2. Mutagenesis

Screen:

Visual: flower morphology, pigment color,

Biochemical pathway mutant

auxotrophy: $his3^-$ in yeast; arg^- in Neurospora; $trp1^-$ in plant

Reporter gene expression (luc bioluminescent protein from firefly)

Selection:

trp pathway: 5-methylanthranilate ---> 5'methyltrp (toxic)

ADH (alcohol dehydrogenase): Allyl alcohol---> acrolein aldehyde (toxic)

Lethal mutations: (such as house keeping genes)

-maintain as heterozygote

-weak hypomorph

-conditional such as temperature-sensitive mutants in T4 phase (p220-221)

Lecture 4: Mutant Characterization

Dominant and recessive mutations

Complementation tests

Biochemical pathways

Epistasis analyses

Read: 206-208; p214-215; 220-224

Fig. 7.14; 7.15; 7.20; 7.25; 7.26; 7.27

Homework#1 will be posted today!

Terminology about different mutations

a) null mutation- complete absence of activity

b) loss-of-function (hypomorph) - reduced activity

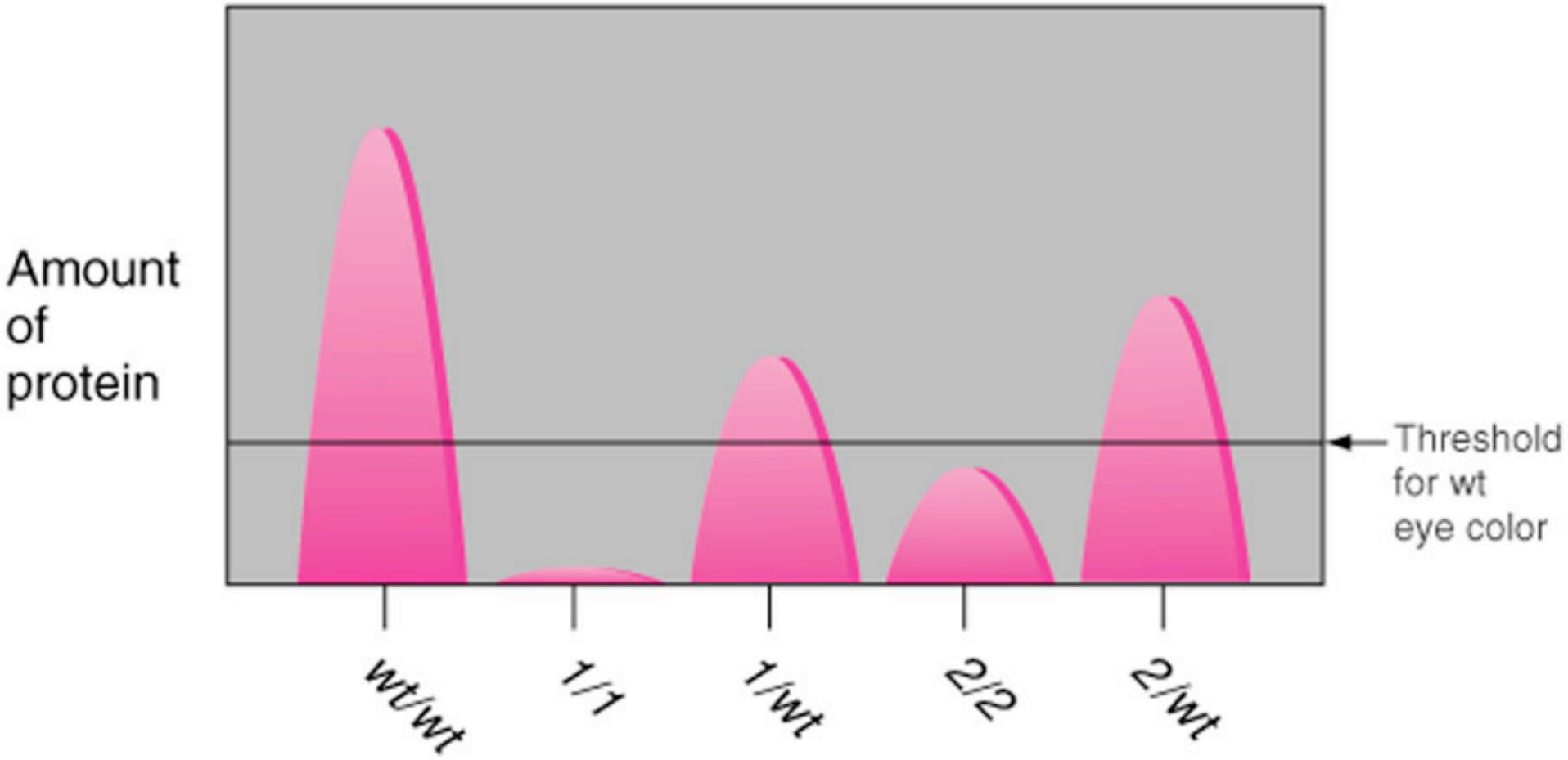
c) gain-of-function (hypermorph)- increased activity

