Information on “Genetics”

TEACHING STAFF

Asunción Contreras and Javier Espinosa.
Asunción Contreras (**coordinator**) is in charge of the organisation and of explaining the general logic and the evaluation system of the Genetics subject (please pay attention on the very first day). Also, regarding making decisions if/when unexpected problems appear.

COURSE ORGANISATION

For the sake of the **learning objectives and outcomes**, minor adjustments in the timetable would affect tutorials, theory and problem discussions.

The documents (including lecture presentations) relevant to the different activities will be available from the Virtual Campus. All of them, as well as the exams will be in **English**. Access will be provided to materials of the “Genética” subject for both students demanding very extensive information and/or students who are not fluent in (rather technical/survival) English.

A total of 50 points could be obtained from “group problems” (answers to problems followed by class discussion, up to 2 points each problem) and another 50 points from exams, each one containing both test and problem(s) at 50%. A total of 20 extra points could be additionally obtained for *very active participation*. All tests will consist of 4 alternative answers (1/3 points penalty for wrong answers)

To pass the course, a total of **50 points** with **a minimum of 20 from the exams** is required.
Plan B: if you obtain more points from any “exam problem” than from the previous “group problems”, then your “exam problem” score will appear also in your “group problems” column. This applies to each of the exams.

July extraordinary exam: 2 problems (5 points) and 25 test questions (5 points). You can pass the course with just 4.3 points/10 if you previously accumulated a minimum of 20 points.

“Group problems” require individual work and subsequent discussions within groups before presenting the “group answers”. Each group will have a minimum of 3 and a maximum of 8 students. Group composition can vary from problem to problem. A “debate” in the **Virtual Campus** will be open to facilitate interactions and technical discussions.

All **students are supposed to know the general logic and the evaluation system** of the Genetics subject, and be aware of the information provided during the activities and on Virtual Campus. Students are expected to be fluent at least on “telegraphic English”. **Active participation** in all types of programmed activities is compulsory. However, the evaluation system is, at the same time, flexible enough to take into account unexpected or unwanted absences or failures. Therefore, there is no need to provide certificates whatsoever.

BIBLIOGRAPHY:

Any Genetics text-book will do, but we strongly recommend:

GRIFFITHS, ANTHONY J.F.; WESSLER, SUSAN R.; CARROLL, SEAN B.; DOEBLEY, JOHN. Introduction to Genetic Analysis. 10th edition (2012). W. H. Freeman.

Web site: http://bcs.whfreeman.com/iga10e/

Registration is free and required to access the exercises.

THEORETICAL CONTENTS AND KEY CONCEPTS

1. **INTRODUCTION TO GENETICS.** The inheritance problem. Model systems. Forward and reverse Genetics. Impact of Genetics on Biology and Society.
2. **GENERAL ASPECTS OF INHERITANCE.** Mendelian Genetics. Genes, chromosomes and heredity. Dominance/recessivity and its variations. Genetics in human pedigrees. Gene interactions and interactions with the environment. Complementation. Metabolic pathways.
3. **GENETIC MAPPING.** Linkage and recombination frequency. Locating genes in the chromosome map. Genetic maps in eukaryotes. Methods for gene mapping in prokaryotes.
4. **POPULATION GENETICS AND EVOLUTION.** Genetic structure of populations. The Hardy-Weinberg equilibrium model. Evolutionary forces: mutation, migration, natural selection and genetic drift. Speciation and evolution.
5. **THE GENERATION OF GENETIC VARIATION**. The molecular nature of the gene. Determination of metabolic pathways. Gene expression and the genetic code. Gene mutations. Chromosome mutations.

PRACTICAL CONTENTS

- **Group tutorials.** With an emphasis on questions and problem solving activities. 3 h.
- **Problems discussion sessions.** Students will present and discuss representative problems. 10 h.
- Specific activities carried out in **Computer** rooms or **Laboratories**.

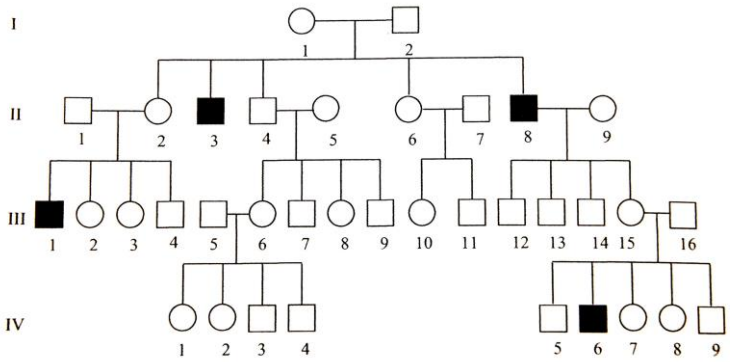
  - ✓ Mendel's Laws and pea plant Genetics. (Computer). 2 h.
  - ✓ Segregation analysis in maize (Laboratory). 3 h.
  - ✓ Sex-Linkage and recombination in *Drosophila melanogaster* (Laboratory). 3 h.
  - ✓ Genetics of ascospore color in *Sordaria* (Laboratory). 3 h.
  - ✓ Genetics of PTC tasting in humans (Laboratory). 3 h (1+2 h).
  - ✓ Population genetics and evolution (Computer). 2 h.
  - ✓ Kariotypes. Detection of chromosomal mutations (Laboratory). 3 h.

Problems

To answer, just think of the most likely form of inheritance for each tree:

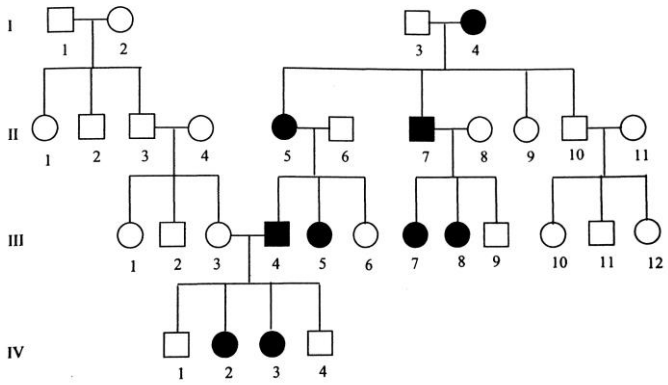
Problem 1. Is the mutant allele dominant o recessive? Why?

X-linked or autosomic? Why?



What is the probability of IV-2 and IV-5 (she is already pregnant) expecting an affected child?

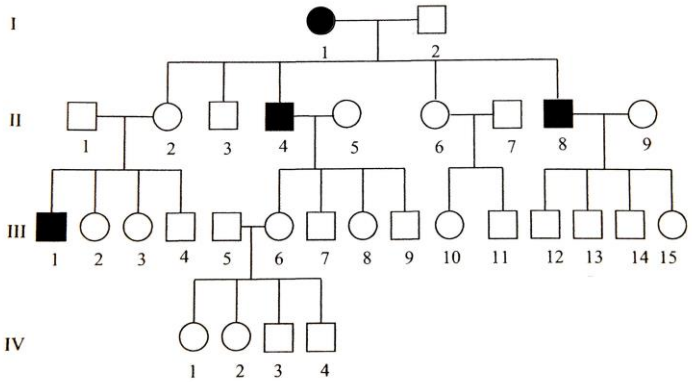
Same question for IV-3 y IV-7 Write down the genotypes of all the grandmothers in the tree.



Problem 2. Is the feature dominant o recessive? ¿linked to X o autosomic) Why?

What is the probability of III-2 and III-8 (already pregnant) expecting an affected son?

Problem 3. What is the most likely form of inheritance?



Why?

What is the probability of III-4 and IV-2 (already pregnant) expecting an affected daughter?

Problem 4. In dogs, dark coat color is dominant over albino and short hair is dominant over long hair. Assume that these effects are caused by two independently assorting genes, and write in the table the genotypes of the parents in each of the crosses shown here, in which D and A stand for the dark and albino phenotypes, respectively, and S and L stand for the short-hair and long-hair phenotypes. Use the symbols C and c for the dark and albino coat-color alleles and the symbols S and s for the short-hair and long-hair alleles, respectively.

