

Біологічна секція “The Importance of G. Gamow's Ideas for Biology of the 21st Century” у 6-ій Гамовській Міжнародній конференції, що проходила в серпні 2019 року в ОНУ імені І. І. Мечникова, присвячена 115-річному ювілею Г. А. Гамова

Кафедрою генетики і молекулярної біології біологічного факультету ОНУ імені І. І. Мечникова у цьому році вперше організовано біологічну секцію у 6-ій Гамовській міжнародній конференції “New Trends in Astrophysics, Cosmology and Radioastronomy after Gamow”, яка проходила спільно з 19-ою Гамовською літньою школою «Astronomy and beyond: Astrophysics, Cosmology, Gravitation, Cosmobiophysics, Radioastronomy and Astrobiology» 11-18 серпня в Одесі. Астрофізичні конференції щорічно проводяться астрофізиками ОНУ. Біологічна секція під назвою “The Importance of G. Gamow's Ideas for Biology of the 21st Century” мала засідання 12.08.2019 та була присвячена 115-річному ювілею Г. А. Гамова.

Георгій Антонович Гамов відомий фізик-теоретик та астрофізик, який народився в Одесі 4 березня 1904 року та навчався у Новоросійському університеті в 1921 році на математичному відділенні фізико-математичного факультету. Він зробив значний вклад в становлення молекулярної біології, вперше поставив проблему генетичного коду. Після відкриття та опублікування моделі дволанцюгової спіралі ДНК Дж. Уотсоном і Ф. Криком. Г. А. Гамов зрозумів, що структура білків в клітині, які складаються із 20 основних амінокислот, повинна бути зашифрована в послідовності з чотирьох нуклеотидів, що входять до складу молекули ДНК. За допомогою арифметичних розрахунків Г. А. Гамов показав, що при поєднанні 4 нуклеотидів трійками складаються 64 різні комбінації, чого цілком достатньо для «запису спадкової інформації».

Серед доповідачів пленарної частини біологічної секції були професор Е. П. Фішер (Гейдельберзький університет, Німеччина) – вчений, що займається історією науки та написав більше 60 книг, серед яких біографія Нобелівського лауреата Макса Дельбрюка. Г. А. Гамов був наставником Макса Дельбрюка в 1931-1932 роках в Копенгагені, де вчені не тільки дискутували, а й здійснювали розрахунки задля вирішення питань з теоретичної фізики – з’ясування структури атомного ядра, визначення енергії нейтронів і електронів, α -розпаду та γ -радіації.

На секції виступили з пленарними доповідями старший науковий співробітник Інституту молекулярної біології і генетики НАН, лауреат стипендії Президента для молодих науковців, к.б.н. О. В. Савицький (м. Київ, Україна), член-кореспондент НААН, д.б.н. С. В. Чеботар – завідувач кафедри генетики та молекулярної біології ОНУ імені Мечникова, провідний науковий співробітник Інституту мікробіології і вірусології імені Д.К. Заболотного НАН, д.б.н. О. М. Громозова (м. Київ, Україна). Активну участь в обговоренні доповідей

брали к.х.н. В.В. Сімірський керівник УП «Госпрозрахункового дослідного виробництва Інституту біоорганічної хімії Національної академії наук Білорусі» (м. Мінськ, Білорусь), д.с.-г.н. Н. А. Мулюкіна – заступник директора з наукової роботи Національного наукового центру «Інституту виноградарства і виноробства імені В. Є. Таїрова», науковий співробітник Селекційно-генетичного інституту – Національного центру насіннезнавства та сортовивчення – О. М. Благодарова

В роботі секції прийняли участь понад 40 науковців з трьох країн: Україна, Німеччина, Білорусь, серед яких було багато молодих талановитих вчених. На секції було заслухано та обговорено 10 наукових доповідей, за результатами досліджень виконаних в 13 провідних наукових установах України, Білорусі, Німеччини, Австрії.

УДК 577.217.32

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ВИСОКОПРОДУКТИВНІ ОБЧИСЛЕННЯ В БІОЛОГІЧНИХ ДОСЛІДЖЕННЯХ

Просторова організація та конформаційні властивості молекул біополімерів лежать в основі практично всіх біологічних процесів. У зв'язку з цим, вирішення багатьох біологічних задач пов'язано саме з необхідністю вивчення тривимірної структури протеїнів і нуклеїнових кислот, а проблема їх просторової організації – є актуальною для молекулярної біології, біофізики, фармакології та медицини. З появою комп'ютерної техніки встановлено більш ніж сто тисяч тривимірних структур протеїнів та їх комплексів, які депоновано у базі даних Protein Data Bank (PDB, <http://www.rcsb.org/>). Однак координати молекул, отримані методом рентгеноструктурного аналізу, є статичними, а їх конформаційні властивості, за наявності фізіологічних умов розчинника, можуть суттєво відрізнитись. Одним із перспективних методів при експериментальних дослідженнях структури молекули в нативному стані є спектроскопія ЯМР (ядерного магнітного резонансу). У випадках, коли експериментальні дані просторової організації досліджуваного протеїну або його структурного комплексу з субстратами відсутні, ефективною альтернативою є методи комп'ютерної структурної біології. Дані методи ще називають комп'ютерним експериментом або експериментом *in silico* [1].

Розглянуто сучасні практичні підходи високопродуктивних обчислень у прикладних біологічних дослідженнях [2-5].

