ISSN 1857–9027
e-ISSN 1857–9949

МАКЕДОНСКА АКАДЕМИЈА НА НАУКИТЕ И УМЕТНОСТИТЕ
ОДДЕЛЕНИЕ ЗА ПРИРОДНО-МАТЕМАТИЧКИ И БИОТЕХНИЧКИ НАУКИ

MACEDONIAN ACADEMY OF SCIENCES AND ARTS
SECTION OF NATURAL, MATHEMATICAL AND BIOTECHNICAL SCIENCES

ПРИЛОЗИ
CONTRIBUTIONS

38
(2)

СКОПЈЕ – SKOPJE
2017

Publisher: Macedonian Academy of Sciences and Arts

*

Printed by: Vinsent Grafika, Skopje

*

Number of copies: 300

*

December 2017

Published twice a year
The Contributions, Sec. Nat. Math. Biotech. Sci. is indexed in:
Chemical Abstracts, Mathematical Reviews, Google Scholar, EBSCO and DOAJ

http://manu.edu.mk/contributions/NMBSci/

Прилози, Одд. прир. мат. биотех. науки, МАНУ	Том	Бр.	стр.	Скопје
	38	2	121–188	2017
Contributions, Sec. Nat. Math. Biotech. Sci., MASA	Vol.	No.	pp.	Skopje

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С О Д Р Ж И Н А

ON THE EVE OF THE GREAT JUBILEE – 50 YEARS OF THE MACEDONIAN ACADEMY OF SCIENCES AND ARTS 1967 – 2017

Taki Fiti

President of the Macedonian Academy of Sciences and Arts

This year the Macedonian Academy of Sciences and Arts (MASA) marks and celebrates a great jubilee – 50 years of existence and work of our highest institution in the field of sciences and arts. Although on 22 February 2017 the 50th anniversary of the enactment of MASA in the Assembly of the Socialistic Republic of Macedonia was marked, and on October 10 it will be 50 years since the solemn establishment of MASA, we proudly emphasize that our roots, the roots of the Macedonian and Slavic cultural and spiritual continuity, are far back, in a time dimension which is measured in centuries. Because the mission of the Ss. Cyril and Methodius, the historical events that made Ohrid, with the famous Ohrid Literary School, already in the IX century to become the center of the Slavic educational and enlightening activity, which then spread throughout all Slavic countries, have fundamentally changed our contribution to the treasury of the European culture and civilization. And furthermore, centuries later, in the middle of the XIX century the Macedonian revival began, with a pleiad of our cultural and national activists. These processes at the beginning of the XX century resulted in the establishment of the Macedonian Scientific and Literary Fellowship in Saint Petersburg, led by Dimitrija Chupovski and Krste Petkov Misirkov, whose rich scientific, literary and cultural activities were a significant reflection of our spiritual continuity and identity, and an event that has marked the dawn of the Macedonian Academy of Sciences and Arts. This continuity will remain in the period between the two world wars, with a pleiad of artists in literature, art, music, philological, economic, legal and technical sciences. A few years after World War II, in 1949, in free Republic of Macedonia, the first state University of "Ss. Cyril and Methodius" was established, within which, in less than two decades, solid personnel resources were created which allowed rapid development of the higher education and scientific activity in our country. It was an event of great importance for the establishment of MASA as the highest institution in the field of sciences and arts.

This millennium pace and continuity in the development of art and scientific thought in our region is an indication and evidence that we are not a nation without its own roots, without its own history, without its own culture, and that the attempts to deny our identity, language, name, no matter where they come from, are residual of the Balkan anachronisms, and essentially speaking, they are absurd and retrograde.

Immediately after the establishment of MASA followed a period of rapid development, diversification and enrichment of its scientific and research activities and artistic work. Almost two decades after the establishment MASA entered the phase of its maturity and has grown and has affirmed as the fundament of the Macedonian science, language, culture and history and as one of the pillars and symbols of the statehood of the Republic of Macedonia.

Today, MASA, according to its integral concept, structure and function, has all the necessary attributes of a modern national academy of European type, and of course, performs the three basic functions typical of the European national academies: creating communication space for confrontation of different views and opinions on important issues in the field of sciences and arts, scientific and research work and advisory role.

The scientific and research activities and artistic work, in fact, constitute the core of the activity of MASA. The number of completed scientific and research projects and projects in the field of arts within MASA is impressive – more than 1,000 projects in the past 50 years. Some of these projects are long-term and are mainly related to the strategic issues of specific national interest, and significant is the number of fundamental and applied research in all fields of science and art represented in the Academy. MASA members in their scientific research increasingly incorporate the international dimension in the work – in the recent years more than 60% of the scientific papers have been published in international journals, most of which have been published in journals with impact factor; 50% of the papers that have been published in

proceedings of scientific and professional meetings are related to meetings held abroad, etc. In addition, the works of our renowned writers and poets, members of MASA, are translated into foreign languages, and their work has found its place in world anthologies. Our prominent painters and sculptors of the older and the younger generation have created and create masterworks that are regularly exhibited at home and abroad. It should be particularly noted that our two research centers – Research Center for Energy and Sustainable Development and the Research Centre for Genetic Engineering and Biotechnology "Georgi D. Efremov", that have gained high reputation in the region and beyond, continue to successfully maintain the attained position. The work of the other research centers also enhances, including the newly established ones, which have begun to work on significant international scientific and research projects.