		Number of progeny			
Parental phenotypes **Genotypes**		D, S	D, L	A, S	A, L
a. D, S × D, S	X	89	31	29	11
b. D, S × D, L	X	18	19	0	0
c. D, S × A, S	X	20	0	21	0
d. A, S × A, S	X	0	0	28	9
e. D, L × D, L	X	0	32	0	10
f. D, S × D, S	X	46	16	0	0
g. D, S × D, L	X	30	31	9	11

Problem 5. In tomatoes, two alleles of one gene determine the character difference of purple (P) versus green (G) stems, and two alleles of a separate, independent gene determine the character difference of "cut" (C) versus "potato" (Po) leaves. The results for five crosses of tomato-plant phenotypes are as follows:

		Number of progeny			
Parental phenotypes	**Genotypes**	P, C	P, Po	G, C	G, Po
P, C × G, C		321	101	310	107
P, C × P, Po		219	207	64	71
P, C × G, C		722	231	0	0
P, C × G, Po		404	0	87	0
P, Po × G, C		70	91	86	77

For each cross in the table write down the most probable genotypes (use your own symbols). Is any of the alleles recessive? If so, which one(s)?
Do the two genes segregate independently?

Which criteria/data have you taken into account to answer?

6. A man of blood-group AB is married to a woman of blood-group A whose father was of blood-group O. To how many different blood groups can their children belong to?

Write down the genotypic and the phenotypic proportions expected in their offspring

What is the probability for the two older sons to be of group B?

10. In a maternity ward, four babies become accidentally mixed up. The ABO types of the four babies are known to be O, A, B, and AB. The ABO types of the four sets of parents are determined. Indicate which baby belongs to each set of parents:

(a) AB × O (b) A × O (c) A × AB (d) O × O

7. Most of the feathers of erminette fowl are light-colored, with an occasional black one, giving a flecked appearance. A cross of two erminettes produced a total of 48 progeny, consisting of 22 erminettes, 14 blacks, and 12 pure whites. What genetic basis of the erminette pattern is suggested?

How would you test your hypotheses?.

8. Radishes may be long, round, or oval and they may be red, white, or purple. You cross a long, white variety with a round, red one and obtain an oval, purple F1. The F2 shows nine phenotypic classes

as follows: 9 long, red; 15 long, purple; 19 oval, red; 32 oval, purple; 8 long, white; 16 round, purple; 8 round, white; 16 oval, white; and 9 round, red. Is there independent assortment of genes for shape and color?

What type of phenotypic segregation are we observing?

Is any of the phenotypes recessive? If so, which one(s)?

Predict the genotypic and phenotypic proportions in the progeny of a cross between a long, purple radish and an oval, purple one.

9.In the multiple-allele series that determines coat color in rabbits, c+ encodes agouti, c^{ch} encodes chinchilla (a beige coat color), and c^{h} encodes Himalayan. Dominance is in the order $c^{+} > c^{ch} > c^{h}$. In a cross of $c+/c^{ch} \times c^{ch}/c^{h}$, what proportion of progeny will be chinchilla?

11. Consider two blood polymorphisms that humans have in addition to the ABO system. Two alleles LM and LN determine the M, N, and MN blood groups. The dominant allele R of a different gene causes a person to have the Rh+ (rhesus positive) phenotype, whereas the homozygote for r is Rh– (rhesusnegative). Two men took a paternity dispute to court, each claiming three children to be their own. The blood groups of the men, the children, and their mother were as follows:

Person	Blood group	
husband	O M Rh+	
wife's lover	AB MN Rh–	
wife	A N Rh+	Possible father(s)
child 1	O MN Rh+	
child 2	A N Rh+	
child 3	A MN Rh–	

From this evidence, can the paternity of the children be established? In the Possible father(s) column specify husband , lover, none, or both.

12. Two normal-looking fruit flies were crossed, and, in the progeny, there were 202 females and 98 males. According to your interpretation of results, write down the genotypic and phenotypic proportions.

Provide a test of your hypothesis.

GENETICS-2014 **EXAM1**

Surname(s):	**Name:**

Answer (concisely and immediately after each question, 12 points) all questions that Rose, the pregnant woman in generation IV is asking you:

1. The type of inheritance of the disease A (thick lines surrounding the symbols), that her husband is suffering (a recent surprise, he has just been diagnosed at the age of 41).

2. Type of inheritance of the disease B (dotted-filled symbols), which is very severe and need a lot of treatment and caring and is afflicting her youngest brother and other relatives.

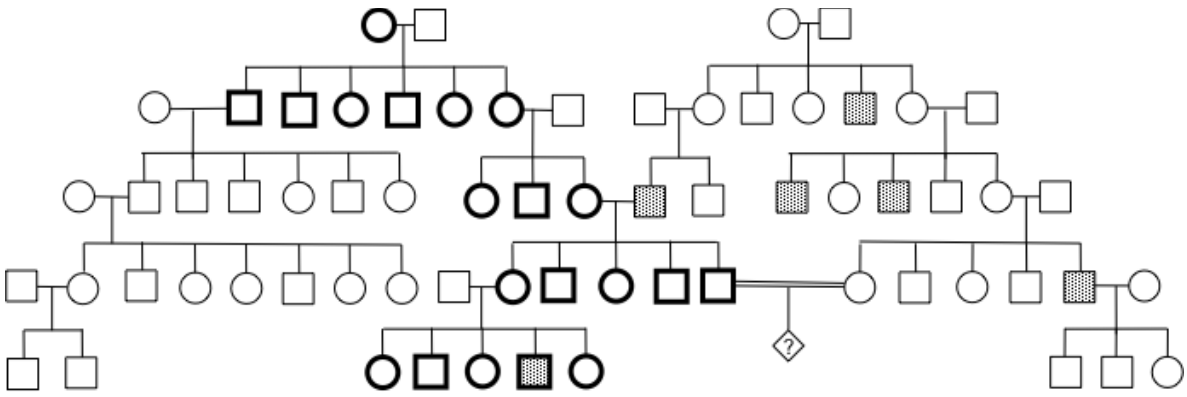
3. Write down (just below the corresponding symbols in the tree) the genotypes of all healthy people that have to necessarily be carriers

4. The probability that the baby that Rose is expecting will suffer from disease A or B

5. For each of the following concepts say whether (yes or no) the info in the tree provides evidence of:
Gene interaction: complementation: incomplete penetrance: gene linkage
(if you find any, please explain)

6. Two of the healthy female relatives, Mary (IV-6) and July (Rose´s only sister) are now saying that they also have some (very mild/non-severe) symptoms of their respective family´s diseases. Do you think it is possible that they are somehow affected by the same mutations as their relatives? Perhaps one or two of them is rather hypochondriac? Please give your opinion for each case.
Mary and disease A:

July and disease B:



EXTRA PROBLEMS

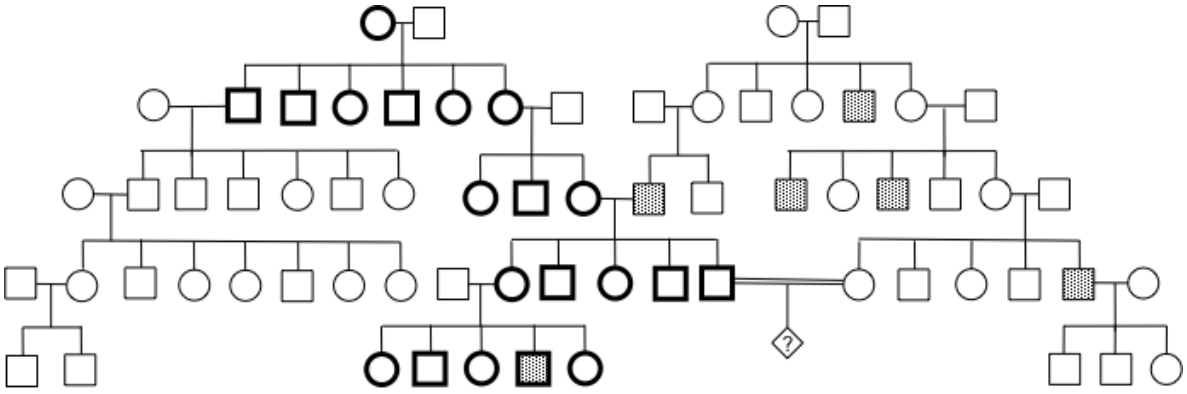
X1(Monday). A brown mouse is mated with two female black mice. When each female has produced several litters of young, the first female has had 48 black and the second female has had 14 black and 11 brown young. Indicate the genotypes of all of the parents.

X2 (Monday). Assume right-handedness (R) dominates over left-handedness (r) in humans, and that brown eyes (B) are dominant over blue (b). A right-handed, blue-eyed man marries a right-handed, brown-eyed woman. One of their two children is right-handed/blue-eyed, while the other is left-handed/brown-eyed. The man marries again, and this time the woman is right-handed and brown-eyed. They have 10 children, all right-handed and brown-eyed. What are the genotypes of the husband and two wives?

