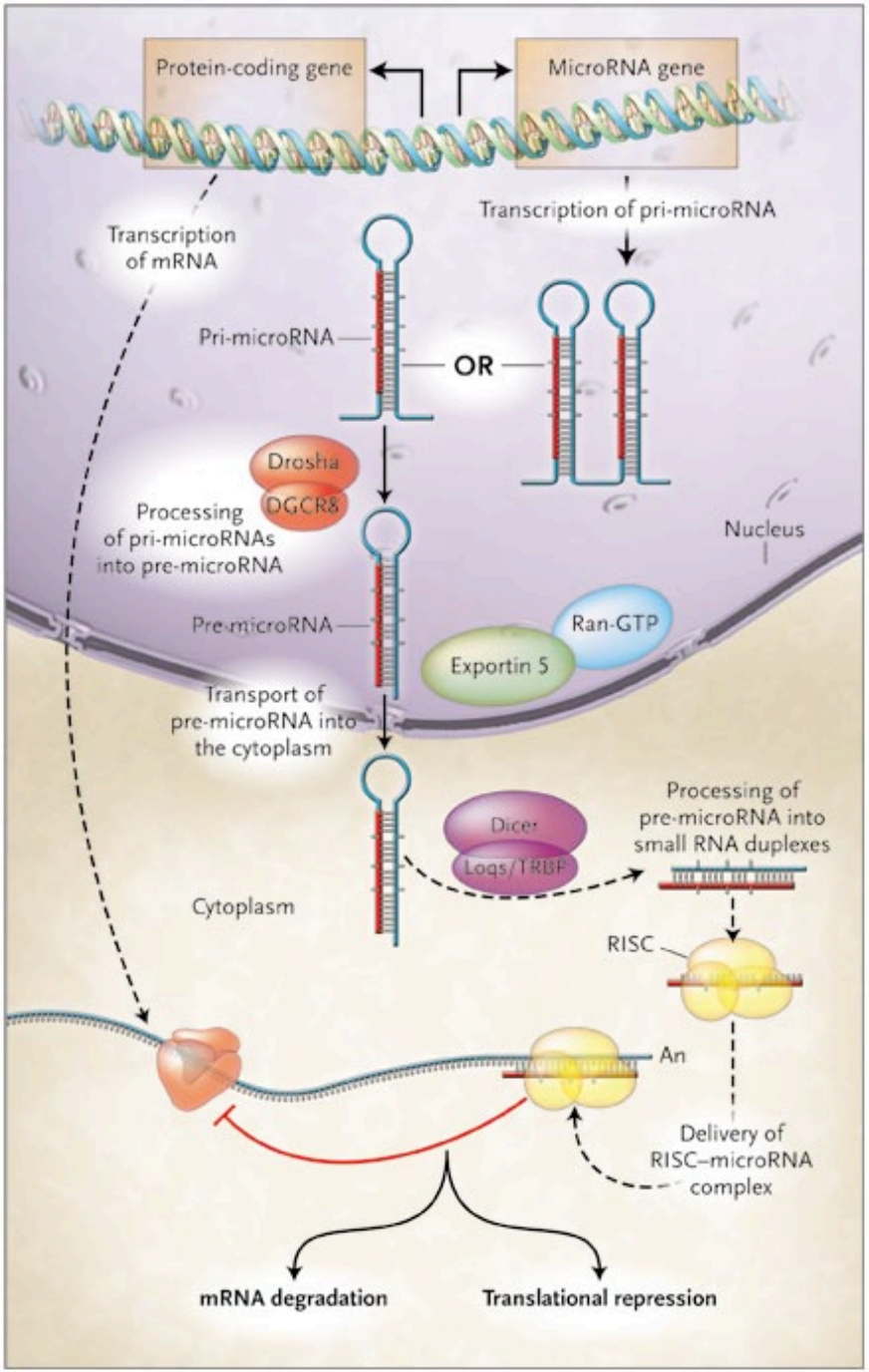

Protein-coding gene
MicroRNA gene
Transcription of pri-microRNA
Transcription of mRNA
Pri-microRNA
OR
Drosha
DGCR8
Processing of pri-microRNAs into pre-microRNA
Nucleus
Pre-microRNA
Ran-GTP
Exportin 5
Transport of pre-microRNA into the cytoplasm
Dicer
Loqs/TRBP
Processing of pre-microRNA into small RNA duplexes
Cytoplasm
RISC
An
Delivery of RISC–microRNA complex
mRNA degradation
Translational repression

RNA interference
post-transcriptional gene suppression (PTGS)