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УДК 577.2

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“MOLECULAR BIOLOGY” – A CREATION OF PHYSICISTS WITH THE HELP OF GEORGE GAMOW

The term “molecular biology” came into use in 1938 when the Rockefeller Foundation supported research activity that was originally called “mathematical biology” to create an exact science of life. Molecular biology became mainstream when it was discovered that one can study genetics with bacteria and their viruses called phages. In 1944 the physicist and Nobel laureate Erwin Schrödinger published his lectures about the question “What is life?” and he suggested that the most important topic were the action of genes that were composed of atoms as was then discovered for the first time. Schrödinger wondered how life managed to escape the second law of thermodynamics with its increasing entropy and he suggested to understand genes as a code script that would supply life with the information it needed to maintain its order. In the following years the nature of genes was shown to consist of DNA (and its double helix) with the genetic information encoded in a sequence of bases in its center. After 1953 it became clear that there had to be a (genetic) code that allows the genes to create the proteins that are responsible for all the chemical reactions going on in a living cell, and at the point Georg Gamow entered the scene with wonderful ideas laid out in many letters to Francis Crick and James Watson that kept the dis-

cussion among scientists going. Eventually the interest of molecular biologists concentrated on a molecule called RNA and Gamow founded an organization called the RNA Tie Club, in order to solve the riddle of the RNA structure and to understand how it builds proteins. Gamow even started an RNA Tie Club letter bearing the slogan “Do or die, or don’t try”. Each member of the Club was assigned an amino acid and Gamow chose Alanine for himself signing the letters with “Ala”. It took some heroic efforts to fully understand the genetic code and biologist needed till the 1960s to establish it eventually learning that the key to the code was the natural occurrence of amino acids as Gamow had suspected from his first involvement on in 1953. The molecular biologists of this period loved Gamow as a colorful personality always ready for practical jokes. The presentation will describe how molecular biology was made by physicists and wonder what that means for the current understanding of life.

УДК 577.2:631[633.111:664.64.016.8]

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APPLICATION OF RESULTS OF SEQUENCING OF THE WHEAT GENOME TO BREEDING PROGRAMS (PERSPECTIVES AND DIFFICULTIES)

During the last decades huge efforts have been made by wheat scientific community to produce a high quality reference sequence of wheat genome cv. Chinese Spring [1, 2]. This work had improved our knowledge about genome organization *Triticum aestivum* L., distribution of gene sequences along chromosomes, presence of different families of repetitive and transposon elements, uneven distribution of recombination events, high proportion of nonsyntenic and duplicated genes, existence of chromosomal domains enriched with co-expressed genes. Chapman et al. [3] provided an additional draft whole genome assembly of a synthetic hexaploid wheat. At the project of IPK whole genome assembly and annotation (a close to reference quality) of Germany winter wheat cultivar ‘Julius’ have been developed, that contribute to better understanding of haplotype diversity and the extent of structural variation in bread wheat [4].

Development of the screening technology 135K array that is based on a new set of SNP markers that have been identified through sequence capture of the wheat Exome and 20K array ideally suited for screening of wheat lines (in genomic selection scheme) by TraitGenetics (www.traitgenetics.com) – are very effective tool for wheat breeding. That permits to develop genomic selection methodology and apply

genome-wide markers with considered random effects on phenotype with the aim to generate transgressive wheat lines.

But even in time when using such effective molecular genetic tools and approaches, wheat breeding lines that have been developed by introgressive hybridization with relative species with the aim to introduce new resistance genes to biotic and abiotic stress are ‘exotic’ material which is difficult to characterize correct by molecular markers. These difficulties observe due to chromosome rearrangement that had induced by introgressive hybridization with species that are carriers homeological chromosomes and among which can occur different type of translocation and apparent spontaneous transfer of chromatin between chromosomes of three A, B and D subgenome. Also for introgressive lines there can be increasing level of chasmogamy due to incorporation to wheat genome chromosomes or their parts from species with chasmogamy reproductive mechanism, for example, like *Secale cereale L.*

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УДК 633.11:631.523:575.116.4

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THE STORAGE PROTEINS ALLELES AS MARKERS OF BREADMAKING QUALITY, IT’S POLYMORPHISM AND DIVERSITY

The storage proteins are exist in grain endosperm of all cereals and are dedicated for growing germ feeding. They are good investigated. At first, structure and biochemical properties, polymorphism, then it’s relationship with human useful attributes, and, at last, genetical control. There are two points of view to storage protein’s alleles as a markers of wheat organism properties (“to be or not to be”) as well as there are two markers concepts (wide and narrow). Narrow concept is that marker

of property may be only this property gene allele. In narrow view storage proteins alleles are markers of bread-making quality, because they are the main material that forms structure of bread. The wide concept supposed that allele of one genetic sign can mark some genetic caused level of other sign. The problem is when we deal with the signs coding by several loci, as many very important cereal varieties properties are. Adaptability and harvest level, frost and diseases resistance – can we investigate these sign markers, or it is a wrong in theory.

To take the answer we are went in two ways. At first, the variability in wheat storage proteins loci alleles content was studied in different selection centres with different climatic zones in Ukraine. And we have take a result that zones has differences in these allele composition, that is consistent with information about connection of allele presence and its influence to some wheat cultivar's properties in general. Then we have analyses more than 4 thousands progenies from two crosses for its storage protein's alleles composition as well as harvest level, frost resistance and bread-making quality. We have received many statistically verified results about markering some sign's levels with protein's alleles, breadmaking quality most of all. Than we have composed ideal allele formula for our zone and chose the best line from 50 ones with it. This line become a variety called Panna, the first theoretical variety, created it theoretical department, it was better than standart in all aspects, it was included in Ukrainian wheat varieties Register, and it was extra-quality variety.

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УДК 530.145.1

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COSMOLOGICAL SINGULARITY AS AN INFORMATIONAL SEED FOR EVERYTHING

G. Gamov is always interested in informational aspects of biological systems and their connection with cosmology. Here it is considered, how to place some amount of matter into the cosmological singularity and to encode its state. Two different approaches are suggested, which give the same result. The expression for the spectral

energy density of the scalar particles, which is initially encoded at the singularity, is deduced. An informational aspect of the problem is discussed. As a result, we have demonstrated that the momentum wave packet could be well-defined at the cosmological singularity so that: 1) some amount of matter could be «placed» at the singularity, and, thereby, 2) some information could be encoded into it. It is not a creation of the particles from vacuum, because a vacuum does not exist before the field oscillators begin to oscillate. Creation of particles from the vacuum is widely considered at 1960th. However, the amount of matter occurs to be very low for the power law expansion including the linear expansion in cosmic time. From the other hand, a vacuum could be defined only after the moment when the field oscillators begin to oscillate, that is relatively far from the singularity. In contrast, in the approach considered it seems evident that one could place any amount of matter and information into the singularity.

