25/10-GENETICS
thousands of organisms have been studied at a genomic level> we can compare
slide 18: the sanger sequencing method allows to carry out 1 sequence reaction at a time and each sequence reaction can read max 700bp
the development of NGS sequencing method brought to always sequencing small fragments, but with this method sequencings of different fragments can be done in parallel
moore's law= developed by computer scientistis, which considers that normally there is a doubling of computer power every 2 years
nowadays to sequence a genom it costs less than 1000 dollars and it takes few days
slide 20:
the human genome is an haploid genome, that contains 20 000 genes. On average in human genome there is a one common variation (polymorphs?) over 1000 between one individual and another
slide 22: 3000 nucleotides (which is only the 0.0001 %) of human genome sequence
only 1.5% of the human genome is made of coding sequences> to be translated into proteins
10%=non coding conserved sequences> they have resisted in mutations> so they must be important (because if sequences resisted to mutations they are likely to be functions for the expression of genes)> in fact they are regulatory sequences
epigenomic sequences> involved in epigenomic marks:
the structure of Dna is threedimensional and sometimes dna can be with proteins creating CROMATINE> usually cannot be expressed when it is closed> but with epigenetic sequences it can be open
epigenetic marks=those changes in the dna that do not involve a change in Dna sequence (mutation)
the great majority of sequences in the dna have some biological function

MENDEL
phenotype=way in which a trait is expressed
by studying how the phenotype is transmitted from the parents to the offspring he laid the foundations of transmission genetics
>before mendel people had already tried to study inheritance and genetics, especially botanics

pre-mendelian theories were based mainly on 2 concepts:
>the pangenesis concept implied that there some substances (gemmules) that were transported from different part of the bodies to the reproduction organs and passed to the offspring
>the concept of blending inheritance: the traits were like liquids: they could be mixed and so the offspring would have sort of an average of the characteristics of the parents

the success of mendel was based on making a complex problem simple
mendel studied only one thing at a time> so that it was less complex> sort of breaking things into different units and study each one individually
true-breeding strains= always maintain its characteristics across generations

they knew that plants had sexual reproduction like mammals:
in pea plants they knew that the male cell (sperm) is contained in pollen grains> and it fertilizes the egg
zygote= male germ cell+ female germ cell

advantage of pea plants: self-fertilization= normally is the sperm of the pollen of the same flower whose egg is fertilized
cross fertilization= (ex: cross a purple flower with a white one)
remove the anthers from the flower before pollination takes place in order to avoid self-fertilization, then take the pollen from another flower and transfer it to the flower
=cross between 2 different varieties
True-breeding varieties
he selected 7 traits> the one which were giving him clearer results
monohybrid crosses =an hybrid is obtained by cross fertilization using two opposite varieties
hybrid= plant obtained by crossing 2 different alternative varieties
"mono"=because they were different only because of 1 trait
F1 generation= first generation=all the seeds looked like only one of the parents >this demonstrates that the concept of blending inheritance is false
F2 generation= obtained by the self fertilization of the f1 generation
>he performed this procedure using all the traits he had selected> on of the 2 varieties (which different by only one trait) always disappeared in the first generation and then it reappeared in a 3:1 ratio
same results with reciprocal crosses: it didn't matter which parent (male or female) had the character and from which parents started

26/10-GENETICS
Mendel's first law:
unit factors are present in pairs> each individual has a pair of unit factor that specifies a certain trait
each parent gives an unit to the offspring
Punnet square: to understand the reason way we should expect a 3:1 ratio for dominant:recessive
inside the square there is the outcome and on the two sides we can write the units present in the two parents(eggs and pollen grains)
in F1 the phenotype is equal to only one parent but the genotypes show that they are actually different from the parents
the dominant trait is the one showed in the phenotype
an allele is one of the two unit factors
Dihybrid crosses : another series of experiment
what happens when there are more than 1 trait? are the trait independent from one another?
the two traits: round(dominant)/wrinkle yellow(dominant)/green
in the f1 generation as expected both the dominant alleles were expressed
2 hypotheses: the two traits are linked > there are 2 possible combinations
or they are independent > 4 possible combinations of the alleles
In the F2 generation he observed new combinations, which were not seen in the parents (parents only have either both dominant or both recessive) >

BASIC PRINCIPLES OF PROBABILITY AND HYPOTHESIS TESTING
Probabilities and predicting the outcome of a genetic cross
Probability
=mathematical measure of likelihood (how likely is something is to happen?)

- empirical probability
- theoretical probability

Example: Consider a typical 6-sided die. What is the probability of rolling a "3"?

Basic notions of probability

$p(A)$ = the probability of outcome A.

The value must lie within the range of 0.0 and 1.0

$p(A,B)$ = the probability of outcome A or outcome B (or both). This is often referred to as **joint probability**.

SUM rule (the "OR" rule)

If outcomes A and B are ***mutually exclusive***, then $p(A,B) = p(A) + p(B)$.
Put another way, the joint probability of outcomes A , B equals the sum of their individual probabilities.