X3 (Friday). At 50, Mary has gone blind as a consequence of a mtDNA mutation. She is now concerned about her two children (Peter and Rose) and 5 grandchildren. Asia and Africa are the daughters of Peter. Albert, Alex and Adela are from Rose. Which ones do you think that are at risk of suffering the same disease?.

Answer (concisely and immediately after each question, 9 points) all questions that Mary, the pregnant woman in generation IV is asking you:

1. The type of inheritance of the S syndrome (characterized by eczema and certain blood problems), which is afflicting her youngest brother and other relatives (dotted-filled symbols)
2. The probability that the baby that she is expecting will suffer the S syndrome
3. The type of inheritance of the D disease (progressive vision loss culminating in blindness) that her husband is suffering
4. The probability that the baby that she is expecting will suffer the D disease (thick lines surrounding the symbols)
5. The probability that her baby will be a healthy girl (*if that was the case, she is going to call her "Miracle"*)
6. If, in feature times, Miracle has her own children, can any of them suffer S syndrome? (Yes or no)
7. Can any of them suffer the D disease? (Yes or no)
8. Could any of them be a carrier of the D disease?
9. Could any of them be a carrier of the S syndrome?



Solutions to class-presented problems

P female male

1 band 3 bands

F1 1(1band): 1(3 bands) 1(1band): 1(2 bands)

Segregation?

Sex linkage, criss-cross?

3 phenotypes?

1 or 2 genes?

P genotypes?

A1A2 x A3

F1 1(1band): 1(3 bands) 1(1band): 1(2 bands)

1 A1A3 : 1 A2A3 1 A1 : 1 A2

Dominance order: A1, A3, A2

Predicting crossing from F1 flies

A2A3 x A1

1 A2A1 : 1 A3A1 1 A2 :1 A3 :

All females 1band 2 male phenotypes:

2 bands 3 bands

Blue sclera and brittle bones in pedigree

One or two genes?

a) Blue sclera: Type of inheritance?

Dominant autosomal?

with some “jumps”:

Informative generations to identify lack of penetrance: I, II, III

II-14 (1 out of 4 with the **A**a phenotype),

III-2, III-14 (2 out of 8 with the **A**a phenotype).

So, penetrance is about 75%

b) Brittle bones? Diagnosed in 6 out of the 16 with blue sclera

An additional symptom?

Pleiotropy? Expressivity differences?

Complementation test to identify *C. elegans* genes making possible the normal/wt movement (smooth gliding motion) instead of wiggle/mutant (in how many genes do we have wiggle mutations?):

1. Make complementation groups (genes)

1,5 2,6,8,10 3,4 7,11,12 9

2. Give names (letters A-E) to the 5 groups/genes

Genotypes examples aaBBCCDDEE (1,5) AAbbCCDDEE (2,6,8,10), etc.

Two phenotypes: mutant (w) or WT (+)

1 and 3 mutants complement (WT or +), 1 and 5 no (w/mutant)

Long 32: sphere 178, disk 270

Total 480 1:6:9 (duplicate interaction)

A-B-	aaB-	A-bb	aabb
9	3	3	1

A and B would contribute similarly to disc-wide shape (otherwise long)

A brown mouse is mated with two female black mice. When each female has produced several litters of young, the first female has had 48 black and the second female has had 14 black and 11 brown young. Indicate the genotypes of all of the parents.

Father: **aa** Female 1: **AA** Female 2: **Aa**

Assume right-handedness (R) dominates over left-handedness (r) in humans, and that brown eyes (B) are dominant over blue (b). A right-handed, blue-eyed man marries a right-handed, brown-eyed woman. One of their two children is right-handed/blue-eyed, while the other is left-handed/brown-eyed. The man marries again, and this time the woman is right-handed and brown-eyed. They have 10 children, all right-handed and brown-eyed. What are the genotypes of the husband and two wives?

Husband: Rr bb **Wife: Rr Bb** **Lover: RR BB**

What are the chances that the child that the wife is expecting will be left-handed/blue-eyed?

1/4 left-handed x 1/2 blue-eyed = 1/8

At 50, Mary has gone blind as a consequence of a mtDNA mutation. She is now concerned about her two children (Peter and Rose) and 5 grandchildren. Asia and Africa are the daughters of Peter. Albert, Alex and Adela are from Rose. Which ones do you think that are at risk of suffering the same disease?.

Peter and Mary, and her children: Albert, Alex and Adela

Additional information and questions to discuss in the laboratory

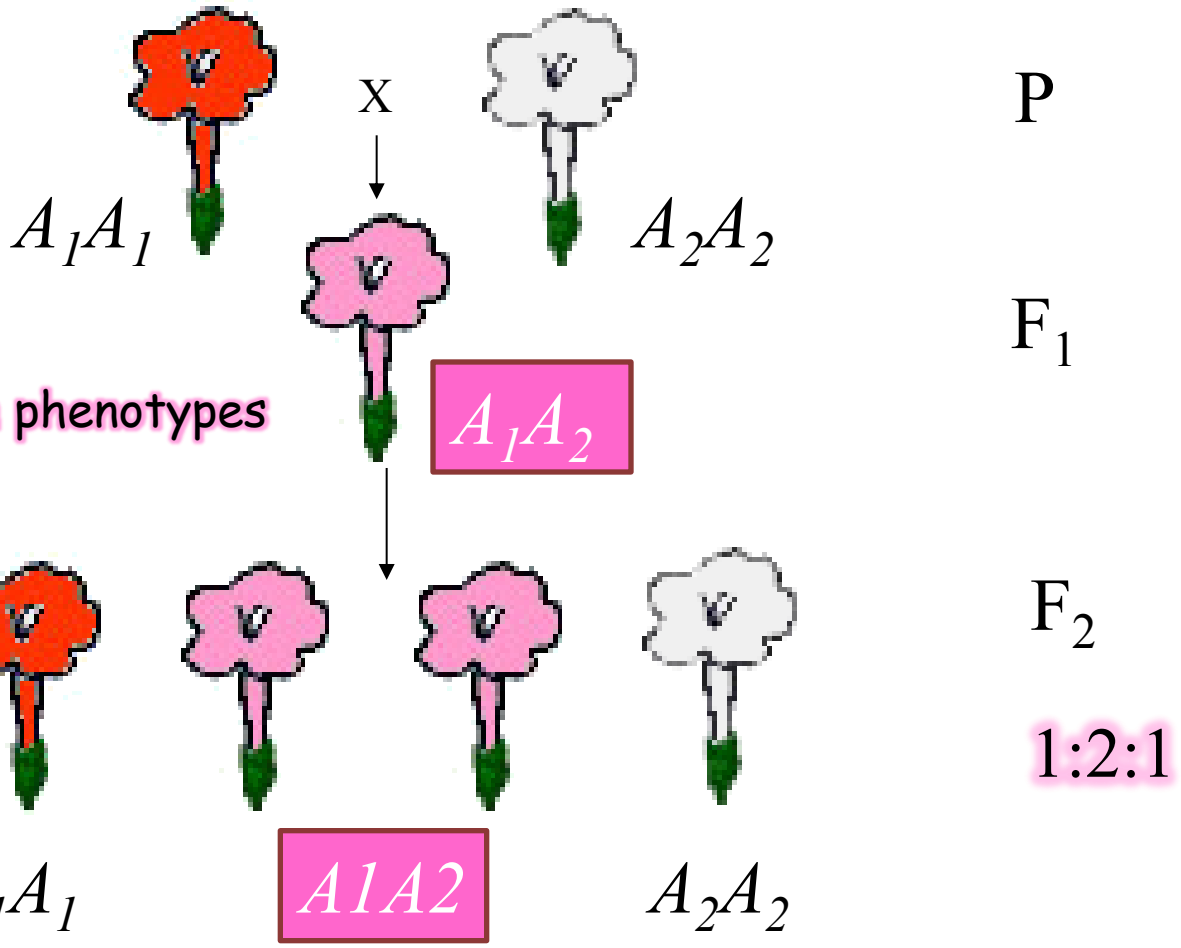
Extensions of mendelian genetics

(Inter)Allelic interactions

Variations on Dominance between alleles of a single gene
Modifications of phenotypic ratios

Incomplete dominance and codominance

Four o´ clock (Mirabilis jalapa)



incomplete/no (or lack of) dominance and codominance

Genetics Mendel´s peas

Access

"Ordenador"
- compartida X:
- "Genetica2013"
- "Programa“
- "Pea Plant Genetics Lab"

