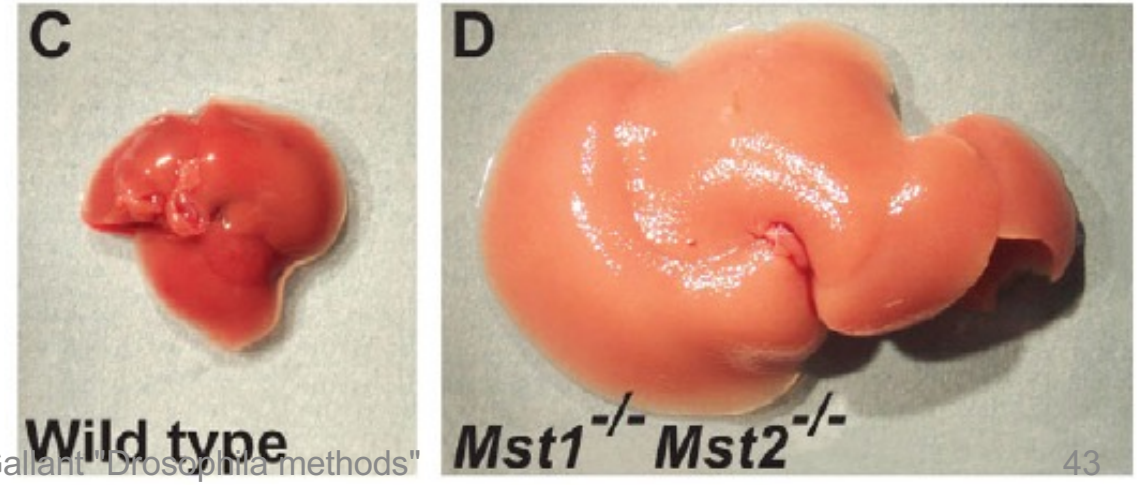
- FRT on homologous chromosomes
- FLP transgene
- Chromosome pairing in somatic cells