(neomorph) - new function of gene

d) suppressors- compensate for other mutations

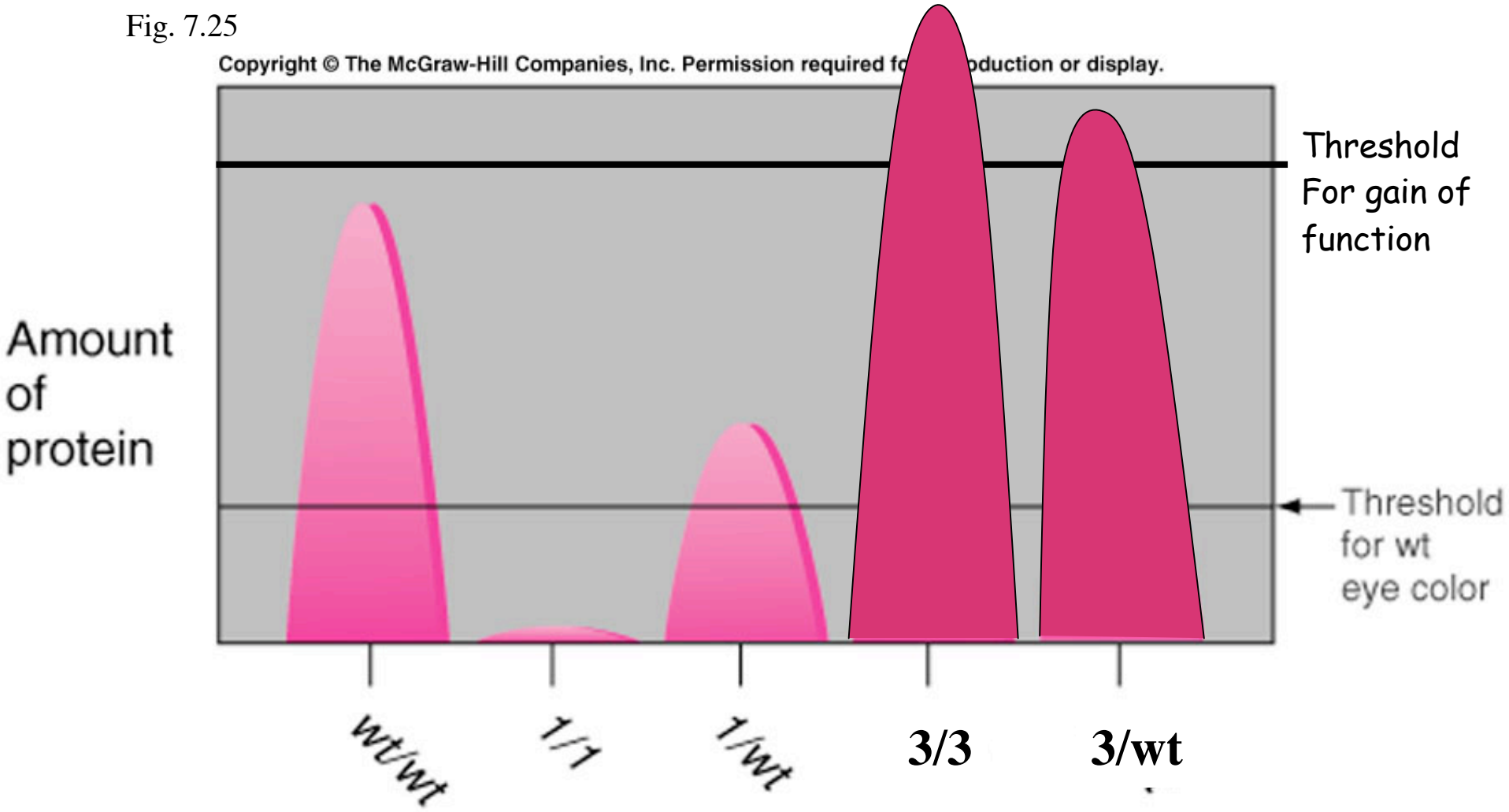
e) enhancer- enhances phenotype of a mutation