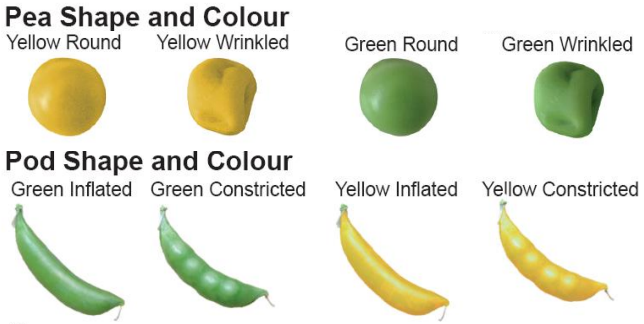
To take away: In "Genetica2013" copy “Pea Plant Genetics Lab"

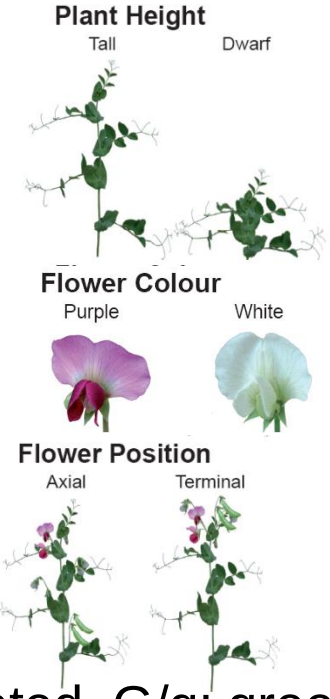
Help/points to remember/greenhouse

Type C (classical)crosses.

Type R (“*retro* “) crosses or backcrosses (test cross or not):

1 of each type of crosses (nºs **1-7, 19-23** and **8-18**).





T/t:tall, W/w: wrinkled, Y/y: yellow, P/p: purple, I/i: inflated, G/g: green, A/a:axial

Assume allele “yellow” is X-linked and recessive. Taking into account genotypes and sexes,

1. How many different crosses can you perform?.
2. For each type of cross determine genotypic proportions in the offspring
3. Idem for phenotypic proportions

Idem for allele “ugly”, which is X-linked and recessive lethal.

Idem for allele “messy”, which is autosomal and recessive lethal.

It is possible to obtain the following genotypic segregations for 1 or 2 genes with 2 alleles each:

1. 1:2:1 for one gene, codominance
2. 9:3:3:1 for 2 genes
3. 2:1 for one gene, complete dominance
4. 3:1 for one gene, complete dominance
5. 1:1 for one gene, complete dominance
6. 9:7 for 2 genes, complete penetrance

For a given pair of 2 genes (2 alleles each), assorting independently, assuming 100% penetrance and no variable expressivity, it is possible to obtain:

a) 4 phenotypes if codominance applies to both genes
b) 6 phenotypes if codominance applies to both genes
c) Up to 9 phenotypes
d) Just 3 phenotypes
e) a 15:1 segregation suggesting gene redundancy

(Inter)Allelic interactions

Variations on dominance between alleles of a single gene

Modifications of phenotypic ratios

Genetic concepts and terminology

Codominance and incomplete dominance
Multiple alleles/allelic series
    Locus/loci
    Polymorphism
Pleiotropic alleles
Lethal alleles
Penetrance and expressivity
Effect of sex on dominance/recessivity

Incomplete dominance and codominance

Four o´ clock (Mirabilis jalapa)

Intermediate or both phenotypes

Incomplete/no (or lack of) dominance and codominance

Incomplete dominance

Rather extreme phenotype

Rather extreme phenotype

Intermediate phenotype

Scale of dominance

Codominance and allelic series

AB	B	A	O
none	anti-A	anti-B	anti -A,-B

The ABO blood groups

4 phenotypes

3 alleles per gene/locus

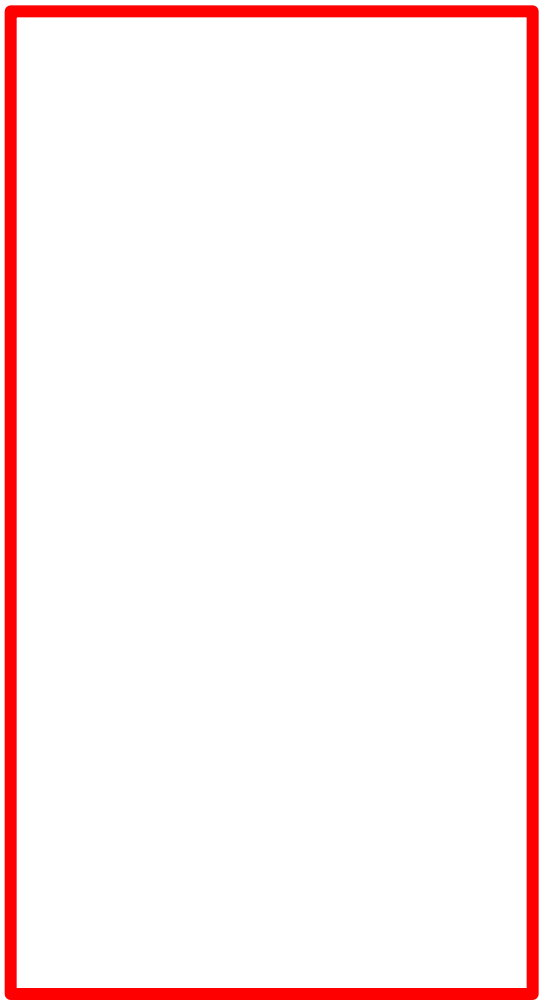
I^A I^B i

All 3 relatively common: polymorphism

Polymorphic locus

How many genotypes?

The ABO allelic series



More allelic interactions and variations on dominance

Sickle-cell anemia, a recessive desease

SEVERE ANEMIA (aa)

Normal (AA y Aa)

Or is it codominant??

Dominance/recessivity, not always that simple

The level of phenotypic detection counts

- ✓ clinical
- ✓ cytological
- ✓ molecular

More variations on Dominance/mendelian ratios

yellow X yellow

AA^y AA^y

2/3 yellow
1/3 wild type

AA AA^y AA^y ~~A^yA^y~~

yellow X wild type

AA^y AA

1/2 yellow
1/2 wild type

Lethality (before birth)

A^y

- ✓ Recessive lethal allele
- ✓ Phenotypic effect on heterozigosis (dominant for color)
- ✓ Pleiotropic (affect different properties)

More variations on Dominance/mendelian ratios

Dominance/recessivity, not always that simple

The same genotype might result in different phenotypes

- ✓ Variable expresitivity
- ✓ Incomplete penetrance

Expressivity: the degree to which a character is expressed

Penetrance: Percentage of individuals with a given genotype that express the associated phenotype (complete is 100%)

Variable expressivity

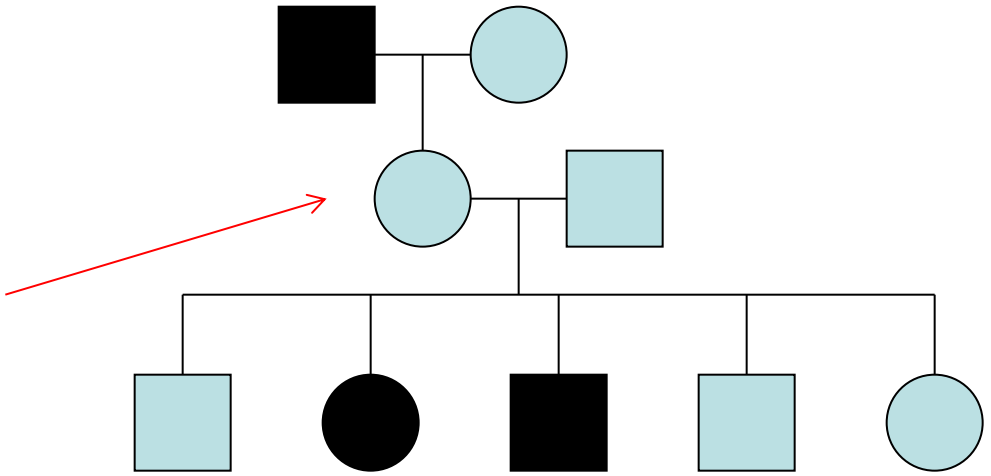
Polydactyly

Different intensity/severity

Variable expressivity

Beagle dogs

Incomplete penetrance



Autosomal dominant character!