Mitotic clones



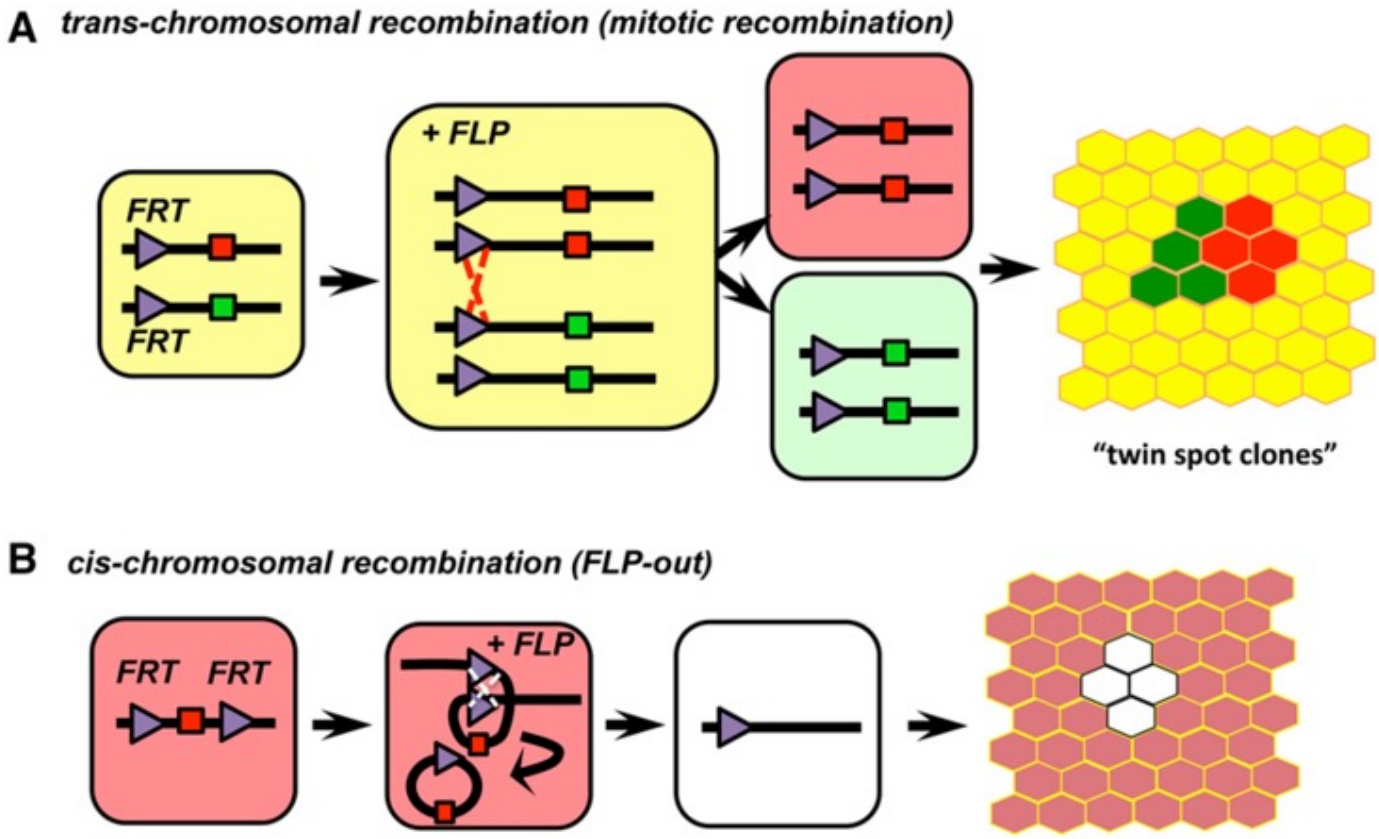