RNAi movie www.nature.com/focus/rnai/animations/index.html

Lecture 19: Drosophila melanogaster

Polytene chromosome

Life cycle

Genetics/Balancer chromosome

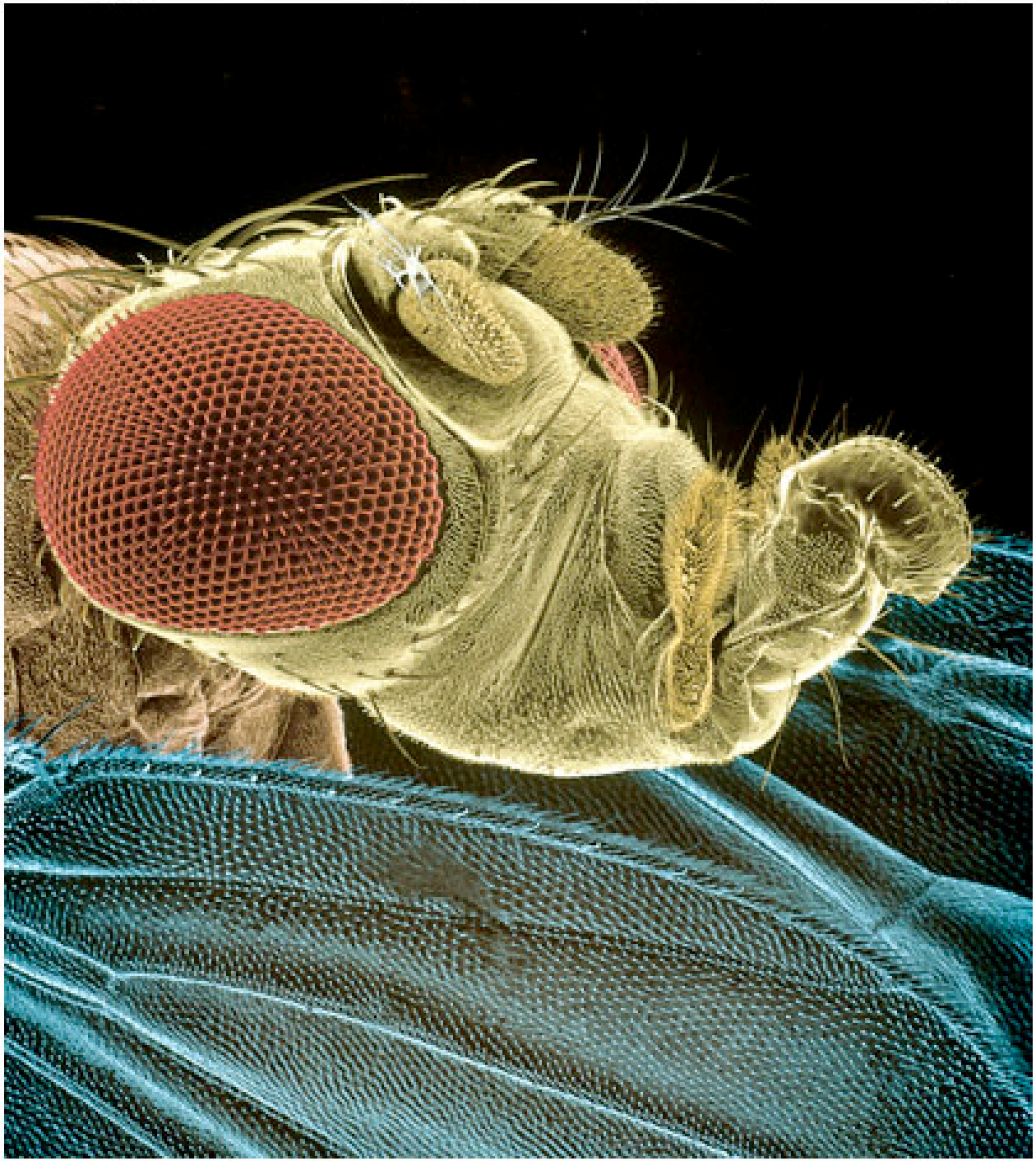
P elements and transformation

Enhancer trap

Genetic mosaic

Read 813-821
Read 140-143
Fig. D1, D3-D10
Fig. 5.25, 5.26

Fig. D.1



Drosophila melanogaster (fruit fly)

Small genome 170 Mb (5% of human genome)

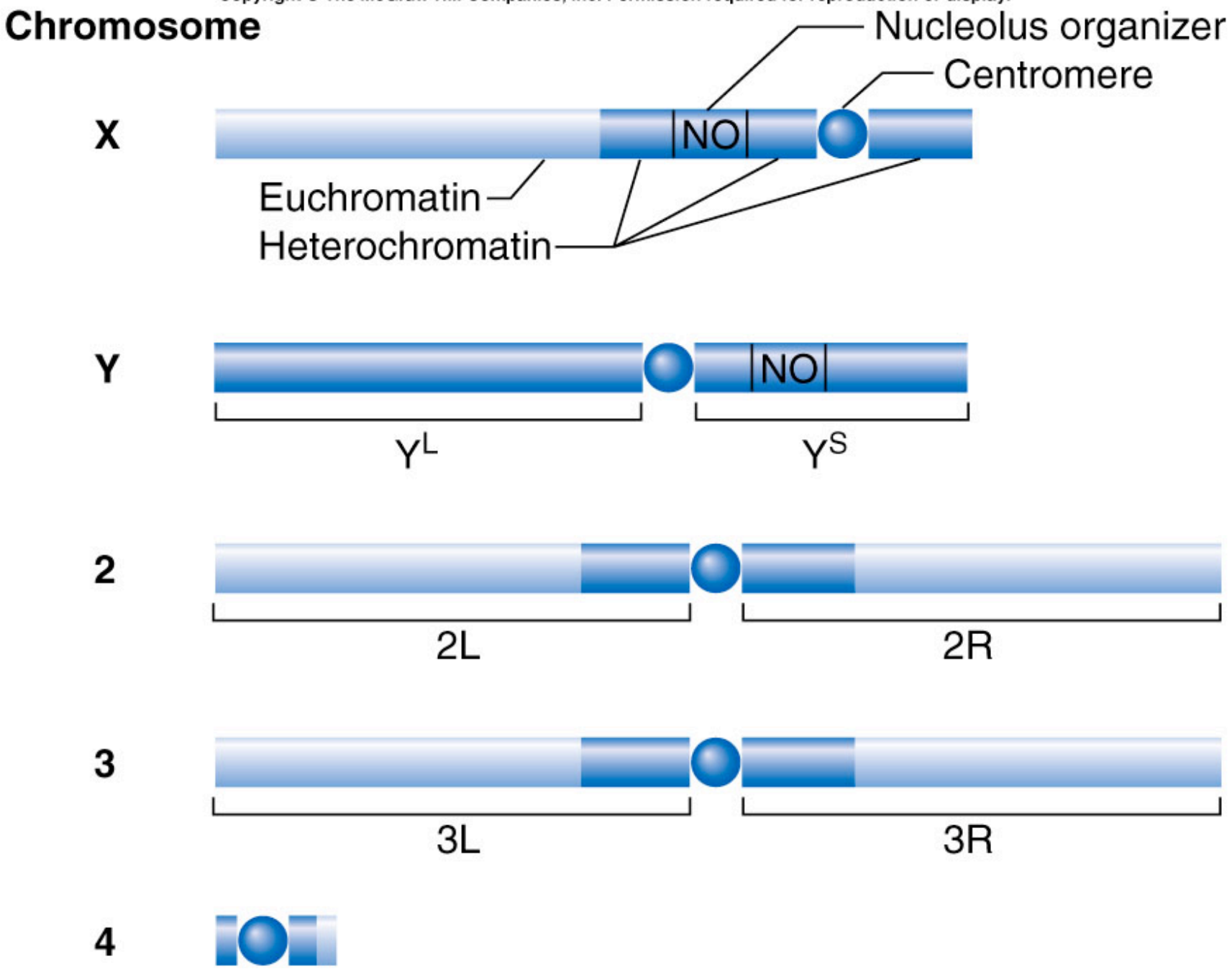
33% repetitive DNA

13,600 genes

Three autosome + X and Y (small chr. number)

