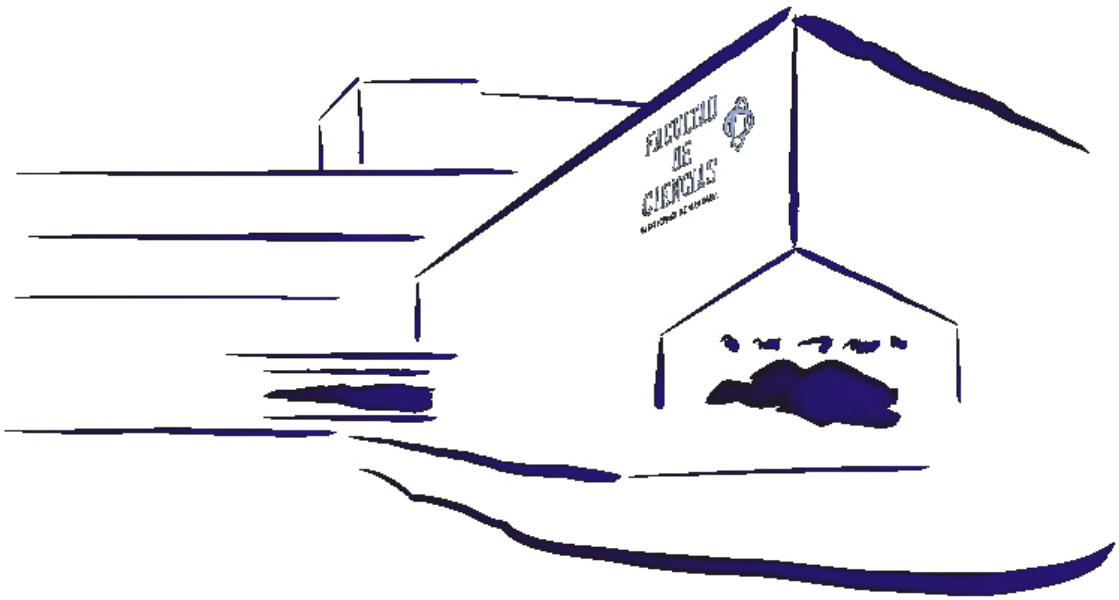

GENETICS I: PROBLEMS AND LABORATORY MANUAL

BIOLOGY DEGREE

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Esta traducción ha sido financiada por el Proyecto de Innovación Docente 22-35 de la Universidad de Granada: "Adaptación del contenido de la asignatura Genética I: de los genes a las poblaciones (2º Grado en Biología) a lengua inglesa".

PROBLEMS

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MENDELIAN GENETICS

1. TROUBLESHOOTING GUIDE

Mono-hybrid crossings

In this set of problems, we will study Mendel's principles of segregation and independent transmission, learn how to make predictions of the results of genetic crosses and understand the usefulness of probability as a tool in genetic analysis. We will start with monohybrid crosses. **Monohybrid crosses** are crosses in which both parents differ in only one characteristic. A monohybrid cross between two pure lines results in F_1 offspring in which all individuals have the phenotype of one of the parents (dominant phenotype) while in F_2, 3/4 of the offspring have this phenotype and 1/4 have the phenotype of the second parent (recessive phenotype). For a hypothetical case in which the character is controlled by a gene with two alleles, one of which determines the dominant phenotype (red flower colour, for example) and the other determines the recessive phenotype (white), we will have:

Red (A) > White (a)

P: Red (AA) x White (aa)
F_1: 100% Red (Aa)
F_2: 1/4 Red (AA): 1/2 Red (Aa): 1/4 White (aa)

The way to discriminate between homozygous and heterozygous red-flowered individuals is by a test cross between these red-flowered individuals and a homozygous recessive (aa) test parent, since the result will be different in each case:

a) Red x White: 100% Red. In this case, the individual with the red phenotype was homozygous AA and the offspring of the test cross will be Aa.

b) Red x White: 50% Red, 50% White. In this case, the individual with the red phenotype was heterozygous Aa and the offspring will be 1/2 AA (red) and 1/2 aa (white).

Probability

Probability expresses the likelihood of a given event occurring. It is calculated as the number of times a particular event occurs divided by the total number of possible outcomes.

Two probabilistic rules are used to predict the proportions of offspring produced by genetic crosses:

- Multiplication rule: states that the probability of two or more independent events occurring simultaneously is calculated by multiplying their independent probabilities.

- Addition rule: states that the probability of different independent and mutually exclusive events occurring is calculated by adding the probabilities of each of them.

To determine the **probability of a particular combination of events** it is useful to employ the following formula:

$$P=\frac{n!}{s!t!}\, p^s\, q^t$$

Where P equals the total probability of an event X with probability p of occurring s times and of an event Y with probability q of occurring t times. Where: s+t = n; p+q = 1.

Dihybrid and polyhybrid crossings

When we analyse the simultaneous inheritance of two or more traits (di-, tri-, polyhybrid crosses) we have to consider, for each gene, the same principles as in a monohybrid cross, i.e.: they can present different allelic alternatives, there are dominance relationships between them and they segregate during meiosis.

The principle of segregation states that two alleles at a locus separate when gametes are formed; the principle of independent transmission states that, when those two alleles separate, their separation is independent of the separation of alleles located at other loci.

This Mendelian principle is only fulfilled in the case of genes located on different chromosomes (or also, as we shall see below, genes located on the same chromosome but far enough apart for crossover to occur at each meiosis).

The following observations should be borne in mind:

a) When the alleles of two loci are separated independently, the dihybrid crosses can be analysed as two independent monohybrid crosses and then the proportions combined.

b) Since they are two independent events, these combinations are calculated using the multiplication rule.

c) In the case of a cross between two dihybrids, the expected ratios are 9:3:3:1.

d) Types of gametes produced in the case of two genes:

Individuals (Genotype)	Gametes	Ratio			
AABB	**AB**	**1**			
AABb	**AB, Ab**	**1/2**	**1/2**		
AAbb	**Ab**	**1**			
AaBB	**AB, aB**	**1/2**	**1/2**		
AaBb	**AB, Ab, aB, ab**	**1/4**	**1/4**	**1/4**	**1/4**
Aabb	**Ab, ab**	**1/2**	**1/2**		
aaBB	**aB**	**1**			
aaBb	**aB, ab**	**1/2**	**1/2**		
aabb	**ab**	**1**			

The same applies in the case of three or more genes, taking into account the following **rule**: according to the principle of Mendelian segregation, a gamete receives only one allele of each gene.

e) To obtain the result of crossing between individuals differing in two or more characters, a Punnet square or a branching diagram (bifurcation method) can be made:

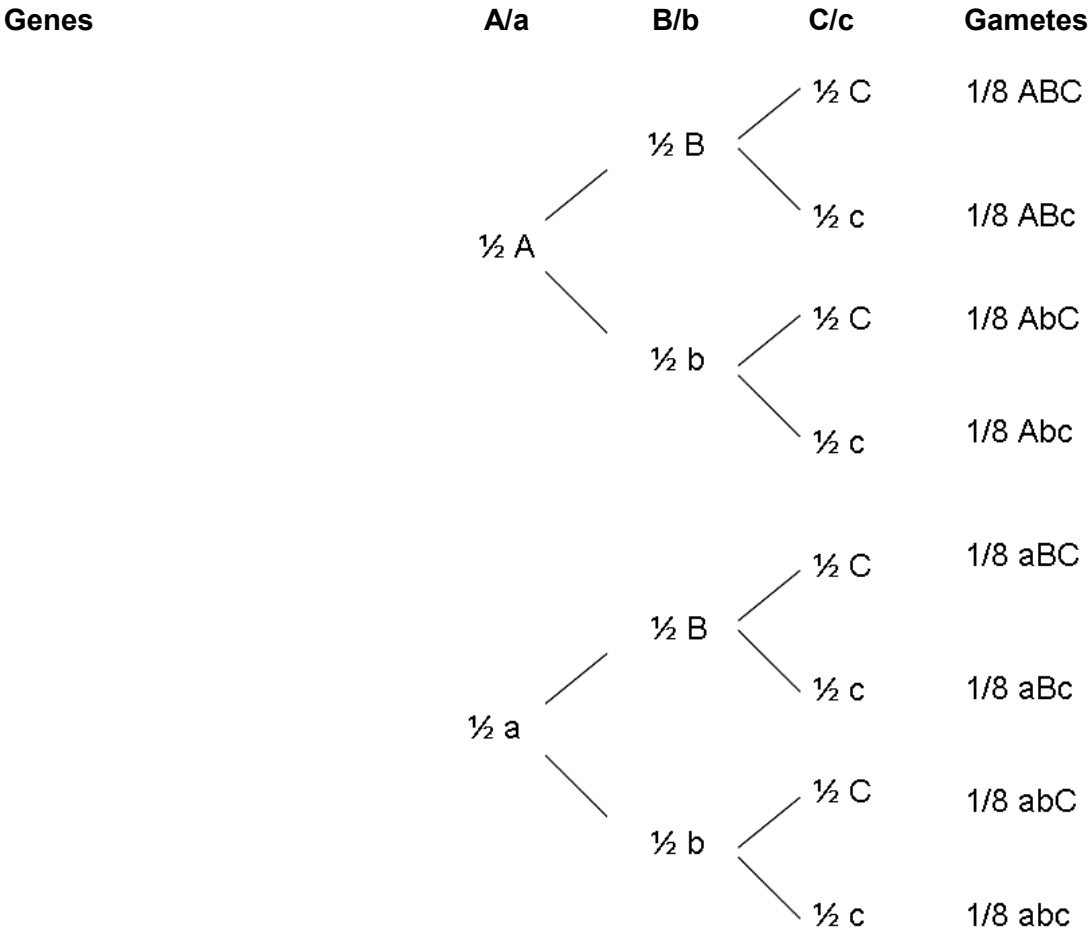
The Punnet square allows us to analyse the genotypic and phenotypic proportions of the offspring. For example, in a dihybrid test cross:

Parents: AaBb x aabb

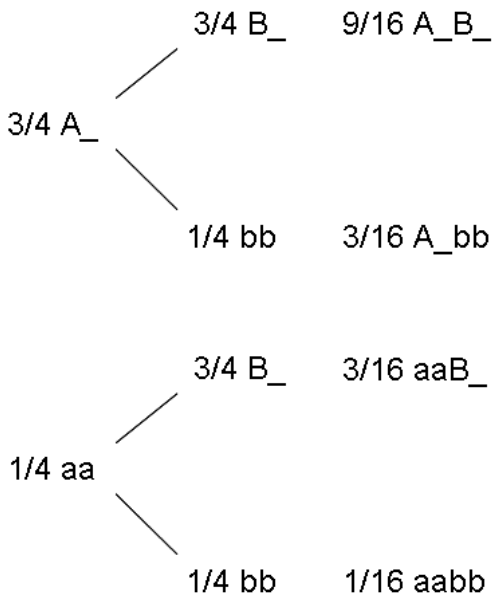
Gametes/Proportion	AB (¼)	Ab (¼)	aB (¼)	ab (¼)
ab (1)	AaBb (¼)	Aabb (¼)	aaBb (¼)	aabb (¼)

It can be seen that the test cross in the case of two genes results in 1:1:1:1 ratio. For three genes it is 1:1:1:1:1:1:1:1, and so on.

The branching diagram (bifurcation method) allows rapid analysis of gamete frequencies or phenotypic frequencies of the offspring. It is useful in the case of more than two genes. Example: the proportion and types of gametes produced by a trihybrid individual will be as follows.



In the same way it can be applied to determine the frequencies of the expected phenotypic classes in the F_2 of a polyhybrid cross. In the case of a dihybrid cross (AaBb x AaBb):



(f) Calculation of probabilities in polyhybrids

They are calculated by applying the general term of a polynomial. For example, in the case of crosses between heterozygotes:

- Calculation of genotypic frequencies: the probabilities of obtaining a homozygous dominant, heterozygous or homozygous recessive offspring in the cross of a monohybrid are 1/4, 1/2 and 1/4, respectively. Generalising this case, for *n* loci, the probability of obtaining an individual whose genotype is dominant for *d* loci, heterozygous for *h* loci and recessive for de *r* loci will be:

$$\frac{n!}{d!\,h!\,r!}(1/4)^d(1/2)^h(1/4)^r$$

Where: d+h+r = n

- Calculation of the phenotypic frequencies: the probabilities of obtaining an offspring with a dominant phenotype or a recessive phenotype from a monohybrid cross are 3/4 and 1/4, respectively. Generalising this case, for *n* loci, the probability of obtaining an individual whose phenotype is dominant for *d* loci, and recessive for *r* loci will be:

$$\frac{n!}{d!\,r!}(3/4)^d(1/4)^r$$

Where: d+ r = n

Chi-square goodness-of-fit test

The **chi-square goodness-of-fit test** is a statistical test that tells us how well the observed values fit the expected values in an experiment. This test does not tell us whether a genetic cross has been performed correctly, whether the results are correct, or whether we have chosen the explanation that best fits our data. Instead, it indicates the probability that the difference between the observed and expected values is due to chance. It is calculated by applying the following formula:

$$\chi^2_{\text{exp}} = \sum \frac{(Observed - Expected)^2}{Expected}$$

Where observed and expected values are considered in absolute values.

The calculated value of χ^2 is then compared with theoretical values having the same degrees of freedom in a χ^2 table. The degrees of freedom represent the number of ways in which the expected classes are free to vary. In the χ^2 test the degrees of freedom equal *n-1*, where *n* is the number of existing phenotypic classes.

In the table, the degrees of freedom are indicated in the left-hand column, while the top column indicates probability. A probability level of 0.05 is normally used, which indicates that if the probability that chance is responsible for the observed deviation is equal to or greater than 0.05, the observed differences are due to chance. When this probability is less than 0.05, chance is not responsible for the deviation and there is a significant difference between the observed and expected values. Thus, if the experimental value of χ^2 is less than the theoretical value for a significance level of 0.05 and a number of degrees of freedom of n-1, we do not reject the hypothesis that we had established *a priori* to explain them and we assume that the observed values conform to the expected ones. Otherwise, we would reject the hypothesis.

X^2 distribution

Degrees of freedom	Probability										
	0,95	0,90	0,80	0,70	0,50	0,30	0,20	0,10	0,05	0,01	0,001
1	0,004	0,02	0,06	0,15	0,46	1,07	1,64	2,71	3,84	6,64	10,83
2	0,10	0,21	0,45	0,71	1,39	2,41	3,22	4,60	5,99	9,21	13,82
3	0,35	0,58	1,01	1,42	2,37	3,66	4,64	6,25	7,82	11,34	16,27
4	0,71	1,06	1,65	2,20	3,36	4,88	5,99	7,78	9,49	13,28	18,47
5	1,14	1,61	2,34	3,00	4,35	6,06	7,29	9,24	11,07	15,09	20,52
6	1,63	2,20	3,07	3,83	5,35	7,23	8,56	10,64	12,59	16,81	22,46
7	2,17	2,83	3,82	4,67	6,35	8,38	9,80	12,02	14,07	18,48	24,32
8	2,73	3,49	4,59	5.53	7,34	9,52	11,03	13,36	15,51	20,09	26,12
9	3,32	4,17	5,38	6,39	8,34	10,66	12,24	14,68	16,92	21,67	27,88
10	3,94	4,86	6,18	7,27	9,34	11,78	13,44	15,99	18,31	23,21	29,59
	Not significant								Significant		

Pedigree analysis

A **pedigree** is a graphical representation of family history showing the inheritance of one or more traits or diseases (phenotypes in general). The purpose is to facilitate genetic analysis of a particular phenotype by examining its inheritance pattern in a particular family.

Commonly encountered symbols are:

Female:

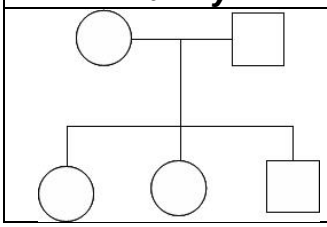
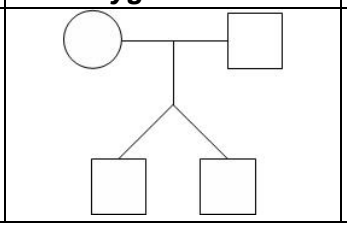
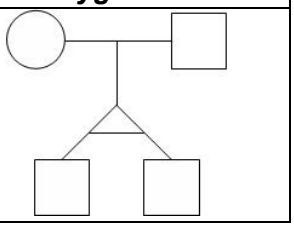
Male:

Sex unknown or not specified:

Presence of the trait:

Absence of the trait:

Representation of two characters:

Family	***Dizygotic twins***	***Monozygotic twins***
		

Generations are usually identified with Roman numerals (I, II, III, IV, V...) and within each generation individuals are identified with Arabic numerals (1, 2, 3, 4, 5...).

2. PROBLEMS SOLVED

Problem 1. Two purebred plants, one long-stemmed and one short-stemmed, were crossed. In F_2 the following phenotypes were obtained: 3/4 long-stemmed and 1/4 short-stemmed. The long stem is dominant over the short stem. What will be the genotype of the parents, the F_1 and the F_2 individuals?

Answer

Let T be the dominant allele that produces long stems and t the recessive allele.

Long stem > short stem

T > t (indicates that T is dominant over t)

The parents are two pure-bred plants, one long-stemmed and one short-stemmed. Therefore, the genotype of the individuals of this cross will be:

P long stem x short stem

TT x tt

F_1 All offspring will be of long stem phenotype and heterozygous (Tt).

F_2 is obtained by self-fertilisation.

F_2 Tt xTt

TT Tt tt tt

3/4 long stem 1/4 short stem

As stated in the problem statement, the following phenotypes are obtained in F_2: 3/4 long stem and 1/4 short stem, corresponding to the genotypes TT and Tt (long stem) and tt (short stem).

Problem 2. In the pea plant the axial position of the flowers is dominant over the terminal position, with "A" representing the allele for the axial position and "a" for the terminal position. If 400 individuals are obtained by crossing two heterozygous plants, how many will have axial position and how many will have terminal position?

Answer

Axial position > terminal position
A>a

The cross of two heterozygous plants will be: Aa x Aa

Genotypic classes and proportion in offspring: AA (1/4); Aa (1/2); aa (1/4)

Phenotypic classes and proportion in offspring: 3/4 axial position (AA + Aa); 1/4 terminal position (aa). Of the total of 400 individuals: 300 will have flowers in axial position and 100 will have flowers in terminal position.

Problem 3. Hot pepper plants were crossed with sweet pepper plants. F_1 was spicy fruit and F_2 produced 32 spicy pepper plants and 10 sweet pepper plants.

a) How many of the spicy plants are expected to be homozygous and how many heterozygous?

b) How do you find out which of the 32 spicy plants are heterozygous?

Answer

P Spicy x sweet
F_1 spicy **(**self-fertilisation to produce F_2)
F_2 232 spicy, 10 sweet

The pungent character is dominant over the sweet character, as crossing the parents (P) results in offspring with a 100% pungent phenotype:

Spicy> sweet
A>a

In addition, the parents have to be pure lines:

P spicy x sweet
AA x aa 100% of the F_1 will be heterozygous plants, phenotyped as follows spicy:

F_1 spicy
Aa F_1 self-fertilisation produces pungent and sweet plants in a 3:1 ratio,

F_2 AA (1/4), Aa (1/2), aa (1/4)

3/4 spicy (AA + Aa) and 1/4 sweet (aa)

The number of plants obtained in F_2 is 32 pungent and 10 sweet, values that are in line with the expected 3:1 ratio.

Among the spicy plants, 1/3 are homozygous and 2/3 heterozygous. Thus, the 32 plants with the pungent phenotype can be of AA (homozygous) or Aa (heterozygous) genotype, while those with the sweet phenotype are aa.

a) There are two genotypic possibilities for the spicy plants: homozygous AA (1/3) and heterozygous Aa (2/3). As 32 spicy plants were obtained in F_2: approximately 11 plants will be AA and 21 plants will be Aa.

b) To find out which spicy plants of F_2 are heterozygous, we make a test cross with sweet plants (aa). In the offspring we will only obtain hot pepper plants if the parent used was homozygous AA, while if it was heterozygous Aa, 1/2 of the offspring will be hot and 1/2 will be sweet:

Spicy x sweet	Spicy x sweet
AA x aa	Aa x aa
↓	↓
All F_1 plants would be hot	1/2 hot (Aa), 1/2 sweet (aa).

Problem 4. Albinism (lack of skin pigmentation) in humans is due to an autosomal recessive allele (a) whereas normal pigmentation is the consequence of a dominant allele (A).

Two normal parents have an albino child. Determine the probability that:

a) The next child is an albino.
b) Both immediate children are albinos.
c) If the parents have two children, that one is albino and the other normal.

Answer

Normal pigmentation > lack of pigmentation or albinism
(A>a)

If two normally pigmented parents have an albino child, it is because both parents must be heterozygous: Aa x Aa

The genotypic and phenotypic proportions of this cross (Aa x Aa) would be:

Genotypic classes and proportion in offspring: AA (1/4), Aa (2/4) and aa (1/4).

Phenotypic classes and proportion in offspring: 3/4 normal pigmentation (AA +Aa) and 1/4 albino (aa).

a) The answer to the first paragraph would be 1/4, since "the probability that the next child will be an albino" is an independent event, it does not influence whether they have already had an albino child before.

b) In this case both immediate offspring are albinos, so the probability that one is an albino "and" that the next is an albino must be taken into account (both events are independent).

Probability of having an albino child (1/4) "and" probability that the next child will be albino (1/4). Remember the multiplication rule (the probability of two or more independent events occurring simultaneously is calculated by multiplying their individual probabilities).

So the final result will be: 1/4 x 1/4 = 1/16.

c) Now we have to calculate the probability of two children, one normal "and" one albino.

Probability of having a normal child (3/4) x probability of an albino child (1/4)

But in this case another alternative has to be taken into account: that the first child is albino and the second is normal [probability of having an albino child (1/4) x probability of a normal one (3/4)].

In other words, as the problem does not establish the birth order of the children, all possibilities must be taken into account:

normal and albino "or" albino and normal

In this exercise we apply the addition rule (the probability of the occurrence of only one of two or more mutually exclusive events is calculated by adding the probabilities of each of them). Therefore, the final result would be:

(3/4) x (1/4) + (1/4) x (3/4) = 6/16 = 3/8

Problem 5. Polydactyly in the human species is due to an autosomal dominant allele. Two polydactylic first cousins, whose common grandparents were normal, wish to have seven children. We want to know the following probabilities:

a) That no child is a polydactyl.
b) The first two are polydactyl and the next five are normal.
c) That three are polydactyl and four are not.
d) If the first three were normal, what is the probability that the fourth would also be normal? And, that the fifth would be polydactylic?

Answer

As polydactyly is a dominant trait and both members of the pair are polydactyl but their common grandparents are normal, both must be heterozygous Aa. Thus, the cross is Aa x Aa and the probability of having a polydactylic offspring (A_) will be 3/4 while the probability of being normal will be 1/4 (aa).

a) The probability of one child being normal is 1/4. The probability that all seven will be normal will be the product of their individual probabilities: $(1/4)^7 = 6.1 \times 10^{-5}$

b) (3/4) x (3/4) x (1/4) x (1/4) x (1/4) x (1/4) x (1/4) = 5.5×10^{-4}

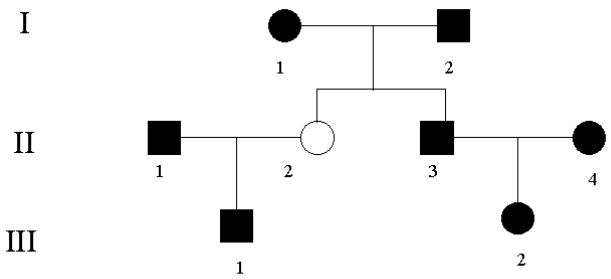
c) In this case, contrary to what happens in section b, in which the order of the descendants was established, there are now different possibilities, as many as possible combinations of cases that fulfil the condition that three of the descendants are polydactylic and four are normal. Thus:

$$P=\frac{n!}{s!t!}\, p^s\, q^t = \frac{7!}{3!4!}\,(3/4)^3\,(1/4)^4 = 1.65 \times 10^{-3}$$

Where P equals the total probability of an event X (being born with polydactyly) *with probability p* (3/4) *of occurring s times* (3 polydactylic offspring) *and of an event Y* (normal) *with probability q* (1/4) *of occurring t times* (4 normal offspring).

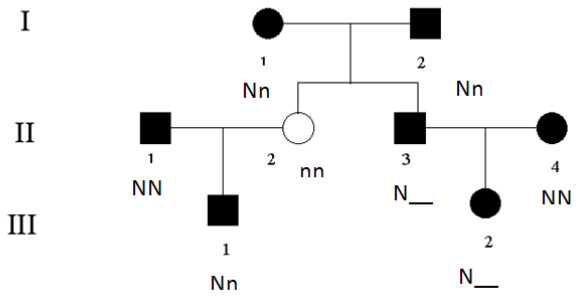
d) They are independent of each other. Therefore, the probability of a heterozygous couple having a polydactylic child is 3/4 and that of having a normal child is 1/4, irrespective of previous offspring.

Problem 6. Black hair in guinea pigs is determined by a dominant N allele and white hair by its recessive n allele. In the following pedigree, unless there is evidence to the contrary, assume that individuals II-1 and II-4 do not carry the recessive allele and calculate the odds that an offspring III-1 x III-2 will have white hair (solid symbols represent black hair).



Answer

The genotypes of each individual in the pedigree shall be:



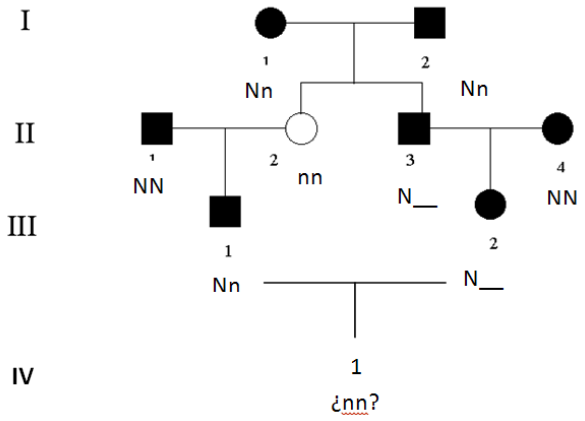
Individuals I-1 and I-2 must both be heterozygous because they have one offspring (individual II-2) with white hair.

Individuals II-1 and II-4 are homozygous dominant for the trait as stated in the problem statement because there is no evidence to the contrary.

The genotype of the II-3 individual can be either NN or Nn, in both cases its phenotype is black haired, but we would have two alternatives for the genotype.

The same is true for the III-2 individual since its genotype can be NN or Nn, which will depend on the genotype of its II-3 parent (whether the latter is homozygous dominant or heterozygous).

The problem asks us to calculate the probability that an offspring of the III-1 x III-2 cross will have white hair:



For the IV-1 individual to have white hair, the III-2 parent must be heterozygous (so that both parents would transmit the "n" allele to the IV-1 offspring).

But for individual III-2 to be heterozygous, individual II-3 must also be heterozygous.

Let's calculate the probabilities that both individuals (III-2 and II-3) have these genotypes.

Cross I-1 x I-2:

Nn x Nn

↓

NN Nn Nn nn

Black hair

Among the offspring with black hair, we will have 1/3 homozygous (AA) and 2/3 heterozygous (Aa). These values correspond to the ratio 1:2 (1/4:2/4) between homozygotes and heterozygotes of the total of 3/4 black-haired offspring resulting from a cross between two heterozygotes.

Thus, the probability that the II-3 individual is heterozygous will be 2/3.Cross II-3 x II-4:

Nn x NN

↓

NN Nn

1/2 1/2

Black hair

From this cross we would obtain 100% of the offspring with black hair, but half of them would be homozygous dominant and the other half heterozygous. Thus, the probability that individual III-2 is heterozygous is 1/2.

Cross III-1 x III-2:

Nn x Nn
↓
NN Nn Nn (3/4 black hair) nn (1/4 white hair)

The probability of obtaining white-haired offspring from this cross is 1/4.

So, the total probability will be: 2/3 x 1/2 x 1/4= 2/24 = 1/12

Problem 7. A black horse of unknown ancestry was mated to a number of purebred red mares. These matings gave 20 red offspring and 25 black offspring.

a) Which of these phenotypic traits is most likely caused by a homozygous recessive?
b) According to your hypothesis, how many individuals of each class would you have expected?
c) Test the hypothesis using the χ^2 method and indicate whether, on the basis of this test, the hypothesis would be accepted or rejected.

Answer

a) The females are purebred, so they will be homozygous for this trait. If they were homozygous dominant, all the offspring should be red. So we assume that the colour in horses is determined by a locus with alleles A (Black) > a (Red) and that the red character is most likely due to the presence in homozygosity of the recessive a allele.

b) The genotypes of the parents and of the F_1 must be as follows:

P: Black Horse Aa x Red Mare aa
↓
F_1: 1/2 Blacks Aa, 1/2 Reds aa

c) The number of observed individuals is:

Blacks Aa: 25
Reds aa: 20
Total: 45

The number of individuals expected according to our hypothesis is:
Blacks Aa: 22.5 (1/2 of 45)
Reds aa: 22.5 (1/2 of 45)
Total: 45

We perform the χ^2 test to see if the observed data conform to the expected data:

$$\chi^2_{\text{exp}} = \sum \frac{(Observed - Expected)^2}{Expected} = (20\text{-}22.5)^2/22.5 + (25\text{-}22.5)^2/22.5 = 0.545$$

The value of the theoretical χ^2 for 1 degree of freedom (number of phenotypic classes minus one) and a significance level of 0.05 is 3.841. Since the experimental χ^2 is lower

than the theoretical χ^2, we do not reject the proposed hypothesis and assume that the observed values are in line with the expected values ($0.3 < p < 0.5$).

Problem 8. In the garden pea (*Pisum sativum*) seed colour is due to two alleles of a gene: the A allele determines yellow colour and is dominant over a which determines green colour. On the other hand, the L allele is responsible for the formation of smooth seeds and is dominant over the L allele that determines rough seeds. Crossing a plant with green and smooth seeds with a plant with yellow and smooth seeds has resulted in offspring consisting of plants with yellow and smooth seeds and plants with yellow and rough seeds. Determine the genotypes of the parents.

Answer

- Parental 1. Phenotype: green and smooth seeds. Genotype: as the green colour is recessive, the plant must be aa. However, the smooth character is dominant, so it must have at least one L_ allele. As its offspring is both smooth (L_) and rough (ll), this parent must be heterozygous (Ll). Therefore, its genotype will be: aaLl

- Phenotype: yellow and smooth seeds. Genotype: as it has the two dominant characters, it must have at least one dominant allele of each gene: A_L_, but we can know its genotype if we look at the F_1: all the descendants have yellow seeds and as the other parent had green seeds (aa), this one must be AA. As for the other character, since in F_1 we find both individuals with smooth and rough seeds, the parent must be heterozygous (Ll). Therefore, its genotype will be: AALl

Problem 9. In guinea pigs, black fur (B) is dominant over albino (b), and rough skin (R) is dominant over smooth skin (r). A black and rough guinea pig crosses with an albino and rough guinea pig and produces the following progeny: 13 black rough, 16 albino rough, 6 black smooth and 5 albino smooth. Identify the genotypes of the parents, using the χ^2 method to test the hypothesis.

Answer

The cross: black-smooth (B_R_) x albino-smooth (bbR_). The fact that in the progeny there are albino-smooth individuals (bbrr) indicates that the genotype of the parents must be: BbRr and bbRr.

If our hypothesis is correct, in the offspring of the cross BbRr x bbRr, we would expect:

Gametes/Proportion	**bR (1/2)**	**br (1/2)**
BR (1/4)	**BbRR (1/8)**	**BbRr (1/8)**
Br (1/4)	**BbRr (1/8)**	**Bbrr (1/8)**
bR (1/4)	**bbRR (1/8)**	**bbRr (1/8)**
br (1/4)	**bbRr (1/8)**	**bbrr (1/8)**

That is to say:

Black-rugged (B_R_): 3/8
Black-smooth (B_rr): 1/8
Albino-rugged (bbR_): 3/8
Albino-smooth (bbrr): 1/8

We apply the χ^2 test to test our hypothesis:

The observed results are:	The expected results are:
Black-rugged: 13	40x3/8 = 15
Black-smooth: 6	40x1/8 = 5
Albino-rugged: 16	40x3/8 = 15
Albino-smooth: 5	40x1/8 = 5

Total: 40

Since there are four phenotypic classes, the number of degrees of freedom is: 4-1= 3.

$$\chi^2_{\exp} = \sum \frac{(Observed - Expected)^2}{Expected} = 0.53;\ (0.9 < p < 0.95)$$

χ^2 theoretical (3; 0,05) = 7,815

The hypothesis is not rejected, so we can conclude that the genotype of the parents is: BbRr x bbRr.

Problem 10. Two abnormal conditions in humans, cataracts and fragile bones, are due to dominant alleles of two different genes. A couple consisting of a man with cataracts and normal bones (whose father had normal eyes) and a woman without cataracts but with fragile bones (whose father had normal bones), wish to have children. Indicate the probability that they will have:

a) A normal offspring
b) A descendant with cataracts and normal bones
c) An offspring with normal eyes and fragile bones
d) A descendant suffering from both diseases

Answer

If we call the alleles of the genes involved C/c and H/h, with C/c being the dominant and recessive alleles of the gene involved in the development of cataracts and H/h being the alleles of the gene involved in bone fragility, the male's genotype is Cchh. As his father has normal eyes, which means he is homozygous for this trait, the male must carry a recessive allele for this trait. The female's genotype is ccHh. As in the male, her father was homozygous recessive for the bone trait and therefore must carry a recessive allele.