II-1 (Aa) is not showing the dominant phenotype

Neurofibromatosis

Extremely variable expressivity
High (almost complete) penetrance

The elephant man

Variations on dominance and sex

Features limited to one sex

Horns (some animals)

Features affected by sex

Environmental influence

c^h (Himalaya) allele from *C* gene determining fur color in mammals: gene product is temperature sensitive

Pigments just in cooler places

Gene interactions

Biochemical pathways

- ✓ Genes encode enzymes
- ✓ Enzymes catalyse the steps in biochemical pathways
- ✓ Therefore, genes control cell chemistry and mutations may disturb crucial processes

Garrod (1909): alkaptonuria

Inborn errors of metabolism

Beadle and Tatum (1941) experiments with *Neurospora*

One-gen-one-enzyme hypothesis

Biochemical pathways and inborn errors of metabolism

Gene interactions

Biochemical pathways

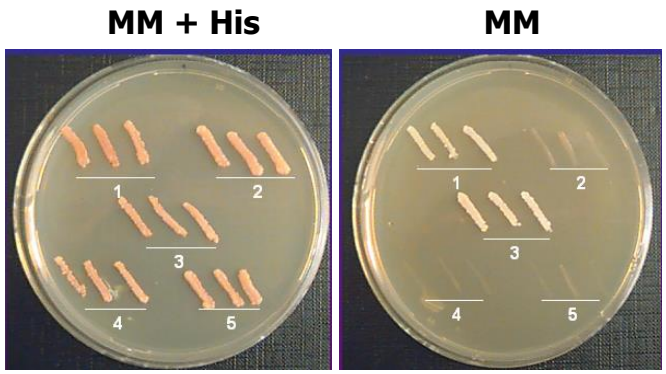
Studying pathways in model microorganisms

Phenotypes in a petri dish

Gene interactions

Biochemical pathways

Studying pathways in model microorganisms
Making mutations in Pathways to synthesize essential products



Saccharomyces cerevisiae

1, 3 are WT: *his*$^+$
PROTOTROPHS

2, 4, 5: *his*$^-$
HISTIDINE AUXOTOTROPHS
(require supplements)

Gene interactions

Biochemical pathways

A Lineal biosynthetic pathway

to synthesize the ESSENTIAL compound E

Gene 1 → Enz 1 → A ⟶ B (Precursor)
Gene 2 → Enz 2 → B ⟶ C (Intermediates)
Gene 3 → Enz 3 → C ⟶ D (Intermediates)
Gene 4 → Enz 4 → D ⟶ E (**Final product**)

Gene 1 → Enz 1 → A ⟶ **B**
Gene 2* → Enz 2 → B ⟶ // C
Gene 3 → Enz 3 → C ⟶ D
Gene 4 → Enz 4 → D ⟶ E

The wt is prototroph
The mutant is auxotroph

- ✓ The mutant in gene2 does not synthesize C, D or E
- ✓ grows with C, D or E
- ✓ **accumulates B**

Inferring pathways: Problem

	Tested compound					
	A	B	C	D	E	G
mutant 1	−	−	−	+	−	+
mutant 2	−	+	−	+	−	+
mutant 3	−	−	−	−	−	+
mutant 4	−	+	+	+	−	+
mutant 5	+	+	+	+	−	+

Gene interactions

Biochemical pathways

Inferring pathways: Problems

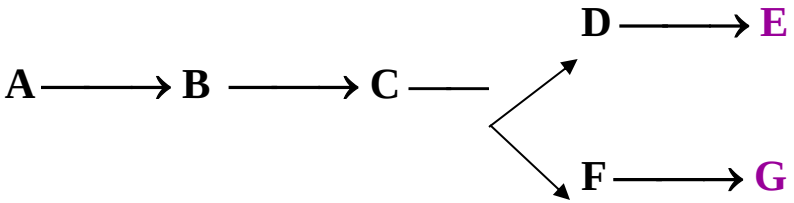
You have isolated four different mutants unable to synthesize the ESSENTIAL compound E for growth. You know that compounds A through D are part of the biosynthetic pathway, but you do not know the order in which they are synthesized in the wild-type, so you test each of the following compounds for its ability to support growth of each mutant.

		Compound added				
		A	B	C	D	E
Mutation	1	-	-	-	-	+
	2	-	+	+	+	+
	3	-	-	+	+	+
	4	-	-	+	-	+

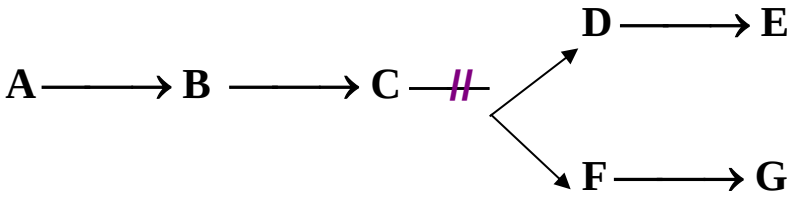
Gene interactions

Biochemical pathways

Branched pathways to synthesize the ESSENTIAL compounds E ad G



Inferring mutant phenotypes



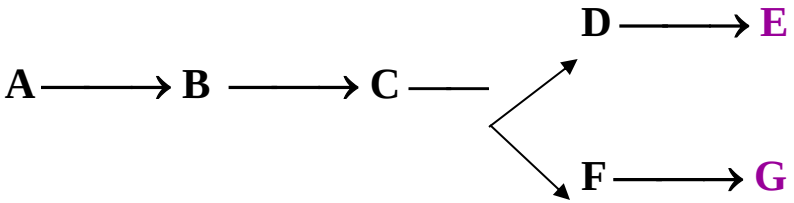
For each single mutant:

- ✓ It accumulates…. ?
- ✓ It does not grow with… ?
- ✓ It grows with…?

Gene interactions

Biochemical pathways

Branched pathways to synthesize the ESSENTIAL compounds E ad G



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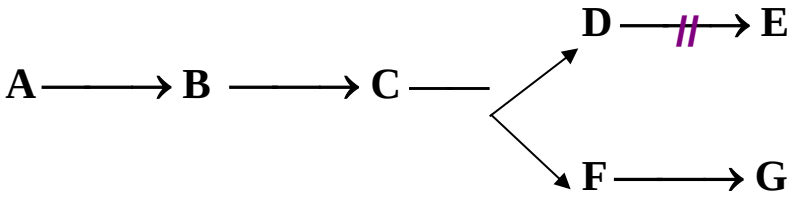

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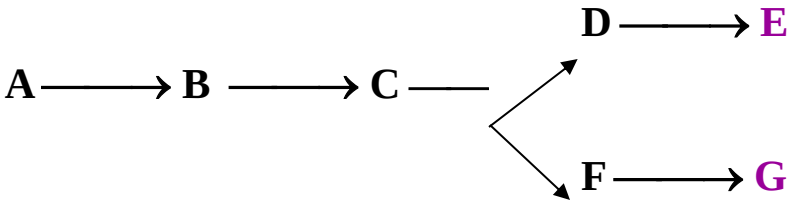

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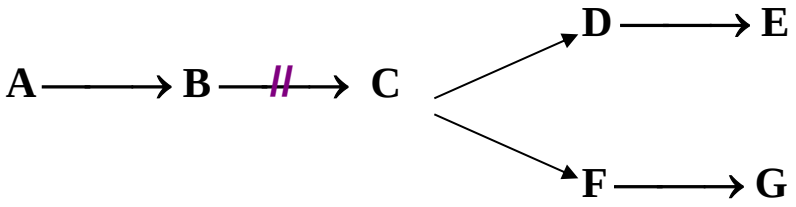

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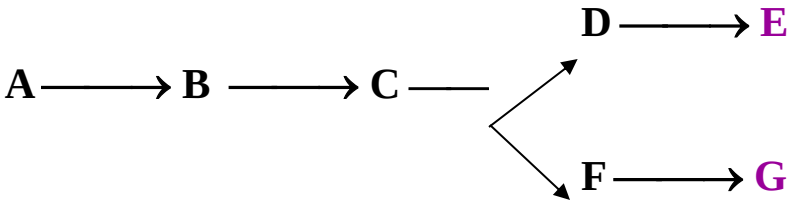
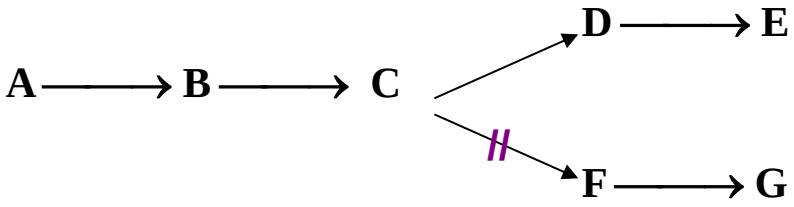
Gene interactions

Biochemical pathways

Branched pathways to synthesize the ESSENTIAL compounds E ad G



Inferring mutant phenotypes



For each single mutant:

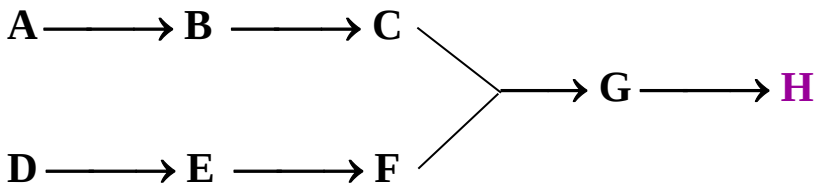
- ✓ It accumulates…. ?
- ✓ It does not grow with… ?
- ✓ It grows with…?