We have considered only an illustrative example with the simple formulas. However, it could be a prototype of how really information about Everything could be stored at the singularity [1].

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УДК 577.113.5 + 582.76.77

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APPLICATION OF 5S rDNA FOR PHYLOGENY RECONSTRUCTION IN *ACER* AND *DIPTERONIA*

The genus *Acer* is one of the biggest ones in the family Sapindaceae. The taxonomic history of the genus is complicated involving numerous rearrangements of species into different sections or even in separate genera. One of the most surprising recent findings is that the related small genus *Dipteronia* (2 species) should be placed into *Acer* [1], although these two genera clearly differs morphologically.

The phylogeny of genus *Acer* still remains poorly studied at the molecular level. The 5S rDNA represents a useful tool for molecular taxonomy [2]. However, the molecular organization of this genomic region remains insufficiently studied for representatives of the genera *Acer* and *Dipteronia*. Therefore, we cloned and sequenced the 5S rDNA intergenetic spacer region (IGS) of six species of genus *Acer* (*A. campestre*, *A. cissifolium*, *A. morofolium*, *A. negundo*, *A. platanoides*, *A. pseudoplatanus*) and one species of *Dipteronia* (*D. sinensis*).

Our data show that the IGS of *Acer/Dipteronia* contain sequence motives, which are similar to that ones, involved in 5S rDNA transcription regulation in species representing other families of angiosperms [3]. These motives include putative “TATA”-box, GC and C elements, which are necessary for transcription initiation, as well as “Oligo-T region” required for termination.

Among the species studied the highest level of IGS similarity – 96.7% – was found between *A. campestre* and *A. platanooides* (section *Platanoidea*) whereas the lowest level – 50.2% – was observed between *A. negundo* (sect. *Negundo*) and *A. pseudoplatanus* (sect. *Acer*). The sequence similarity between *D. sinensis* and species of *Acer* ranges from 64.5 (*A. morofolium*) to 91.4% (*A. cissifolium*). On the phylogenetic ML-dendrogram *D. sinensis*, *A. cissifolium* and *A. negundo* were combined in the same clade with the boot-strap support of 73. Hence, our novel data strongly support the proposition that the species of *Dipteronia* should be replaced into genus *Acer* in spite of morphological difference.

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УДК 577.113.5 + 582.632

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5S RIBOSOMAL DNA OF DISTANTLY RELATED *QUERCUS* SPECIES: MOLECULAR ORGANIZATION AND TAXONOMIC APPLICATION

The genus *Quercus* (oak) is a widespread and economically important genus of tree plants. The intrageneric taxonomy of this group remains controversial due to widely distributed interspecific hybridization and convergent similarity of morphological traits. Further progress in this problem requires application of molecular methods. Still, cpDNA-based markers were mainly used in taxonomic studies of *Quercus*. However, considering the role of reticular evolution in oaks phylogeny [1], wider use of nuclear markers appears to be desirable.

Taking into account that the comparison of 5S rDNA was successfully used in the molecular taxonomy of plants [2], we cloned and sequenced this genomic region for representatives of three taxonomically distant *Quercus* species: *Q. acutissima* (sect. *Cerris*) and *Q. glauca* (sect. *Cyclobalanopsis*) from East Asia and *Q. texana* (sect. *Lobatae*) from North America. We also identified 5S rDNA in the genome sequences available in the Genbank database for North American species *Q. lobata* (sect. *Quercus*) and for the related genus *Castanea*. The novel sequences were aligned with the previously published 5S rDNA sequences of western Eurasian representatives of sections *Quercus*, *Cerris* and *Illex*.

It was shown that the 5S rDNA repeated units demonstrate a high level of intragenomic sequence similarity in representatives of genus *Quercus*. At the same time, numerous nucleotide substitutions and indels were found by comparison of intergenic spacers (IGS) of different species. In *Quercus*, the potential external elements of the 5S rDNA promoter differ from those in other families of dicotyledonous plants [3]. The results of the comparative sequence analysis of the 5S rDNA IGS support the existing taxonomy of the genus and indicate the isolated position of sect. *Cyclobalanopsis*, which can be considered as a separate subgenus.

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УДК 577.21:616.6

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NOVEL MUTATIONS IDENTIFIED AFTER WES AND PREDICTED ENCODED PROTEINS ALTERATIONS IN PATIENTS WITH DSD

Disorders of sex development (DSD) include diverse clinical phenotypes (1 in 4500 live births) and represent a major pediatric concern [1]. There are more than 80 known genes involved in DSD pathogenesis and about 960 possibly implicated candidates.

The aim of the research was to identify new DSD gene candidates using whole exome sequencing in trios (DSD-affected child and both parents) with following bioinformatical analysis of discovered SNPs.

Paternal substitution 15:41862801 G/T and de novo insertion 15:41865665 -/GT-GGGCGTTCGG (GRCh37) in *TYRO3* gene were detected in 46, XY, female with gonadal dysgenesis. Substitution is resulted in loss of canonical splice site on the beginning of exon 12, and as a result, deletion of exon 12 from transcript, that leads to the junction of exons 11 and 13 in the sequence of mRNA transcript and creation of premature stop codon. If cryptic site use, exon 12 will be 7 nucleotides shorter, this leads to frame shift and premature stop codon and loss of kinase domain in TYRO3 protein. Insertion in exon 17 leads to duplication of 4 amino acids and thereby elongation of α -helix in kinase domain.

Maternal substitution 15:42977290 C/T (GRCh37), Arg1172Cys and paternal deletion 15:42979360 AGCACA/- (GRCh37), 1862Ser and 1863Thr were found in *STARD9* gene (both in the region with protein's unknown functional activity) in 46, XY, female with gonadal dysgenesis. It may cause the changes in tertiary structure of STARD9, including location of StAR domain which initiates steroid production by mediating the delivery of cholesterol to the first enzyme in steroidogenic pathway.