$p(AB)$ = the probability of simultaneous outcomes A and B.

PRODUCT rule (the "AND" rule)

If outcomes A and B are ***independent***, then $p(AB) = p(A) \times p(B)$.

Examples

- Consider a cross between two heterozygous (*Aa*) individuals. What are the odds of getting an *aa* individual in the next generation?

(probability of mother contributing *a*) x (probability of father contributing *a*) = (1/2)·(1/2)=1/4

- Consider a cross between two heterozygous (*Aa*) individuals. What is the fraction of that will have the dominant phenotype?

Possible genotypes
- AA P= ¼
- Aa P= ¼
- aA P= ¼

chance of any of these events = ¼ + ¼ + ¼ = 3/4

- Suppose you have crossed two plants with genotypes: A/a; b/b; C/c; D/d; E/e and A/a; B/b; C/c; d/d; E/e

what is the probability of getting a progeny plant with all recessive phenotypes?

1/4 *1/2*1/4*1/2*1/4=1/256

- In a cross between heterozygous pea plants: SsYy x SsYy

What is the chance of getting a progeny plant that either shows both dominant phenotypes (smooth and yellow seeds) or both recessive phenotypes (wrinkled and green seeds)?

9/16 + 1/16 = 10/16

Conditional probability

Conditional probability -> measure of the probability of an event given that another event has occurred.

A = event of interest

B = conditioning event

Probability of A given B -> **P (A|B)**

If $P(A|B) = P(A)$ or $P(B|A) = P(B)$, then events A and B are said to be independent

$$P(A|B) = \frac{P(A \cap B)}{P(B)}$$

Given two events A and B, the conditional probability of A given B is defined as the quotient of the probability of the **joint of events A and B**, and the **probability of B**

If event B is necessary for A, then P A∩B = P(A) and P (A|B) = P(A)/P(B)

Conditional probability example

In a monohybrid cross of **tall** plant x **short** plant, all F1 offspring is **tall**
if F1 individuals are cross-fertized, what is the probability that a tall plant in the F2 progeny is also heterozygote?
A = heterozygote
B = conditioning event = Tall
This may be visualized as restricting the sample space to *B* = Tall

ALL EXERCISES ON VIRTUALE EXCEPT 1

HYPOTHESIS TESTING

Population and samples

Population=the set of all possible states of a random variable. The size may be infinite or finite.
Random sample= subset of the population; its size is always finite.
From the random sample we can try to estimate the parameters of the population

HYPOTHESIS TESTING

>Formulate a scientific idea: a hypothesis (Ho)
>Apply a statistical test to accept or reject the hypothesis with high confidence, based on the observed data
> The statistical test is used to contrast the **observed** results (our data) to **expected** results (those predicted by the hypothesis being tested)
>*pi-value*= the probability of obtaining a value of a test statistic greater or equal to the value observed in the sample (under Ho) just by chance
When the probability calculated from statistical test is high, it is assumed that chance alone produced the difference. When the probability is low, it is assumed that a significant factor other than chance produced the deviation (there is something wrong)

HYPOTHESIS TESTING

Chi-square test (goodness of fit test)

ex=Mendel's dihybrid cross:
1)observed data
2)formulated a hypothesis
3)are the data consisted with the hypothesis?
From the table we can see there is a little deviation,
but in Mendel's time this theory on hypothesis testing wasn't invented yet and he was okay with the results.
>Hugo de Vries experiment> similar to Mendel but using another plant
HYPOTHESIS TESTING- chi-square test
CATEGORICAL (qualitative) data=a commonly used statistical method to determine goodness of fit is the **chi square test** (**χ2**) (Pearson's test)
1)Formulate a Null Hypothesis: **Ho**

2)Evaluate the **goodness of fit** between the observed data and the data that are predicted from a hypothesis
3)Reject or not reject the Ho.
4)If not rejected, any observed deviations are attributed to chance alone

The chi-square statistics is used to used to estimate how frequently the observed deviation can be expected to occur strictly as a result of chance

o is the observed value for a given category
e is the expected value for that category under H0

The chi-square **p-value** indicates the probability that **chance alone** produced the deviation between the expected and the observed values
p-value high>it is assumed that chance alone produced the difference
p-value low> it is assumed that a significant factor other than chance produced the deviation

HYPOTHESIS TESTING
>Set a **significance level (usually** α = 0.05 or 5%)
> **calculate P-value** (the probability of observing a test statistic as extreme as the results really observed during the test, assuming the null hypothesis is true
>if the p-value is less than the significance level, we reject the null hypothesis

Hypothesis testing - Chi-square test

$$\chi^2 = \Sigma \frac{(o - e)^2}{e}$$

cumulative probability (chance of obtaining a sample statistic as extreme as the test statistic)

Different chi-square distributions depending on the degrees of freedom
degrees of freedom = number of independently varying parameters
=n of independent classes (n-1)

>on the top there are the corresponding p values, on the left the degrees of freedom
>in this table are reported all the critical values corresponding to a p-value