Fig. 7.25



1: null mutation; 2: hypomorphic mutation
Both 1 and 2 are recessive

Fig. 7.25



3: hypermorphic mutation
3 is dominant

The underlying nature of recessive or dominant mutations

Recessive

hypomorph: reduced level or a protein with a weak function

Null: complete loss of function

Dominant

hypermorph: increased level or more effective activity

neomorphic: new function

dominant-negative: poisonous effect

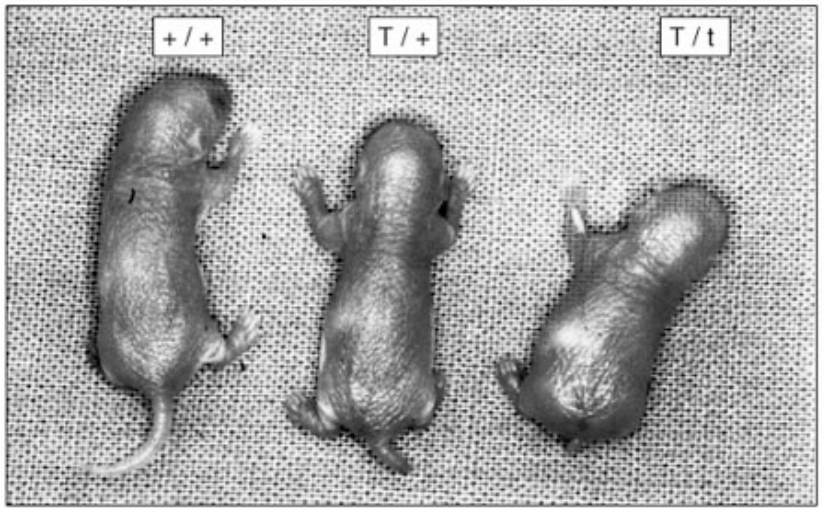
haploid-insufficient

Ectopic expression

Semi-dominant

Fig. 7.27

(a) Haploinsufficiency



(c) *Kinky:* A dominant negative mutation

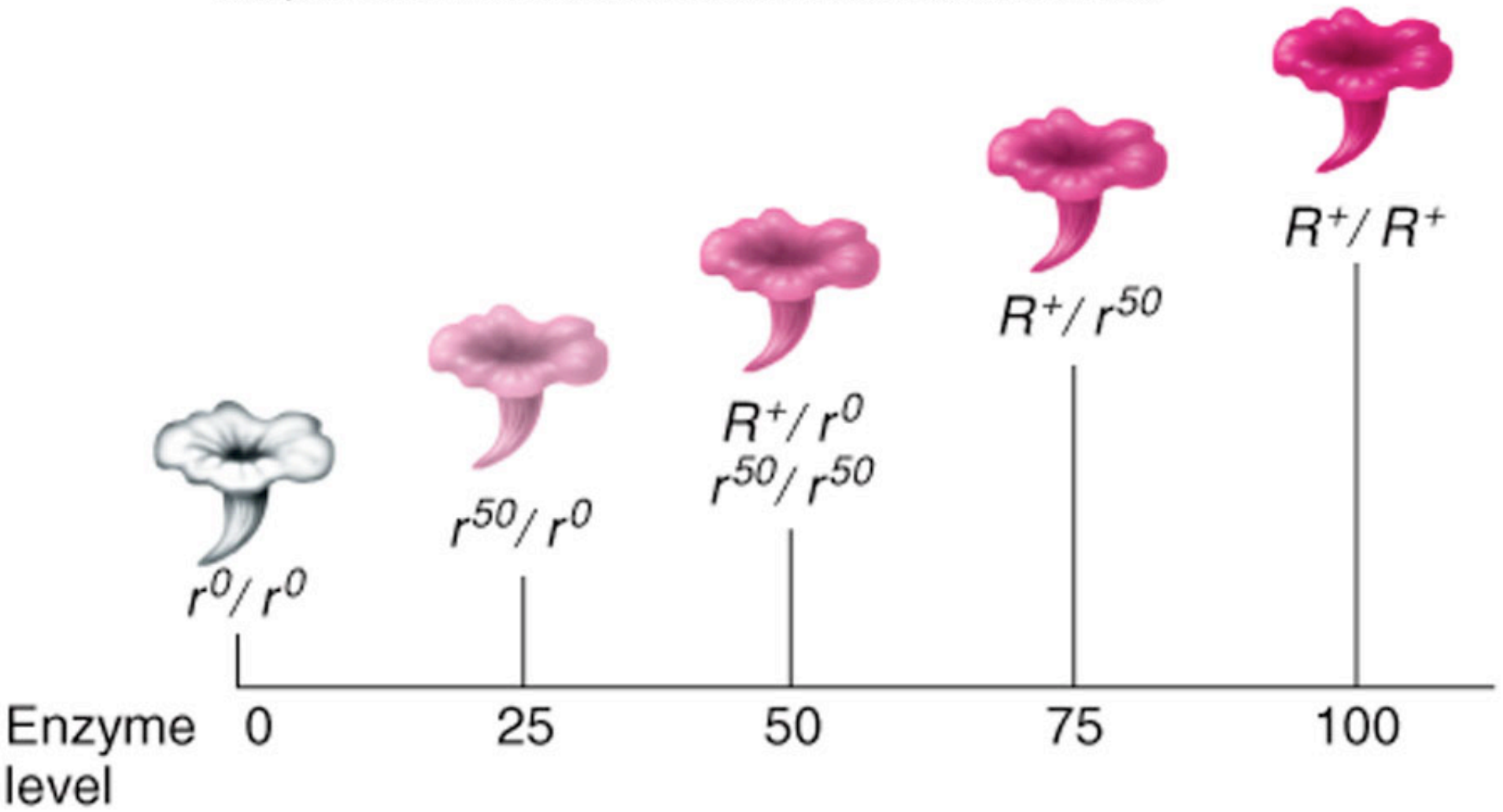
(b) Dominant negative mutations

Functional enzyme	Nonfunctional enzyme			
$d^+d^+d^+d^+$	$d^+d^+d^+D$	$d^+d^+D\ d^+$	$d^+\ D\ d^+d^+$	$D\ d^+d^+d^+$
	$d^+d^+D\ D$	$d^+D\ D\ d^+$	$d^+\ D\ d^+D$	$D\ d^+D\ d^+$
	$D\ d^+d^+D$	$D\ D\ d^+d^+$	$d^+\ D\ D\ D$	$D\ d^+D\ D$
	$D\ D\ d^+D$	$D\ D\ D\ d^+$	$D\ D\ D\ D$	

D = dominant mutant subunit
d^+ = wild-type subunit

(d) A result of ectopic expression

Fig. 7.26



r^0: null; r^{50}: hypomorph; R^+: wild type

Determine recessive or dominant nature of the mutation

c_1c_1 (Colorless mutant) ⊗ +/+ (Red: WT)

↓

$c_1/+$ (red: recessive)
(colorless: dominant)

↓ ⊗ (self)

		Recessive	**dominant**
1/4	c_1/c_1	colorless	colorless
2/4	$c_1/+$	red	colorless
1/4	+ /+	red	red