The gametes produced by each of them are:

Male: Ch and ch
Female: cH and ch

The result of the cross between the two would be:

Gametes/Proportion	**cH (1/2)**	**ch (1/2)**
Ch (1/2)	**CcHh (1/4)**	**Cchh (1/4)**
ch (1/2)	**ccHh (1/4)**	**cchh (1/4)**

Thus:

a) The probability of having a normal offspring, i.e. a double homozygous recessive (cchh), is 1/4.

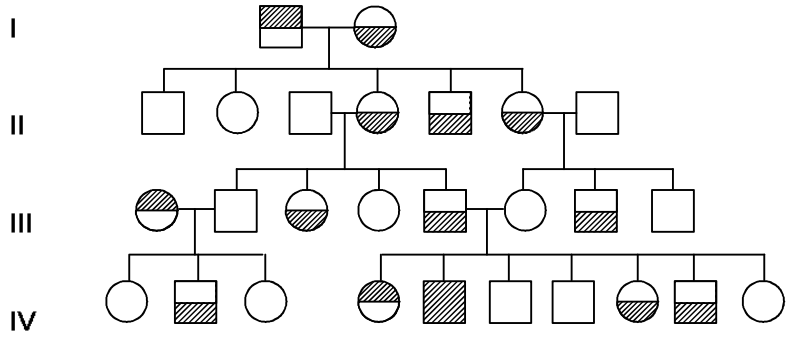
b) In this case we are asked for the probability of an offspring with cataracts and normal bones, so the genotype is Cc or CC for cataracts and hh for normal bones. As can be seen in Punnet's table, only one of the four possible offspring (Cchh) fulfils the conditions, so the frequency is 1/4.

c) An offspring with normal eyes and fragile bones has ccHh or ccHH as possible genotypes. If we look at the table, we see that, in this cross, only the ccHh genotype is obtained, with a probability of 1/4.

d) Finally, we are asked for the probability of an individual being born with both diseases. This means that the individual can have CCHH, CCHh, CcHH or CcHh as possible genotypes. Only one of these (CcHh) can occur in this cross. The probability of obtaining it is 1/4.

Problem 11. The following pedigree shows the transmission of two characters in a single family. Character 1 is indicated by shading the upper half of the symbol and character 2 is indicated by shading the lower half. Using alphabetic symbols for the genes involved (e.g. A/a for characteristic 1 and B/b for characteristic 2) answer the following questions:

a) What type of inheritance is involved in each of the traits?
b) Determine the genotypes of all individuals in the pedigree as far as possible.
c) What genotypes and phenotypes and what proportions would be expected in the mating between individuals IV-3 and IV-5?



Answer

a) Trait I: Two individuals without the shaded trait (III-5 and III-6) have a daughter (IV-4) and a son (IV-5) with the shaded trait, so it must be recessive.

Character 2: Two individuals without the shaded trait (III-1 x III-2) have an offspring with the shaded trait (IV-2). Likewise, it must be recessive.

In both cases, there are only two phenotypes and the genealogy can be interpreted by considering that in the inheritance of both traits the traits (phenotypes) shown in shading are determined by recessive alleles of two autosomal A/a and B/b genes.

b) The genotypes of the individuals shall be:

I-1, aaBb I-2, A_bb

II-1, AaBb II-2, AaBb II-3, A_Bb
II-4, Aabb II-5, Aabb II-6, Aabb

II-7, A_Bb

III-1, aaBb III-2, A_Bb III-3, A_bb
III-4, A_Bb III-5, Aabb III-6, AaBb
III-7, A_bb III-8, A_Bb

IV-I, AaB_ IV-2, Aabb IV-3, AaB_,
IV-4, aaBb IV-5, aabb IV-6, A_Bb
IV-7, A_Bb IV-8, A_bb IV-9, A_bb
IV-10, A_Bb

c) IV-3 is of genotype AaBB or AaBb. The probability of each of these combinations will depend on the genotypic constitution of their parents:

III-1 x III-2
aaBb A_Bb
↓
IV-3 AaB_

As we know that IV-3 is dominant phenotype, it will be AaBB with probability 1/3 and AaBb with probability 2/3. It does not matter what his parents are like for the A,a locus, nor that we do not know exactly what his mother's genotype is for this locus, since we know that IV-3 is Aa with probability 1.

Therefore, and given that IV-5 is aabb, the two possible crosses will be:

(1/3) AaBB x aabb
↓
1/2 AaBb
1/2 aaBb

(2/3) AaBb x aabb
↓
1/4 AaBb
1/4 aaBb
1/4 Aabb
1/4 aabb

The first crossing has a probability of 1/3 and the second of 2/3. Combining them, we will obtain the solution to the question:

Genotypes	**Proportions**	**Phenotypes**
AaBb	1/2 x 1/3 + 1/4 x 2/3 = 1/3	Dominant for A and B (AB)
aaBb	1/2 x 1/3 + 1/4 x 2/3 = 1/3	Dominant for B (aB)
Aabb	1/4 x 2/3 = 1/6	Dominant for A (Ab)
aabb	1/4 x 2/3 = 1/6	Recessive for A and B (ab)

Problem 12. In cockerels and hens, the trait feathery feet (F) is dominant over the trait clean feet (f) and the pea crest (P) over the single crest (p). Two cocks A and B are crossed with two hens C and D. All four birds have feathered feet and pea crests. Cock A with both hens has all feathered and pea-crested offspring. Cock B with hen C has offspring with feathered or clean feet, but all with pea crests. Cock B with hen D has all feathery offspring, but some with pea crests and some with single crests. What are the genotypes of the four parent birds and the offspring of each cross?

Answer

The parent individuals, A, B, C and D, because they have feathery legs and pea crests, will be F_P_. The other two alleles will have to be discovered by studying each cross:

Crosses A x C and A x D (F_P_ x F_P_)

All the offspring are feathery with pea crests: F_P_. The fact that there are no individuals with clean legs (ff) indicates that it is highly unlikely that both parents carry the f allele (however, one of them could carry it in each cross). For the same reason, as there are no individuals with single crests (pp), we must think that the p allele will be carried by a maximum of one parent in each cross.

No further conclusions can be drawn from these crossings at this stage.

Crossing B x C (F_P_ x F_P_)

The offspring are of two types: feathery-legged and pea-crested (F_P_) and clean-legged and pea-crested (ffP_).

This leads us to affirm that B and C are heterozygous for the F gene (Ff), since the combination ff is found in their offspring. Regarding the second locus, as in the previous crosses, it will be very unlikely that both parents carry the recessive allele, although one of them could be a carrier of the recessive allele.

Crossover B x D (FfP_ x F_P_)

The descendants are: pea-crested feathered (F_P_) or single-crested feathered (F_pp).

It follows, by a reasoning similar to the above, that B and D carry the p allele, and that only one of them will carry the f allele, in this case, B. Therefore, the genotype of B is definitely FfPp and that of the female D is FFPp.

Reviewing the cross B x C, now that we know the genotype of B we can state that the genotype of female C is FfPP.

Turning now to the crosses A x C and A x D, since C carries the f allele, A will probably not carry it, nor will it carry the p allele, since it is carried by D. Thus, the genotype of A will therefore be FFPP.

Thus, the crosses will have been:

A x C: FFPP x FfPP
↓
FFPP All feathered with pea crest
FfPP

A x D: FFPP x FFPp
↓
FFPP All feathered with pea crest
FFPp

B x C: FfPp x FfPP
↓
FFPP Plumose with pea crest
FFPp

FfPP
FfPp

ffPP Clean-footed and pea crest
ffPp

B x D: FfPp x FFPp
↓

FFPP Plumose with pea crest
FfPP
FFPp
FfPp

Ffpp Feathered with simple crest
Ffpp

Problem 13. If an individual heterozygous for five independent loci (AaBbCcDdEe) is self-fertilised**:**

a) How many genetically distinct gametes can it produce?
b) What number of different genotypes will appear in the offspring?
c) What is the expected frequency of heterozygous individuals for three loci and homozygous dominant individuals for the remaining two loci?
d) What is the expected frequency of AaBbCcDDEE offspring?

Answer

a) The individual is a pentaheterozygote, i.e. heterozygous for five loci. As the genes are independent, the segregation of each gene is an event independent of the others. Let us reason as follows: If we take into account, for example, only the gene A, a, we can conclude that a heterozygous individual will produce two different types of gametes, one with the A allele and one with the a allele. The same result will be obtained if we do it with the rest of the genes since the individual is heterozygous for all 5 genes. The result, since they are independent events, will be: 2x2x2x2x2, i.e. $2^5 = 32$.

b) The offspring of a pentahybrid cross, for each gene, conforms to the Mendelian genotypic proportions: 1/4: 1/2: 1/4. For example, for the gene A/a, 1/4 AA, 1/2 Aa, 1/4 aa, we see that there are 3 genotypic classes. The same happens for the rest of the genes and as the five genes are independent, the number of different genotypes is: $3x3x3x3x3 = 3^5 = 243$

c) Since the question does not specify which genes should be heterozygotes and which should be homozygotes, we can calculate genotypic frequencies using the probability function of the binomial distribution:

$$\text{Probability} \begin{pmatrix} \text{D homozygous loci XX} \\ \text{h heterozigous loci Xx} \\ \text{d homozygous loci xx} \end{pmatrix} = \frac{n!}{d!h!r!} (1/4)^d \ (1/2)^h \ (1/4)^r$$

where n = number of loci

That, in the case of the question posed in the statement, it turns out to be:

$$\text{Probability} \begin{pmatrix} \text{2 homozygous loci XX} \\ \text{3 heterozygous loci Xx} \\ \text{0 homocygous loci xx} \end{pmatrix} = \frac{5!}{2!3!0!}\,(1/4)^2\,(1/2)^3\,(1/4)^0 = \frac{10}{128} = \frac{5}{64}$$

d) In this case, the question includes the detailed description of the genotype of each of the following five genes, so for the calculation of frequencies we will multiply the probabilities of the genotypes at each locus:

$$P(\text{AaBbCcDDEE}) = P(\text{Aa}) \cdot P(\text{Bb}) \cdot P(\text{Cc}) \cdot P(\text{DD}) \cdot P(\text{EE}) = \frac{1}{2} \cdot \frac{1}{2} \cdot \frac{1}{2} \cdot \frac{1}{4} \cdot \frac{1}{4} = \frac{1}{128}$$

3. PROBLEMS TO BE SOLVED

Problem 1. Mendel discovered that in peas the axial position of the flowers is dominant over the terminal position. We represent by "A" the allele for the axial position and by "a" for the terminal position. Determine the types of progeny produced by each of the following crosses:

a) AA x aa;
b) AA x Aa;
c) Aa x aa;
d) Aa x Aa.

Problem 2. Brachydactyly is a rare dominant human trait that causes shortening of the fingers. Several studies have shown that approximately half of the offspring of brachydactyl x normal marriages are brachydactyl. What proportion of brachydactyl offspring would be expected between two heterozygous individuals?

Problem 3. Curly coats in dogs dominate over smooth coats. From a cross between a pair of curly-haired dogs, a curly-haired puppy was produced. What type of cross could be made to test whether the puppy is heterozygous for this trait?

Problem 4. In experiment A, a white-haired guinea pig is crossed with a black-haired guinea pig and all the offspring are white-haired. In experiment B, a white-haired guinea pig is also crossed with a black-haired guinea pig, but this time the offspring consist of 5 black-haired and 5 white-haired guinea pigs. What will the genotypes of the parents and the offspring be in the different experiments?

Problem 5. In cats, the black coat is dominant over the grey coat. A black-haired female, whose mother is grey, is mated to a grey male. If the resulting litter consists of six kittens, what is the probability that three are black and three are grey?

Problem 6. The absence of molars in the human species is due to an autosomal dominant allele. From the marriage of two first cousins without molars and whose

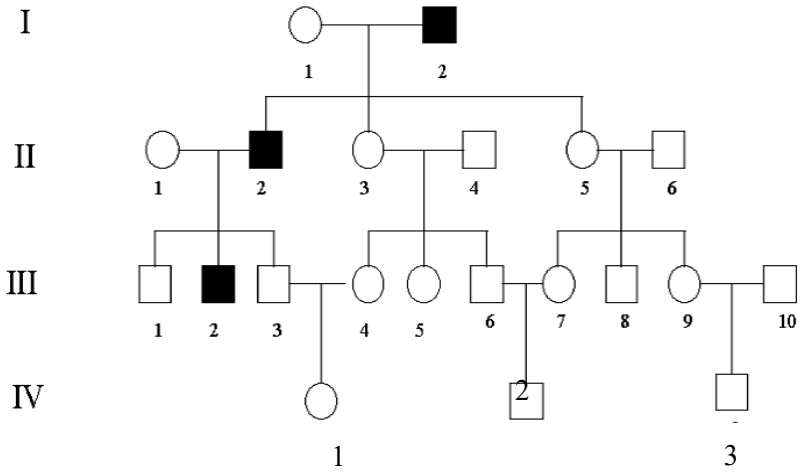
common grandparents were normal, five children are born. We want to know the following probabilities:

a) All children without molars
b) The two oldest without molars and the three youngest with molars
c) Three with molars and two without
d) If the first four are normal, what is the probability that the fifth is also normal and that the fifth does not have molars?

Problem 7. The recessive r allele of a gene is the primary cause of red hair colour in the human species. Dark hair is due to the dominant allele R. In the pedigree shown below assume, unless there is evidence to the contrary, that individuals who marry into this family do not carry the r allele. Calculate the probability that a child from these marriages will have red hair:

a) III-3 x III-9
b) III-4 x III-10
c) IV-1 x IV-2
d) IV-1 x IV-3

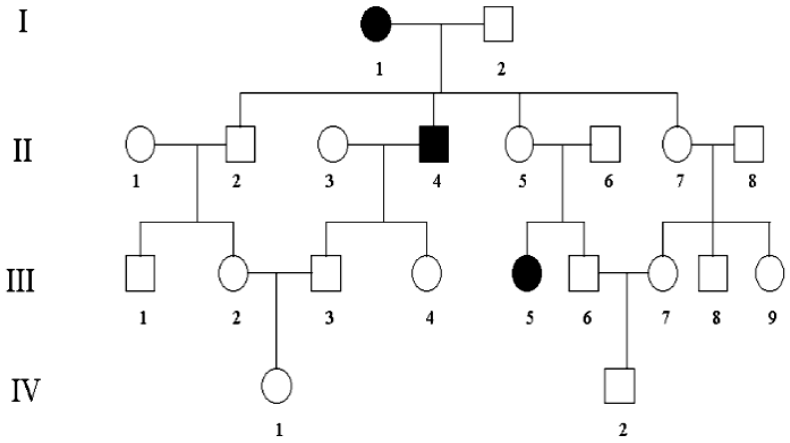
Black symbols represent red hair; white symbols, dark hair.



Problem 8. The allele that determines the spotted coat in rabbits (S) is dominant to the allele for solid colour (s). In the following pedigree consider that those individuals that have mated with members of this family do not carry the allele for solid colour (s), unless proven otherwise. Calculate the probabilities that solid-coloured rabbits are born in the following matings:

a) III-1 x III-9
b) III-1 x III-5
c) III-3 x III-5
d) III-4 x III-6
e) III-6 x III-9
f) IV-1 x IV-2
g) III-9 x IV-2
h) III-5 x IV-2
i) III-6 x IV-1

Black symbols represent solid-coloured animals; white symbols represent mottled animals.



Problem 9. A black coat in cocker spaniels is governed by the dominant B allele of a locus and red colour by its recessive b allele. The uniform pattern of colour is governed by the dominant allele of a locus S, which is independently transmitted, and the mottled pattern by its recessive allele s. A black-haired, uniform black male mates with a red-haired, mottled female and they produce a litter of six pups: two uniform black, two mottled red, one mottled black and one uniform red. Determine the genotypes of the parents.

Problem 10. A leguminous plant with tall stem, yellow legume and round seed is crossed with a dwarf, green round plant, giving 3/8 tall, green round plants, 3/8 dwarf, green round plants, 1/8 tall, green rough plants and 1/8 dwarf, green rough plants. Determine the genotypes of the parents.

Problem 11. At F_2 of two maize varieties, obtained by crossing lines differing in two genes, the following phenotypic segregations were obtained:

	AB	Ab	aB	ab
Variety a	117	26	18	7
Variety b	82	12	33	8

Is the deviation from the 9:3:3:1 segregation significant in each case?

Problem 12. Dark hair (R) in *Homo sapiens* is dominant over red hair (r). Brown eye colour (M) dominates over blue (m). A couple consisting of a brown-eyed, dark-haired man and a dark-haired, but blue-eyed woman had two children, one brown-eyed and red-haired and the other blue-eyed and dark-haired. Determine the genotypes of the parents and those of the offspring by reasoning the answer.

Problem 13. The crossing of two wild-type *Drosophila* individuals gave entirely wild-type F_1 offspring. Each F_1 fly was subsequently crossed with reduced wing (vestigial mut. -vg-) and black body (ebony mut. -e-) flies. The following results were obtained:

1/4 of the crosses gave: wild, vestigial, ebony, vestigial-ebony in proportion 1:1:1:1.
1/4 of the crosses gave: an offspring in which all were wild.
1/4 of the crosses gave: wild, vestigial, in the ratio 1:1.

1/4 of the crosses gave: wild, ebony, in the ratio 1:1.

What are the genotypes of the two individuals of the parental generation?

Problem 14. Given that in tomatoes, red is dominant over yellow and normal size is dominant over dwarf, if homozygous red hybrid normal size tomatoes are crossed with a yellow dwarf variety, what proportion of the F_2 red tomatoes will be dwarf?

Problem 15. If two pairs of alleles are transmitted independently, with A being dominant over a and B over b, what is the probability of obtaining: (a) an Ab gamete from an AaBb individual, (b) an AABB zygote from an AaBB x AaBb cross, (c) an Ab gamete from an AABb individual, (d) an AABB zygote from an aabb x AABB cross, (e) an AB phenotype from an AaBb x AaBb cross, (f) an AB phenotype from an AaBb x AABB cross, and (g) an aB phenotype from an AaBb x AaBB cross?

Problem 16. The normal cleft leg trait in pigs is produced by the recessive allele m, while the dominant allele M produces a mule-legged condition. White coat colour is determined by a dominant allele of another B locus and black by its recessive b allele. A white mule-legged pig is crossed with a female of the same phenotype. Among the offspring there are six white pigs with normal hoof; seven black pigs with mule foot; fifteen white pigs with mule foot and three black pigs with normal hoof. If a backcross is made between one of the parents and the F_1 individuals with black with cloven hoof, what phenotypic frequency could be expected among the offspring?

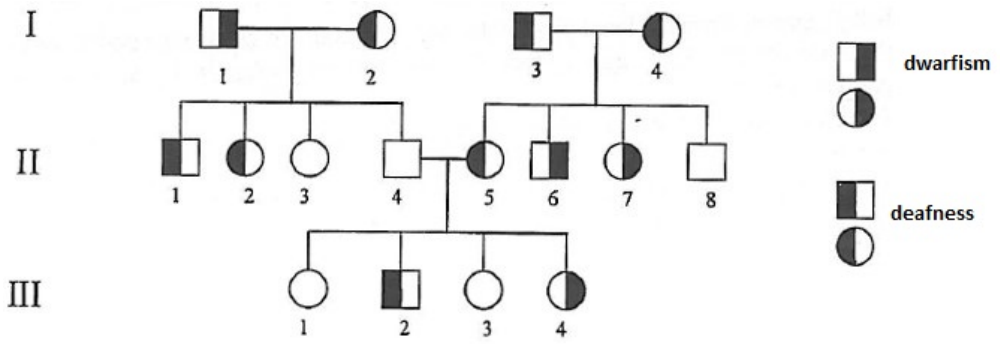
Problem 17. A plant heterozygous for 4 independent genes (Aa Bb Cc Dd) is self-fertilising. Determine the expected frequency of the following genotypes in the progeny of this plant:1) aa bb cc dd; 2) aa bb Cc Dd; 3) Aa Bb Cc Dd.

Problem 18. A plant has been obtained which is heterozygous for 6 independent loci (AaBbCcDdEeFf), each with two alleles and complete dominance. Assuming that the plant is self-fertilised, there is a desire to know:

a) What is the probability that an offspring will be triple heterozygous?
b) What is the probability that an offspring is heterozygous for four loci and homozygous recessive for the other two loci?
c) What is the probability that an offspring will be homozygous AA and heterozygous for the remaining loci?
d) How many different genotypes can be formed that are heterozygous for two loci?

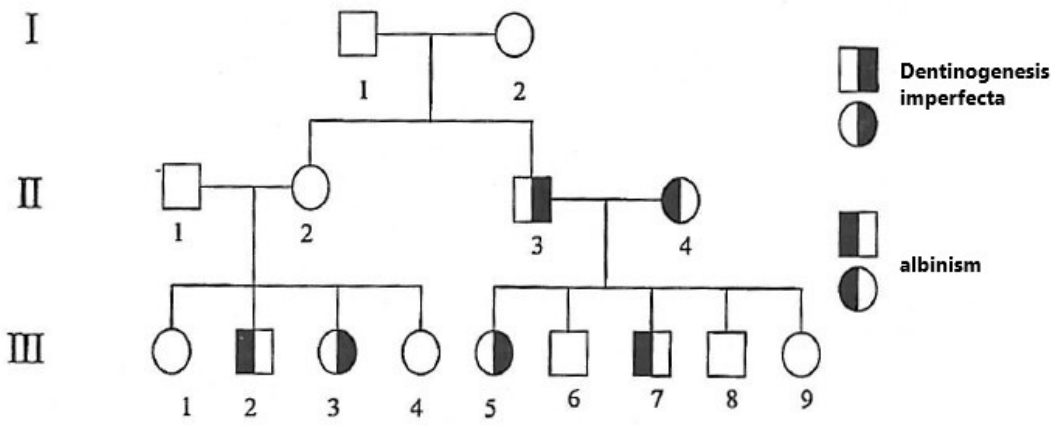
Problem 19. A couple heterozygous for 5 independent genes has a child. If we call A,B,C,D,E, the dominant alleles and a,b,c,d,e, the recessive alleles of these loci, what is the probability that the child will have a dominant phenotype for 3 of these traits? And an ABcde phenotype?

Problem 20. The following genealogy shows a family affected by two diseases that occur infrequently in humans and are known to be due to genes located on different chromosomes.



a) What is the type of inheritance of each of these diseases (determine the genotypes of all the individuals in the genealogy)?
b) Calculate the probability that the first offspring of II-1 and III-4 is:
1) healthy
2) affected by deafness
3) affected by dwarfism
4) affected only by either of the two diseases.
c) If II-1 and III-4 have two offspring, what is the probability that each is affected by a different disease?
d) If they have 3 children, what is the probability that two are affected by deafness and the other by diastrophic dwarfism?

Problem 21. The following genealogy shows a family affected by two diseases that occur infrequently in humans, dentinogenesis imperfecta and albinism, and which are due to genes located on different chromosomes.



a) What is the type of inheritance of each of these diseases?
b) Determine the genotypes of all individuals in the pedigree
c) Calculate the probability that the first descendant of III-3 and III-7 is:
1) healthy
2) affected by the two diseases
d) If III-3 and III-7 have four offspring, what is the probability that
1) two are affected by dentinogenesis?
2) only the third is albino?

Problem 22. Coat colour in mice is known to be determined by several genes. The presence of a yellow band of pigment near the tip of the coat is called the "agouti" pattern and is produced by the dominant A allele. The recessive condition at this locus (aa) does not have this subapical band and is known as non-agouti. The dominant allele of another locus B produces black and the recessive genotype bb produces brown. The

homozygous cc genotype restricts pigment production to the extremities of the body in a so-called Himalayan pattern, while the dominant C allele allows pigment to be distributed over the entire body. When crossing purebred agoutis, brown, Himalayan pattern mice with purebred, non-agoutis, black, non-Himalayan mice, (a) what are the expected phenotypic proportions in F_1 and F_2? (b) What percentage of the fully coloured, black, agouti F_2 would be expected to be of genotype AaBBCc? (c) What percentage of all Himalayan mice in F_2 might be expected to show brown pigment? (d) What percentage of all agoutis in F_2 might be expected to show black pigment?

4. SOLUTIONS TO PROBLEMS

Problem 1
a) 100% Aa (axial position phenotype)
b) 50% AA (axial position phenotype); 50% Aa (axial position phenotype)
c) 50% Aa (axial position phenotype); 50% aa (terminal position phenotype)
d) 25% AA (axial position phenotype); 50% Aa (axial position phenotype); 25% aa (terminal position phenotype)

Problem 2
75% Brachydactyls (BB and Bb)

Problem 3
A test cross with a smooth-haired female.

Problem 4
Experiment A: BB (white) x bb (black): 100% Bb (white)
Experiment B: Bb (white) x bb (black): 50% Bb (white), 50% bb (black)

Problem 5. 5/16

Problem 6
a) 243/1024=23.7%
b) 9/1024 = 0.8%
c) 90/1024 = 8.8%
d) normal 1/4; no molars 3/4

Problem 7
Genotypes:
I-1: Rr I-2: rr
II-1: Rr II-2: rr II-3: Rr II-4: RR II-5: Rr II-6: RR
III-1: Rr III-2: rr III-3: Rr III-4: R_ III-5: R_ III-6: R_ III-7: R_ III-8: R_ III-9: R_ III-10: RR
IV-1: R_ IV-2: R_ IV-3: R_

a) 1/8
b) 0
c) 1/4 x 7/12 x 5/12 = 0.061
d) 1/4 x 7/12 x 1/4 = 0.036

Problem 8
Genotypes:
I-1: ss I-2: Ss
II-1: SS II-2: Ss II-3: SS II-4: ss II-5: Ss II-6: Ss II-7: Ss II-8: SS

III-1: S_ III-2: S_ III-3: Ss III-4: Ss III-5: ss III-6: S_ III-7: S_ III-8: S_ III-9: S_
IV-1: S_ IV-2: S_

a) 1/16
b) 1/4
c) 1/2
d) 1/6
e) 1/12
f) 0.069
g) 0.059
h) 0.236
i) 0.097

Problem 9. BbSs x bbss

Problem 10. From the data provided it is not possible to know which of the two phenotypes affecting stem height (tall or dwarf) is dominant.
V = green legume; v = yellow legume
R = round seed; r = rough seed.
Thus, one possibility would be:

Tall stem, yellow legume and round seed: AavvRr
Dwarf stem, green legume and round seed: aaVVRr

Alternatively:

Tall stem, yellow legume and round seed: aavvRr
Dwarf stem, green legume and round seed: AaVVRr

Problem 11. The deviation of the variety **a** is significant with respect to the 9:3:3:1 ratio; Experimental $\chi^2_{3\,df}$ = 13.27. (P<0.005). The theoretical $\chi^2_{3\,df}$ = 7.81.
The deviation of variety **b** is also significant with respect to the 9:3:3:1 ratio; Experimental $\chi^2_{3\,df}$ = 9.84. (P: 0.02-0.01). The theoretical $\chi^2_{3\,df}$ = 7.81.

Problem 12. Male: MmRr. Female: mmRr. Children: Mmrr, mmR_

Problem 13. vg^+ vg^+ e^+ e^+, vg^+ vg e^+ e

Problem 14.

Parental individuals: RrNN (Normal Reds) x rrnn (Dwarf Yellows)
F_1: Rr Nn (normal reds), rrNn (normal yellows)
F_2: The proportion of red tomatoes that are dwarf is 1/4.

Problem 15. a) 1/4; b) 1/8; c) 1/2; d) 0; e) 9/16; f) 1; g) 1/4

Problem 16. 1/4 white-normal:1/4 white-mule:1/4 black-normal:1/4 black-mule.

Problem 17.
1) $(1/4)^4 = 0.0039$
2) $(1/2)^2\ (1/4)^2 = 0.0156$
3) $(1/2)^4 = 0.0625$

Problem 18.
a) 20/64=0.3125 b) 15/256=0.0586 c) 1/128=0.0078 d) 240

Problem 19.
(a) P = 0.26
(b) P = 0.009

Problem 20.
1) Deafness: autosomal dominant; Dwarfism: autosomal recessive.

S (deafness) > s and E > e (dwarfism)
I-1: eess; I-2: E_Ss; I-3: EeSs; I-4: EeSs
II-1: EeSs; II-2: EeSs; II-3: Eess; II-4: Eess; II-5: EeSs; II-6: eess; II-7: eess; II-8: E_ss
III-1: E_ss; III-2: E_Ss; III-3: E_ss; III-4: eess

2) a) 1/4 b) 1/2 c) 1/2 d) 1/2
3) 1/8
4) 3/64

Problem 21.
a) Albinism and dentinogenesis are autosomal recessive.
(b) D>d (dentinogenesis imperfecta) and A>a (albinism)

I-1: DdA_; I-2: DdA_
II-1: DdAa; II-2: DdAa; II-3: ddAa; II-4: Ddaa
III-1: D_A_; III-2: D_aa; III-3: ddA_; III-4: D_A_; III-5: ddAa; III-6: DdAa; III-7: Ddaa; III-8: DdAa; III-9: DdAa

c) 1) 1/3; 2) 1/6
d) 1) 3/8=0.375; 2) 8/81=0.0987

Problem 22.
(a) Genotype of parents: AAbbcc x aaBBCC; F_1: all AaBbCc (agouti-black-non-Himalayan); F_2: the proportions of a trihybrid: 27:9:9:9:3:3:3:1
b) 2/3x1/3x2/3 = 4/27 (14.8%)
c) 25%
d) 75%

EXTENSIONS OF MENDELIAN GENETICS

1. PROBLEM-SOLVING GUIDE

Sex-linked genes

These genes are located on the differential segment of the X chromosome and show different phenotypic ratios in reciprocal crosses. The father transmits them to his daughters and the mother to her sons and daughters.

How to determine if a trait is due to a **sex-linked recessive** mutation?

a) Males exhibit the trait more frequently than females.
b) If the father does not have the trait, and the mother is heterozygous (carrier), half of the sons will have the trait and none of their daughters.
c) If the father has the trait, and the mother is homozygous normal, none of the sons will have the trait, and all daughters will be carriers.

How to determine if a trait is due to a **sex-linked dominant** mutation?

a) If the father has the trait, all daughters will have the trait.
b) If the father does not have the trait, half of the daughters and sons of a heterozygous female will have the trait.

Holandric genes

These genes are located on the differential segment of the Y chromosome; they are exclusive to males of XX/XY sex-determinant species. In species with ZZ/ZW sex determination, the genes on the differential segment of the W chromosome are equivalent to the holandric genes and, in this case, are called hologynic.

Sex-influenced genes

They are autosomal genes, but the dominance relationships between alleles are different in males and females. Homozygotes, both females and males, have the same phenotype, but heterozygotes will have one phenotype or another depending on the sex. In these cases, although they are autosomal, the sex of the individuals must not be omitted when establishing their phenotype.

Sex-limited genes

These are usually autosomal genes that are transmitted through both sexes but are expressed only in one sex, i.e., the trait is present only in males or only in females. An example of this type of character is the presence of showy plumage in male birds, while females have mimetic colors.

Incomplete dominance and codominance

When there is incomplete dominance or codominance, the phenotypic proportions coincide with the genotypic proportions. In monohybrid crosses, these proportions are:

1/4, 1/2, 1/4. In the case of **incomplete dominance**, the phenotype of the heterozygote (ratio 1/2) is intermediate between the two parental homozygote phenotypes. In the case of **codominance**, the heterozygote has both phenotypes.

Multiple allelism

Genes with more than two alleles also have Mendelian segregation. Each diploid individual has only two alleles even if there are many different alleles of that gene in the population. They can be distinguished because more than two different phenotypes for that trait are observed in the population. The complementation test is what allows us to know if two mutant phenotypes are due to alleles of the same gene, or if the mutations are in different genes.

Lethal alleles

Lethal alleles are alleles that cause the death of the organism that carries them. In the evolutionary sense, lethal alleles are alleles that lead to "genetic death", i.e. the inability to reproduce of the individuals that have them. In these terms, a lethal mutant allele, which for example prevents zygote development, and another lethal mutant allele that leads to sterility are in genetic terms the same since both cause the individuals carrying them not to contribute to the next generation.

Some alleles may have similar or different effects on a particular phenotype and lethality, in terms of dominance. For example, in phenylketonuria, the mutant allele responsible for defective enzyme activity is recessive. Individuals with both copies of the mutated gene have a phenylketonuric phenotype and in practice do not reproduce. The allele is recessive for the phenotype "phenylketonuric" and recessive for the character "lethality" (although individuals are viable in practice it is understood that they leave no offspring). Affected offspring of carrier couples will be 1 in 4.

If the allele is dominant for the "lethality" trait, a single copy leads to the inability to reproduce (or death) in which case the allele is lost in that generation and does not carry over to the next.

On the other hand, the allele for yellow tail color in mice is dominant over agouti which is recessive. But in double doses the allele for the yellow color of the tail causes lethality, so the gene that controls the character "tail color" in mice is a gene with pleiotropic effects since it influences the character "tail color" and the character "viability of the individual" or "lethality". For the trait "tail color" it is dominant and for the trait "lethality" it is recessive, so the allele is dominant with a recessive lethal effect.

When crossing mice of dominant phenotype, with yellow tail, these dominant alleles with recessive lethal effect distort the Mendelian phenotypic proportions of 3/4 of the offspring with dominant phenotype and 1/4 with recessive phenotype, and proportions of 2/3:1/3 yellow:agouti are obtained.

Another consequence of this phenomenon is that by definition yellow-tailed mice are heterozygous and therefore pure lines of yellow-tailed individuals cannot be obtained. The consequence is that when crossing yellow-tailed individuals with each other, recessive phenotype individuals (agoutis) will always appear in the offspring, with predictable proportions of 1 in 2.

