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Abstracts of the 8th Baltic Genetics Congress

The 8th Baltic Genetics Congress was held in Kaunas from 22 to 24 March 2023. Its main focus was consolidation of the scientists of the Baltic countries working in various fields of genetics. The idea of the congresses of Baltic geneticist arose after the three Baltic countries had regained their independence and the academies of sciences of these countries decided that geneticists from the Baltic countries should be brought together. The first Baltic Genetics Congress took place in Vilnius in 1992; since then, four congresses have already been organised in Lithuania, and this congress is the second hosted in Kaunas.

At the 8th Baltic Genetics Congress, over 100 participants from the Baltic countries, Spain, the USA, the United Kingdom, Romania, Italy, Norway, Poland, and Ukraine took the opportunity to exchange ideas, views, and knowledge in population and evolutionary genetics, epigenetics, genomics, developmental genetics, bacterial, yeast, viral, plant, animal, and human genetics. The most important research results shared at the 8th Baltic Genetics Congress are presented here in 95 abstracts (three plenary talks, 27 oral and 65 poster presentations).

We are sincerely grateful to the Lithuanian Academy of Sciences for editorial support.

Algimantas Paulauskas
Editor-in-Chief

MOLECULAR CHARACTERISATION AND PHYLOGENETIC ANALYSIS OF THE HONEYBEE (APIS MELLIFERA) MITE BORNE PATHOGEN DWV-A AND DWV-B ISOLATED FROM LITHUANIA

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Deformed wing virus (DWV) is known as one of the main viruses which affects honeybees' health globally and has close association with *Varroa destructor* mites. The virus has two widespread genotypes: DWV-A, and DWV-B (VDV-1) mostly transmitted by *Varroa destructor* mites. In this study we collected early-stage pupae samples from 72 apiaries of eight regions of Lithuania and initially investigated the prevalence of *Varroa destructor* mites. Samples were collected in the beginning of summer of 2021, and prevalence of *Varroa destructor* infection in beehives reached 29.2%. Around 1156 mites from 125 hives were collected in total. The presence of DWV-A and DWV-B (VDV-1) pathogens was examined by real-time PCR targeting the *CRPV-capsid* region. Prevalence of DWV-B in mites was 56% and DWV-A was detected in 12.8% of mite samples. Also, five examined mite samples had dual virus infection. Prevalence of DWV-B in pooled brood samples was 24.67% and DWV-A was detected in 25.97% of samples. Four dual virus infections were defined in pooled brood samples as well. Molecular characterisation of virus detected in mites was based on sequence analysis of RNA-dependent RNA polymerase (*RdRp*) region. Phylogenetic analysis demonstrated the presence of high DWV-A and DWV-B genotype variety in Lithuania.

Keywords: honeybees, Lithuania, deformed wing virus, *Varroa destructor* mites, DWV-B, DWV-A

DEVELOPMENT OF TRANSCRIPTION FACTOR-BASED BIOSENSORS FOR L-AND D-LACTIC ACIDS

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Lactic acid is an important platform chemical used in food, agriculture, cosmetic, pharmaceutical, and chemical industries. Optically pure forms of L- and D-lactic acids are essential to obtain high-quality polylactic acid, a biodegradable polymer that has the potential to replace traditional petroleum-based plastics. Naturally, L- and D-lactic acids are produced by lactic acid bacteria (*L. lactis*, *L. paracasei*, and *E. hirae*), *Bacillus* species (*B. coagulans*, *B. subtilis*), and filamentous fungi (*R. oryzae*). However, in order to achieve technologically and economically sustainable production, the complex selection for best producing bacterial strains and multifactorial process optimisation are often required. Inducible gene expression systems and their application in transcription factor-based biosensors are useful tools for screening and developing microbial strains with improved production of chemical compounds including L- and D-lactic acids. We identify and characterise the L- and D-lactic acid-inducible systems from *Escherichia coli* MG1655, *Cupriavidus necator* H16, and *Pseudomonas* spp. The *Ec*LldR/P_{lldP} and *Cn*GntR/$P_{H16\_RS19190}$-inducible systems are specific to the L-lactic acid and exhibit 19- and 24-fold induction, respectively. The *Pa*PdhR/P_{lldP}, *Pf*PdhR/P_{lldP}, and *Pl*PdhR/P_{lldP} systems have similar affinities to both L- and D-lactic acids. This study provides insight into the understanding of L- and D-lactic acid-inducible systems that can be used as diagnostic devices in synthetic biology and contributes to the development of microbial cell factories.

Keywords: transcription factor-based biosensor, inducible gene expression system, L-lactic acid, D-lactic acid

EVALUATION OF NANOPARTICLE GENOTOXICITY AND CYTOTOXICITY IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES *IN VITRO*

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Due to their unique properties, nanoparticles (NPs) are widely used in different technologies. However, one of the main concerns with nanoparticle exposure is their genotoxic potential. The most frequently used techniques to detect DNA damage induced by nanoparticles are micronuclei (MN) and comet assays. These two methods are often used in combination due to their advantages over each other, where the comet assay detects general DNA damage, such as DNA strand breaks and alkali-labile sites, and MN assay can evaluate the clastogenic and aneugenic effects. In this study, we investigated the genotoxicity and cytotoxicity of differently sized cobalt oxide (Co_3O_4), silver (Ag), and gold (Au) NPs, also silica (SiO_2) and polystyrene NPs in human peripheral blood lymphocytes *in vitro*. The lymphocytes of three healthy donors (for the MN assay), and the lymphocytes of five healthy donors (for the comet assay and cytotoxicity evaluation) were treated with different NPs. It was shown that the most cytotoxic were 10 nm Au nanorods, 13 nm, and 35 nm Ag nanospheres, while other NPs did not significantly impact cell viability. The comet assay results revealed that the highest amount of primary DNA damage was induced by both size cobalt oxide NPs, silver (35 nm), gold (5 nm), and polystyrene NPs. Finally, the MN assay results showed that cobalt oxide, silver, and polystyrene NPs statistically significantly increased the frequency of MN. Overall, smaller NPs induced more DNA damage that was statistically significant compared to the larger ones. Also, metal and metal oxide NPs were more cytotoxic and genotoxic compared to non-metal NPs.

Keywords: nanomaterials, comet assay, micronucleus assay, genotoxicity, cytotoxicity

IDENTIFICATION OF *SARCOCYSTIS* PARASITES FROM ANIMAL CARCASS AND ENVIRONMENTAL SAMPLES USING *cox1* AS GENETIC MARKER

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Protozoan *Sarcocystis* parasites are characterised by an obligatory two-host life cycle and can infect animals and humans. There has been much debate about the classification of several species infecting farm animals as well as their specificity to the intermediate host. As morphological analysis is not commonly sufficient to identify various *Sarcocystis* species, the need for molecular and genetics studies is constantly increasing. However, such detailed investigations were mostly conducted on animal carcasses. Genome databases mainly contain sequences of 18S rRNA gene of *Sarcocystis*. Nevertheless, our recent studies and studies worldwide have revealed that *cox1* is a more suitable target to separate taxonomically related *Sarcocystis* spp. having farm animals as intermediate hosts. Since the number of *cox1* sequences of some species of *Sarcocystis* is not high in the databases, consequently choosing appropriate markers can be difficult. Therefore, the aim of this work was to select suitable primers for the identification of *Sarcocystis* spp. from the carcass and environmental samples. During the study, various combinations of primers were tested, changing their length, composition, binding site, and specificity for one or a group of species. The nested PCR method using species-specific primers was found to be the most suitable, with a 350–400 bp fragment after the second step. Appropriate primers were selected for the identification of nine *Sarcocystis* species infecting domestic animals. Selected species were confirmed by sequencing. The genetically-based method developed in the current study allowed to establish that *Sarcocystis* parasites are widespread in the natural environment in Lithuania and may infect livestock.

Keywords: *Sarcocystis*, protozoa, *cox1*, environmental samples, molecular methods

A DIFFERENT LOOK AT POPULATION GENETICS: CAN OFFSPRING FROM A SINGLE PLANT REPRESENT WHOLE POPULATION? A STUDY OF THE SAND PINK (*DIANTHUS ARENARIUS* L.)

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Sand pink (*Dianthus arenarius* L.) is an allogamous perennial species belonging to the Caryophyllaceae family. It commonly grows in Europe and Asia, mostly in the Baltic Sea region. As it grows mainly in open sand and sandy soil, sand pink habitats include grey sand dunes, continental dunes, and seldom dry pine forests. In Latvia, the species has aggravated distribution. Like many species of Caryophyllaceae family, its fruit contains up to 30 seeds in a capsule. The sand pink is listed in the EU Habitats Directive (Annex II, 92/42/EEC). In Latvia, it is one of the specially protected species listed in the 'Red Book'. The aim of this study was to determine whether the genetic diversity of a single plant offspring represents the genetic diversity of the population. Retrotransposon-based molecular marker system, iPBS (inter Primer Binding Site), was used to determine genetic diversity. First, genetic diversity was determined in three sand pink populations located in Latvia: Kolka, Pāvilosta, and Užava. DNA was extracted and analysed from 22 individual leaf samples. Secondly, the genetic diversity of the offspring of each population was determined. To do this, 11 seeds from two capsules of the same parent plant were germinated *in vitro*. Seedlings (10–14 days old) were used for DNA extraction. Two primers, iPBS2242 and iPBS2239, were applied to conduct genetic analysis. It was determined that offspring polymorphism was alike to the whole population's polymorphism, and the genetic distance between two groups was low. Therefore, it could be concluded that one plant offspring can represent a whole population.

Keywords: Sand pink, *Dianthus arenarius* L., iPBS, molecular markers, genetic diversity, retrotransposons

Acknowledgements: the study was funded by the project 'Biological studies of living organisms in urban, rural and aquatic ecosystems' (AAP2016/B034).

GENETIC STRUCTURE OF *DAPHNIA CUCULLATA* SARS, 1862 NATIVE POPULATION IN EASTERN LATVIA LAKES

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We used *Daphnia cucullata* as a model organism in research for the irst time in four deepest Latvian lakes from Boreal biogeographical region lakes Svente, Riča, Dridzis and Geranimovas-Ilzas in order to find out what genetic diversity of *Daphnia cucullata* population is according to different parameters in similar lakes using microsatellite markers. We have determined the most appropriate microsatellite markers for genetic studies of *Daphnia cucullata* population. The average level of the observed heterozygosity in the *Daphnia cucullata* populations ranged from 0.042 to 0.167, while the average level of the expected heterozygosity ranged from 0.119 to 0.440. In all *Daphnia cucullata* populations the average observed and expected level of heterozygosity (according to Hardy-Weinberg) was different, but these differences were insignificant ($p < 0.001$). Number of polymorphic loci of *Daphnia cucullata* population in the investigated lakes ranged from 33% to 100%. The lowest number of polymorphic microsatellite loci of *Daphnia cucullata* population was found in the Lake Drīdzis (33%), while the highest number of polymorphic microsatellite loci was found in the lakes Geraņimovas-Ilzas (83%) and Riča (100%). The highest FST values were between *Daphnia cucullata* populations of lakes Riča and Svente (0.501) and lakes Svente and Geraņimovas-Ilzas (0.487). The lowest FST values were between *Daphnia cucullata* populations of lakes Riča and Geraņimovas-Ilzas (0.077). The smallest genetic distance (D) (Nei, 1978) in the *Daphnia cucullata* populations was observed between lakes Riča and Geranimovas-Ilzas (0.163), while the greatest genetic distance was found between lakes Drīdzis and Geraņimovas-Ilzas (0.701) and between lakes Riča and Drīdzis (0.563). It was showed, that the populations which were geographically far from each other, and whose lakes were not connected with each other, were the most similar.

Keywords: Cladocera, *Daphnia cucullata*, Eastern Latvia Lakes, genetic structure, microsatellite-PCR

Acknowledgements: research was supported by the ESF project Formation of Interdisciplinarity Research Group for Securing the Sustainibility of Salmonid Lakes in Latvia No. 2009/0214/1DP/1.1.1.2.0/09/APIA/VIAA/089 and ESF Project No. 8.2.2.0/20/I/003 'Strengthening of Professional Competence of Daugavpils University Academic Personnel of Strategic Specialization Branches 3rd Call'.

MOLECULAR IDENTIFICATION OF MESOSTIGMATID (ACARI: MESOSTIGMATA) MITES PARASITISING BATS IN LITHUANIA

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The mesostigmatid (Acari: Mesostigmata) mites are one of the most important groups of bat parasites (Mammalia: Chiroptera). For the large diversity, small size, and morphometric characteristics variability, molecular diagnosis is highly valuable for the species identification of these small-size mites. The aim of this study was to phylogenetically analyse bat-parasitising mites of Macronyssidae and Spinturnicidae families in Lithuania based on the nuclear (28S ribosomal RNA) gene region. The analyses showed that sequences of Spinturnicidae mites were identical to each other and most similar (92.1%) to the *Meristaspis* sp. (FJ911797) sequence. Meanwhile, sequences of the Macronyssidae mites were most similar (96.5%) to the *Steatonyssus occidentalis* (GU440594) sequence. Among the obtained *28S rRNA* sequences of Macronyssidae and Spinturnicidae mites separately, two genotypes were identified. This study provides new information on the phylogenetic relationships of Macronyssidae and Spinturnicidae mites collected from different species of bats in Lithuania.

Keywords: Macronyssidae, Spinturnicidae, bats, Lithuania

MITOCHONDRIAL DNA DIVERSITY OF EUROPEAN POND TURTLE (*EMYS ORBICULARIS*) POPULATION IN LITHUANIA

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The European pond turtle (*Emys orbicularis*) (Linnaeus 1758) is a species that is widely distributed in Europe and listed as endangered in many countries. The northernmost limit of the spread of this species passes through Lithuania and any significant negative change in its environment could lead to the extinction of their populations. Only one autochthonous and reproducing population is currently known in the southern part of Lithuania. Moreover, the pond turtle is an endangered and protected species in Lithuania, listed in the Lithuanian Red Book. In this study, we aimed to estimate the genetic diversity of European pond turtles in Lithuania using sequences of the mtDNA cytochrome b (*cyt b*). Genetic material was collected from a total of 69 saliva and egg samples, and an 881bp fragment of the mtDNA control region was amplified and sequenced. The analysis of the mtDNA sequences revealed that only one haplotype (Ia) was determined for the *E. orbicularis* inhabiting Lithuania. Additionally, the phylogenetic analysis demonstrated that the turtle population in Lithuania was assigned to lineage I distributed in eastern Europe and Asia Minor. The results of this investigation show that strict genetic monitoring of turtles is necessary in order to establish and protect the European pond turtle population in Lithuania.

Keywords: *Emys orbicularis*, endangered species, mtDNA cytb, low diversity, conservation

CFH GENE rs1061170 AND rs1410996 POLYMORPHISMS, AND CFH SERUM LEVELS ASSOCIATION WITH PITUITARY ADENOMA

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Pituitary adenomas are usually benign, non-metastatic tumours. Although most PA are not malignant, up to 35% of tumours can spread to surrounding tissues. PA accounts for about 15% of all intracranial tumours and is the third most common type of central nervous system tumour after meningioma and glioma. The prevalence of PA in the general population is about 17%. While thousands of genetic variants, including single nucleotide polymorphisms (SNPs), are related to different types of cancer, the molecular mechanisms of the diseases are still not fully understood. Complement factor H (CFH) is a primary regulator of the alternative complement pathway that controls C3 activation and modulates innate immune responses. C3a stimulates anterior pituitary hormone release and activates the hypothalamic-pituitary-adrenal axis, a key regulator of inflammation. The complement molecules modulate systemic inflammatory responses through communication with the pituitary gland. Since the *CFH* gene is associated with inflammation, it may also be linked to cancer development and progression. The *CFH* gene is located on the long arm of chromosome 1 (1q32). Our aim was to determine *CFH* rs1061170, and rs1410996 as well as CFH serum levels in PA patients and healthy subjects and relate the obtained results to PA hormonal activity, invasiveness, and recurrence. We found that the *CFH* rs1061170 CC genotype and C allele were statistically significantly less frequent in the hormonal active PA group than in the control subjects ($p = 0.024$, $p = 0.030$, respectively). Moreover, *CFH* rs1061170 genotypes (TT, TC, CC) showed a statistically significant difference between the non-invasive PA and control group ($p = 0.039$). Also, the T allele of this polymorphism was statistically significantly more frequent in patients with non-invasive PA than in the control group ($p = 0.034$). We evaluated CFH serum level, but no statistically significant differences were found ($p = 0.596$). Furthermore, *CFH* rs1410996 showed no statistically significant differences.

Keywords: pituitary adenoma, *CFH*, rs1061170, rs1410996, serum levels, invasiveness, hormonal activity, relapse

NOVEL MicroRNA-BASED DIAGNOSTIC AND THERAPEUTIC APPROACHES TO PERIODONTAL DISEASES

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This study aimed to identify periodontal disease-associated microRNAs (miRNAs) in gingival tissue and bodily fluids and to evaluate the potential of miRNA-based diagnostics and therapeutics in periodontitis (PD) and periimplantitis. Microarray analysis of gingival tissue samples (N = 16) revealed 140 upregulated miRNAs in PD-affected tissues as compared to healthy controls. Following a thorough validation, miR-140-3p, -145-5p, -146a-5p, and -195-5p were selected for further analysis in gingival crevicular fluid (GCF) (N = 210), plasma (N = 221), and saliva (N = 173) samples by means of quantitative reverse transcription PCR. Severe forms of PD were associated with increased levels of GCF miR-140-3p and -145-5p. PD-affected patients had higher plasma levels of miR-140-3p as compared to periodontally-healthy controls. The correlation between increased levels of salivary miR-146a-5p and periodontal outcome parameters, indicating worse clinical status of PD, were observed. The best diagnostic performance was demonstrated by a combination of miR-140-3p, -145-5p, -146a-5p for diagnostics of both severe PD (AUC = 0.709, $P < 0.001$) and mild to moderate PD (AUC = 0.612, $P = 0.018$). Functional analysis of PD-specific miRNAs was performed by transfection of human bone marrow mesenchymal stem cells with inhibitors of selected miRNAs (antagomiRs). Analysis revealed that inhibitors effectively decreased the expression levels of respective miRNAs in cells cultured on both cell culture plastic and medical titanium. The two most potent antagomiRs decreased expression levels of miR-140-3p and -145-5p by approximately two-fold. The study demonstrated that miR-140-3p, -145-5p and -146a-5p may serve as potential non-invasive diagnostic biomarkers for PD. However, further studies are essential in order to apply miRNA inhibition technology in treating peri-implant diseases.

