

**MINISTRY OF SCIENCE AND HIGHER EDUCATION
RUSSIAN FEDERATION**

**FEDERAL STATE AUTONOMOUS EDUCATIONAL INSTITUTION OF
HIGHER EDUCATION
NORTH-CAUCASUS FEDERAL UNIVERSITY**

Methodological Guidelines
for Students on arranging and performing laboratory works
in the Discipline of Biology: Introduction to Genetics
for Students majoring (Specialty) in 31.05.01 General Medicine

Laboratory Work No. 1

Theme 1. Mendel's Laws.

Objective of the work: to consolidate theoretical knowledge of the laws of heredity discovered by G. Mendel.

Equipment and materials: notebooks, pens, pencils, two pairs of coins of different denominations.

Procedure

Small samples and populations can show significant deviations from expected genetic patterns.

Task 1. Imagine that you can grow only four plants in each generation. Let's check if this is enough to demonstrate a 3:1 segregation.

You have a pea variety with a dwarf stem and purple flowers, and a second variety with a tall stem and white flowers. In the F1 generation, it produced all four plants with a tall stem and purple flowers.

Simulate what kind of offspring can be obtained in subsequent generations using the coins provided to you.

For each trait and each plant, you need to simultaneously toss two coins. To avoid confusion, use different pairs of coins for the height trait and the flower color trait. Designate one side of the coin as the dominant allele and the other as the recessive allele. Then the combination of the sides of the two coins will correspond to the genotype of an individual plant for one gene. Based on your simulation, fill in Table 1 below using the following allele designations: tall height – T, dwarfism – t, purple flowers – P, white flowers – p. Genes P and T are inherited independently of each other.

Write below the crossing scheme for these pea varieties.

P: x F1: F2:

Table 1. Characteristics of the simulated F2 generation.

	Plant 1	Plant 2	Plant 3	Plant 4
Genotype obtained during simulation for genes P and T				
Phenotype corresponding to the genotype (describe in words)				
Theoretical probability of this phenotype				

What is the probability that in a sample size of 4 individuals in the offspring from a cross between two heterozygotes Aa x Aa, three descendants will have the dominant phenotype and one will have the recessive phenotype? To solve this, you need to find the probability of one elementary combination (for example, the first plant is recessive, and the second, third, and fourth are dominant) and the number of these elementary combinations. For the number of elementary combinations, you can use the combination formula:

$$C_{mn} = n! / (m!(n-m)!)$$

n is the population size, m is the size of the sample taken from the population, n! is the product of natural numbers from 1 to n.

Answer the questions: 1. What is the probability of one elementary combination? 2. What is the number of elementary combinations in this case? 3. What is the probability of obtaining 3 dominant phenotypes and 1 recessive in a sample of 4 individuals?

Task 2. Now imagine that we have the opportunity to test Mendel's third law on a larger sample. We are given 160 plants from the F₂ generation. Among them were 11 dwarf plants with white flowers, 26 dwarf plants with purple flowers, 31 tall plants with white flowers and 92 tall plants with purple flowers.

Compare the obtained results with the expected ones using the χ^2 criterion.

Fill in Table 2, calculate the value of the χ^2 criterion and evaluate the degree of correspondence between the observed data and the expected segregation. To calculate the χ^2 criterion, use the formula:

$$\chi^2 = (O - E)^2 / E$$

O is the observed number of individuals in a specific phenotypic class, and E is the theoretically expected number of individuals in the same phenotypic class. Use the critical value $\chi^2 = 7.81$ for comparison.

Table 2. Characteristics of the given F₂ generation.

Phenotypic class	Dwarf white	Dwarf purple	Tall white	Tall purple
Observed (O)	11	26	31	92
Expected (E)				

Answer the questions: 1. What is the value of χ^2 ? 2. Does it exceed the critical value of $\chi^2 = 7.81$? 3. Can we confirm the assumption of independent inheritance of the stem height and flower color traits?

Laboratory Work No. 2

Theme 2. Gene interaction. Inheritance of the AB0 blood group system

Objective of the work: to verify in practice the features of inheritance of the AB0 blood group system.

Equipment and materials: notebooks, pens, pencils, rulers.

The AB0 blood group system was discovered in 1901 by K. Landsteiner. Under the AB0 system, four main blood groups (phenotypes) are distinguished, namely: 0 (I), A (II), B (III), AB (IV).

The gene locus for these alleles is located on the long arm of chromosome 9. The main products of the first of these genes — genes A and B, but not gene 0 — are specific glycosyltransferase enzymes.

They determine the presence of agglutinogens on the surface of erythrocyte cell membranes.

Human blood plasma may contain anti-A and anti-B antibodies (α -, β -hemagglutinins). If blood contains (during a transfusion) both erythrocytes with A antigens and anti-A antibodies in the blood plasma, agglutination of erythrocytes occurs, the same happens in the presence of B antigens and anti-B antibodies.

Thus, there are 4 permissible combinations of phenotype with 6 possible genotypes: which of them is characteristic of a given person determines their blood group.

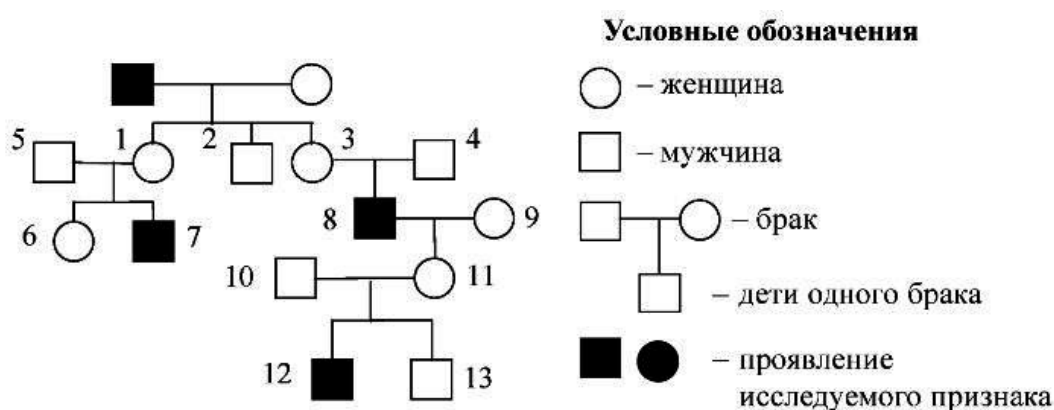
The presence of antigens on erythrocytes is determined by 3 types of genes: I^A – dominant, codes for the formation of antigen A, I^B — dominant, codes for the formation of antigen B, I^O – recessive, does not code for the formation of antigens:

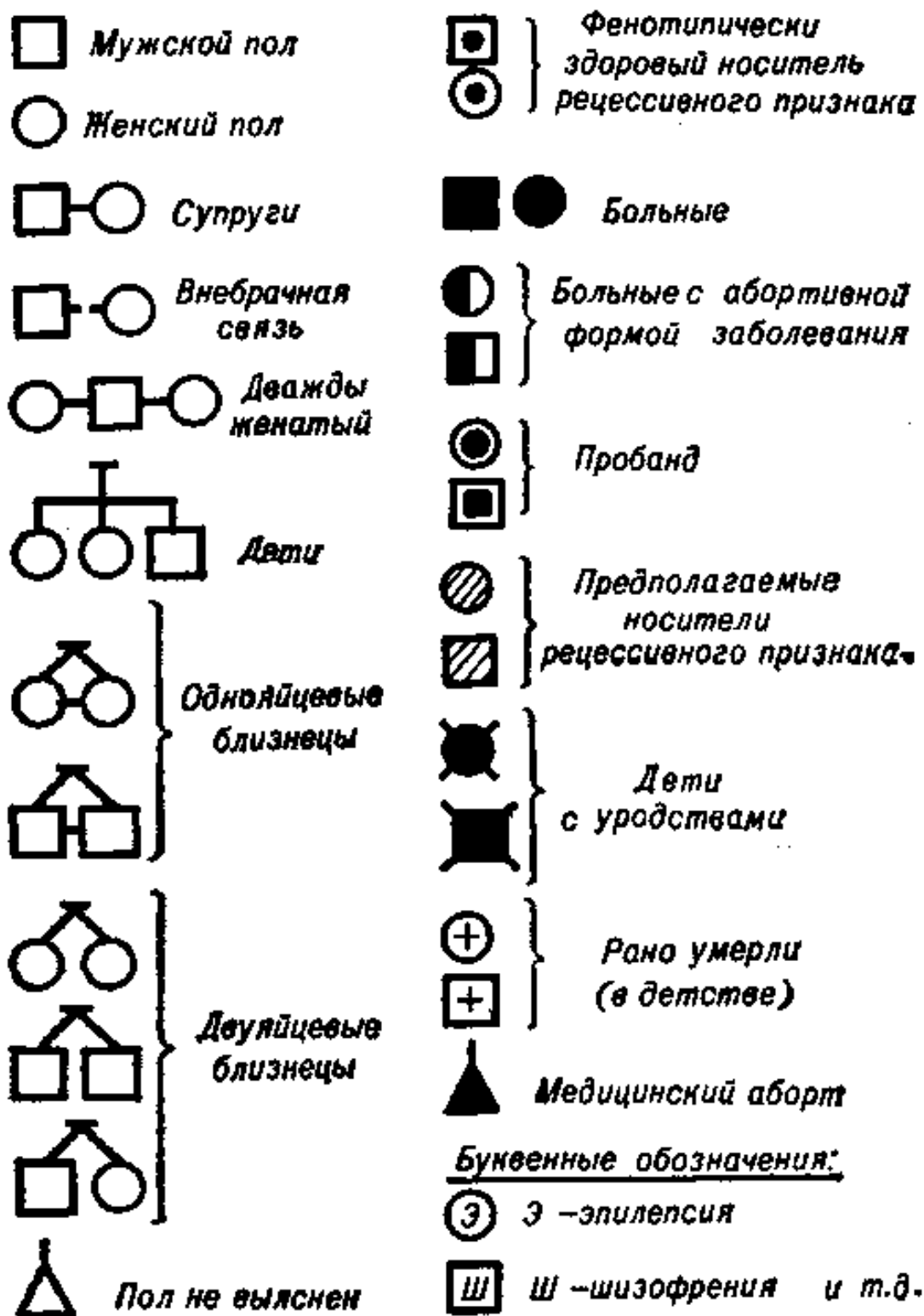
- 0 (I) $\alpha\beta$ – genes $I^O I^O$, no A and B hemagglutinogens on erythrocytes, α - and β -hemagglutinins in plasma (universal donors of erythrocyte mass in the absence of incompatibility with other blood group systems).
- A (II) β – genes $I^A I^A$ or $I^A I^O$, A hemagglutinogens on erythrocytes, β -hemagglutinins in plasma.
- B (III) α – genes $I^B I^B$ or $I^B I^O$, B hemagglutinogens on erythrocytes, α -hemagglutinins in plasma.
- AB (IV) $\alpha\beta$ – genes $I^A I^B$, A and B hemagglutinogens on erythrocytes, no α - and β -hemagglutinins in plasma (universal donors of blood plasma in the absence of incompatibility with other blood group systems).

Thus, the inheritance of the AB0 blood group system occurs according to the codominant-recessive type.

Procedure

Task 1. Create your own pedigree (or the pedigree of an acquaintance) for the AB0 blood group system, involving the maximum possible number of relatives, using the following symbols:





Task 2. Based on available data, try to determine their genotypes. If someone's blood group is not reliably known, try to guess what it might be and with what probability.

Task 3. Compare your data with the data of your classmates. Which blood group is most common in your group? Calculate the frequencies of the IA, IB, IO alleles in your group.

Answer the questions: 1. Do the observed data correspond to generally accepted ideas about the inheritance of blood groups? 2. How can the observed differences from the theoretically expected ones be explained, if any?

Laboratory Work No. 3

Theme 3. Cell cycle. Mitosis.

Objective of the work: Consolidate knowledge about the division of somatic and germ cells.

Equipment and materials: notebooks, pens, pencils, microscopes, a set of “onion root mitosis” microslides.

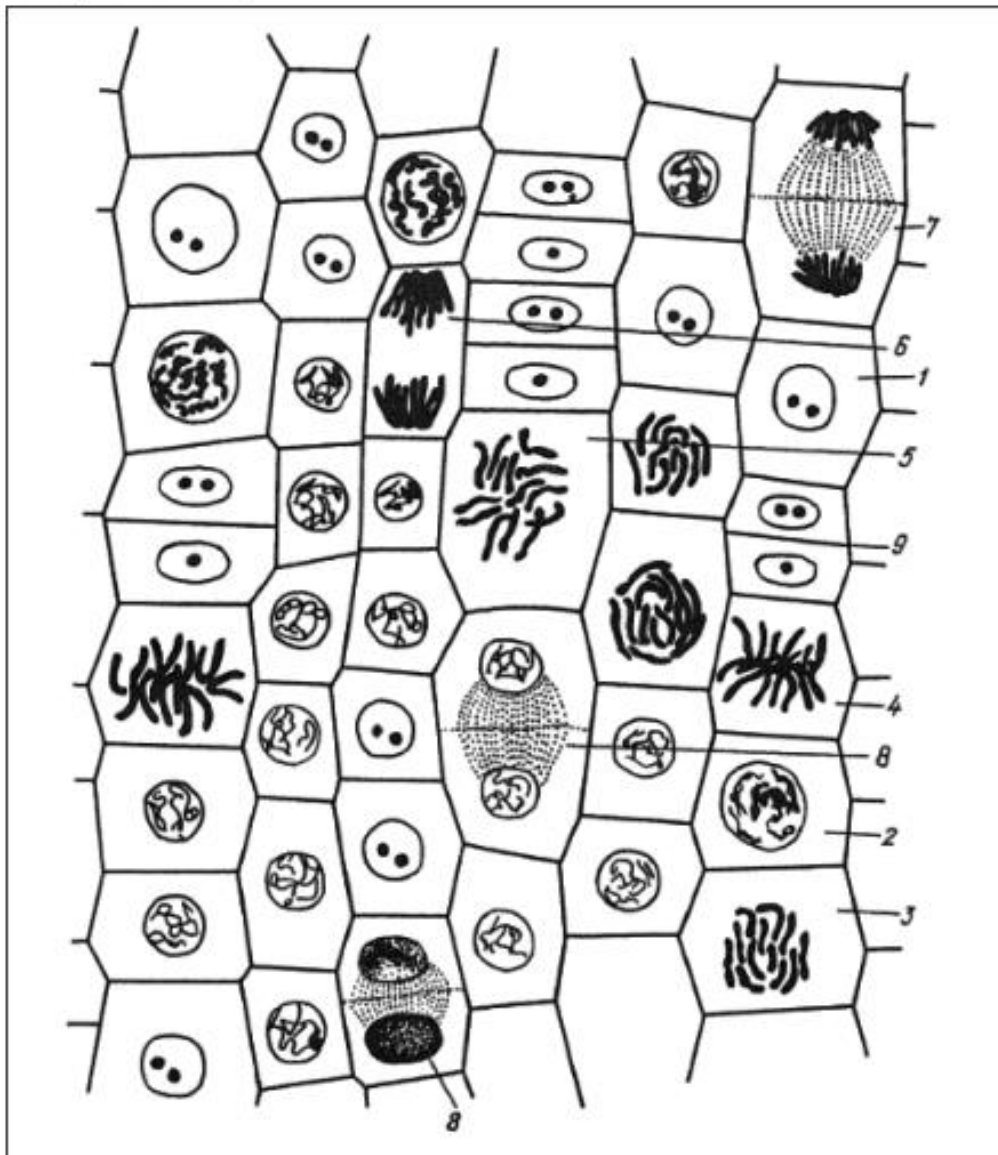
Mitosis is the indirect division of a cell, the most common method of reproduction for eukaryotic cells. The biological significance of mitosis lies in the strictly equal distribution of chromosomes between daughter nuclei, which ensures the formation of genetically identical daughter cells and preserves continuity in a series of cell generations.

Phases of mitosis:

Prophase: * Occurs condensation of chromosomes inside the nucleus. * Disintegration of the nuclear envelope. * Centrioles (if present) move to the poles of the cell, and the formation of the spindle apparatus begins in the cell cytoplasm. The spindle apparatus consists of microtubules made of the tubulin protein. * Kinetochores mature on the chromosomes, and they attach to the microtubules.

Metaphase: * Kinetochores attach the chromosomes to the spindle apparatus and align them along the cell equator into the metaphase plate.

Схема митотического цикла в кончике корешка лука



Anaphase: * Microtubules shorten, pulling the chromosomes to the poles of the cell. * Polar microtubules elongate, preparing the cell for cytokinesis.

Telophase: * Chromosomes reach the poles. * Kinetochores disappear. * The nuclear envelope is restored. * The nucleus is formed. * Chromosomes decondense. * Cytokinesis occurs.