Gene Interaction and Epistasis

When two genes are involved in the determination of the same trait, it is said that there is a phenomenon of gene interaction. The first thing to distinguish is whether the two

genes that control the trait do so independently (genes that act in separate metabolic pathways) in which case it is a **NON-EPISTATIC GENE INTERACTION**. In this type of interaction, if two genes are involved and each of them has two alleles with complete dominance of one over the other, then four possible phenotypes arise (two for each gene).

If the genes act in the same pathway and the product of one gene is the substrate of the next (through the enzymes encoded by the genes logically), then it is an **EPISTATIC GENE INTERACTION.**

If the dominant allele of one of the two genes involved determines the phenotype, it is **dominant epistasis**.

If the recessive allele (in double dosage) of one of the genes involved determines the phenotype, it is **recessive epistasis.**

If the recessive alleles of the two genes (in double dosage both, aa_ _ or _ _ bb) result in the same phenotype, then it is **duplicate recessive epistasis**.

If each of the two dominant alleles of each of the two genes (A_ or B_) results in the same phenotype, then it is **duplicate dominant epistasis**.

If the dominant allele of one of the genes and the recessive allele of the other (A_ y _ _ bb) result in the same phenotype, then it is **dominant inhibitory epistasis**.

When we cross two diheterocygotes (and only if we cross two diheterozygotes) we obtain a 9:3:3:1 ratio in the offspring. If there is epistasis, this ratio will be modified as follows:

If there is **dominant epistasis**, we obtain a 12:3:1 ratio (three phenotypes) in the dihybrid cross.

If there is **recessive epistasis**, we obtain a 9:3:4 ratio (three phenotypes) in the dihybrid cross.

If there is **duplicate recessive epistasis** we obtain a 9:7 ratio (two phenotypes) ratio in the dihybrid cross.

If there is **duplicate dominant epistasis,** we obtain a 15:1 ratio (two phenotypes) in the dihybrid cross.

If there is **dominant inhibitory epistasis,** we obtain a 13:3 ratio (two phenotypes) in the dihybrid cross.

It is important to identify the gene interaction between two genes **by how they determine the phenotypes and not by learning the proportions that appear when crossing two diheterozygotes.** The epistatic relationships that two genes maintain are the same if two diheterozygotes are crossed as if any other two genotypes are crossed, and the mentioned proportions occur only when two diheterozygotes are crossed.

2. PROBLEMS SOLVED

Problem 1. Hypophosphatemia leads to a type of rickets in which patients do not respond to normal doses of vitamin D. This disorder is caused by a sex-linked dominant allele. What phenotypes would be expected amongst the sons and daughters of the following marriages?
a) Affected man and normal woman.
b) Affected woman who is the daughter of the previous marriage and normal man.

Answer

a) If the rickets-associated allele is called "A" and the wildtype allele "a",

affected man x normal woman
X^AY X^aX^a
↓
X^AX^a X^aY
Affected daughters Normal sons

b)

X^AX^a x X^aY
↓

X^AX^a	X^aX^a	X^AY	X^aY
Daughters		Sons	
Affected	Normal	Affected	Normal

Problem 2. A male hemizygous for a sex-linked recessive mutation is crossed with a female heterozygous for such mutation.

a) What is the probability of having a child with a mutant phenotype in this cross?
If this couple has 10 children,
b) what is the probability that a randomly chosen daughter will have the mutant phenotype?
c) what is the probability that 7 of them will be mutant males and 3 normal females?

Answer

X^aY x X^AX^a
↓

X^AX^a	X^aX^a	X^AY	X^aY
1/4	1/4	1/4	1/4
normal	mutant	normal	mutant

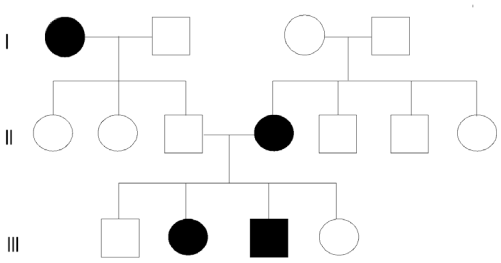
a) 1/4.
b) 1/2, as we know that it is female and half of them are mutants.
c) Considering that the probability of a mutant male being born is 1/4, and that of a normal female is also 1/4, the total probability will be:

$$P = \frac{10!}{7!\,3!} (1/4)^7 (1/4)^3$$

Problem 3. The following pedigree shows the inheritance of a small dorsal spot in a certain variety of wild partridge. Knowing that the associated allele is sex-linked and recessive, determine:

a) the probability that a male offspring of the cross between III-1 and III-2 will show the spot;
b) the probability that the cross between II-4 and II-5 will produce three offspring without

the spot and two with it.



Answer

As the partridge is a bird and, therefore, females represent the heterogametic sex and males the homogametic sex:

female ZW / male ZZ

Thus, the possible genotypes will be:

Female with spot: Z^aW
Female without spot: Z^AW
Male with spot: Z^aZ^a
Male without spot: Z^AZ^A o Z^AZ^a

In the pedigree:

I-1, Z^aW	I-2, Z^AZ^-
I-3, Z^AW	I-4, Z^AZ^a
II-1, Z^AW	II-2, Z^AW
II-3, Z^AZ^a	II-4, Z^aW
II-5, Z^AZ^-	II-6, Z^AZ^-
II-7, Z^AW	
III-1, Z^AZ^a	III-2, Z^aW
III-3, Z^aZ^a	III-4, Z^AW

a) The cross shall be:

III-1 (Z^AZ^a) x III-2 (Z^aW)

↓

Z^AZ^a	Z^aZ^a	Z^AW	Z^aW
1/4	1/4	1/4	1/4
male without spot	male with spot	female without spot	female with spot

The probability of a male carrying the spot will be 1/2 (half of the males).

b) As II-5 can be Z^AZ^A (P = 1/2) or Z^AZ^a (P = 1/2), there will be two possible crosses:

1)

	(1/2) II-5	x	II-4
	Z^AZ^A		Z^aW
	↓		
	Z^AZ^a		Z^AW
	1/2		1/2
	male		female
	without spot		with spot

This cross cannot be considered because it does not produce offspring with spot (remember that the problem asks the probability of 3 offspring being born without spot and two with it).

2)

	(1/2) II-5		x	II-4			
	Z^AZ^a			Z^aW			
	↓						
Z^AZ^a		Z^aZ^a		Z^AW		Z^aW	
1/4		1/4		1/4		1/4	
male		male		female		female	
without spot		with spot		without spot		with spot	

The probability of one offspring with spot will be:

P = 1/2

And the probability of one offspring without spot will be:

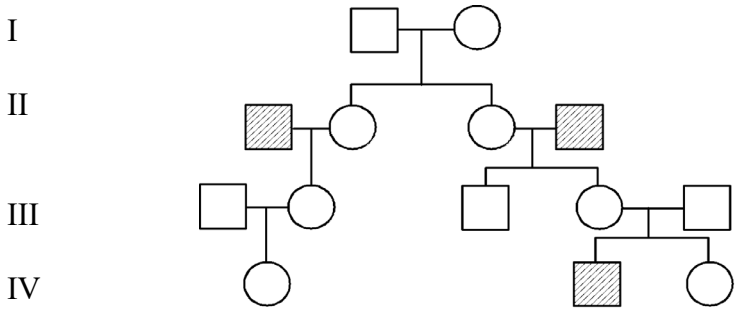
P = 1/2

Therefore, the required probability will be:

$$P_T = \frac{5!}{3!\,2!} (1/2)^3 (1/2)^2 = 10\ (1/2)^3\ (1/2)^2$$

Problem 4. The following pedigree illustrates the inheritance of the trait "flattened ear" in Ursids, with affected individuals highlighted in black.

a) Determine the type of inheritance, considering the following possibilities: holandric gene, sex-linked gene (dominant or recessive trait-associated allele).
b) What is the probability that IV-1 and IV-2 will have an affected offspring (of either sex)?
c) What is the probability that one of their female cubs will be normal?
d) What is the probability that they will have a normal female cub?
e) If they have six cubs, what is the probability that two of them will be affected males, one a normal female, two normal males, and one an affected female?
f) What is the probability that the first two will be affected males, the third a normal female, the next two normal males, and the last one an affected female?



Answer

a) *Holandric gene*: This is not possible, as it would have to be passed on from parents (males) to offspring (also males). II-1, for example, manifests the trait, but not his father, and II-4 shows the trait but not his male cub.

Dominant allele of a sex-linked gene: This is also not possible, as the female cubs of affected males (who receive their X from them) and the mothers of these males (who have passed on their X) should display the trait. For example, II-1, who shows the trait, has a female cub who does not manifest it.

Recessive allele of a sex-linked gene: This may be the case, as affected males receive the X from their mother and pass it on to their female offspring, but none of these females manifest the trait, as they are heterozygous. Accordingly, if we designate the allele for the normal aspect as X^A, and the allele for the trait as X^a (affected individuals), being $X^A > X^a$, the genotypes of the different individuals in the pedigree will be:

I-1, X^AY I-2, X^AX^-

II-1, X^aY II-2, $X^A X^-$
II-3, X^AX^- II-4, X^aY

III-1, X^AY III-2, X^AX^a
III-3, X^AY III-4, X^AX^a
III-5, X^AY

IV-1, X^AX^- IV-2, X^aY
IV-3, X^AX^-

b) The proposed cross is:

IV-1 x IV-2
X^AX^- X^aY

To find out the possible genotypes of IV-1 and their probabilities, we may analyse the cross of its parents:

III-1 x III-2
X^AY X^AX^a

↓

X^AX^A X^AX^a X^AY X^aY

1/2 1/2 (we know that it is a female)

There will be two possible crosses:

(1/2) IV-1 x IV-2 → X^AX^A x X^aY → X^AX^a (1/2) X^AY

all normal

(1/2) IV-1 x IV-2 → X^AX^a x X^aY → X^AX^a (1/4) X^aX^a (1/4) X^AY (1/4) X^aY (1/4)

normal affected normal affected

Hence, the probability we are asked for will be:

P = P (IV-1 x IV-2 → affected) =

= P (IV-1 X^AX^A) x P (IV-2 X^aY) x P (X^AX^A x X^aY → affected) +

+ P (IV-1 X^AX^a) x P (IV-2 X^aY) x P (X^AX^a x X^aY → affected) =

= 1/2 x 1 x 0 + 1/2 x 1 x 1/2 = 1/4

c) If IV-1 is X^AX^A, all her female offspring will be normal. If, otherwise, is X^AX^a, half of their female offspring will be normal:

P = 1/2 x 1 + 1/2 x 1/2 = 1/2 + 1/4 = 3/4

d) In the first case, half of the offspring will be normal females, and, in the second case, one quarter of the offspring will be normal females:

P = 1/2 x 1/2 + 1/2 x 1/4 = 1/4 + 1/8 = 3/8

e) $P_T = \frac{6!}{2!\,1!\,2!\,1!} \times (P_1)^2 \times (P_2)^1 \times (P_3)^2 \times (P_4)^1$

Where: P_1 = Prob. of having an affected male
P_2 = Prob. of having a normal female
P_3 = Prob. of having a normal male
P_4 = Prob. of having an affected female

P_1 = 1/2 x 0 + 1/2 x 1/4 = 1/8
P_2 = 1/2 x 1/2 + 1/2 x 1/4 = 1/4 + 1/8 = 3/8
P_3 = 1/2 x 1/2 + 1/2 x 1/4 = 1/4 + 1/8 = 3/8
P_4 = 1/2 x 0 + 1/2 x 1/4 = 1/8

$P_T = \frac{6!}{2!\,1!\,2!\,1!} \times (1/8)^2 \times (3/8)^1 \times (3/8)^2 \times (1/8)^1 = 180 \times 27 \times (1/8)^6 = 4.860 \times (1/8)^6 = 0.02$

f) $P = (1/8)^2 \times (3/8)^1 \times (3/8)^2 \times (1/8)^1 = 27 \times (1/8)^6 = 0.0001$

Problem 5. Long-eared goats mated to short-eared goats produce offspring with medium ear size in the F_1 generation. The ratio observed in the F_2 generation is 1/4 long, 1/2 medium, and 1/4 short ears on both males and females. Beardless male goats mated to bearded female goats produce bearded male progeny and beardless females. F_2 males have a proportion of 3/4 bearded and 1/4 beardless, while F_2 females have a proportion of 3/4 beardless and 1/4 bearded.

What is the type of inheritance of these two traits?

Answer

a) Ear size is determined by an autosomal gene with two alleles with intermediate inheritance (also called incomplete dominance). This is deduced, firstly, by observing that the proportions coincide in males and females and, secondly, because in the F_1 an intermediate phenotype appears, which is the most frequent class in the F_2, and which would correspond to heterozygotes. By assigning arbitrary symbols, we can identify each phenotype with its corresponding genotype:

LL = long
CC = short
LC = intermediate

The cross was:

P: long x short
LL CC
↓
F_1: LC intermediate
↓
F_2: LL LC LC CC
1/4 1/2 1/4
long intermediate short

The presence or absence of beard seems to be an autosomal Mendelian sex-influenced trait. The bearded condition would be dominant in males and recessive in females, while the beardless manifestation would be dominant in females and recessive in males. This conclusion is reached from the observation of the F_2, where the proportions are reversed from males to females. If we assign alphabetic symbols:

B = bearded
I = beardless
B > I in males
B < I in females

Therefore, the cross of the example was:

P: beardless male x bearded female
II BB
↓
F_1: IB: bearded males
beardless females
↓
F_2: II IB IB BB
Males 1/4 3/4
beardless bearded

Females 3/4 1/4
beardless bearded

Problem 6. A woman with AMRh+ phenotype has a daughter with AMRh- phenotype.

a) Which genotypes would allow you to rule out an individual as a possible father?
b) Which phenotypes?

Answer

The system that determines the MN blood group has two main alleles, $\mathbf{L^M}$ and $\mathbf{L^N}$, which are codominant. The AB0 group is determined by the allelic series $\mathbf{I^A = I^B > i}$, and the most commonly characterised Rh group is due to the expression of an autosomal gene with two alleles: **D**, which produces the Rh+ phenotype, and **d**, which determines the Rh- phenotype (with D being dominant over d).

The woman in this problem is AM+ and her daughter AM-. Therefore, their genotypes are:

Mother: $I^A_L^ML^M$ Dd Daughter: $I^A_L^M\ L^M$ dd

Consequently, we would rule out any individual with the following genotypes/phenotypes as a possible father:

AB0 group: $\mathbf{I^BI^B}$ genotype, because he would have passed on the $\mathbf{I^B}$ allele to the daughter, and she would have manifested it. We could not rule out, in principle, any individual with the $\mathbf{I^AI^A}$, $\mathbf{I^Ai}$, $\mathbf{I^AI^B}$, $\mathbf{I^Bi}$, or **ii** genotypes. As for the phenotypes, we could not rule out any of them because the allele combinations of the possible genotypes produce the four phenotypes (A, B, AB, and 0).

MN group: $\mathbf{L^NL^N}$ genotype (N phenotype), because the real father passed on the $\mathbf{L^M}$ allele to the daughter. Individuals with $\mathbf{L^ML^M}$ (M group) or $\mathbf{L^ML^N}$ (MN group) genotypes could not be ruled out.

Rh group: **DD**, because his father had to pass on a **d** allele**.** As individuals with **Dd** (Rh+ group) or **dd** (Rh-) genotypes could not be ruled out, we could not discard any phenotype in principle.

Problem 7. In humans, the MN blood groups are defined by a gene with two codominant alleles, M and N, so that the MM genotype leads to the M group, the MN genotype to the MN group, and the NN combination to the N group. A man, whose parents had the M group (one of them) and the N group (the other one), marries a woman of unknown MN phenotype with whom he has a child with the M group.

a) What can be asserted about the genotype of the mother?
b) If it is also known that the maternal grandfather had the N group, what is the probability that the next child of this couple will have a different group than its sibling?
c) Knowing that both members of the couple are heterozygous for a gene that causes a certain skin disease in recessive homozygosity, what is the probability that, if they have three children, one of them will have the MN group and will manifest the disease, and the other two will have a group other than MN and will not manifest the disease?

Answer

a) The child has the MM genotype because he shows the M group. His father is heterozygous MN**,** and regarding his mother we can only know that she has an M allele that was passed on to him.
b) If the maternal grandfather had the N group, his genotype was NN and, therefore, the

woman must be MN**.** The probability that the heterozygous couple will have a child with a group other than M (that is, MN or N) will be 3/4:

$$\begin{array}{c} MN \times MN \\ \downarrow \\ \underline{MM} \quad \underline{MN \quad MN} \quad \underline{NN} \\ 1/4 \qquad 1/2 \qquad 1/4 \end{array}$$

c) The cross was MNAa x MNAa, and the probability we are asked for:

$$P = \frac{3!}{1!\,2!} (P_1)^1 \ (P_2)^2$$

P_1 = P (MN group and with the disease) = 1/2 x 1/4 = 1/8
P_2 = P (MN group and without the disease) = 1/2 x 3/4 = 3/8

Substituting, $P = (3/8)^3$

Problem 8. Indicate whether two persons with the AB, Rh+ phenotype could have the following offspring, stating the possible genotypes in such case:

a) A Rh+
b) A Rh-
c) 0 Rh+
d) 0 Rh-

Answer

The proposed cross was:

AB Rh+ x AB Rh+
$I^AI^BD_$ $I^AI^BD_$

a) Yes, $I^AI^AD_$ genotype

b) Yes, I^AI^Add genotype

c) and **d)** It is not possible, since the offspring should be ii because it has the 0 group.

Problem 9. Different types of fur colouring can be observed in guinea pigs, such as black, albino, cream, and sepia. Determine the most likely genotypes of the parents in the following crosses.

	Cross	black	sepia	cream	albino
1	black x black	227			
2	black x albino	10	9		
3	crema x cream			34	11
4	sepia x cream		24	11	12
5	black x albino	13		12	
6	black x cream	19	20		
7	black x sepia	18	20		
8	sepia x sepia		26	9	
9	cream x albino			15	7

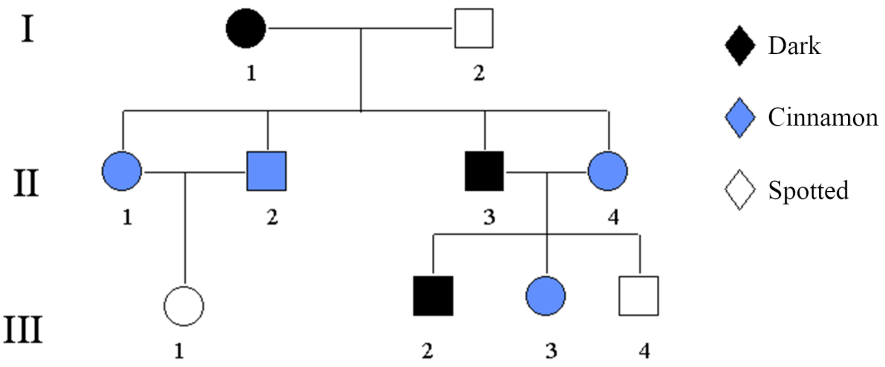
Answer

The fact that different phenotypes appear for the same trait may lead us to believe that there could be more than one *locus* involved in the determination of the trait. To elucidate this, we must take into account that of all the crosses carried out, none has more than three different types of offspring, which would be the case for some of them if there were two genes involved. The complementation test or the allelism test would resolve this question.

As it can be observed, there are four possible phenotypes that are possibly due to four different alleles. Thus, according to the results of the crosses above, we can state that black (C) is dominant over all, sepia (c^s) over cream, and sepia and cream (c^c) over albino (c) (black > sepia > cream > albino). Accordingly, the genotypes are as follows:

1.- CC x C-
2.- Cc^s x cc
3.- c^cc x c^cc
4.- c^sc x c^cc
5.- Cc^c x cc
6.- Cc^s x c^cc^c or c^cc
7.- Cc^s x c^sc^s, c^sc^c or c^sc
8.- c^sc^c x c^sc^c
9.- c^cc x cc

Problem 10. A series of multiple alleles determines the distribution of coat pigments in dogs. The A^s allele produces a uniform distribution of the dark pigment over the body; the a^y allele reduces the intensity of pigmentation resulting in cinnamon-coloured dogs; and the a^t allele produces spotted coats, such as cinnamon and white, cinnamon and brown, etc. The dominance hierarchy is $A^s > a^y > a^t$. Given the following pedigree:



a) Determine as much genotypes as possible.
b) Calculate the probabilities that the mating of III-1 and III-2 will produce spotted offspring.
c) What proportion of the dark pigmented offspring obtained from the cross between I-1 and II-3 will be heterozygous?

Answer

a)

I-1: A^sa^y I-2: a^ta^t

II-1: $a^{y}a^{t}$ II-2: $a^{y}a^{t}$ II-3: $A^{s}a^{t}$ II-4: $a^{y}a^{t}$

III-1: $a^{t}a^{t}$ III-2: $A^{s}_$ III-3: $a^{y}a^{t}$ III-4: $a^{t}a^{t}$

b) The III-2 individual can be either $A^{s}a^{t}$ or $A^{s}a^{y}$ with a probability of 1/2 in each case. If III-2 were $A^{s}a^{t}$, only spotted offspring would be produced.
Therefore, the cross should be:

(1/2) $A^{s}a^{t}$ x $a^{t}a^{t}$

↓

1/2 $A^{s}a^{t}$, 1/2 $a^{t}a^{t}$

P= 1/2 x 1/2= 1/4

c) The cross is: $A^{s}a^{y}$ x $A^{s}a^{t}$

↓

$A^{s}A^{s}$ $A^{s}a^{y}$ $A^{s}a^{t}$ $a^{t}a^{t}$

Dark pigment

Of the 75% with dark pigment, 2/3 are heterozygous.

Problem 11. A short-tailed mutant mouse appeared in a certain strain of mice. When this short-tailed mutant was crossed with normal long-tailed mice, the F_1 consisted of 6 short-tailed and 5 long-tailed mice. Two of the F_1 short-tailed mice were selected and crossed to produce an F_2 comprising 8 short-tailed and 4 long-tailed mice.

a) What hypothesis would you propose to explain this type of inheritance?
b) What phenotypic proportions would be expected when crossing the short-tailed and long-tailed mice of the last offspring?

Answer

a) This type of inheritance may be explained if the short tail mutation is dominant for tail length and recessive for lethality, so that there will never be viable homozygotes for the short tail and all short-tailed individuals will be heterozygous. The short tail is produced by the *C_* genotypes whereas the *cc* genotype results in long tails, as crossing short- and long-tailed mice produces both phenotypes in a proportion of 2:1.

b) The genotypes of the F_2 individuals are: Cc (short tail) and cc (long tail). Therefore, the offspring of this cross will be 1/2 Cc (short tail) and 1/2 cc (long tail).

Problem 12. In the fox, the two alleles of a certain gene, *P* and *p*, produce platinum- and silver-coloured fur, respectively. Crossing platinum-coloured foxes with each other always results in both platinum- and silver-coloured foxes, so that no pure platinum-coloured lines can be obtained.

a) How would you explain these results?
b) What phenotypic proportions would be expected from a cross between platinum- and silver-coloured individuals?
c) What phenotypic proportions would be expected from a cross between platinum-coloured individuals?

Answer

a) Since silver-coloured foxes always appear in the offspring of a cross between platinum-coloured foxes and, therefore, no pure platinum-coloured lines can be obtained (meaning that there are no PP individuals), the interpretation that best explains these results is that the allele for platinum coat colour (P_) is dominant over the allele for silver coat colour (pp) but recessive for the lethality trait.

b) The cross is between Pp (platinum) and pp (silver) genotypes. Hence, the offspring will be composed of half platinum-coloured (Pp) and half silver-coloured (pp) individuals.

c) The cross is between Pp individuals. Thus, the genotypes of the offspring were: 1/4 PP, 2/4 Pp, and 1/4 pp. However, as the PP indiviuals are not viable, the offspring had a phenotypic proportion of 2/3 platinum and 1/3 silver.

Problem 13. In hens, the following four crest shapes are determined by the interaction between 2 genes (R,r and P,p), where:

R_pp determines a ROSETTE shaped crest
rrP_ determines a PEA shaped crest
R_P_ determines a WALNUT shaped crest
rrpp determines a SIMPLE shaped crest

The mating between walnut-crested and rosette-crested birds produced the following F_1 offspring: 4 simple-crested, 5 pea-crested, 13 rosette-crested, and 12 walnut-crested.

What are the most likely genotypes of the parental birds?

Answer

Considering the presence of the 4 phenotypes in the offspring, a non-epistatic genetic interaction may be happening between these two genes. Since simple-crested individuals appear in the F_1, the walnut-crested individuals must necessarily be diheterozygous (RrPp). Similarly, as both rosette- and pea-crested individuals also appear in the offspring, the rosette-crested individuals must be heterozygous for the first gene (Rrpp).

Problem 14. In *Drosophila*, the wild-type eye is red because of the presence of two pigments: drosopterin and xanthommatin (which are synthesised in two different metabolic pathways). When the autosomal recessive mutants *brown* (without drosopterin and with xanthommatin) and *scarlet* (without xanthommatin and with drosopterin) are crossed, all F_1 individuals have red eyes and the F_2 shows the following phenotypic distribution:

9 red : 3 scarlet : 3 brown : 1 white

a) What type of gene interaction occurs between these two genes?
b) What are the genotypes of the parental individuals used in this cross?

Answer

a) Taking into account that the two pigments are synthesised in different metabolic pathways, 4 possible phenotypes can be manifested as the problem states: 1) having both pigments (xanthommatin and drosopterin, resulting in red colour), 2) having only drosopterin (scarlet, which corresponds to bright red), 3) having only xanthommatin (brown), and 4) having no pigment (white eyes). Therefore, the eye colour trait in *Drosophila* is determined by two biallelic genes, in which the two alleles of each gene show a relationship of complete dominance of one over the other. As they are involved

in separate metabolic pathways, the type of gene interaction is non-epistatic.

b) Parental individuals: bwbw st^+st^+ (brown) x bw^+bw^+st st (scarlet)
F_1: bw^+bw st^+ st (red eyes)
F_2: 9 red: 3 scarlet: 3 brown: 1 white

Problem 15. Brewbaker observed that F_1 inbred plants from crosses between two lines of white flowering clovers (*Trifolium repens*) resulted in an F_2 consisting of 5 red:75 white flowering clovers. No lethality was indicated.

a) Using the simplest explanation, how many genes may be involved in such crosses?
b) Define the implicated alleles with symbols and elucidate the genotype of the F_2 red plants.

Answer

a) 75 white/5 red corresponds to a 15:1 ratio, which is the proportion observed when two genes have a duplicate dominant epistasis relationship and two diheterozygotes are crossed (the dominant alleles of each of the two genes involved determine the same phenotype).

b) Parental individuals: AAbb x aaBB
F_1: AaBb
F_2: **9** A_B_(white) **3** A_bb (white) **3** aaB_(white) **1** aabb (red)
15 white: 1 red

Problem 16. Mating between rats of identical genotype produced the following offspring: 14 cream coloured : 47 black coloured : 19 albino.

a) What is the approximate proportion of the offspring?
b) What type of epistasis may be responsible for such phenotypic distribution?
c) What are the genotypes of the parental individuals and the offspring?

Answer

a) If it is assumed that there are two genes involved with two alleles each, and we calculate the ratio over a total of 16 F_1 individuals, the phenotypic distribution is as follows: 9.4 : 3.8 : 2.8, which is close to a 9:4:3 ratio.
b) These genes have a recessive epistasis relationship, since the observed proportions are the typical obtained by crossing two diheterozygotes for two genes with this type of epistasis.
c) The parental rats are diheterozygous (AaBb) and the offspring have the following genotypes: **9** A_B_ (black), **3** A_bb (cream), **3** aaB_(albino), **1** aabb (albino).

Problem 17. The dominant allele (B) of a gene determines the white colour of pumpkin fruit and the recessive allele (b) the coloured fruit. The yellow fruit is determined by the dominant allele (V) of an independently distributed hypostatic gene, and the green fruit by its recessive allele (v). When dihybrid plants are crossed, the offspring shows a phenotypic distribution of 12 white : 3 yellow : 1 green.

a) What type of epistasis occurs between these two genes?
b) What proportion of fruit colour is expected in the following crosses?

b1) Bbvv x Bbvv?
b2) Bbvv x bbVv?

c) If the cross between two plants results in an offspring producing 1/2 yellow fruits and 1/2 green fruits, what are the genotypes and phenotypes of the parental individuals?

Answer

a) If the B allele is present the fruits are white. On the contrary, if it is not present they are coloured. Hence, there is a dominant epistasis relationship between the two genes.

b1) Bbvv x Bbvv: 1/4 BBvv (white), 2/4 Bbvv (white), 1/4 bbvv (green). Then, 3/4 have white fruits and 1/4 green.

b2) The Bbvv individual produces two types of gametes: Bv and bv. Similarly, the bbVv individual also produces two types of gametes: bV and bv. Then:

	½ Bv	½ bv
½ bV	¼ BbVv	¼ bbVv
½ bv	¼ Bbvv	¼ bbvv

Therefore, 2/4 of the offspring produce white fruits, 1/4 yellow fruits, and 1/4 green fruits.

c) Since coloured fruits require the presence of the bb genotype, and the problem states that the fruits of the offspring are all coloured (half yellow and half green), the individuals that are crossed must be bb. Similarly, as they produce plants with green and yellow fruits in equal proportion, the genotypes of the second gene must be Vv and vv. Hence, the genotypes determining such phenotypic proportions are bbVv and bbvv.

Problem 18. Two white flowering pea plants were crossed producing a purple flowering F_1. A random cross between two F_1 plants produced an offspring consisting of 96 plants, of which 53 were purple flowering and 43 were white flowering.

a) What phenotypic proportion is approximately expected in the F_2?
b) What were the most likely genotypes of the parental plants?

Answer

a) A ratio of 9 purple : 7 white is expected accordingly with the observed frequencies (53:47). Such distribution corresponds to a duplicate recessive epistasis.

b) The white flowering plants are AAbb and aaBB. Hence, F_1 individuals are diheterozygous (AaBb, producing purple flowers), and the ratio of the F_2 will be **9** A_B_ (purple) : **3** A_bb (white) : **3** aaB_ (white) : **1** aabb (white). That is, 9 purple : 7 white.

Problem 19. The red colour of wheat is due to the R_B_ genotype and the white colour to the double recessive rrbb. Both rrB_ and R_bb genotypes determine brown colour. If a homozygous red line is crossed with a white line:

a) What phenotypic ratio is expected in both the F_1 and F_2 generations?
b) If the brown F_2 individuals are artificially crossed at random, what phenotypic proportions are expected in the offspring?

Answer

a) Since it is stated that the parental individuals are homozygous, the cross was RRBB x rrbb and, thus, the F_1 will be diheterozygous (RrBb) showing a red phenotype.

The F_2 obtained by crossing F_1 individuals (RrBb x RrBb) will have the following phenotypes and ratio:

9 red (R_B_) : 6 brown (3 R_bb 3 rrB_) : 1 white (rrbb)

b) The F_2 brown individuals will have the following genotypes and proportions:

1 RRbb : 2 Rrbb : 1 rrBB : 2 rrBb. The gamete pool produced by these individuals will be as follows:

1 RRbb will produce 1 Rb
2 Rrbb will produce 1 Rb and 1 rb
1 rrBB will produce 1 rB

2 rrBb will produce 1 rB and 1rb

Therefore, if we add up:

Rb gametes: 1+1 (out of a total of "6 gametes") = 2/6 = 1/3 Rb
rB gametes: 1+1 (out of a total of "6 gametes") = 2/6 = 1/3 rB
rb gametes: 1+1 (out of a total of "6 gametes") = 2/6 = 1/3 rb

Thus, the cross will be:

	1/3 Rb	1/3 rB	1/3 rb
1/3 Rb	1/9 RRbb (brown)	1/9 RrBb (red)	1/9 Rrbb (brown)
1/3 rB	1/9 RrBb (red)	1/9 rrBB(brown)	1/9 rrBb (brown)
1/3 rb	1/9 Rrbb (brown)	1/9 rrBb (brown)	1/9 rrbb (white)

6/9 brown, **2/9** red, and **1/9** white.

3. PROBLEMS TO SOLVE

Problem 1. A couple, both of whose members have normal vision, have a color-blind child.

a) What are the genotypes of the parents?
b) What are the sex and genotype of the child?

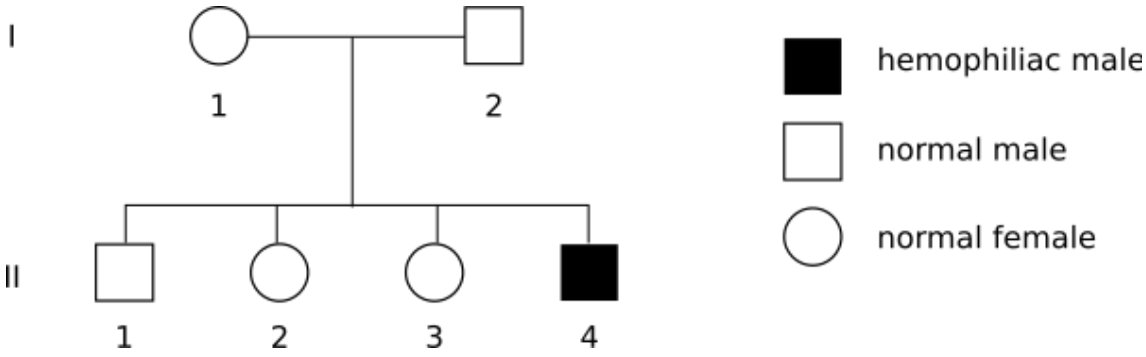
Problem 2. A normal-sighted woman whose father is colorblind marries a man whose mother was colorblind. What genotypes will the offspring of this couple have if …

a) they are male?
b) they are female?