Keywords: microRNA, antagomiR, periodontitis, periimplantitis, epigenetics

APPLICATION OF GENETIC MARKERS TO REVEAL THE EFFECTS OF ELECTROMAGNETIC RADIATION ON *LEMNA MINOR* AND *DROSOPHILA MELANOGASTER*

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To evaluate the effect of electromagnetic radiation on model organisms, the axenic laboratory line of the common duckweed (*Lemna minor*) and fruit flies (*Drosophila melanogaster*) of the Oregon line were chosen as convenient model organisms. To obtain several generations of organisms steadily affected by experimentally enhanced electromagnetic field (EMF), duckweeds were grown on a standard medium in Petri dishes, and placed inside the Helmholtz coil generating low frequency (50 Hz) EMF. Fruit flies, grown in tubes placed inside the Helmholtz coil, were similarly affected by EMF. Control groups included fruit flies and duckweeds grown under the same conditions but placed distantly from the source of electromagnetic radiation (EMR). The growth parameters of *L. minor* (number of fronds in each Petri dish, fronds area, and growth rate) were recorded weekly. The plants that were not replanted to Petri dishes with replenished medium were fixed in ethanol to obtain DNA sequences of ascorbate peroxidase (APx), glutathione peroxidase (GPx), and catalase (Cat) genes to confirm or reject the proposition that EMR may generate new point mutations. The number of dead pupas and insects that reached the imago stage was recorded after each new generation of *D. melanogaster* was obtained. One male and one female representing each new generation of the control group and the group affected by LF EMF were preserved in ethanol to obtain DNA for amplification and sequencing of non-coding region of Y chromosome indicated as loci *kl-5* known to participate in the process of spermatogenesis. The results of the performed experiments revealed the growth-inhibiting effect of the increased electromagnetic radiation (when magnetic flux density reached 1.3–1.4 mT) in the group of the common duckweed exposed to EMF. A significantly higher number of variations in DNA sequences of *L. minor* clones directly affected by LF EMR in comparison to control was revealed mainly at the introns of APx ($P = 0.011$), GPx ($P = 0.009$) and Cat ($P = 0.044$) genes. The appearance and accumulation of point mutations as transitions or transversions of nucleotides became evident starting from the tenth week of the experiment. The number of *D. melanogaster* that reached imago stage remained high in the control group after the fifth generation of fruit flies was obtained. Contrary to the control group, different results were obtained in the group of fruit flies affected by LF EMR: no larvae, pupa, or insect that reached the imago stage were observed after the same time passed from the beginning of the experiment. It revealed a negative effect of EMR, leading to a reproductive failure at the fourth generation of *D. melanogaster* when magnetic flux density was set to 0.75 mT. Appearance of double peaks in chromatograms of DNA sequences representing fruit flies grown inside the Helmholtz coil contrary to the absence of double peaks at the same nucleotide positions among fruit flies representing the control group could be related to the effects of enhanced electromagnetic radiation eventually causing disturbance of spermatogenesis leading to the reproductive failure.

Keywords: low frequency electromagnetic field, *Lemna minor*, *Drosophila melanogaster*, ascorbate peroxidase, glutathione peroxidase, catalase, reproductive failure

GENOMICS OF SEX-LIMITED EXPERIMENTAL EVOLUTION ON A HERMAPHRODITE

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The evolution of gonochorism from hermaphroditism can be linked to the evolution of sex chromosomes and sex-biased and sex-specific gene expression to allow both sexes to reach their fitness optimum. There is evidence that sexual selection drives the evolution of male-biased genes and the Y chromosome in particular. We therefore investigated changes in gene expression and allele frequencies in four replicate populations of *Macrostomum lignano*, a simultaneous hermaphrodite, which we exposed to sex-limited experimental evolution for male- or female-specific fitness by using green fluorescent protein (GFP) as a sex-determining gene. We performed pool-seq of RNA and DNA from worms after more than 20 and 40 generations of selection, respectively. We found that female-selected (F) lines had changed the most in their gene expression and detected significant allele frequency differences between the selection regimes across the genome, and on the scaffold where the GFP locus is located. These results show an indication of sexual specialisation across the genome. Additionally, we found higher numbers of structural variants in F lines and fewer in the male-selected (M) lines, a possible indication of increased recombination rate in F lines and purifying selection in M lines. Moreover, we found that the mitochondrial genome had higher coverage in the M line, which can be linked to changes in gene expression associated to metabolism and increased sexual activity observed in another study. As predicted, we found that expression of genes previously identified as testis-biased candidates tended to be downregulated in F lines. We did not find any significant expression differences for previously identified candidates of other sex-specific organs, but this may simply reflect that few transcripts have been characterised in this way. In conclusion, our experiment suggests that changes in testis-biased gene expression is important in the early evolution of sex chromosomes and gonochorism. This is an important contribution, since much previous research in this area in animals comes from either theoretical models or comparative studies of already old sex chromosomes.

Keywords: *Macrostomum lignano*, experimental evolution, sexual selection, genomics, evolution of gonochorism, hermaphrodite, sex chromosome evolution

THE GENETIC STRUCTURE OF THE POPULATION OF *APHELOCHEIRUS AESTIVALIS* INHABITING RIVERS IN NORTHERN EUROPE

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The genetic structure of a population determines the amount and distribution of genetic variation within and among populations of selected species. Analyses of that structure contribute to understanding population dynamics, occurrence trends, and genetic relationships among populations. In the present study, we determined the genetic structure of five populations of selected model species, the riverine water bug *Aphelocheirus aestivalis* (Insecta: Heteroptera). Samples were collected from populations inhabiting rivers in northern Poland, Lithuania, Estonia and Finland, respectively. The preliminary insight into the genetic structure of selected populations was based on the analyses of eight polymorphic microsatellite loci. As a result, we found that all selected microsatellite loci were highly polymorphic in the tested samples. The number of alleles per locus per sample varied between 4 and 20. Moreover, private alleles were observed in each sample tested. Further analyses of F_{ST} values revealed, in general, a low level of genetic differentiation among samples. Only the sample collected from the Porvoonjoki River (Finland) was identified as genetically differentiated from other samples at a moderate level (values of F_{ST} in the range of 0.051–0.070). Principal Coordinates Analysis (PCoA) revealed that samples collected from the Šventoji (Lithuania) and the Łyna (Poland) rivers were more similar to each other than to the other analysed samples. The presented study is part of a research grant. Analyses will be continued on a wider spatial scale using mitochondrial and nuclear markers. Results will be also analysed in the context of the prevalence of known endosymbiotic bacteria infecting populations of *A. aestivalis*.

Keywords: microsatellites, genetic structure, *Aphelocheirus aestivalis*, phylogenetic relationships

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DIAPORTHE SPP.: IDENTIFICATION AND GENETIC CHARACTERISATION

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The genus *Diaporthe* Nitsche (1870) represents a widely spread group of phytopathogenic fungi that can cause severe diebacks, cankers, leaf spots, decay, blights, and wilts. Causal agents of diseases could lead to serious economic and ecological losses of many plants. The aim of this study was to identify and to characterise pathogenic fungi of the genus *Diaporthe* isolated from three different woody *Fabaceae* family plants in Lithuania. Plant material of three genera (*Cytisus*, *Robinia* and *Caragana*) was collected between 2017 and 2021 and 664 microscopic fungi from 133 (out of 159) plants were isolated. The representatives of the morphogroups were studied using molecular methods. Several markers were used to identify organisms to species. Fragment of nuclear ribosomal internal transcribed spacer region (ITS), actin gene (ACT), calmodulin (CAL) gene, translation elongation factor 1-α (TEF1) gene, beta-tubulin (TUB) gene were amplified by polymerase chain reaction (PCR). To determine identity to species, isolates were first analysed individually with all loci separately. All five loci were then combined, and phylogenetic analysis of fungal isolates was performed. Sequences generated in this study were deposited in GenBank. A dependence on plant genus and *Diaporthe* species was observed. Phylogenetic analysis showed that *Diaporthe oncostoma* species were found on *Robinia* plants, *Diaporthe* sp. (*Diaporthe caraganae*) and several isolates of *D. oncostoma* were obtained from *Caragana* plants. Differently from other plants, *C. scoparius* has three other species of the genus *Diaporthe*. They are similar to *D. eres*, *D. forlicesenica*, and *Diaporthe* sp. (predicted as a new species after sequences analysis).

Keywords: *Diaporthe*, *Fabaceae*, invasive, molecular identification, plant pathogens

GLOBAL WARMING AND GENETIC CHALLENGES FOR FOREST TREES IN NORTHERN EUROPE

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Long-living forest trees are failing to adapt timely in response to the rapidly changing environment. This raises several challenges, particularly to forest trees. This paper reviews major issues and results from research projects aimed to tackle these challenges. The first challenge is preserving genetic diversity within species as an adaptability reserve. This is becoming a problem in the regions where commercially intensive forestry prevails, such as in northern Europe. Here the forest strategy needs to find optima between forest use and genetic diversity conservation. Such optima will be most efficient if based on the understanding of the function of genetic diversity within and among populations as well as the effects of commercial management of genetic diversity of forest tree populations. The second challenge is related to the spreading of species northwards. Here the concerns are on the genetic diversity and inbreeding levels of the front edge populations, namely, these populations will be the founders of future gene pools. We are facing the advancement of exotic species *Fagus sylvatica*, *Acer pseudoplatanus*, *Quercus petrea* and *Larix decidua* to Lithuania as well as spread of native *Tilia cordata*, *Acer platanoides* into pine and spruce dominated forests. Shall we merely observe or facilitate the genetically sound spreading? The third challenge is efficient improvement of the adaptability of forest trees via tree breeding. Modeling of new selection approaches and new adaptive threats such as pest insects and desiccation stress is discussed.

Keywords: forestry, tree breeding, genetic diversity, forest sustainability, climate change

GENETIC DIVERSITY AND EFFECTIVE POPULATION SIZE OF GREY WOLF POPULATION IN LITHUANIA

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We studied the genetic diversity of the Lithuanian wolf population based on 16 highly polymorphic autosomal microsatellite loci in 186 culled wolves. The results showed that the Lithuanian wolf population experienced a significant decline in effective population size in the recent past. This may lead to an increase of inbreeding in more confined territorial groups. The ratio of the coalescence effective population size to the demographic population size was as low as 0.13 whereas the ratio of the variance effective population size to the demographic population size is was 0.53. This result indicates a rapid recovery of allelic diversity and, at the same time, potential risks of inbreeding. Expected heterozygosity varied 0.72–0.76; strong geneflow (estimated with a coalesce algorithm), high allelic richness, abundance of rare alleles, low inbreeding, current effective population size is above 50% of the demographic population size) were observed. The geographical distribution of population inbreeding had a structure. The populations in the region below Vilnius had consistency higher inbreeding levels, which may be due to anthropogenic barriers for migration. According to the geographic distribution of rare alleles and the covalence migration estimates, it is likely that the genetic flow follows the north-eastern part of Lithuania, south-westwards towards Kėdainiai, and that it is bordered to the east by the Lithuanian-Belarusian border. The AMOVA revealed weak genetic differentiation between regions and weak but significant differentiation between geographical populations of wolves in Lithuania. The Bayesian genetic structure analysis showed a structure with three genetic groups are likely to exist in the country. Although the genetic groups are geographically mixed, one of them has a significantly lower inbreeding coefficient and its geographic location may indicate a direction of gene migration from the north-east towards the south-east.

Keywords: genetic diversity, wildlife, *Canis lupus*, inbreeding

OPTIC NEURITIS: THE INFLUENCE OF RELATIVE LEUKOCYTE TELOMERE LENGTH AND TRF1 EXPRESSION

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Optic neuritis (ON) is a disease associated with primary inflammation of the optic nerve. It may be associated with a number of systemic autoimmune diseases. TRF1 (telomeric repeat binding factor) plays a critical role in telomere protection, sister telomere resolution, and telomere length alteration. Telomere length shortening is the result of the combined effects of oxidative stress, inflammation, and repetitive cell replication on telomeres, thus linking telomere length, chronological aging, and associated diseases. In this article, we show that there is no association between relative leukocyte telomere length and serum TRF1 levels and optic neuritis. The study enrolled patients with ON and a random sample of a healthy population. The relative leukocyte telomere length for each sample was determined by qPCR. Serum TRF1 levels were determined by enzyme-linked immunosorbent assay (ELISA) using the (Telomeric Repeat Binding Factor (NIMA-Interacting) 1) ELISA kit. Results were analysed using the statistical analysis method of IBM SPSS Statistics 27.0. We found that relative leukocyte telomere length was not statistically significant between control and ON groups (median (IQR): 0.505 (0.396) vs. 0.591 (0.873), $p = 0.183$). Also, analysis of serum TRF1 levels in control and ON groups revealed no statistically significant results between these groups (mean (SD): 0.934 (0.207) vs. 0.928 (0.151), $p = 0.916$). Although TRF1 is thought to play a protective role in telomere length shortening, our study found that serum TRF1 levels and relative leukocyte telomere length were not associated with optic neuritis.

Keywords: optic neuritis, telomere length, TRF1

MECHANISMS AFFECTING FLUORIDE TOLERANCE IN SOIL BACTERIA *PSEUDOMONAS PUTIDA*

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Organofluorides are increasingly used in various areas of life (agrochemicals, medications, waterproof clothing). However, production of organofluorides is burdensome for the environment. When an enzyme capable of incorporating fluoride into organic compounds was found in 2002, it paved a new path for synthesising organofluorides. As a soil bacterium, *Pseudomonas putida* has a versatile and robust metabolism capable of withstanding harsh environments, and there is an interest in its use as a cell factory to produce fluoropolymers. Based on the literature, *P. putida* can tolerate high fluoride concentration due to fluoride ion transporter called CrcB. Conducting experiments with *P. putida* strain lacking CrcB transporter it was noticed that spontaneous NaF-tolerant mutants arose from the *DcrcB* background of *P. putida* KT2440. This indicates that there are some other molecular mechanisms contributing to fluoride tolerance of *P. putida*. To identify the factors affecting the fluoride tolerance in *P. putida*, a transposon mutagenesis was carried out. We found a Cro/CI type transcriptional regulator PP_3125, deletion of which enabled CrcB-deficient *P. putida* cells to grow on NaF concentrations ten times higher than a normal CrcB deficient strain. By whole genome sequencing of the natural NaF-tolerant mutants showed that NaF tolerance was acquired by large genomic deletions (17 598–340 046 bp), whereas these deletions included the previously described *PP_3125* gene. Label-free proteomics analysis of *P. putida* strains lacking PP_3125 regulator revealed that an expression of protein PP_2036 was upregulated significantly. Since little is known about the PP_3125 transcriptional regulator and genes *PP_2036* and *PP_2037*, further studies are needed to describe their roles in NaF tolerance of *P. putida*.

Keywords: CrcB, fluoride, *Pseudomonas putida*

THE ROLE OF TELOMERIC REPEAT BINDING FACTOR 1 (*TERF1*) IN THE HORMONAL ACTIVITY OF PITUITARY ADENOMAS

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Pituitary adenomas (PA) are among the most common intracranial tumours, which are responsible for various hormonal dysfunctions. They are classified according to their histologic features and hormonal activity. PAs may be non-functioning or secreting one or more hormones. Telomeric repeat binding factor 1 (TERF1) is a protein that has been shown to play a role in the DNA damage response. Also, polymorphisms in the *TERF1* gene have been associated with shortened telomeres. Shortened telomeres are a risk factor for several age-related diseases, including cancer. To better understand the underlying mechanisms of functioning PA and to develop new therapeutic strategies, we investigated the genetic factor that might be related to the hormonal activity of these tumours. The study involved 330 subjects: 110 patients with PA and 220 healthy subjects. Single nucleotide polymorphism (rs1545827) was performed using RT-PCR. Results were analysed using IBM SPSS Statistics 27.0. After analysing the distribution of genotypes and alleles in the functioning PA group and the control group we found that *TERF1* rs1545827 CC genotype and C allele were statistically significantly more frequent in patients with functioning PA than in the control group (65.0% vs. 36.8, $p < 0.001$, 89.0% vs. 62.0%, $p < 0.001$, respectively). In contrast, the CT genotype was statistically significantly less frequent in the functioning PA group than in the control group (30.0% vs. 50.5%, $p = 0.005$). After analysing the influence of *TERF1* rs1545827 on disease manifestation, we found that CT *vs*. TT and TT *vs*. CC genotypes were associated with 3- and 4.5-fold decreased odds of functioning PA occurrence, according to the most robust-codominant model (OR = 0.337; 95%. CI: 0180-0.631, $p = 0.001$; OR = 0.223; 95% CI: 0.064–0.777; $p = 0.019$, respectively). Also, each T allele decreased the odds of functioning PA occurrence by 2.6-fold (OR = 0.392; 95% CI: 0.236–0.650; $p < 0.001$). Current evidence might indicate a protective role of *TERF1* rs1545827 in functioning PA development.

Keywords: pituitary adenoma, TERF1, rs1545827

NATIONAL GENETIC RESOURCES OF PLANTS IN LITHUANIA

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The study and conservation of genetic resources of plants (GRP) in Lithuania has a long tradition. Since 1994, the efforts on GRP have been concentrated within the National Programme on Plant Genetic Resources. In 2001, the Seimas of the Republic of Lithuania adopted the Law on National Genetic Resources of Plants, which regulates the accumulation, preservation, and use of National Genetic Resources of Plants and stipulates provisions for the sustainable use of these resources, their protection against devastation extinction, and destruction as well as to saving the biological diversity. According to the provisions of this law, the genetic resources of plants that possess ecological, selective, and economic value for the Republic of Lithuania are selected and included in the central database of the National Genetic Resources of Plants. They include plant cultivars, plant populations or their parts, single plants or their groups, or reproductional parts of plants (seeds, pollen, embryos, meristematic tissues, buds, sprouts). The Plant Gene Bank (now the State Forest Service) with coordination centres for different plant groups (agricultural plants, forest trees, fruits and vegetables, ornamental plants, and medical plants) has been established. At the end of 2022, there were 5914 accessions on the National List of Genetic Resources of Plants in Lithuania. In 2022, the State Forest Service signed an agreement with NordGen, the Nordic Centre for Genetic Resources. According to this agreement, NordGen will store the seeds of 123 accessions of Lithuanian genetic resources of plants in the Svalbard Global Seed Vault.

Keywords: plant, genetic resources

GENETICS METHODS AND MODEL ORGANISMS FOR BIOTESTING

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The aim of this study was to create a biological testing system that would ensure comprehensive identification of the biologically active components and novel properties (protection against the effects of various electromagnetic fields) of innovative 3D bio-textile during its development. Well-studied species with a short life cycle sensitive to environmental changes were chosen as test-organisms. Initially the impact of succinate (amber), SiO_2, Al_2O_3, and Ag nanoparticles (NP) on the development of fruit fly (*Drosophila melanogaster)* was determined. Fertilised eggs of the wild type line of fruit flies were grown placed in NP (0.01% or 0.1%) containing media. The viability, locomotor activity, body size, and external morphology were examined. No clear deterioration of the given parameters was observed in the NP groups. In tests of the effect of different sizes (100 and 1000 nm) silica NP on *Lemna minor*, a direct relationship was found between the size and concentration of nanoparticles and the growth parameters and cell apoptosis. The immature pollen cultures in one nuclei stage (barley and cyclamens) were used as highly sensitive model system to test all set of NPs. The flow cytometry method was used to measure the responses of experimentally affected gametic cells and control (non-affected) by laser (488 nm) irradiation. Statistically significant differences of cellular reactions were found dependent on the type of NPs incorporated into medium on which the cells were grown. After performing these exterior tests, the impact of NP on cell responses to oxidative stress using biochemical and molecular markers was studied.