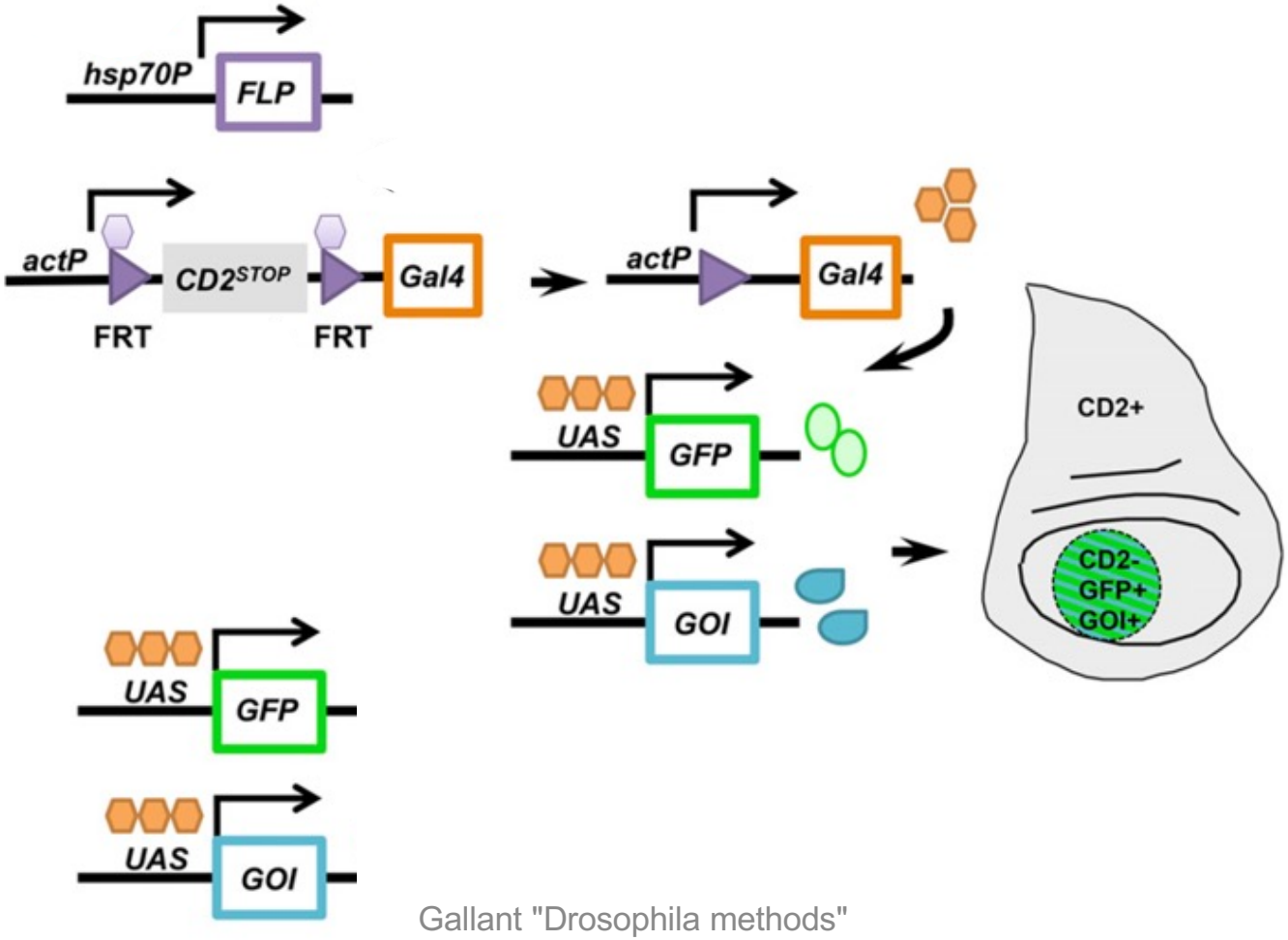
Wu et al. (2003), *Cell 114*, 445.