Mutation in *WT1* gene was identified in 46, XX, male, with ovotestis. De novo SNP 11:032413528 T/C (GRCh37) impairs recognition by the spliceosome protein SRp40, leads to retaining of *WT1* gene's intronic 9-10 sequence in mRNA, and therefore formation of premature stop codon, resulting in a lack of 19 amino acids in DNA-binding domain of WT1 protein.

New candidate gene mutations, described in our study, may be used to update DSD-target diagnostic panels after Sanger sequencing validation. To reveal functional significance of identified mutations in candidate DSD-causing genes mammalian cellular *ex vivo* models will be established.

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УДК 582.282.23:576.311.31.004.12]:551.590.2

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SENSITIVITY OF MICROORGANISMS TO THE COSMIC WEATHER CHANGE

To date, a large amount of factual material has been compiled on the influence of cosmophysical factors, the so-called space weather, on biological processes. But the issues of the nature and mechanism of action of these factors remain poorly understood. Microorganisms are an excellent object of investigation of these effects, since they are highly sensitive to environmental changes, participate in almost all processes taking place in the biosphere and can be considered as a relatively simple model. One of the processes that demonstrate the connection between biological and cosmophysical rhythms is the reaction of metachromasy (MTX) of volutin granules of microorganisms (bio-astronomic effect of Chizevsky-Velchover). When we stain the cells with methylene blue this structures usually colored in blue, sometimes became violet or red - this is the so called reaction of methachromasy. During more than 10 years of monitoring this reaction in standard laboratory conditions we first established that methachromasy rhythmic is associated with the typical periods of Space-Physics events [1].

The one-way ANOVA showed highly significant (close to the line) connection of the metachromasy index with cosmic rays ($F(2) = 151.14$ ($p < 10^{-7}$)) the feedback with the solar activity, close to the inverse relationship with geomagnetic activity, and nonlinear coupling with the solar wind speed. It was shown that when the flow of charged particles (Solar Wind) increases the growth rate of yeast decreases while sensitivity to antibiotics - increases. We try to study the mechanism of this relation and established that one of the main receptor of these signals may be inorganic polyphosphates. These biomolecules are present at all stages of evolution and characterized by polyfunctional action [2]. It is possible to suggest that study of the mechanism of the metachromasy reaction of volutin granules of yeast cells may lead to the explanation of a lot of phenomena of space biology. Apparently, the continuation of this work requires the combined efforts of specialists in different fields of knowledge.

Now we research the sensitivity of bioluminescent bacteria to changes in the intensity of the earth's magnetic field.

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УДК 612.9-092:579.887.111:612.215:612.085.2

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THE ROLE OF SURFACTANT PROTEIN IN THE MODELING IN VITRO PATHOGENIC PROPERTIES OF MYCOPLASMA PNEUMONIA

The toxin associated with respiratory distress syndrome *Mycoplasma pneumoniae* interacts with a high degree of affinity with the surfactant protein A (SP-A) and has a direct cytopathic effect on pulmonary cells epithelium. It is important to study the mechanisms of realization of the pathogenic potential of *Mycoplasma pneumoniae*, determining its pathogenic potential, associated with the surfactant protein A.

The aim of the study was to determine the role of SP-A in the manifestation of pathogenic properties of *M. pneumoniae*.

The In vitro model was the cell line of human lung carcinoma A549, which activated by *M. pneumoniae* and recombinant CARDS toxin (rCARDS), human SP-A used for cell preincubation. The relative expression of cytokine genes TNF- α , RANTES, IL-6 and IL-33 was evaluated by comparing CT ($\Delta\Delta$ CT) using GAPDH as a reference gene.

The presence of SP-A was accompanied by increased expression of A549 TNF- α , IL-6, RANTES cells and limited the production of IL-33 ($p < 0.05$) associated with Allergy. Adding to the environment of the cultivation of SP-A and subsequent infection of *Mycoplasma pneumoniae* led to a significant decrease in expression of the CC chemokine RANTES in 24 hours. The effect of SP-A in respect of restrictions on the production of IL-33 in *Mycoplasma* infection may be significant in reducing the risk of formation of allergic response during infection of *Mycoplasma pneumoniae*.

SP-A regulates the interaction between *M. pneumoniae*, rCARDS and A549 cells by modulating cytokine expression. The study of the effect of surfactant protein A on the pathogenic properties of *Mycoplasma pneumoniae* on human bronchial epithelial cells brings clarity to the understanding of the mechanisms of realization of the pathogenic potential of *Mycoplasma pneumoniae*: on the one hand, *Mycoplasma pneumoniae* interacts with SP-A, which can contribute to the penetration of the pathogen into the human cell, on the other hand, SP-A binds free CARDS molecules and thus can reduce the amount of the active form of one of the main factors of pathogenicity *Mycoplasma pneumoniae* – CARDS-toxin.

УДК 616.857-037-078.088.7

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THE STUDY OF THE DOPAMINE-BETA-HYDROXYLASE DBH3 GENE POLYMORPHISM IN PATIENTS WITH MIGRAINE WITH AURA

Dopamine β -hydroxylase (DBH) is a key enzyme in the development of neurological diseases, particularly migraines. In the literature there is no data on the effect of DBH3 on the development of the disease: it is known only about the association of polymorphic gene variants with changes in the level of DBH in the serum of patients with migraine with aura (MA).

The aim of the work was developing a method for detecting DBH3 gene polymorphism in patients with migraine on the basis of PCR.

Peripheral blood samples from 40 patients with MA and 40 healthy individuals (K) comparable by sex and age were used as biological material. Genomic DNA was isolated using TRIzol reagent ("Invitrogen", USA). Oligonucleotides were designed using the online application Primer3 V. 0.4.0 and the online algorithm mfold/DNAfold. Statistical data processing was carried out using the application package "SPSS version 16" (SPSS Inc.). To solve the problem of comparing two independent groups of qualitative variables, the criterion χ^2 was used. The significance level $p < 0.05$ is accepted as critical.