Fig. Mitotic cycle in onion root tip cells (according to "Botany". V.G. Khrzhanovsky. S.F. Ponomarenko, 1988): 1 — interphase, 2-9 — mitosis (2-3 — prophase, 4-5 — metaphase, 6-7 — anaphase, 8 — telophase, 9 — cytokinesis).

Procedure

Task 1. Examine the "Onion root mitosis" microslide under a microscope. Find cells in the following phases of the cell cycle: * Interphase * Early prophase * Late prophase * Metaphase * Anaphase * Telophase

Call the instructor and show them all stages of the life cycle (or photograph them and show the instructor later). Draw them in your notebook, making the appropriate labels. Indicate two-chromatid chromosomes, single-chromatid chromosomes, cell poles, and spindle fibers.

Task 2. Find a cell in the late prophase stage of mitosis. Count the number of spiralized chromosomes. Answer the questions: 1. How many DNA molecules are currently in the onion root cell? 2. What is the diploid set of chromosomes for an onion? 3. How many linkage groups does an onion have?

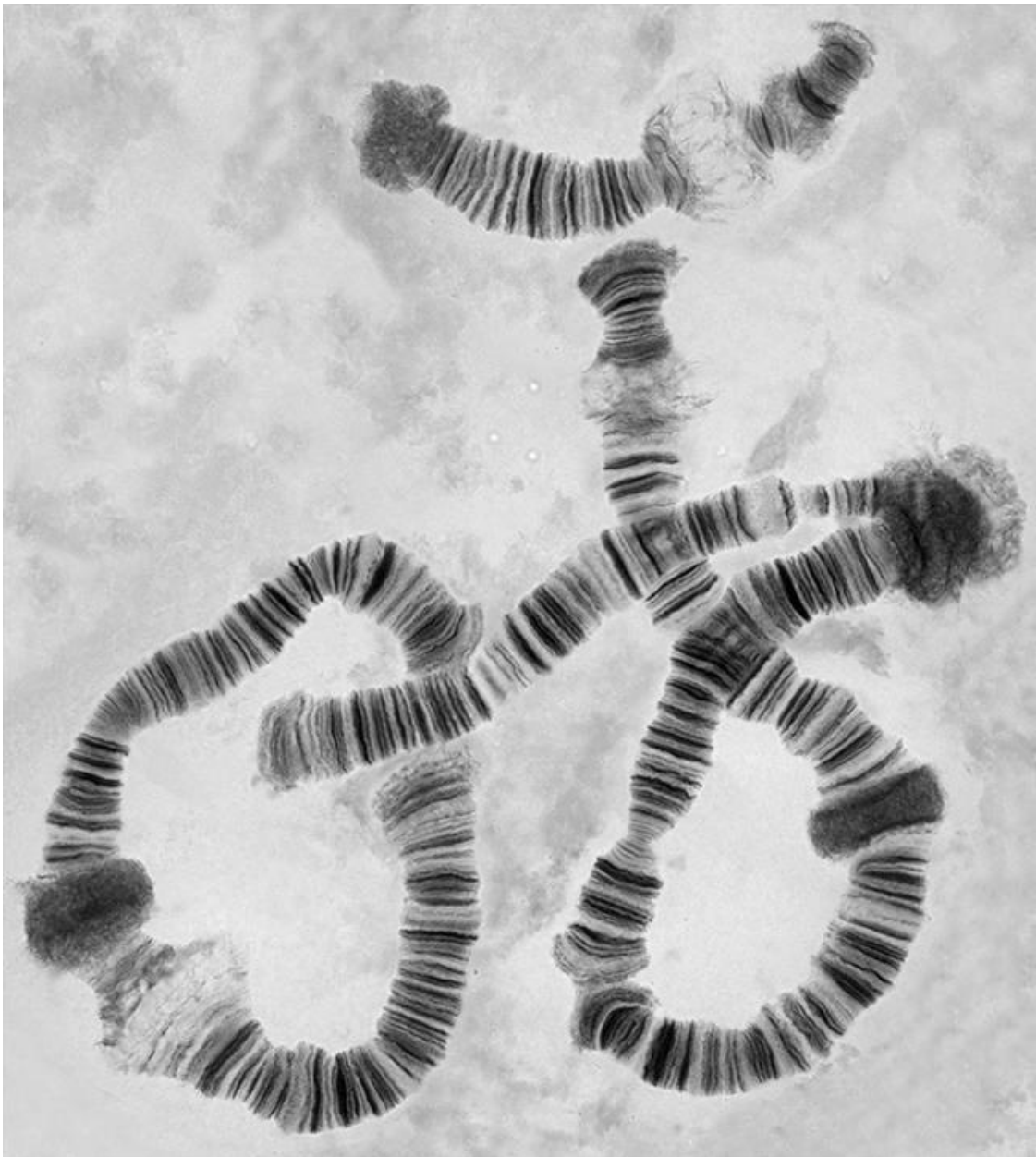
Laboratory Work No. 4

Theme: Preparation of temporary mounts of polytene chromosomes from Chironomidae salivary glands

Objective of the work: independently prepare a mount of polytene chromosomes from the salivary glands of a non-biting midge (Chironomidae), verify the presence of giant chromosomes.

Equipment and materials: binocular, microscope, alcohol lamp, bath, microscope slides and coverslips, tweezers, blade, dissecting needles, cotton wool, 2% acetocarmine, 96% ethanol.

Polytene chromosomes are giant interphase chromosomes that arise in certain types of specialized cells as a result of two processes: firstly, multiple DNA replication not followed by cell division, and secondly, lateral conjugation of chromatids. Cells containing polytene chromosomes lose their ability to divide, they are differentiated and actively secreting, that is, polytenization of chromosomes is a way to increase the number of gene copies for synthesizing a specific product. Polytene chromosomes can be observed in Diptera, in plant cells associated with embryo development, and in ciliates during macronucleus formation.



A fundamental difference from other types of chromosomes is that polytene chromosomes are interphase chromosomes, whereas all others can only be observed during mitotic or meiotic cell division.

The reason for the appearance of polytene chromosomes is DNA replication in cells not accompanied by their division. This leads to the accumulation of newly built DNA strands. These strands are tightly connected to each other along their length. In addition, somatic synapsis of homologous chromosomes occurs in the salivary glands, that is, not only sister chromatids conjugate with each other, but also homologous chromosomes of each pair conjugate with each other. Thus, a haploid number of chromosomes can be observed in the cells of the salivary glands.

The characteristic shape and size of polytene chromosomes are achieved due to their maximum despiralization and multiple reproduction of chromosomes without their subsequent segregation, that is, they form as a result of endomitosis.

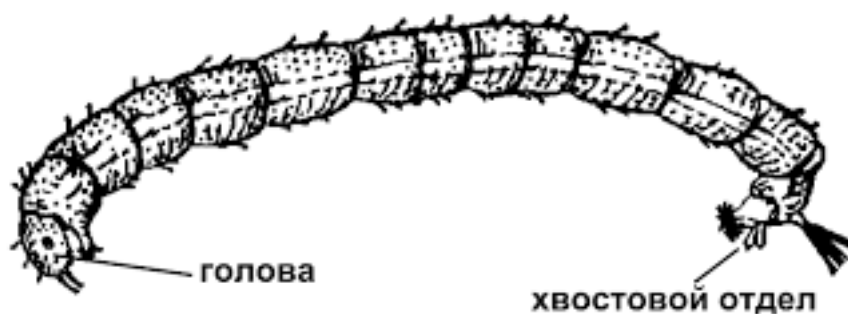
Polytene chromosomes have a characteristic transverse banding, caused by the presence of areas of denser spiralization of the chromonemata – chromomeres. The dark regions

(i.e. chromomeres) contain spiralized inactive chromatin, while the light bands indicate a region with increased transcriptional activity. The clear distinction between dark bands and light interband regions is explained by the non-segregation of daughter chromonemata. For this reason, all features of a single chromonema, including the chromomere pattern, become more vividly expressed.

In essence, polytene chromosomes represent a pair of giant homologous chromosomes in a state of perfectly precise somatic conjugation. At the same time, the bands and interband regions of the homologs are located strictly parallel and closely aligned. Such conjugation is not characteristic of the vast majority of somatic cells.

Procedure:

1. Degrease the microscope slides with a cotton swab moistened with ethanol.
2. Place the microscope slide under the binocular and fix it with clips.