In its half-century of existence and work MASA developed a rich publishing activity. Since its establishment until today around 700 titles have been published – monographs, results of scientific projects, proceedings from scientific meetings, music releases, facsimile and jubilee publications, joint publications with other academies and scientific institutions, publications of solemn meetings, special issues of the departments of MASA etc. A special contribution to the publishing activities of MASA provides the "Trifun Kostovski" Foundation that has been existing and working for 18 years.

MASA proactively follows the changes and the new trends in the scope of the advisory function of the modern European national economies, and in that context the obligations arising from the project SAPEA - Science Advice for Policy by European Academies, initiated by the European Commission in order to intensify the cooperation of the European academies within their advisory role. Through the publication of the results of our scientific and research work, their presentation to the wider scientific and professional public in the country, to the government officials, etc., MASA participates in the policy-making in the field of sciences and arts and in the overall development of the country. The maintenance of the independence of MASA in carrying out the advisory role is our highest priority and principle.

In the recent years MASA has developed extensive international cooperation that contributes to the affirmation of the Macedonian scientific and artistic work and to the increasing of the reputation of MASA and of the Republic of Macedonia in international scale. Today, our Academy cooperates with more than 30 foreign academies and scientific societies and is a member of 7 international associations of academies. In the recent years the cooperation with the academies from the neighboring countries has been intensified, as well as with the Leibniz Society of Sciences from Berlin, and also, within the so-called Berlin process (Joint Science Conference of Western Balkans Process / Berlin Process) the cooperation with the German National Academy of Sciences – Leopoldina, with the French Academy of Sciences, the academies of Southeast Europe and others.

Due to the results achieved in its work, MASA and its members have won a number of high national and international awards. In the past 50 years, MASA has won around 90 awards and recognitions – charters, plaques, certificates of appreciation, medals and decorations from national and international scientific, educational, artistic and other institutions. Particularly, it should be noted that MASA has been awarded with the high decoration Order of the Republic of Macedonia for the contribution to the development of the scientific and research activity and artistic creativity of importance to the development and affirmation of the Macedonian science and state, which is awarded by the President of the Republic of Macedonia, as well as the prestigious Samuel Mitja Rapoport award of the Leibniz Society of Sciences from Berlin, which, for the first time, has been awarded to MASA. Today, 22 members of MASA have the status of foreign, corresponding and honorary members, as well as holders of honorary PhDs at around 60 foreign academies, scientific societies and universities.

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The developmental trajectory of MASA unambiguously confirms that the Academy, in its 50 years of existence and work, faced with periods of heights, but also periods of descents and turbulences that are most directly linked to the situation in the Macedonian society, i.e. with crisis periods of different nature – the dissolution of the former common state (SFR Yugoslavia), problems with the recognition of the international status of the country after its independence, the embargoes and the blockades of the country in the early transition years, the internal conflict in 2001 and the political crisis in the last two-three years. In such crises and tense periods the criticism for the Academy grew – that MASA is an institution closed in itself, that MASA stays away from the current issues and developments in the country, and so on. On the one hand, it is a result of the insufficient understanding of the social role of the Academy – MASA is the highest scientific institution, where hasty reactions of columnist 'type', with daily political features are not characteristic. On the contrary, MASA uses facts and arguments. The basic activity of MASA, the results achieved in the

scientific research and the artistic work is our identification within the national and international professional and scientific community, and beyond, within our society. On the other hand, this criticism and perception of MASA has a real basis in the fact that MASA, as opposed to the huge opus of implemented scientific and research and artistic projects still insufficiently affirms the results of its scientific and artistic production to the public. It is our weakness that we must overcome in the future. Of course, we cannot and must not "turn a blind eye" to the other weaknesses and omissions which, at least from time to time, we have faced with over the past 50 years and which we will face with in the future – insufficient scientific criticism of the events in the field of sciences and arts, insufficient resistance to political influence etc. On the contrary, in the future, we will have to clearly identify the weaknesses and the oversights in our work and to find out the right approaches to overcome them.