Problem 3. Assume a sex-linked trait in exotic birds such that its recessive allele *a* determines white tail feathers and *A* determines colored tail feathers. If a heterozygous male is crossed with a white-feathered female and eight offspring are obtained, what is the probability that six of them will have colored tail feathers?

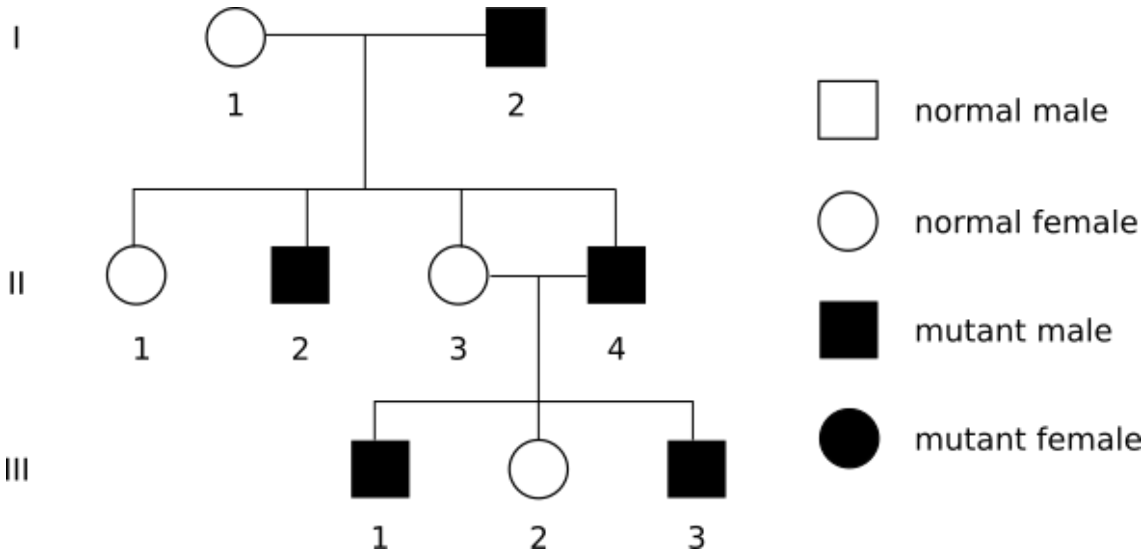
Problem 4. The following pedigree shows a case of hemophilia A, a disease due to the recessive allele of a sex-linked gene.

a) If II-2 marries a normal man, what is the probability that her first child will be a hemophiliac male?
b) Assuming that her first child is hemophilic, what is the chance that her second child will be a hemophiliac male?
c) If II-3 marries a hemophiliac male, what is the probability that her first offspring will be normal?
d) If I-1's mother was phenotypically normal, what genotype did his father have?
e) If I-1's mother was hemophiliac, what was the phenotype of I-1's father?



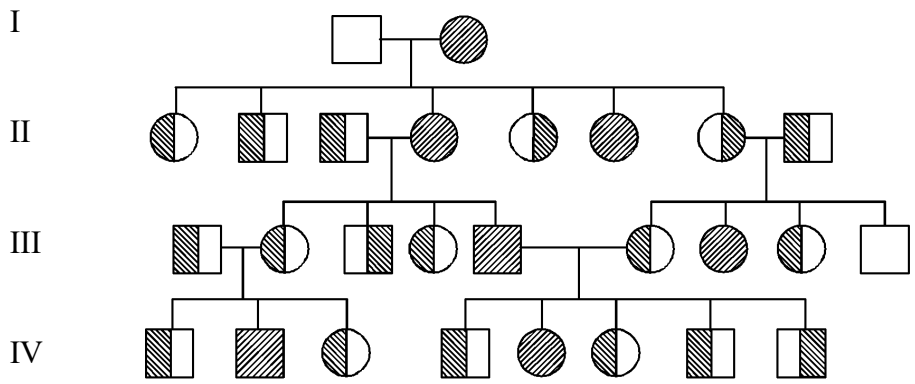
Problem 5. Given the following pedigree,

a) Is it compatible with the presence of a holandric gene?
b) Is it compatible with the presence of a sex-linked recessive allele?
c) If mating between III-2 and III-3 produces a mutant female, which of the two hypotheses above is applicable? Using appropriate symbols assign genotype to each of the individuals in the pedigree.



Problem 6. The following pedigree shows the transmission of two human traits in a family. The individuals shaded on the left side show nasal asymmetry, and those shaded on the right side show deformation of the auricle.

a) What type of inheritance is involved in each of the traits?
b) Determine as far as possible the genotypes of all the individuals in the pedigree.
c) What is the probability that from the cross IV-1 x IV-6 a daughter with nasal asymmetry and normal auricle is born?



Problem 7. There is a couple consisting of a non-bald female (whose mother was bald), and a bald male whose father was not bald.

a) What will be the genotype of all these individuals?
b) What proportion of bald and non-bald individuals would we expect in the offspring of this couple?

Problem 8.- In cattle, white color is determined by a dominant C^c allele in males and recessive in females. The allele for red color C^R behaves as dominant in females and recessive in males. From a cross between a red male and a homozygous white female,

a) What phenotypic and genotypic proportions would you expect in F_1 and F_2?
b) If a homozygous white cow has a red calf, what sex will the calf be?
c) What genotype is not possible from the male parent in (b)?

Problem 9. The flowers of certain plants can be red, pink, or white. Red flowers crossed with white flowers produce only pink flowers. When plants with pink flowers were crossed, they produced 113 red, 129 white, and 242 pink. The hypothesis is that these colors are produced by a single gene locus with alleles that exhibit intermediate inheritance. Test this hypothesis using the $\chi 2$ test.

Problem 10. A dominant allele (L) determines short fur in the guinea pig, and a recessive allele (l) determines long fur. Alleles with intermediate inheritance at an independent locus determine the color, being: c^yc^y= yellow; c^yc^w = cream; c^wc^w = white. From the cross of two dihybrid rabbits with short fur and cream color, predict the expected phenotypic frequency in the offspring.

Problem 11. The shape of radishes can be long, round, or oval. The color can be red, blue and purple. A long and blue variety is crossed with another round and red variety, producing an oval and purple F_1. The F_2 obtained was: 9 long, red; 15 long, purple; 19 oval, red; 32 oval, purple; 8 long, blue; 16 round, purple; 8 round, blue; 16 oval, blue; 9 round, red.

a) How many allelic pairs are involved in determining shape and color?
b) What phenotypes would you expect in crosses between F_1 and each of its parents?
c) If oval-purple radishes were commercially preferred, which radish lines should be maintained to produce the most of these radishes and why?

Problem 12. A man with blood group A has a child of blood group 0 with a woman of blood group B. a) What are the genotypes of these three people? b) What other genotypes, and with what frequencies, can be expected in the children of this couple?

Problem 13. An AB- man and a 0+ woman have a B+ child.

a) Determine the possible genotypes of each of the individuals.
b) Explain whether either parent could donate blood to the child.

Problem 14. The following five mothers, (a) through (e), with the given phenotypes, each had a child whose phenotype is described. Select the possible fathers of each of these children from among the five males for whom the genotypes are given.

Mother	Mother's phenotype	Child's phenotype	Possible father's genotypes
a	A M Rh+	O M Rh+	1. I^Ai L^ML^N rr
b	B N rh-	B N rh-	2. I^Bi L^ML^N RR
c	O M rh-	A MN Rh+	3. ii L^NL^N rr
d	A N Rh+	AB MN Rh+	4. ii L^ML^M rr
e	AB MN rh-	AB M rh-	5. I^AI^A L^ML^N RR

Problem 15. In a case of disputed paternity, the mother's blood group phenotype is A MN rh- and the son's phenotype is B N Rh+. Write down all the possible blood group phenotypes that the father may have.

Problem 16. A woman and a man have four biological children with the following genotypes: ii RR L^ML^N; I^Ai Rr L^NL^N; ii RR L^NL^N; I^Bi rr L^ML^M. What are the genotypes of the parents?

Problem 17. The inheritance of skin color in cattle is determined by a series of multiple alleles with the following dominance hierarchy: $S > s^h > s^c > s$. The "S" allele puts a band of white around the middle of the animal that is called the Dutch belt; the "s^h" allele produces the Hereford-type spots; the solid color is the result of the "s^c" allele, and the Holstein-type spots are due to the "s" allele. Homozygous Dutch-belted males are crossed with females with Holstein-type spots. F_1 females are crossed with Hereford-type spotted males with s^hs^c genotype. Predict the genotypic frequencies in the offspring.

Problem 18. Yellow mice with curved tails are crossed with each other. The offspring show a ratio of 6 yellows with curved tails: 2 yellows with normal tails: 3 agoutis with curved tails: 1 agouti with normal tail.

a) Which of the two traits is associated with a lethal allele?

b) Is the curved tail determined by a dominant or recessive gene?

c) What is the genotype of the yellow mice with the curved tail?

d) What are the genotypes of the four phenotypic types?

Problem 19. A sex-linked mutant character called "notch" (N) is lethal in Drosophila when present in hemizygous males (there can be no homozygous NN females as they would have to inherit the dominant from the father). Heterozygous females (Nn) have small notches on their wing tips. Homozygous recessive females (nn) and hemizygous males (n) have normal wings (wild type).

a) Calculate the expected viable phenotypic ratios in F_1 and F_2 without considering sex, when common type males are crossed with notched females.
b) What is the ratio of viable males/viable females in F_1 and F_2?
c) What is the ratio of viable notched animals / common type in F_1 and F_2?

Problem 20. Crossing Mexican bald dogs with each other results in offspring consisting of bald dogs and normal dogs in a 2:1 ratio. By crossing Mexican bald dogs and normal dogs, the offspring have a phenotypic ratio of 1:1 of "Mexican bald" and "normal".

a) Do you think it would be possible to obtain a pure breed of bald dogs? Why?
b) What phenotypic ratios would be expected from a cross between the "Mexican bald" and "normal" individuals obtained in the 2:1 ratio of the problem statement?

Problem 21. In the corn snake, skin coloration is controlled by two genes with two alleles each with complete dominance (*A,a* and *B,b*). Each gene, through the enzymes encoded by it, controls the synthesis of a different pigment in separate metabolic pathways. One of these two genes produces an orange pigment (*A_* presence of orange pigment and *aa* absence of orange pigment) and the other gene produces a black pigment (*B_* presence of pigment and *bb* absence of black pigment). These two genes are transmitted independently.

a) What type of interaction do these two genes have?
b) A wild-type snake (with both pigments) is crossed with an albino snake (without pigment) and the F_1 is all wild-type colored. What phenotypic composition will the F_2 have?

Problem 22. In bluebell, the wild color of the flowers is blue. All the intermediaries in the pathway to the anthocyanin (blue) pigment are colorless. Two white mutant strains are available, when crossing these two strains between each other, the F_1 flowers are all blue. When you cross these F_1 plants with each other, the F_2 is composed of plants with blue flowers and plants with white flowers in the ratio 9 blue: 7 white.

a) What type of inheritance would explain these results?
b) What genotypes do the white-flowered plants used in the cross have?
c) What genotypes do the F_1 plants have?
d) What genotypes would two F_2 plants have to have in order to obtain plants with blue and white flowers in the ratio 1 blue: 3 white when crossed?

Problem 23. In a metabolic pathway leading to the synthesis of a blue pigment, we start from a colorless precursor that by the action of a first gene is transformed into a magenta intermediary (*A_* transforms and *aa* does not transform). This intermediary by the action of a second gene is transformed into a blue pigment (*B_* transforms and *bb* does not transform).

a) Identify the type of genetic interaction between these two genes.
b) When crossing an individual with magenta flowers with an individual with white flowers (colorless), the offspring have magenta flowers and when crossing them with each other, 3/4 of the offspring are magenta and 1/4 are colorless. Determine the genotypes of these plants.
c) What genotypes would two blue plants have to have in order for the offspring obtained by crossing them to be 3/4 blue and 1/4 magenta?

Problem 24. In the Labrador dog, coat color is controlled by the action of two genes with two alleles each (*B*,*b* and *E*,*e*). Individuals with *B_* genotypes have black color while *bb* individuals have a brown color. The second gene (*E*,*e*) causes the black or brown pigment produced by the first gene to be deposited in the fur so that in *E_* individuals the pigment is deposited in the fur while in *ee* individuals the pigment is not deposited in the fur and they have a golden color.

a) Identify the type of gene interaction that these two genes maintain.
b) Black individuals were crossed with pure breeds of golden color and the offspring presented a phenotypic composition 1 black: 1 brown: 2 golden. What genotypes did the parents have?
c) What phenotypic composition is expected when crossing the black individuals of the previous F_1?

Problem 25. The synthesis of a given pigment in a metabolic pathway is a two-step process controlled by two genes with two alleles each (*A*,*a* and *B*,*b*). The enzyme encoded by the first gene needs a double dose (*aa*) to be able to transform a colorless precursor into a colorless intermediate substance. On this intermediate substance acts the enzyme encoded by the second gene, which also needs a double dose (*bb*) to be able to transform the intermediate substance into a red pigment.

a) What type of interaction do these two genes maintain?
b) What phenotypic proportions are expected from a cross between a plant diheterozygous for these two genes and a plant with red flowers?
c) What phenotypic proportions are expected from a cross between two diheterozygous plants?

Problem 26. A gene with two alleles encodes an enzyme that transforms a colorless precursor into an intermediate substance that is also colorless. At least one functional allele (*A_*) of the first gene is needed for the reaction to take place. A second gene transforms the intermediate substance into pigment. This second gene has two alleles: *B* (red pigment) and *b* (yellow pigment).

a) What type of gene interaction do these two genes have?
b) Crossing two lines, one with red flowers and the other with white flowers results in an F_1 consisting of red, yellow, and white flowers in a 1:1:2 ratio. What genotypes do the two parental lines have?

Problem 27. A gene with two alleles encodes an enzyme that transforms a colorless precursor into an intermediate substance that is also colorless. Both functional alleles of that first gene are needed for the reaction to take place (*A_* does not transform and *aa* transforms). A second gene transforms the intermediate substance into green pigment. This second gene also has two alleles: *B_* which does not transform the intermediate into pigment and *bb* which transforms the intermediate into green pigment).

a) What type of gene interaction do these two genes have, and why?
b) Crossing a white-flowered line with a green-flowered line produces an F_1 with white and green flowers in the ratio of 3/4 white and 1/4 green. What genotypes do these two lines have?

Problem 28. In *Drosophila*, purple eye color is due to the recessive allele of a gene (*pr*). The recessive allele (*s*) of another unlinked gene suppresses the mutant phenotype "purple eyes" (*pr/pr*). Thus an individual of genotype *pr/pr s/s* has red eyes (wild-type phenotype in *Drosophila* as far as eye color is concerned).

a) What is the gene interaction between these two genes?
b) If a fly with purple eyes and not carrying the suppressor allele is crossed with a fly homozygous for red eyes and homozygous for the suppressor, what will be the phenotypic composition of the offspring?
c) What are the genotypes of two flies whose offspring is 3/4 red, and 1/4 purple?
d) What are the genotypes of two flies whose offspring is 13 red:3 purple?

Problem 29. Pineapple leaves can have three phenotypes: "spiny", "spiny-tipped" and "spineless". When crossing pure lines of "spiny-tipped" individuals with "spiny" individuals all F_1 had "spiny-tipped" and F_2 had a phenotypic ratio of 3 "spiny-tipped":1 "spiny". When crossing pure lines of "spineless" individuals with "spiny tip" individuals, F_1 was all "spineless" and F_2 "spineless"/"spiny tip" in a 3:1 ratio. Finally, pure lines of thornless individuals were crossed with thorny individuals, F_1 was all thornless and F_2 12 "spineless": 3 "spiny tip" and 1 "spiny".

a) How many genes are involved in the character "pineapple leaf shape"?
b) Assign symbols to these genes
c) Do these genes have any interaction?
d) Determine the phenotypic proportions expected when crossing the "spineless" individuals of the last cross in the statement with each other

4. SOLUTIONS TO PROBLEMS

Problem 1.
a) Mother $X^+ X^d$; Father X^+Y;
b) Son: $X^d Y$

Problem 2.
a) Male: 50% colorblind, 50% normal vision
b) Female: 50% colorblind, 50% normal vision (carriers)

Problem 3.

$$P = \frac{8!}{6!2!} (1/2)^6 (1/2)^2 = 28 (1/2)^8$$

Problem 4.
a) 1/8

b) 1/4
c) 3/4
d) Normal or hemophiliac
e) Normal

Problem 5
a) Yes
b) Yes
c) The second hypothesis (recessive sex-linked)

Problem 6.
a)
Nasal Asymmetry: dominant, sex-linked.
Deformation of the auricle: recessive, autosomal

b)
I-1, X^aYDd I-2, X^AX^add

II-1, X^AX^aDd II-2, X^AYDd
II-3, X^AYDd II-4, X^AX^add
II-5, X^aX^add II-6, X^AX^add
II-7, X^aX^add II-8, X^AYDd

III-1, X^AYDd III-2, X^AX^-Dd
III-3, X^aYdd III-4, X^AX^-Dd
III-5, X^AYdd III-6, X^AX^aDd
III-7, X^AX^add III-8, X^AX^aDd
III-9, X^aYDd

IV-1, $X^AYD_$ IV-2, X^AYdd
IV-3, $X^AX^-D_$ IV-4, X^AYDd
IV-5, X^AX^-dd IV-6, X^AX^-Dd
IV-7, X^AYDd IV-8, X^aYdd

c) 5/12

Problem 7.
a) Non-bald female (C_1 C_2), bald mother (C_1 C_1), bald man (C_1 C_2), his non-bald father (C_2 C_2)
b) 3/4 of the sons and 1/4 of the daughters will be bald.

Problem 8.
a)
F_1: red females, white males
F_2: Females: 25% white, 75% red; Males: 25% red, 75% white
b) Female
c) C^cC^c

Problem 9.
$\chi^2_{experimental} = 1.057 < \chi^2_{theoretical}$ Intermediate inheritance

Problem 10.
Short yellow fur: 3/16
Short cream fur: 6/16

Short white fur: 3/16
Long yellow fur: 1/16
Long cream fur: 2/16
Long white fur: 1/16

Problem 11.
a) 2 allelic pairs FL/FR CB/CR

b) FLFR CBCR x FLFL CBCB
¼ long blue; ¼ long purple; ¼ oval blue; ¼ oval purple

FLFR CBCR x FRFR CRCR
¼ round red; ¼ oval purple; ¼ oval red; ¼ round purple

c) Oval-purple homozygous, so that all of the offspring is the commercially preferred variety.

Problem 12.
a) Male: I^A i. Female: I^Bi. Son: ii
b)

Genotype	I^AI^B	I^Ai	I^Bi	ii
Phenotype	AB	A	B	0
Frequency	1/4	1/4	1/4	1/4

Problem 13.

a) Father AB-: I^AI^Bdd
Mother 0+: ii DD o iiDd
Son B+: I^BiDd
b) Only the mother can donate blood to the child.

Problem 14.
a) Possible fathers: 1, 2 y 4
b) Possible fathers: 1 y 3
c) Possible fathers: 5
d) Possible fathers: 2
e) Possible fathers: 1

Problem 15.
There are 12 possible fathers: I^B_ L^N_ R_

Problem 16.
I^Ai L^ML^N Rr x I^Bi L^ML^N Rr

Problem 17.
50% Dutch belt: Ss^h Ss^c
25% Hereford-type spots: s^hs
25% Solid color: s^cs

Problem 18.
a) Yellow is dominant for fur color, and recessive lethal.
b) Curved tail is dominant
c) AaCc
d) AA lethal; Aa yellow; aa agouti. C_ curved tail; cc normal tail

Problem 19.
a) F_1: 2/3 wild-type, 1/3 notched; F_2: 6/7 wild-type, 1/7 notched
b) F_1: 2 female:1 male; F_2: 3 male: 4 female
c) F_1: 1:2; F_2: 1:6;

Problem 20.
a) It would not be possible, because "bald" is homozygous lethal. All balds are heterozygous.
b) 1 normal: 1 bald

Problem 21.
a) Non-epistatic gene interaction
b) 9 wild-type: 3 orange : 3 black :1 albino

Problem 22.
a) Duplicate Dominant Epistasis
b) AAbb and aaBB
c) AaBb
d) AaBb and aabb or also Aabb and aa Bb

Problem 23.
a) Recessive epistasis
b) AAbb and aabb
c) AABb and AaBb

Problem 24.
a) Recessive epistasis
b) BbEe and bbee
c) 9 black: 4 golden: 3 brown

Problem 25.
a) Duplicate Dominant Epistasis
b) 3/4 white y 1/4 red
c) 15/16 white, 1/16 red

Problem 26.
a) Recessive epistasis
b) AaBb and aabb

Problem 27.
a) Duplicate Dominant Epistasis
b) AaBb and aabb

Problem 28.
a) Dominant Inhibitory Epistasis
b) All of them with red eyes
c) PdpdSs y pdpdss (both red eyes)
d) PdpdSs y PdpdSs

Problem 29.

a) Two genes with two alleles with complete dominance of one allele over the other.

b) A_ “spineless”, B_ “spiny-tipped” and bb “spiny”.

c) There is an epistatic interaction. If allele A is present, the phenotype is always “spineless”, no matter what the genotype of the second gene is. Therefore there is a dominant epistatic interaction of A over B.

d) 32/36 “spineless”, 3/36 “spiny-tipped”, 1/36 “spiny”

LINKAGE, RECOMBINATION AND GENETIC MAPS

1. PROBLEM-SOLVING GUIDE (TROUBLESHOOTING GUIDE)

General considerations

In the first place, we must always keep in mind that in order to use linkage and recombination for genetic mapping, we must rely on crosses between di-hybrid or tri-hybrid individuals (generally obtained by crossing two pure breeds) and recessive homozygote for all genes under consideration (test cross). Any other type of cross will generally not allow this type of genetic analysis. In a test cross, the distribution of phenotypes in the offspring closely reflects the distribution of gametes produced by the hybrid, as the homozygote contributes only recessive gametes.

There are different types of problems in this section, depending on the number of genes to analyse simultaneously and the approach we take. In relation to the number of genes, the most typical problems are di-hybrid and tri-hybrid crosses, although we can also handle more than three genes in the same problem. Regarding approach, we can find problems with a direct approach, in which we will be asked to predict the type and frequency of offspring expected in a test cross, knowing the total number of offspring and the genetic distances, and problems with an inverse approach, in which we will be asked to calculate the genetic distances from the data of a test cross. These problems can also be complicated in those cases where there is sex linkage or some genetic interaction between them. Let's see how we should approach the resolution of each of these types of linkage and recombination problems in diploid organisms.

Problems with an inverse approach

Di-hybrid cross

We will be given the number of offspring that have appeared from each of the 4 possible phenotypes in a di-hybrid test cross and we will be asked to calculate the genetic distance between the genes in question.

First, we must verify that the two genes are linked, checking that the offspring do not conform to a 1:1:1:1 distribution, that is, that all the phenotypes do not appear with equal frequency. If necessary, because the differences are not obvious, or because the problem statement requires it, we can demonstrate the linkage by applying a χ^2 statistical test. Then we will be able to find out (or verify) which are the recombinant gametes produced by the di-hybrid, which we will identify in the phenotypes with the lowest frequency in the offspring. We can then calculate the recombinants frequency by applying the formula: **P = Nº recombinants / N**. The genetic distance will result from multiplying that frequency by 100 and expressing it in centi-Morgans (cM) or in map units (u.m.).

(See Solved Problem 2)

Tri-hybrid cross

We will be provided with the number of offspring that have appeared from each of the 8

possible phenotypes in a test cross of a tri-hybrid. We will be asked to calculate the genetic distance between the genes in question.

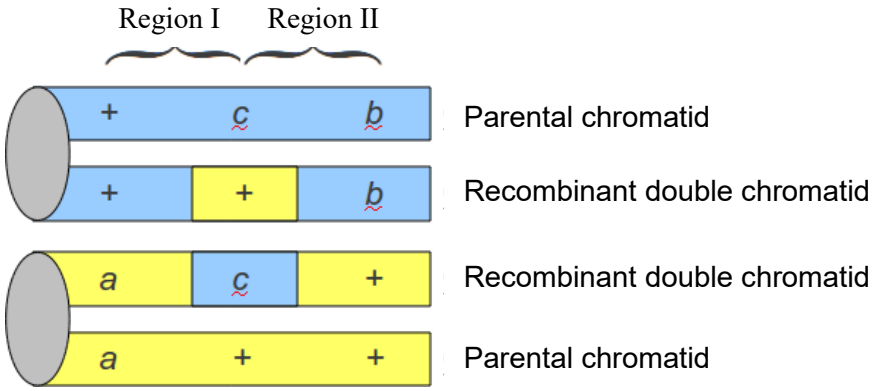
However, in a tri-hybrid cross it can be the case that 1) all three genes are independent, 2) two are linked and the third is independent, or 3) all three genes are linked. Each of these possibilities can be analysed by a χ^2 statistical test if necessary or required. To check whether the three genes are independent, we will test the hypothesis that they are not linked, which implies an expected distribution 1:1:1:1:1:1:1:1, meaning that all types of individuals in the offspring appear with equal frequency. To check if two genes are linked and the other is independent, we need to carry out independence tests using χ^2 test for each of the three pairs of genes, for which we will have to build three tables that gather the observed numbers of each of the offspring, considering only two genes and not three. For example, from the table of offspring in exercise 4 of our list of problems, we can extract another one in which we exclusively consider the genes +/a and +/b:

Phenotype	Number
+ + +	92
+ + c	25
+ b +	1
+ b c	399
a b c	77
a + +	426
a + c	3
a b +	31
TOTAL	1054

Phenotype	Number
++	92+25=117
+b	1+399=400
a+	426+3=429
ab	77+31=108
TOTAL	105 4

With these new data we can apply a χ^2 test to check if +/a and +/b are linked to each other or not. After repeating with the other two pairs of genes (+/a with +/c and +/b with +/c), we will know the linkage relationships between the three genes.

If all three genes are linked, then we can calculate the genetic distances between them. First, we will need to know the relative order of the genes on the chromosome. To do this, we have to compare the genotypes of the parental gametes, which we will recognize in the offspring as the most frequent phenotypes (+bc and a++, in the table on the left), with those of the double recombinants, which will be the least frequent (+b+ and a+c). If we compare them two by two, we will realise that between +bc (parental) and +b+ (recombinant) only the position of the alleles of the gene +/c changes, and that between a++ (parental) and a+c (recombinant), only the position of the alleles of the +/c gene changes. This indicates that gene +/c is between the other two, since in a double recombinant (when there is recombination in regions I and II), only the central gene changes chromatid, as we can see in this diagram:



Once the order of the genes is established, we can calculate the distances from the centre to each of the end genes. We will call P_1 the distance between the genes that define region I, according to the following formula, where RI is the number of recombinants in region I, DR is the number of double recombinants and N is the total number of individuals analysed.

$$P_1 = \frac{RI + DR}{N}$$ In the same way, $$P_2 = \frac{RII + DR}{N}$$

To calculate the distance between the two extreme genes (a and b) simply add the map distances between a and c and between c and b.

$P = P_1 + P_2$

Coincidence Coefficient (CC):

$$CC = \frac{\text{Observed frequency of double recombinants}}{\text{Expected frequency of double recombinants}} = \frac{DRO / N}{P_1 \times P_2}$$

where DRO is the total number of double recombinants observed.

The interference coefficient (I) will have the complementary value of CC, that is:
I = 1 – CC.

(See Solved Problem 4)

Problems with a direct approach

Di-hybrid cross

We will be provided with linkage data between two genes, including their genetic distance and the genotype of the di-hybrid, and asked to calculate the expected number of each of the 4 possible phenotypes, among a total of N offspring.

First we will calculate the number of recombinant individuals, clearing R from recombinant frequency formula: P = N recombinants / N, so **N recombinants = P x N** (we calculate P by dividing the genetic distance by 100; for example, at a distance of 20 cM corresponds to a value of P=0.2). Of the total number of recombinants calculated in this way, half will correspond to each of the two types of possible recombinants.

To calculate the number of parents, we only have to subtract the total number of recombinants calculated before from N. Of the result, half will correspond to each of the two types of possible parents.

(See Solved Problem 3)

Tri-hybrid cross

We will be provided with linkage data between three genes, including their genetic distances and the genotype of the tri-hybrid, and asked to calculate the expected number of each of the 8 possible phenotypes, among a total of N offspring.

In the first place we will have to define what the 8 possible genotypes are, derived from the test cross of a tri-hybrid, whose relative frequencies will depend on the genotype of the tri-hybrid. If it is a trihybrid in the mating phase, like the one in solved problem 5, its genotype will be: +++/abc, and, therefore, it will produce these 8 different types of gametes, distributed as follows:

Gamete type	Genotype
Parental	+++
	abc
Recombinant region I (a-b)	+bc
	a++
Recombinant region II (b-c)	++c
	ab+
Recombinant doubles	+b+
	a+c

If any of the genes are in repulsion phase, your genotype might look like this: ++c/ab+ and, therefore, will produce the same 8 different types of gametes, but distributed in this other way:

Gamete type	Genotype
Recombinant region II (b-c)	+++
	abc
Recombinant doubles	+bc
	a++
Parental	++c
	ab+
Recombinant region I (a-b)	+b+
	a+c

In other cases, the same way would be used to classify the phenotypes of the descendants. Once this is done, the expected number of each genotype is calculated according to this order: 1st) double recombinants, 2nd) single recombinants and 3rd) parental.

The expected number of double recombinants is cleared from the coincidence coefficient formula (see section 1b), so: DRO = CC x P_1 x P_2 x N. Of these, half will be of each of the two types of doubles possible recombinants.

The numbers of single recombinants of each type are solved from the formulas for the genetic distances of regions I and II (see 1b). Thus: RI = P_1 x N – DR and RII = P_2 x N – DR. In both cases, half will be from each of the two possible single recombinant types.

The number of parents is obtained by subtracting from the total number of recombinants of regions I and II and of double recombinants: Parental = N – RI – RII – DR. again, half will be from each of the two possible parental types.

(See Solved Problem 5)

Problems with more than three genes

They can have a direct or inverse approach but, in any case, this type of problem is solved by subdividing the data into groups of three genes, each of which is solved independently as we have seen previously for tri-hybrid crosses. Once this is done, the data for all the genes is reassembled. The resolution of these problems is not conceptually difficult, but it is long and systematic.

2. PROBLEMS SOLVED

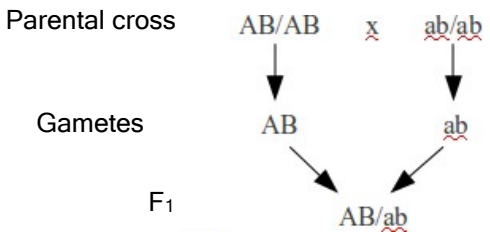
Problem 1. Suppose that the recessive allele a induces the formation of white flowers (normal flowers are red) and the recessive allele b causes wrinkled leaves (normal leaves are smooth) in an ornamental plant species. Considering that these genes are located on the same chromosome at a distance of 30 centi-Morgans (cM), if we cross AABB individuals with other aabb:

a) What genotypes and phenotypes would appear in the F_1?
b) What would be the frequencies of these phenotypes and genotypes?
c) What types of gametes will F_1 females produce?

d) What will be their relative frequencies?
e) If we were to cross F_1 female plants with aabb male plants, what would be the frequencies of the resulting phenotypes?

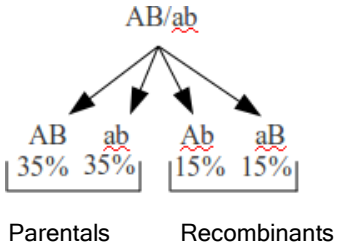
Answer

a)



By having both dominant alleles, F_1 individuals will be normal.

b) 100% of the offspring in the F_1 will be dihybrids in mating phase and normal.



c) and d) Two different parental gametes (70% total; 35% of each type) and two recombinants (30% total; 15% of each type) are produced.

e)

	Female gametes and their frequencies			
	AB 0.35	**ab** 0.35	**Ab** 0.15	**aB** 0.15
Male gametes **ab** 1.0	**AB/ab** 0.35	**ab/ab** 0.35	**Ab/ab** 0.15	**aB/ab** 0.15
Phenotypes	Normal	White flowers and wrinkled leaves	Wrinkled leaves	White flowers

Problem 2. From the crossing of a dihybrid individual in the repulsion phase (Ab/aB) with a double homozygous recessive (ab/ab), 1000 descendants are obtained with the following phenotypes and numbers: 408 Ab, 392 aB, 106 AB and 94 ab.

a) Are these genes linked?
b) If so, at what genetic distance are they?

Answer

a) It is evident, observing the results of the cross that the four types of possible gametes that the dihybrid can form do not appear in the same proportion of 1/4. A χ^2 statistical test would probably indicate that this distribution of offspring phenotypes departs significantly from the 1:1:1:1 expected for independent genes. Therefore, we conclude that these genes are linked.

b) The genetic distance is estimated by the frequency of recombinant gametes produced by the dihybrid. In this case, the recombinant gametes are AB and ab, since in the dihybrid they are in the repulsion phase (the parental gametes would therefore have these genotypes: Ab and aB). The frequency of individuals of recombinant phenotype (from recombinant gametes) would be:

$$P = \frac{106 + 94}{408 + 392 + 106 + 94} = 0{,}2$$

Therefore, the genetic distance between these two genes is 20 centi-Morgan (cM) or map units.