Giant polytene chromosome

Fast life cycle (10 days)



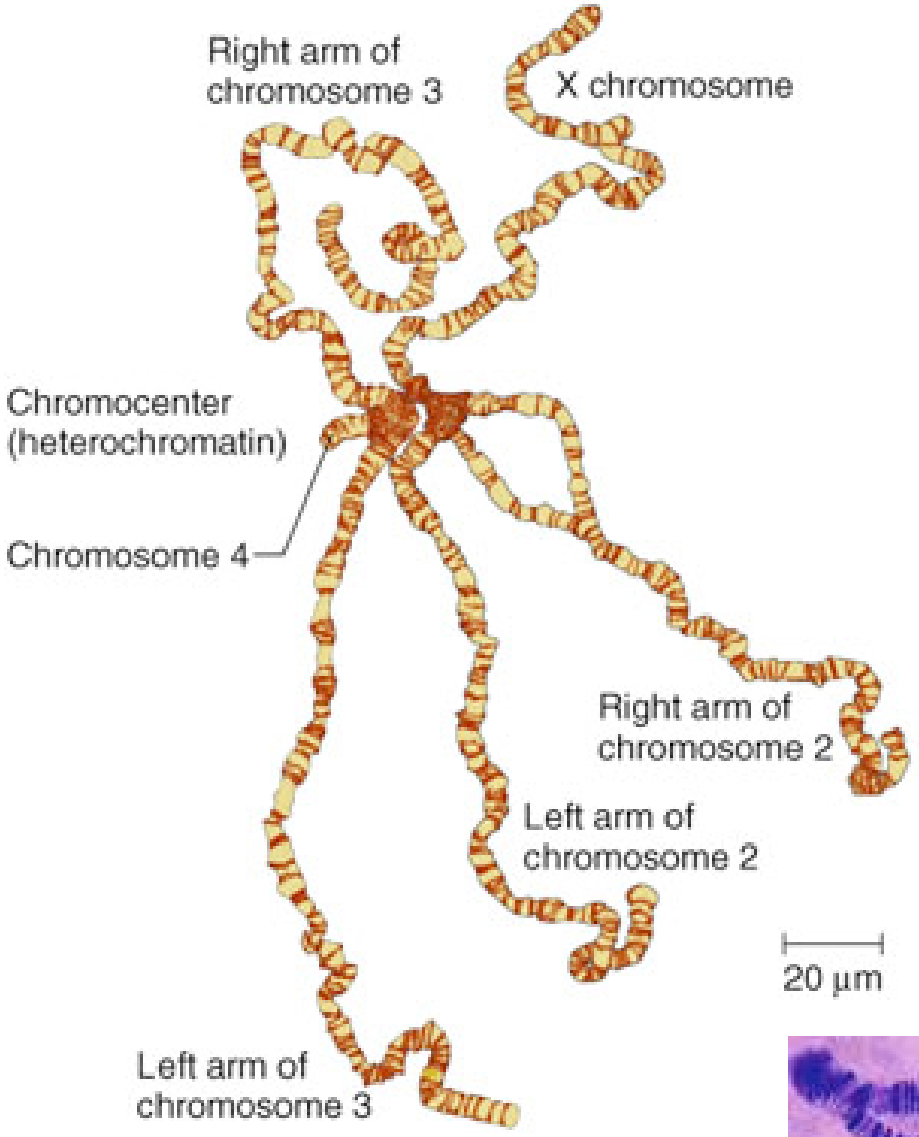


Fig. 12.15

Giant polytene chromosomes of larval salivary gland are key tools

- Replicate 10-11 times
- 1024-2048 sister chromatids stay associated under perfect lateral register
- Homologous chromosome stay tightly synapsed
- Chromocenter – common region where centromeres coalesce