3. Place a non-biting midge larva on a dry, degreased slide.
4. Determine the position of the head and tail under the binocular. Find the 2nd and 3rd segments from the head side.
5. Holding the head with tweezers, make an incision with a blade between the 2nd and 3rd segments and immediately separate the front part from the rest of the body.
6. Carefully extract the salivary gland using a dissecting needle. The salivary glands of the non-biting midge are paired formations resembling a bunch of grapes, located on both sides of the esophagus.
7. Remove body parts from the microscope slide. Leave the salivary glands on the slide in their own hemolymph.
8. Check the integrity of the salivary gland under a microscope at 4x magnification. Secretory cells with nuclei are clearly visible on the mount.
9. Stain and fix the mount: 9.1. Drop 5-7 drops of 2% acetocarmine onto the microscope slide with the salivary gland. 9.2. Heat the mount over an alcohol lamp flame for 2 minutes, preventing the stain from overheating or drying out.
10. Cover the mount with a dry coverslip so as not to introduce air bubbles. Place filter paper over the glass and press lightly with your finger.
11. Examine and draw the stained mount under the microscope at 4x objective magnification. Secretory cells with nuclei are clearly visible on the mount. Draw the general view of the non-biting midge's salivary gland.
12. Switch the microscope to 10x objective magnification, examine and draw the nuclei of the salivary gland secretory cells.
13. Switch the microscope to 40x objective magnification, examine and draw ribbons of polytene chromosomes with euchromatic and heterochromatic regions within the nuclei.

14. Draw conclusions regarding the laboratory work.

Laboratory Work No. 5

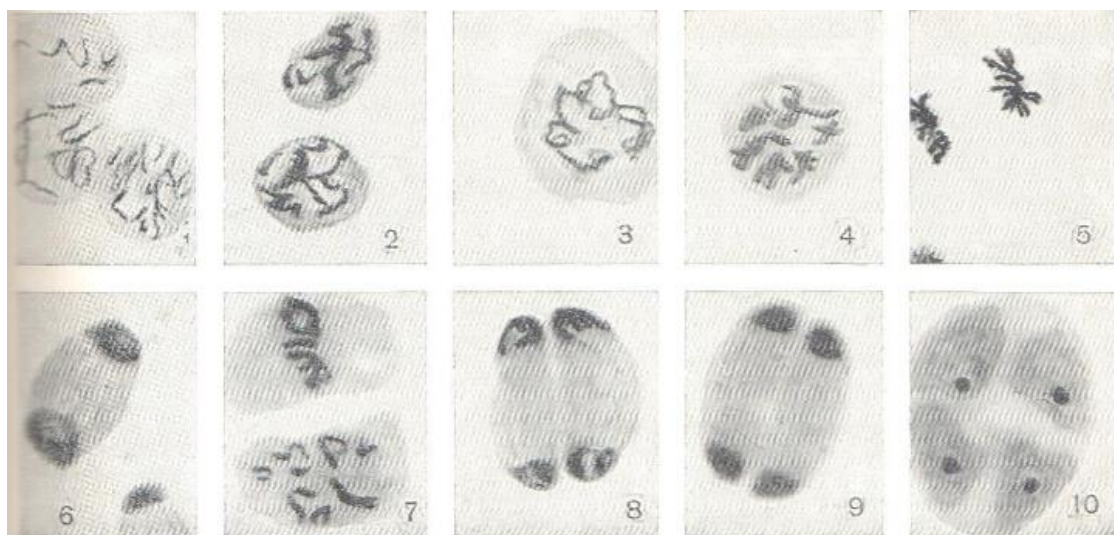
Theme: Study of meiosis in anthers of flowering plants

Objective of the work: study the phases and stages of meiosis during the formation of germ cells in plants.

Equipment and materials: buds of daylily, onion, lupine, tradescantia fixed with acetic alcohol (fixation time 10-12 hours) and stored in 70% alcohol; microscopes, dissecting needles, microscope slides and coverslips, pipettes, filter paper strips, matches, tweezers, porcelain dishes for distributing plant objects, Petri dishes, stain (acetocarmine).

Most higher plants reproduce sexually. Sexual reproduction is associated with the formation of haploid cells and the subsequent fusion of male and female gametes. For the formation of haploid cells, there is a mechanism by which the number of chromosomes is halved, i.e., the cell transitions from a diploid state to a haploid state. This mechanism is carried out during a special division - meiosis. It is defined as a special type of division of differentiating germ cells or spores.

Meiosis consists of two subsequent divisions: reductional and equational. They are not equivalent, although they have the same phases: prophase, metaphase, anaphase, and telophase. During these divisions, four haploid cells are formed from one diploid cell, and the chromosomes duplicate only once. Compared to mitosis, meiosis has a number of distinctive features. One of them is that 99.7% of DNA is synthesized in the premeiotic interphase (as a result of which each chromosome becomes two-chromatid), and its remaining amount is formed in the prophase of the reductional division of meiosis - prophase I. During this same phase of meiosis, conjugation (temporary pairing) of homologous chromosomes occurs, leading to the formation of homologous pairs of chromosomes - bivalents. Thus, in anaphase I, members of each pair of homologous chromosomes diverge to the poles of the cell. At each pole, there is one homolog from the pair, and altogether – a haploid number of two-chromatid chromosomes. The process of separation of each pair of chromosomes proceeds independently of others, which creates the possibility for various combinations of chromosomes from the original diploid cell in the gametes.



Micrographs of meiosis stages in onion (*Allium cepa*) anthers: 1–3 – prophase I (1 – leptotema, 2 – pachynema, 3 – diplotema); 4 – metaphase I; 5 – anaphase I; 6 – telophase I; 7 – metaphase II; 8 – anaphase II; 9 – telophase II; 10 – pollen tetrad.

In the prophase of the first meiotic division, another important process occurs – crossing over – the exchange of homologous regions of non-sister chromatids of homologous chromosomes. Crossing over leads to a significant change in their gene composition, as a result of which the chromatids become recombined, creating the possibility for hereditary variation in the offspring.

Thus, thanks to meiosis, cells transition from a diploid to a haploid level, and in addition, the hereditary variability of gametes is ensured through various combinations of chromosomes, as well as the crossing over process.

Procedure:

1. Take a bud, and using a dissecting needle and tweezers, extract an anther about 2 mm long from it.
2. Place the anther in the center of the microscope slide and thoroughly crush it to a powder state using the tip of the dissecting needle.
3. Drop 2-3 drops of stain onto the crushed anther and cover the preparation with a Petri dish to prevent the stain from evaporating. Stain for 8-10 minutes.
4. Cover the object with a coverslip. To arrange the archesporium cells in a single layer, gently press on the coverslip with the end of a match.
5. Remove excess stain with a strip of filter paper. Under no circumstances should you draw the stain out from under the coverslip.
6. Place the finished microslide under the binocular microscope.
7. Using the instructional card of meiosis stages in onion anthers, find several division phases on your microslides. Draw them in your notebooks, supplementing them with labels.
8. Fill in the table “Differences between the phases of reductional division of meiosis and the phases of mitosis”.

Table. Differences between the phases of reductional division of meiosis and the phases of mitosis

Phase of mitosis	Event	Phase of meiosis I	Event
Prophase		Prophase I	
Metaphase		Metaphase I	
Anaphase		Anaphase I	
Telophase		Telophase I	

