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EXPLORING GENETIC DIVERSITY USING GENOMIC

Abstract: In this article, I will broadly address the application of genomic technologies in assessing biological diversity, including various organisms ranging from Chelicerates to plants. I will focus on the application of genomic technologies in the development of new bio-nano materials, and genomics of Montenegrin grapevine evolution.

INTRODUCTION

The nucleic acid sequence in the DNA molecule determines the hereditary and biochemical properties of one organism. The complete genome sequence of an organism contains all the information necessary for the development of the organism, its behavior, physiology, biochemical processes that can be carried out by the organism, its resistance to disease and hereditary properties that will be transmitted to the offspring. The sequence of the complete genome summarizes the „user manual" of a particular organism and represents the basis for understanding the life functions and the evolution of the organism, containing data on the history and origin of the organism. Sequencing and genomic analysis of the DNA sequences experienced an explosion after the publication of the *Homo sapiens* genome sequence in 2001. Bearing in mind the complexity of the sequencing process and the analysis of the genome, the question is why this complex analysis actually serves. First, this analysis gives us the „user's manual" for each organism and the genetic program for how individual organisms function. This genomic knowledge is the base knowledge-based economy and bioeconomy. This genomic knowledge is the basis for the development of new

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technologies including medicine and health, agriculture and food industry, veterinary medicine, industrial biotechnology, ecology, environmental protection, forensic medicine, and production safety. Genome sequencing technology has created a whole range of genomic disciplines integrating biology, informatics, and mathematics into computational biology and system biology. The three basic branches of genomics are structural genomics (which includes mapping and sequencing), comparative genomics (which researches diversity and genome evolution), and functional genomics which analyses the function of genes in biological systems. New „OMICS“ technologies such as proteomics (which studies proteins), metabolomics (which studies metabolites), and transcriptomics (which explores RNA expression in various tissues) represent advances in our understanding of the genome. New applications include archaeological genomics, where our history is „read“ through a history recorded in DNA, agricultural genomics, and environmental genomics.

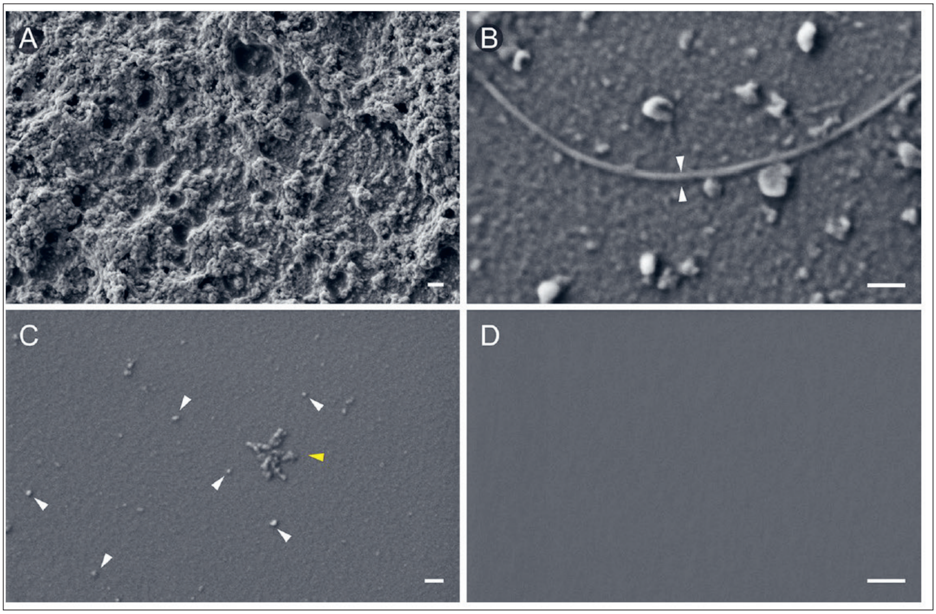
GENOMICS IN THE GENERATION OF NEW BIOMATERIALS

The development of modern technologies relies heavily on novel materials with applications in a wide range of fields, including medicine and pharmacology, food production, engineering, and catalysis. Specific characteristics of materials necessary for particular applications include properties such as mechanical strength, elasticity, biocompatibility, biodegradability, size, density, and a combination of biochemical and physical characteristics that are often found in biological materials. Materials such as spider and silkworm silk have served as a basis for the development of specific materials used in pharmacology, diagnostics, and regenerative medicine1–3, but have also inspired the development of biomimetic synthetic materials4–6. Nanomaterials are emerging as an important element for modern therapeutic treatments. For effective drug delivery, potential nano-carriers must be biocompatible, biodegradable, non-toxic, and non-immunogenic, and need to allow versatile conjugation of specific drug load and delivery in a particular cellular compartment 7–9. Currently, there are numerous nanomaterials on the market with often confusing nomenclature, such as „natural“ biomaterials that can be of organic origin (spider or silkworm silk) or inorganic origin (titanium dioxide, nano-silver and many others), synthetic biomimetic materials (polymers, plastic), and materials produced by recombinant-technology. However, the application of nanomaterials in modern life has raised concerns about their environmental safety and potential for adverse effects on human health10.