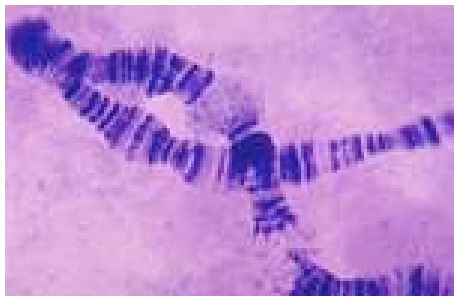
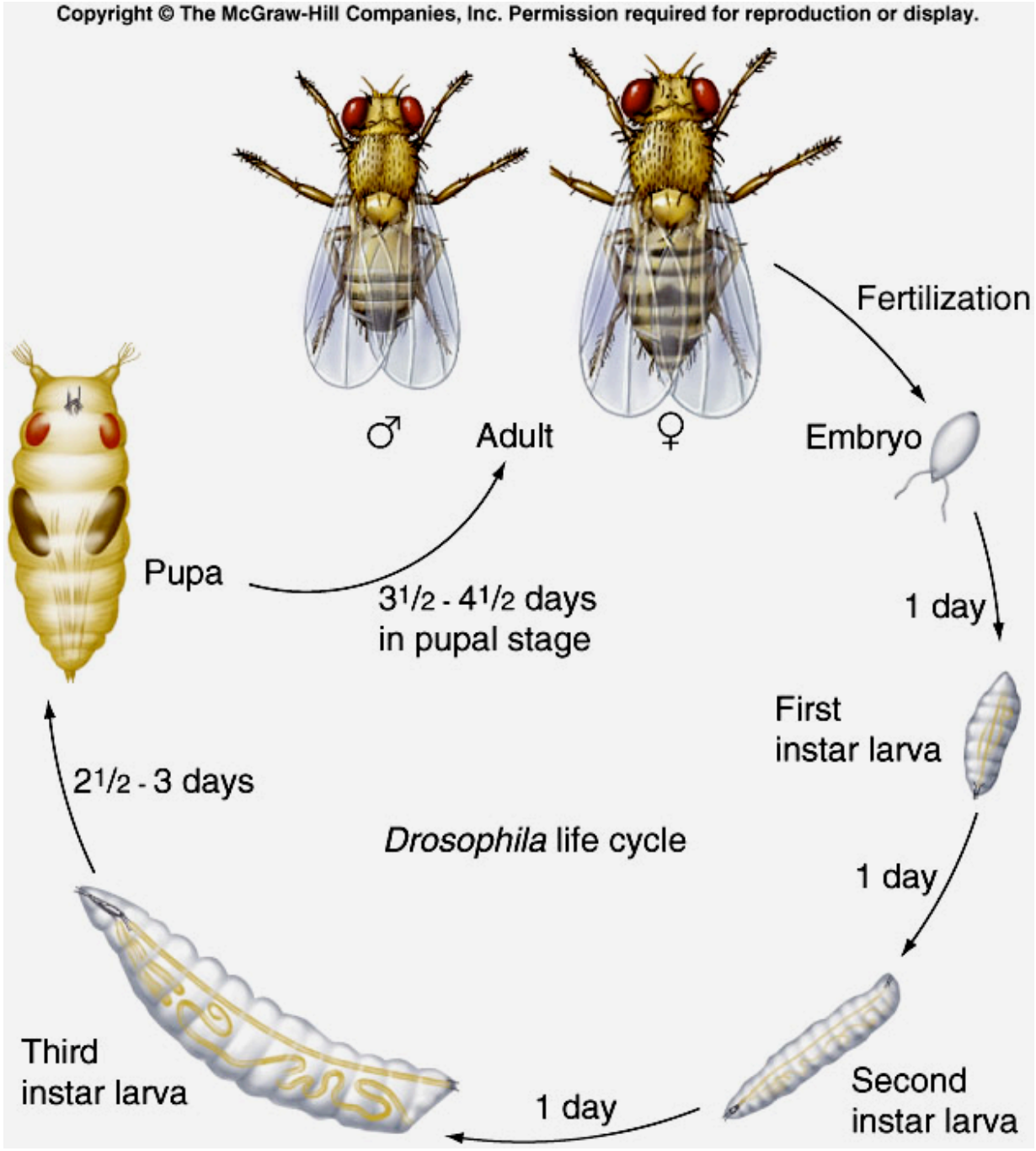


Fig. D.4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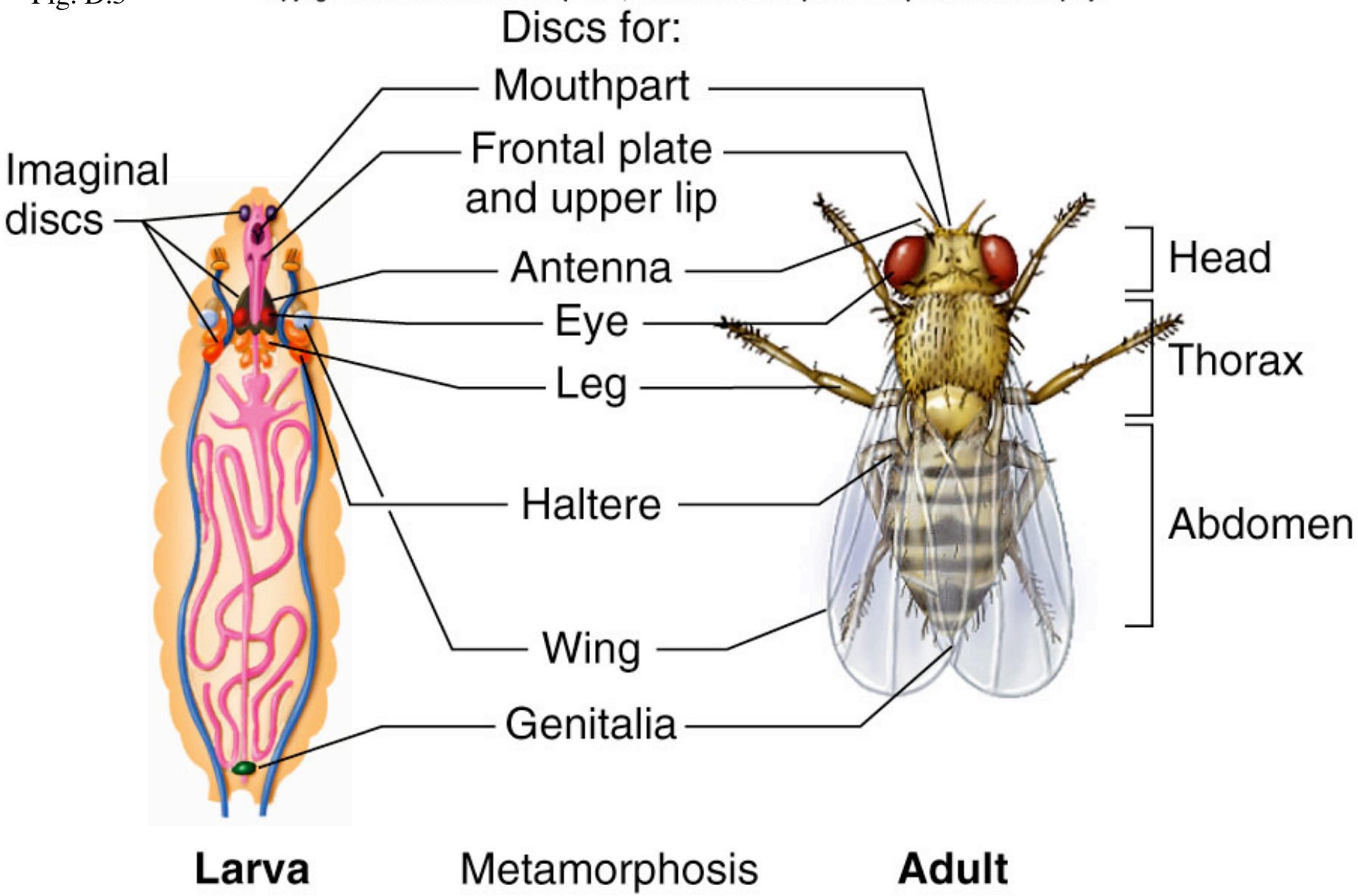
♂
Adult
♀
Fertilization
Embryo
1 day
First instar larva
1 day
Second instar larva
1 day
Third instar larva
2½ - 3 days
Pupa
3½ - 4½ days in pupal stage
Drosophila life cycle