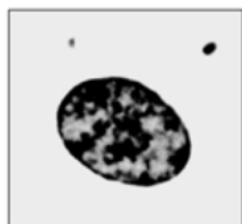
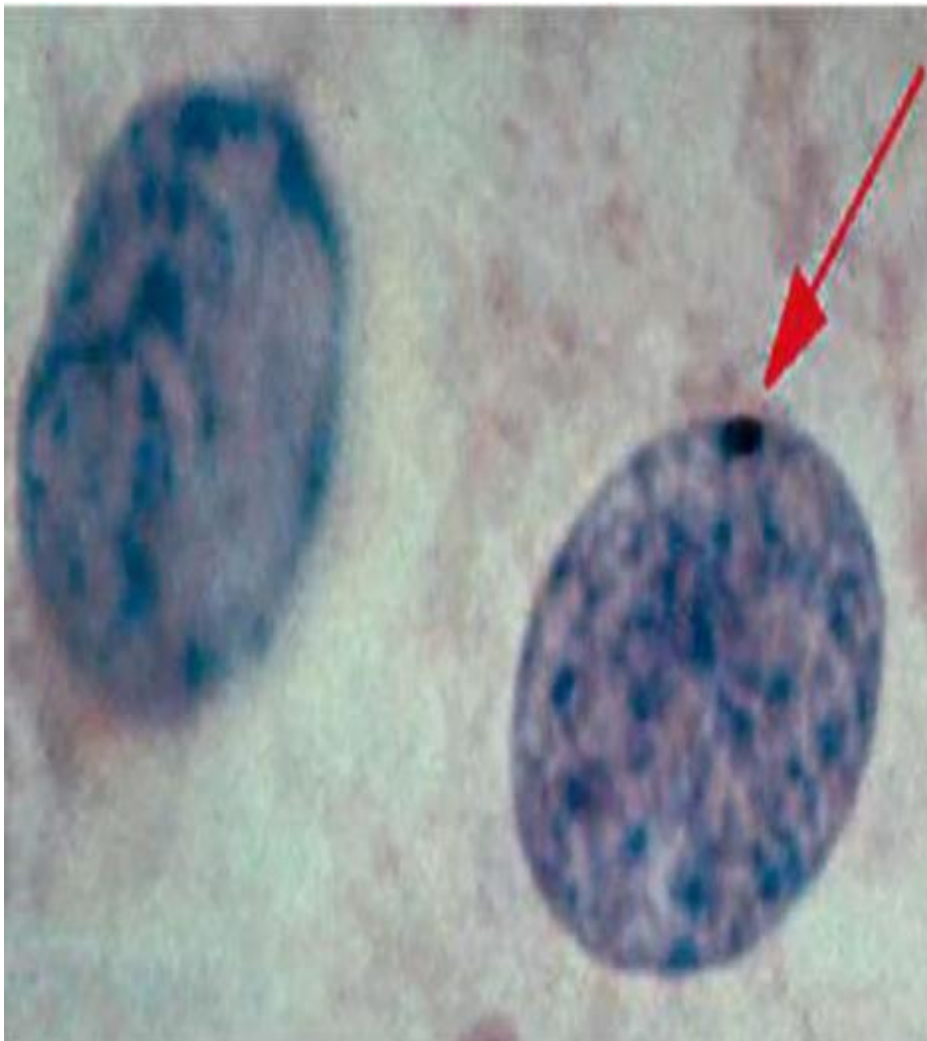
Laboratory Work No. 6

Theme: Determination of sex X-chromatin

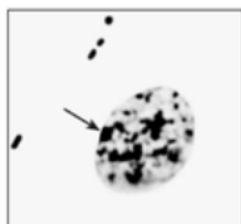
Objective of the work: learn how to make a buccal epithelium preparation; analyze the preparation under a microscope; count the number of cells having sex chromatin.

Equipment and materials: water, acetocarmine, test tube rack, sterile wipes, cotton swabs, microscope, dissecting needle, microscope slides and coverslips, pipette, medical gloves.

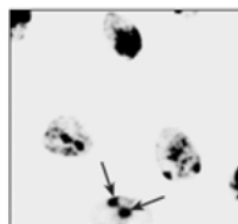
Sex chromatin (Barr body) is condensed chromatin (heterochromatin) containing the DNA of one of the two X chromosomes in the nuclei of female cells. In medical practice, for rapid diagnosis, sex chromatin is determined in the epithelial cells of the buccal mucosa or in a peripheral blood smear. In the nuclei of epithelial cells, sex chromatin is detected as a compact clump of chromatin called a Barr body. In peripheral blood leukocytes, sex chromatin is detected in the form of “drumsticks” – clumps of chromatin connected by a thin thread to the cell nucleus. The study of sex chromatin, apart from determining gender, also allows establishing anomalies in the number of X chromosomes.



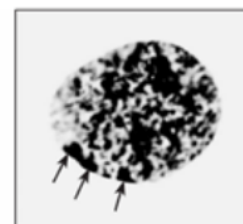
XV
Тельце Барра отсутствует
(нормальный мужчина)



XX
1 тельце Барра
(нормальная женщина)



XXX
2 тельца Барра



XXXX
3 тельца Барра

Procedure:

Preparation for sample collection Before starting the laboratory work, rinse your mouth with a small amount of water. Do not eat, drink, or chew gum before taking the sample.

Preparation of a temporary microslide: 1. Put medical gloves on your hands. 2. Take a clean cotton swab, avoiding touching the swab to anything other than the area for collecting the biomaterial. 3. Rub the mucosa intensely in the recess between the left gum and cheek with the cotton swab for 30 seconds, pressing the swab against the inner surface of the cheek. 4. With your mouth open, press your palm against your left cheek from the outside. The movements of the swab should be clearly felt by the palm from the outside. It is necessary to rub as large an area of the inner surface of the cheek as possible. 5. Perform the same manipulations with the right cheek. During the rubbing process, periodically rotate the swab around its axis so that it contacts the mucous membrane over the entire surface of the swab. 6. Next, degrease the surface of the microscope slide with a sterile wipe, dry it, and place the obtained biomaterial in the form of a smear. 7. Drop acetocarmine onto the smear and cover with a coverslip.

Study of buccal epithelial cells: 1. The finished preparation is placed under the microscope and examined at low magnification to detect epithelial cells (cells have large nuclei). 2. After that, it is necessary to examine individual cells in the preparation for the presence or absence of sex chromatin, which is located under the nuclear envelope in the form of intensely stained clumps. Magnification used is (40×10). 3. In the laboratory workbook, it is necessary to make a schematic drawing "Buccal epithelial cell". Indicate the boundaries of the nucleus, nucleoplasm, chromatin, and Barr body. 4. Count the number of Barr bodies in the nuclei of 50 epithelial cells. Count only nuclei with clear contours that have an intact nuclear envelope. 5. Enter all results into the table. Draw a conclusion about the sex of the investigated individual. In the case of a female karyotype (46, XX), sex chromatin is usually detected in 20-70% of normal buccal epithelial cells, while in individuals with a male karyotype (46, XY) it is found very rarely, so it is necessary to examine the nuclei of 50-100 cells for a reliable result.

Stage of work	What was seen	Conclusion
Sex chromatin		
Examination of epithelial cells for the presence of Barr bodies.	1. Number of studied cells - ... 2. Number of epithelial cells with Barr bodies - ...	

General conclusion: 1. Did you manage to detect sex chromatin in buccal epithelial cells?
2. Determine the sex based on the results of the cytological examination.

Laboratory Work No. 7

Theme: Molecular basis of heredity. Extraction of genomic DNA from buccal epithelium cells.

Objective of the work: To carry out the procedure for extracting DNA from cells and tissues, and to familiarize oneself with the method of enzymatic DNA extraction.

Equipment and materials: Sterile swab-probe in a tube, automatic pipette (100-1000 µl), sterile tips (100-1000 µl), laminar flow hood, vortex, high-speed centrifuge (for 1.5 ml tubes), thermostat for microtubes (1.5 ml), NanoDrop 2.0 spectrophotometer, microtubes

(DNase and RNase free 1.5 µl), ice pack, “GenPrep” reagent (Syntol LLC), scissors, tweezers, electric loop and instrument sterilizer, thin permanent marker, -30°C freezer.

Procedure

1. Heat the thermostat to 70°C.
2. Using a probe, the student should take a sample of their own buccal epithelium (from the inner surface of the cheek).
3. Using scissors, the end of the probe with buccal epithelium samples is cut off and placed in a 1.5 ml tube. The scissors are flamed in an electric loop and instrument sterilizer before each use. The tube containing the probe tip is placed in a rack.
4. Add 300 µl of “GenPrep” reagent to the sample. To do this, set the volume to 300 µl on an automatic 100-1000 µl pipette, put a filter tip (100-1000 µl) on the pipette, and transfer the required volume of solution into the 1.5 ml tube. The solution must be stored on an ice pack cooled in a refrigerator to 6-8°C.
5. Close the tube with a cap, vortex for 5-10 seconds, and centrifuge to spin down drops. Place the tubes in the thermostat and incubate for 15 minutes at 70°C, vortexing the tubes and briefly centrifuging every 2-3 minutes.
6. Remove and dispose of the cut ends of the probes from the tubes. The tweezers are flamed in an electric loop and instrument sterilizer before use.
7. Check the quantity and quality of the extracted DNA using a NanoDrop 2.0 spectrophotometer. Record its concentration and A260/A280 and A260/A230 values on the sample label.
8. Centrifuge the tube with the sample to spin down drops. Dispose of the probes as Class B medical waste.
9. Sign the tubes with a marker and put them in a Zip Lock bag. Label the bag with samples; students must fill out a questionnaire and consent form for submitting genetic material.
10. Place the bag of samples in the -30°C freezer.
11. Complete the report in the notebook.