Problem 3. If two genes A/a and B/b are linked at a genetic distance of 28 cM, how would the 1000 offspring of the following cross be distributed: AB/ab x ab/ab?

Answer

Since this is a test cross, the composition of the offspring will depend exclusively on the types of gametes produced by the dihybrid. In this case, the gametes and their frequencies would be:

	Parental 72%		Recombinant 28%	
Gametes	AB	ab	Ab	aB

Frequencies 0.36 0.36 0.14 0.14

To calculate the expected number of offspring of each type in a total of 1000, we only have to multiply the frequency of each gamete by this number, resulting in:

	Offspring			
	Parental		Recombinants	
Genotypes	*AB/ab*	*ab/a b*	*Ab/ab*	*aB/ab*
Phenotypes	AB	ab	Ab	aB
Number	360	360	140	140

Problem 4. Suppose there are three linked genes, +/a, +/b and +/c, whose order on the chromosome is not known. A trihybrid is crossed with the triple homozygous recessive, obtaining the following offspring:

+ + +	92
+ + *c*	25
+ *b* +	1
+ *b c*	399
a b c	77
a + +	426
a + *c*	3
a b +	31
TOTAL	1054

a) What genotype did the trihybrid have?
b) In what order are the genes on the chromosome?
c) What are the genetic distances between all of them?

Answer

a) The genotype of the trihybrid is deduced from the phenotypes of the most frequent offspring, since these will derive from parental gametes. These are the +bc and a++ phenotypes, whose genotypes should be: +bc/abc and a++/abc since they come from the crossing of +bc and a++ gametes, respectively, with the abc gametes produced by the recessive triple homozygote. Consequently, the genotype of the trihybrid would be:

+bc/a++.

b) The order is a-c-b, it is deduced by observing the parents (+bc/abc, a++/abc) with the double recombinants (a+c/abc, +b+/abc) It is observed that the gene that changes position is the c/+, so it is the central gene.

The distance between +/a and +/c would be:

P_1= 92+77+1+3/ 1054 = 16.41 cM

and the distance between +/c and +/b would be:

P_2= 25+31+1+3/ 1054= 5.69 cM

To calculate the distance between the two extreme genes (a-b):

P_1+P_2= 16.41+5.69= 22.1 cM

Problem 5. Let three genes be +/a, +/b and +/c that are linked in that order and separated by the following distances: Pa-b = 30 cM and Pb-c = 20 cM. In that chromosomal region, the interference has a value of 0.5. After testing trihybrids in mating phase, a total of 10,000 offspring are tested.
What number will appear from each of the different types of offspring?

Answer

Mating phase trihybrids have this genotype: +++/abc. Therefore, they will be able to produce 8 different types of gametes, distributed as follows:

Gamete type	Genotype
Parental	+++
	abc
Recombinant región I (a-b)	+bc
	a++
Recombinant región II (b-c)	++c
	ab+
Recombinant doubles	+b+
	a+c

DRO = 0.5 x 0.3 x 0.2 x 10,000 = 300, of which 150 (half) will be phenotype +b+ and the other 150 will be a+c.

RI = 0.3 x 10,000 – 300 = 2,700, of which half (1,350) will be +bc and half (1,350) will be a++.

RII = 0.2 x 10,000 – 300 = 1,700, of which half (850) will be ++c and half (850) will be ab+.

Parental = 10,000 – 2,700 – 1,700 – 300 = 5,300, of which 2,650 will be +++ and as many will be abc.

The results can be arranged in the following table:

Phenotype	+++	abc	+bc	a++	++c	ab+	+b+	a+c	TOTAL
Nº expected	2650	2650	1350	1350	850	850	150	150	10000

3. PROBLEMS TO SOLVE

Problem 1. In a certain dioecious plant species, the recessive allele l induces the formation of lanceolate leaves and the recessive allele p causes leaves without pigment. The two genes are located on the same chromosome and show 30% recombination. An LLPP individual is crossed with another llpp.

a) What genotypes and phenotypes would appear in the F_1?
b) What frequencies would these phenotypes and genotypes have?
c) What types of gametes will F_1 females produce?
d) What will be their relative frequencies?
e) If we crossed the F_1 females with llpp males, what would be the frequencies of the resulting phenotypes?

Problem 2. The black body colour of *Drosophila* is determined by the allele recessive e and the vestigial wings by the recessive allele vg of another gene linked to the first at a distance of approximately 20 map units. What phenotypes and frequencies are expected to appear in:

a) A cross between heterozygous females whose alleles are in the repulsion configuration with heterozygous males whose alleles are in coupling configuration.
b) A reciprocal cross to the one in the previous section.
c) A cross in which both heterozygous parents present their alleles in the repulsion configuration.

Problem 3. In a certain mammal, eye colour is determined by the interaction of two linked genes, A/a and B/b. This is a double recessive epistatic interaction, so that the simultaneous presence of the dominant alleles of A and B produces black eyes, while double or simple recessive individuals have light eyes. Homozygous black individuals (AB/AB) are crossed with other double recessives (ab/ab) and the descendants of the F_1 are subjected to a test cross, obtaining 1,255 animals with black eyes and 1,777 with light eyes. On the other hand, Ab/Ab individuals were crossed with other aB/aB and the F_1 was subjected to a test cross, obtaining 147 black-eyed and 1,540 light-eyed animals in the offspring. Calculate the genetic distance between these two genes.

Problem 4. The colour of the flower of a certain plant is determined by a gene whose alleles B and b determine, respectively, red colour and white colour. The size of the plant is determined by the E/e gene, linked to the previous one, whose alleles determine the normal plant (E) and the dwarf plant (e). The following table shows the phenotypes and their frequencies of test crosses of two heterozygous plants:

Plant	**I**	**II**
Red flowers and normal plant	88	23
Red flowers and dwarf plant	12	170
White flowers and normal plant	8	190
White flowers and dwarf plant	92	17
Total	200	400

a) What are the genotypes of heterozygous plants I and II?
b) What is the genetic distance between these genes?
c) If the two heterozygous plants are crossed with each other, what frequency of offspring with white flowers and dwarf size would be expected?

Problem 5. Genes A and B, located on the same chromosome, are at a genetic distance of 20 map units. Genes C and D, located on another chromosome, are 30 units apart.

A homozygous dominant AABBCCDD individual is crossed with a homozygous recessive aabbccdd. An F_1 individual is then crossed with the recessive parent. What genotypes and with what frequencies will appear in the offspring?

Problem 6. The recessive alleles, e and vg, of 2 linked genes in *Drosophila* produce black bodies and vestigial wings, respectively. When common-type flies are crossed with recessive double mutants, F_1 individuals are coupling configuration dihybrids. Subsequently, the test cross of the F_1 gave the following results: 1,930 wild type, 1,888 black and vestigial, 412 black and 370 vestigial.

a) Calculate the distance between loci e and vg.

Another gene (cn), whose recessive allele lies between the e and vg loci, produces cinnabar eyes. When flies of the wild phenotype are crossed with flies of the triple mutant phenotype, a trihybrid F_1 is obtained, which, subjected to a test cross, gave the following results: 664 wild flies, 652 black, cinnabar and vestigial, 72 black and cinnabar, 68 vestigial, 70 black, 61 cinnabar, and vestigial, 4 black and vestigial and 8 cinnabar.

b) Calculate the map distance between the three genes.
c) Do the distances e-vg calculated in part a) and b) coincide? Why?
d) What is the coefficient of coincidence?
e) And the interference?

Problem 7. In *Drosophila*, the characters curved wings and lack of antennas are due to the action of the corresponding recessive alleles of two different loci.

In the F_1 of mating a curved-winged female with a male without antennas, all females were normal and all males curved-winged. The segregation obtained in the F_2 was as follows:

Phenotype	Females	Males
Normal	390	129
Curved wings	378	243
Without antennas		240
Curved wings and without antennas		132
Total	768	744

From these data, what can we say about the location of these genes?

Problem 8. Suppose three linked genes, +/a, +/b and +/c, not necessarily in that order. After subjecting a trihybrid to a test cross, the following phenotypic frequencies were obtained:

Phenotype	Number
+ + +	88
+ + *c*	21
+ *b* +	2
+ *b c*	339
a b c	55
a + +	355
a + *c*	2
a b +	17
Total	879

a) What are the genotypes of the homozygous parents used to obtain the trihybrid?
b) Determine the relative order of these genes on the chromosome.
c) Calculate the genetic distances between them.

Problem 9. Recessive alleles r, c, and n of three linked genes produce, respectively, kidney-shaped eyes, cardinal eyes, and a black body in one species of insect. Homozygous females with kidney-shaped and cardinal eyes are mated with homozygous black males. The trihybrid females of the F_1 are subjected to the test cross and among the 4,000 descendants analysed the following types and numbers are found:

1,761 kidney cardinal, 1,773 black, 128 kidney black, 138 cardinal, 97 kidney, 89 black cardinal, 8 common type, and 6 black cardinal kidney.

a) Calculate the order and map distances.

Problem 10. The recessive allele of the hairpin gene (h) produces separate elytra in a putative beetle species. The recessive allele of another gene, tall (a), determines a greater length of the legs. The recessive allele of a third gene, called maroon (g), produces maroon body colour. Common-type heterozygous females for all three loci were crossed with common-type males. The F_1 data appears below:

Females	All common type	
Males	57 maroon, tall	2 maroon
	419 maroon, fork	439 tall
	60 fork	13 common type
	1 tall, fork	9 maroon, tall, fork

a) What type of sex determination chromosome system would this species be likely to have?
b) Which gene is in the middle?
c) What is the distance between the h and a loci?
d) What is the distance between loci h and g?
e) What is the value of the interference coefficient?

Problem 11. Suppose three pairs of alleles in a species of bird: +/n, +/o, and +/p. The n, o, p alleles are all recessive and sex-linked. On the Z chromosome they are found in the n-o-p order, with the distance between n and o being 12 map units and between o and p being 10 map units. For this region of the Z chromosome, the coincidence coefficient is 0.5. In a cross between a normal male of genotype ++p/no+ and females nop/W:

Predict the types and frequencies of the genotypes that are expected to appear among a total of 200 randomly chosen females of the offspring.

Problem 12. In a certain organism there are two strains, A and B, whose genotypes are OOPPQQ and ooppqq respectively. The offspring of the A X B cross were backcrossed with B and an offspring with the following distribution was obtained:

OPQ 100	Opq 25
opQ 100	oPq 25
OPq 100	oPQ 25
opq 100	OpQ 25

Determine the linkage relationships between these three loci, knowing that they belong to the same linkage group.

Problem 13. A strain homozygous for four different recessive mutations a, b, c, and d, located on the same chromosome (but not necessarily in this order), is available which crosses with a strain homozygous for the dominant wild-type alleles. Next, the F_1 females are crossed with homozygous recessive males and 2,000 F_2 individuals are counted, obtaining the following results:

Phenotype	Number	Phenotype	Number
a b c d	668	a b c +	97
a b + d	5	a+ c d	2
a + c +	69	a b + +	1
a + + +	145	a + + d	0
+ + + +	689	+ + + d	98
+ + c +	5	+ b + +	1
+ b + d	76	+ + c d	1
+ b c d	143	+ b c +	0

a) Determine the distances between the four genes and draw the partial genetic map for them.
b) What is the coefficient of interference that occurs between the genes located in first and third position?
c) And between the genes located in second and fourth position?
d) Is the number of recombinant triples the same as expected?

Problem 14. In a certain species, five recessive mutations were isolated: a, b, c, d, and e, two of which (b and e) map within five units of each other. In order to establish the linkage relationships between the five corresponding loci, two different crosses were made, whose offspring presented the following phenotypic compositions:

AaDdEe x aaddee		BbCcDd x bbccdd	
ADE	43	BCD	305
ADe	171	BcD	111
AdE	20	bCD	10
aDE	3	Bcd	8
Ade	2	bcD	60
aDe	25	BCd	72
adE	184	bCd	117
ade	52	bcd	317

Determine, from these data, a) the order of the five loci, and b) the genetic distances between them.

4. SOLUTIONS TO PROBLEMS

Problem 1.

a) Wild-type (normal) phenotype, with heterozygous LP/lp genotype
b) All individuals in F_1 will be like this
c) LP, lp, Lp and lP
d) LP 0.35 lp 0.35 lp 0.15 lp 0.15
e) Wild 35%; Lanceolate-no pigment 35%; lanceolate15%; No pigment 15%

Problem 2.

a) Wild 0.55; black 0.2; black-vestigial 0.05; vestigial 0.2
b) Wild 0.5; black 0.25; black-vestigial 0; vestigial 0.25
c) Wild 0.5; black 0.25; black-vestigial 0; vestigial 0.25

Problem 3.

17.33 map units or centi-Morgans (cM)

Problem 4.

a) Plant I: BE/be; Plant II: Be/bE
b) In both cases, 10 cM
c) 0.0225

Problem 5.

ABCD	0.14	ABcd	0.14	ABCd	0.06	ABcD	0.06
AbCD	0.14	abcd	0.14	AbCd	0.06	abcD	0.06
AbCD	0.035	Abcd	0.035	AbCd	0.015	AbcD	0.015
aBCD	0.035	aBcd	0.035	aBCd	0.015	aBcD	0.015

Problem 6.

a) 17 cM
b) Distance e-cn = 8.94 cM; cn-vg distance = 9.51 cM
c) Double crossovers between two genes go undetected, so map distances between distant genes tend to underestimate genetic distances.
d) CC = 0.883
e) I = 0.117

Problem 7.

The genes are located on the X chromosome and linked at a distance of 35.1 cM.

Problem 8.

a) The genotypes of the parents are: (+bc/+bc) and (a++/a++).
b) The order is: a - c - b
c) Distance a-c = 16.72 cM; c-b distance = 4.78 cM; a-b distance = 20.59 cM.

Problem 9.

a) The three genes are linked.
b) The relative order is: r - n - c. Distance r-n = 7 cM; n-c distance = 5 cM.

Problem 10.

a) This species will probably have an XX/XY or XX/X0 sex determination chromosome system, since the data indicate that the genes in question are located on an X-type sex chromosome.
b) The h gene is between the other two.
c) The distance between h and a is 2.5 cM.
d) The distance between h and g is 12 cM.
e) There is no interference in this chromosomal region: I = 0

Problem 11.

Type of male gametes	**Genotype of F_1 females**	**Expected number**
Recombinant Doubles	+*op*/ W	0.6
	n++/ W	0.6
Recombinant Region I	+*o*+/ W	11.4
	n+*p*/ W	11.4
Recombinant Region II	+++/ W	9.4
	nop/W	9.4
Parental	++*p*/ W	78.6
	no+/ W	78.6

Problem 12.

O and P are linked at a distance of 20 cM.

Problem 13.

a) distance a-c 15 cM; distance c-b 8 cM; distance b-d 10 cM
b) I_{ab}= 0.5
c) I_{cd} = 0.688
d) No. 2.4 expected and 2 observed.

Problem 14.

a) order: a - d - b - e - c
b) distance a-d 10 cM; distance d-b 15 cM; distance b-e 5 cM; distance e-c 20 cM

QUANTITATIVE GENETICS

1. QUANTITATIVE GENETICS PROBLEM SOLVING GUIDE

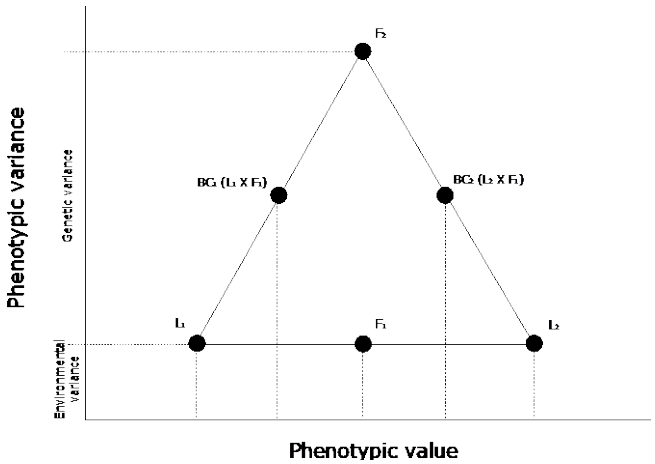
The **phenotypic value** of a trait for an individual is the measure of that trait in that individual. Thus, if a person's height is 1.78 m, that will be his or her phenotypic value. The mean and variance can be calculated for a set of individuals (a population, a sample, a generation, etc.). The mean for that trait will be the average phenotypic value, and the variance will correspond to the phenotypic variance or total variance. This **phenotypic variance** can be partitioned into several components:

$$\sigma^2_P = \sigma^2_G + \sigma^2_E + \sigma^2_{GxE}$$

σ^2_P represents the total phenotypic variance, σ^2_G the **variance of genetic origin**, σ^2_E the **variance of environmental origin**, and σ^2_{GxE} the variance due to the genotype-environment interaction (or gene-environment interaction or G×E). This latter variance is usually deemed zero if there is no clear evidence of its existence. The superscript 2 does not indicate that these are squared variances but only squared deviations, that is, variances. Sometimes variances are represented by a capital V followed by a subscript identifying the type of variance (e.g., V_T or V_P as total or phenotypic variance, V_G as genetic variance, ...).

Likewise, the genetic variance can be decomposed into several subcomponents, such as additive genetic variance, genetic variance due to dominance, or due to epistasis.

When the phenotypic values and variances for two inbred or pure lines (L_1 and L_2) and F_2 are known, it is interesting to construct a graph with the mean phenotypic value on the abscissa and the variance on the ordinates. This graph can be very useful for obtaining the heritability values and variance components. If the trait behaves in a purely additive way, the phenotypic value for the means of F_1 and F_2 will be the same and coincide with the mean value between the two inbred lines. Since these are two pure lines, their genetic variances will be zero, and all the variances shown by these lines will be due to the environment.



Broad sense heritability (H^2) is calculated as the ratio of the genetic variance to the total variance. As in the case of variances, the superscript 2 does not indicate squared.

$$H^2 = \frac{V_G}{V_P} = \frac{\sigma^2_G}{\sigma^2_G + \sigma^2_E + \sigma^2_{GXE}}$$

Narrow sense heritability (h^2) is calculated as the ratio of the additive genetic variance to the total phenotypic variance:

$$h^2 = \frac{V_{G\ additive}}{V_P} = \frac{\sigma_A^2}{\sigma_G^2 + \sigma_E^2 + \sigma_{GXE}^2}$$

There are several methods for calculating heritability. One is by estimating the values of the genetic and environmental variances (for example, by creating a pure line and estimating in it the value of the phenotypic variance that will correspond to the value of the environmental variance, since pure lines have no genetic variance). Another is based on resemblance between relatives. A third method uses the estimation of various parameters during a process of artificial selection or breeding. In the latter case, it would be sufficient to obtain h^2 from the breeder equation ($\Delta Z = h^2 S$), where ΔZ refers to selection progress (or response to selection, the change in the mean over one complete generation) and S is the selection differential (the average difference between the parent generation and the selected parents):

$$h^2 = \frac{selection\ progress}{selection\ differential} = \frac{|x_1 - x_0|}{|x_s - x_0|}$$

where the values of x are the mean values for the trait in the initial generation before selection (x_0), the selected individuals (x_S), and the generation resulting from selection (x_1).

When the **number of genes contributing to a quantitative trait** is not very high, it is possible to obtain an approximation of this number. For this, it is necessary to know the phenotypic value of two pure lines that differ in the trait in question and the genetic variance. Thus, the minimum number of genes (n) in which two lines differ will be:

$$n = \frac{(L_1 - L_2)^2}{8\sigma_G^2}$$

where L_1 and L_2 are the phenotypic values of each line and σ^2_G is the variance of genetic origin. This estimate will always be a minimum estimate.

Suppose it is known that several bi-allelic loci (n) contribute in an additive and equal way to determine a trait. In this case, the **phenotypic value of the various genotypes** for these loci can be deduced. For each of these loci, there would be two alleles (A and a) contributing a particular value to the trait, such that the one represented by the capital letter would be the one with the highest contribution. Thus, the phenotypic value (PV) would be

$$PV = x\ M + (2n - x)m$$

where x is the number of alleles of maximum contribution, ($2n$- x) is the number of alleles of minor contribution, M is the contribution to the phenotype of the alleles of maximum contribution, and m is the contribution of the alleles of minimum contribution.

The distribution of phenotypes from the lowest phenotypic value (aa bb cc dd ee ...) to the highest phenotypic value (AA BB CC DD EE ...) in a progeny such as F_2 would follow a binomial of the form:

$$\begin{matrix} frequency\ of\ the\ class\ with \\ x\ aleles\ of\ maximum\ contribution \end{matrix} = \frac{2n!}{x!\ (2n - x)!}\ A^x a^{2n-x}$$

where A and a are the frequency of those alleles in the progeny. For the case of an F_2, these values are equivalent to ½

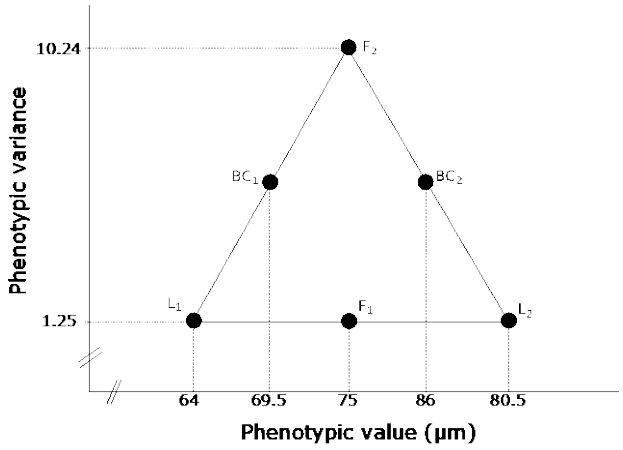
2. SOLVED PROBLEMS

Problem 1.

From a natural sample of *Drosophila suboscura*, two lines differing in wing length, estimated as the length of several of the veins that show the wings of these insects, were obtained using selection. In one of the lines, males had an average wing length of 64 µm; in the other line, wings were 86 µm long. The phenotypic variance observed in each line was 1.25 μm^2. When crossed, the phenotypic variance observed in the F_2 was 10.24 μm^2.

Assuming that wing length in males follows an entirely additive polygenic inheritance and that the two lines can be considered pure, (a) determine the phenotypic value for this trait in F_1, F_2 and (b) calculate the heritability. (c) Determine the phenotypic values of the backcrosses between F_1 and the parental lines.

To solve this problem, it is illustrative to represent the phenotypic values and variances in a graph where phenotypic value (as abscissas) and phenotypic variance (as ordinates) are plotted against each other:



If the trait behaves in an entirely additive way, then the phenotypic value for the means of F_1 and F_2 will be the same and will coincide with the mean value between the two pure lines. Thus, the mean phenotypic value will be 75 µm for both F_1 and F_2.

If two pure lines are involved, the genetic variance will be zero, and the variance shown by these lines will be due to the environment. In this problem, this variance is equal to 1.25 μm^2. In F_1, all individuals are genetically homogeneous and their genetic variance will also be zero. However, new genetic combinations are produced in the F_2, and this generation will show both genetic and environmental variance. The genetic variance in the F_2 will be the difference between the total variance of F_2 and the environmental variance (that shown by L_1, L_2, and F_1). In this case, the genetic variance is

$$V_G = V_{PF_2} - V_{PF_1} = 10.24 - 1.25 = 8.99\ \mu m^2$$

And if we know the genetic variance and the total variance (the phenotypic variance of F_2), then the heritability (H^2) is calculated as:

$$H^2 = \frac{V_G}{V_P} = \frac{8.99}{10.24} = 0.878$$

From the Figure, it is easy to determine the total variance and the phenotypic value of the progenies obtained in the backcrosses between the F_1 and the parental lines. If we call these progenies BC_1 (backcross L_1 x F_1) and BC_2 (backcross L_2 x F_1), the phenotypic value of BC_1 will lie at half the distance between the phenotypic values of L_1 and F_1, and similarly for BC_2. In addition, the phenotypic variance for these progenies will lie at half the distance between the variances of L_1 and F_2, as indicated in the Figure. Thus, the phenotypic value for BC_1 will be 69.5 µm and 80.5 µm for BC_2.

Results:

(a) Mean phenotypic value of F_1: 75 µm
Mean phenotypic value of F_2: 75 µm
(b) Heritability: 0.878
(c) Mean phenotypic value of BC_1: 69.5 µm
Mean phenotypic value of BC_2: 80.5 µm

Problem 2.

From the data in the previous problem, is it possible to determine the number of loci controlling the wing length trait in males?

It is possible to obtain a minimum estimate of this number. Thus, the number of loci contributing to this quantitative trait in which these lines of Drosophila differ can be calculated as

$$n = \frac{(L_1 - L_2)^2}{8\sigma_G^2}$$

where L_1 is the phenotypic value of line 1 (64 µm), L_2 is the phenotypic value of line 2 (86 µm), and where the genotypic variance is obtained as the difference between the variance in the F_1 (or those of pure lines) and the variance in the F_2. For the pure lines and F_1, we only find the environmental variance, while genetic and environmental variances contribute to the variance in the F_2; therefore, its difference is equivalent to the genetic variance.

$$n = \frac{(L_1 - L_2)^2}{8\sigma_G^2} = \frac{(64 - 86)^2}{8 \times (10.24 - 1.25)} = 6.73$$

After rounding, we can conclude that a minimum of 7 loci controls the trait.

Result: at least 7 loci.

Problem 3.

In a series of experiments with mice to modify plasma cholesterol concentrations, Weiburst (Genetics 73: 303-12; 1973) found that the heritability estimated using selection experiments was 0.42. Suppose in a population of mice with a cholesterol level of 2.16 mg ml^{-1}, those with the lowest cholesterol were selected as parents of the next generation (mean 2.00 mg ml^{-1}). What will be the average of this trait in the next generation?

This is a typical artificial selection problem. A set of individuals with favourable values of the trait of interest is selected as breeders from a population or group of individuals. From the problem, it is inferred that someone wants to make a selection in favour of cholesterol reduction, so individuals with low plasma cholesterol values were selected. From the heritability formula obtained from selection experiments

$$h^2 = \frac{selection\ progress}{selection\ differential} = \frac{|x_1 - x_0|}{|x_s - x_0|}$$

we can solve the selection progress

$$selection\ progress = h^2 \times\ selection\ differential = h^2 \times\ |x_s - x_0|$$

For this specific problem, it shall be the case that $h^2 = 0.42$, the mean of the selected individuals is $x_s = 2.00$ mg ml^{-1} and the mean of the original population is $x_0 = 2.16$ mg ml^{-1}.

$$selection\ progress = h^2 \times\ selection\ differential = 0.42 \times\ |2.00 - 2.16| = 0.0672$$

which would correspond to the selection progress. In this case, we are dealing with a reduction in cholesterol levels, and therefore the value in the next generation will be

$$PV_{x_1} = x_0 - selection\ progress = 2.16 - 0.0672 = 2.09$$

Result:

Mean plasma cholesterol in the next generation: 2.09 mg ml^{-1}.

Problem 4.

Two pure lines of maize showing mean heights of 120 and 180 cm have been obtained. Assume that this trait is controlled by five genes that contribute equally and additively, that the line with the lowest height shows genotype aa bb cc dd ee, and that the one with the highest height shows genotype AA BB CC DD EE. When crossing both lines, what phenotypic value will the F_1 have? What will the phenotypic distribution be like in F_2?

We can actually calculate what the average phenotypic value (PV) of the various genotypes will be since the environment will also modify the phenotypic value of each particular individual. If the inbred lines have PVs of 120 and 180 cm, we can calculate how much each allele contributes to the value of the trait:

Contribution of the "lowercase" or minimum contribution alleles — In the case of the aa bb cc dd ee line (120 cm), each lowercase allele will contribute 120 cm/10 alleles= 12 cm.

Contribution of the "uppercase" or maximum contribution alleles — In the case of the AA BB CC DD EE line (180 cm), each uppercase allele will contribute 180 cm/10 alleles = 18 cm.

The F_1 will be of genotype Aa Bb Cc Dd Ee; therefore, its PV will be 5 × 12 cm + 2 × 18 cm = 150 cm, corresponding to the mean phenotypic value between the two pure lines.

F_2 will also have a mean PV of 150 cm, but we are asked to calculate the distribution of phenotypes and not just the mean value. To do this, it is appropriate to think that in F_2 there will be a distribution of phenotypic classes that differ in the presence of uppercase alleles. Thus we will have classes of 0 capital alleles, 1 capital allele, 2 capital alleles.... until we reach the class of 10 capital alleles. The development of the binomial gives the frequency of these classes:

$$\begin{matrix} frequency\ of\ the\ class\ with \\ x\ aleles\ of\ maximum\ contribution \end{matrix} = \frac{2n!}{x!\,(2n-x)!}\,A^x a^{2n-x}$$

where *n* is the number of loci, *x* represents the number of uppercase alleles, and *A* and *a* are the probability of finding the uppercase or lowercase allele in a genotype. These probabilities (*A* and a) have a value of ½ in the case of an F_2.

	Frequency	Phenotypic value (cm)
With 0 alleles of maximum contribution (aa bb cc dd ee)	$\frac{1}{1024}$	120
With 1 alleles of maximum contribution	$\frac{10}{1024}$	126
With 2 alleles of maximum contribution	$\frac{45}{1024}$	132
With 3 alleles of maximum contribution	$\frac{120}{1024}$	138
With 4 alleles of maximum contribution	$\frac{210}{1024}$	144
With 5 alleles of maximum contribution	$\frac{252}{1024}$	150
With 6 alleles of maximum contribution	$\frac{210}{1024}$	156
With 7 alleles of maximum contribution	$\frac{120}{1024}$	162
With 7 alleles of maximum contribution	$\frac{45}{1024}$	168
With 7 alleles of maximum contribution	$\frac{10}{1024}$	174
With 10 alelles of máximum contribution (AA BB CC DD EE)	$\frac{1}{1024}$	180

Problem 5.

From a population of maize in which the mean ear length was 18 cm and the phenotypic variance for this trait was 5 cm, a pure line was obtained with ears of 15 cm (mean value) and a variance of 2 cm. Calculate the heritability for ear length.

The pure lines show no variation of genetic origin, implying that all the phenotypic variation they show is of environmental origin. Thus, the pure line of the problem shows a total phenotypic variance equal to the environmental variance (V_E= 2 cm).

However, the original population shows a total phenotypic variance (V_T) that is the sum of the genetic variance and the environmental variance: $V_T = V_G + V_E = 5$ cm.

From this expression, the value of the genetic variance can be obtained as $V_G = 5$ cm - V_E , being V_E = 2cm, and therefore the genetic variance V_G = 3 cm.

Knowing both variances (genetic and environmental), obtaining the value of heritability is straightforward.

$$H^2 = \frac{V_G}{V_T} = \frac{3\ cm}{3\ cm + 2\ cm} = 0.6$$

3. PROBLEMS TO BE SOLVED

Problem 1.

After obtaining two pure lines of a given insect, they were crossed, and F_1 and F_2 were produced. The following results were obtained for the wing length trait:

	Mean wing length in mm	Phenotypic variance in mm^2
Line 1	4.90	0.08
Line 2	10.71	0.07
F_1	8.82	0.10
F_2	9.13	1.16

Assuming that there are no dominance or epistatic effects in this trait and that all individuals were grown under the same laboratory conditions:

(a) At how many loci involved in this trait do lines 1 and 2 differ?

(b) How many loci control the expression of the wing length trait in this insect?

Problem 2.

If three independently segregating loci with two alleles each (A/a, B/b, C/c), determine the height in a plant to the extent that the presence of the alleles represented by the capital letters adds 2 cm to the base height of 2 cm.

a) Give the height expected in the F_1 from a cross between the homozygous strains: AABBCC (14 cm) x aabbcc (2 cm).

b) Give the distribution of heights (phenotypes and frequencies) expected in an F_1 x F_1 cross.

(c) What proportion of this F_2 will have the same height as the parental strains?

d) What proportion of the F_2 being homozygous would have the same height as the F_1.

Problem 3.

Suppose that the difference between a barley strain that produces 4 g per plant and one that produces 10 g is due to three equal and cumulatively acting multiple factors,

AABBCC. Cross one strain with the other.

a) How much will the F_1 phenotypes produce?

b) How much will the F_2 phenotypes produce?

Problem 4.

Suppose that, in pumpkins, the difference in fruit weight between a 1350 g and a 2700 g type is due to three genes A/a, B/b and C/c, where each dominant allele contributes 225 g of fruit weight. Cross a 1350 g plant (aabbcc) with a 2700 g plant.

a) What will the F_1 phenotypes weigh?

b) What about the F_2 phenotypes?

Problem 5.

Two maize inbred lines (AABBCCDDEE and aabbccddee) that differ at five independent loci for ear length have a phenotypic value of 25 cm and 15 cm, respectively.

An F_2 of 512 plants is obtained, and it is desired to know how many of them will have ears of 19 cm.

Problem 6.

A particular breed of rabbits has an average body weight of 3,600 g. Another breed has an average of 1,875 g. Mating between these two breeds produces an intermediate F_1 with a standard deviation of 162 g. The variability of the F_2 is higher, as its standard deviation is 230 g.

Calculate the number of genes contributing to the body weight of the adult rabbits.

Problem 7.

From a population of *Drosophila melanogaster* that had variable wing length with mean $x_0 = 3.415$ and variance $V_{p0} = 0.650$, a pure line was obtained by inbreeding that had mean $x = 3.180$ and variance $V_p = 0.403$ for this trait.

Calculate the heritability for this wing length trait in this population.

Problem 8.