Indeed, it has been shown that many engineered nanomaterials cause undesired effects in living organisms. For example, TiO2 affects circadian rhythm, immune and inflammatory response and basal metabolism, SiO2 affects immune response and genes involved in inflammation processes and polystyrene nanomaterials are associated with effects on apoptosis, inflammation, and basal metabolism11. Thus, biomaterials of natural origin are believed to have superior characteristics relative to synthetic materials. They are often biocompatible and biodegradable, and, as proteinaceous molecules, can be easily functionalized for specific applications. Silks are a family of proteinaceous materials secreted by many arthropods for different biological functions. The most studied examples of such organic materials are spider silk and silkworm silk. However, recent studies have uncovered „silk-like" materials from other arthropods including numerous insect species12, mites13 and even some marine species such as mussels14 and barnacles15.

All these silk proteins, encoded by fibroin genes, contain common structural motifs, such as the β-pleated sheet, generating unique silk properties depending on slight differences in individual sequences. The majority of these naturally produced silk threads are in the micron range of fibre thickness and are used for construction of cocoons, prey capturing, egg sac production, adherence to substrates or forming the pedicel of eggs16. Mechanical properties of these silk fibres can be expressed in terms their Young's modulus, which characterizes the deformation due to applied stress, and ranges from 7 GPa in silkworm to 13.5 GPa in the spider *Nephila clavipes* to a high Young's modulus of 28 GPa in the bugworm *Eumeta variegate*17. Another little-studied group that spins silk are phytophagous spider mites (Tetranychide). They belong to acari, in which the majority of species do not produce silk; however, spider mites from genus *Tetranychus* have evolved the production of versatile silk used for locomotion and dispersal from plant to plant18–20, protection from predators 18,20,21, sheltering from climatic conditions22, as a surface for egg laying23, and for communication via pheromone and social-clue deposition24,25. Silk fibers from Tetranychus urticae have a striking characteristic: they have diameters on the nanometre scale, representing the thinnest natural silk fiber produced by silk-spinning arthropods13,26,27.

This nano-silk displays an extraordinarily high Young's modulus that is almost double that of spider N. clavipes silk13,26 and is in the range of bagworm silk17, potentially representing a natural nano biomaterials with valuable characteristics for technology and medicine. The T. urticae genome sequencing project allowed the isolation of fibroin gene sequences; however, due to the limited amount of silk production by *T. urticae* and its fine structure, it was not possible to characterize this promising biomaterial. To overcome the limitation of lowsilk production rate, we established a culture of the related species, gorse spider mite, *Tetranychus lintearius*, that produces copious amounts of silk (Tl-S) (Fig. 1A), and using semi-industrial production generated a sufficient amount of silk for biochemical and physical characterization. We have shown that *T. lintearius* silk has thickness and physical characteristics similar to *T. urticae* silk. We characterized native Tl-S and produced nano-particles generated from Tl-S (Tl-SN) and compared then to *Bombyx mori* silkworm native silk fibroin (Bm-SF) and nanoparticles (Bm-SFN) as the standard in the field. Exposure of mouse fibroblasts to Tl-S-derived biofilm and nanoparticles demonstrated that this cell line can grow successfully in the culture at a comparable level to that seen in the presence of Bm-SF derived nanoparticles and biofilm. Finally, we fluorescently labeled Tl-SN and showed that they can enter the cytoplasm of cultured cells. These experiments suggest that Tl-S is a new cytocompatible material and a potential source of natural nanoparticles with potential for various applications, including pharmacology and biomedicine.