Keywords: 3D bio-textile, succinate nanoparticles, SiO_2 nanoparticles, Al_2O_3 nanoparticles, Ag nanoparticles

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CHARACTERISATION OF THE *DICTYOCAULUS* SPECIES NEMATODE FROM RED DEER IN LITHUANIA

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Dictyocaulus sp. are parasitic lungworms. They can cause pneumonia in ungulates and can affect productivity of ungulates as much as cause other diseases. The aim of this study was to characterise *Dictyocaulus* spp. using different genetic markers. Fifteen red deer (C*ervus elaphus*) were analysed for the presence of lungworms in 2021–2022. The lungworms were collected from trachea, bronchi, and bronchioles. DNA was extracted from the adult lungworms by using a QIAGEN QIAamp DNA Mini Kit. The *cox1*, *SSU*, and *cytB* genes were amplified by PCR and sequenced. A total of 168 lungworms were collected from red deer. Phylogenetic analysis of the amino acid sequences inferred from individual genes showed high similarity (98.5–99.8%) with *Dictyocaulus cervi*. Analysis of *cox1* gene sequences showed high genetic diversity. *D. cervi* sequences revealed the presence of ten haplotypes and grouped in five distinct clades. The data of *SSU* and *cytB* sequences showed 99% similarity with the published sequences of *D. cervi* and revealed two haplotypes in both genes. This study was the first phylogenetic analysis of lungworms collected from red deer in Lithuania. More studies should be conducted.

Keywords: nematode, *Dictyocaulus* sp., red deer, Lithuania

CHANGING GENETIC DIVERSITY OF *RUTILUS RUTILUS* IN THE CONTEXT OF ANTHROPOGENIC ACTIVITIES

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One of the most abundant fish species *Rutilus rutilus* is widely distributed in Lithuania, and its potential to adapt to environmental changes attracted our interest. Unfortunately, the understanding of how anthropogenic activities can affect the genetic diversity of this species is poor. We studied three populations of roaches (samples collected in the Neris and the Žeimena rivers, and Lake Drūkšiai) over a period of five years (2017–2022) to determine genetic diversity based on mtDNA sequence analysis. Phylogenetic relationships established in haplotype network revealed sharing of most frequent haplotype between studied samples except the sample from Lake Drūkšiai collected in 2017. Moreover, most haplotypes detected in the samples from Lake Drūkšiai representing genetic diversity characteristic of 2017 population were not detected in 2022 either in Lake Drūkšiai or in roach samples representing populations of the Neris and the Žeimena rivers. Obvious differences of haplotype diversity detected when comparing two samples of Lake Drūkšiai may be related to recovery of the ecosystem previously affected by operating nuclear power plant significantly rising the temperature of this water body. Otherwise, post-glacial relations between hydro-system of the Neris River and Lake Drūkšiai can be predicted as some common haplotypes still are present in both currently isolated ecosystems. The phenomenon causing significant changes of haplotypic diversity in roach population is going to be studied more comprehensively including other informative genetic markers and specimens representing periods before and during, operation of the nuclear power plant and after its closing.

Keywords: *Rutilus Rutilus*, D-loop marker, anthropogenic activities, genetic diversity

PHYLOGEOGRAPHIC CONTEXT OF PUTATIVE PATERNAL LINEAGES OF THE RURIKS AND THE GEDIMINIDS IN MASSIVELY RESEQUENCED EASTERN BALTIC AND FENNOSCANDIAN POOL OF Y CHROMOSOMES

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The Ruriks, believed to be of Scandinavian origin, reigned in Novgorod from AD 862 and in Kyiv from 882. Their dynasty ruled over Russian principalities until the beginning of the 17th century. Paternal lineages of a number of their descendants have been studied and many of them were shown to carry the mutation Y10931 within N3a3-L550 clade. Volkov and Seslavin (2019) suggested Swedish origin of this lineage. Gediminas was the founder of the Gediminid lineage, and their dynastic branches reigned from the 14th to the 16th century in Lithuania and Poland. Their descendants carry the marker L551 – another branch within the N3a3-L550 lineage. Haplogroup N3a3a is the most numerous shared paternal lineage among Estonians, Latvians, and Lithuanians. Thus, with thousands of high-coverage chrY sequences (~10 Mb) from Estonia and neighbouring countries at hand, we set off to disentangle the putative Gediminas and Rurik lineages in this geographic context. Interestingly, we see that an overwhelmingly 'Estonian clade' N3a3a-FGC14542 and N3a3a-Y4351, carried by males mainly of Fennoscandian origin, split from each other ~2450 years ago (ya). This is almost 30 generations before Rurik's lineage N3a3a-Y10931 arises. The Gediminid lineage N3a3a-L551 diversifies ~1800 ya and is carried mainly by contemporary males from the south-eastern shores of the Baltic Sea. In contrast to the Ruriks, the carriers of L551 seem to be absent among Scandinavians and present only in traces among Estonians. Hence, though the Ruriks and the Gediminids share common patrilieal descent, it long predates their dynastic eras and their later ancestries differ in their phylogeography within N3a3-L550.

Keywords: population genetics, haploid genome, uniparental, Y chromosome, demographic history, Baltic region, prehistory

MATERNAL CONTACTS AROUND AND ACROSS THE BALTIC: THOUSANDS OF COMPLETE mtDNA SEQUENCES REVEAL CONTRASTS IN SHARED MATERNAL LINEAGES AMONG POPULATIONS FROM THE SHORES OF THE BALTIC SEA

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Supported by the flourishing ancient DNA and whole genome sequencing field, research on modern European uniparental lineages has demonstrated a rift between maternal and paternal genealogies. Male-mediated expansions in continental Europe tend to coalesce in the Bronze Age with contrasting geographical patterns, whereas maternal lineages show a late Palaeolithic origin with no evident geographic patterns. Here we undertake a large-scale population-based examination of the uniparental gene pool of the eastern Baltic Sea region by juxtaposing male and female phylogenies. For this purpose, we collected over 6000 complete mtDNA and re-sequenced Y-chromosomes from Estonia, Finland, Sweden, Latvia, and Poland with additional modern and ancient haploid genomes from published sources. We reconstructed dated phylogenies of all these sequences. In case of maternal lineages, population-based large-scale sample sizes allow us to detect inter- and intrapopulation clusters within sub haplogroups. In contrast with the general notion of primarily homogenous mitochondrial gene pool of Europe, the fully resolved maternal phylogenetic trees from the circum-Baltic region exhibit a pattern previously associated with male haplogroup N. Almost 40% of Finnish mtDNA sequences belong to clusters common to linguistically related Estonians, whereas below 8% are shared with geographically close Swedish population. In Estonia, roughly 15% of mtDNA sequences belong to shared Finnish-Estonian clusters, whereas 7% form common clusters with the Latvian samples. Despite known Swedish settlements from recent history, less than 2% of Estonian mtDNA samples form common clusters with Swedes. More extensive sampling of 'Baltic maternal lineages' may allow further adjustment in finding and dating 'our common mothers'.

Keywords: population genetics, haploid genome, uniparental, maternal, paternal, mtDNA, mitochondria, Y chromosome, demographic history, Baltic region, prehistory

POWDERY MILDEW RESISTANCE IN ESTONIAN-GROWN WHEAT

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Powdery mildew affects wheat production in the Baltic region. Breeding for resistance and cultivation of resistant wheat varieties is a cost-effective and environmentally safe control measure. Effective combinations of adult plant and seedling resistance genes need to be used in breeding for durable resistance. In this study we used a collection of 39 powdery mildew isolates polymorphic to common resistance genes and a selection of 21 spring and 24 winter wheat varieties for resistance testing. As a result of a controlled seedling inoculation experiment, resistance patterns for each wheat genotype were constructed. Comparing resistance patterns of wheat varieties to controls with known *Pm* genes, the presence of resistance genes was postulated and confirmed by using specific DNA markers to *Pm1a*, *Pm3d*, and *Pm6.* In addition, we screened the material for adult plant resistance gene *Pm38/Lr34/YR18/Ltn1*. Using both phenotypic data and DNA markers, we identified the presence of *Pm1a* gene in 11 spring wheat varieties and *Pm6* in two spring and eight winter wheat varieties. We confirmed the presence of *Pm3d* in ten spring and in one winter wheat variety and *Pm38* in two winter wheat varieties. According to our data, *Pm1a*, *Pm3d*, and *Pm6* are partially effective against the local powdery mildew population and the protective effect can be increased by a combination of more than two *Pm* genes. Seedling resistance can be combined with adult plant resistance *Pm38/Lr34/YR18/Ltn1* locus using MAS. Results of the seedling resistance test coupled with DNA marker analysis in addition to field trial data aid to making informed decisions during wheat breeding process.

Keywords: wheat, powdery mildew, disease resistance, *Pm* genes

GENETIC DIVERSITY OF ENDOPHYTIC BACTERIA ISOLATED FROM EXTREME ENVIRONMENTS

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Plants harbour a wide diversity of microorganisms which play a crucial role in their growth, survival, and establishment by conferring enhanced resistance to abiotic stress allowing plants to grow in extreme conditions. The aim of our study was to identify endophytic bacteria associated with *Deschampcia antarctica* and estimate their plant growth-promoting potential. Twelve endophytic bacterial cultures were isolated from *D. antarctica* sampled during the 25th Ukrainian Antarctic Expedition (January-April 2020) and identified by the 16S rRNA molecular approach and followed phylogenetic analysis using GenBank database and Blast software. The most abundant genus among isolated endophytic bacteria was *Pseudomonas* (five isolates 10.1, 23.1, 24.4, 26.2, 26.4). Based on bootstrap analysis it was shown that 10.1 isolate was 81% related to *P. salomonii*. According to General Time Reversible model, 10.1 and 26.2 isolates were organized to one group, 23.1 to another, while 26.4 and 24.4 were the most evolutionary distanced. Therest of the isolates were represented by *Psychrobacter*, *Agreia*, *Hafnia*, *Pseuarthrobacter*, *Bacillus*, *Brachybacterium*, and *Kocuria* species. That could be the evidence of rich genetic biodiversity of endophytic bacteria isolated from extreme environments.

Keywords: identification, endophytic bacteria, extreme environments, biodiversity

CHANGES IN THE GENETIC DIVERSITY OF WILD BOARS IN THE TERRITORY AFFECTED BY AFRICAN SWINE FEVER

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Wild boar (*Sus scrofa*) is one of the most widely distributed mammals. In Lithuania, an exponential growth of the wild boar population has been observed since 1935. However, after the outbreak of African swine fever (ASF) in 2014, the wild boar population began to decline. According to the 2010 records, there were over 56,000 wild boars, but in 2019 the amount was less than 20,000. The population before and during the outbreak of ASF was divided into subpopulations by regions and investigated using the microsatellite analysis. Studies of the genetic structure of the wild boar population before ASF showed the presence of only one population in the entire study area. This can be proof that there are no barriers preventing wild boars from migrating throughout the territory of Lithuania. High genetic number of subpopulations and low level of differences between subgroups indicate migration and gene flow between localities. A study of population genetic structure during the outbreak found that subpopulations in the western region were genetically distinct from others in the region. The resulting cluster of the western region includes the territory where no outbreaks of ASF were detected during the study period. The analysed wild boar population before and during ASF outbreak showed a high level of genetic variation. Significant deviations from Hardy-Weinberg equilibrium were observed in all subpopulations, mainly due to lack of heterozygotes. The genetics of the wild boar population in Lithuania can be influenced by the selective hunting strategy implemented both before and during the outbreak.

Keywords: wild boar, African swine fever, microsatellite, genetic diversity

GENETIC DIVERSITY OF *LYTHRUM SALICARIA* POPULATIONS IN RELATION TO THE RIPARIAN ENVIRONMENT

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Riparian habitats are very valuable and important parts of the ecosystem but nowadays are suffering from various anthropogenic factors such as urban and agrarian pollution and mechanical interventions by tourists. *Lythrum salicaria* L. (purple loosestrife) is a perennial herb growing in wet meadows, swamps, and along the banks of ditches and lakes. It is native to Europe, temperate Asia, and Northwest Africa. Genetic diversity of *L. salicaria* populations has never been examined in the Baltic countries. The aim of present study was to measure genetic diversity of *L. salicaria* populations from Lithuania in relation to the parameters of the riparian environment. Fifteen populations of *L. salicaria* growing in the margins of river basins of the Nemunas, seaside and the Lielupė were selected. Four amplified fragment length polymorphism markers were employed for genetic analysis. The mean percentage of polymorphic loci was 57.2. The Mantel test showed a correlation between population genetics and the geographic distance. Populations of *L. salicaria* had greater genetic diversity within populations rather than among. According to the Bayesian gene clustering, populations of *L. salicaria* are admixtures of two genetic clusters. Genetic parameters were related to river fragments differing in riverbed origin, river basins, type of land use and cover. Analysis of molecular variance revealed significant differentiation between populations according to the distinct river basin, and between populations from natural and regulated fragments of the rivers.

Keywords: purple loosestrife, AFLP, river regulation, land use, aquatic plants, riparian ecosystem

GENETIC INVESTIGATIONS OF *SARCOCYSTIS* SPECIES IN *LARUS* AND CORVIDAE BIRDS

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Sarcocystis are cyst-forming coccidians with an obligatory two-host life cycle. Sarcocysts are formed in muscles of intermediate hosts (prey) and sporocysts develop in small intestines of definite hosts (predator). DNA sequence determination and comparison are essential for the identification of these parasites. The current study aimed to evaluate genetic variability of parasites detected in birds of the genus *Larus* and family Corvidae based on detection and sequence analysis of *18S* rRNA, *28S* rRNA, *ITS1* and *cox1* genetic markers. Between 2015 and 2021, muscle samples of 271 birds and 161 intestine samples collected from Lithuania were examined for the presence of *Sarcocystis*. By microscopical methods, *Sarcocystis* spp. prevalence was 27.3% and 26.7% in muscles and intestines, respectively. Presence of seven *Sarcocystis* species detected in the muscles of birds were confirmed based on *28S* rDNA and *ITS1* sequence analysis. Calculated similarity values corresponding to intraspecific and interspecific genetic diversity did not overlap and the highest percentage attributed to the category of intraspecific variation reaching up to 3.0% was observed for *S. halieti* species within *ITS1*. The observed level of intraspecific variability of the latter parasite species may be related to the highest number of intermediate hosts species detected in the current study. *Sarcocystis* spp. identified in muscles of examined birds were phylogenetically placed together with species transmitted via birds of prey. Comparison of *ITS1* or *cox1* sequences revealed the presence of 12 *Sarcocystis* spp. in the intestines of investigated birds. This is the first comprehensive report of *Sarcocystis* species genetic diversity in corvids and larids.

Keywords: *Sarcocystis*, Corvidae, *Larus*, *28S* rRNA, *ITS1*, genetic variability

SELECTION OF PLASTID DNA MARKERS FOR COMPARISON OF POPULATIONS OF *ECHINOCYSTIS LOBATA* (MICHX.) TORR. ET A. GRAY

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As a representative of *Cucurbitaceae*, the wild cucumber (*Echinocystis lobata*) is a herbaceous annual vine climbing with the help of branched tendrils and forming spiny capsules. The species grows in wetlands (riverbanks, lakesides) and wastelands. The wild cucumber is cultivated in the gardens due to numerous fragrant whitish flowers and eye catching fruits. It is native to North America but is recognized as invasive species in many European countries. Romania is proposed as the first country of *E. lobata* settlement in Europe, where it has first arrived from North America in the early 20th century. Within last decades it has been recognized as an invasive species of Lithuania intensively spreading along riverbanks. Till now, molecular data about *E. lobata* is rather scarce. The aim of this study was to select chloroplast DNA markers for analysis of *E. lobata* populations. DNA markers such as *ycf1.59*, *ycf1.70*, *atpB*, *rbcL*, *atpI*, *atpH*, *F71*, *R1516*, *R1661* were tested. Chloroplast-amplified DNA fragments were visualised on 1% agarose gel, cut out, and cleaned with GeneJET PCR Purification Kit. For selected populations and individuals, the best conditions (reaction mix, temperatures, the number of cycles) of polymerase chain reaction were determined. Finally, three (*atpB-rbcL*, *atpI-atpH*, *F71- R1516*) out of five cpDNA primer pairs generated pure enough and readable DNA fragments for sequencing.

Keywords: *Cucurbitaceae*, wild cucumber, cpDNA, molecular markers, invasive species

MicroRNA EXPRESSION IN 4NQO-TREATED LEUKOCYTES OF TYPE 1 DIABETES MELLITUS PATIENTS

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Type 1 diabetes mellitus (T1DM), or insulin-dependent diabetes, is a condition characterised by T cell-mediated destruction of pancreatic cells. The resulting hyperglycaemia is believed to increase the formation of reactive oxygen species leading to oxidative stress (OS) thus increasing the risk of diabetic complications. However, the underlying epigenetic regulatory mechanisms acting in response to OS remain poorly understood. Recent studies indicate several microRNAs (miRNAs) that participate in regulating β-cell function during T1DM pathogenesis; thus, they can be putative biomarkers for early diagnostics and prognosis. The current study sought to assess the expression of four selected miRNAs – miR-16-5p, miR-17-5p, miR-106a-5p, and miR-223-3p – in leukocytes treated with 4-nitroquinoline 1-oxide (4NQO), which induces OS in cells. The samples were obtained from 40 patients with advanced T1DM and 21 non-diabetic patients (NDP). MiRNA expression levels were quantified by means of real-time PCR using the TaqMan-based approach. MiRNA expression did not differ between T1DM and NDP; however, there were some associations with disease-related complications. Expression levels of all four miRNAs after 4NQO treatment were generally lower in individuals diagnosed with nephropathy than in those without this condition (all $p < 0.050$). MiR-16-5p expression in 4NQO-treated leukocytes was also lower in the cases with chronic kidney disease ($p = 000$). Moreover, lower miR-223-3p levels were associated with poorly managed T2DM ($p = 0.043$). Our preliminary findings suggest that the analysed miRNAs may play a role in the development of kidney-related T1DM complications. To confirm these findings, larger independent cohort analyses followed by functional experiments are required.

Keywords: type 1 diabetes mellitus, miRNA, 4-nitroquinoline 1-oxide, oxidative stress

PHYLOGENETIC ANALYSIS OF LAELAPIDAE MITES (MESOSTIGMATA: DERMANYSSOIDEA) BASED ON COI GENE

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The mitochondrial cytochrome c oxidase subunit I (COI) gene is a molecular marker frequently used for the taxonomical identification of species and the determination of intra- and interspecific variation in arthropods, including mesostigmatic mites. However, there is a lack of data on the phylogeny of morphologically and ecologically diverse mites from the Laelapidae family. In the present study, six species of laelapid mites collected from six rodent species in Lithuania, Slovakia, the Czech Republic, and Norway have been molecularly characterised based on the COI gene. The obtained molecular data from 57 specimens of mites were used to discriminate between species and investigate the phylogenetic relationships and genetic diversity among Laelapidae mites from four genera. The mean genetic distance between laelapid mites calculated in this study was 0.1215. The high genetic diversity (Hd = 0.870) was found among *Laelaps agilis* mites with nine *COI* haplotypes detected. Two haplotypes of *Laelaps jettmari* and *Hyperlaelaps microti* were detected, whereas only one haplotype was found among *Laelaps hilaris*, *Haemogamasus nidi*, and *Myonyssus gigas*. COI sequences of four mite species were firstly registered in the NCBI database.