For detection the replacement of A \rightarrow G in position 444, the following oligonucleotide primers were selected:

DBH3-f-5'-TCCTTCATGCCTGGAGCCCAGTGCTTGTCT-3'

DBH3-r-5'-GACAGGAAAGGTACTATGACATTGGCACAG-3'

The composition of the reaction mixture (final volume – 10 μ l): 1 μ l genomic DNA (20 μ g/ μ l), 0.4 μ l of each primer (5 mm), 0.1 μ l Taq polymerase (5 U/ μ l), 5 μ l Master-Mix, 13.0 μ l DEPC. The conditions of thermocycling: 94°C – 30 s; 35 cycles 94°C – 30 s, 60°C 30 s, 72°C – 30 s; 72°C – 2 min.

The PCR product was treated with *Eco*NI restrictase and analyzed by electrophoresis in 3% agarose gel. The PCR product had a size of 207 b.p. DBH3 allele A were not exposed to EcoNI, while alleles G were split into fragments 169 and 38 b.p.

Statistically significant ($p < 0.05$) differences on the basis of "DBH3 genotype" in the group of male patients with MA were revealed (table 1).

Table 1

Relative (%) and absolute (n) frequencies of the genotypes at the polymorphic locus DBH3

Genotype	MA, male	MA, women	K, male	K, women
AA	15,00% (n=3)	30,00% (n=6)	30,00% (n=6)	30,00% (n=6)
AG	60,00% (n=12)	40,00% (n=8)	35,00% (n=7)	40,00% (n=8)
GG	25,00% (n=5)	30,00% (n=6)	35,00% (n=7)	30,00% (n=6)

Further research to identify polymorphic variants of the gene DBH 3 will establish the pathogenic mechanisms of migraine with aura and the contribution of genetic factors in this pathology.

УДК 616.314.17-002-078

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MICROBIOLOGICAL CHARACTERISTICS OF PERIODONTOPATHOGENIC FLORA IN PATIENTS WITH INFLAMMATORY-DESTRUCTIVE DISEASES OF PERIODONTIUM

The development of periodontitis is most frequently associated with an increase in the number and persistence in periodontal tissues bacteria of the first order (*Porphyromonas gingivalis* (P.g.), *Tannerella forsythensis* (T.f.), *Aggregatibacter actinomycetemcomitans* (A.a.)) and second order (*Prevotella intermedia* (P.i.) and *Treponema denticola* (T.d.)).

The aim of the work – to study the species composition of periodontopathogenic microflora of gingival pockets in patients with inflammatory and destructive periodontal diseases by using PCR method in real time.

The research included 55 patients: group 1 consisted of 15 patients with chronic complex periodontitis, group 2 – 12 patients with chronic simple periodontitis, group 3 – 12 patients with chronic complex periodontitis with aggressive course, group 4 – 16 patients with chronic gingivitis. The contents of the dentogingival pocket were used as a biological material. The significance level $p < 0.05$ is accepted as critical.

Results and discussion. Periodontopathogenic flora was detected in $92.31 \pm 7.96\%$ of cases with periodontitis; in $68.75 \pm 7.82\%$ of cases with gingivitis, pathogens

were detected in both mono- and mixed state. Frequency of detection of periodontopathogenic microorganisms: group 1 – mono-infection (60,0%), mixed-infection (33,33%), absence of microbial DNA (6,64%); group 2 – mono-infection (50,0%), mixed-infection (33,33%), absence of microbial DNA (16,66%); group 3 – mono-infection (33,33%), mixed-infection (66,67%), absence of microbial DNA (0,0%); group 4 – mono-infection (43,75%), mixed-infection (25,00%), absence of microbial DNA (31,25%).

The high frequency of detection of periodontal flora in the contents of the gingival pockets indicates the etiological role of these microorganisms in the development of periodontal diseases. The PCR method, which has high analytical characteristics and the speed of obtaining the result, should be used in the algorithm of examination of patients of dental profile.

УДК 616.5-002-056.43:616.521-036.1:616.5-078.088.7

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THE RELATIONSHIP OF CLINICAL DATA AND SKIN MICROFLORA MOLECULAR-GENETIC IDENTIFICATION RESULTS IN PATIENTS WITH ATOPIC DERMATITIS AND ECZEMA

The microbial landscape of the skin in patients with atopic dermatitis (AD) and eczema differs significantly from the microflora of healthy people. A distinctive feature of blood pressure and eczema is the increased colonization of the skin of patients by microorganisms and high susceptibility to infectious agents.

The aim of the study was to analyze the relationship of clinical and anamnestic data and the results of molecular genetic identification of individual representatives of the skin microflora in patients with blood pressure and eczema.

107 patients were examined: group 1–79 patients with atopic dermatitis (L20.0–L20.9); group 2–28 patients with eczema (L30.0–L30.9). The control group (group 3) included 30 healthy individuals. As methods of clinical and instrumental examination of patients were used: examination of patients; visual assessment using the SCORAD evaluation scale, confirmed by photodocumentation, dermatoscopy.

Molecular genetic identification of the microflora of the skin (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *E. floccosum*, *M. furfur*, *M. restricta*, *M. obtusa*, *M. globosa*, *M. sympodialis*, *M. pachydermatis*, *T. interdigitale*) was performed using PCR method in real time [1].

When carrying out molecular genetic analysis of the skin scrapings of patients revealed the presence of *C. albicans* (74,77% (n=80)), *C. glabrata* (37,38% (n=40)),

C. parapsilosis (15,89% (n=17)), *E. floccosum* (about 4.67% (n=5)), *M. furfur* (of 35.51% (n=38)), *M. restricta* (of 7.48% (n=8)), *M. obtusa* (12,15% (n=13)), *M. globose* (22,43% (n=24)), *M. sympodialis* (of 44.86% (n=48)), *M. pachydermatis* (5,61% (n=6)), *T. interdigitale* (about 4.67% (n=5)).