In a strain of *Drosophila melanogaster* showing a variable number of extra chaetae, selection was made to increase this number, choosing as parents from the initial strain G_0 to form the following generation G_1, those individuals that presented a higher number

of kinetochores, until completing 25 % of the individuals of this strain. The observed data in both generations are shown below:

	Number of extra chaetae															Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
G_0			2	9	12	21	34	30	17	13	4	2				144
G_1					3	11	12	15	27	16	10	5	2			101

a) Calculate the heritability for the trait "number of extra chaetae" in this generation.

b) Would the heritability for this trait vary in successive generations of selection? Please give reasons for the answer.

Problem 9.

In a particular population of pigs, it has been estimated that fat thickness has a heritability of 80%. The average fat thickness of this population is 1.2 inches, and the average of the individuals selected from this population as parents of the next generation is 0.8 inches.

What is the expected average in the next generation?

Problem 10.

In a large population of hens, the mean weight is 2.98 kg with a variance of 1.278. From these hens, we have selected a pure line for weight which shows a mean weight of 3.57 kg with a variance of 0.813. To obtain the next generation, we select only the individuals of highest weight of the two populations, with the population selected from the large population having a mean of 4.45 kg and the population of hens selected from the pure line having a mean of 4.57 kg. Determine the average weight of the offspring obtained in the selected large population and the selected pure line.

4. SOLUTIONS TO PROBLEMS

Problem 1.

a) 4 loci.

b) At least 4 loci.

Problem 2.

a) 8 cm.

b)

	Frequency	Phenotypic value (cm)
With 0 alleles of maximum	1/64	2
With 1 alleles of maximum	6/64	4
With 2 alleles of maximum	15/64	6
With 3 alleles of maximum	20/64	8

With 4 alleles of maximum	15/64	10
With 5 alleles of maximum	6/64	12
With 6 alleles of maximum	1/64	14

c) 2/64

d) Zero.

Problem 3.

a) F_1 is genetically homogeneous and with a mean value of 7 g.

b) In the F_2 there are 7 phenotypic classes (if the environmental variance is very low):

	Frequency	Phenotypic value (g)
With 0 alleles of maximum	1/64	4
With 1 alleles of maximum	6/64	5
With 2 alleles of maximum	15/64	6
With 3 alleles of maximum	20/64	7
With 4 alleles of maximum	15/64	8
With 5 alleles of maximum	6/64	9
With 6 alleles of maximum	1/64	10

Problem 4.

a) F_1: AaBbCc 2025 g.

b)

	Frequency	Phenotypic value (g)
With 0 alleles of maximum	1/64	1350
With 1 alleles of maximum	6/64	1575
With 2 alleles of maximum	15/64	1800
With 3 alleles of maximum	20/64	2025
With 4 alleles of maximum	15/64	2250
With 5 alleles of maximum	6/64	2475
With 6 alleles of maximum	1/64	2700

Problem 5.

Approximately 105 individuals.

Problem 6.

14 loci.

Problem 7.

$H^2 = 0.38$

Problem 8.

a) $h^2 = 0.585$

b) As the selection process progresses, the genetic variance becomes smaller and smaller, and therefore, the heritability will also be smaller and smaller.

Problem 9.

The expected average in the next generation is 0.88 inches.

Problem 10.

The average weight of the offspring of the selected individuals will be 3.51 kg.

POPULATION GENETICS

1. GUIDE TO SOLVING POPULATION GENETICS PROBLEMS

Population structure

The calculation of genotypic frequencies and allelic frequencies for a locus with two alleles (A_1 and A_2, for example) in a given population is performed as follows:

Genotypic frequencies:

To calculate the frequency of each genotype in the population, the number of individuals presenting a given genotype is divided by the total number of individuals in that population. Thus, if D is the frequency of homozygotes for one allele (A_1, for example), R the frequency of homozygotes for the other allele (A_2) and H the frequency of heterozygotes, it shall be the case that D+H+R=1.
This calculation is simpler if it is a locus with codominant alleles since the number of individuals belonging to each phenotypic class coincides with that of each genotypic class. In the case of a locus with dominance of one allele over another, if we do not have any instrument to differentiatedifferent individuals with dominant phenotype (that is, those that are homozygous for the dominant allele versus those that are heterozygous), it is impossible to determine the genotypic frequencies (and allelic frequencies) unless the population is in Hardy-Weinberg equilibrium (as we will see further on).

Allelic frequencies:

To calculate the frequency of each allele in the population, we will have to divide the number of copies of each allele (A_1 and A_2) by the total number of copies of all existing alleles in the population for the locus considered.
Thus, if p is the frequency of the A_1 allele and q is the frequency of the A_2 allele:
p = 2D + H/2D+2H+2R = 2 (D + ½ H)/2(D + H + R); p = D + ½ H
q = 2R + H/2D+2H+2R = 2 (R + ½ H)/2(D + H + R); q = R + ½ H
[Recall that D + H + R = 1]

Hardy-Weinberg equilibrium:

In a panmictic population (i.e., where individuals mate randomly), large in size and in the absence of evolutionary forces (mutation, migration, drift, natural selection), the process of inheritance, by itself, does not change the allelic frequencies or genotypic frequencies of a given locus. Moreover, equilibrium genotypic frequencies are achieved in a single generation of random mating and the allelic frequencies of the next generation will be the same as those of the parental generation. In such an equilibrium situation, the genotypic frequencies are given by the square of the sum of the allelic frequencies [$(p + q)^2 = p^2 + 2pq + q^2$].

In equilibrium:

p^2 = frequency of A_1A_1 individuals
$2pq$ = frequency of A_1A_2 individuals
q^2 = frequency of A_2A_2 individuals

Estimation of equilibrium frequencies in natural populations.

Autosomal genes, codominance: all genotypes can be phenotypically distinguished. Therefore, we calculate the genotypic frequencies observed in the population and, from

them, we calculate p and q (allelic frequencies). The calculation of allelic frequencies allows us to estimate what the expected genotypic frequencies would be in an equilibrium situation (p^2, 2pq, q^2). If the observed frequencies coincide with the expected frequencies, we can assume that the population is in Hardy-Weinberg equilibrium. If there are differences between observed and expected values, we test the significance of the differences using a χ^2 test with the null hypothesis being the existence of Hardy-Weinberg equilibrium:

$$\chi^2_{\exp} = \sum \frac{(Observados - Esperados)^2}{Esperados}$$

To evaluate Hardy-Weinberg equilibrium, the number of degrees of freedom is not equal to the number of phenotypic classes minus one, but to the number of phenotypic classes minus the number of alleles. This is because, knowing the frequency of an allele (or the frequency of a phenotype or genotype) and the total number of individuals, the frequencies of all genotypes can be known, since the expected values are based on the observed allelic frequencies.

Autosomal genes, dominance: The heterozygous genotype (Aa) cannot be phenotypically distinguished from the homozygous dominant (AA). Therefore, allelic frequencies cannot be obtained directly, since two of the genotypic frequencies are unknown.

However, we could calculate the frequency q as the square root of q^2, which would be the frequency of the recessive homozygotes. Of course, this can be done only in the case where the population is in Hardy-Weinberg equilibrium.
In this case:

q = frequency of allele a = $\sqrt{q^2}$

And, therefore, p (frequency of allele A) would be: 1 - q.

From these calculations, we could now determine the genotypic frequencies in the equilibrium (p^2, 2pq, q^2)

Sex-linked genes: In sex-linked genes, the equilibrium genotypic frequencies for individuals of the homogametic sex (generally females) match those of the autosomal genes, since they receive one allele from each of their parents. In contrast, the heterogametic sex (males) receive their single X chromosome from their mother, so the two genotypic frequencies in this sex coincide with the respective allelic frequencies in the females of the preceding generation, p and q.

p = frequency of allele A
q = frequency of allele a

Therefore:

Frequency of males with dominant phenotype (hemizygous for the dominant allele): p
Frequency of males with recessive phenotype (hemizygous for the recessive allele): q
Frequency of females with homozygous dominant genotype: p^2
Frequency of heterozygous females: 2pq
Frequency of females with homozygous recessive genotype: q^2

For male genotypes, these frequencies are the proportions among all males; for female genotypes, these frequencies are the proportions among all females.

Mutation

To calculate the frequency at which an A allele is found after a given number of generations by mutation from A → a, the formula to be applied is:

$p_t = p_0 (1 - u)t$

Where p_t is the frequency of allele A after t generations, p_0 is the initial frequency of A in the population and u is the mutation rate.

If there is a direct mutation rate u (A → a) and a reverse mutation rate v (a → A), the equilibrium frequencies of the two alleles A and a of a locus, are estimated as follows:

$p_e = v / (u + v)$
$q_e = u / (u + v)$

Migration

If we call m the proportion of individuals passing from one population (population I) to another (population II) in each generation, 1 - m will be the proportion of initial individuals in the receiving population (population II). If we call P the frequency of a given allele in population I and p0 its frequency in population II, it shall be the case that in population II the frequency of the allele after a migration event, in the absence of other factors, will be p1:

$p_1 = (1 - m)p_0 + mP = p_0 - m (p_0 - P)$

The following expression allows us to calculate the number of generations (t) required for a given change in gene frequencies due to migration:

$$(1 - m)^t = (p_t - P) / (p_0 - P).$$

From this we can clear pt in order to estimate the frequency of a given allele after t generations.

Selection

Fitness (w) is a measure of the reproductive efficiency of a genotype. For convenience, w = 1 for the genotype with the highest reproductive efficiency, while the efficiencies of the other genotypes will be given relative to the first genotype. Thus, one way to estimate the biological efficiencies of each genotype is to divide the mean number of offspring of each genotype by the mean number of offspring produced by the genotype with the highest progeny.

The selection coefficient (s) is calculated as 1 - w:

After one generation of selection, in a sufficiently large population and with random mating, if we apply the general model of natural selection, it shall be the case that the expected relative genotypic frequencies will be:

Frequency of genotype AA: $p^2 w_{AA} / \overline{W}$
Frequency of genotype Aa: $2pq w_{Aa} / \overline{W}$
Frequency of genotype aa: $q^2 w_{aa} / \overline{W}$

Where $\overline{W}$ (average fitness of the three genotypes) = $p^2 w_{AA} + 2pq w_{Aa} + q^2 w_{aa}$

And, therefore,

$p' = p^2w_{AA} + 1/2\ 2pqw_{Aa}$
$q' = q^2w_{aa} + 1/2\ 2pqw_{Aa}$

2. SOLVED PROBLEMS

Problem 1. In a human population of 1200 individuals, analysis for the MN system blood group revealed the existence of 365 M individuals, 556 MN individuals and 279 N individuals. What are the genotypic and allelic frequencies in this population for the MN locus?

Answer

The blood group of the MN system is determined by the presence of antigens of two types encoded by two codominant alleles of one gene, L^M and L^N:

M individuals are $L^M\ L^M$ homozygotes.
MN individuals are $L^M\ L^N$ heterozygotes.
N individuals are $L^N\ L^N$ homozygous.

Since there is codominance, the phenotypic classes and their frequencies coincide with the genotypic ones, so to calculate the genotypic frequencies we only have to divide the number of individuals that have each phenotype by the total number of individuals in the population:

Genotypic frequency = number of individuals with the genotype/total number of individuals in the population.

D = Frequency of the $L^M\ L^M$ genotype = 365/1200 = 0.304
H = Frequency of the $L^M\ L^N$ genotype = 556/1200 = 0.463
R = Frequency of the $L^N\ L^N$ genotype = 279/1200 = 0.233

Such that: D + H + R = 1.

To calculate the allelic frequencies, we will divide the number of copies of each allele (L^M and L^N) by the total number of copies of all the alleles existing in the population for this locus.

Thus, if p is the frequency of the L^M allele and q is the frequency of the L^N allele:

p = 2D + H/2D+2H+2R = 2 (D + 1/2 H)/2(D + H + R) = D + 1/2 H
q = 2R + H/2D+2H+2R = 2 (R + 1/2 H)/2(D + H + R) = R + 1/2 H

[Recall that D + H + R = 1]

That is:

p = 0.304 + 1/2 0.463 = 0.5355
q = 0.233 + 1/2 0.463 = 0.4645

[Remember that p + q = 1, and that q = 1 - p].

Problem 2. In a population, for locus A, there are 200 A_1A_1 individuals, 522 A_2A_2 individuals and 678 A_1A_2 individuals. Is this population in Hardy-Weinberg equilibrium?

Test the hypothesis using the χ2 statistical test.

Answer

The observed genotypic frequencies are [N = total number of individuals = 1400]:

D = frequency of A_1A_1 individuals = 200/1400 = 0.143
H = frequency of A_1A_2 individuals = 678/1400 = 0.484
R = frequency of A_2A_2 individuals = 522/1400 = 0.373

Then:

p = frequency of the A_1 allele = D + 1/2 H = 0.143 + 1/2 0.484 = 0.385
q = frequency of the A_2 allele = R + 1/2 H = 0.373 + 1/2 0.484 = 0.615

In equilibrium, the expected genotypic frequencies would be:

p^2 = frequency of A_1A_1 individuals = $(0.385)^2$ = 0.148
2pq = frequency of A_1A_2 individuals = 2 (0.385) (0.615) = 0.474
q^2 = frequency of individuals A_2A_2 = $(0.615)^2$ = 0.378

[Recall that $p^2 + 2pq + q^2 = 1$].

In absolute values, the expected number of individuals would be:

Frequency of A_1A_1 individuals = 0.148 x 1400 = 207
Frequency of A_1A_2 individuals = 0.474 x 1400 = 664
Frequency of A_2A_2 individuals = 0.378 x 1400 = 529

The observed and expected frequencies are similar, so it could be assumed that the population is in equilibrium, something that we are going to check by means of the χ2 statistical test:

$$\chi^2_{exp} = \sum \frac{(Observados - Esperados)^2}{Esperados} = \frac{(200-207)^2}{207} + \frac{(678-664)^2}{664} + \frac{(522-529)^2}{529} =$$

= 0.237 + 0.295 + 0.093 = 0.625

To evaluate Hardy-Weinberg equilibrium, the number of degrees of freedom is not equal to the number of phenotypic classes minus one, but to the number of phenotypic classes minus the number of alleles. This is because, knowing the frequency of an allele (or the frequency of a phenotype or genotype) and the total number of individuals, the frequencies of all genotypes can be known, since the expected values are based on the observed allelic frequencies.

Therefore, the theoretical χ2 with which this experimental χ2 must be compared is the one corresponding to 1 degree of freedom (3 phenotypic classes - 2 alleles). The value of the theoretical χ2 for a significance level of 0.05 is 3.84:

DISTRIBUCION DE χ^2

Grados de libertad	Probabilidad										
	0,95	0,90	0,80	0,70	0,50	0,30	0,20	0,10	0,05	0,01	0,001
1	0,004	0,02	0,06	0,15	0,46	1,07	1,64	2,71	3,84	6,64	10,83
2	0,10	0,21	0,45	0,71	1,39	2,41	3,22	4,60	5,99	9,21	13,82
3	0,35	0,58	1,01	1,42	2,37	3,66	4,64	6,25	7,82	11,34	16,27
4	0,71	1,06	1,65	2,20	3,36	4,88	5,99	7,78	9,49	13,28	18,47
5	1,14	1,61	2,34	3,00	4,35	6,06	7,29	9,24	11,07	15,09	20,52
6	1,63	2,20	3,07	3,83	5,35	7,23	8,56	10,64	12,59	16,81	22,46
7	2,17	2,83	3,82	4,67	6,35	8,38	9,80	12,02	14,07	18,48	24,32
8	2,73	3,49	4,59	5.53	7,34	9,52	11,03	13,36	15,51	20,09	26,12
9	3,32	4,17	5,38	6,39	8,34	10,66	12,24	14,68	16,92	21,67	27,88
10	3,94	4,86	6,18	7,27	9,34	11,78	13,44	15,99	18,31	23,21	29,59
	No significativo								Significativo		

Since the experimental χ^2 is lower than the theoretical χ^2, we do not reject the equilibrium hypothesis and assume that the observed values conform to those expected in equilibrium ($0.3 < p < 0.5$).

Problem 3. In a panmictic population composed of 1000 individuals, the frequencies of the genotypes for the autosomal locus A are the following: 90 individuals A_1A_1, 100 A_1A_2 and 810 A_2A_2. Determine whether the population is in Hardy-Weinberg equilibrium, testing the hypothesis usingthe χ^2 test.

Answer

The observed genotypic frequencies are:

D = frequency of A_1A_1 individuals = 90/1000 = 0.09
H = frequency of A_1A_2 individuals = 100/1000 = 0.10
R = frequency of A_2A_2 individuals = 810/1000 = 0.81

Given these genotypic frequencies, the allelic frequencies would be:

p= allelic frequency of the A_1 allele= D + 1/2H = 0.09 + 1/20.10 = 0.14
q= allelic frequency of the A_2 allele= R + 1/2H = 0.81 + 1/20.10 = 0.86

In equilibrium, the expected genotypic frequencies would be:

p^2= frequency of A_1A_1 individuals = $(0.14)^2$= 0.02
2pq= frequency of A_1A_2 individuals = 2 (0.14) (0.86) = 0.24
q^2= frequency of A_2A_2 individuals = $(0.86)^2$= 0.74

The expected values would be:

Frequency of A_1A_1 individuals = 0.02 x 1000 = 20
Frequency of A_1A_2 individuals = 0.24 x 1000 = 240
Frequency of A_2A_2 individuals = 0.74 x 1000 = 740
The observed and expected values are very different so it could be assumed that the population is not in equilibrium, something that we are going to check by means of the χ^2 statistical test:

$$\chi^2_{exp} = \sum \frac{(Observados - Esperados)^2}{Esperados} = \frac{(90-20)^2}{20} + \frac{(100-240)^2}{240} + \frac{(810-740)^2}{740} =$$

= 245 + 81.66 + 6.62 = 333.28

The value of the theoretical χ^2 for a significance level of 0.05 is 3.84. Since the experimental χ^2 is much larger than the χ^2 theoretical, we reject the equilibrium hypothesis.

Problem 4. In a human population in Hardy-Weinberg equilibrium, the frequency of individuals affected by an autosomal recessive disease is 4 per 10000 individuals. What would be the allelic and the genotypic frequencies in this population?

Answer

There is no codominance at this locus. Instead, from the statement, we can conclude that there is a phenotype caused by homozygosity of a recessive allele while the heterozygous genotype (Aa) cannot be phenotypically distinguished from the homozygous dominant (AA). Therefore, allelic frequencies cannot be obtained directly, since two of the genotypic frequencies are unknown. However, we could calculate the frequency q as the square root of q^2, which would be the frequency of the recessive homozygotes. This can be done only in the case that the population is in Hardy-Weinberg equilibrium, as happens in this population.

The frequency of sick people is 4 per 10000 inhabitants. Thus:

q^2= frequency of recessive homozygotes = 4/10000 = 0.0004;
q= frequency of the allele a = 0.02 ($\sqrt{0.0004}$)

And, therefore, p (frequency of the A allele) would be: 1 - q = 1 - 0.02 = 0.98

With these allelic frequencies, the equilibrium genotypic frequencies would be:

p^2 = frequency of AA = 0.9604
2pq = frequency of Aa = 0.0392
q^2 = frequency of aa = 0.0004

Problem 5. In a human population the frequency of a disease resulting from an autosomal recessive allele is 4%. Assuming the population is in equilibrium, determine the probability that a healthy couple will have a sick child.

Answer

As in the previous problem, since we assume that the population is in equilibrium, we know the frequency of the homozygous recessive individuals (q^2), which allows us to determine the values of p and q:

q^2= 0.04
q= frequency of the allele a = $\sqrt{0.04}$ = 0.2

And, therefore, p (frequency of the allele A) would be: 1 - q= 1 - 0.2 = 0.8

With these allelic frequencies, the equilibrium genotypic frequencies would be:

p^2 = frequency of AA = 0.64
2pq = frequency of Aa = 0.32
q^2 = frequency of aa = 0.04

Since what we are asked is the probability that a healthy couple has a sick child and for that to happen both members of the couple must be heterozygous, we have to determine first the frequency of heterozygotes among the total number of healthy individuals:

Frequency of heterozygotes among healthy individuals = $2pq/2pq+p^2$= 0.32/0.32+0.64 = 0.33

Being both parents heterozygous, the probability of having a sick child would be 1/4.

So, the probability of having a sick child for that couple would be:

P = P(Aa) x P(Aa) x P(Aa x Aa → aa) = 0.33 x 0.33 x 0.25 = 0.027.

Therefore, the probability would be 2.7%.

Problem 6. A human population is sampled and 36 out of 10,000 women are found to be colour blind (X^dX^d). Knowing that the population is in equilibrium:

(a) Calculate the frequency of healthy and colour-blind males and the frequency of healthy homozygous female and healthy female carriers.
b) How many colour-blind males should be expected for each female with the disease?

Answer

a) For sex-linked genes, the equilibrium genotypic frequencies for individuals of the homogametic sex (in this case, females) match those of autosomal genes, since they receive one allele from each of their parents. In contrast, the heterogametic sex (in human, males) receive their single X chromosome from their mother, so the two genotypic frequencies in this sex match the respective allelic frequencies in the females of the preceding generation, p and q.

Thus, if the colour-blind frequency is 36/10000:

q^2 = 0.0036
q = frequency of allele d = $\sqrt{0.0036}$ = 0.06
p = frequency of allele D = 1 - q = 0.94

And, therefore:

Healthy males = p = 0.94 (94% of males)
Colour-blind males = q = 0.06 (6% of males)

Homozygous healthy females= p^2= $(0.94)^2$ = 0.8836 (88.36% of females)
Healthy female carriers = 2pq = 2(0.94)(0.06) = 0.1128 (11.28% of females)
Colour-blind females= q^2=0.0036 (0.36% of females)

b) Colour-blind men/colour-blind women = 0.06/0.0036 = 16.66

Problem 7. At a certain locus the mutation rate of A → a is 10-6, the rate of backmutation being negligible. What will be the frequency of A after 10, 100 and 100,000 generations of mutation, if we start from an initial frequency of 0.5?

Answer

If p0 is the initial frequency of an A allele that mutates to the a allele with a frequency u per generation, in the next generation the frequency of A (p_1) will be:

$p_1 = p_0 - up_0 = p_0 (1 - u)$

In one more generation, the new frequency of A will be:

$p_2 = p_1 - up_1 = p_1 (1 - u) = p_0 (1 - u) (1 - u) = p_0 (1 - u)^2$

In t generations,

$p_t = p_0 (1 - u)^t$

Therefore, in the problem at hand, given that p0 = 0.5 and u = 10-6, after 10 generations, the frequency of A will be:

$p_{10} = 0.5 (1 - 10\text{-}6)^{10} = 0.499995$

In 100 generations:

$p_{100} = 0.5 (1 - 10\text{-}6)^{100} = 0.4999995$

In 100,000 generations:

$p_{100,000} = 0.5 (1 - 10\text{-}6)^{100,000} = 0.4524$

Problem 8. Assuming that the direct and reverse mutation rates at a certain locus in Drosophila melanogaster are:

$A \rightarrow a$: 2×10^{-5}
$a \rightarrow A$: 6×10^{-7}

What are the expected equilibrium allele frequencies if no other process intervenes?

Answer

If the mutation rate $A \rightarrow a$ is u and the mutation rate $a \rightarrow A$ is v, and the initial frequencies of A and a being, p0 and q0, respectively, after one generation of mutation the frequency of A will be:

$p_1 = p_0 - up_0 + vq_0$

If we represent by Δ_p the change in the frequency of A:

$\Delta_p = p_1 - p_0 = p_0 - up_0 + vq_0 - p_0 = vq_0 - up_0$

At equilibrium, there should be no change in allele frequencies; therefore, $\Delta_p = 0$. Calling p_e and q_e the equilibrium allele frequencies and knowing that $p_e + q_e = 1$,

$\Delta p_e = vq_e - up_e = 0.$

Thus: $up_e = vq_e$

And, therefore: $up_e = v(1 - p_e) = v - vp_e$; $p_e = v / (u + v)$

In the same way: $vq_e = u(1 - q_e) = u - uq_e$; $q_e = u / (u + v)$

In our problem,

$p_e = 6 \times 10^{-7} / 2 \times 10^{-5} + 6 \times 10^{-7} = 0.029$

$q_e = 2 \times 10^{-5} / 2 \times 10^{-5} + 6 \times 10^{-7} = 0.971$

Problem 9. In a population that maintains its size constant over generations, the frequency of an allele at an autosomal locus at a given time is 0.4. The rate of migration into that population from a neighbouring population where the frequency of that allele is 0.6 is 20%.

a) What will be the frequency of the allele in question one generation later?
b) What will it be after 5 generations?
c) Will there ever come a time when, under these conditions, the gene frequencies will not change?

Answer

If we call m the proportion of individuals passing from one population (population I) to another (population II) at a given time, 1 - m will be the proportion of initial individuals in the resident population (population II). If we call P the frequency of a given allele in population I and p0 its frequency in population II, it shall be the case that in population II the frequency of the allele after a migration event, in the absence of other factors, will be p1:

$p_1 = (1 - m)p_0 + mP = p_0 - m(p_0 - P)$

The change in allele frequencies due to migration (Δ_p) will be:

$\Delta_p = m(p_0 - P)$

[So when p_0 and P are equalized, there will be no more changes in population II even if migration continues].

The difference between the allele frequencies in both populations will be:

$p_1 - P = p_0 - m(p_0 - P) - P = p_0 - mp_0 + mP - P = (1 - m)p_0 - (1 - m)P = (1 - m)(p_0 - P)$

In the same way, after 2 generations of migration:

$p_2 - P = (1 - m)^2 (p_0 - P)$.

Therefore, the difference between the gene frequencies of the two populations after t generations of migration will be given by the formula:

$p_t - P = (1 - m)^t (p_0 - P)$

from where:

$(1 - m)^t = (p_t - P) / (p_0 - P)$.

This expression allows us to calculate the number of generations necessary for a given change in gene frequencies due to migration.

In the problem at hand:

(a) $p_1 = p_0 - m\ (p_0 - P) = 0.4 - 0.2\ (0.4 - 0.6) = 0.44$

b) $(1 - m)^t = (p_t - P) / (p_0 - P) \rightarrow (1 - 0.2)^5 = (p^5 - 0.6) / (0.4 - 0.6) \rightarrow p^5 = 0.534$

c) When allele frequencies are equalized, once the frequency of the allele in the recipient population rises to 0.6 (value of its frequency in the donor population), although there is still gene flow, this frequency will not be modified ($\Delta_p = 0$; $p_0 = P$):

$p_t = p_t\text{-}1 - m\ (p_{t-1} - P)$

If $p_t\text{-}1 = P$, then $p_t = p_{t-1}$

Problem 10. In a large, randomly mated population of insects, the progeny produced on average by individuals belonging to each of the three possible genotypic classes for a locus with two alleles (A and a) have been analysed and the following results have been obtained:

Genotype Average offspring

AA 150
Aa 120
aa 75

a) What is the value of the fitness in each case?
b) What is the value of the selection coefficient for each of the genotypes?
c) Being the frequency p of allele A equal to 0.6, what will be its frequency in the next generation?

Answer

a) Fitness (w) is a measure of the reproductive efficiency of a genotype. For convenience, w = 1 for the genotype with the highest reproductive efficiency, while the efficiencies of the other genotypes will be given relative to the first. Thus, one way to estimate the biological efficiencies of these three genotypes is by dividing the mean number of offspring of each genotype by the mean number of offspring produced by the genotype with the highest progeny:

Fitness of AA (w_{AA}): 150/150 = 1
Fitness of Aa (w_{Aa}): 120/150 = 0.8
Fitness of aa (w_{aa}): 75/150 = 0.5

b) The selection coefficient (s) is calculated as 1 - w:

Selection coefficient against the genotype AA: s = 1 - 1 = 0
Selection coefficient against genotype Aa: s = 1 - 0.8 = 0.2
Selection coefficient against genotype aa: s = 1 - 0.5 = 0.5

c) After one generation of selection, applying the general model of natural selection, it shall be the case that the expected relative genotypic frequencies will be:

Frequency of genotype AA: $p^2 w_{AA} / \overline{W}$

Frequency of the genotype Aa: $2pqw_{Aa}/\overline{W}$

Frequency of genotype aa: $q^2w_{aa}/\overline{W}$

Where $\overline{W}$ (average fitness of the three genotypes) = $p^2w_{AA} + 2pqw_{Aa} + q^2w_{aa}$ = (0.6)2(1) + 2(0.6)(0.4)(0.8) + (0.4)2(0.5) = 0.36 + 0.384 + 0.08 = 0.824

[Given that p = 0.6 and q = 0.4]

Thus:

Frequency of genotype AA: $p^2w_{AA}/\overline{W}$ = 0.36/0.824 = 0.437

Frequency of genotype Aa: $2pqw_{Aa}/\overline{W}$ = 0.384/0.824 = 0.466

Frequency of genotype aa: $q^2w_{aa}/\overline{W}$ = 0.08/=0.824 = 0.097

And, therefore,

p = 0.437 + 1/2 0.466 = 0.67
q= 0.097 + 1/2 0.466 = 0.33

3. PROBLEMS TO SOLVE

Problem 1. A population is initiated with the following genotypic frequencies: 0.24 AA, 0.32 Aa, 0.44 aa. In a Hardy-Weinberg equilibrium situation (random mating, very large population size, and no evolutionary forces), what will the genotypic frequencies be in the next generation?

Problem 2. In a sample of 1,000 people, 326, 470, and 204 individuals were found to have blood groups M, MN, and N, respectively.
(a) Calculate the allele frequencies and the Hardy-Weinberg expected genotypic frequencies.
b) Use the χ^2 test to determine whether the expected and observed quantities are in statistical agreement.

Problem 3. There are two allelic variants, A and a, of the gene causing an inherited disease in humans, such that aa individuals have the disease, while Aa heterozygotes have mild symptoms of the disease, with AA being normal. In a population of 10,000 individuals, 4 showed full manifestation of the disease and 400 showed mild symptoms. Determine whether or not the population is in Hardy-Weinberg equilibrium for the locus causing this disease.

Problem 4. What is the frequency of Aa heterozygotes in a population in Hardy-Weinberg equilibrium in which the frequency of the dominant phenotype is 0.19?

Problem 5. In a population of 10,000 individuals in which the frequency of the dominant allele for a given trait is 0.7, how many individuals are expected to show a dominant phenotype if the population is in Hardy-Weinberg equilibrium?

Problem 6. A trait is determined by two codominant alleles, A_1 and A_2. In a population of 200,000 individuals, 64% are A_1A_1 homozygotes. If the population is in Hardy-Weinberg equilibrium, how many individuals are expected to be A_2A_2 homozygotes and how many heterozygotes?

Problem 7. The frequency of albinos in a human population in Hardy-Weinberg equilibrium is 1 per 10,000 individuals. What would be the allele frequencies and genotypic frequencies in this population?

Problem 8. A human population is sampled and 1 in 10,000 individuals is found to be phenylketonuric. Phenylketonuria is a disease resulting from an autosomal recessive allele. Assuming that the population is in equilibrium, determine the probability that a healthy couple will have a phenylketonuric child.

Problem 9. Abnormal hemoglobin is determined by the Hb^S allele, the normal allele being Hb^A. Homozygotes for Hb^S suffer from severe anemia. Assuming that a human population is in Hardy-Weinberg equilibrium for this locus and that individuals affected with severe anemia occur at a frequency of 1 per 1,000: what is the proportion of healthy but disease-carrying individuals?

Problem 10. The most common form of hemophilia is due to a sex-linked recessive allele with a frequency of 0.0001 in a human population at Hardy-Weinberg equilibrium.
(a) What are the expected frequencies of the two male genotypes and the three female genotypes in the population?
b) How many hemophilic males should be expected for each hemophilic female?

Problem 11. Seventy percent of males in a human population at Hardy-Weinberg equilibrium show dominant phenotype for a sex-linked locus. What percentage of females in that population will show dominant phenotype?

Problem 12. At a certain locus, the mutation rate of A → a is 10^{-5}, with the rate of backmutation being negligible. What will be the frequency of A after 10, 100, and 100,000 generations of mutation, if we start from an initial frequency of 0.3?

Problem 13. Assuming that the direct (A → a) and inverse (a → A) mutation rates at a certain locus of *Paravespula vulgaris* are 10^{-6} and 10^{-8}, respectively, what are the expected equilibrium allele frequencies if no other process intervenes?

Problem 14. In a population that maintains constant size over generations, the frequency of an allele at an autosomal locus at a given time is 0.2. The rate of migration into that population from a neighbouring population where the frequency of that allele is 0.7 is 10%.

a) What will be the frequency of the allele in question one generation later?
b) What will it be after 10 generations?

Problem 15. In a human population, for a given A/a locus, the frequency of the A allele (p) is 0.5 and for each offspring that individuals with AA genotype have, heterozygotes also have on average one offspring while aa individuals produce on average 0.8 offspring.

a) What is the value of the fitness in each case?
b) What is the value of the selection coefficient for each of the genotypes?
c) What will be the frequency of each genotype in the next generation?

d) What will be the frequency of allele A in the next generation?

Problem 16. The a allele of a human gene is recessive lethal, whereas A in homozygosis produces a 40% reduction in fertility.

a) What is the value of the selection coefficient for each of the genotypes?
b) What is the value of fitness in each case?
c) How much will the allele frequencies (p and q) be worth after one generation of selection if the initial frequencies were 0.8 and 0.2, respectively?

Problem 17. In a large, randomly mated population of rodents, the progeny produced on average by individuals belonging to each of the three possible genotypic classes for a locus with two alleles (A and a) have been analysed and the following results obtained:

Genotype Average offspring

AA 8
Aa 12
aa 25

a) What is the value of fitness in each case?
b) What is the value of the selection coefficient for each of the genotypes?
c) Being the frequency p of allele A equal to 0.9, what will be its frequency in the next generation?