Keywords: Laelapidae mites, cytochrome oxidase subunit I gene, phylogenetic analysis, genetic diversity

COUNTING THE LINEAGES OF SOUTH-EASTERN BALTIC FOUNDING FATHERS WITHIN HAPLOGROUP N3a3-CTS10760 LANDSCAPE

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All known human male lineages in the world are part of the global human chromosome Y (chrY) phylogenetic tree. Massively parallel short-read sequencing (SRS) can now routinely access ~10 Mb of chrY which allows reconstruction of time-calibrated phylogenies of modern paternal lineages with unprecedented precision. The initial surge of SRS chrY mostly focused on decoding chrY phylogenetic structures globally, followed by numerous more specialised regional and haplogroup-focused studies. We sequenced and analysed chrY from several thousands of individuals around the Baltic, the neighbouring areas, and elsewhere. Here we focus mostly on chrY haplogroup (hg) N3a, in particular N3a3-CTS10760, that uniquely brings together a considerable proportion of Estonian, Latvian and Lithuanian patrilineal genetic heritage, albeit present, at lower frequencies, also among their neighbours. Using coalescence analysis, we pinpoint more than a dozen successful founding fathers in our Estonian dataset, who established their still well-visible pedigrees, often more than several thousands of years ago. Most remarkably, the analysis reveals a distinct pattern within such ancient pedigrees: some of them share common ancestry with Finns and other Scandinavians, but not with N3a3 variants south of Estonia, whereas others are, as distinctly, phylogenetically closer to the variation among Latvians, Lithuanians, Belarusians, and Poles. These results force us to critically rethink scenarios about the historic male-line demography within superficially smooth 'N3a3-CTS10760 landscape of maleness' in our shared Lithuanian-Latvian-Estonian spatial surrounding.

Keywords: Y chromosome, phylogenetic trees, coalescence analyses, sequencing, Baltic paternal lineages, N3a3-CTS10760

DNA MARKER-BASED FOREST GENETIC MONITORING (FGM) SYSTEM IN LITHUANIA: A CASE STUDY ON *PINUS SYLVESTRIS*

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The Scots pine is economically and ecologically the most important forest tree species covering 34.5% of the total forest area in Lithuania. The ongoing climate and environmental change has been recognised as one of the main threats to forest trees and ecosystems. Thus, genetic diversity is of paramount importance and serves as a base for the evolution of forests tree species and their adaptation to climate change. The objective of Forest Genetic Monitoring (FGM) is to assess the current status of genetic resources and quantify relevant changes at a temporal scale in order to preserve long-term adaptive evolutionary potential. The European Forest genetic recourses programme (EUFORGEN) emphasises the importance of FGM and presents revised FGM methods. Furthermore, EUFORGEN underlines that the system for FGM of the forest genetic conservation units would be an invaluable tool for conservation and sustainable use of forest genetic resources. Therefore, in our study we are presenting DNA marker-based case study of FGM of the Scots pine in two forest genetic reserves (Marcinkonys and Braziūkai). We genotyped 350 trees in total: 175 adults (100 BRAZ_T and 75 MARC_T) and 175 saplings of natural regeneration (100 BRAZ_V and 75 MARC_V). Based on on 11 microsatellite markers, our results show how DNA markers can be employed for DNA-based FGM system in Lithuania. The FGM project and the case study on the Scots pine will be presented.

Keywords: *Pinus sylvestris*, microsatellites, DNA, FGM, Forest Genetic Recourses, FGR

IDENTIFICATION OF *ALNUS GLUTINOSA* L. AND *A. INCANA* (L.) MOENCH. HYBRIDS IN NATURAL FORESTS OF LITHUANIA USING DNA MICROSATELLITE MARKERS

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Two alder species (*Alnus glutinosa* and *Alnus incana*) naturally occur in Lithuania and are considered ecologically and economically important forest tree species that cover 13.8% of the total forest area. Natural interspecific hybridization among the species of the same subgenus *Alnus* can be considered controversial because it can increase genetic variation upon which natural selection may act but, at the same time, it can cause genetic erosion and interrupt species integrity. Therefore, the objective of our study was to assess the likelihood of spontaneous hybridizations between native alders in natural forest stands of Lithuania based on 15 variable nuclear microsatellite markers. Based on the morphological traits, we assigned the total of 189 trees to the three groups of *Alnus incana*, *Alnus glutinosa*, and putative hybrids and then genotyped them. The genetic variation and differentiation enabled us to identify interspecific hybrids and to assess genetic diversity among the groups of pure species and hybrids. Furthermore, our results identified two microsatellite loci as species-specific and discriminating well between the studied species. Overall, our results are important for genetic conservation and breeding programs of *Alnus* spp. The results of the *Alnus* spp. hybridisation study will be presented.

Keywords: DNA, black alder, hybridization, grey alder, SSR, microsatellites, genetic variation

ASSESSMENT OF THE GENETIC STRUCTURE AND DIVERSITY OFNORWAY SPRUCE (*PICEA ABIES* (L.)) POPULATIONS IN LITHUANIA

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Being a key species in northern Europe and covering 21.1% of total forest area in Lithuania, the Norway spruce is a fast-growing evergreen coniferous tree of high ecological and economical importance. Due to changing environmental and climatic conditions, large areas of the Norway spruce are becoming vulnerable to pests and diseases. The European Union Forest Strategy 2030 is in line with the EU Biodiversity Strategy 2030 and is underlining the importance of biodiversity in forest ecosystems and recognises the importance of increasing resilience and adaption of EU forests to climate change.It therefore becomes important to assess and monitor the level of the genetic diversity of forest tree populations as an integral part of biodiversity. Thus, phenotypic evaluation in hand with DNA marker-based knowledge is essential to assess the genetic diversity, structure, and variation of Norway spruce populations in Lithuania. Collected knowledge on phenotypic and genetic variation of spruce populations in Lithuania might help to manage forests in a more sustainable manner and to increase their resilience. The aim of the study was to determine the genetic structure, diversity, effective population size, and the degree of inbreeding of naturally occurring Norway spruce populations in Lithuania based on DNA markers and to investigate possible links between morphological/phenotypic traits and DNA polymorphism. The project and its methodology will be presented.

Keywords: *Picea abies* (L.), microsatellites, genetic variation, phenotypic traits, phenology

OBTAINING IMPROVED EXPOSURE ESTIMATES OF CHEMICAL MATERIAL STRESSORS RELATED TO EPIGENETIC EFFECTS IN ENVIRONMENTAL SPECIES BY INCORPORATING SOIL FACTORS

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Although knowledge of exposure to both natural and anthropogenic environmental stressors causing adverse effects via epigenetic mechanisms in humans is well documented, understanding the way exposure to chemical materials dispersed in the environment may induce epigenetic effects in environmental species is still poor. As research on emerging chemical material stressors of ecosystem species has expanded, epigenetic effects have been noted with species exposure to several classes of substances, including metals, persistent organic pollutants, nanomaterials, and micro-plastics. These classes of materials are often encountered by organisms differently in soils and understanding of often complex environmental (material-soil) exposure interactions is sought for a more holistic interpretation of the epigenetic effect. Natural soils have the potential to mitigate or magnify any epigenetic effects by varying the exposure of environmental species to chemical materials, which also undergo transformations over time (aging) in soils. Suggestions for obtaining improved exposure estimates of chemical material stressors related to epigenetic effects in environmental species that incorporate soil factors will be presented. The goal of this research is to improve understanding of how environmental species are impacted by emerging chemical material contaminants in the environment.

Keywords: ecological epigenetics, chemical material stressors, soil factors, emerging environmental contaminants

ESTABLISHING GAMETIC CELLS IN *IN VITRO* CULTURES OF SPRING WHEAT HYBRIDS (*TRITICUM DURUM* × *TRITICUM AESTIVUM*) AND OBTAINING DOUBLED HAPLOIDS

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As the demand for wheat in both the food and livestock industries is high, breeders around the world are working to create even more new varieties to meet the demand of producers. Special attention is paid to yield quality, disease resistance, and ecological plasticity. Interspecific hybrids of soft wheat (*Triticum aestivum*) and durum wheat (*Triticum durum*) are considered as breeding material with great potential. Classic methods of breeding take more than 12 years to create a new variety so it is important to improve and develop the breeding methods that speed up the breeding process. One of the most effective methods is the use of doubled haploids (DH) that shorten the time required for homozygosity. The aim of this work was to receive green plants-regenerants to obtain double haploids from the interspecific hybrids (*Triticum durum* × *Triticum aestivum*) using an anther culture. Flow cytometry was used to verify wheat polyploidy. The methodology developed at the Laboratory of Environmental Genetics of Institute of Biology, University of Latvia, for soft wheat with modifications was used. In the course of the work, 16 green plants-regenerants were obtained. Embryos were formed from six of 12 hybrids and the genotype was confirmed to be a major limiting factor of the androgenesis process. Using an anther culture, the number of albino plants is higher than the number of green plants-regenerants. In the obtained DH, both hexaploid and tetraploid cells were found in different proportions; green regenerating plants with a triploid or diploid set of chromosomes were not found. Seeds of fertile DH plants formed DH lines and were passed to the Stende Research Centre of the Institute of Agricultural Resources and Economics for involving them in the breeding process.

Keywords: wheat, anther culture, doubled haploids, interspecific hybrids, flow cytometry

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THE DIFFERENCE IS IN THE SIMILARITY: ACOMPARISON OF THE LENGTH AND AREA OF Y CHROMOSOME IN THREE SPECIES: WISENT (*BISON BONASUS*), BISON (*BISON BISON*), AND CATTLE (*BOS TAURUS*)

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The wisent (*Bison bonasus*), bison (*Bison bison*), and cattle (*Bos taurus*) are classified into two different genera: *Bison* and *Bos*, but they belong to one family, *Bovidae*. This close genetic relationship between the said species opens up many research avenues. Based on cytogenetic studies, the basic difference – the morphology of the Y chromosome – between the analysed species was demonstrated. In order to determine the differences in the morphology of the Y chromosome in these three species, photographic documentation (C bands) and measurements of the Y chromosome were performed. The measurements were used to determine the length and area of the Y chromosome: real and relative. The average relative length of the Y chromosome in the wisent was 1.0137%, in the bison 1.177%, and in the cattle 0.8884%. The real length of the Y chromosome of the wisent was 1.5627 μm, and in the bison 1.6202 μm, and they were shorter than the chromosome Y of the cattle, which was 2.0566 μm. The analysis of the second parameter – the area of the Y chromosome– showed that in the cattle and the bison, the relative area is the same (0.8105%). This corresponds with the real area of the Y chromosome, which is very similar (bison – 1.7793 μm^2, cattle – 1.7795 μm^2). An interesting observation is that the obtained value of the relative area of the Y chromosome in the wisent is significantly higher (0.8542%) than in the other species, although, at the same time, this chromosome has a smaller real area in the wisent (1.7441 μm^2).

Keywords: cytogenetic, chromosome Y, European bison, American bison, domestic cattle

ESTONIAN NIPT PRACTICE: FETAL CELL-FREE DNA ENRICHMENT INCREASES THE SENSITIVITY AND ROBUSTNESS OF THE TEST

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Non-Invasive Prenatal Testing (NIPT) has been a part of Estonia's reimbursed first-trimester pregnant screening since 2020. The advanced NIPT service is spared regionally over 45+ clinics, and the NIPT analytical competence has concentrated in the Tartu laboratory, the Competence Centre on Health Technologies. We recently launched IVD-certified NIPT test protocol to overcome sensitivity and low foetal fraction challenges in routine clinical practice. The protocol performs foetal origin cell-free DNA (cfDNA) enrichment, increasing foetal fraction by an average of 3.6× compared to conventional NIPT before whole-genome Illumina sequencing. The advantage of cfDNA increase, in the range of 20–30%, is directly associated with the increased sensitivity of the test to detect clinically significant chromosomal deletions – microdeletions. Currently, the test screens eight microdeletions, but the number is not limited in the future. In addition, the cfDNA enrichment overcomes the low foetal fraction issue. The new protocol reduces the need for re-sampling to 0.5% making the screening test robust and recommendable for overweight patients. We conducted a retrospective study of 50 obese patients with one or two previous failed NIPT trials. The applied NIPT protocol succeeded in 100% of the studied samples and provided a foetal fraction higher than 4% for all cases. In conclusion, the foetal-origin cfDNA enrichment protocol has been successfully implemented into the Estonian NIPT screening program. The need for re-testing has been reduced five times, clinically relevant microdeletions are screened routinely, and the test performs well with overweight patients. The commercial name of the NIPT test presented here is NIPTIFY Focus Plus.

Keywords: NIPT, foetal fraction, enrichment, microdeletions

STUDY OF THE GENETIC DIVERSITY OF THE CLOUDBERRY *RUBUS CHAMAEMORUS* L. BASED ON IPBS MOLECULAR MARKER SYSTEM

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Cloudberry (*Rubus chamaemorus* L.) is a perennial plant species from the genus Rosaceae with subarctic and boreal circumpolar distribution mainly in the Northern Hemisphere. In Europe, cloudberry is widespread in Fennoscandia and the Baltic countries. In Central Europe, cloudberry is found in small, isolated populations distributed in mountainous regions and can be observed as a glacial relict. In Belarus, cloudberry occurs only in the northern part of the country, and it is considered a critically endangered species with a high protection level. In this study, iPBS (inter-primer binding site) retrotransposon-based molecular marker system was used to assess the genetic diversity and the genetic structure of population of Latvian and Belarus natural populations of cloudberry. In total 276 samples from 12 populations, eight from Latvia and four from Belarus, were analysed by comparison of iPBS polymorphism results. Based on molecular data analysis, genetic differentiation of cloudberry subpopulations was evaluated by calculating the following parameters: the percentage of polymorphic bands (P), effective numbers of alleles (Ne), the average number of (Na), Shannon's Information Index (I), Nei's genetic diversity (He), total genetic diversity (Ht), the mean within-population genetic diversity (Hs), genetic differentiation among different populations (Gst), and the gene flow (Nm). A low rate of gene exchange between studied cloudberry populations (Nm = 0.95) was found. The obtained value of the correlation index between genetic and geographic distances ($r = 0.32$) indicates that despite a restricted level of the gene flow among cloudberry populations, genetic differentiation depended on geographic distances, and possibly on specific habitats.

Keywords: cloudberry, *Rubus chamaemorus*, iPBS, molecular markers, genetic diversity, retrotransposons

Acknowledgements: the study was funded by the project 'Evaluation of the cloudberry (*Rubus chamaemorus* L.) genetic resources of Latvia as a background for the breeding program and conservation' (2019–2021) (project No. in Latvia LV-BY/2020/4).

RIVER REGULATION IMPACT ON THE GENETIC DIVERSITY OF *PHALARIS ARUNDINACEA* POPULATIONS: THE CASE STUDY OF THE MERKYS BASIN, LITHUANIA

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The reed canary grass (*Phalaris arundinacea* L.) is a perennial grass species, which grows along the margins of rivers, lakes, and streams, native to Europe and Asia. It is widely distributed along the riverbanks and lakesides in Lithuania and the neighbouring Baltic states. Genetic diversity of *P. arundinacea* populations has never been examined in this region. Similar to what has happened in many places of Lithuania, the Merkys River basin underwent anthropogenic regulation in the mid-20th century. Long-term consequences of such perturbations are not known. The present study aimed at evaluation of the genetic diversity of *P. arundinacea* populations from natural and regulated fragments of the Merkys River basin. Fourteen populations of *P. arundinacea* were selected. To measure genetic parameters of populations, 14 single sequence repeat (SSR) markers were used. Populations of *P. arundinacea* had greater genetic diversity within populations rather than among. The mean percentage of polymorphic loci was lower for populations from regulated rivers (40.0%) compared to the populations from natural fragments of the rivers (53.1%). Principal coordinate analysis distinguished populations from the parts of regulated river. According to the Bayesian gene grouping, populations of *P. arundinacea* are admixtures of three genetic clusters. The first group comprised downstream populations (the Varėnė, the Verseka, the Upper Grūda, the Lower Grūda, the Upper Merkys, the Lower Merkys); upstream populations (the Upper Šalčia, the Beržė, the Lower Šalčia, the Visinčia, the Lower Šaltykščia, the Nedilė) belonged to the second cluster, and the third cluster comprised two populations from regulated riverbed sites (the Taurupis and the Upper Šaltykščia). Our results suggest that river regulation might have effect on the genetic diversity of populations of *P. arundinacea.*

Keywords: reed canary grass, microsatellites, SSR, river regulation

LONG-TERM EFFECTS OF THE EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC RADIATION (50 Hz, 500 μT) ON THE *DROSOPHILA MELANOGASTER*

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Nowadays, the rapid development of wireless communication devices in combination with other moder technologies increases electromagnetic radiation and is a source of electromagnetic pollution that affects living organisms. This study was aimed to determine the long-term influence of extremely low-frequency (50 Hz, 500 μT) electromagnetic field (LF-EMF) on reproductive and biochemical parameters of model organisms, fruit flies (*D. melanogaster*). The equal number of tubes with fruit flies of both sexes were divided in control and experimental groups grown on the same medium. The experimental group was placed in a Helmhotz coil, which generated LF-EMF continuously. The number of offspring and reproductive capacity in each generation were counted and compared between control and experimental groups. Steadily increasing differences between control and experimental groups became significant after the fifth generation (F5) was obtained: the reproductive capacity was reduced by half in the group affected by LF-EMF. To determine potential effects of LF-EMF on reproductivity and the possible impact on DNA, we sequenced the promoter and intron part of the PHGPx1 gene involved in the defensive enzymatic mechanisms neutralising reactive oxygen species. The appearance of point mutations in the progenies of generation F7 was detected in DNA sequences representing the group affected by LF EMF. The first part of the experiment was terminated after progeny representing the 15th generation (F15) of both experimental and control groups were obtained. Only one family (tube) survived in the LF-EMF group as opposed to numerous survived families in the control group. The survivors representing F15 were used to design the second part of the experiment. The catalase activity was measured and compared between groups of fruit flies divided to previously unaffected and experimentally affected by LF-EMF groups. Catalase activity was increased by approximately about 25% in the experimental group in comparison to control. Trying out the binary food choice assay, the visible changes between all groups were not found.

Keywords: LF–EMF, *Drosophila melanogaster*, reproductive capacity, catalase

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GENETIC ANCESTRY DYNAMICS DURING THE LATE STONE AGE PERIOD IN THE WESTERN PART OF THE EASTERN EUROPEAN PLAIN

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Studies of ancient DNA (aDNA) from individuals that lived in European territory during the last ten thousand years suggest that three ancestral populations – European Mesolithic hunter-gatherers (HG), Anatolian Neolithic farmers, and Bronze Age Steppe nomads –made a major contribution to the genetic make-up of modern Europeans. Limited available data from Eastern Europe suggest that demographic processes here might differ substantially from the better studied western part. To fill in the existing gap in the available aDNA material from Eastern Europe and to contribute to better understanding of regional human history, we sequenced aDNA from individuals dated to a time transect of approximately 5000 years (from the first half of the eighth millennium to 2500 BC) from the current territory of Belarus. We found that the oldest sample from Belarusian territory had Eastern HG-like (EHG) ancestry, whilst the genetics ancestry changes to Western HG-like (WHG) or takes intermediate WHG/EHG form in analysed individuals from subsequent periods (until 3000 BC). Individuals dated between 3000–2500 BC had ancestries typical of both Early European Farmers (EEF) and Late Neolithic European populations. Altogether, our data (1) extend the spatial range of EHG ancestry westward as compared to previously known, (2) indicate either presence of complex genetic structure in the Late Stone Age populations in Eastern Europe or suggest several waves of migrations that lead to mixing of ancestries, and (3) support the spread of EEF-like ancestry as far northeast as the current territory of Belarus.

Keywords: human ancient DNA, Eastern Europe, Stone Age, Neolithic, Bronze Age

GENETIC CHARACTERISATION OF THE PHYTOPATHOGENIC BACTERIA *XANTHOMONAS* SPP.