With the use of statistical analysis (independence criterion – χ^2 -Pearson), significant differences in the frequency of detection of all studied pathogens between the main and control groups ($p < 0.05$), as well as between groups 1 and 3 ($p < 0.05$) and groups 2 and 3 were established. In the biological material of the patients of the main study group, pathogens were present in the form of mixed infection. Significant relationships were established between the presence of 3 or more pathogens in the biological material of patients and the prevalence of the skin process 40-80% ($\chi^2=71,26$; $p=0.007$), the intensity of clinical manifestations with a degree of severity of 2-3 points ($\chi^2=52.18$; $p=0.025$), the degree of severity of subjective symptoms from 5 to 10 points ($\chi^2=29.65$; $p=0.031$). The presence of significant associations was also found between the presence of 4 or more pathogens in the scraping of the patient's skin and the severe course of the disease ($\chi^2=64.75$; $p=0.034$).

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УДК 633.11:572.22

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ANALYSIS OF THE SMALL RNA-SEQ DATA OF THE BREAD WHEAT LINES WITH INTROGRESSIONS FROM AMBLYOPYRUM MUTICUM

Modern high-throughput methods of the transcriptomes analyses are tend to generate large data sets, which are impossible to interpret without bioinformatics methods. The small RNA-seq method is used to study miRNAs – short regulatory sequences widely distributed in plants. The results of small RNA-seq are stored in the FASTQ files which record millions of sequences each representing individual molecule. Data analysis workflow vary depending on the aims of the study, but the main steps are common: identification of known small RNAs, identification of novel small RNAs, looking for sequences differentially expressed among groups of genotypes, target prediction and annotation.

In this study we used bioinformatics instruments for analysis of the data obtained

after sequencing of the small RNAs libraries of five genotypes: common wheat *Triticum aestivum* L. variety Aurora ($2n = 6x = 42$, AABBDD), genome substitution amphidiploid Aurotica ($2n=6x=42$, AABBTT) with TT genome from *Amblyopyrum muticum*, and three dark colored hairy glumed plants from the *T. aestivum* / *A. muticum* introgressive lines, the development of which is described in [1].

After trimming adapters, raw reads were filtered by length and quality score with FilterFASTQ tool. Processed reads were aligned with the Bowtie [2] against known pre-miRNA sequences of *B. distachyon* and *T. aestivum* deposited in the miRBase. Additionally, processed reads were mapped to the wheat genome with the miRPlant software to find novel miRNA sequences [3]. Known miRNAs and predicted novel miRNAs were analyzed for the differential expression between libraries with limma-voom algorithm [4]. The target genes for the DE predicted miRNAs were predicted with psRNATarget [5], the transcription factors were identified amongst the targets with the BLAST [6] against Gramineae sequences from Plants Transcription Factor Database.

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УДК 616-001-078.088.7

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GENE EXPRESSION PROFILE EVALUATION IN TRAUMATOLOGY

Early diagnosis of inflammatory diseases of the musculoskeletal system is necessary to avoid destructive processes that can lead to a significant deterioration in the quality of life, early disability and premature death. In this regard, expectations for gene research are high.

The aim of the study was to assess the levels of gene expression of individual genes in patients with inflammatory and non-inflammatory diseases of traumatology profile.

Studies were conducted in patients with rheumatoid arthritis (RA), reactive arthritis (Rea) and osteoarthritis (OA). The sample size of each group is 30 patients. Biological material for the study – samples of synovial tissue of the knee joint. To analyze expression levels, we selected the following genes: genes of fibroblast growth factor $\beta 2$ (TGF- $\beta 2$), genes of collagen 4 (*col4a1*) MATRIX components, genes of vascular cell adhesion molecules (VCAM-1) and intercellular adhesion molecules (ICAM-1) of synoviocytes. Calculation of expression levels was carried out relative to house-keeping gene HGUS: for comparing the normalized level of expression taken from the level of 0.8-1.0.

By determining the levels of TGF- $\beta 2$ gene expression, an increase in this indicator (>5.9) was found in the group of patients with OA, in the group of patients with RA the levels of normalized expression were <0.6 , whereas in Rea – within 0.8-1.0. The decrease in gene expression levels of COL4A1 collagen was observed in OA (<0.3 mm), the increase in RA ($>9,4$), the value of the expression levels of this gene in Pea is made of 0.8-1.0. The expression of genes of adhesion molecules ICAM-1 and VCAM-1 was enhanced both in RA (>14.2 and >13.8 , respectively) and in Rea (4.3-8.6 and 3.6-7.9, respectively), while in OA these parameters were within the "established norm" (0.8-1.0).

The study of gene expression opens up new opportunities for practical traumatology. Clinical questions and expectations focused on the study of molecular markers and gene expression profile for initial diagnosis, gene expression profiles in this initial stage of the disease can provide important information about the triggering mechanisms of the pathological process.

УДК 612.015.34:611.778:57.088.7

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MOLECULAR-GENETIC METHOD FOR DETECTION EXPRESSION LEVELS OF THE XENOBIOTICS BIOTRANSFORMATION GENES IN THE PATIENTS SKIN

Metabolism of xenobiotics is formed in the process of evolution of the adaptation mechanism of the body, aimed at neutralizing toxic substances. This process is genetically determined and on the one hand universal, and on the other has individual characteristics for each person.

The aim of the work was to adapt the molecular genetic method to determine the levels of expression of xenobiotic biotransformation genes (*UGT1A7*, *HMOX2*, *BLVRA*, *CCL13*, *APOBR*, *ABCC2*, *GSTP1*) in scrapings of deep layers of the skin of patients.