Laboratory Work No. 8

Theme: Study of cytoplasmic heredity

Objective of the work: to study the phenomenon of cytoplasmic heredity, its mechanisms, and its role in the transmission of genetic information. To master methods for analyzing the inheritance of traits associated with cytoplasmic genes.

Theoretical part

Cytoplasmic heredity is the transmission of genetic information through organelles located in the cell cytoplasm (mitochondria and chloroplasts), rather than through nuclear chromosomes. Main features: 1. **Mitochondrial DNA (mtDNA):** A circular DNA molecule containing genes necessary for the functioning of mitochondria. Inherited maternally. 2. **Chloroplast DNA (cpDNA):** A circular DNA molecule containing genes necessary for photosynthesis. Inherited maternally in most plants. 3. **Inheritance features:** Does not obey Mendel’s laws. Manifests as a maternal effect.

Equipment and materials: microscope, preparations of plant cells (e.g., Elodea leaves), preparations of animal cells (e.g., oral mucosa cells), stains (e.g., methylene blue), DNA

extraction kit, PCR thermal cycler, primers for mitochondrial DNA amplification, agarose gel, electrophoresis unit, UV transilluminator.

Procedure

Part 1: Observation of cytoplasmic organelles. Prepare a temporary preparation of Elodea leaf cells: Place an Elodea leaf on a microscope slide. Add a drop of water and cover with a coverslip. Examine the preparation under the microscope at 400x magnification. Find the chloroplasts and draw them.

Prepare a preparation of oral mucosa cells: Using a cotton swab, take a smear from the inside of the cheek. Apply the smear to a microscope slide and stain with methylene blue. Examine the preparation under a microscope and find cells with mitochondria. Draw them.

Part 2: Extraction of mitochondrial DNA (if a kit is available). Perform cell lysis. Separate mitochondria using centrifugation. Extract DNA from mitochondria. Evaluate the concentration and purity of the DNA using a spectrophotometer.

Part 3: Amplification of mitochondrial DNA. Prepare the PCR reaction mix: Add DNA, primers, Taq polymerase, and nucleotides. Perform amplification in a PCR thermal cycler: * Denaturation: 95°C, 5 minutes. * 30 cycles: denaturation (95°C, 30 sec), annealing (55°C, 30 sec), elongation (72°C, 1 min). * Final elongation: 72°C, 10 minutes.

Perform electrophoresis of PCR products in agarose gel: Prepare a 1% agarose gel. Add ethidium bromide for DNA visualization. Load the samples and marker into the gel wells. Run electrophoresis at 100 V for 30–40 minutes. Visualize the results using a UV transilluminator.

Part 4: Analysis of results. Capture an image of the gel. Determine the sizes of the amplified fragments by comparing them with the marker. Draw conclusions about the presence and size of mitochondrial DNA.

Control questions: 1. What is cytoplasmic heredity? 2. Which organelles participate in cytoplasmic heredity? 3. Why is mitochondrial DNA inherited maternally? 4. What methods are used to study mitochondrial DNA? 5. How does cytoplasmic heredity affect the inheritance of traits?

Laboratory Work No. 9

Theme: PCR analysis as a method for detecting mutations

Objective of the work: the use of polymerase chain reaction (PCR), DNA restriction, and electrophoresis methods to detect mutations (polymorphisms) in animals and plants.

Equipment and materials: DNA extraction kit (e.g., Qiagen DNeasy Blood & Tissue Kit), primers for amplifying the target DNA region, PCR reagents (Taq polymerase, PCR buffer, dNTPs, MgCl₂), restriction enzymes and corresponding buffers, agarose gel, ethidium bromide (or another intercalating dye), electrophoresis unit and power supply, transilluminator for DNA visualization, animal tissue or blood samples.

Procedure

DNA Extraction. Using a commercial DNA extraction kit, extract DNA from animal tissue or blood samples according to the manufacturer's instructions. Assess the quality and

quantity of the DNA using a spectrophotometer (e.g., Nanodrop) or a fluorometer (e.g., Qubit). Prepare the DNA samples for further analysis.

Polymerase Chain Reaction (PCR). Prepare the PCR reaction mix, you can use a ready-made mixture: * DNA template: 50–100 ng. * Primers: 10 pmol of each. * dNTPs: 200 μ M of each. * Taq polymerase: 1 unit. * PCR buffer: 1x. * $MgCl_2$: 1.5–2.5 mM. * Water: up to a total volume of 25 μ l.

Perform amplification in a thermal cycler according to the following protocol: * Denaturation: 95°C, 5 min. * 30 cycles: Denaturation: 95°C, 30 sec. Annealing: 55–60°C (depending on primers), 30 sec. Elongation: 72°C, 30–60 sec (depending on amplicon length). * Final elongation: 72°C, 5 min.

Verify the success of the amplification via agarose gel electrophoresis (see step 4).

DNA Restriction. Prepare the restriction reaction mix: * PCR product: 10 μ l. * Restriction enzyme: 1 unit. * Restriction buffer: 1x. * Water: up to a total volume of 20 μ l.

Incubate the mixture at the optimal temperature for the selected restriction enzyme (usually 37°C) for 1–2 hours. Stop the reaction by heating the mixture to 65°C for 10 minutes (if necessary).

Agarose Gel Electrophoresis. Prepare a 1.5–2% agarose gel, adding ethidium bromide (0.5 μ g/ml) for DNA visualization.

Load the samples into the gel wells: * PCR product before restriction. * PCR product after restriction. * Molecular weight marker (e.g., 100 bp ladder).

Run the electrophoresis at a voltage of 80–100 V for 30–60 minutes. Visualize the results using a transilluminator.

Expected results: Successful amplification of the target DNA region (a clear band is visible on the gel). After restriction: If the mutation is absent, the expected fragment pattern will be observed. If the mutation is present, the fragment pattern will differ from the expected one (e.g., absence of cutting or the appearance of additional bands).

Task: Compare the obtained fragment patterns with the expected results for normal and mutant alleles. Determine the presence or absence of a genetic anomaly. Draw conclusions about the possible consequences of the detected anomaly for the animal.

Laboratory Work No. 10

Theme: Structure of the DNA molecule. Transfer of genetic information in the cell

Objective of the work: study the structure of the DNA molecule, its role in storing and transferring genetic information, as well as the processes of replication, transcription, and translation.

Theoretical part:

DNA (deoxyribonucleic acid) is a molecule consisting of two strands twisted into a double helix. Each strand consists of nucleotides, which include: A phosphate group. Deoxyribose (sugar). A nitrogenous base (adenine (A), thymine (T), guanine (G), cytosine (C)). DNA

strands are complementary: adenine pairs with thymine (A-T), guanine — with cytosine (G-C) via hydrogen bonds.

Transfer of genetic information: 1. **Replication:** the process of DNA duplication before cell division. 2. **Transcription:** synthesis of mRNA on a DNA template. 3. **Translation:** synthesis of protein on an mRNA template involving ribosomes and tRNA.

Equipment and materials: 1. DNA model (or image of DNA structure). 2. Computer with access to databases (e.g., NCBI, GenBank). 3. Sequence analysis software (e.g., SnapGene, BioEdit, etc.). 4. Table of the genetic code. 5. Examples of DNA and RNA sequences.

Procedure

1. **Study of DNA structure.** Examine the DNA model or its image. Pay attention to: The double helix. Nucleotide composition (A, T, G, C). Complementarity of strands (A-T, G-C). Determine which chemical groups form the “backbone” of the DNA molecule (phosphate and deoxyribose). Explain the role of hydrogen bonds in stabilizing the double helix.
2. **Modeling DNA replication.** Using a DNA sequence (e.g., 5'-ATGCCGTA-3'), build a complementary strand. 2.2. Explain how enzymes (DNA polymerase, helicase) participate in the replication process. Model the replication process by separating the strands and synthesizing new complementary strands.
3. **Modeling transcription.** Using the same DNA sequence, build a complementary mRNA strand. Note that in RNA, thymine (T) is replaced by uracil (U). Explain the role of RNA polymerase in the transcription process.
4. **Modeling translation.** Using the mRNA sequence, divide it into codons. Determine the amino acid sequence using the genetic code table. Explain the role of ribosomes, tRNA, and anticodons in the translation process.
5. **Comparison of DNA and RNA.** Compare the structure of DNA and RNA: Sugar type (deoxyribose in DNA, ribose in RNA). Presence of thymine (T) in DNA and uracil (U) in RNA. Single-stranded (RNA) vs. double-stranded (DNA) structure. Discuss the functions of DNA (information storage) and RNA (information transfer and protein synthesis).