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Bacteria of the genus *Xanthomonas* are one of the most scientifically significant and economically damaging microorganisms. The geographic range of this pathogen isimpacted by climate change and rising air temperature, therefore more and more *Xanthomonas* bacteria are found in Lithuania as well. By using molecular methods, these pathogens may be quickly and precisely identified and characterised, which can aid in halting the spread of diseases brought on by the *Xanthomonas* bacteria in Lithuania. *Xanthomonas arboricola* pv. *juglandis* has already been found in Lithuania on walnuts (*Juglans* spp.); in this study, *Xanthomonas* was detected in plants of *Fabaceae* and *Poaceae* family. Six bacteria out of 366 were identified as belonging to *Xanthomonas* genus after PCR with X1 and X2 primers and 16S rRNR sequencing. Analysis of the 'house-keeping' genes *fyuA*, *gyrB* and *rpoD* helped to identify two *Xanthomonas* species: *Xanthomonas arboricola* (isolates no. DC167, AP534, AP540, AP618, MK11.3) with all three present genes and *Xanthomonas translucens* (isolate no. AP468) with absent *fyuA* gene. Analysis of the type 3 effector (T3E) genes *xopA*, *xopR*, *xopG* and *avrBs2* allowed determining the amount of *Xanthomonas arboricola* pathovars; however, *avrBs2* gene was present only in DC167, AP618, and MK11.3 isolates, which separated *Xanthomonas arboricola* into at least two pathovars.

Keywords: phytopathogenic bacteria, *Xanthomonas*, MLSA, T3E, *Fabaceae*, *Poaceae*

DEVELOPMENT OF GALLIC ACID INDUCIBLE SYSTEM-BASED WHOLE-CELL BIOSENSORS

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Gallic acid, a member of phenolic acids, is a significant secondary plant metabolite outstanding for its cytotoxic, anti-inflammatory, anti-microbial, and free-radical scavenging properties. This hydroxybenzoic acid and its derivatives are used in various industries including pharmaceutical, chemical, food, cosmetics, ink, and dye, and its demand is constantly growing. Its conventional production is based on hydrolysis of tannins and extraction from plants, environmentally hazardous, expensive, and low-yielding processes, which are aimed to be replaced by microbial engineering-based biosynthesis. To ameliorate the detection of synthesized gallic acid, we characterised the transcription factor-based inducible system *Pp*GalR/P_{galB} and demonstrated its activation in *Pseudomonas putida* KT2440 by the extracellularly added gallic acid in *a* dose-dependent manner. Subsequent research indicated that characterised *Pp*GalR/P_{galB} system is not activated in non-native hosts. Since *P. putida* carrying gallic acid degradation pathway is necessary for system's induction, the catabolic product of gallic acid was identified as an effector molecule, interacting with the transcription factor GalR and activating the gene expression. The inducible system was engineered and developed for detection of gallic acid using non-native hosts *E. coli* and *C. necator*. Besides, *P. putida*-based biosensor was employed to measure gallic acid in extracts of green tea (*Camellia sinensis*) leaves. This study for the first time reports the strategy for developing gallic acid-inducible whole-cell biosensors using bacterial species outside *Pseudomonas* genus.

Keywords: gallic acid, inducible gene expression system, transcription factor, biosensor

POTENTIAL EVOLUTIONARY TRAJECTORIES OF *ORCHIS XCOLEMANII* HYBRID ZONE

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Despite the highly specialised pollination strategies, hybridisation is a common phenomenon among Mediterranean deceptive orchids. Food-deceptive species sire a progeny of F_1 unfertile plants, which work as a late post-zygotic barrier. Conversely, when pre-zygotic barriers of sexually deceptive (*Ophrys*) species are absent, the hybrids are fertile, and an extensive introgression may occur. Here, we performed molecular analysis and hand pollination treatments to characterise a hybrid zone of two food-deceptive species, *O. mascula* and *O. pauciflora*. Hybrids (called *O. xcolemanii*) have shown different amounts of parental nrDNA strongly supporting them being F_2 and/or successive hybrid generations. Comparable high levels of reproductive success were detected in natural conditions and in experimental crosses suggesting the absence of effective reproductive barriers either between hybrids either between hybrids or parental species. Considering ecological and distributional features of *O. xcolemanii* across its distribution range, we hypothesise that these populations have originated by secondary contact in the periglacial belt of Apennines. Moreover, the rare and localised *O. pauciflora* could benefit from a genetic enrichment by hybridising with a widespread related species. *O. xcolemanii* is not a dead-end population but may have a role as a potential reserve of adaptive variability and is an unusual stage along the speciation process.

Keywords: food-deceptive orchids, hybrid zones, homoploid hybrid speciation, reproductive barriers

THE GENETIC STATUS OF THE EUROPEAN BISON (*BISON BONASUS*) POPULATION IN LITHUANIA

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The European bison (*Bison bonasus*) specie had a dramatic bottleneck effect during WWII. The restoration of the Lithuanian bison population began at the end of the XX century when the Lithuanian population was restored with ten individuals from the Prioksko-Terrasny reserve in Russia and one free-roaming individual captured in Lithuania. There are three subpopulations in the center region of Lithuania and one new is forming in the southern part of Lithuania. The aim of this study was to analyse the genetic diversity and genetic structure of the European bison population in Lithuania. The investigation, which was performed using 13 microsatellite markers, revealed that the Lithuanian population has a different genetic structure compared with the other populations. In addition, two different haplotypes were identified in the Lithuanian population using partial mitochondrial DNA sequencing. The results of this study are crucial for sustaining the conservation status of the European bison population in Lithuania.

Keywords: bison, Lithuania, microsatellite, mtDNA, genetic

BACTERIAL GENES AND HOST METABOLITES IN PATIENTS WITH ISCHEMIC HEART DISEASE

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Ischemic heart disease (IHD) is the most prevalent among cardiovascular diseases. The main cause of IHD is atherosclerosis, which is a multifactorial inflammatory disease of blood vessels. Recent studies have shown that bacteria might have a significant impact on the pathogenesis and progression of atherosclerosis. Thus, the aim of this study was to determine the distribution of biofilm-encoding *Enterobacteriacea* genes; additionally, protein LL-37 and arachidonic acid (ARA) metabolites produced by host in immune response to ischemic heart disease. The study included 75 patients with IHD randomly selected for the study. Bacterial *16S r-DNA*, and *wcaF*, *papC*, *sdhC* genes were detected in whole blood by using a novel real-time PCR methodology adapted in the Laboratory of Molecular Cardiology of Lithuanian University of Health Sciences (LUHS). Reference sequences were used to validate the results. Concentration of LL-37 protein was measured with ELISA. The analysis of ARA metabolites in blood plasma was performed at the Institute of Pharmaceutical Technologies of LUHS. Bacterial *16S r-DNA* was detected in 31%, and genes specific for *Enterobacteriaceae* (*wcaF*, *sdhC*) in 20% (n = 15/75) of the represented study patients. Patients younger than 65 years old had *Enterobacteriaceae* genes detected in their blood more frequently than patients aged 65 years and above (p = 0.018). *Enterobacteriaceae* genes were also found more frequently in patients with type 2 diabetes (p = 0.048). Concentrations of LL-37 were higher in patients with *16S r-DNA* and biofilm-specific genes in the blood. Additionally, concentrations of ARA metabolites 5S-HETE and 12S-HETE correlated with LL-37 protein concentrations.

Keywords: *Enterobacteriaceae*, bacterial DNA, blood direct PCR, ischemic heart disease

GENETIC DIVERSITY OF *CTENOCEPHALIDES FELIS* FLEA SPECIES IN LITHUANIA

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The cat flea, *Ctenocephalides felis*, is the most common flea species parasitising domestic pets worldwide. It is a vector for different agents of infectious diseases. Recent studies demonstrated the association between genetic characteristics of *C. felis* and their ability to transmit vector-borne pathogens. The aim of this study was to estimate the genetic diversity of *C. felis* from cats in Lithuania based on different genetic markers. A total of 92 *C. felis* fleas were collected from domestic cats. DNA from fleas was extracted from each specimen individually using 2.5% ammonium hydroxide solution. PCR targeting the *COI*, *18S rRNA*, and *28S rRNA* genes were amplified using specific primers. The results of the molecular analysis confirmed the morphological identification of *C. felis* flea species. Phylogenetic analysis based on the *COI* gene revealed the separation of the two clades and genetic variability within the *C. felis* species. Meanwhile, the phylogenetic analysis based on *18S* and *28S* genes did not show a genetic variation of *C. felis* fleas in Lithuania. In conclusion, the *COI* gene sequences are suitable for phylogenetic relationship analysis at the intraspecies level. Further genetic studies should be required to fully evaluate the level of genetic diversity of *C. felis* flea species in Lithuania.

Keywords: cat flea, *Ctenocephalides felis*, genetic marker, Lithuania

GENOTYPING OF *CYPRINUS CARPIO* IN PARENTAL AND OFFSPRING GENERATIONS OF LITHUANIAN CARP STRAINS UTILISING MICROSATELLITE MARKERS

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In the last several years, the Šilavotas Subdivision of the Department of Inland Waters and Aquaculture, which was responsible for the scientific and practical application of cultivating strains of the common carp (*Cyprinus carpio* L.), has suffered a devastating loss of carp breeding stock due to unfavourable climate conditions. It may have caused a loss of genetic diversity of the cultivated carp strains. The goal of this research was to investigate the genetic diversity of the offspring of Šilavotas and Bubiai carp strains by utilising 11 microsatellite loci markers and to reconstruct and assess the paternal link between the assessed offspring generation and their paternal generation. It was found that representatives of the offspring generation of the Lithuanian carp strain were characterised by low genetic diversity: the number of alleles per locus varied from two to four. All 11 loci were analysed for deviations from Hardy Weinberg equilibrium, and it was determined that five out of 11 loci deviated from the equilibrium. However, when determining the mean expected and observed heterozygosity of the sample, it was found that the observed heterozygosity (0.514) differed from the expected heterozygosity (0.505) by a small margin, which suggests that the sample group, as a whole, is within the equilibrium, and does not show signs of inbreeding. After testing the possible paternal link between paternal and offspring generations, it was determined that 14 out of 17 paternal generation individuals could have partaken in the crossbreeding process, while 22 out of 30 offspring generation individuals could potentially be offspring of the aforementioned parents.

Keywords: *Cyprinus carpio*, carp strains, microsatellite marker, genetic diversity

HISTONE METHYLATION-MODIFYING GENE EXPRESSION IN PROSTATE CANCER

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Prostate adenocarcinoma (PCa) is the most prevalent male malignancy worldwide and one of the leading causes of cancer-associated death in men. Accumulating evidence points to the importance of epigenetic alterations (e.g., histone methylation, HM) as key events in prostate carcinogenesis. In this study, expression of HM-associated genes was analysed in two cohorts aiming to evaluate their potential clinical applications. Quantitative PCR-based approach was used for quantification of the selected genes in the Lithuanian cohort (N = 121), whereas next-generation sequencing data was obtained for the analysis of the Cancer Genome Atlas dataset (TCGA, N = 333). After several screening steps, seven HM genes were selected for more thorough analysis by single-gene assay experiments. Expression of *KMT1E, KDM5A* and *KMT5A* differed among PCa and non-tumour tissues (all $p < 0.0500$), with diagnostic specificity for PCa reaching up to 87.2% ($p < 0.0001$) in the Lithuanian cohort. Moreover, *KDM5A* and *KMT1E* emerged as predictors of disease progression-free survival, adding to the prognostic potential of clinicopathological parameters. These associations were also observed in the TCGA cohort. In conclusion, the present study showed the potential clinical utility of HM gene deregulation in PCa. However, further validation of our findings is required in other independent cohorts.

Keywords: prostate cancer, gene expression, histone demethylase, histone methyltransferase, biochemical disease recurrence

DEVELOPMENT AND CHARACTERISATION OF INDOLE-RESPONSIVE WHOLE-CELL BIOSENSOR BASED ON THE INDUCIBLE GENE EXPRESSION SYSTEM FROM *PSEUDOMONAS PUTIDA* KT2440

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Indole is a biologically active compound naturally occurring in plants and some bacteria. It is an important specialty chemical used as a precursor by the pharmaceutical and chemical industries and in agriculture. Recently, indole has been identified as an important signaling molecule for bacteria in the mammalian gut and biofilm formation. The regulation of indole biosynthesis has been studied in several bacterial species; however, this has been limited by the lack of *in vivo* tools suitable for indole production, import, and export monitoring. The genetically encoded biosensors have been shown to be useful for real-time quantitative metabolite analysis. For the first time to our knowledge, this study presents the identification and characterisation of a transcription factor-based indole-inducible system *Pp*TrpI/$P_{PP\_RS00425}$ from *Pseudomonas putida* KT2440. Indole whole-cell biosensors based on *Escherichia coli* and *Cupriavidus necator* strains are developed and validated. The specificity and dynamics of biosensors in response to indole and its structurally similar derivatives are investigated. The gene expression system *Pp*-TrpI/$P_{PP\_RS00425}$ is shown to be specifically induced up to 639.6-fold by indole, exhibiting a linear response in the concentration range from approximately 0.4 to 5 mM. The results of this study form the basis for the use of whole-cell biosensors in indole metabolism-relevant bacterial species screening and characterisation.

Keywords: indole, L-tryptophan, inducible gene expression system, *Pseudomonas putida*, whole-cell biosensor

PREVALENCE OF PANTON-VALENTINE LEUKOCIDIN TOXIN ENCODING GENE AMONG CLINICAL STRAINS OF *STAPHYLOCOCCUS AUREUS*

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Infections caused by *Staphylococcus aureus* are among the major causes of morbidity and mortality worldwide. Panton-Valentine leukocidin (PVL) gene is produced by *S. aureus*. PVL is mainly associated with methicillin-resistant *S. aureus* (MRSA) infections, and they are more virulent and highly transmissible strains. The aim of this study was to determine antibiotic resistance and detection of the Panton-Valentine leukocidin (*lukF-PV*) gene. The bacterial collection used for the study was retrieved by Microbiology and Virology Institute from clinical substances of patients from various hospitals in Lithuania. The study sample consisted of 159 clinical strains of *S. aureus*. The disk diffusion method was used for antibiotic resistance evaluation, and the polymerase chain reaction (PGR) method determined the prevalence of Panton-Valentine leukocidin toxin encoding gene (*lukF-PV*). After the evaluation of antibiotic resistance of *S. aureus* strains, benzylpenicillin resistance appeared most frequently among MRSA. All MRSA strains were resistant to cefoxitin and benzylpenicillin, high ciprofloxacin resistance appeared as well, and more than half were characterised as gentamicin-resistant. Prevalence of the Panton-Valentine leukocidin toxin encoding gene among the tested strains was low, but it was determined that *luk S/F-PV* gene among MRSA was almost twice as prevalent as in methicillin-sensitive strains of *S. aureus*.

Keywords: *Staphylococcus aureus*, Panton-Valentine leukocidin, antibiotic resistance, methicillin-resistant *S. aureus*

INVESTIGATION OF DROUGHT STRESS RESPONSE OF LITHUANIAN BARLEY CULTIVARS

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Since its domestication approximately 10,000 years ago, barley has remained a staple cereal crop for food, feed, and brewing. Advances in genetics have also established barley as a valuable model organism for understanding the genetic basis of cereal crop phenotypes. Climate change, now more than ever, is a threat poised to disrupt global livelihoods. It is imperative to develop conservation strategies, chief among which are breeding programmes for climate-resilient crops. Given these circumstances, our team set out to investigate Lithuanian barley varieties to determine the most drought tolerant ones. Six local and two foreign barley cultivars were examined utilising biochemical, morphological, and genetic analysis techniques. Drought stress was induced for ten days using a 15% PEG-6000 growing medium. Acidic ninhydrin assay, Evans blue staining, and Relative Water Content (RWC) methods were used to determine free proline content, root cell viability, and tissue water upkeep, respectively. Proline analysis revealed a 40–90% increase in stress group plant proline content. Cultivars 'Noja' and 'Rusnė' exhibited the lowest change between control and stress samples. In RWC measurements, cultivars 'Kirsna' and 'Rusnė' performed the best, demonstrating only approximately a 2% decrease in RWC. In cell viability assays, cultivars 'Alisa', 'Arka', and 'Rusnė' demonstrated the best results, with only a marginal decrease in root cell viability. Several genes of interest, including delta-1-pyrroline-5-carboxylate synthase (*P5CS1*) and others, associated with drought tolerance phenotype, were sequenced and are being analysed. Depending on the results of the genetic analysis, future investigations may include sequencing of additional genes or gene expression analysis.

Keywords: barley, drought stress, climate change, cereal breeding, relative water content, cell viability assay, free proline, gene sequencing

IN VITRO SEED CULTURE IN BREEDING AND GENETIC RESEARCH

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The aim of this study was elaboration of *in vitro* seed culture techniques for various species guided by the specificity of the species and the achievable goal. *In vitro* seed cultures have a wide application in various stages of the breeding, from the acquisition and updating of the starting material to propagation and are used as raw material in genetic studies. One of the significant aspect of seed cultures is suitability for the recovery of genetic resources from old, practically non-germinating seeds material. Seeds samples of *Trifolium pratense* (16–34 years old), *Trifolium hybridum* (24–33 years old), *Medicago sativa* (44 years old), and *Medicago* sp. (14–19 years old) were sprouted, and the genotypes were recovered with elaborate methods. *In vitro* seedlings of *T. pratense* were used to obtain tetraploid plants from well adapted local diploid varieties. The most suitable colchicine treatment and cultivation conditions were found. Also, the micropropagation method for *T. pratense* plants was developed. Ploidy level and genomic stability of cloned plants were detected using flow cytometry and iPBS molecular marker system. *Cannabis sativa* seed culture was established for seedling molecular sexing by *SCAR* markers. The female plants were selected for micropropagation. For long-germinating seeds, a method was developed to increase germination and reduce germination time: so, the *Rubus chamaemorus* seeds, which usually germinate up to seven years after ripening, produced seedlings within 1–2 months of their introduction into *in vitro* cultures.

Keywords: seeds *in vitro* culture, flow cytometry, molecular marker, breeding

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PREVALENCE OF *WOLBACHIA* IN POPULATIONS OF *APHELOCHEIRUS AESTIVALIS* INHABITING RIVERS IN SOUTHERN EUROPE AND ITS POTENTIAL IMPACT ON THE HOST'S GENETIC DIVERSITY

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The bacterium *Wolbachia* infects about 52% of aquatic species. Since *Wolbachia* is co-inherited with mitochondria, natural selection acting over the bacterium will also affect mitochondria. Depending on the infection context, this hitchhiking effect may increase or decrease the host's genetic diversity at the mitochondrial level and thus cause problems in phylogeny inference. In the present study, *Aphelocheirus aestivalis* (Insecta: Heteroptera) was selected as a model species. The riverine water bug is the only reported species of the monogeneric family Aphelocheiridae occurring in most parts of Europe. Its limited dispersal abilities are predicted to limit the gene flow between populations, promoting population fragmentation, genetic divergence, local adaptation, and endemism. Here, we determined the phylogenetic relationships among six populations of *A. aestivalis* inhabiting rivers in Southern Europe (Hungary and Romania). Analyses were performed on both mitochondrial and nuclear levels (*cox1* and nine microsatellite loci, respectively). Moreover, each sample was screened for the presence of *Wolbachia*. As a result, *Wolbachia* was found in each selected sample and at least 60% of tested specimens were infected per sample. The highest percentage of specimens infected with the bacterium was observed in the sample collected from the Caraș river in Romania (93%). Analyses performed at the mitochondrial and nuclear levels revealed close relationships among selected populations. Those close relationships may be connected with various factors, e.g., common origin and belonging to a single phylogenetic lineage. Thus, the hypothesis on the *Wolbachia*-induced reduction of *A. aestivalis* genetic diversity seems less likely.