The studies were performed using scrapings of deep layers of skin from patients with atopic dermatitis (n=3) and eczema (n=3), as well as practically healthy individuals in the control group (n=3). The allocation of RNA and the reverse transcription reaction was performed using sets of reagents "Art RNA MiniSpin" and "ArtMix-RT revertaza" (LLC "ArtBioTech", RB). The resulting cDNA was used for the production of TaqMan PCR in real time using the reagent "Quick-Load Taq 2X Master Mix" (Primtech, RB), specially selected pairs of primers and probes for each gene, including house-keeping genes, on the thermal cyclers "Rotor-Gene-6000" ("Corbett research", Australia).

At the first stage of the study, pairs of primers (forward and reverse) and sequences of TaqMan probes for *UGT1A7*, *HMOX2*, *BLVRA*, *CCL13*, *APOBR*, *ABCC2*, *GSTP1* genes were selected using Vector NTI software. Next, we conducted the optimization of the composition of the reaction mixture and the mode of amplification for the detection of expression of each of the tested genes.

In 3 samples, negative results were obtained to identify the expression of the gene *ABCC2*, in 3 samples – the gene *GSTP1*. In all other cases, the threshold cycle values obtained by performing monoplex PCR in real time for target genes ranged from 18.64 to 32.04.

In order to assess the level of amplification of nonspecific DNA fragments, an electrophoretic analysis of amplicons obtained during PCR was additionally carried out, the results of which confirmed the presence of specific DNA fragments in all analyzed samples. The high analytical specificity of the adapted method was also confirmed by the method of sequence analysis of amplicons extracted from the gel after electrophoresis (genetic analyzer ABI Prism 310 ("Applied Biosystems",

USA)). To normalize the values of target gene expression levels, the gene *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) was chosen as a reference gene, since it was the lowest value of the coefficient of variation – 6.1%.

УДК 633.854.78:575.2227

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EARLY MATURITY LINES AS THE BASE OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) HYBRID BREEDING IN BELARUS

Sunflower (*Helianthus annuus* L.) is non-traditional for Belarus crop. Since 1995 years in our Institute was started the breeding program for creation the ultra-early and early maturity sunflower hybrids with vegetation period 80-100 days with high oil content.

In present two hundred thirty early maturity sunflower lines were created and four F₁ sunflower hybrids were including in national list.

Investigation our material by SSR-markers shows low genetic diversity of sterility maintainer lines. In order to expand genetic base our material we introduced new hybrids from USA, Serbia, Swiss, France, Germany, Moldova and Russia.

The high productivity forms with short vegetation period were selected. Plant heights, sunflower head diameter, 1000 seed weight, seed yield, oil content, vegetation period, resistant to major diseases (sclerotinia, rust, verticillium wilt, septoria, downy mildew) were analyzed. In results of multiple selection and self-pollination (2016-2018) fertility restoration lines M215/16Rf, M217/16Rf, M226(6)/16Rf, M67/17Rf, M92/17Rf, M111/17Rf, M58/18Rf, M67/18Rf, M76/18Rf, M148/18Rf, M153/18Rf and sterility maintainer lines M294/16B, M296/16B, M68/17B, M103/17B were obtained.

Using the new fertility restoration lines allowed to get the high productivity hybrids (seed yield 3,5-4 t/ha, vegetation period – 90-95 days). These previously results allow us to see good perspective of new breeding material.

Now two F₁ sunflower hybrids «Krok» and «Azimut» are testing by State Inspection for Testing and Protection of Plant Varieties of the Republic of Belarus.

Early maturity, high productivity and high oil content are allowed to use our hybrids outside traditional zone sunflower growing.

УДК 577.2:633.1

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PHOTOPERIODIC SENSITIVITY AND GENETIC POLYMORPHISM OF *Ppd-1* GENES IN UKRAINIAN WHEAT VARIETIES AND LINES

Solar radiation is one of the main regulator of the various physicochemical and photobiological processes in plants. A huge number of experimental and theoretical works are devoted to the study of wheat photoperiodism – the reaction to the daily rhythm of lighting, expressed in a change in the duration of growth and development of plants.

The successful reproductive cycle of wheat plants largely depends on earing and flowering in favorable conditions. Information about the genetic control of these processes and the molecular interactions in their background is necessary for better understanding of the mechanisms of adaptability of wheat. The reaction to the photoperiod, vernalization (the influence of low temperatures) and ambient temperature are the main exogenous factors that affect the transition to flowering.

The response of wheat to the photoperiod is regulated by the *Ppd-1* system, which includes genes located on the chromosomes of the second homeologues group. Dominant *Ppd-1* genes reduce the photoperiodic sensitivity of wheat plants. *Ppd-D1* is the key gene that determines the photoperiodic reaction of common wheat (*Triticum aestivum* L.). An analysis of the geographical distribution of *Ppd* genes in the world showed that winter wheat varieties that were grown in the more northern latitudes were highly sensitive to the photoperiod, while varieties of southern latitudes reacted poorly on the decrease in daylight hours. Breeders have chosen the *Ppd* genotypes, that were the best adapted for certain environmental conditions, during selection of plants with the most suitable agronomical important traits for the growing region.

The aim of the work was to study genetic polymorphism and photoperiodic sensitivity of modern Ukrainian varieties and lines of bread winter wheat.

The photoperiod sensitivity and genetic polymorphism at *Ppd-1* system were investigated for analogue-lines (BC₇) created in PBGI on two different genetic backgrounds of the well-known Ukrainian varieties “Kooperatorka” and “Stepnyak” and modern winter wheat varieties from The V.M. Remeslo Myronivka Institute of Wheat (MIP; 4964' N; 3108' E) – Beregynya myronivs'ka (2016), Economka

(2008), Zymoyarka (2007), Kryzhynka (2002), Legenda myronivs'ka (2012), Myronivs'ka zolotovercha (at the varietal testing), Myronivs'ka 65 (2000), Myronivs'ka storichna (2009), Oberig myronivs'ky (2014), Pamyati Remesla (2009), Svitanok myronivs'ky (2014), Juviliyar myronivs'ky (2009). For studies of photoperiodic sensitivity of lines and varieties, experiment was conducted in 2015 and 2019. The sprouted seeds were irrigated for 60 days, planted in vegetable vessels on the open air on April, 16 and grown for natural and artificially reduced (12 hours) photoperiod for 30 plants (2 vegetable vessels) of each grade in each of the experimental variants. The photoperiod was carried out by closing the plants with a box of dark film from 7 to 75 days after discharging. The expiration date of each plant was noted by the label. The data analysis was performed with variation statistics.