Part 1: 1. Given the DNA sequence: 5'-ATG CCT GAA TTT GCA TAA-3' Write the complementary DNA strand. *Hint:* use the complementarity rule (A-T, G-C).

2. Based on the complementary DNA strand, write the sequence of mRNA. *Hint:* in RNA, thymine (T) is replaced by uracil (U).

Part 2: Determination of amino acid sequence 3. Using the genetic code table, determine the amino acid sequence encoded in the mRNA. *Example:* the AUG codon encodes methionine (Met).

4. Find the start and stop codons in the mRNA sequence. Explain their role.

Part 3: Analysis of the properties of the genetic code 5. Choose one of the codons and find in the genetic code table all possible codons that encode the same amino acid. *Example:* the amino acid “leucine” is encoded by the codons UUA, UUG, CUU, CUC, CUA, CUG

Таблица генетического кода					
Первое основание	Второе основание				Третье основание
	У(А)	Ц(Г)	А(Т)	Г(Ц)	
У(А)	Фен	Сер	Тир	Цис	У (А)
	Фен	Сер	Тир	Цис	Ц (Г)
	Лей	Сер	-	-	А (Т)
	Лей	Сер	-	Три	Г (Ц)
Ц(Г)	Лей	Про	Гис	Арг	У (А)
	Лей	Про	Гис	Арг	Ц (Г)
	Лей	Про	Глн	Арг	А (Т)
	Лей	Про	Глн	Арг	Г (Ц)
А(Т)	Иле	Тре	Аси	Сер	У (А)
	Иле	Тре	Аси	Сер	Ц (Г)
	Иле	Тре	Лиз	Арг	А (Т)
	Мет	Тре	Лиз	Арг	Г (Ц)
Г(Ц)	Вал	Ала	Асп	Гли	У (А)
	Вал	Ала	Асп	Гли	Ц (Г)
	Вал	Ала	Глу	Гли	А (Т)
	Вал	Ала	Глу	Гли	Г (Ц)

Laboratory Work No. 11

Theme: Linked inheritance. Crossing over

Objective of the work: study the phenomenon of linked inheritance of genes and the role of crossing over in the process of genetic material recombination. Perform an analysis of the inheritance of linked genes using model organisms.

Theoretical part

Linked inheritance. Genes located on the same chromosome are inherited together and are called linked. This phenomenon was discovered by Thomas Morgan when studying the inheritance of traits in *Drosophila*. Linked inheritance is disrupted as a result of crossing over — the exchange of regions between homologous chromosomes during prophase I of meiosis.

Crossing over. Crossing over leads to allele recombination and increases genetic diversity. The frequency of crossing over depends on the distance between genes: the further apart the genes are located, the higher the probability of crossing over between them. The frequency of crossing over is measured in map units or centimorgans (1 centimorgan = 1% crossing over).

Equipment and materials: data on the results of hybrid crosses (e.g., in *Drosophila* or plants), tables for recording crossing results, calculator for calculating crossing over frequency, software for constructing genetic maps (e.g., MapChart or others).

Procedure

Analysis of linked inheritance. Study the results of a hybrid cross of organisms differing in two or more linked traits (e.g., body color and wing shape in *Drosophila*). Record the phenotypes of the offspring and their numbers. Determine which combinations of traits are parental and which are the result of crossing over.

Calculation of crossing over frequency. Using the data on the offspring, calculate the crossing over frequency using the formula:

$$\text{Частота кроссинговера (\%)} = \frac{\text{Число рекомбинантных особей}}{\text{Общее число потомков}} \times 100$$

Determine the distance between genes in centimorgans (1% crossing over = 1 centimorgan).

Construction of a genetic map. Based on the calculated crossing over frequency, build a genetic map of the chromosome, indicating the location of the genes and the distance between them. Explain how the distance between genes affects the probability of crossing over.

Comparison with independent inheritance. Compare the results of linked inheritance with the expected results for independent inheritance (according to Mendel's law). Explain why linked genes do not yield a 9:3:3:1 Mendelian ratio in a dihybrid cross.

Expected results: Identification of linked inheritance of genes based on offspring analysis. Calculation of crossing over frequency and determination of the distance between genes. Construction of a genetic chromosome map. Understanding the role of crossing over in genetic diversity.

Laboratory Work No. 12

Theme: Structure and organization of the genome

Objective of the work: study the structure and organization of the genome using the example of model organisms. Analysis of genome components, including coding and non-coding sequences, as well as their functional features.

Theoretical part

The genome is the entirety of an organism's genetic information encoded in DNA (or RNA in some viruses). The genome includes: * **Coding sequences:** genes encoding proteins and functional RNAs. * **Non-coding sequences:** introns, regulatory elements (promoters, enhancers), repeats (satellites, transposons).

Organization of the genome: In prokaryotes: the genome is represented by a single circular DNA molecule compactly organized in the nucleoid. Genes are often organized into operons. In eukaryotes: the genome is divided into several linear chromosomes packed into chromatin. It contains introns and exons, regulatory elements, and a large number of repeats.

Equipment and materials: computer with access to databases (e.g., NCBI, Ensembl), sequence analysis software (e.g., SnapGene, Geneious), examples of genomic sequences (e.g., bacterium *E. coli*, human *Homo sapiens*, *Ovis aries*, etc.), tables with characteristics of genomes from different organisms.

Procedure

1. **Analysis of the prokaryotic genome.** Using the NCBI database, find the genome sequence of a prokaryotic organism (e.g., *E. coli*). Study the genome structure: Determine the genome size. Find the genes. Identify regulatory elements (promoters, operators). Compare the organization of the prokaryotic genome with expected features (lack of introns, compactness).
2. **Analysis of the eukaryotic genome.** Using the Ensembl database, find the genome sequence of a eukaryotic organism (e.g., human). Study the genome structure: Determine the genome size and the number of chromosomes. Find examples of genes with introns and exons. Identify regulatory elements (promoters, enhancers). Find repeats (satellites, transposons). Compare the organization of the eukaryotic genome with prokaryotes.
3. **Comparison of genomes of different organisms.** Choose two organisms (e.g., a bacterium and a human, or an animal). Compare their genomes by the following parameters:
 - Genome size.
 - Number of genes.
 - Presence of introns and exons.
 - Proportion of non-coding sequences. Explain the differences in genome organization.
4. **Functional genome analysis.** Choose a specific gene (e.g., the hemoglobin gene in humans). Perform an analysis of its structure: Find coding (exons) and non-coding (introns) regions. Determine the regulatory elements (promoter, enhancer). Explain how gene structure affects its function.

Expected results: Understanding the structure and organization of the genome in prokaryotes and eukaryotes. Ability to analyze genomic sequences and identify functional elements. Comparison of genomes from different organisms and explanation of their differences.

Laboratory Work No. 13

Theme: Determination of allele and genotype frequencies in a population on the example of taste sensitivity to phenylthiocarbamide (PTC)

Objective of the work: determination of allele and genotype frequencies in a population using the example of taste sensitivity to phenylthiocarbamide (PTC).

Equipment and materials: phenylthiocarbamide, distilled water, vials with a cap and dropper.

Theoretical part

Phenylthiocarbamide (phenylthiourea, PTC) is a white crystalline substance that some people (about 70%) find bitter to taste, while others find it tasteless.

Thus, the substance makes it possible to identify hereditary determined human polymorphism caused by the absence (or inactivity) of one of the receptor proteins. The body's response to this substance is determined by a single gene; the ability to taste phenylthiocarbamide is dominant over the inability to discern its taste.

Sensitivity to PTC is a genetic marker for a predisposition to gastric and duodenal ulcers.

Procedure

Task 1. Prepare PTC solutions. 1. In 100 ml of boiled water, dissolve a 260 mg portion of PTC (the salt is poorly soluble, so prolonged mixing of the solution is required!). To save resources, you can take 130 mg of salt per 50 ml of water. This yields the initial solution (dilution 0). 2. Pour 2 ml of the resulting solution into a penicillin vial and add the same amount of distilled water (dilution 1). 3. Pour 2 ml of the solution of dilution 1 into the next vial and add 2 ml of distilled water (dilution 2). 4. Similarly, prepare all subsequent dilutions (3–15), using the solutions of the previous dilutions. 5. Equip each vial with a cap and a dropper. Number the vials, caps, and droppers. Do not move droppers from one vial to another!

Task 2. Conduct an experiment in a group on sensitivity to PTC. The experimenter asks the test subject to open their mouth and stick out their tongue. They draw the solution with a dropper and drop 1-2 drops onto the tongue (caution: do not touch the tongue!). The test is carried out in the direction of increasing concentration, i.e., starting from the 15th dilution down to the 0th.