Keywords: *Wolbachia*, mitochondrial markers, microsatellites, phylogeny, *Aphelocheirus aestivalis*

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AGRONOMICAL AND MORPHOLOGICAL CHARACTERISATION OF PERSPECTIVE FLAX GENOTYPES IN PLANT BREEDING IN LATVIA

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Flax (*Linum usitatissimum* L.) is an important eco-friendly and multipurpose crop. Studies in Latvia are focused on increasing the diversity of genetic resources of flax by samples well adapted for growing in sustainable agro-ecosystems. The aim of this study was to characterise new flax lines and identify the most promising genotypes for fibre flax breeding. Eighteen fibre flax lines were evaluated for agronomically important traits; variety 'Vilani' was used as the standard variety. All studied genotypes were created by hybridisation with old varieties of the Latvian origin suitable for growing in the Latvian conditions. They were repatriated from gene banks abroad. Those lines are characterised by spring growth habits and annual type of life. Field investigations were carried out from 2021 to 2022. The lines differed in the colours of flower petals, including white, light blue, and blue. The capsules for all genotypes were tightly closed and of brown seed colour. The vegetation period for 33% of samples was identified as short and for 67% as medium length. The flax genotype 'N1-1' exhibited a significantly higher stem yield (497.5 $g \cdot m^{-2}$) and plant technical height (59.8 cm) in comparison with the standard variety.

Keywords: genotypes, morphological traits, stem yield, vegetation period

STRONG GENETIC STRUCTURE OF THE EURASIAN BLUE HONEYSUCKLE (*LONICERA CERULEA*)

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The blue honeysuckle, *Lonicera caerulea* L. s.l. (Caprifoliaceae, Caeruleae), is a highly polymorphic species native to the Northern Hemisphere that exhibits a large range from eastern Scandinavia to Kamchatka in Eurasia and is widespread in North America. Currently, the blue honeysuckle is considered a promising berry crop. We evaluated the genetic diversity of native *L. caerulea* populations from the western (Baltic countries) and eastern (the Russian Far East and Japan) edges of the Eurasian range using inter-simple sequence repeat (ISSR) and chloroplast DNA (*psb*A-*trn*H and *trn*L-*trn*F) markers. The genetic relationships of populations and genotypes were analysed using principal coordinate and cluster analyses (neighbour joining and Bayesian clustering). Our study showed clustering of individuals and populations according to geographic origin. The analyses of ISSR polymorphisms in populations confirmed the opinion of other authors that geographically separated blue honeysuckle populations are genetically divergent. The revealed high population differentiation reflects the complex intraspecific taxonomic structure of this honeysuckle species. However, sequence analysis of cpDNA regions revealed no phylogeographic structure among the populations, which indicates the integrity of diverged populations of one species. We also found that the eastern populations of blue honeysuckle had significantly greater genetic diversity parameters than the populations from the Baltic region. This finding correlates with the endangered status of the blue honeysuckle in the Baltic countries. As economic interest in *L. cerulea* continues to increase, studies on the genetic structure of the blue honeysuckle will provide information about processes occurring in geographically isolated populations that are potential sources of genetic diversity.

Keywords: *Lonicera*, DNA polymorphism, population genetic structure, speciation, ISSR markers, chloroplast DNA regions

DIFFERENTIATION OF GENETIC LINES OF THE EUROPEAN BISON *BISON BONASUS* BASED ON STR LOCI

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The European bison is a species reintroduced into nature based on only twelve founders for the Lowland-Caucasian line (LC) and even fewer (seven from twelve) for the Lowland (LB) animals. One of the principles that has been applied in the protection of this species is the spatial separation of genetic lines. This requires the selection of individuals and verification of their belonging to one of the two genetic lines. Over the years, our team has conducted genetic monitoring of the European population of the wisent. Here we present the use of ten microsatellite loci for 2227 individuals divided into two genetic lines (LC and LB). The mean number of alleles was 2.7 (SE 0.3). The observed heterozygosity was lower than the expected heterozygosity in both genetic lines. Those parameters (Ho, He) were higher in the LC line than in the LB line. The difference between the lines was noticeable in the allele proportion and based on PCA and structure analysis, although the number of markers was not enough to clearly distinguish these two genetic lines.

Keywords: microsatellites, genetic diversity, genetic distance, *Bison bonasus*, genetic drift

EUROPEAN EEL (*ANGUILLA ANGUILLA*) MITOCHONDRIAL DNA DIVERSITY IN A PART OF THE BALTIC LAKELAND

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Anguilla anguilla (Linnaeus, 1758) is a unique catadromous fish species in the Latvian fauna. Eels inhabit freshwater, brackish, and coastal freshwater and spawn in the Sargasso Sea. In recent decades, its population has decreased not only in Latvia, but also in Europe. Currently, its position has been recognised as critical and a range of normative acts have been adopted for its restoration, such as Regulation EC 1100/2007. The Baltic Lakeland area represents naturally recruited and introduced eels. Only some bodies of water are freely accessible to natural migration of eels in Latvia. Natural migration is prevented by multiple obstacles, such as dams.

The estimation of the genetic structure of populations is important for efficient management of naturally recruited and introduced eels. Two mitochondrial regions were used to investigate the genetic structure within and between eel samples from different waterbodies. Variation of sequencies of mtDNA cyt b and D-loop region were established. The diversity of haplotypes was studied in ten waterbodies from a part of the Baltic Lakeland, namely Lake Sīvers, Lake Usmas, Lake Ķišezers, Lake Liepājas, Lake Alūksnes, Lake Rāznas, Lake Vialikija Švakšty, Lake Svir, Lake Myadzyel, and the Myadzelka river.

Haplotype variation was different in all investigated waterbodies. New haplotypes were found for both mitochondrial regions for eels in Lake Sīvers and Lake Usmas. The detected sequences characterised *Anguilla rostrata* in Lake Alūksnes.

Keywords: *Anguilla anguilla*, cyt *b*, D-loop, genetic diversity, haplotype

NEW INSIGHTS INTO THE ELECTROTRANSFER MECHANISM OF CHARGED MOLECULES

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Electroporation is a biophysical technique that is commonly used to enhance the permeability of cell membranes to exogenous biomolecules through the application of external electric fields. This technique is routinely employed for the transfer of small molecules, chemotherapeutic agents, and nucleic acids (DNA or RNA). The electrotransfer mechanism of charged molecules depends on the molecular size. Small molecules such as propidium iodide enter the cell by diffusion from both sides of the electroporated membrane. The entry of significantly larger pDNA molecules is a multi-step process that involves the electrophoretic movement of DNA, the interaction with the cell side facing the cathode and formation of aggregates, translocation to the cytoplasm, and migration towards the nucleus. Electrophoresis is also believed to be the mode of entry for oligonucleotides and siRNA. Therefore, the entry of molecules is limited to the duration of pulse application and occurs only on the side of the cell facing the cathode. Since both pDNA and siRNA molecules enter the cytoplasm at the cathode-oriented side of the cell, the aim of this study was to investigate the impact of simultaneous electrotransfer using confocal fluorescence microscopy. Our results demonstrated that the electrotransfer of oligonucleotides and siRNA is driven by both electrophoresis and post-pulsation diffusion from both sides of the electroporated membrane. What is more, we discovered that pDNA negatively affects the electrotransfer efficiency of siRNA molecules in a concentration-dependent manner. Altogether, the study proposes novel fundamental insights into the electrotransfer mechanism of charged molecules.

Keywords: oligonucleotides, siRNA, electrotransfer, electroporation, nucleic acids

VITAMIN D-BINDING PROTEIN GENE POLYMORPHISMS ARE ASSOCIATED WITH MULTIPLE SCLEROSIS IN THE LATVIAN POPULATION

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Vitamin D is a nutrient and hormone that plays an important role in the pathogenesis of different autoimmunity-related diseases including multiple sclerosis (MS). Vitamin D acts by binding to a specific Vitamin D-binding protein (*VDBP*), which is involved in vitamin D transport and storage. The genetic variations of the *VDBP* gene have been studied as a potential risk factor for MS. These results need further investigation by genotyping in a case/control study. This study aimed to identify the potential association of *VDBP* gene polymorphisms with MS in the Latvian population. The *VDBP* (rs7041 and rs4588) were genotyped in 296 patients and 253 healthy individuals by the restriction enzyme site polymorphism method. Statistical analysis was performed with SPSS.25 Statistical Package. Significant associations with MS for both *VDBP* gene loci were found for common alleles and for homozygotes with common alleles included, but homozygotes with rare alleles were identified as clinical protective factors (rs4588, $p < 0.001$ and rs7041, $p < 0.01$, respectively); The two-locus risk genotype GG-CC (including common alleles) and the risk haplotype G-C confirm the associations found ($p < 0.01$). We present evidence that the *VDBP* (rs7041 and rs4588) may contribute to the risk of multiple sclerosis in the Latvian disease cohort.

Keywords: vitamin D-binding protein gene, polymorphisms, multiple sclerosis

Acknowledegements: the study was funded by UL project No. 1.1.1.2/VIAA/4/20/718 'The role of vitamin D and its receptor gene polymorphisms in the modulation of intestinal inflammation in patients with relapsing and progressive forms of multiple sclerosis' and ERDF project No. 1.1.1.1/16/A/016.

POPULATION GENETICS RESEARCH IN THE BALTIC COUNTRIES

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Population genetics seeks to understand how and why the frequencies of alleles and genotypes change over time within and between populations. It is the branch of biology that provides the deepest and clearest understanding of how the evolutionary change occurs. The use of genetic markers to study ecosystem health and function is a burgeoning line of scientific research. Studying the distribution and change in allele and gene frequencies helps us understand how populations are impacted by environmental changes. Genetic structures of populations have recently been combined with data sets obtained in related disciplines such as conservation biology, ecology, biogeography, and evolutionary biology. Several excellent review articles have been published that generally focus on European and American scientists. Here, we present selected aspects of population genetics, genome evolution, and molecular phylogeny with an emphasis on contributions by the researchers of the Baltic countries.

Keywords: population genetics, genome evolution, molecular phylogeny, Baltic countries

PHYLOGENETIC CHARACTERISATION OF SARS-COV-2 INFECTION IN FARMED MINKS FROM LITHUANIA

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In Lithuania, the first outbreak of SARS-CoV-2 infection in farmed American minks was reported in Jonava region in November 2020, with further SARS-CoV-2 infection confirmed in other mink farms in different regions of Lithuania. In 2021, samples from dead and apparently healthy minks (the nasopharyngeal swabs) and their environment were collected in 15 mink farms located in 12 districts of the country. Full-length virus genome sequencing was performed in Illumina MiSeq Sequencer. The genomes were assembled using the Illumina BaseSpace software DRAGEN COVID Lineage (v3.5.9). The Fasta format of whole genome sequences was used for clade and lineage assignment using online tools Nextclade and GISAD. Multisequence alignment was performed with Nextclade. Using MEGA X software, the resulting alignment was used to build a maximum likelihood phylogenetic tree under the GTR+G+I model with 1,000 bootstrap repeats. A total of 276 variable nucleotides were detected among the analysed 47 SARS-CoV-2 genome sequences derived from minks. The average diversity was the highest in the S protein (0.0263). The D614G mutation in the spike protein was found in all sequences. Phylogenetic analysis revealed the circulation of seven GISAID clades (GH, GRY, GR, GV, G, GK, and O) or four Nextstrain clades (20I, 20E, 20C, and 21J) in the minks during the study period. Analysed strains belonged to ten lineages (Nextclade; GISAID). Most infections were caused by Nextstrain clade 21J (Delta) strains: 72.4% of sequences belonged to the five different lineages from this clade. The distribution of CoV-2 variants in minks and humans in Lithuania over clades and locations and phylogenetic relationships between SARS-CoV-2 strains derived from minks in Lithuania, Latvia, Poland, Denmark, Netherlands, and France were analysed.

Keywords: SARS-CoV-2, phylogenetic analysis, mink farm, clade, lineage, Lithuania

ASSOCIATION OF POLYMORPHISMS IN CAST GENES WITH RESIDUAL INDICATORS OF FEED EFFICIENCY IN LATVIAN DARK HEAD SHEEP

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Feed efficiency in sheep is a crucial economic trait. Residual indicators of feed efficiencyare the following: residual feed intake (RFI) shows the economic value of feed consumption; residual weight gain (RWG) is associated with faster growth rates; residual intake and weight gain (RIG) offers the benefits of both components of feed efficiency. Improving the residual performance in the breeding process has an economic benefit. Sire rams with better RFI and RWG produce offspring with a higher value of this indicator. With the rapid development of research on sheep genome, there are more opportunities for breeding in the field of animal husbandry using genetic markers associated with various traits. Calpastatin (CAST) plays a key role in skeletal muscle development and is involved in protein metabolism, muscle growth, and fat deposition. This study aimed to analyse *CAST* gene polymorphisms and their relationship with the residual indicators of feed efficiency in the Latvian dark head (LT, *Latvijas tumšgalve*) breed. Exons of the *CAST* gene and their flanking intron regions were sequenced for 48 controlled fattened offspring of LT sires. Of the 43 polymorphisms found, 23 were found to form four groups with variations of complete linkage disequilibrium. SNPs: rs408766737, rs409957235, rs161885105, rs408766737, and rs161885105 showed a statistically significant association with RIF, RWG and/or RIG. Therefore, *CAST* gene SNPs have the potential to be molecular markers for RIF, RWG, and/or RIG indicators.

Keywords: calpastatin, feed efficiency, residual feed intake, Latvian dark head, sheep breeding

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GENETICAL VARIABILITY AND STRUCTURE OF TWO SPECIES OF FAMILY COLUMBIDAE IN EUROPE AND MOROCCO

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Population genetic structure of two widespread Palearctic species belonging to family Columbidae was investigated covering breeding and wintering areas. The Woodpigeon (*Columba palumbus*), the first studied species, is abundant and increasing in numbers whereas population of the second species, the European Turtle Dove (*Streptopelia turtur*), is considered declining and is listed as *Vulnerable* by the IUCN. The previous study carried out in western and southern Europe suggested that this species is panmictic across Europe. Between 2014 and 2020, 606 Woodpigeons collected in Sweden, European Russia, Belarus, Lithuania, Belgium, Spain, Portugal, Hungary, the coastal region of the Black Sea in Russia and northern Caucasus region, as well as 258 European Turtle Doves sampled in Ukraine, Spain, and Morocco were examined using mtDNA D-loop sequence analysis. Based on D-loop, higher genetic variability was determined for European Turtle Doves (Hd = 0.938 ± 0.008, π = 0.01613 ± 0.00036) than for Woodpigeons (Hd = 0.886 ± 0.010, π = 0.00638 ± 0.00026). A relatively higher genetic variability in the European Turtle Dove can be explained by two phylogenetic lineages identified for this species within mtDNA. The genetic divergence was determined between the Turtle Doves sampled in Morocco and Ukraine discriminating them from Spanish samples. Therefore, different conservation plans should be applied for this species in defined specific regions. The pair-wise Φ_{ST} comparisons, SAMOVA, and PCoA analyses indicated that different management units encompassing migratory populations of Woodpigeons using three main flyways (eastern Atlantic, central European-Mediterranean, and the Black Sea) and for the resident populations located in north and south Iberia should be established.

Keywords: *Columba palumbus*, *Streptopelia turtur*, mtDNA, genetic variability, population genetic structure, conservation

BARLEY *TWEAKY SPIKE* MUTATION ASSOCIATION WITH PHYTOHORMONES AND MUTATION IDENTIFICATION WORKFLOW USING WGS

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The *tweaky spike* (*tw*) pleiotropic mutant, developed by chemical mutagenesis, is characterised by alterations in various parts of the spike, increased levels of free radicals and altered amino acid composition in the grains, as well as immunodeficiency and genetic instability. Exploration of the genetic nature and regulation of *tw* mutation may expand general understanding of barley reproductive development and the range of targets for genetic manipulations. This study aimed to explore phytohormone association with *tw* mutation by treating *tw* mutants and *WT* with phytohormones (gibberellin, cytokinin, melatonin, and auxin). Four features of *tw* mutants were selected for phenotype analysis: spikeletless gaps, the presence of an overdeveloped tip of the spike (a 'crown'), lodicule transformations, and variation in flower organ number. Only auxin had a significant effect on all the tested features of *tw* phenotype compared to control ($p < 0.05$) while other phytohormones altered mutant phenotype frequencies only in one or two features. This data suggests that the *tw* mutation mainly disrupts auxin physiology, and mild effects of other phytohormones on *tw* phenotype might be a side effect of auxin gradient changes in *tw* barley spikes. To explore genetic background of *tw* mutation, we created a recombinant $tw_2 \times WT$ and $tw \times WT$ F_2 population. Segregation analysis showed that *tw* mutation is monogenic and WGS was performed on recombinants of *tw* phenotype (50 individuals) ant WT (46 individuals) populations. Further analysis includes SNP ratio analysis and validation of candidate genes in other allelic *tw* mutants of independent origin.

Keywords: phytohormones, barley, flower structure, tweaky spike mutation

DIFFERENCES BETWEEN TWO HORSE POPULATIONS BASED ON GENETIC AND GENOMIC INBREEDING

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The aim of the study was to estimate the inbreeding between two horse populations using genomic and pedigree data. For the study, Trakehner and Žemaitukai horses were genotyped by GGP Equine 70k chip (Illumina Infinium). An expected (Hexp) and observed heterozygosity (Hobs) was calculated. Two methods were applied to estimate the inbreeding. Pedigree data were used to calculate genetic inbreeding. A run of homozygosity (ROH) analyses was the basis to estimate genomic inbreeding (FROH). From the total of 66.560 SNP markers, up to 55.770 were located on autosomes of Trakehner and only 48.228 on Žemaitukai horses. The determined Hexp and Hobs was 0.302 and 0.323, respectively, in Trakehner horses and 0.243 and 0.266, respectively, in Žemaitukai horses. In Trakehner horses the shortest segments of ROH showed the highest proportion across all detected ROHs (88.96%), whereas the lowest distribution was found for the ROHs > 16 Mb (0.06%). The average FROH varied from 20.0 (ROH 0 – 2 Mb) to 1.4 (ROH > 16 Mb). In Žemaitukai horses, the average FROH varied from 18.9 (ROH 0 – 2 Mb) to 1.9 (ROH > 16 Mb). The shortest ROH segments showed the highest proportion across all the ROHs detected in both horse groups, which indicated older inbreeding. The genomic inbreeding coefficient determined in the category of FROH > 16 Mb in comparison with genealogical inbreeding showed a narrower diversity of the Trakehner population, it was also confirmed by Hexp and Hobs. In addition, genomic inbreeding could cause rapid reduction of diversity and needs special attention in the conservation of their genetic variation.