CHROMOSOMES
MEIOSIS AND MITOSIS (chapter 3 book)

The discovery of chromosomes

1900>rediscovery of Mendel's work (H. DeVries, C. Correns, E. von Tschermak-Seysenegg)

>1879> Walter Flemming discovered chromosomes and cell division (mitosis)
He saw that when cells were dividing inside the cells there were this colored structure (which were then called chromosomes), which had probably something to do with the passing of genetic material within daughter cells, as the structures were dividing: one part in each of the 2 daughter cells

Chromosome theory of inheritance

>1902 **Walter Sutton** and **Theodor Boveri** independently described chromosomes during **meiosis**, cell division in germ cells
>Sutton proposed the **chromosomal theory of inheritance>** "the association of paternal and maternal chromosomes in pairs and their subsequent separation during the reduction division...may constitute the physical basis of the Mendelian law of heredity."
>Confirmed by the work of Morgan and colleagues using *Drosophila*

>PROCARIOTES: circular chromosome in the cytoplasm (nucleoid)

>EUCARIOTES>linear chromosomes in the nucleus
>Eukaryotic chromosomes are packaged into chromatin
>the structure of chromatin is highly compact, in order to be expressed chromatin must be open the opening and closing of chromatin must be regulated
>epigenetics is one of the mechanisms with which chromatin is made more or less accessible

Cell division in prokariotes (bacteria)

>Cells divide by **binary fission** (simple) >the chromosome replicates to produce two identical copies
>These two copies segregate from each other, with one copy going to each daughter cell.

Cell division in eukaryotes

Two different cell division processes

Mitosis

- takes place in **somatic cells (**all cells expect germ cells)
- produces two daughter cells with the same genetic component as the parent cell.
- Chromosomes are replicated prior to mitosis (S phase) and are divided so that each daughter cell receives a copy of every chromosome.

Meiosis

- takes place in **germ cells** (cells which form the male and female gamete)
- produces **haploid gametes** (gametes with half the chromosomes of the parental cells) from **diploid** cells
- Chromosomes replication prior to meiosis (S phase) followed by two rounds of nuclear and cell division
- daughter cell receives half of the chromosomes (one

of each pair of homologous chromosomes)

- generates **diversity**: daughter cells are different from

mother cell

The chromosome complement

- Cells from individuals of the same species contain the same number of chromosomes
- In the nucleus of **somatic** cell chromosomes are usually present in pairs: **homologous chromosomes** (in diploid organisms), each deriving from a parent
- The **gametes** that unite in fertilization to produce the diploid somatic cells have nuclei that contain only one set of chromosomes (**haploid**), consisting of one member of each pair

• **n** = **haploid** number; **2n diploid** number

Human >n = 23 (46 total chromosomes) Dog>n = 39 (78 total chromosomes)
Drosophila>n = 4 (8 total chromosomes) Garden pea>n = 7 (14 total chromosomes)

Diploid versus haploid: 2n versus n

- somatic cells are **diploid** (2n) (each chromosome pair has one maternal and one paternal copy)
- Meiosis> generates **haploid** (*n*) gametes
  - In Drosophila,*2n*=8, *n*=4
  - In humans,*2n*=46 and *n*=23
- Two gametes fuse during fertilization to form a **zygote**.
- Zygotes are diploid (2n) – carry two matching sets of chromosomes

Cytogenetics: chromosomes under the microscope

=genetic field that allows to observate chromosomes under the microscope
Chromosome bending=technique to study the structure of chromosome
SLIDE 6 Karyotipe= picture of the chromosomes under the microscope
the two sister chromatids are joined together by the kinetochore

Homologous chromosomes and sister chromatids

>sister chromatids are identical copies of a replicated chromosome
>homologs are copies of the same chromosome (one from father, one from mother) can have different alleles for some genes
>nonhomologs carry completely unrelated sets of genes

Chromosomes have distictive shapes and sizes

The cell cycle

alternation between phase of division and phase of non-division
before mitosis cells must replicate their chromosomes
G phases=checkpoint= to make sure it is the right time for the cell to divide

>late s phase > the DNA double helix is replicated and so the DNA content will be 4C
>each daughter phase will receive a single chromatin and so we go back to the G1 phase

Mitosis in brief

S phase> DNA replication takes place: each chromosome becomes two sister chromatids attached to each other. Chromosomes are extended forming chromatin
Prophase> chromosome start to condense and so to become visible under the microscope
Metaphase> chromosomes line up at the equatorial plane
Anaphase> sister chromatids are pulled to the opposite poles by microtubules
Telophase> chromatids are separated to the opposite poles. Nuclear membrane reforms and cell divide-at the end 2 daughter cells will be generated, having the same genetic material as the starting cell

The nuclear spindle

>Spindle fibers play a fundamental role in movement and separation of chromosome in mitosis and meiosis

Kinetochore and sister chromatid separation

Meiosis in brief

aim= create gametes that are haploid from a diploid cell> done by equally dividing the 2 homologous chromosomes