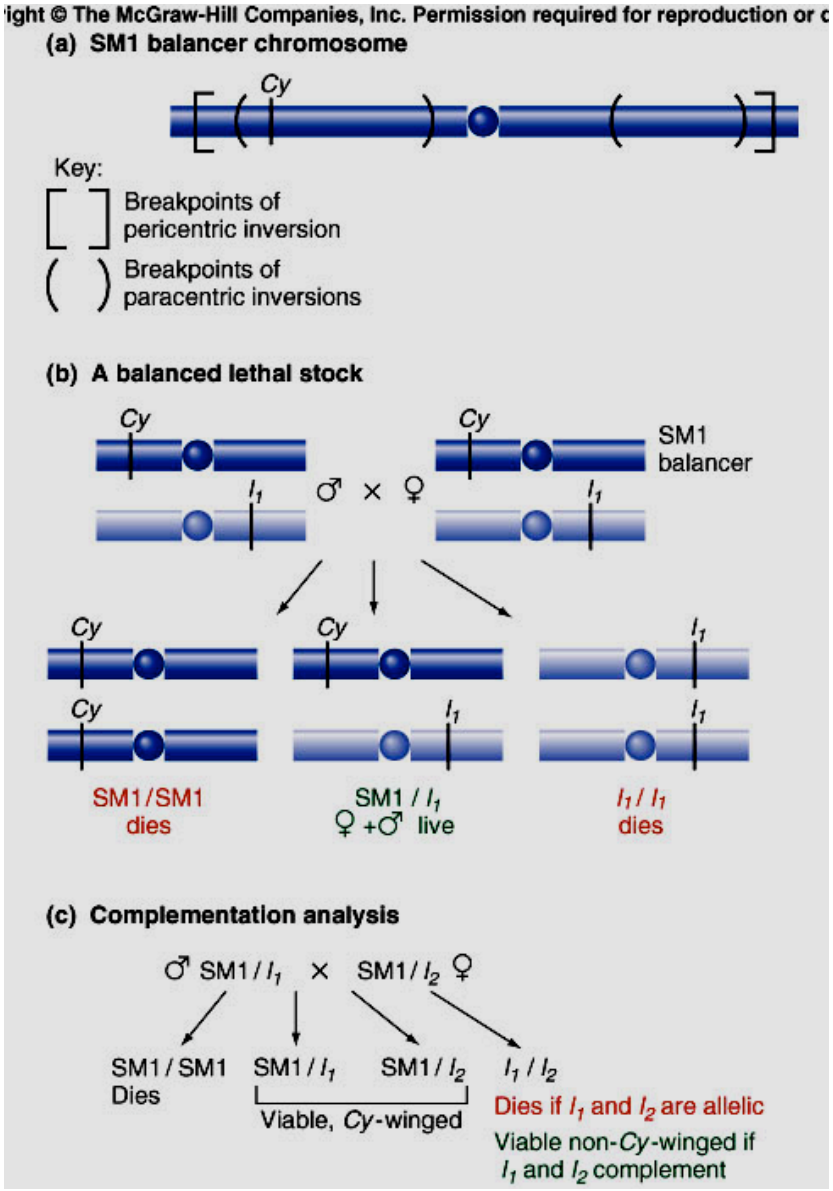
Fig. D.5



Techniques of genetic analysis

- In Drosophila, crossing over occurs only in females
  - Absence of crossing over in males has considerable technical significance
    - Maintain linkage relationships by inheritance through male parent
  - In females, only a moderate amount of crossing over occurs
    - 1-2 crossovers per meiosis
    - No crossing over in heterochromatin or 4^{th} chromosome

Balancer chromosomes help preserve linkage



- Balancers carry multiple, overlapping inversions
- Most contain a dominant marker and recessive lethal mutation that prevents survival of homozygotes
- Useful in genetic manipulations and mutant screens

Fig. D.6

P-element transposons are critical tools in molecular genetics

- Hybrid dysgenesis
  - Males from Drosophila strains carrying P elements crossed to females that lack P elements
  - P element becomes highly mobile in germ line of F1 hybrids
  - Chromosome breakage reduces fertility in hybrids
  - Progeny of F1 flies carry many new mutations induced by P element insertions
  - Molecular details
    - P element primary transcript encodes transposase that catalyzes transposition
    - Cross between P and M strain causes hybrid dysgenesis
    - Cross between P and P strain does not
      - Eggs produced by P female have repressor protein that prevents transposition
      - Repressor coded for by alternatively spliced P element mRNA