Keywords: heterozygosity, genomic inbreeding, genetic inbreeding, Trakehner, Žemaitukai

GENETIC DIVERSITY OF *BABESIA CANIS* STRAINS IN THE BALTIC COUNTRIES

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Canine babesiosis caused by haemoprotozoan *Babesia* parasites is an emerging and rapidly expanding tick-borne disease in central and north-eastern Europe, with the vast majority of clinical babesiosis cases in European dogs caused by *Babesia canis*. In the last decade, the endemic area of *B. canis* has expanded in the Baltic countries due to the rapid expansion of its vector, *Dermacentor reticulatus* tick. Genetic variability is important in the survival of *Babesia* parasites in their vertebrate hosts and could play an important role in the survival and distribution of particular *B. canis* genotypes to new areas in the face of anthropogenic pressure and changing climate. The present study aimed to investigate the genetic diversity and distribution of *B. canis* strains in the Baltic region and compare patterns in the prevalence and geographical distribution of *B. canis* genotypes in different European regions. Blood samples from dogs suspected of babesiosis were collected for molecular analysis. PCR-RFLP and sequence analyses based on a partial region of 18S rRNA and Bc28.1 genes were used for *B. canis* genotyping. The molecular characterisation of *B. canis* isolates indicates the presence of genetically heterogenic *B. canis* strains in Lithuania. The distribution and prevalence of *B. canis* genotypes in Lithuania are specific and differ from findings obtained in Latvia and other regions of the north-eastern and central Europe.

Keywords: *Babesia canis*, canine babesiosis, PCR-RFLP, 18S rRNA; Bc28.1

PHYLOGENETIC CHARACTERISATION OF TICK-BORNE ENCEPHALITIS VIRUS FROM LITHUANIA

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The Baltic countries is the region in Europe where tick-borne encephalitis (TBE) is most endemic, with the highest notification rate by cases of TBE reported in Lithuania. Ticks from the Ixodidae family are the main vectors of the TBE virus (TBEV) in Europe. This study describes TBEV strains derived from two tick species, *Ixodes ricinus* and *Dermacentor reticulatus*, and provides a genotypic characterisation of viral isolates from Lithuania. TBEV isolates derived from ticks collected in seven counties of Lithuania were characterised based on sequence analysis of the partial E protein and NS3 genes. The obtained 56 sequences were compared with selected 68 TBEV strains from the NCBI database. Phylogenetic analyses of the E and NS3 gene sequences derived from 29 Lithuanian TBEV isolates revealed that they were specific for Lithuania and all belong to the European subtype with a maximum identity of 98.70% and 96.32% to the reference strain Neudoerlf (U27495), respectively. The TBEV isolates showed a significant regional genetic diversity: 11 variants with 37 variable nucleotides were detected among the E gene sequences of 462 bp, and 14 variants with 61 variable nucleotides were detected among the NS3 gene sequences of 761 bp. Amino acid sequence analysis of the Lithuanian TBEV isolates revealed the presence of five amino acid substitutions in the E protein gene and seven in the NS3 gene. No correlation was found between TBEV genotypes and tick species. However, genetic differences were observed between strains from different locations, while strains from the same location showed high similarity.

Keywords: tick-borne encephalitis virus, ticks, phylogenetic analysis, E protein gene, NS3 gene

STUDY OF OLIGO- AND POLYNUCLEOTIDE AND PROPIDIUM IODIDE ELECTROTRANSFER EFFICIENCY

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Electroporation (EP) is a method used to controllably reduce the barrier function of cell plasma membrane by applying electric pulsation. In this way, hydrophilic molecules such as drugs or poly- and oligonucleotides may be introduced into the cell. In the latter instance, a new gene may be inserted or expression of native genes may be modulated, respectively. Despite abundance of scientific literature concerning various applications of this technique both *in vitro* and *in vivo*, the character of electrotransfection itself has not been thoroughly investigated. Thus, the aim of this study was to analyse the aspects of electrotransfer of plasmid DNA, oligonucleotides, and propidium iodide (PI) and the influence of electroporation parameters on electrotransfer efficiency. Electroporation was carried out using steel electrodes and CHO-K1 cells. Suspended cells were affected by low-voltage (500 V/cm, 5 ms) or high-voltage (1400 V/cm, 100 μs) pulsation. PI, labelled oligonucleotide and pEGFP electrotransfer efficiency was detected using flow cytometry; in the case of the latter, GFP expression was assayed 24 h following the experiment. The results demonstrate that PI electrotrasfer happens irrespectively of used EP parameters and is observed after the pulsation is removed. Oligonucleotide electrotransfer appears to primarily rely on electrophoretic force induced by electric pulsation, but further analysis may be necessary. The highest pDNA electrotransfection rate was achieved using HV pulsation, while LV pulses considerably reduced the number of viable cells and achieved insignificant transfection rates, suggesting a different nature of molecule intake compared with oligonucleotide and PI electrotransfer.

Keywords: electroporation, electrotransfection

WHAT IS mtDNA ATP6 MARKER POTENTIAL FOR THE RESEARCH IN INTRASPECIFIC GENETIC DIVERSITY OF THE EURASIAN PERCH (*PERCA FLUVIATILIS*) WITHIN PHYLOGEOGRAPHIC AND ANTHROPOGENIC ACTIVITY CONTEXTS?

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So far, the anthropogenic effects on intraspecific genetic diversity of freshwater fish species have been poorly studied. To accumulate molecular data related to the phylogeographic features that are essential for determination of anthropogenic influence causing changes of genetic diversity, appropriate model fish species should be chosen and its genetic diversity using various molecular markers should be studied comprehensively. To test mtDNA ATP6 potential to reveal intraspecific genetic diversity of the Eurasian perch (*Perca fluviatilis*) in the Baltic Sea Region and neighbouring countries, a total of 193 perch from Lithuania, Latvia, Belarus, and Ukraine was investigated using newly created primers. Thirteen different haplotypes were identified after direct sequencing of mtDNA ATP6 gene fragment (627 bp). Most of these haplotypes were singletons, but four haplotypes, named A, B, C, and D, had higher frequencies forming central haplotypes of corresponding haplogroups in haplotype network. Principal Component Analysis grouped perch samples into three distinct groups (I–III) showing non-accidental geographic distribution that encompassed representatives of previously described four mtDNA D-loop genetic groups (I–IV). In general, we found similar but less complex tendencies of genetic variation of the perch in the studied macrogeographic region using mtDNA ATP6 compared to previous studies based on different mtDNA markers. To reveal the anthropogenic impact on perch genetic structure, perch samples collected from freshwater hydrosystems serving as coolers of nuclear or thermal power plants requires more comprehensive research focusing on the perch and other abundant fish species, collection of temporal samples, and continuous genetic monitoring.

Keywords: genetic diversity, haplogroup, haplotype, mtDNA, fish, ATP6

URINARY MIRNA ASSOCIATION WITH TREATMENT RESISTANCE IN TRIPLE-NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype. The main characteristics of TNBC are lack of oestrogen, progesterone and human epidermal growth factor 2 receptors. Chemotherapy remains the main treatment strategy. However, severe side effects and unpredictable treatment effectiveness remain the main concern of chemotherapy. Many different microRNAs (miRs) are frequently dysregulated and can be secreted into the circulatory system. It suggests that miRNAs in urine could serve as a promising tool for identifying non-invasive biomarkers for TNBC. Therefore, the purpose of this study was to identify miRNAs implicated in TNBC chemoresistance and may be used to anticipate treatment outcomes. In this study, 186 TNBC urine samples (93 patients) were evaluated for three urinary miR levels (miR-200a-3p, miR-210-3p, miR-125a-5p) by means of RT-qPCR. Samples were collected before and six months after treatment with paclitaxel combined with carboplatin, or four cycles of doxorubicin combined with cyclophosphamide. Analysis showed that miR-200a-3p and miR-125a-5p were significantly upregulated in the residual disease group compared to the non-residual disease group ($p = 0.019$, $p = 0.350$, respectively). Moreover, miR-200a-3p was significantly upregulated in the incomplete response treatment group compared to the complete response group ($p = 0.018$). Finally, a significantly decreased level of miR-200a-3p was observed in patients who had DNA-damage repair germline mutations compared to the group which did not ($p = 0.034$). Overall, results suggest that miR-200a-3p and miR-125a-5p are involved in processes of chemotherapy resistance. The detection of the changes in miRNA level in urine samples from TNBC patients could be used as a completely non-invasive way to predict the effectiveness of treatment.

Keywords: TNBC, microRNA, urine

MOLECULAR CHARACTERISATION OF *BARTONELLA* SPP. IN CATS IN LITHUANIA

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Domestic pets are susceptible to infection with various species of *Bartonella* and may be involved in the transmission of these pathogens to humans. Domestic cats (*Felis catus*) are considered the main reservoir of three zoonotic *Bartonella* species: *B. henseale*, *B. clarridgeiae* (both of which can cause cat scratch disease), and *B. koehlerae* (a causative agent of endocarditis in humans). The aim of the present study was to investigate the presence of *Bartonella* infections in stray and pet cats in Lithuania and characterise *Bartonella* isolates based on sequence analysis of 16S-23S rRNA ITS region. Blood samples were taken from 163 cats presented in pet clinics and animal shelters. *Bartonella* DNA was detected in 4.9% (8/163) of cat samples. A total of eight *Bartonella* 16S-23S rRNA ITS region sequences were analysed. Phylogenetic analysis of eight *Bartonella* isolates demonstrated that the cats were infected with *B. henselae*, *B. clarridgeiae*, and *Bartonella* sp. Closely related to *Bartonella schoenbuchensis*. *B. schoenbuchensis* strains are associated with ruminants and have not yet been documented as infecting cats in Europe. The phylogenetic tree of 16S-23S rRNA ITS region of *Bartonella* spp. Showed three well-supported clusters. The 16S-23S rRNA ITS region sequences of *B. henselae*, *B. clarridgeiae*, and *Bartonella* sp. derived from cats in this study were heterogenic. Among the Lithuanian *B. henselae* isolates, two genotypes with five variable nucleotides were detected. Two cat specimens harboured two different *B. clarridgeiae* genotypes (differing at two nucleotide positions). This study is the first report on the prevalence and molecular characterisation of *Bartonella* spp. in cats in Lithuania.

Keywords: *Bartonella*, molecular characterisation, cats, genotypes

INSIGHTS INTO LATVIAN HUMAN GENOME INITIATIVES: FROM THE POPULATION DIVERSITY TO DISEASE-SPECIFIC POLYGENIC RISKS

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Understanding population-specific genome variability is an obvious step needed to implement genome-based applicability into personalised medicine. As a pilot project on the way towards national genome reference, we performed whole-genome sequencing of 800 individuals selected from the Genome Database of Latvian Population (LGDB). We provide the first information on the composition of variants obtained from this experiment. Next-generation sequencing was performed using the DNBSEQ-T10 instrument. The results were compared to genotypes obtained by an Illumina GWAS array from the same individuals with high concordance between both platforms. We extended our sample including 4500 genotyped samples from the LGDB to evaluate the genetic structure and ancestry admixture of the Latvian population. We observed a distinct population stratification of the Latvian sample based on ethnic and regional origin. To a high degree, self-reported ethnicity correlated with the composition of ancestral populations in admixture analysis compared with data from neighbouring countries. The group of people of Latvian ethnicity displayed an unusually high proportion of Western Hunter-gatherer ancestry compared to other populations. We also demonstrate significantly improved imputation of low-frequency variants using a Latvian haplotype reference panel and compare the different polygenic risk scores on their performance to predict type 2 diabetes This study offers the most extensive to-date analysis of genetic variation in Latvia providing a background for a public reference resource.

Keywords: Latvian genome, genetic variation, type 2 diabetes, admixture analysis

DEVELOPMENT OF SPIKE KNOTS: EXPERIENCE FROM THE PROGENY TRIALS

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Growth of coniferous trees in the Baltic region is expected to increase, at least with the boost from assisted migration. However, with the production of biomass alone we will not be able to outcompete other regions of the world with notably larger annual tree increments. Therefore, stem and wood quality traits need also to be considered in tree breeding. We aimed to evaluate the family effect on the incidence of spike knots and its relation to growth traits based on repeated inventories and sample trees from open-pollinated progeny trials. A higher incidence of spike knots was associated with better height growth and the presence of lammas shoots, but family-level associations with growth were not distinct. The family had statistically significant effect on growth and spike knots, yet a mainly weak correlation was observed between both traits. The presence of spike knots decreased with time (age of trees), which indicates that results of inventories at juvenile age might be misleading. The heritability of spike knots was low. Although the presence of lammas shoots was low, it showed a strong positive genotypic relationship with the formation of spike knots in the next growing season. The potential to select fast growing families with low probability of spike knots was indicated.

Keywords: ramicorns, stem quality, tree breeding

MOLECULAR DIAGNOSIS OF PARASITIC WORMS IN LEECHES (HIRUDINIDA)

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Molecular diagnosis was applied to identify parasitic worms in leeches, which are intermediate hosts and possibly participate in the transmission of parasites in molluscs, fishes, and birds. The knowledge of molecular diversity and taxonomic affiliation of the parasites occurring in leeches remains poorly known. In the period of 2020–2022, 794 leeches belonging to nine species were collected in lakes, rivers, and ponds in Lithuania and examined for the presence of parasites. The compression method was applied for the detection of metacercaria (larvae stage of flukes) and cysticercoids (larvae stage of tapeworms) in leeches. The parasites found were isolated from the leeches and examined using light microscopy. For more accurate identification molecular analysis was done using the 28S rDNA partial gene (primers digl2, 1500R), the second internal transcribed spacer region (ITS2) (primers BD1, BD2, NLR, NLF) region, and the cytochrome c oxidase (COI) fragment (primers JB3, JB13) using sequences analysis. The obtained sequences and currently available molecular data in GenBank showed 100% similarity with *Posthodiplostomum cuticula* (Nordmann, 1832), 99% with *Cotylurus syrius* (Dubois, 1934), and 87% with *Microsomacanthus* sp. (Hymenolepididae).

Keywords: molecular detection, leeches, *Posthodiplostomum cuticula*, intermediate hosts

GENETIC DIVERSITY OF MITOCHONDRIAL CYTOCHROME B GENE OF *PIPISTRELLUS* BATS IN LITHUANIA

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Bats (Chiroptera) are distributed worldwide and are the second largest order of mammals (next to rodents). Currently, 15 bat species belonging to seven genera have been identified in Lithuania. One of the most common genera is *Pipistrellus*, which includes three species: *Pipistrellus nathusii*, *P. pygmaeus*, and *P. pipistrellus.* Bats are recognised as a reservoirs or carriers of numerous species of viruses, bacteria, and protozoan parasites, some of them with zoonotic potential to infect other animals or humans. While most studies are focusing on bat-associated microorganisms, little research is done on the genetic diversity of bats themselves. The aim of this study was to identify bat species and characterise their genetic diversity and their phylogenetic relationship. A total number of 22 blood samples (*Pipistrellus pygmaeus* (n = 12), *P. nathusii* (n = 10)) were collected in 2020–2021. Amplification of 800 bp partial cytochrome b (cytb) gene was applied. The sequence analysis by BLAST and phylogeny revealed two bat species: *P. nathusii* (n = 10) and *P. pygmaeus* (n = 12). Furthermore, a comparison of sequences showed an intraspecific genetic variation in the studied bat species. Several specific mitochondrial haplotypes of *P. nathusii* and *P. pygmaeus* in bats from Lithuania were detected.

Keywords: bats, *Pipistrellus*, genetic diversity, Lithuania

SOME ASPECTS OF BACTERIOPHAGES ISOLATED FROM ANTARCTICA

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The most widespread viruses on the planet are bacteriophages. Phages play an important role in the ecosystem as they mediate lateral gene transfer, alter host metabolism, and redistribute bacterial biochemical compounds, in addition to obligate bacterial parasitism. In this study, we examined the presence of bacteriophages in samples of vascular plants isolated from Antarctica. We isolated bacteriophages from samples of Antarctic plants and soil by a direct method using a stock culture. For DNA purification, we used the rapid spin column method to analyse a broad spectrum of bacteriophages propagated in bacteria grown in liquid cultures. The bacteriophages isolated from various plant and soil samples were identical to Dickeya phage phiDP10.3 to varying degrees: the samples were 86.01%, 86.89%, 86.91%, and 93.57% identical, which was obtained using the BLAST package. Multiple alignment and phylogenetic analysis of the obtained sequences were performed using the MEGA package. The sequences of the isolated phages and the sequences of the three genes of Dickeya phage phiDP10.3 from GenBank were compared. The samples with 93.57%, 86.91%, and 86.01% identity are the closest to gene 2 of the tag locus. Furthermore, the sample with the lowest identity is closest to gene 1 of the tag locus. This suggests that evolutionary processes are reflected in mutations of genes 1 and 2 of the tag locus. The obtained results can provide a good basis for further research and clarification of the evolutionary relationship between bacteriophages and plants inhabiting the Antarctic. Similar studies may be able to establish the nature of frost resistance.

Keywords: bacteriophages, Antarctic plants, Antarctic soil, Dickeya phage

GENETIC DIVERSITY OF *BORRELIA* STRAINS CIRCULATING IN URBAN AREAS IN LITHUANIA

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Causative agents of Lyme borreliosis (LB) are spirochetes from *Borrelia burgdorferi* s. l. complex whose lifecycle is restricted to cycling between *Ixodes* ticks and vertebrate hosts. To date, over 20 genospecies of the *B. burgdorferi* s.l. complex have been identified worldwide, and eight of them were found to be associated with human LB. The identification of *Borrelia* spp. is complicated because of the high diversity of species and strain, complex transmission cycles, a broad range of vertebrate hosts and tick vectors, and a wide range of clinical symptoms. *Borrelia burgdorferi* s. l. bacteria have a complex genome which is unique among other bacteria as it is highly segmented and includes linear and circular DNA molecules. To date, molecular multilocus typing techniques and specific and sensitive molecular tools are required for precise diagnostic of *Borrelia* strains which could provide useful information on the molecular ecology and population structure of *B. burgdorferi* s. l. The present study aimed to investigate the presence of *Borrelia* infections in *Ixodes ricinus* ticks collected from urban areas in Lithuania and characterise *Borrelia* strains using three different targets of bacteria genome: linear plasmid *ospA* gene, chromosomal flagellin (*fla*) gene, and 16S (rrs) – 23S (rrlA) rRNA intergenic spacer (IGS) region. *Borrelia* DNA was detected in 36% (193/536) of collected ticks. Multilocus sequence analysis revealed the presence of three species from *Borrelia burgdorferi* s. l. complex with *B. afzelii* the most prevalent, followed by *B. garinii* and *B. burgdorferi* s. s. Phylogenetic analysis demonstrated inter- and intraspecific diversity among *Borrelia* isolates. Moreover, molecular analysis based on the *fla* gene and IGS region allowed identifying *B. miyamotoi*, which belongs to the *Borrelia* relapsing fever group.

Keywords: Borrelia burgdorferi s. l., Borrelia miyamotoi, ticks, ospA gene, fla gene, IGS region, urban areas

PANGENOMIC ARCHITECTURES ARE PRESENT IN MULTIPLE MARINE INVERTEBRATE SPECIES. BIOLOGICAL IMPLICATIONS AND NOVEL ANALYSIS TOOLS

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Advances in whole-genome resequencing techniques for non-model species have uncovered a greater prevalence of genomic structural variation among eukaryotes than previously assumed. Large insertions and deletions, previously believed to mainly occur in non-coding regions, appear to frequently impact protein-coding genes, which can appear in a hemizygous state in diploid organisms. At a population level, these genes may be absent in certain individuals ('dispensable' genes), defining by contrast a larger set of genes that are present in all individuals ('core' genes). This phenomenon is known as gene presence-absence variation (PAV). The union set of core and dispensable genes constitutes the so-called pan-genome, with the dispensable fraction potentially playing a significant role in local adaptation and phenotypic diversity. We describe the pangenomic architecture of two ecologically and commercially important bivalve species: *Mytilus galloprovincialis* and *Crassostrea gigas.* Although at different levels both species are characterised by massive gene PAV, widespread hemizygosis, and an open pangenome, whose dispensable fraction is strongly enriched in genes involved in immune response and survival. Although the phenomenon is still poorly studied, our results suggest that it might be present in many marine invertebrates. We discuss the biological implication of this phenomenon in terms of the capability of adaptation of these species to variable marine coastal environments and report a bioinformatic pipeline for the *de-novo* assembly of bivalve pangenomes and the assessment of gene PAV based on resequencing data.