Clones: cis / trans



Germani et al. 2018

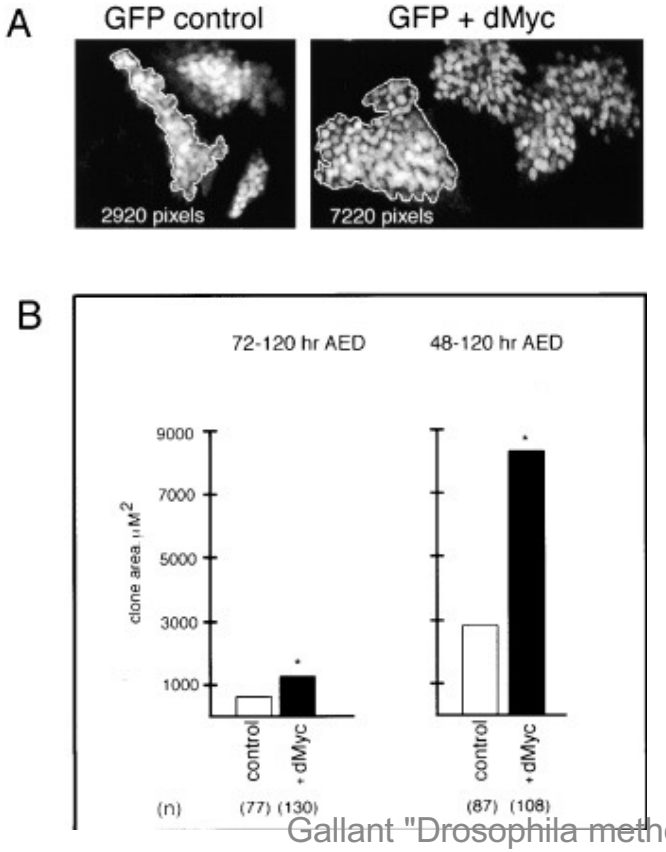
Clones: FLP-out GAL4



Germani et al. 2018

Clones: FLP-out GAL4

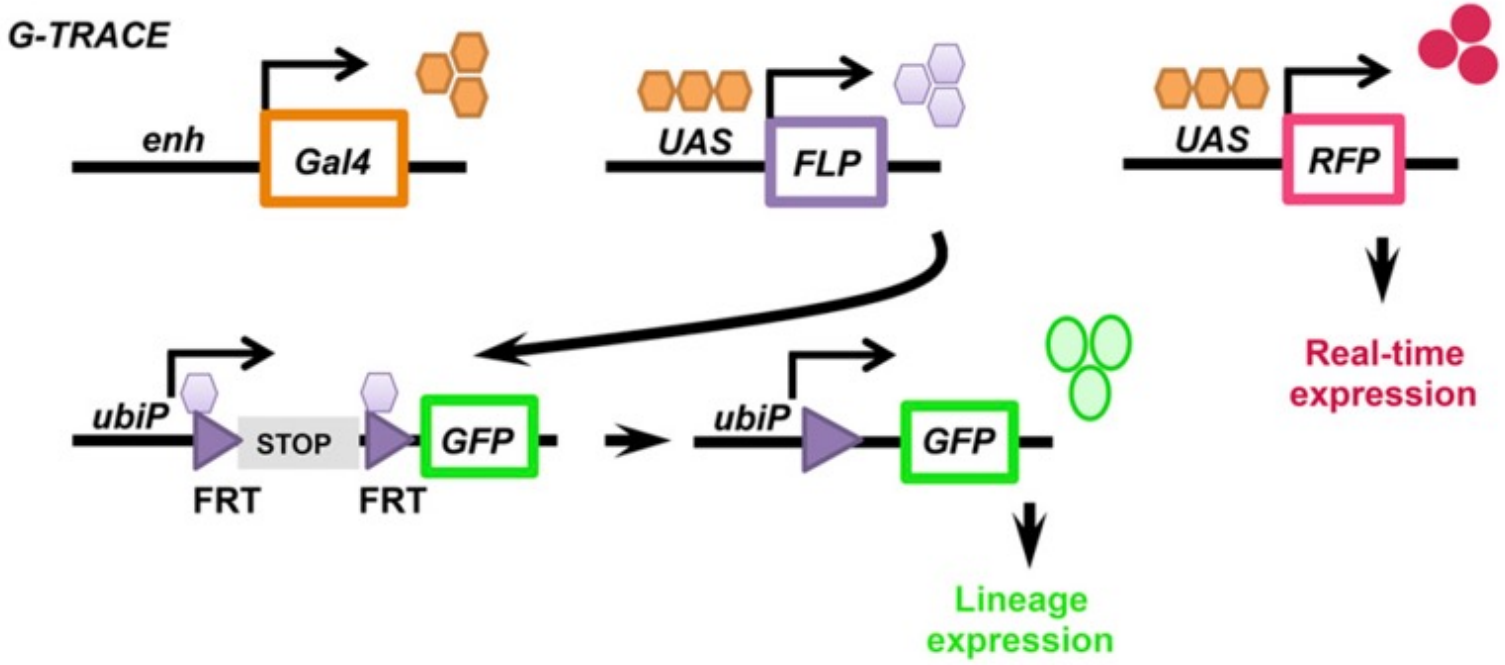
- Following the behaviour of a clone of cells over time:
  - Growth, proliferation, differentiation