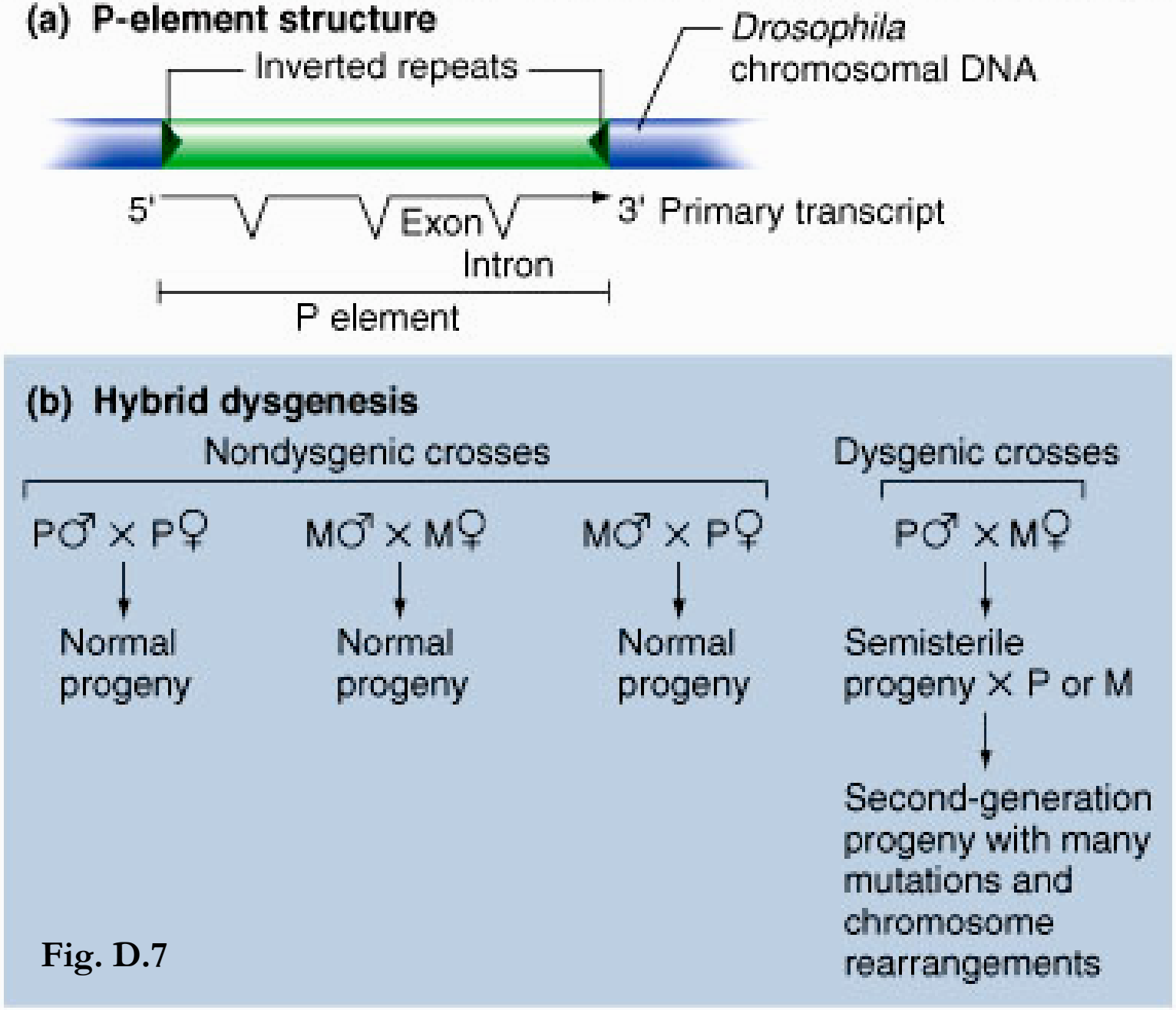
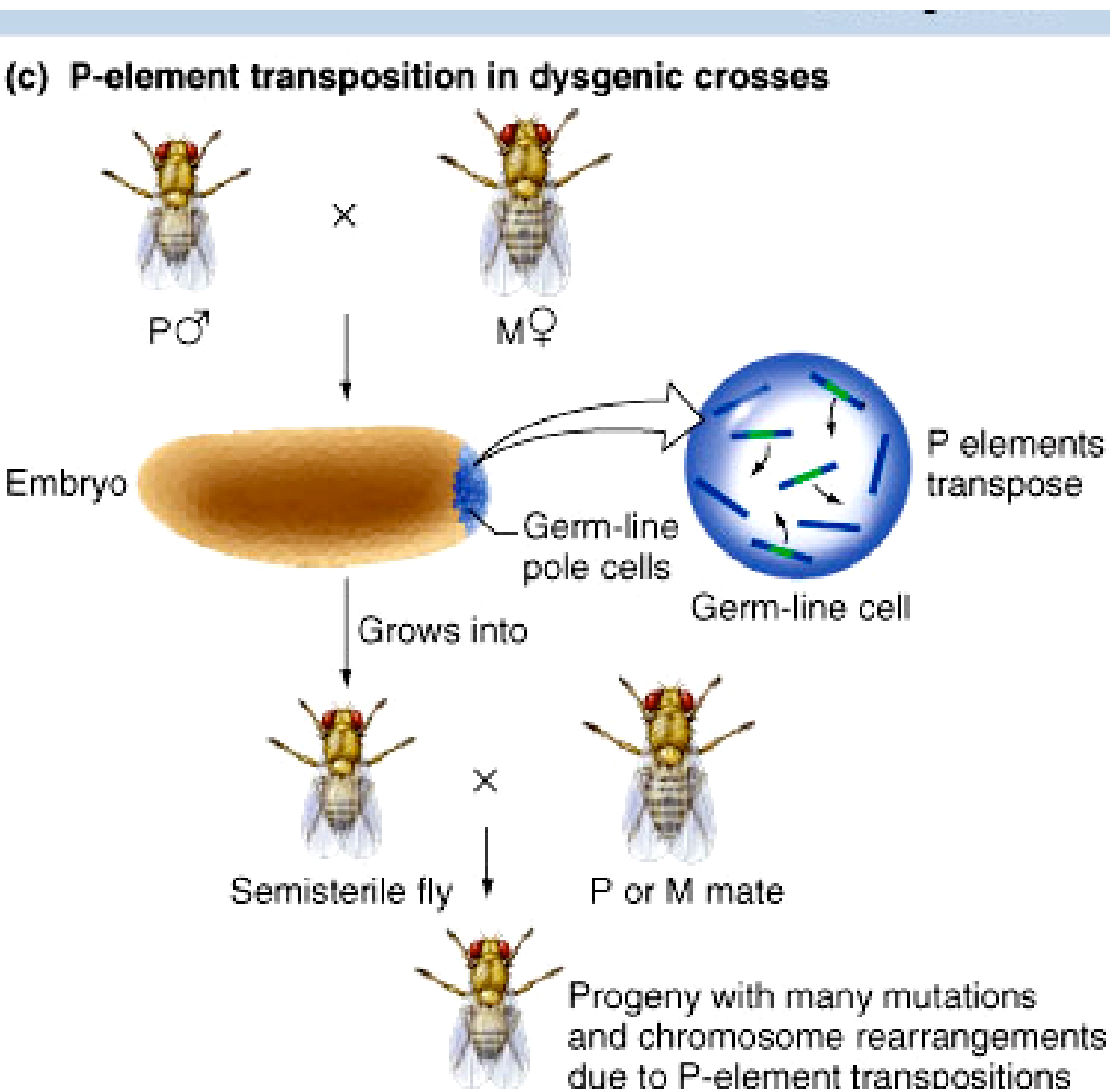