Complementation tests among Drosophila eye mutants

Fig. 7.14

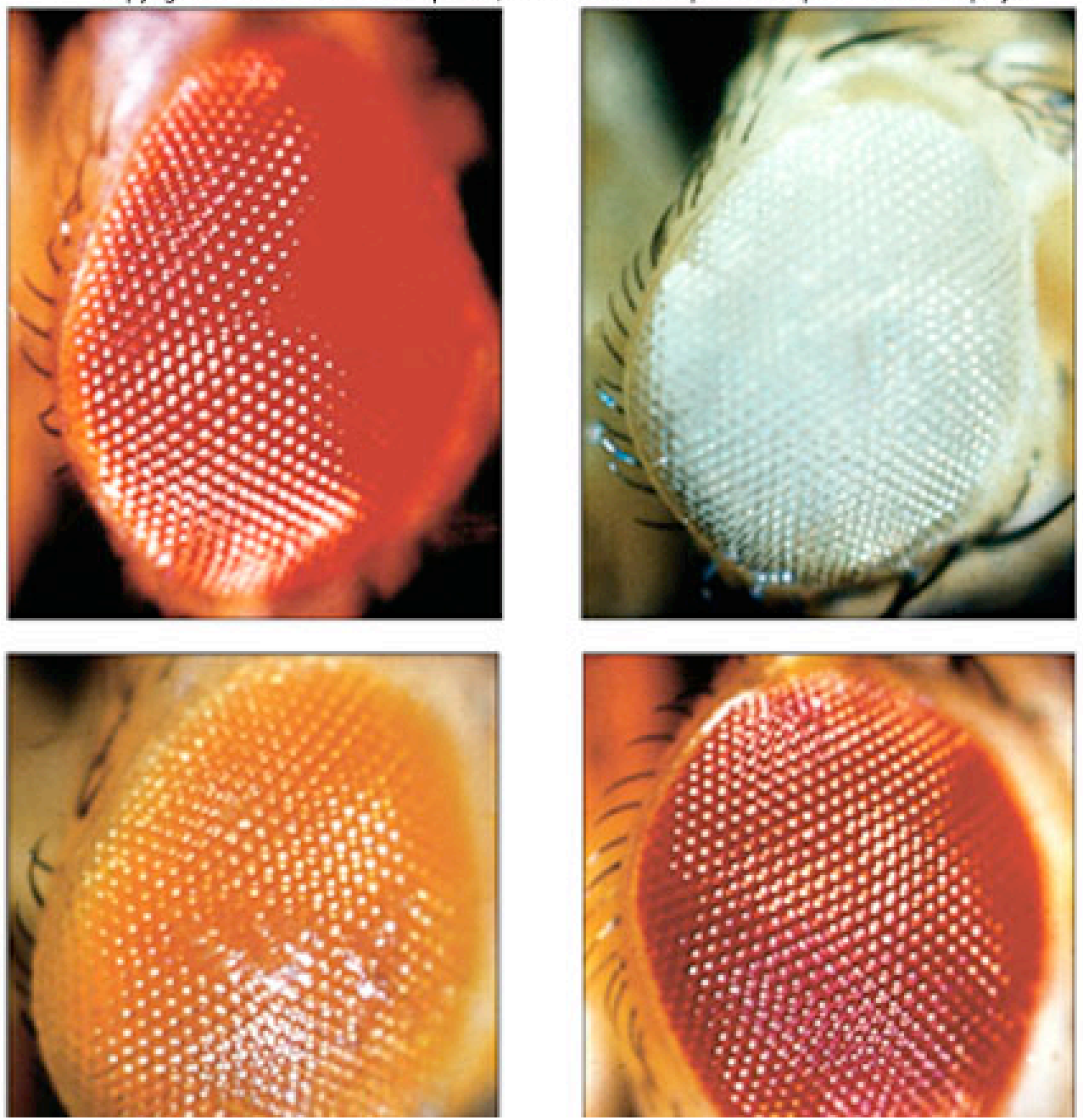
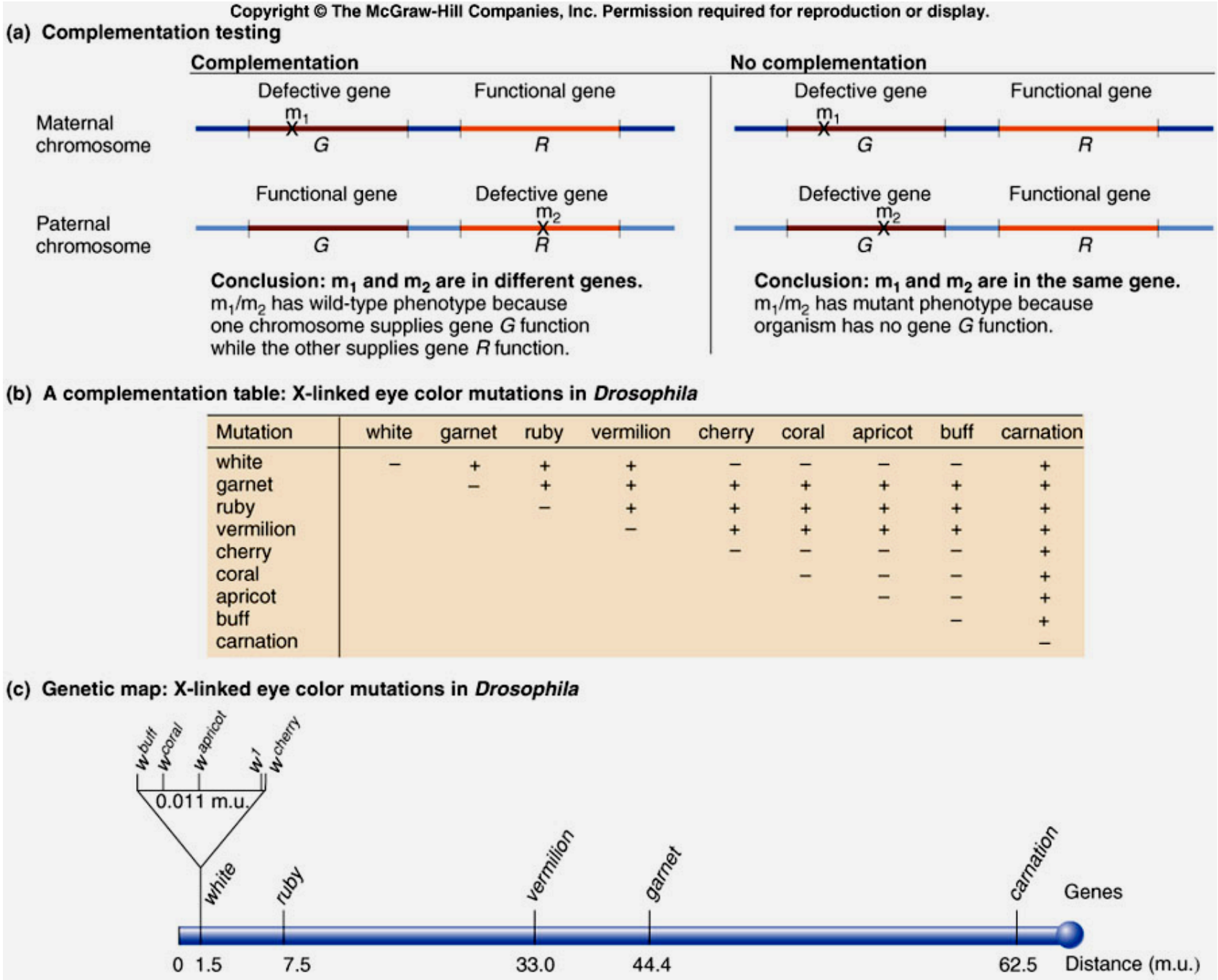