4. SOLUTIONS TO PROBLEMS

Problem 1. For a single autosomal gene, if mating is random, equilibrium is reached in a single generation, regardless of the values of the starting allele frequencies. Therefore, the genotypic frequencies we are asked for will be those of the equilibrium and will be calculated as a function of the allele frequencies:

Frequency of A = p = 0.24 + 1/2 0.32 = 0.4
Frequency of a = q = 0.4 + 1/2 0.32 = 0.6

Frequency of AA = p^2 = 0.16
Frequency of Aa = 2pq = 0.48
Frequency of aa = q^2 = 0.36

Problem 2.
a)
D = Frequency of LMLM = 326/1000 = 0.326
H = Frequency of LMLN = 470/1000 = 0.470
R = Frequency of LNLN = 204/1000 = 0.204

p = Frequency of LM allele = 0.326 + 1/2 0.470 = 0.561
q = Frequency of the LN allele = 0.204 + 1/2 0.470 = 0.439

p^2 = Expected LMLM allele frequency at equilibrium = 0.3147 (x 1000 = 315)
2pq = Expected LMLN frequency at equilibrium = 0.4925 (x 1000 = 492)
q^2 = Expected LNLN frequency at equilibrium = 0.1927 (x 1000 = 193)

b)
The theoretical χ^2 value (1 degree of freedom) for a significance level of 0.05 is 3.84. Since the experimental χ^2 is smaller than the theoretical χ^2, we do not reject the equilibrium hypothesis and assume that the observed values conform to those expected in equilibrium ($0.1 < p < 0.2$).

Problem 3. According to the data in the statement, the genotypic and allele frequencies for this locus in this population are:

D (Frequency of AA) = 0.9596; H (Frequency of Aa) = 0.04; R (Frequency of aa) = 0.0004.

p (Frequency of A) = 0.9796; q (Frequency of a) = 0.0204.

In an equilibrium situation, the expected frequencies would be:

p^2 = 0.9596 (9596 individuals out of the total 10000 existing in the population).
2pq = 0.04 (400 individuals out of the total of 10000 in the population).
q^2 = 0.0004 (4 individuals out of the total 10000 in the population).

The observed values coincide with the expected values. Therefore, the population is in Hardy-Weinberg equilibrium.

Problem 4. If the frequency of individuals with dominant phenotype is 0.19, the frequency of individuals with recessive phenotype will be 0.81. Given that the population is in equilibrium:

q^2 = 0.81; q = 0.9; p = 0.1

p^2 = 0.01; 2pq = 0.18; q^2 = 0.81

The frequency of heterozygotes Aa (2pq): 18%.

Given that p = 0.7 (q = 0.3) and the population is in equilibrium, the expected frequency of individuals with dominant phenotype is the frequency of AA homozygotes (p^2) plus that of Aa heterozygotes (2pq):

p^2 = 0.49
2pq = 0.42

The frequency of individuals with dominant phenotype will be 0.91, that is, out of a total of 10000 individuals, 9100 will show this phenotype.

If we call p the frequency of the A_1 allele and q the frequency of the A_2 allele, it shall be the case that in this population in equilibrium, the frequency of A_1A_1 will be p^2, that of A_1A_2 will be 2pq and that of A_2A_2 will be q^2:

p^2 = 0.64; p = = = 0.8; q = 1 - p = 0.2;

2pq = 0.32; q^2 = 0.04

Thus, out of a total of 200000 individuals, 32% will be heterozygotes (64000) and 4% will be A_2A_2 homozygotes (8000). The remaining 128000 (64%) will be the A_1A_1 indicated in the problem statement.

Problem 7.

q^2 = 0.0001; q = 0.01; p = 0.99

p^2 = 0.9801; 2pq = 0.0198; q^2 = 0.0001

Problem 8.

q = 0.01; p = 0.99; p^2 = 0.9801; 2pq = 0.0198; q^2 = 0.0001.

Frequency of heterozygotes among healthy = $2pq/2pq+p^2$ = 0.0198/0.9998 = 0.0194

P = P(Aa) x P(Aa) x P(Aa x Aa → aa) = (0.0194)(0.0194)(0.25) = 0.000098

Problem 9.

q= 0.032; p=0.968;

p^2=0.937; 2pq= 0.062; q^2=0.001

The proportion of healthy but disease-carrying individuals (2pq) is 6.2%.

Problem 10.

a)
Males:
Normal: p = 0.9999
Hemophiliacs: q = 0.0001

Females:
Normal homozygous: p^2 = 0.9998
Normal heterozygous: 2pq = 1.9998 x 10^{-4}
Hemophilic: q^2 = 1 x 10^{-8}

b)
Hemophilic Males/Hemophilic Females = 1 x 10^{-4}/1 x 10^{-8} = 10000

Problem 11.

Males:
Dominant phenotype: 0.7
Recessive phenotype: 0.3

For males, being hemizygous for sex-linked genes, the genotypic frequencies match the allele frequencies:

p (frequency of A) = 0.7; q (frequency of a) = 0.3.

In equilibrium, females will be distributed by genotypic classes as follows:

Homozygous for the dominant allele: p^2 = 0.49.
Heterozygous: 2pq = 0.42
Homozygous for the recessive allele: q^2 = 0.09

And by phenotypic classes:
Dominant phenotype: 0.49 + 0.42 = 0.91
Recessive phenotype: 0.09

Problem 12.

$p_t = p_0 (1 - u)^t$

$p_{10} = 0.3 (1 - 10^{-5})^{10} = 0.2999$

$p_{100} = 0.3 (1 - 10^{-5})^{100} = 0.2997$

$p_{100000} = 0.3\ (1 - 10^{-5})^{100000} = 0.1104$

Problem 13.

$p_e = v / (u + v) = 10^{-8}/10^{-6} + 10^{-8} = 0.0099$

$q_e = u / (u + v) = 10^{-6}/10^{-6} + 10^{-8} = 0.9911$

Problem 14.

a) $p_1 = p_0 - m\ (p_0 - P) = 0.2 - 0.1\ (0.2 - 0.7) = 0.25$

b) $(1 - m)t = (p_t - P) / (p_0 - P) \rightarrow (1 - 0.1)10 = (p_{10} - 0.7) / (0.2 - 0.7) \rightarrow p_{10} = 0.53$

Problem 15.

a) $w_{AA} = 1$; $w_{Aa} = 1$; $w_{aa} = 0.8$
b) $s_{AA} = 0$; $s_{Aa} = 0$; $s_{aa} = 0.2$
c) (mean fitness of the three genotypes) = $p^2w_{AA} + 2pqw_{Aa} + q^2w_{aa} = 0.25 + 0.5 + 0.20 = 0.95$

Thus:

Frequency of genotype AA: $p^2w_{AA}/\overline{W} = 0.25/0.95 = 0.263$
Frequency of genotype Aa: $2pqw_{Aa}/\overline{W} = 0.5/0.95 = 0.526$
Frequency of genotype aa: $q^2w_{aa}/\overline{W} = 0.2/=0.95 = 0.211$

d) Therefore,

p = 0.263 + 1/2 0.526 = 0.526
q= 0.211 + 1/2 0.526 = 0.474

Problem 16.

a) $s_{AA} = 0.4$; $s_{Aa} = 0$; $s_{aa} = 1$
b) $w_{AA} = 0.6$; $w_{Aa} = 1$; $w_{aa} = 0$

(c) (average fitness of the three genotypes) = $p^2w_{AA} + 2pqw_{Aa} + q^2w_{aa} = 0.384 + 0.32 + 0 = 0.704$.

Thus:

Frequency of genotype AA: $p^2w_{AA}/\overline{W} = 0.384/0.704 = 0.5454$
Frequency of genotype Aa: $2pqw_{Aa}/\overline{W} = 0.32/0.704 = 0.4545$
Frequency of genotype aa: $q^2w_{aa}/\overline{W} = 0/=0.704 = 0$

Therefore,

p = 0.5454 + 1/2 0.4545 = 0.773
q= 0 + 1/2 0.4545 = 0.227

Problem 17.

a)
Fitness of AA (w_{AA}): 8/25 = 0.32
Fitness of Aa (w_{Aa}): 12/25 = 0.48
Fitness of aa (w_{aa}): 25/25 = 1

b)
Selection coefficient against the AA genotype: s = 1 - 0.32 = 0.68
Selection coefficient against the Aa genotype: s = 1 - 0.48 = 0.52
Coefficient of selection against genotype aa: s = 1 - 1 = 0

c)
(average fitness of the three genotypes) = $p^2w_{AA} + 2pqw_{Aa} + q^2w_{aa}$ = 0.2592 + 0.0864 + 0.01 = 0.3556.

Thus:

Frequency of genotype AA: $p^2w_{AA}/\overline{W}$ = 0.2592/0.3556 = 0.729
Frequency of genotype Aa: $2pqw_{Aa}/\overline{W}$ = 0.0864/0.3556 = 0.243
Frequency of genotype aa: $q^2w_{aa}/\overline{W}$ = 0.01/0.3556 = 0.028

And, therefore,

p = 0. 729 + 1/2 0.243 = 0.85
q= 0.028 + 1/2 0.243 = 0.15

LABORATORY MANUAL

MICROSCOPE OPERATION AND MAINTENANCE

Rules of use

As the microscope is a precision device, it is very convenient to ensure good performance and a long life by following a series of procedures:

1. Switch on the light.

2. Place the preparation on the stage and center the stained area on the axis of the objective, looking from the outside.

3. Open the diaphragm of the condenser and place it in its highest position.

4. Place an objective of low power (10X) as close as possible to the preparation, without touching it. Carry out this operation by lowering the tube using the coarse adjustment knob, observing the microscope laterally.

5. Observe through the eyepiece.

6. Slowly raise the objective, moving the coarse adjustment knob until the image appears sharp.

7. Once focused with the 10X objective, do not touch the coarse adjustment knob, so that focusing with the following objectives will be performed using only the fine adjustment knob.

8. Proceed to observe the entire preparation. To do this, move the stage with the stage control knobs from left to right and from top to bottom, taking care not to pass through the same point of the preparation twice. The coordinates of those cells it is interesting to observe using higher magnification objectives are recorded using the two Vernier scales (see below).

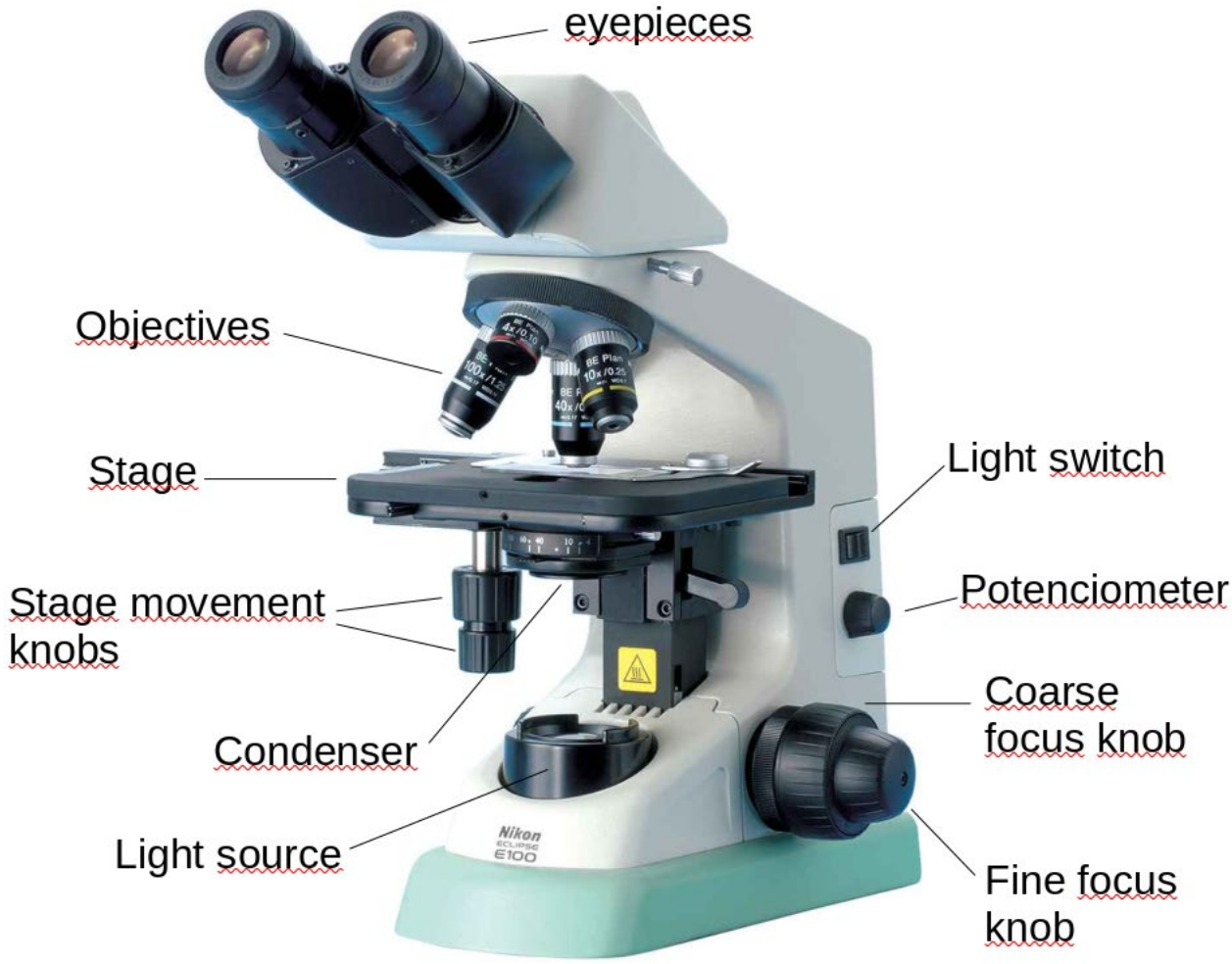
9. Once the observation of the preparation is completed, the cells whose coordinates were recorded can be observed with higher magnification objectives. With the 40X objective it is necessary to take the precaution, as with the immersion objective of 100X, of never touching the preparation with the distal lens. For more detailed observations, the 100X immersion objective should be used, placing a drop of immersion oil on the preparation.

10. Rectify the focus after every objetive change, if necessary, using only the micrometer screw.

11. Always move any element of the microscope smoothly and slowly.

12. Once the observation is finished:

- Attach the 10X objective.
- Remove the preparation.
- Wash the slide and coverslip with tap water.
- Disconnect the microscope.
- Put the cover on the microscope.



Recommendations

The microscope should not be left on when not in use, but should not be switched on and off at short intervals.

The microscope should not be slid on the table, especially when it is hot, to avoid vibrations.

Proper contrast with each objective is achieved by raising and lowering the condenser and opening and closing the condenser.

Recording the coordinates of an object observed using the microscope

For a better study of a microscopic preparation, a method should be followed that allows the analysis of the entire preparation, without leaving any areas unobserved. This can be done by making successive horizontal passes starting on one side of the coverslip and ending on the opposite side.

In the course of the observation we may find a number of interesting cells, either for further observation or to be photographed. Therefore, it is necessary to record their exact position in order to be able to locate them again later. To do this, we will make use of the graduated (Vernier) scales of the microscope stage.

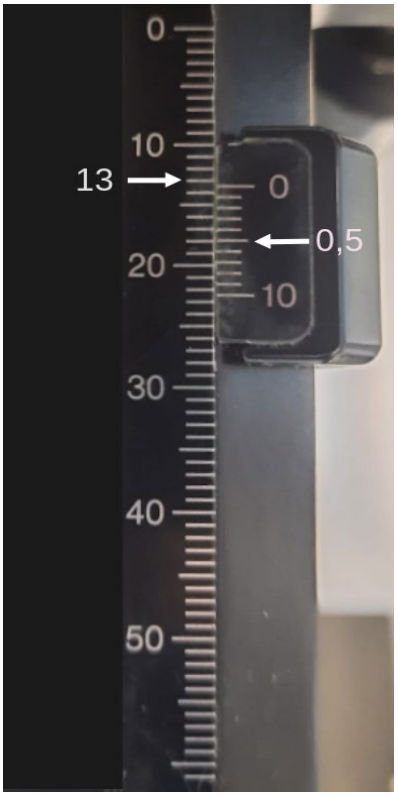
To record the coordinates, please proceed as follows:

1. The cell whose coordinates are to be recorded is placed in the center of the field of the microscope.

2. On the abscissa (horizontal) axis there is a small fixed scale, ranging from 0 to 10 (nonius) and a large scale, whose numbering depends on the microscope model, which slides on it.

The reading value comprises a whole number part and a decimal number part. The former is indicated by the division of the large scale immediately before the 0 of the nonius. If this 0 were to coincide exactly with a division of the large scale, there would be no decimal part. The decimal part would be given by the division of the nonius that coincides exactly with any division of the large scale (see example figure below).

3. On the ordinate (vertical) axis there are also two scales. The large scale slides over a fixed small scale, which ranges from 0 to 10. The reading of the coordinates is undertaken in the same way as on the abscissa axis. In this way, we obtain the coordinates that will allow us to find the cells we want to observe again.



Reading value: 13.5

MITOSIS

1. OBJECTIVE

The objective of this practical lecture is to know the structure of chromosomes by studying the morphology they show during the mitotic process, also learning how to elaborate a karyotype, which is a fundamental tool for describing any species cytogenetically. By visualizing the features of the different phases of mitosis under the microscope, we will be able to understand how the chromosome number is maintained during the development of an individual, the regeneration of tissues or in asexual reproductive processes.

2. THEORETICAL BACKGROUND

Cell division consists of two fundamental processes: mitosis, or division of the nucleus, and cytokinesis, or division of the cytoplasm. Both processes are independent but must occur coordinately. This results in two daughter cells with a chromosomal endowment identical to each other and to that of the parental cell. Thus, mitosis is the stable mechanism for cells to accurately distribute genetic information between daughter cells during cell divisions. To facilitate this distribution, the chromosomes are condensed, making their morphology apparent. This allows us to know at that moment how many chromosomes a species has, where the centromere (or primary constriction) is located, the number of chromosome arms or the existence of secondary constrictions and chromosomal satellites.

When a cell is not dividing, it is said to be in interphase, which corresponds to the time lapse between two successive mitoses. During this period, the chromatin is decondensed and there is a great metabolic activity because this is when most of the genes are expressed. Nevertheless, each cell type only expresses those genes necessary for its specific function.

An important event of interphase is DNA replication, which occurs in the period called S, after which the chromosomes already have two identical replicas called sister chromatids. This S phase is preceded by the G1 period and followed by the G2 period in which there is cell growth, transcriptional activity and the cell prepares to divide. After G2, mitosis is initiated. When the cell is not going to divide again after a mitotic cycle, it remains in the G0 phase.

Accordingly, the cell cycle includes the following phases (Figure 1):

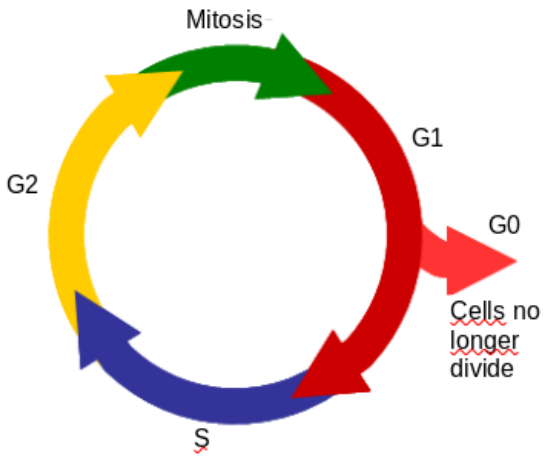


Figure 1: Cell cycle

Once initiated, the mitotic process proceeds continuously without interruption, but a number of significant key events take place that are necessary for the correct distribution of the genetic information. Based on these events, four stages have arbitrarily been established:

- Prophase
- Metaphase
- Anaphase
- Telophase

The chronology and characteristics of the key mitotic events described below are common to most studied organisms. However, exceptions have been described in some species affecting the timing of the initiation of chromosome condensation (there are species in which chromosomes never condense), the disappearance of the nuclear membrane or the establishment of the metaphase plate. Anaphase seems to be the most conserved stage among different organisms.

Prophase

During this period, the chromatin fiber, which has been organizing itself into increasingly complex folds, appears as visible chromosomes that gradually condense. There are 2n chromosomes in the cell and each chromosome consists of two sister chromatids with equal information and morphology. These are joined together at the centromere and along the chromosome arms by protein complexes. At the end of prophase, the nucleoli are disorganized and the nuclear membrane disappears, the components of which are dispersed in the nucleus.

Metaphase

The chromosomes are now free in the cytoplasm and the centromeres of each chromosome contact the spindle fibers, which are organized in the microtubule organizing center (MTOC), formed by the centrioles, which acts as the center of attraction of the chromosomes towards the poles, and the amorphous pericentriolar mass. The intervention of spindle fibers and other chromosome movement proteins allows the chromosomes to organize themselves into the so-called metaphase plate. Each sister chromatid is oriented towards a different pole, which ensures that the genetic information of each chromosome is distributed to the two daughter cells. The chromosomes reach their maximum degree of condensation and their morphology becomes evident (Figure 2). Therefore, it is at this stage that all the morphological features of the chromosomes can be best studied. These features may be useful, for example, to identify homologues and make a karyotype, or to carry out comparative studies between phylogenetically close species and learn about their karyotypic evolution.

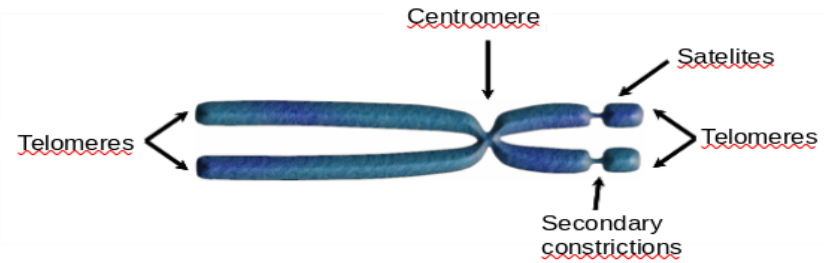


Figure 2: Morphological elements of a chromosome

The centromere is the primary constriction that appears in all chromosomes and is the place where the kinetochores, the protein structure to which the mitotic spindle fibers are attached, are associated. Its position defines the number of arms of a chromosome. If it is located very near to one end of the chromosome, the chromosome will have only one arm, and if it is in any other position, chromosomes with two arms result. The kinetochore functions as an "interface" between the centromere and the spindle fibers.

Secondary constrictions are seen on some chromosomes, usually associated with the nucleolar organizer regions (NOR), where the genes for ribosomal RNA are located. The chromosome fragment that runs from the secondary constriction to the telomere is called the chromosomal satellite. Telomeres constitute the chromosomal ends, there being one on each chromatid arm, and play a critical role in maintaining the integrity of the chromosome.

Anaphase

Anaphase is generally the shortest stage of mitosis. Each centromere splits in two and the proteins that held the sister chromatids together are disorganized, allowing them to segregate (migrate) to opposite poles. At each cell pole, a group of chromosomes (2n) containing a single chromatid oriented toward the corresponding pole become visible.

Telophase

The chromosomes grouped at each pole begin to decondense and the nucleoli and nuclear membrane are rearranged from pre-existing and newly synthesized components. Cytokinesis completes cell division at the end of this stage, including the distribution of organelles and other cytoplasmic components between the two daughter cells, although it is not as precise as mitosis. The entire process of cell division results in two cells with 2n chromosomes each.

In this practical lecture, we will use cytogenetic techniques to observe the following genetic phenomena:

- DNA and chromatin replication: chromosomes with two chromatids.

- Conservation of hereditary material and constancy of chromosome number: segregation of the sister chromatids of each chromosome during anaphase.

3. RELATED LESSONS IN THE THEORETICAL PROGRAM

This project is mainly related to the contents of lessons 1, 2 and 3 of the syllabus of the subject “Genetics I” of the Biology degree.

4. METODOLOGY

The material we need to study mitosis must necessarily be an actively proliferative tissue. In small animals, bone marrow from long bones, spleen, gastric cecum and cell cultures such as, for example, lymphocytes, are used. In plants, the apical end of the roots (apical meristem) is frequently used, but other materials such as leaf apices or flower ovaries can be used. In this practical lecture we are going to use roots from individuals of the species *Muscari comosum*.

The bulbs of this species have been kept in hydroponic culture in order to let them to develop roots. Once grown, the ends of the roots were cut off and treated with 0.05% colchicine for 4h, which prevents the formation of the mitotic spindle and thus cause the

accumulation of cells in metaphase, where we will see a better chromosome morphology. Colchicine has an additional effect, that is an extra condensation of the chromosomes, which increases the frequence of cells with well separated chromosomes. The roots are then fixed by immersing them in a fixative composed of a mixture of ethanol and glacial acetic acid in a 3:1 ratio. This coagulates the cell contents so that they retain their shape, structure and composition. The roots of *M. comosum* are also subjected to hydrolysis in 1N ClH at 60°C for 1 min to relax the DNA structure and facilitate subsequent staining of the chromosomes with basic dyes (e.g. orcein), that react with the aldehyde groups of the nucleotides.

Obtaining the preparations

1. Stain the roots with 2% acetic acid orcein for 10-15 min.
2. Remove any remaining orcein with a filter paper and wash lightly by adding a drop of 45% acetic acid to remove the excess of orcein.
3. On a clean slide placed on a piece of filter paper, place a new drop of orcein of about 1 cm in diameter.
4. Using the handled needle and the lancet, cut the tip of the apex, which is recognized by its darker colour and its point-shaped end. The meristem is easily detached from the root, so if there is difficulty in performing this operation, it means that it is not the meristematic apex.
5. Remove the rest of the root, leaving only the apex on the drop of orcein. With the base of the needle handle, macerate it until it splits into several smaller pieces.
6. Place a clean and degreased coverslip on the orcein drop and proceed to squash the material. To do this, hold one edge with a piece of filter paper and tap gently with the tip of the needle, causing trapped air bubbles and excess dye to be expelled. Place a piece of filter paper on the coverslip and press it with your thumb, preventing the coverslip from sliding on the slide.

Chromosome observation and imaging

Once we have obtained the chromosomal preparation, we may observe it under the microscope for study. The features to be recorded for the cell types you will be able to observe are as follows (Figure 3):

- Interphase (non-mitotic cells):
  - Decondensed chromatin.
  - Number and size of nucleoli.

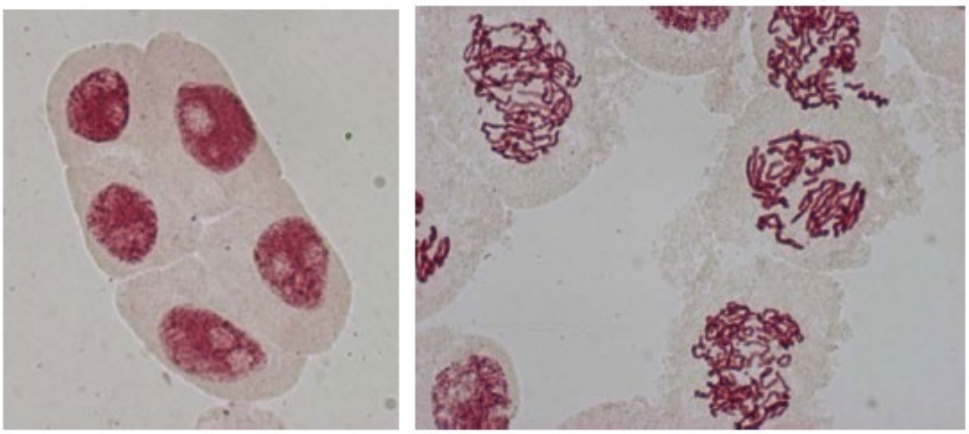
- Prophase:
  - Beginning of chromosome condensation.
  - Nucleoli.

- Metaphase:
  - Arrangement of chromosomes in the metaphasic plate.
  - Number of chromosomes and all details that can be identified on chromosome morphology: centromere, chromosome arms, chromatid number, secondary constrictions and satellites.
  - From a microphotograph of this phase, the karyotype of a species can be studied (see below).

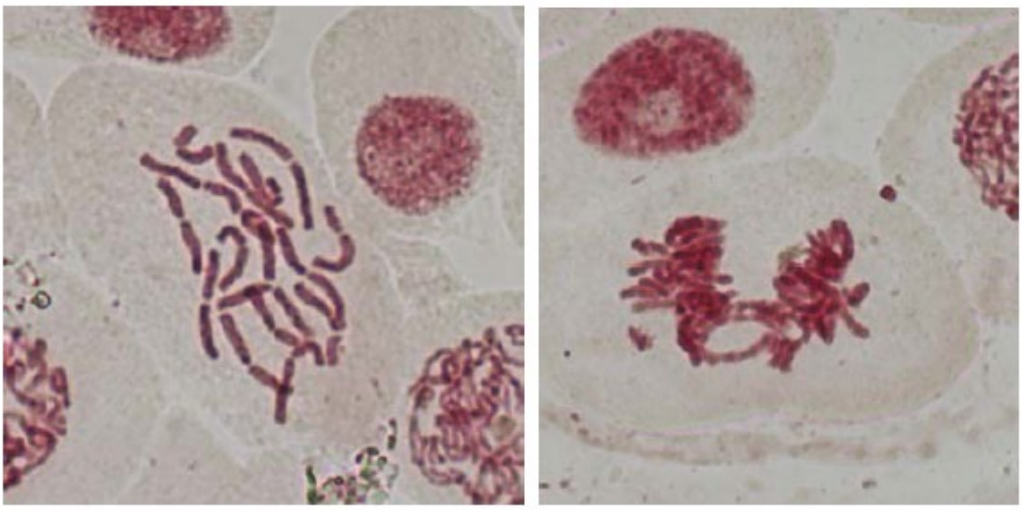
- Anaphase:
  - Orientation of centromeres and chromosomes toward the cell poles.
  - Migration and segregation of sister chromatids.
  - Simple (single chromatid) structure of chromosomes.

- Telophase:
  - Compact grouping of chromosomes at both cell poles.
  - Beginning of chromosome decondensation.
  - Appearance of the septum between the two newly formed nuclei.

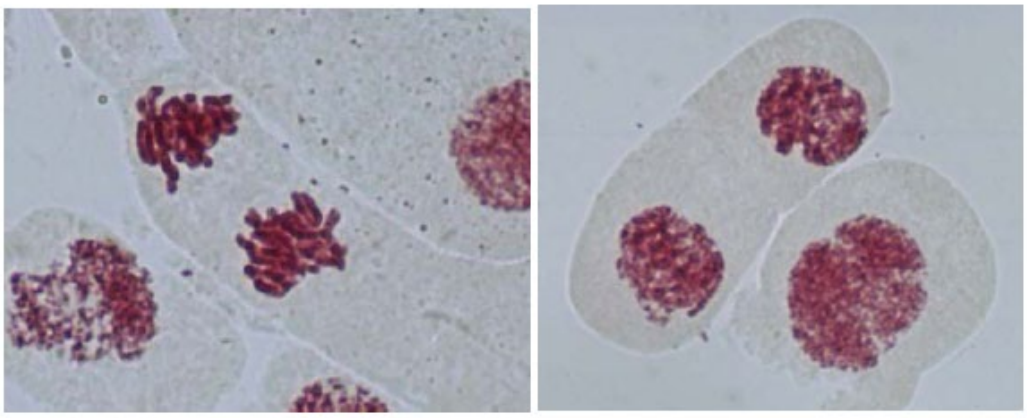
During the study, photographs of cells in metaphase can be taken using a camera attached to the microscope. To do this, we select a cell that contains all the chromosomes without overlapping, in order to correctly visualize their morphology and number. The images are then analysed using image processing software to perform a karyotype.



Interphase Prophase



Metaphase Anaphase



Telophase Daughter cells

Figure 3: Mitosis phase

Performing a karyotype

The features of the chromosomes in metaphase, including their number, size, centromere position, number and position of secondary constriction, is a constant for all normal cells of every individual of a species. To perform a karyotype from a photograph

of a cell in metaphase, chromosomes must matched by size, centromere position and secondary constrictions. Using image editing software, the chromosomes are sorted and arranged similarly to what is observed in Figure 4, taking into account the following rules:

- Cromosomes are placed in vertical position.
- Centromeres will be placed on a horizontal line.
- The long arms will be placed below the horizontal line.
- Chromosome pairs are arranged in order from largest to smallest into groups of similar morphology (seen below).

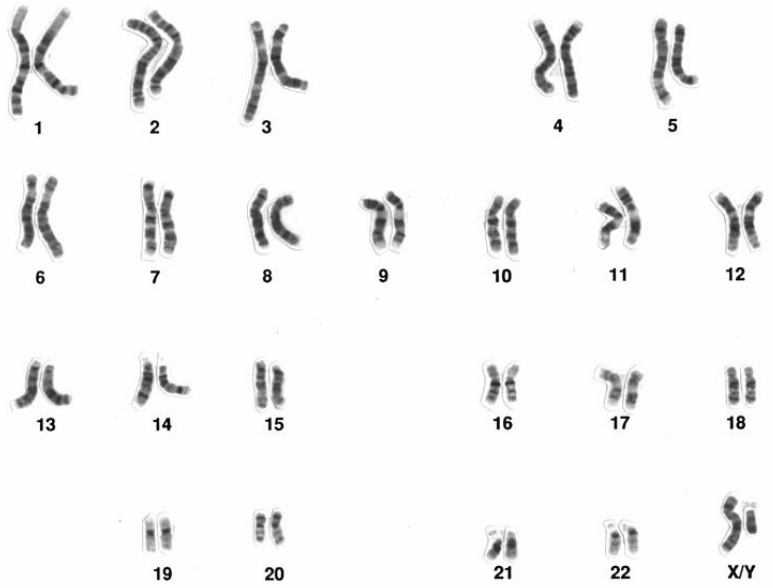


Figure 4: Human karyotype (G-bands)
Source: https://upload.wikimedia.org/wikipedia/commons/5/53/NHGRI_human_male_karyotype.png
National Human Genome Research Institute

The following table should be filled with the measurements taken on the chromosomes:

Chromosome pairs	1		2		3…	
Measurements	A	B	A	B	A	B
Length of the long arm						
Length of the short arm						
Total length						
Mean of the two homologues						
% of contribution to the karyotype						
r = long arm / short arm						

The following parameters are calculated as follows:

% of contribution to the karyotype: adding the average length of all the pairs, the total average length of the karyotype is obtained. Referring to this value, the relative percentage of each pair must be calculated.

r = arm ratio: this parameter indicates the relative length between the arms of each chromosome and permits us to classify them as follows (Figure 5):

1 < r < 1.7: metacentric chromosome. The two arms are approximately equal.