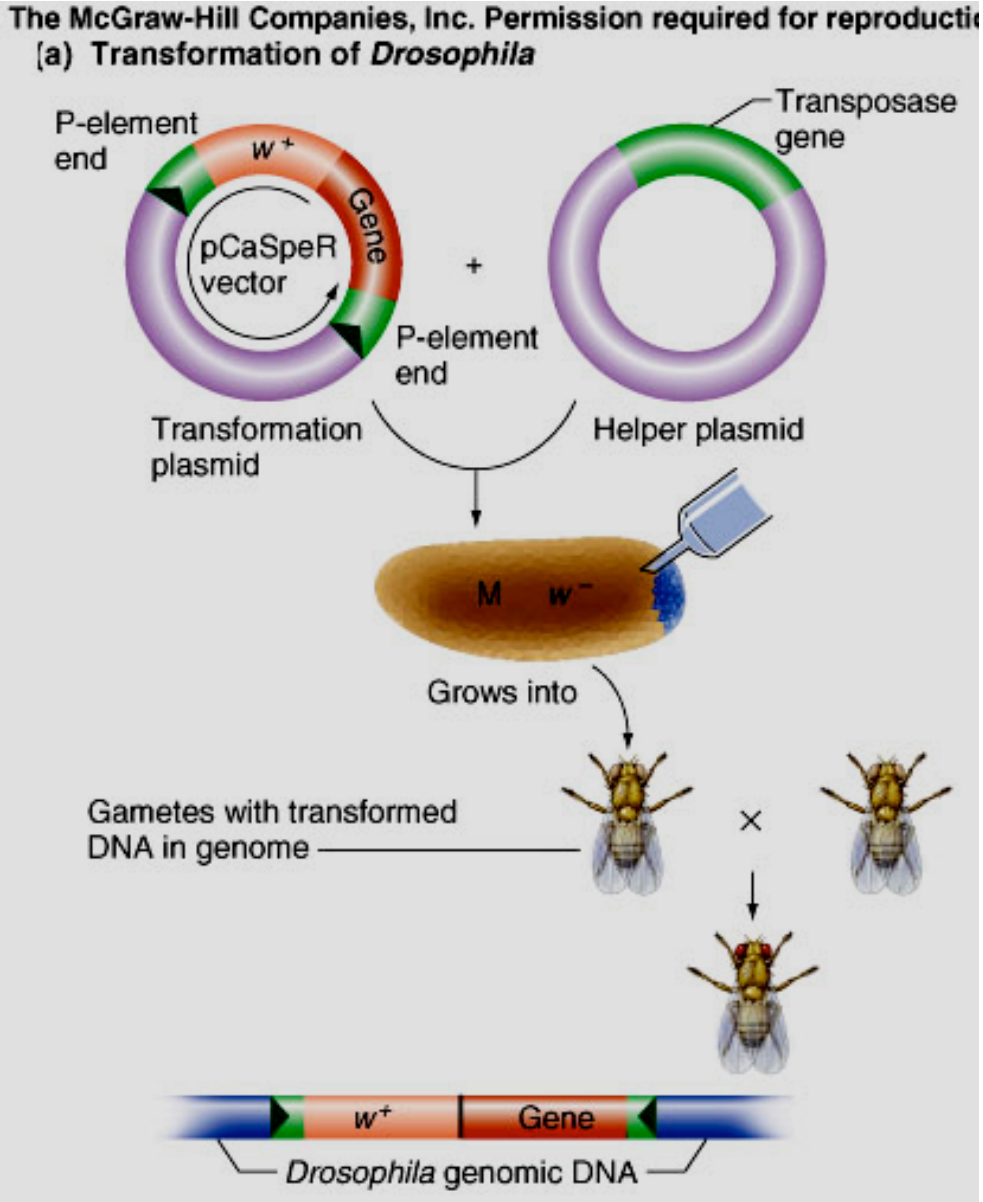