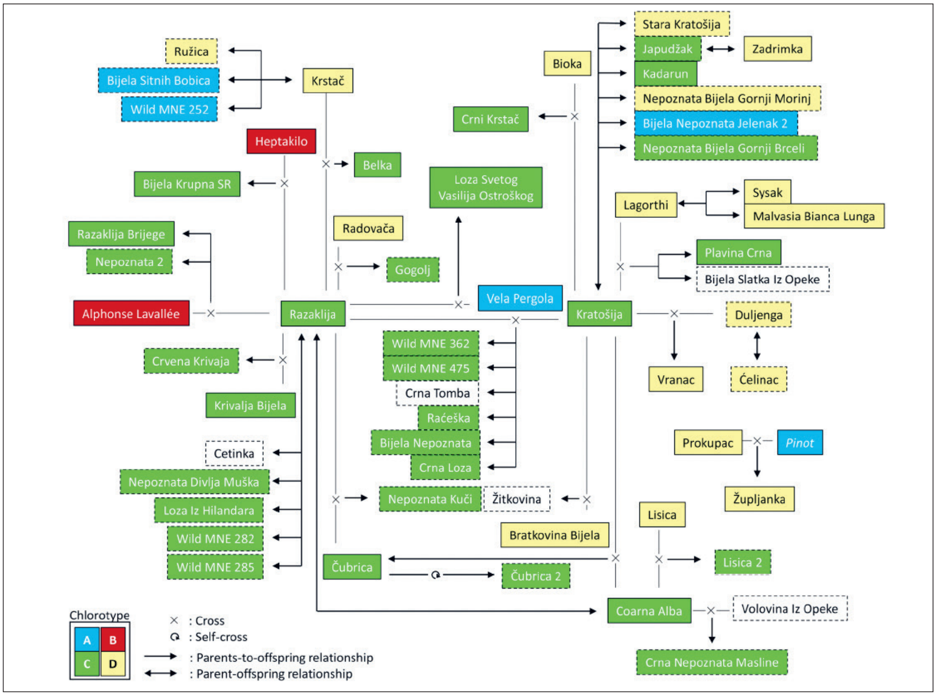


Focused Ion Beam milling combined with Scanning Electron Microscopy (FIB-SEM) of T. lintearius nanoparticles at different concentrations. (A) 10X dilution of T. lintearius nanoparticles showing dense conglomerates of particles. (B) 100X dilution shows spontaneous formation of silk fibers (arrowheads). (C) At 1000X dilution individual nanoparticles show diameter of ca. 20 nm (arrowheads) (D) Silicone wafer with no sample loaded (control). Scale bar: a—200 nm, b–d—100 nm.

IMPORTANCE OF GENOMICS FOR MONTENEGRO: AN EXAMPLE OF THE GRAPEVINE GENOMICS

Montenegro has incredible genetic diversity and it requires focused and strategic investment in genomics because without it the diversity and richness of species that Montenegro has could not be valorized or will be used by someone else in the development of new technologies. The peak of the iceberg and the potential in this area reveals the largest genomic research in Montenegro performed so far, the analysis of the genomic diversity of grapevine that was carried out in cooperation between Plantaze, the Ministry of Agriculture and the Ministry of Science of Montenegro and supported by CANU in cooperation with the world's leading institute for Grapevine Research (ICVV) from La Rioja, Spain. This research using the methods of genomic analysis 28, comparative genomics and genetic pedigrees analysis showed that Montenegro has 64 unknown grape varieties (so far the largest number in the surrounding) and their complete pedigree and their genetic relationships have been established. The central variety that is the parent of Montenegrin vine varieties is Kratosija, which shows great diversity and historical data from the Budva Statut from 1427 suggests that it is a variety cultivated in this area for centuries.

According to the pedigree structure of the Montenegrin grape varieties, this wine region has a similar structure with traditional wine regions such as Bordoux or Burgundy in France, where individual varieties such as Cabernet franc or Pinot noir are the basis of local pedigrees indicating the old traditional viticulture. This research, which is more extensively discussed in this Proceeding, is a starting point for further analysis that has the potential to place Montenegro in important international touristic wine destination but also to create a scientific and technological basis for the exploitation of these findings. The sequencing of the Vranac genome revealed its origin from the father Kratosija variety and the mother Duljenga and opened the pathway to genomics and metabolomics that should determine the structure of the Vranac genome and metabolites such as resveratrol and many complex polyphenols and tannins specific for this variety. The next step of this research is determination of the origin of the American variety Zinfandel by genomic technologies. Until now, the similarity of Zinfandel, Primitivo, Crljenak Kastelanski / Pribidgarag / Tribidraga was determined with a small number of molecular markers and there was no material analysed from Montenegro where Kratosija showed the highest biodiversity, being one of the prime candidates for the origin of Zinfandel. This great biodiversity suggests that Montenegro is the center of origin for this variety



First-order genetic relationships (trios and duos) detected for cultivated and wild grape varieties sampled in Montenegro. Chlorotypes (A, B, C or D) are indicated with different colors, according to the inserted code. If white, no information on chlorotype was available. Unidentified and unique genotypes in the ICVVSNP database are shown in boxes with broken borders. These genetic relationships were obtained with the likelihood-based method implemented in Cervus v. 3.0 for parentage analysis, on the basis of SNP genetic data.

that was later distributed world-wide. Thus, the next step in this project is the genomic analysis to determine from where Zinfandel was introduced to America. By using genomics, Montenegro has managed to preserve 64 unknown grapevine varieties from disappearing, determine their pedigrees and genetic relations and ensure the development of autochthonous viticulture and wine production for the next centuries. This pedigree of Montenegrin varieties has great significance for archeobotanics. The first paper on ancient DNA from grapevine seeds (Ramos-Madrigal et al. 2019) what published a while ago, showing that the grapeseed DNA discovered from the year 1050 corresponds to DNA of the modern variety Sauvignon Blanc, demonstrating that this variety has been grown in France for almost 1000 years. This discovery has an incredible significance for Montenegro, because the pedigree of Montenegrin grapevine varieties and the numerous archaeological findings from the Greek, Roman, and Medieval periods could allow a deeper understanding of the genetic history of Montenegrin varieties that would represent an incredible quantum leap for the history and genetics of the vines in Montenegro and the world. Research on the genetic diversity of the grapevine is the first serious step in the application of genomics in Montenegro, which could make it one of the backbones of the future societal and technological development.

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