Johnston et al. 1999

Germani et al. 2018

Clones: cell/enhancer development



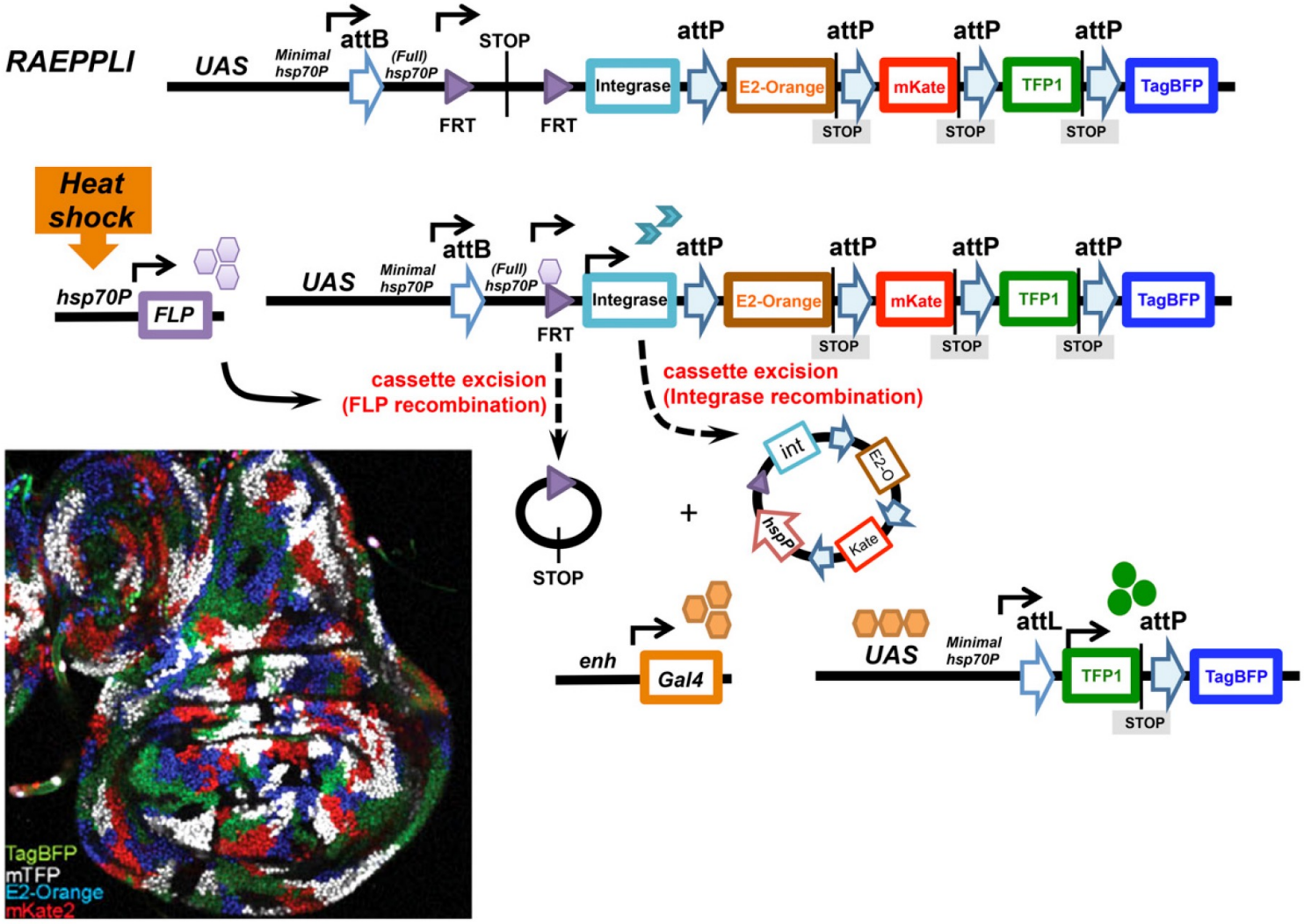
Germani et al. 2018

Clones: cell/enhancer development

- In which cells was the enhancer active at any time of development?
- Where is the enhancer active at the time of analysis?

Germani et al. 2018

Clones: lineage tracing



Germani et al. 2018

Summary *Drosophila*

- *Drosophila* biology
- *Drosophila* genetics
- Methods:
  - Transgenesis
  - CRISPR-based mutagenesis/misexpression
  - Binary overexpression:
    - the GAL4/UAS system
    - GAL4-, UAS-dsRNA, and other collections
    - Other expression systems, combinations
  - Mitotic clones (mosaicism)