Fig. D.7


(c) P-element transposition in dysgenic crosses
P♂
×
M♀
Embryo
Germ-line
pole cells
P elements
transpose
Germ-line cell
Grows into
Semisterile fly
×
P or M mate
Progeny with many mutations
and chromosome rearrangements
due to P-element transpositions

Transformation: the introduction of cloned DNA into flies



- P-elements used as vectors
- Insert fly DNA into intact P element and then into plasmid
- Inject into syncytial embryos from M strain mothers
- Cross to P males
- Mimicking hybrid dysgenesis

Fig. D.8a

P-element as a tool for mutagenizing and tagging genes

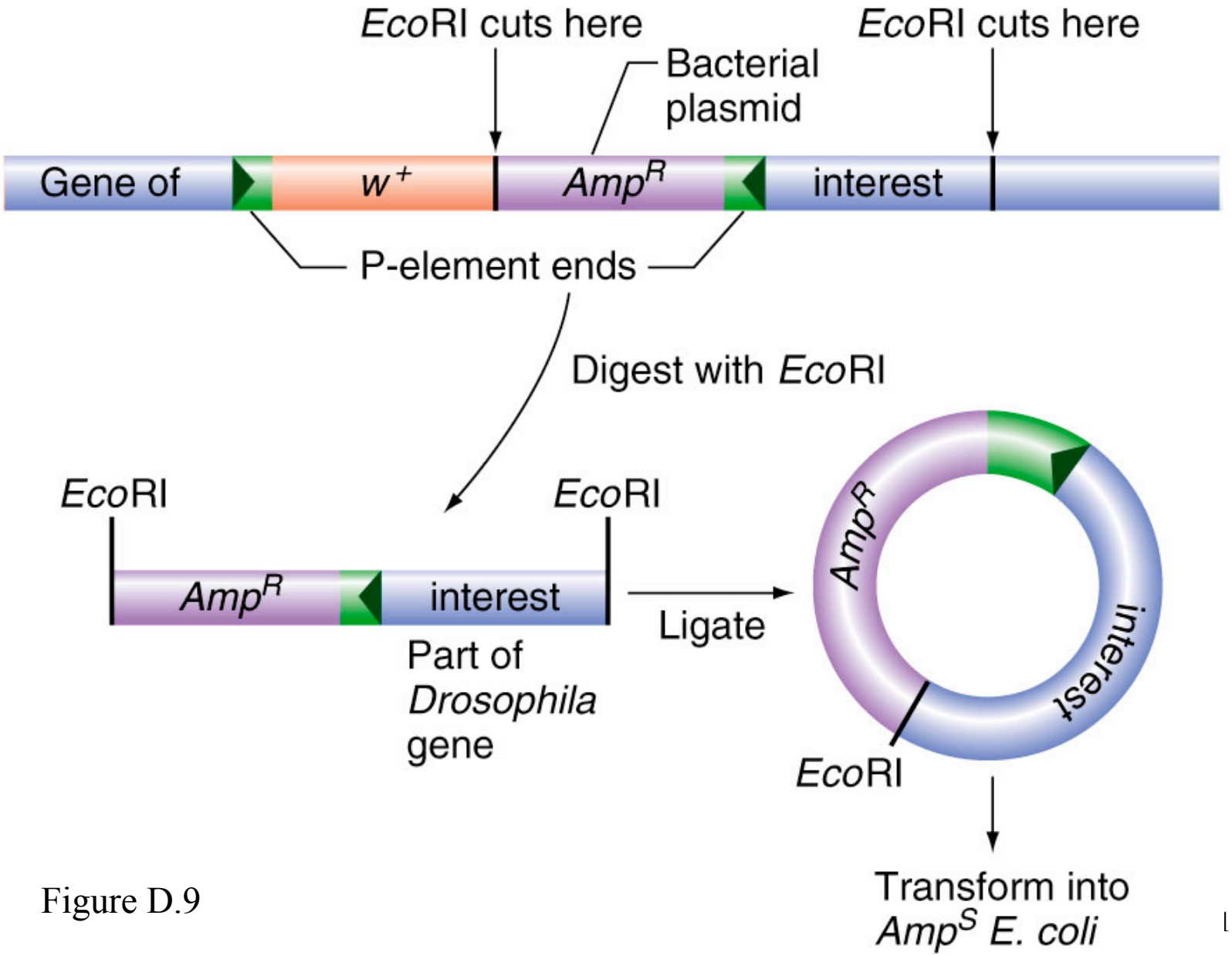


Figure D.9

Enhancer trapping to identify genes by expression pattern

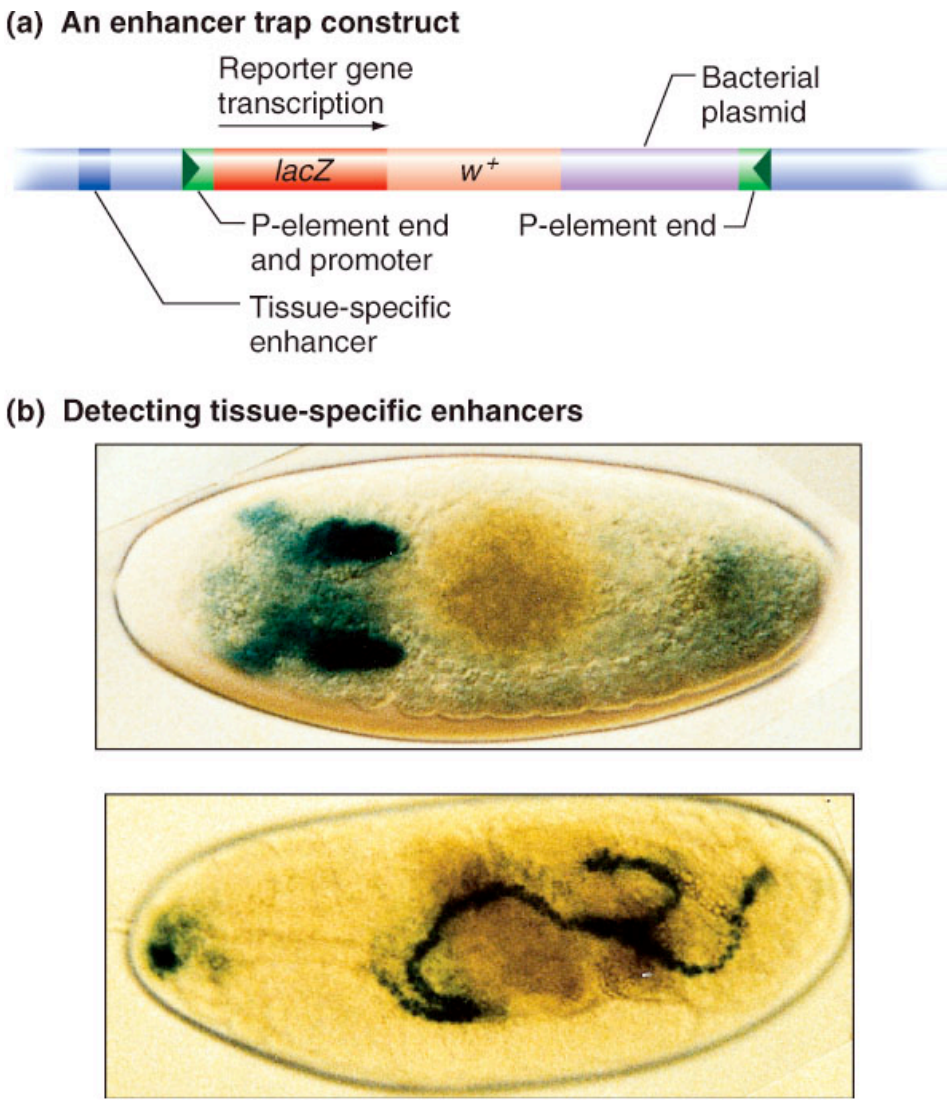


Fig. D.10

- P element with *lacZ* gene downstream of promoter
- When mobilized, 65% of new insertions express *lacZ* reporter during development
- Promoter can only activate transcription if under control of enhancers of genes near insertion site
- Detects genes turned on in certain tissues
- Genes isolated by plasmid rescue