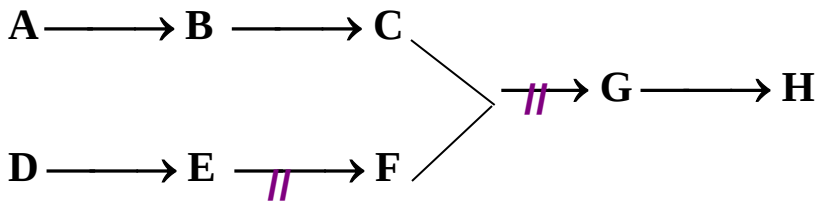
Gene interactions

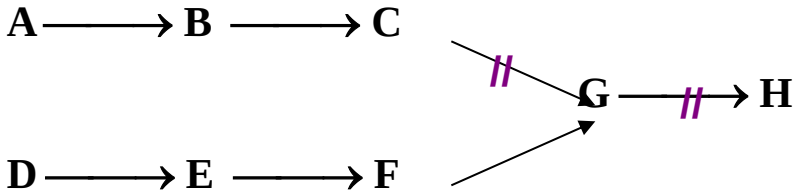
Biochemical pathways

Branched pathways | Inferring mutant phenotypes

to synthesize the ESSENTIAL compound H







Are all the mutants auxotrophs??

Gene interactions

Biochemical pathways

Inferring pathways: Problem 1

compound added to the media

mutant	A	B	C	D	E	F	G	H	E+G
1	-	-	-	-	+	-	-	-	+
2	-	-	-	-	-	-	-	+	+
3	+	-	-	-	-	-	-	+	+
4	-	-	-	-	+	+	-	-	+
5	-	+	-	+	-	-	+	-	+
6	-	+	-	-	-	-	+	-	+
7	-	-	-	-	-	-	+	-	+

(+) means growth (-) means not growth

Draw the metabolic pathway compatible with the data

Gene interactions

Biochemical pathways

Inferring pathways: Problem 2

Certain bacteria requires amino acid X to grow. 8 auxotroph mutants have been isolated and their ability to grow on minimal medium and the accumulated compounds was determined :

mutant	compound added to the media									Accumulated compounds
	C	E	F	G	H	I	J	K	X	
1	–	–	+	+	–	–	+	–	+	–
3	–	–	+	–	–	–	–	–	+	J
4	–	–	+	–	+	–	–	–	+	C, I
5	–	+	+	–	–	+	–	–	+	H, K
6	–	–	–	–	–	–	–	–	+	F
8	–	–	+	–	–	–	–	–	+	H, I
9	–	–	+	–	–	+	–	–	+	E, H
10	–	–	+	–	–	–	+	–	+	G

Draw the metabolic pathway compatible with the data

Which mutants will be able to grow in MM when supplied with compounds H and J?

Which mutants will be able to grow in MM supplied with compounds C and K?

In which media supplied with a unique compound will a double mutant 4 and 9 grow?

Which compounds will accumulate this double mutant?

Gene interactions

Biochemical pathways

Inferring pathways: Problem 3

Six *Sordaria fimicola* strains, each one with a different mutation, are unable to grow on minimal media (MM), unless it is supplied with one or various compounds.

mutant	Supplemented compounds											
	A	B	C	D	E	F	G	H	A+E	A+H	C+E	C+H
1	+	–	–	–	–	–	–	–	+	+	–	–
2	–	–	–	+	–	–	–	–	+	+	+	+
3	–	+	–	+	–	–	–	–	+	+	+	+
4	–	–	–	–	–	–	–	–	+	+	+	+
5	–	–	–	+	–	–	+	–	+	+	+	+
6	–	+	–	+	–	+	–	–	+	+	+	+
7	–	–	–	–	–	–	–	+	–	+	–	+

Draw the pathway

Which mutants will be able to grow in MM when supplied with C + G compounds?

which compounds will accumulate a double mutant carrying mutations 2 and 3?

In which media supplied with a unique compound will the previous mutant grow?

Gene interactions

Genes (products) in pathways

Biological pathways: Biochemical, also regulatory

Genetic interactions: Key concepts

- Complementation
- Supression
- Epistasis

physical interactions between gene products, or not

Gene interactions and regulatory pathways

Interaction between a regulatory protein and its target

Gene interactions and regulatory pathways

Interaction between a regulatory protein and its target

Gene interactions and regulatory pathways

Interaction between a regulatory protein and its target

Gene interactions and regulatory pathways

Interaction between a regulatory protein and its target

Allelic interactions in pathways (steps)

A (+) → funcional Enzyme

a (m) → non funcional (null mutation)

A/a

X —//→ Y

wt phenotype: *AA, Aa*

Mutant phenotype: *aa*

Allelic interactions in Biological pathways

"Complementation" of function by the **wt** dominant allele

A (+) → functional Enzyme
a (m) → non functional (null mutation)

Gene interactions in Biological pathways

Complementation of function when combining two different mutants

A and B (+) → functional products

a and b *(m1, m2)* non functional

Complementation : a wild type phenotype from combining

two haploid genomes carrying different recessive mutations

(**wt** alleles in the same cells!)

AaBb

Intergenic **Complementation**

haploid genomes: gametes or not

Gene interactions in Biological pathways

Complementation of function when combining two different mutant lines

A and B (+) → functional products

a and b *(m1, m2)* non functional

sweet peas

AaBb

How many mutant/white pure lines?

With single gene mutations?

Gene interactions in Biological pathways

Complementation in haploid eukaryotes

N. crassa

Gene interactions in Biological pathways

Complementation

In bacteria

They also have a social life

and *sex* (without meiosis)

merodiploids or partial diploids:

chromosome

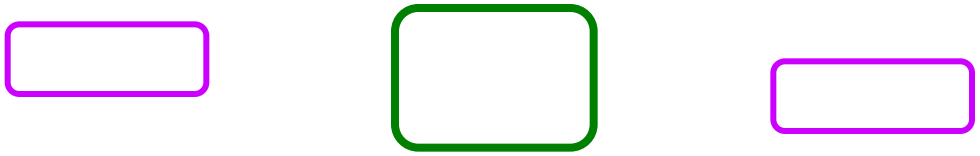
plasmid

Gene interactions in Biological pathways

Complementation

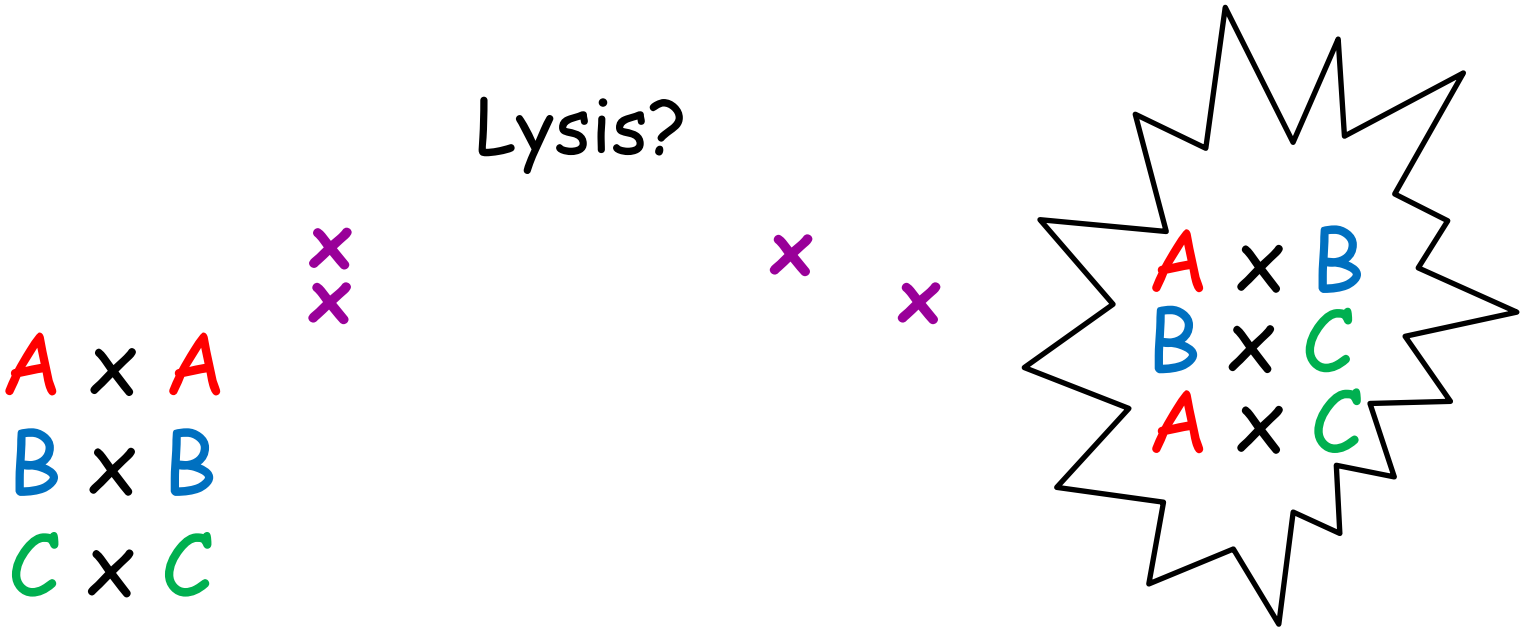
In bacteriophages

Phage T2



Lytic plaques/lysis halos

Lysis?