By using PCR analysis with molecular markers we have identified the alleles of photoperiod sensitivity genes in the analogue-lines and winter wheat varieties. In the recurrent lines Kooperatorka and Stepnyak 1 we revealed allele *Ppd-D1b*. The allele *Ppd-D1a* was detected in the early analogue-lines Kooperatorka rannya and Stepnyak 1 ranniy. The ratio of dominant and recessive alleles of the *Ppd-D1* gene in MIP varieties was 76% and 34%, respectively. There was no polymorphism at *Ppd-B1* and *Ppd-A1* loci, all lines and varieties were carriers of the recessive alleles *b*.

Lines with the *Ppd-D1b* allele had a strong reaction to shortening of the day light length which manifested in the heading delay for 14.2 days for the Kooperatorka and 11.7 days for Stepnyak 1 on the shortened photoperiod. The presence of the *Ppd-D1a* allele in the genotype significantly decreased the sensitivity to the photoperiod. The difference in the length of the period from germination to heading on a natural and shortened photoperiod in the lines Kooperatorka rannya and Stepnyak 1 ranniy reached 4.6 and 5.8 days, respectively. We also analyzed differences in the length of the period before heading between pairs of lines and between groups of MIP varieties, which differed by the *Ppd-D1* gene alleles, on the natural and shortened photoperiod. The difference in heading time was 2-3 days on a natural photoperiod, on the shorter photoperiod it increased to 8-12.6 days. It should be noted that significant delay in vegetation was observed on the reduced day for plants with recessive alleles: some of the plants remained in the exit into the tube phase, while plants from line Kooperatorka stay on in the tillering phase.

УДК 575.174.015.3:664.641.12

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POLYMORPHISM OF γ -GLIADIN LOCI *Gli-A1*, *Gli-B1* and *Gli-D1* IN BREAD WHEAT VARIETIES THAT HAVE DIFFERENT ELECTROPHORETIC VARIANTS OF GLIADINS

Collection of 44 bread wheat varieties (*Triticum aestivum* L.) from different countries that have been characterized by different electrophoretic specters of allelic variants of gliadins by E. Metakovsky [2018] were analyzed by using PCR with allele-specific primers, which were recommended Zhang et al. [2003] for *Gli-A1*, *Gli-B1*, *Gli-D1* loci.

We have revealed – three different amplification fragments with primers to *Gli-B1.1* allele and five fragments with primers to *Gli-B1.2* allele among tested varieties.

For wheat varieties with allelic variant of gliadins – *Gli-B1b* and *Gli-B1n* the amplification fragment 369 bp was detected with the primers to *Gli-B1.1* allele; the *Gli-B1q* allelic variant of gliadin was corresponded to amplification fragment – 375 bp. For wheat varieties with *Gli-B1i*, *Gli-B1j*, *Gli-B1m*, *Gli-B1o*, *Gli-B1r* electrophoretic allelic variants of gliadins we have detected 400 bp amplification fragment with primers to *Gli-B1.1* allele. Wheat varieties that have *Gli-B1f* allelic variant of gliadins also have fragment of amplification 397 bp according to PCR with allele-specific primers to *Gli-B1.2* allele. In our experiment the *Gli-B1d* allelic variant of gliadins was corresponded to fragment of amplification 409 bp that have been developed with primers to *Gli-B1.2* allele, but Polischuk et al. [2010] have shown that *Gli-B1d* corresponded to *Gli-B1.1* allele. The *Gli-Ba* and *Gli-B1p* allelic variants of gliadins correspond to 21 bp and *Gli-B1e* allelic variant of gliadins match to 391 bp PCR-fragment, which have been developed in allele-specific PCR with primers to *Gli-B1.2* allele. For wheat varieties with *Gli-B1c* allelic variant of gliadins amplification fragments 400 bp or 397 bp were detected and similar for varieties with *Gli-B1h* we detected fragments – 400 or 409 bp with primers to *Gli-B1.2* allele. In some varieties *Gli-B1g* matches 400 bp fragments of *Gli-B1.1* allele and for other varieties with *Gli-B1g* fragment 397 bp was amplified with primers to *Gli-B1.2* allele. Similar situation was with *Gli-B1k* allelic variant of gliadins, which for number wheat varieties was corresponded to amplification fragments 400 bp of *Gli-B1.1* or 397 bp of *Gli-B1.2* alleles for some other wheat varieties.

We did not reveal clear correspondence between allelic variants of gliadins and

amplification fragments that have been developed by allele-specific PCR for *Gli-A1* and *Gli-D1* loci among tested varieties. For the loci we have observed six heterogeneous varieties and seven varieties with two alleles *Gli-A1.1* and *Gli-A1.2* together and 12 varieties with *Gli-D1.1* and *Gli-D1.2* alleles together in each studied genotype of the variety. In this case we have used BLAST service to find sequences which were used by Zhang et al. [2003] for primer developing and compared that sequences with another in the database. We have searched the same sequences with different alleles of *Gli-A1* and *Gli-D1* loci and plenty of the similar sequences with some different mutations. But the most interesting results we have got for *Gli-A1* locus. We have found a big sequence MG560140.1 (5335195 bp) published by Huo et al. [2018], which include two copies of *Gli-A1.1* allele sequence, that amplified in PCR and EF426565.1 (157918 bp) published by Gao et al. [2007] containing *Gli-A1.1* and *Gli-A1.2* sequences together. It could be the reason why we have observed two alleles together in some varieties. But for *Gli-D1* locus we did not found analogous big sequences.

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