Fig. 7.15



Mutation	white	garnet	ruby	vermilion	cherry	coral	apricot	buff	carnation
white	–	+	+	+	–	–	–	–	+
garnet		–	+	+	+	+	+	+	+
ruby			–	+	+	+	+	+	+
vermilion				–	+	+	+	+	+
cherry					–	–	–	–	+
coral						–	–	–	+
apricot							–	–	+
buff								–	+
carnation									–

Determine allelism by complementation tests

Pairwise crosses between homozygotes and examine F1 for phenotype
only applicable for recessive mutations

male	c_1c_1	c_2c_2	c_3c_3	c_4c_4	c_5c_5	c_6c_6
Colorless	**red**	**red**	**colorless**	**red**	**red**	c_1c_1
colorless	**colorless**	**red**	**red**	**red**		c_2c_2
colorless	**red**	**red**	**red**			c_3c_3
colorless	**red**	**red**				c_4c_4
colorless	**colorless**					c_5c_5
colorless						c_6c_6

female

Maize kernel mutants:

c_1-c_6: **colorless, recessive**
wt: red

Three complementation groups:

1. c_1, c_4
2. c_2, c_3
3. c_5, c_6

"Complementation group" equals "Gene"

If two mutations failed to complement,

they are alleles of the same gene

they are allelic to each other

they belong to the same complementation group

If two mutations complements each other,

they are alleles of different genes

they are not allelic to each other

they belong to different complementation groups