If the test subject says they feel a taste, you can do one more test (the next in increasing concentration) to verify. If they state that the intensity of the taste (bitter) is increasing, the previous dilution number is recorded.

Sometimes a false sensation arises (sweet taste, sour, etc., or an increase in bitterness is not observed with an increase in concentration) – in this case, the test for taste sensitivity is continued.

If a person feels dilution 0, the result is recorded as such – “0”. If they do not feel even the initial (0th) dilution, then “n/f” – “not felt” is recorded.

Record the obtained results in the table:

Subject No.	PTC Sensitivity Level (from 0 to 15)
-------------	--------------------------------------

1

2

3

4

...

Task 3. Calculation of the distribution of alleles for sensitivity and non-sensitivity to PTC in the “population”. Using the data from the previous task, calculate the frequency q of the allele for non-sensitivity to PTC. Remember that non-sensitivity is a recessive trait.

To do this, use the Hardy-Weinberg equation: $(pA + qa)^2 = p^2AA + 2pqAa + q^2aa = 1$

- Calculate the frequency of the dominant allele $p = 1 - q$.
- Determine the frequency of homozygotes for the dominant allele (p^2).

- Calculate the frequency of heterozygous genotypes ($2pq$).

List of Main Literature:

1. Genetics and genomics in medicine / Ronald Doron Cohn, Stephen W. Scherer, Ada Hamosh - Elsevier. – 2024. – 612 p.

List of Additional Literature:

1. Genetics: analysis & principles, Sixth edition / Robert J. Brooker - New York NY : McGraw-Hill Education. -2018. – 872 p.
2. Lewin's GENES XII / Jocelyn E. Krebs, Elliott S. Goldstein, Stephen T. Kilpatrick. - Jones & Bartlett Learning . – 2018. – 1025 p.

**MINISTRY OF SCIENCE AND HIGHER EDUCATION
RUSSIAN FEDERATION**

**FEDERAL STATE AUTONOMOUS EDUCATIONAL INSTITUTION OF
HIGHER EDUCATION
NORTH-CAUCASUS FEDERAL UNIVERSITY**

Methodological Guidelines
for Students on arranging and performing independent work
in the Discipline of Biology: Introduction to Genetics
for Students majoring (Specialty) in 31.05.01 General Medicine

Methodological Recommendations for Studying Theoretical Material

When performing the independent study of theoretical material, the student must follow the rules as set below. The working curriculum of the respective academic discipline shall be recommended to be used as the basis for such activity. According to the schedule of classroom lessons and independent work, a different number of hours is to be allocated for studying individual topics. A significant portion of the student's independent work should be allocated to mastering the material on the covered topic, writing a summary (notes) on the specified theoretical blocks, and searching for additional literature based on the NCFU library and via the Internet system. The lecture material shall not cover all theoretical material; the greater part of it must be mastered through independent work. Given the above, working with the recommended literature is mandatory. Special attention must be paid to the content of the main provisions and conclusions, the explanation of phenomena and facts, and the understanding of the practical application of the theoretical issues under consideration. In the process of this work, the student should strive to understand and memorize the main provisions of the material being considered, the examples illustrating it, as well as to understand the illustrative material. Preparation should be completed by drawing up a plan (summary/notes) on the material (issue) being studied. This allows for the creation of a concentrated, concise representation of the issues being studied. First, studying the recommended literature is to be done. Special attention must be paid to the content of the main provisions and conclusions, the explanation of phenomena and facts, and the understanding of the practical application of the theoretical issues under consideration.

During this work, the student should strive to understand and memorize the main provisions of the material being considered, the examples illustrating it, as well as to understand the illustrative material. Preparation should be completed by drawing up a plan (summary/notes) on the material (issue) being studied. This allows for the creation of a concentrated, concise representation of the issues being studied. If necessary, one should seek (advice) consultation from the instructor. When going to a consultation, it is necessary to thoroughly think through the questions that require clarification. Keeping records contributes to turning reading into an active process, mobilizing motor memory along with visual memory. The following should be born in mind: a student who systematically keeps records creates their own individual stock of auxiliary materials for a quick review of what has been read and for the mobilization of accumulated knowledge. Records are especially important and useful when they reflect the thoughts that arose during independent work.

It is important to develop the students' capacity to compare sources and to think through the material being studied.

Improving note-taking skills among students is of great importance.

The instructor may recommend the following main forms of recording to students: a plan (simple and detailed), excerpts, and theses (abstracts).

The results of note-taking can be presented in various forms.

- A plan is an outline of the read material, a brief (or detailed) list of questions reflecting the structure and sequence of the material.
- A detailed plan fully replaces a summary.
- A summary (notes) is a systematized, logical presentation of the source material. Four types of summaries are distinguished:
 - Outline-summary (Plan-notes) – this is an expanded, detailed plan in which sufficiently detailed records are provided for those points of the plan that need explanation.
 - Textual summary – this is the reproduction of the most important provisions and facts of the source.
 - Free summary – these are clearly and briefly formulated (stated) main provisions resulting from a deep understanding of the material. It may contain excerpts, quotations, and theses; part of the material may be represented by a plan.
 - Theme-based summary – this is compiled on the basis of studying a number of sources and gives a more or less exhaustive answer according to a certain scheme (issue).

Methodological Guidelines for the Preparation for Practical Activities

When preparing for practical activities, the recommendation implies studying the control questions for the given lesson and the theoretical material from one's own notes.

The main form of work during the lessons is an interview (discussion). Each student must be ready to speak on all issues raised in the plan and demonstrate maximum activity during their consideration.

The speech must be constructed freely, convincingly, and with arguments. The instructor ensures that the speech is not reduced to a reproductive level (simple reproduction of the text); simple reading of the summary is also not allowed.

It is necessary for the speaker to show their own attitude towards what they are talking about, express their personal opinion and understanding, substantiate it, and be able to draw the correct conclusions from what has been said. In doing so, the student may refer to the entries in the summary, directly to the primary sources, use knowledge of previously studied disciplines, etc. In addition, to obtain a grade at an advanced level, students solve situational tasks based on the results of the mastered topics.

During the execution of practical works, a defense of practical skills is provided, the full list of which is specified in the fund of assessment tools.

Methodological Recommendations for the Preparation of Presentations and Reports

The report must contain elements of novelty; along with the fundamental aspect, an analysis of the current state of the problem under study must be carried out, as well as inclusion in regional issues. The report must include an introduction, a theoretical part, a conclusion, a list of literature, and a presentation that reveals the essence of the topic in graphs, pictures, diagrams, and formulas.

The report is recommended to be submitted on 1 printed sheet. The text of the work must be printed with 1.5 line spacing on one side of a standard sheet (A4) of white paper.

The text and other printed elements of the work must be black, and the contours of letters and signs must be clear, without halos and shading. The font must be Times New Roman, size 14. The titles of chapters and paragraphs are highlighted in bold. The sheet with text must have margins: left – 30 mm, right – 10 mm, top – 20 mm, bottom – 20 mm.

Page numbering of the text is done in the lower right corner of the sheet. It is necessary to number pages starting from the page where the Introduction is printed, on which the number 3 is placed. After that, all pages, including appendices, are numbered.

A space equal to two intervals is placed between the title of the chapter and the title of the paragraph of this chapter, and the title of the paragraph must not be separated from the text of this paragraph by a space. The titles of paragraphs shall be separated from the text of the previous paragraph by a space equal to two intervals. Each chapter, as well as the introduction, conclusions, appendices, and the list of used literature, begin on a new page. The word Chapter is not to be written. Chapters have ordinal numbers within the entire work, designated by Arabic numerals (for example: 1, 2, 3), followed by a period.

The word **PARAGRAPH** or the paragraph symbol (§) is not placed in the title. Paragraphs have ordinal numbers within the chapters, designated by Arabic numerals (for example: 1.1. and 1.2.).

Headings of chapters and paragraphs in the text must be centered; a period shall not be placed at the end of the title of the chapter and paragraph. It is not allowed to hyphenate (transfer) a part of a word in a heading. 4.5. The numbering of tables and figures can be running (continuous) or relate to the number of the chapter and paragraph. For example, if a table or a figure is included in the text of the first paragraph of the second chapter, the numbering is as follows: Table 2.1.1., Fig. 2.1.1.

The last digit means the ordinal number of the table (or figure) in this paragraph.

The table is to be placed as the next page after the first mention of it in the text.