1.7 < r < 3: submetacentric chromosome. There is a clear difference in length between the arms, but it is not excessive.

3 < r < 7: acrocentric or subtelocentric chromosome. One of the arms is much shorter than the other.

7 < r: telocentric chromosome. The centromere is very close to one of the telomeres, meaning it has only one visible arm.

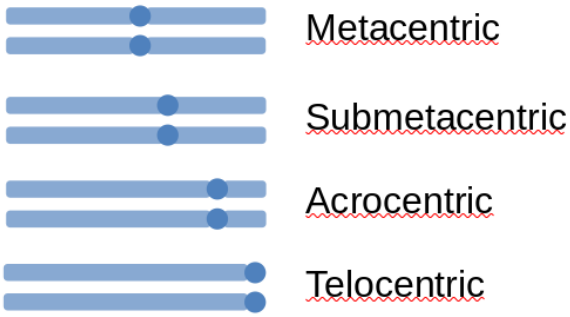


Figure 5: Types of chromosomes according to their arm ratio

Idiogram

Using the data recorded in the table, an idiogram, which is a haploid graphical representation of the karyotype, can be made (Figure 6). The length of the bars is determined by the average length of the homologues.

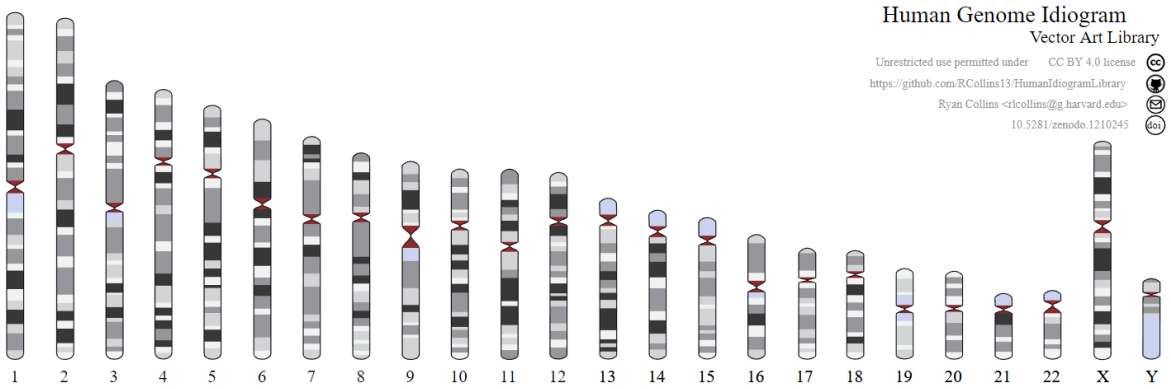


Figure 6: Idiogram of the human karyotype (G-bands). Source: https://commons.wikimedia.org/wiki/File:Human_genome_idiogram.svg. Ryan L. Collins

5. QUESTIONS

1. How many cells of each type have you observed?
2. Which treatment is applied to accumulate metaphase cells in the preparations?
3. Fixative is used to relax the DNA structure. True or false?

4. Indicate the chromosome number of the species studied.
5. How many chromosomal types does the karyotype of *M. comosum* contain, according to the position of their centromeres?

MEIOSIS

1. OBJECTIVE

During the development of this laboratory exercise we will learn about the cytogenetic particularities that determine the biological significance of meiosis. Visualising the characteristics of the different phases of meiosis under the microscope, we will gradually understand the physical basis of the inheritance of characters (genes are transmitted with chromosomes) and how chromosome number is maintained in a species through generations. Chromosomal behaviour observation during meiosis will allow us to relate the pairing and segregation of homologous chromosomes to the constancy of the chromosomal number in a species over generations and the combination of paternal and maternal characters that occurs in any individual.

2. THEORETICAL BACKGROUND

Meiosis is a process that consists of two consecutive cell divisions without DNA replication between them. Its function is different from that of mitosis since meiosis reduces the chromosome number by half, obtaining haploid gametes in organisms with sexual reproduction. This reduction is achived when chromosomes with the same type of genetic information, or homologues, pair first and then segregate to different cell poles. During chromosome pairing and synapsis, both homologues can recombine, that is, exchange chromosome segments, generating new allelic combinations on the resulting chromosomes. The different possible combinations of paternal and maternal chromosomes that may be included in a gamete, due to the segregation of homologues, is another additional source of genetic variability.

Meiosis consists of two cell divisions called meiosis I (reductional) and meiosis II (equational). As stated above, meiosis I separates homologous chromosomes and combines genetic information and the second division (meiosis II) evenly distributes the chromatids that had replicated in the premeiotic interphase.

In meiosis, four stages can be observed in each of the two divisions: prophase, metaphase, anaphase, and telophase.

The characteristics of each stage are as follows:

MEIOSIS I

Prophase I. A long and complex stage where one of the most important aspects of the meiotic process occurs: crossing over and recombination. It is divided into five substages: leptotene, zygotene, pachytene, diplotene, and diakinesis.

Leptotene. The key event is the onset of the chromosome condensation that appears as a tangle within the nucleus. At this time, the chromosomes have two chromatids but are not yet visible under the microscope.

Zygotene. This is the stage where the phenomenon of synapsis or chromosomal pairing occurs. Homologous chromosomes are associated along their entire length, which allows them to later exchange chromosomal segments (crossover) and recombine. Each pair of paired homologues constitutes what is known as a bivalent, consisting of four chromatids.

Pachytene. The chromosomal condensation level is greater and the bivalents appear shorter and thicker, with the homologues remaining attached along their entire length. Crossing over and recombination occur at this stage.

Diplotene. The level of bivalents condensation increases. Homologous chromosomes begin to separate at centromere level, staying joined by contact points called chiasmata that are the cytogenetic manifestation of crossing over. However, the sister chromatids of each homologue remain attached. The number of chiasmata varies between species, populations, individuals and cell type. The bivalent size also determines the number of chiasmata present in it. In the human female meiosis, for example, an average of two to three chiasmata are observed per bivalent, with the large chromosomes showing a greater number of chiasmata.

Diakinesis. The bivalents already show a very high level of condensation, appearing as thick bodies. The centromeres of each homologous pair initiate co-orientation toward opposite poles. At the end of diakinesis, and therefore of prophase I, the nucleoli and the nuclear membrane become disorganized, just as in mitotic prophase.

Metaphase I. The bivalents exhibit their maximum condensation degree. The centromeres of homologous chromosomes attach to the spindle fibers reorganizing at the metaphase plate. In contrast to mitotic metaphase, paired chromosomes, that is, bivalents (n bivalents) are arranged on the equatorial plate rather than single chromosomes (2n).

Anaphase I. Homologous chromosomes segregate to opposite poles. This event is extremely important since it results in a chromosome number reduction (n chromosomes at each pole). Sister chromatids of each chromosome still remain attached but only at the centromere level, unlike in mitosis where centromeres divide and both chromatids separate completely and segregate in mitotic anaphase.

Telophase I. When segregation of homologous chromosomes is complete, they cluster at both cell poles. The chromosomes decondense and nucleoli and nuclear membrane reassemble.

Finally, cytokinesis occurs giving rise to two daughter cells.

MEIOSIS II

The second meiotic division is very similar to the mitotic process but there are some fundamental differences. When meiosis II begins, the chromosomes are already replicated showing two chromatids since there is no DNA replication in the previous interphase (or interkinesis). This second division also consists of four stages: prophase II, metaphase II, anaphase II and telophase II.

Prophase II. This is a short-term stage where n chromosomes appear with both chromatids diverging, as if repelling each other, and joined only by their centromere giving them a cross shape. The nucleoli and nuclear membrane disassemble.

Metaphase II. The n chromosomes attach to the spindle fibers and organise themselves into the metaphase plate.

Anaphase II. Each centromere divides and the sister chromatids segregate towards opposite poles. At each cell pole we will observe n chromosomes with a single chromatid.

Telophase II. Migration of the n single-chromatid chromosomes ends and they begin to decondense. The nucleoli and nuclear membrane reappear. Cytokinesis takes place.

At the end of the entire meiotic process, four haploid cells with n chromosomes are obtained.

Meiosis observation that is carried out in this lab exercise will allow us to observe important genetic phenomena:

- Reduction of chromosome number during the formation of haploid gametes: observation of the segregation of homologous chromosomes in anaphase I and sister chromatids in anaphase II.

- Generation of genetic variability during meiosis:

  a) Random combination of paternal and maternal chromosomes: observation of segregation of paternal and maternal chromosomes for each chromosome type during anaphase I
  b) Genetic recombination between homologous chromosomes: observation of pairing and chiasmata between homologous chromosomes.

Summary of chromosome number and DNA amount in each stage:

Chromosome number	Cell type	DNA amount
2n	Spermatogonium (mitosis)	4C
2n	Primary spermatocyte (1) from leptotene to telophase I	4C
n	Secondary spermatocytes (2) from interkinesis to telophase II	2C
n	Spermatids (4)	C
n	Spermatozoa (4)	C

3. RELATED TOPICS FROM THE THEORY SYLLABUS

This lab exercise is mainly related to the contents of units 1 and 2 of the Genetics I course of the Biology degree, whose contents are the following:

Unit 1. Mendelian genetic analysis: The method of Mendelian genetic analysis. Principle of segregation. Principle of independent transmission. Genealogical trees. Calculation of probabilities. Statistical testing of segregations: chi-square test.

Unit 2. Chromosomal basis of inheritance: Genes and chromosomes. Mitosis and meiosis. Genetic significance of mitosis and meiosis.

4. METHODOLOGY

Preparation of meiotic chromosome spread

The material necessary for the study of meiosis are the tissues where the production of male and female gametes takes place, which in animals are the testes and ovaries, respectively. The production of ovules occurs through the oogenesis process and that of spermatozoa through spermatogenesis.

In higher plants the reproductive organ is the flower, which may contain both female (gynoecium) and male (androecium) reproductive organs, or there may be exclusively female or male flowers on different plant individual (dioecious) or on the same individual (monoecious). Male gametes or pollen grains (microspores) are generated in the anthers

and the female gametes or ovules (megaspores) in the ovary.

In this lab exercise we are going to study spermatogenesis in grasshoppers, since in these species the nuclei show few and large chromosomes, facilitating their study.

Chromosomal preparations will be performed with fixed testis follicles from male grasshoppers.

In order to obtain the necessary material for the preparations, we have previously anesthetized a male with ethyl acetate and proceeded to dissect it in order to extract the testicular mass where the testis follicles are located. Subsequently, the testicles are fixed in ethanol:acetic acid in a 3:1 ratio. After one hour, the material is ready for study.

The methodology for obtaining chromosome preparations varies depending on the characteristics of the material used. Chromosome preparations of testis follicles are made by squashing, following a somewhat different procedure from that used for the study of mitosis in plants. In this case, the procedure is as follows:

1. Clean a degreased slide and place on filter paper.
2. Deposit a small drop of lacto-propionic orcein in the center of the slide and place two follicles on it.
3. Macerate the follicles by crushing them directly with the flat end of a metal or plastic object (a pen, a sleeved needle, a lancet, etc).
4. Drop a coverslip onto the orcein cell suspensión.
5. Remove any trapped air bubbles by holding the coverslip with filter paper at one of its corners and applying slight pressure with the tip of the lancet.
6. Crush the material by placing a piece of filter paper on the coverslip and holding the slide with one hand and exerting strong pressure on the coverslip with the thumb of the other hand. Care should be taken to ensure that the coverslip does not slip.

Chromosome observation

The student must locate, study and make and interpretative drawing of the main stages of meiosis.

The most significant events to observe in each stage are (Figures 1 and 2):

- Leptotene:

  Tangled appearance of chromosomes.
  The filaments are simple.
  An intensely stained body is observed: X chromosome. Males are X0 and females are XX.

- Zygotene:

  Shorter and less tangled fibers.
  Double appearance of the filaments as a consequence of pairing and sinapsis of homologous chromosomes (bivalent).
  X chromosome (univalent) continues to appear more stained and without definite shape.

- Pachytene:

  The bivalent number can be counted.
  The filaments are much thicker.

X chromosome is still highly condensed and is usualy folded on itself.

- Diplotene:

  Bivalents are even shorter and thicker.
  Chiasmata, or points of contact between homologous, are observed.
  X chromosome stretches during this stage, but remains more condensed than the rest.

- Diakinesis:

  Bivalents are now much shorter and thicker, presenting a more rounded shape.
  X chromosome continues to be more condensed than the rest.

- Metaphase I:

  Bivalents are maximally condensed and therefore their edges or contours are sharp.
  X chromosome usualy appears somewhat more decondensed at this stage and stains less than the bivalents.

- Anaphase I:

  Homologous chromosomes of each bivalent are observed migrating towards opposite poles.
  X chromosome, as a univalent, will go to one of the poles.

- Telophase I:

  Chromosomes are grouped at the poles.
  Chromosomes are more decondensed.

- Interkinesis

- Prophase II:

  Haploid number of chromosomes.
  Divergent chromatids with a cross shape.

- Metaphase II:

  Chromosomes more condensed, shorter and thicker. Sister chromatids separated except at the centromere.

- Anaphase II:

  Segregation/migration of sister chromatids towards each pole.

- Telophase II:

  Chromosomes cluster at cell poles and begin to decondense.

In order to observe the different stages of meiosis in grasshoppers, it is necessary to study more than one chromosome preparation since within the testis follicles there are functional subunits that are called cysts or set of cells in the same meiotic stage. For this reason, in a single chromosome preparation we will only be able to observe 3 or 4 different meiosis stages.

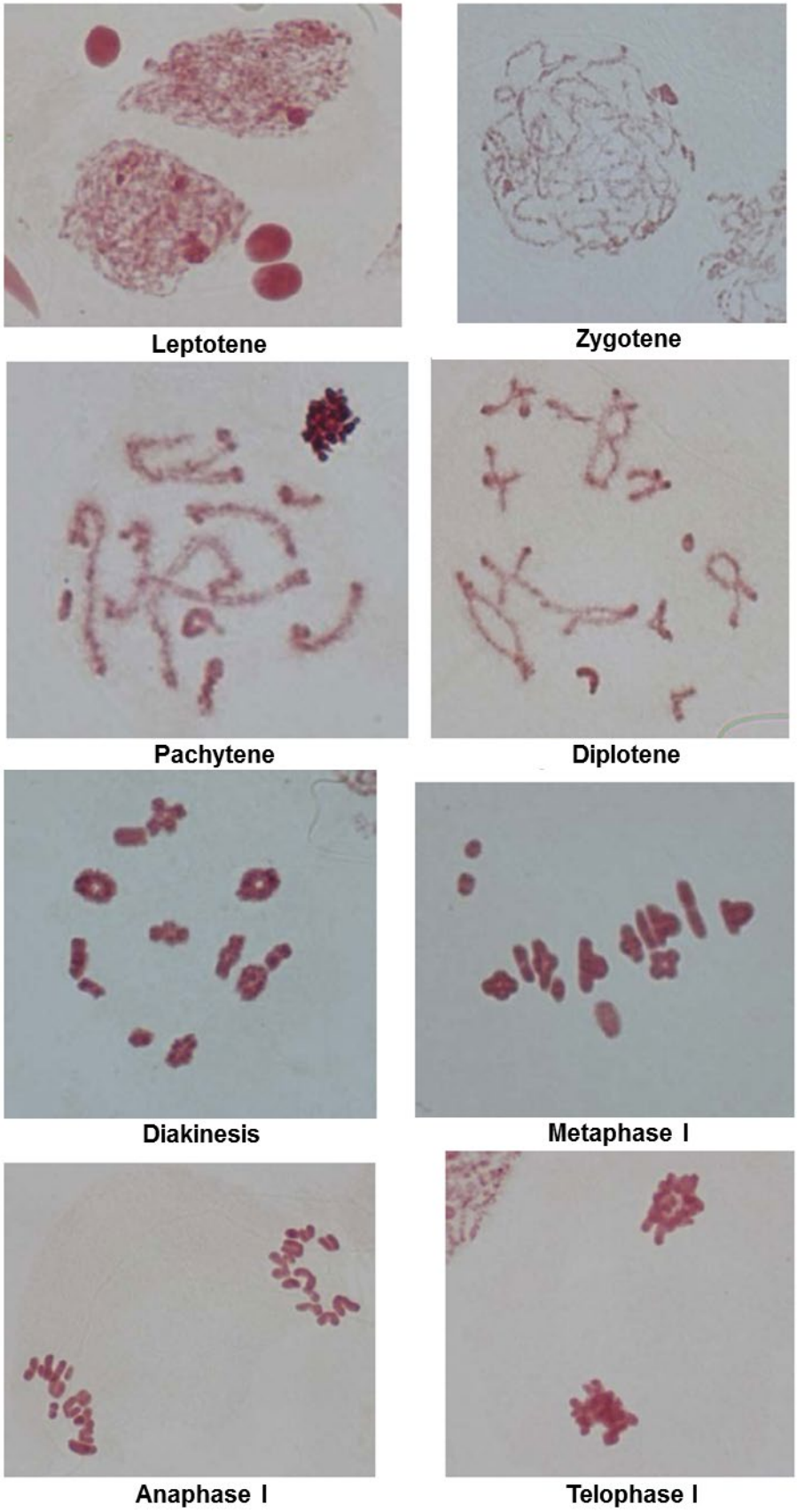


Figure 1: Steps of meiosis I

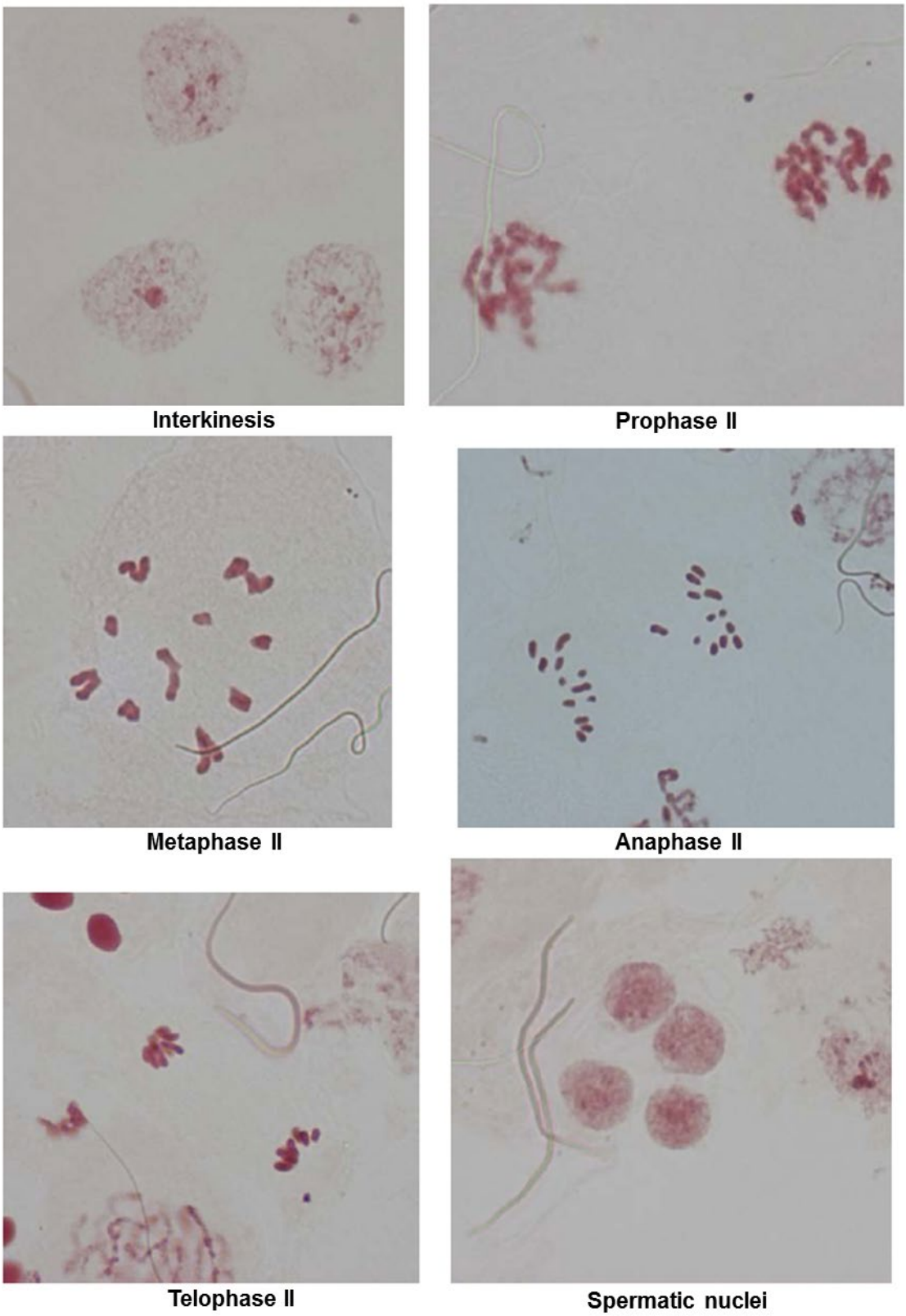


Figure 2: Steps of meiosis II

5. QUESTIONS

1. Describe the differences between meiosis I and meiosis II
2. Do the two observed poles of anaphase I have the same chromosome number? What about the poles of anaphase II?
3. Indicate the number of bivalents that can be counted in a cell
4. Indicate the number of chiasmata observed in diplotene
5. How do we distinguish the X chomosome from the autosomes?

EXPERIMENTAL DROSOPHILA CROSSES

EXPERIMENTAL DROSOPHILA CROSSES

OBJECTIVES

The objectives of this practice are twofold:

1. To deduce and apply Mendelian principles, as the fundamental basis of heredity, and to understand their exceptions. Thus, the type of inheritance of a character can be determined by performing controlled crosses and analyzing the phenotypic frequencies of the offspring obtained (cross 1).
2. To understand the phenomenon of linkage by calculating the relative distance between two genes on the same chromosome that are responsible for two mutations in Drosophila melanogaster. Use of recombination frequencies for the realization of genetic maps (cross 2).

2. TASKS

To collect, analyse and process the data obtained in the laboratory from the offspring of two experimental crosses. The first, in relation to objective 1, using single mutants of Drosophila melanogaster and the second, in relation to objective 2, with double mutants. Finally, relevant conclusions will be drawn by answering the questions in the questionnaire at the end of this section.

3. RELATED THEORY PROGRAM TOPICS

This project is mainly related to the contents of Units 1, 2, 3 and 4, whose contents are as follows:

Unit 1. Mendelian genetic analysis: The method of Mendelian genetic analysis. Principle of segregation. Principle of independent transmission. Genealogical trees. Calculation of probabilities. Statistical testing of segregations: χ^2 test.

Unit 2. Chromosomal basis of inheritance: mitosis and meiosis. Genetic significance of mitosis and meiosis. Chromosomal theory of inheritance and its proof.

Unit 3. Extensions of Mendelism: Genes on sex chromosomes. Variations in dominance relationships. Multiple allelism. Lethal genes. Pleiotropy. Gene interaction and epistasis. Allelism test: complementation. Penetrance and expressivity. Sex-limited or sex-limited traits. Gene-environment interaction. Cytoplasmic inheritance. Maternal effect.

Unit 4. Ligation and recombination. Genetic maps: Linkage. Recombination. Recombination frequency and its significance. Map distances. Genetic maps: two-point and three-point maps. Interference and coincidence coefficient. Somatic recombination. Molecular mechanism of homologous recombination.

4. BASIC NOTIONS ABOUT DROSOPHILA CULTURE:

Differentiation between sexes: the fruit fly presents a clear sexual dimorphism as described below. For the correct development of the practice it is necessary to be familiar with the morphological characteristics of both sexes. This will allow us to differentiate both sexes easily (Figure 1).

Features of females:
a) They are usually larger than males, although this may vary according to the stage of development the individual is at.
b) Abdomen with a pointed end and thicker than that of the male.
c) Dorsum of the abdomen with dark transverse bands separated from each other up to the end of the abdomen.

Features of males:
(a) Generally smaller in size than females.
b) End of the abdomen rounded.
c) The last transverse bands of the abdomen are fused, giving a dark appearance at the end of the abdomen.
d) They have a sexual comb (modified chelae) on the first tarsal segment of the first pair of legs.

Life cycle: The life cycle of this organism lasts about two weeks. The different stages of the life cycle are described below.

a) Egg: White and covered with a thick envelope called chorion, with small appendages at the upper end that serve to float in a liquid medium.

b) Larva: This is the growth phase. White in colour, it lives in the medium in which it perforates channels. It has three larval stages, with two molts.

c) Pupa: When the larva prepares to pupate, it moves away from the humidity of the culture medium and climbs up the walls of the glass tube to a relatively dry area where it fixes. The prepupa presents a white and flexible skin with spiracles, progressively hardening (white pupa stage) and becoming darker with time (mature pupa).

d) Imago - This phase begins when the fly emerges from the pupal envelope. It usually measures 2 to 3 mm in length and weighs 0.8 to 1.5 mg, depending on whether it is male or female, respectively. The adult is not pigmented until 3-4 hours after hatching. In addition, the wings are folded during the first hour of life. Sexual maturity is reached by 6 hours after hatching.

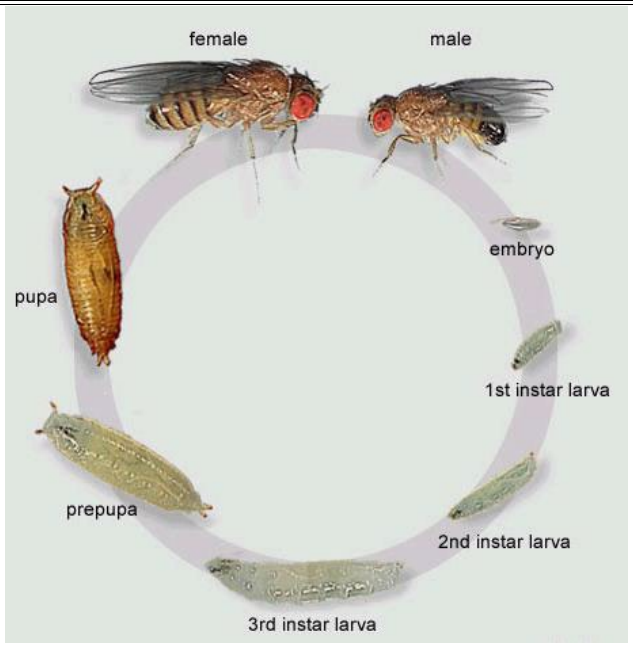


Figure 1.- Morphology and life cycle of the fruit fly.

5. METHODOLOGY

The complete protocol to be used throughout this activity is as follows:

1. Material needed:

-Flies with the sepia mutation (cross 1).
-Flies with the miniature mutation (cross 1)
- Double mutant flies white/miniature (cross 2)
- Wild flies (cross 2)
-Glass tubes.
-Cotton plugs.
-Marker pens and labels.
-Yeast plate with yeast.
-Dissection material.
-Watch glasses.
-Blotting paper.
-Ethyl ether.
-Magnifying glass.
-Oven between 25-28ºC.

2. Development of the practice

First week (These procedures will be carried out by department personnel. Attendance is optional):

Cross 1: Obtaining virgin miniature females: From a vial with pupae of miniature mutants, all adults are extracted and 4 hours are left to elapse until the flies emerge. The males are separated from the females (of these, those that have just emerged have meconium and a lightly pigmented body, indicating that they are virgins). Those that have been emerged for a longer time do not have meconium but remain virgins until 8 hours later, when they begin to mate.

To perform the crossing, two miniature virgin females are taken for each sepia male and are introduced in the same vial.

Cross 2: Virgin white/miniature females are obtained and crossed with wild males, following the already known protocol.

Second week (This procedure will be carried out by department personnel. Attendance is optional):

Adults of the parental generation are removed so that they do not mate with the offspring. In the cross 2, F_1 individuals are maintained in the vials so they can produce a new generation F_2.

Third week: (This will take place in the practice to be carried out by all students)

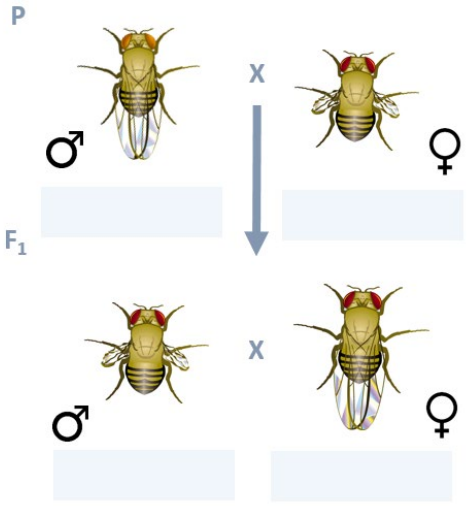
Observation of F_1 (cross 1) and F_2 (cross 2), annotation and interpretation of results of both crosses.

6. QUESTIONS

In relation to cross 1:

1. Determine the type of inheritance of the characters studied.

2. Assign a genotype to the individuals of the following scheme, taking into account the two characters.



3. Indicate the number of individuals obtained from each genotype.

4. Taking into account only the miniature trait: what conclusions can be drawn about the result of this cross? Could it be related to any of Mendel's Laws?

5. Considering only the sepia characteristic: what conclusions can be drawn about the result of this cross? Could it be related to any of Mendel's Laws?

6. Considering the results of both traits at the same time globally: what conclusions can be drawn? With which Mendel's Law could the combined study of the inheritance of both traits be related?

7. Test the hypothesis using a χ^2 test.

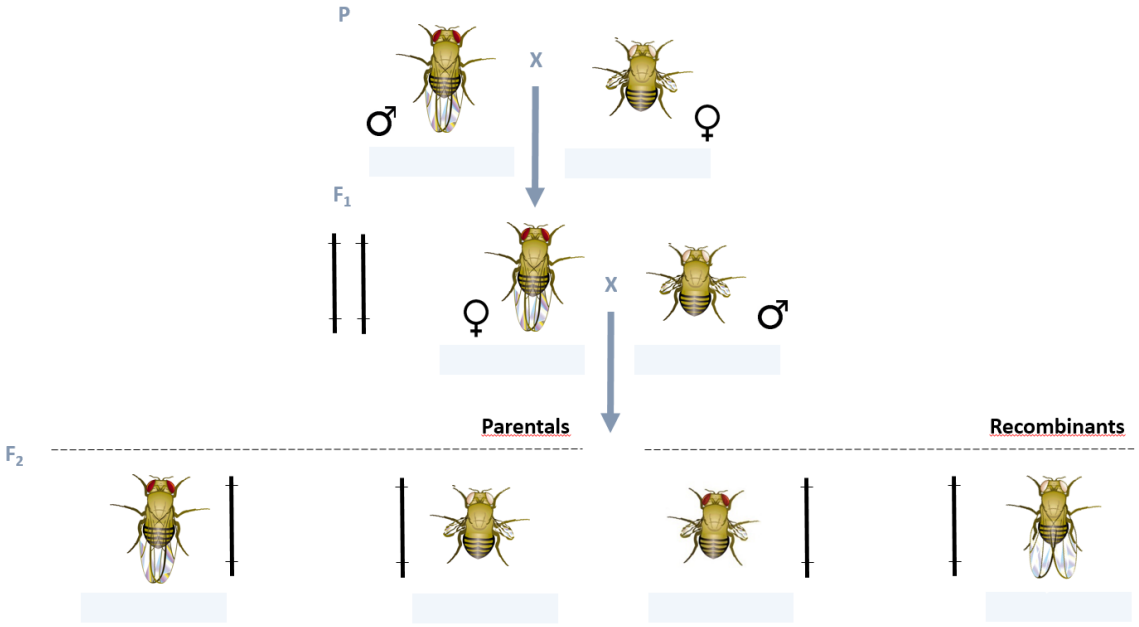
8. Could the proposed hypothesis be extrapolated to any pair of genes that we want to consider?

9. What offspring and in what proportions would be expected in a hypothetical F_2?

10. What is the relationship between the principles of inheritance that we have just deduced and the events that take place during the processes of cell division?

In relation to cross 2:

1. Assign a genotype to the individuals in this scheme taking into account the two characters.



2. Indicate the number of individuals obtained from each genotype and determine the type of inheritance of the characters studied.

3. What conclusions can be obtained from the result of the crosses carried out?

4. What is the position of the genes responsible for the white and miniature mutations? How far apart are they?

5. If, instead of using a white/miniature mutant in the crosses, we had used a vestigial/ebony mutant: would we have obtained similar results? Why?

6. Starting from a population of yellow males and white females: design an experiment to calculate the distance between the genes responsible for both mutations. Use the following scheme to help you.

7. Predict the offspring of a hypothetical F_2 obtained from a reciprocal cross of the test cross we have made. Reason your answer.

8. What is the relationship between a linkage map and the events that take place during cell division processes?