Keywords: marine invertebrate, pangenomic architecture, *Mytilus galloprovincialis*, *Crassostrea gigas*

STUDY OF INTERGENERIC HYBRIDS BETWEEN *LOLIUM PERENNE* AND *FESTUCA GIGANTEA* AIMING TO REVEAL THE ORIGIN OF *F. GIGANTEA*

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Earlier crossing tests and recent DNA maker studies show *F. gigantea* (2n = 6x = 42) species to be closely related to diploid *F. pratensis* (2n = 2x = 14) and some diploid *Lolium* spp., *L. multiflorum*, and *L. perenne* (2n = 2x = 14). The aim of this study was to reveal the origin of *F. gigantea* by the assessment of its hybrids with the putative progenitor species. We produced three hybrids between *L. perenne* (female plant) × *F. gigantea* (2n = 4x = 28), which were multiplied and evaluated in the field trial. In order to determine the morphological traits of these intergeneric hybrids and compare them to parents, we evaluated inflorescence type, growth habitus, the length and width of the leaf, and the length of the leaf auricles. The hybrids were very strong, well-growing plants morphologically closer to the *F. gigantea* parent and exceeding it in height. Genomic *in situ* hybridization (GISH) is an effective method that uses the genomic DNA of one parent to differentiate its chromosomes from other species in the hybrid by fluorescent labelling. Therefore, GISH has the ability to dissect the genome structure and determine progenitor species in allopolyploids. We applied rhodamine labelled *L. perenne* genomic probe in the metaphase chromosome spreads of *L. perenne* × *F. gigantea* (2n = 4x = 28). Strong hybridisation was detected on seven chromosomes coming from *L. perenne*. In addition, *L. perenne* florescent probe highlighted (intermedium signal) a set of 14 chromosomes and discriminated them for the remaining seven showing no signal. The same *L. perenne* fluorescent probe highlighted a set of 28 chromosomes and 14 chromosomes had no signal in the metaphase plates of *F. gigantea* chromosomes. These results provide information on possible subgenome composition of allohexaploid *F. gigantea* showing two of its subgenomes to be closely related to *L. perenne*.

Keywords: *Festuca gigantea*, alloxeaploid, GISH, subgenome composition

MUTAGENIC EFFECTS OF HERBICIDES TO ANIMALS

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Ecotoxicological research on the harmful effects of agricultural chemicals on fauna is necessary given the expansion of crops associated with the use of these products. Mutagenic anomalies have been observed in the native wildlife in different countries, however, we do not know how this affects Lithuanian wildlife. Over the past decade, the comet assay or single-cell gel electrophoresis (SCGE) has become one of the standard methods for assessing DNA damage, with applications in genotoxicity testing, biomonitoring and molecular epidemiology, and fundamental research in ecogenotoxicology. Significant differences in DNA damage among specimens collected from intensive chemical agricultural fields and non-agricultural fields in all parameters of the comet assay have been found. Agricultural landscapes can harbour many amphibian species, which tend to respond negatively to agricultural chemicals at different life stages. Amphibians, especially anurans, are broadly used as test animals and bioindicators in evaluating the effects of pollutants in aquatic and agricultural ecosystems.

Keywords: mutagenic effect, genetic anomalies, herbicides, pesticide exposure, genetic damages

GENE EDITING IN PLANTS

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The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system has provided scientists with a powerful tool for generation of site-specific mutations in genes of interest. This system has been shown to work in over 70 different crop species from apple to wheat. It requires expression of Cas (CRISPR-associated) nuclease and guide RNA (gRNA) in plant cells. Efficiency of the CRISPR/Cas approach depends on several factors: (1) which Cas nuclease is used and optimisation of Cas sequences to plant codons; (2) which promoters are used to drive the expression of Cas genes, and/or expression of gRNA cassette; (3) the choice of target site and construction of gRNAs, and (4) on the type of transgene delivery to host/plant genome. Potential effect of Cas9 and Cas12/Cpf1 nucleases and the presence or absence of plant introns in Cas genes and the number of nuclear localization signals (NLSs) on the efficiency of site-specific mutagenesis will be discussed. We will also discuss the role of promoters that drive expression of Cas nuclease or gRNA cassettes. In addition, we will focus on the type of transgene delivery into plant genome. To test the effect of these factors on the efficiency of site-specific mutagenesis, we used barley as a model crop and *HvTB1* as a gene of interest, a homolog of maize major domestication locus *teosinte branched1* controlling branching architecture. Our preliminary data on *HvTB1* gene editing will be presented.

Keywords: gene editing, CRISPR/Cas, barley, *teosinte branched1*

MOLECULAR DIVERSITY OF LITHUANIAN POPULATIONS OF THE SMALL BALSAM (*IMPATIENS PARVIFLORA* DC.)

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The small balsam (*Impatiens parviflora* DC.) is a highly invasive species. The native range of the species is Central Asia. In Europe, it was introduced in the mid-19th century; it spread rapidly and nowadays is one of the most common herbaceous alien plants found in European deciduous forests. In Lithuania, this species is invading parks, groves and forests, especially areas of intensive tourism and human activities. Amplified fragment length polymorphism (AFLP) markers were chosen for this study, because they offer a large number of informative DNA loci and no prior knowledge is needed about the genome of the organism to apply this method. The aim of our research was to determine the genetic diversity of Lithuanian populations of the invasive species *I. parviflora* by amplified fragment length polymorphism markers. Individuals of *I. parviflora* were sampled from 21 populations in south-eastern, central, and north-western regions of Lithuania. AFLP loci analysis revealed low polymorphism of Lithuanian populations of *I. parviflora*. According to hierarchical analysis of molecular variance (AMOVA), much higher molecular variance within populations was determined compared to the variance among populations. *Impatiens parviflora* populations did not differentiate depending on biotope, geography, or road types. For Lithuanian populations of *I. parviflora*, many genetic clusters at AFLP loci were found by Bayesian analysis.

Keywords: *Balsaminaceae*, *Impatiens parviflora*, invasive species, AFLP markers, genetic diversity

PROPAGATION OF RYE AND WHEAT GENETIC RESOURCES OF LATVIAN ORIGIN FOR NICHE PRODUCTS

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Three ray varieties and more than 20 spring and winter wheat varieties and prospective lines were created in the Stende breeding unit between 1922 and 1950. Since selection was carried out in local conditions, they are adapted to specific growing conditions and stresses. Such genotypes are recognised as a very valuable source of genetic diversity with some unique traits often lost in modern varieties. Their quality allows food industry to produce new niche products. Shortly after regaining state independence, in 1993, the Latvian Society of Geneticists and Breeders initiated an inventory of Latvian breeders' work collections and repatriation of the genetic material of Latvian origin from gene banks abroad. Since 1997, local crop varieties and other valuable breeding material are kept in the Latvian Gene Bank. The maintained number of seeds of each sample is not very high and it is not meant for direct commercial use. To make this genetic resource available to farmers, a project was conducted to propagate and evaluate rye and wheat varieties created in Latvia from 1922 to 1950. Yield, grain quality, resistance to pathogens, lodging resistance, and backing quality were evaluated in organic conditions in the Stende Research Centre of the Institute of Agricultural Resources and Economics and in the 'Brīvzemnieki' organic farm. The best wheat and rye varieties that are appropriate for organic agriculture will be included in the catalogue of conservation varieties and available for legal propagation and use for baking high-quality bread and other bakery products.

Keywords: rye, wheat, conservation varieties, gene banks, bread

LOW COVERAGE WGS-ASSISTED INVESTIGATION OF UP-STREAM FLANKING SEQUENCES OF SCOTS PINE CANDIDATE GENES FOR RESISTANCE AGAINST ROOT ROT

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For non-model organisms, transcriptome data are often available, but a reference genome or a high-quality draft genome sequence is seldom available. This is the case for *Pinus sylvestris* L., a species of high economic and ecological significance. This research continues a study into transcriptome dynamics in the Scots pine in response to inoculation with the root rot-causing fungus *Heterobasidion annosum*. We decided to study the up-stream flanking sequences of Scots pine candidate genes for resistance against root rot to identify regulatory regions that could influence and coordinate gene expression. Initially, linear DNA amplification in combination with massive parallel sequencing was used with very limited success. To clarify the reasons behind the problems encountered using this approach, we decided to attempt a whole genome sequencing (WGS) using a long-read sequencing technology. Given the large size of the Scots pine genome (~24 Gbp), a very low genome sequence coverage was expected. Using the best reads ($L \geq 30$ kb, $q \geq 10$, $n \sim 867$ k), preliminary results show 84% of reference transcripts mapping with the sequencing reads, including primer binding sites used in linear DNA amplification- based approaches. Of the reference sequences, 14% mapped to the long reads but without covering the primer binding sites. We are still producing and analysing new WGS data that will provide additional information and genome coverage. Nonetheless, our results demonstrate the usefulness of long read sequencing for the analysis of plant species with limited genomic resources, and even low genome coverage can provide valuable information to complement the existing data and knowledge.

Keywords: *Pinus sylvestris* genome, resistance-linked genes, massive parallel sequencing, gene regulatory regions

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MOLECULAR IDENTIFICATION OF *SARCOCYSTIS* SPP. IN THE INTESTINES OF MUSTELIDS FROM LITHUANIA

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Members of the genus *Sarcocystis* are cyst forming coccidian parasites that infect mammals, birds, and reptiles. These parasites are characterised by an obligatory prey-predator two-host life cycle. Sporocysts develop in the small intestine of definitive hosts, while sarcocysts are formed in the muscles of intermediate hosts. Definitive hosts are known for less than half of *Sarcocystis* species. Sporocysts of different *Sarcocystis* species can be distinguished only by molecular methods. Members of the family Mustelidae are opportunistic predators, and their diet consists of birds, various mammals, fish, amphibians, reptiles, invertebrates, fruits, and carcasses of dead animal. They are widespread in Lithuania and occur in all habitats. The role of mustelids as possible definitive hosts of *Sarcocystis* spp. are not well revealed. In the present study, we aimed to clarify whether mustelids can spread (1) *Sarcocystis* species whose intermediate hosts are birds, and (2) *Sarcocystis* species using ungulates as intermediate hosts. We examined 115 small intestine samples of mustelids for *Sarcocystis* spp. After dissection of the animal, the intestinal epithelium was scraped to extract DNA from the sample, followed by nested PCR with species-specific primers and sequencing of selected samples. In this work, 16 species of *Sarcocystis* (*S. bertrami*, *S. bovifelis*, *S. capreolicanis*, *S. capracanis*, *S. cruzi*, *S. elongata*, *S. entzerothi*, *S. hirsuta*, *S. hominis*, *S. japonica*, *S. linearis*, *S. morae*, *S. rileyi*, *S. silva*, *S. truncate*, and undescribed *Sarcocystis* sp. closely related to *S. wenzeli*) were for the first time identified in intestines of mustelids. Horse, goat, roe deer, red deer, moose, fallow deer, sika deer, chicken, duck, and cattle serve as intermediate hosts of these *Sarcocystis* species found. The detection rates of avian *Sarcocystis* species were significantly lower (11%) comparing to species using ungulates as intermediate hosts (86%). The research carried out so far is important for disclosing potential definitive hosts of *Sarcocystis* species under the natural conditions.

Keywords: *Sarcocystis*, Mustelidae, genetic identification

GENETIC AND SOCIAL DIVERSITY IN IRON AGE SCYTHIAN COMMUNITIES OF EASTERN EUROPE

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One of the most famous nomadic groups of the Iron Age (IA) were Scythians whose cultural continuum stretched through Eurasian steppes from Central Asia to Central Europe between the eighth and the third centuries BCE. Recent broad-scale studies into ancient DNA characterised the Scythian gene pool as a mixture of preceding Bronze Age Yamna-related ancestry and East Asian components, with independent origins for eastern and western groups, but continuous gene flow between them. However, plenty of local aspects such as social structure, individual life-trajectories, and migrant-local interaction have still remained unresolved. To address these questions, we are launching the interdisciplinary project integrating genomic, isotopic, and archaeological evidence to learn about the structure and social stratification of the IA groups in Ukraine. The key site of the study is the Bil'sk fortified settlement (BFS) representing one of the largest IA complexes in Eastern Europe (the 8th–3rd centuries BCE). The collection of 40 new osteological samples is compiled to study the genetic structure of different social groups of the BFS. We plan to compare the ancestry profiles of early and late elite groups of Scythians and of local sedentary non-elite groups to reveal their possible interactions and admixture. The genetic evidence will be complemented with stable isotope analysis to study the diet and mobility of elite/locals and male/female groups. Also, we will analyse the genetic affinities of the BFS with earlier and later ancient groups and model population continuity between IA and modern individuals from forest-steppe Ukraine.

Keywords: ancient DNA, human population genetics, Iron Age, Eastern Europe, Scythians

IDENTIFYING POTENTIAL CARF/SAVED-TM PROTEINS IN TYPE III CRISPR-CAS SYSTEMS THROUGH BIOINFORMATIC ANALYSIS

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The CRISPR-Cas system is a prokaryotic adaptive immune system that provides protection against invasion of such genetic elements as viruses and plasmids. It is composed of two main components: the CRISPR array, which contains spacers that are derived from previous invaders, and the Cas proteins, which are responsible for providing immunity against these invaders. The presence of different CARF and SAVED domain-containing associated proteins to type III CRISPR-Cas systems, which bind cyclic adenylates, and their potential role in the immunity mechanism was identified. A signature protein for type III CRISPR-Cas systems Cas10 produces cyclic adenylates after it recognizes foreign nucleic acid, CARF and SAVED domains bind these cyclic adenylates, and are activated. Most of the so far studied associated proteins were shown to be unspecific nucleases. Among them, multiple transmembrane proteins (CARF/SAVED-TM) were identified. However, their function in the immunity mechanism is least understood. This study aimed to determine real CARF/SAVED-TM candidates and characterise them prior laboratory work from bioinformatically identified ones through a comprehensive bioinformatic analysis.

Keywords: CRISPR-Cas, CARF and SAVED domain, transmembrane proteins, bioinformatic search, gene analysis

SNP PANELS DESIGN FOR THE MANAGEMENT OF THE WISENT POPULATION

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Genetic monitoring is an important part of population management in the conservation of endangered species. Years of microsatellite analysis led us to search for more effective molecular tools. Besides estimating genetic diversity, it is also important to be able to assign a genetic line if pedigree data are missing or to perform parentage and individual identification. In our study, we create a custom-designed SNP (Single Nucleotide Polymorphism) microarray to genotype a large number of wisents, totalling 455 samples from two genetic lines. The results allowed us to select highly informative SNP markers divided into separate panels. Here we present a panel of 50 SNPs for individual and parentage identification and a second panel of 30 SNPs for assessing membership of the genetic line. Selected SNP panels can be used together or independently, depending on the research purpose.

Keywords: Single Nucleotide Polymorphism, wisent, population management, genetic lines, individual identification, parentage control

ASSESSMENT OF GENOME STABILITY OF DOGS

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Genome instabilities can lead to a wide variety of diseases and poor health parameters. Many endogenous and exogenous factors influence the level of damage to genetic material. Genome integrity depends on factors such as the fidelity of DNA replication, normal DNA organization in the chromosomes, and repair mechanisms. The aim of the study was to assess the stability of the genome of dogs using cytogenetic tests sister chromatid exchange, fragile sites, bleomycin, and comet. The study assessed the chromosome stability of Molossoid dogs. Cytogenetic assays are very sensitive and can be used as biomarkers of normal DNA replication and repair potential and maintenance of control over the entire cell cycle. The use of these tests for a more accurate assessment of the genome stability and integrity of animals enable observation of the number of chromosomal instabilities generated in a given individual. The introduction of cytogenetic tests will enable a more precise assessment of the stability and integrity of the genome of animals and make it possible to observe the number of chromosomal instabilities generated in a given individual, which may be indicative of its health potential.

Keywords: genome, dogs, cytogenetic test

HEIGHT GROWTH OF AN IMPROVED CONIFEROUS FOREST: A CASE STUDY IN LATVIA

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Bioeconomy will be the backbone of economies in Northern Europe. Incorporated in tree breeding, assisted gene migration is its essential part. Altered stand dynamics of improved trees should be identified and incorporated in growth models to accurately reflect its results. Such advanced models can be used for the assessment of different alternatives, e.g., in relation to the best strategies for increased climate change mitigation effect. Our study assessed forms of the King-Prodan height growth function based on the remeasured National Forest Inventory plots in Latvia to predict the growth of improved coniferous trees in categories 'qualified' and 'tested' using height measurements from progenies of more than 350 open-pollinated families per species (the Scots pine and the Norway spruce). Both categories had steeper growth trajectories at young age compared to natural regeneration (mean growth on unimproved material). The growth of the category 'tested' for pine exceeded that of the category 'qualified' across the modelled age range, while trajectories mainly overlapped for the spruce on lower site indices (representing only 20% of the current area occupied by this tree species). The functions with category-specific multipliers reflect more accurately the actual growth of improved stands, advancing planning of timely management activities (thinning) as well as the strategies on the initial stand density to reduce the effect of natural disturbances. The single model with category-specific set of multipliers may be easily applicable in practice or incorporated in growth simulators without increased complexity for end-users. Improved planting stock is designed for high site indices, and developed models demonstrate high accuracy in such conditions.

Keywords: tree breeding, adaptation, dynamic modelling, forest reproductive material

GENOMIC GC% CONTENT RELATIONSHIPS BETWEEN BACTERIOPHAGES AND THEIR HOSTS

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The growth of next-generation sequencing availability has resulted in the elucidation of complete genome sequences for thousands of different bacteriophages infecting a wide array of hosts. It has been proposed that bacteriophages may benefit from having their genome GC content resembling that of their host's chromosome, although many examples of phage-host pairs exhibiting high differences in their genomic GC% were documented throughout the years. In this study, we use a dataset comprising all available complete genome entries available at the INDSC for phages infecting representatives of the 30 most popular phage isolation host genera and take a closer look at the GC% relationship between the genomes of phages and their respective indicated hosts. Only few of the selected bacterial genera have not shown a significant difference between the GC% content of their representatives and the phages infecting them. For most of the bacterial genera examined, although lower on average, phages demonstrated surprisingly broad possible genome GC% contents when compared to their hosts. A closer look at the phages with an unambiguous species-level host designation resulted in a strong positive correlation between the genome GC% contents of phages and their hosts (Pearson and Spearman's correlation coefficients = 0.88, $p = 0$), and a simple linear regression equation 'Phage GC% = 4.09 + 0.84*Host GC%' could explain 77% of the phage GC% content variation (R squared = 0.77). The results obtained demonstrate that the GC% content of a phage should not necessarily be relatively similar to that of its host for successful infection and replication.

Keywords: bacteriophages, prokaryotes, complete genomes, genomics, GC% content