Gene interactions in Biological pathways

Complementation

Inferring gene (numbers) involved in processes

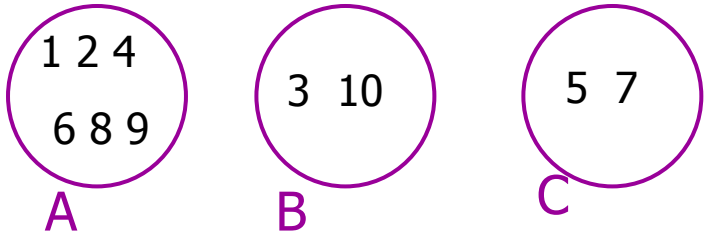
In bacteriophages

10 independently obtained phage stocks defective in lysis

How many different genes required for lysis?

→Infection in pairs

	2	3	4	5	6	7	8	9	10
1	–	+	–	+	–	+	–	–	+
2		+	–	+	–	+	–	–	+
3			+	+	+	+	+	+	–
4				+	–	+	–	–	+
5					+	–	+	+	+
6						+	–	–	+
7							+	+	+
8								–	+
9									+



3 complementation groups, each one identifies a gene

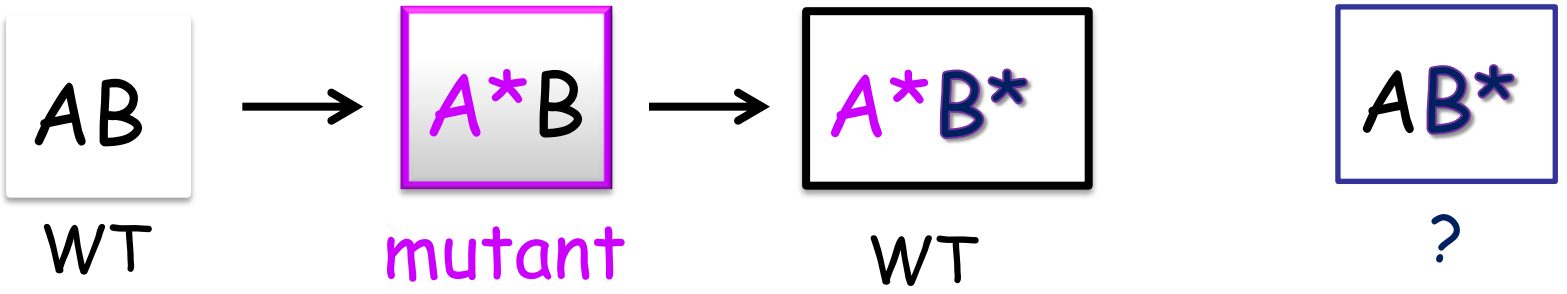
Gene interactions in Biological pathways

Suppression, a rare type of "complementation" or "rescue of function" of a given mutant by an specific mutant allele of a different gene

A → functional product

A* mutant/non functional

B* suppressor type phenotype



Suppression: a second mutation in a <u>different gene</u> restores the wild type phenotype to a mutant

haploid or diploids (easier with haploids)

Gene interactions: Suppression

protein-protein interactions

Gene interactions: Suppression

protein-protein interactions

Gene interactions: Suppression

protein-protein interactions

Gene interactions: Suppression

protein-protein interactions

Gene interactions in diploids

Complementation, epistasis and others

Genes involved in the same processes:

changes on the 9:3:3:1 di-hybrid Mendelian ratio

No gene interaction

2 genes affecting the same character: color

- Gene

Fur color of many mammals

- *A*: pigment localization
- *B*: pigment color

AA, *Aa* } "Agouti" color

aa solid

BB, *Bb* } Black

bb brown

How many phenotypes?

No gene interaction

Dihybrid crosses: 4 pure lines, 4 colours

P

AAbb cinnamon X *aaBB* black

or

AABB agouti X *aabb* brown

F_1 *AaBb* agouti

F_2

9 *A_B_* agouti
3 *A_bb* cinnamon
3 *aaB_* black
1 *aabb* brown

4 phenotypes from a dyhibrid cross

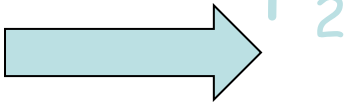
Gene interactions: Complementation

Pea flower color

Only 2 phenotypes from a dyhibrid cross

2 white pure "mutant" lines

One dominant allele from each of the 2 genes required for purple



9	*W_D_*	purple
7	3 *W_dd*	
	3 *wwD_*	white
	1 *wwdd*	

9:7: also called "double recessive *epistasis*"

2 genes determining the same character: color

Gene interactions: Complementation

Gene interactions: Suppression

Gene products from A and B work in the same pathway
Specific mutations in b **suppress** the a (mutant) phenotype

A-B- 9	
A-bb 3	**13**
aaB- 3	**3**
aabb 1	

A-B- 9	
A-bb 3	**10**
aaB- 3	**6**
aabb 1	

A (Wild type)
a (mutant phenotype)

b (wild type allele)
B (suppressor, but wt phenotype)

A (WT)
a (mutant)

B (WT)
b (mutant)

Only 2 phenotypes from a dyhibrid cross

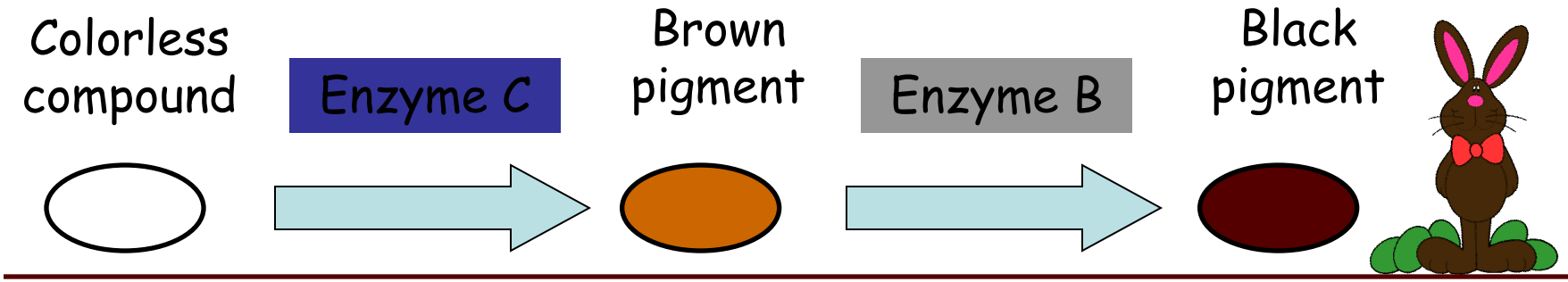
Molecular basis/examples:

A (not a) sinthezises essential compound X, wt b performs a similar reaction, supressor B can make enough X

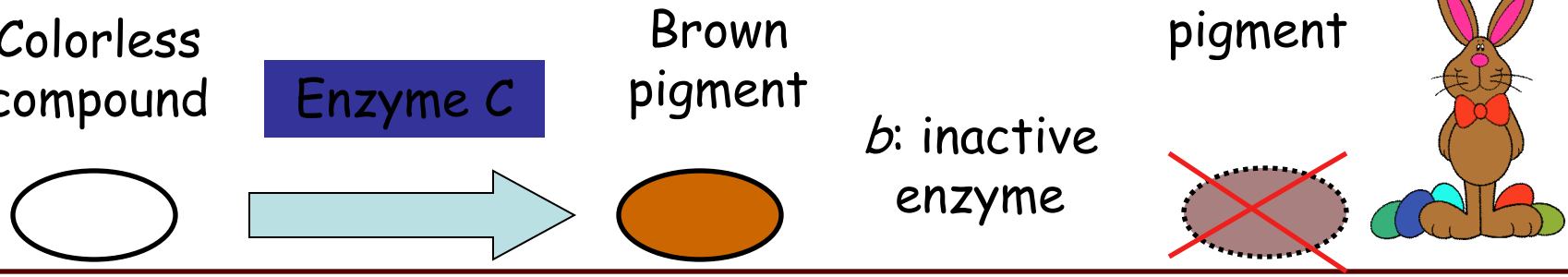
A and B products interact physically a and b too!

Recessive epistasis

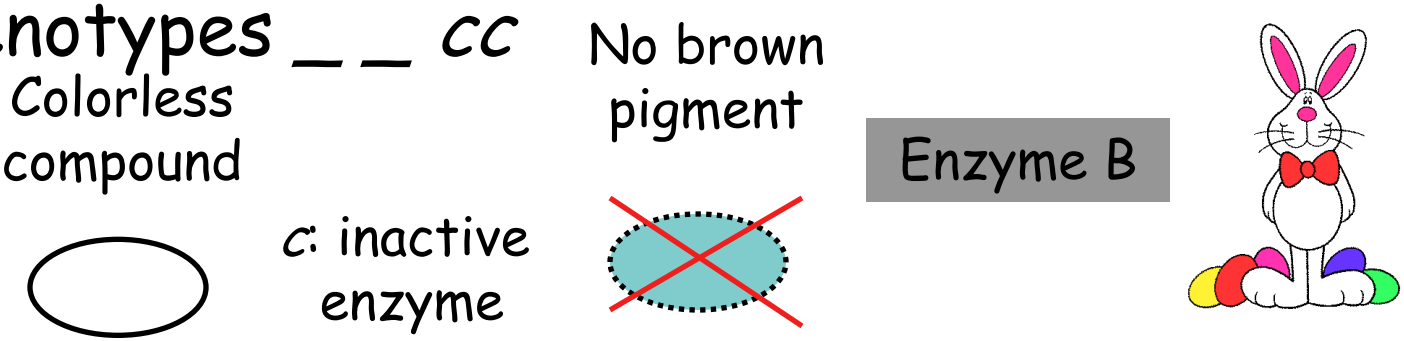
- Genotypes *B_C_*



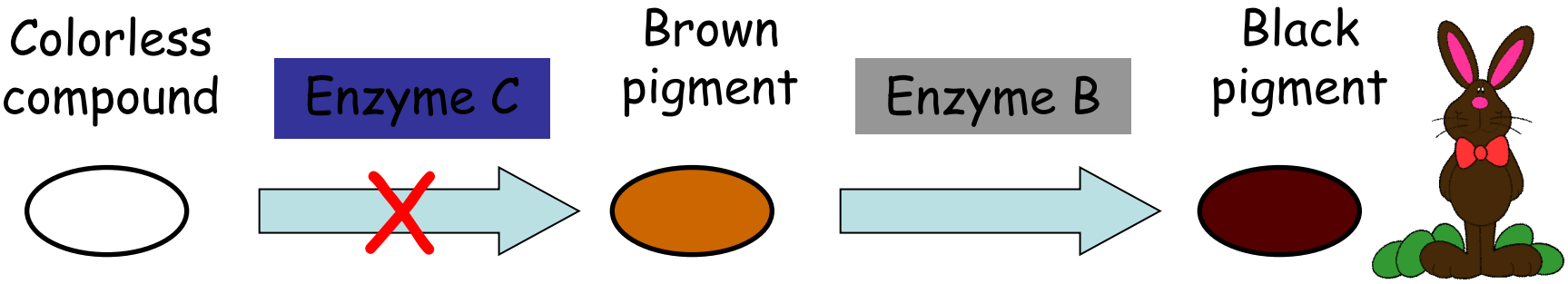
- Genotypes *bbC_*



- Genotypes *_ _ cc*



Gene interactions:**Epistasis**



c: inactive/null allele (no enzyme activity)

the *epistatic gene* *C/c* acts upstream (more important/more drastic phenotypes)

Gene interactions: **Epistasis**

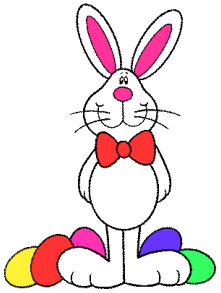
- The action of one gene (*epistatic*) conceals/masks the expression of other gene (hypostatic)

- In a biochemical (regulatory or developmental) pathway the *epistatic genes* act before (upstream) the genes concealed

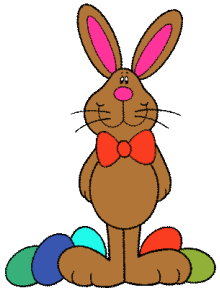
- 3 phenotypes from a dyhibrid cross

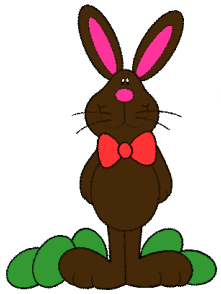
Dihybrid crosses

4 pure lines, 3 colours

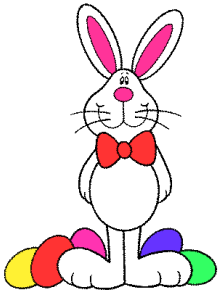


BBcc albino × *bbCC* brown



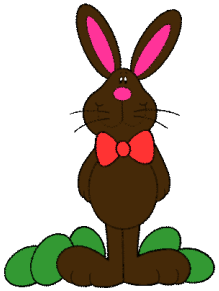


BBCC black × *bbcc* albino



Epistasis

F_1



BbCc black

F_2

9	*B_C_*	black
3	*bbC_*	brown
4	3 *B_cc*	albino
	1 *bbcc*	albino

Establishing a mode of inherentance

example

P Red X Green

F_1 Yellow

F_2 140 Yellow
48 Red
52 Green

One or 2 genes involved?

	Epistasis	Incomplete dom.
	9 Yellow	2 Yellow
	3 Red	1 Red
	4 Green	1 Green
Expected number	135	120
	45	60
	60	60

The χ^2 test (Chi-squared)

Null hypothesis: it is a ratio 9:3:4

Classes	O	E	$(O-E)^2$	$(O-E)^2/E$
Yellow	140	240X9/16=135	25	0.19
Red	48	240X3/16=45	9	0.2
Green	52	240X4/16=60	64	1.23
Total	240			Σ= 1.62

Degrees of freedom= 2

- Degrees of freedom: number of phenotypic classes minus the numer of parameters extracted from the sample. 3-1(total)=2

p: probability of getting, by chance, an equal or higher deviation

1.62: between 0.5 and 0.1 (50%-10%)

df \ p	0.995	0.975	0.9	0.5	0.1	0.05	0.025	0.01	0.005	df
1	.000	.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879	1
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597	2
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838	3
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860	4
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750	5
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548	6
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278	7
8	1.344	2.180	3.490	7.344	13.362	15.507	17.535	20.090	21.955	8
9	1.735	2.700	4.168	8.343	14.684	16.919	19.023	21.666	23.589	9
10	2.156	3.247	4.865	9.342	15.987	18.307	20.483	23.209	25.188	10
11	2.603	3.816	5.578	10.341	17.275	19.675	21.920	24.725	26.757	11
12	3.074	4.404	6.304	11.340	18.549	21.026	23.337	26.217	28.300	12
13	3.565	5.009	7.042	12.340	19.812	22.362	24.736	27.688	29.819	13
14	4.075	5.629	7.790	13.339	21.064	23.685	26.119	29.141	31.319	14
15	4.601	6.262	8.547	14.339	22.307	24.996	27.488	30.578	32.801	15

Hypothesis accepted

χ^2 test of other hypothesis

Null hypothesis: it is a 1:2:1 ratio

Classes	O	E	$(O-E)^2$	$(O-E)^2/E$
Yellow	140	240X2/4=120	400	3.33
Red	48	240X1/4=60	144	2.4
Green	52	240X1/4=60	64	1.07
Total	240			Σ= 6.8

Degrees of freedom= 2

- 6.8>5.991
- Hypothesis rejected, since the probability of getting such a deviation is less than 5%

Limit: p=0.05

- When the probability of getting an equal or higher deviation to observed is less than 5%, the hypothesis is rejected

- Then, another hypothesis must be formulated and tested afterwards