Today we live in a world of great science. The strong development of sciences, the new technological model based on information and communication technologies, the new wave of entrepreneurial restructuring of economies and societies, the globalization of the world economic activity, opened new perspectives to the economic growth and the development of individual countries and of the world economy as a whole. However, these processes, by their nature, are contradictory. The latest global financial and economic crisis of 2007–2009 revealed the contradictions of the globalization and the discontent of the people from it – the uneven distribution of wealth and power among individual countries, destruction of the resources and the environment worldwide, exhaustion of power of the existing technology and development models. These processes resulted in other problems – refugee and migration crises, strengthening of the regional and national protectionism despite the efforts to liberalize the international trade, fencing of the countries with walls at the beginning of the new millennium, changes in the economic and technological power and of the geo-strategic position and importance of entire regions and continents, etc. Nevertheless, one thing is a fact – societies that aspire to grow into societies and knowledge-based economies more easily deal with all the above mentioned problems, challenges and risks of the modern world. Of course, moving towards a development knowledge-based model assumes large investments of resources in education, science, research and development and in culture, simultaneously accompanied by well-conceived and devised strategies on development of these crucial areas of the human spirit and civilizational endurance. Hence, this fact, undoubtedly, emphasizes the special significance of the national academies of sciences and arts in achieving this objective.

In the recent years the Republic of Macedonia has been facing with the most difficult political and social crisis in the period after its independence. We are facing a crisis of the institutions, breach of the principles of the rule of law, the phenomenon of "captured state", a decline in the process of democratization of the society and falling behind on the road to the Euro-Atlantic integration processes. The problems that are now in the focus of our reality will require major reforms, much knowledge, energy and political will to overcome them. In this sense, and in this context, the role of MASA and of the overall scientific potential of the country in overcoming the crisis is also particularly important.

The above summarized evaluations and considerations about the development of MASA in the past 50 years, about the achievements in the realization of its basic activity, about the problems it faced and faces with, about the major challenges arising from the new age and which are determined with the changes in the international and national environment, they alone define the main priorities of our Academy in the forthcoming period:

– Our long-term goals are contained in the mission and vision of MASA as the highest institution in the field of sciences and arts. The mission of MASA is through the development of the basic functions that are characteristic for all modern national academies of European type, to give its full contribution to the inclusion of the Macedonian science and art in the modern European and world trends, and our vision is the Republic of Macedonia to become an advanced society based on science and knowledge;

– In the forthcoming years the focus of the scientific and research activity and artistic work of MASA, in cooperation with the other scientific and research institutions in the country and with government experts, will be particularly focused on the elaboration of issues and topics that are most directly related to the sources of the current political and social crisis in the country in order to offer possible solutions, approaches and policies to overcome it;

– The issues related to the Euro-Atlantic integration processes of the Republic of Macedonia, their continuous and persistent scientific monitoring and elaboration and active participation of MASA members in the preparation for the accession negotiations with the EU will remain a high priority on the agenda of

MASA. Our ultimate goal is the Republic of Macedonia to become a democratic, economically prosperous and multicultural European country.

– The increasing incorporation of the international dimension in the scientific and artistic work of MASA, through the cooperation with foreign academies, scientific societies and other scientific institutions, through application and work on scientific projects financed by the European funds and the funds of other international financial institutions, also remains our important priority.

Let us congratulate ourselves on the great jubilee – 50 years of the Macedonian Academy of Sciences and Arts.

ПРИЛОЗИ, Одделение за природно-математички и биотехнички науки, МАНУ, том **38**, бр. 2, стр. 129–135 (2017)
CONTRIBUTIONS, Section of Natural, Mathematical and Biotechnical Sciences, MASA, Vol. **38,** No. 2, pp. 129–135 (2017)

Received: September 28, 2017
Accepted: November 20, 2017

ISSN 1857–9027
e-ISSN 1857–9949
UDC: 630*113:551.33(497)
DOI: 10.20903/csnmbs.masa.2017.38.2.108

Short review

STUDIES ON THE GLACIAL REFUGIA OF FOREST TREES ON BALKAN PENINSULA

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During the Ice Age the southern parts of Europe, including Balkans, remained practically unaffected by glaciations and served as refugia for many plant and animal species. However, even within the framework of each glacial refugia, the conditions were not homogeneous and isolated "hot spots" appeared that had led to "refugia within refugia". The territory of Balkan Peninsula could be subdivided into different zones, which are of different importance from the point of view of formation of the present day biodiversity. Tree species are particularly suitable for studies of inter-glacial and post-glacial migrations, due to their important ecological role in shaping the environmental conditions. Today, studying of these issues is greatly facilitated by introducing of genetic markers, allowing precise tracing of post-glacial migrations. The topic is less studied in the Balkan Peninsula, even though the region is considered one of the most important European refugia. A brief review of the more important studies in this respect is presented and the prospects and importance of such studies is discussed.

Key words: glacial refugia; Balkans; forest trees; genetic markers; migrations

INTRODUCTION

The glacial refugia are considered safe havens [1], where biota survived periods of glaciation during the Last Glacial Maximum (LGM) in Europe – until 12000 before present [2]. During the glaciations, Northern and part of Central Europe were covered by glaciers, up to several km thick, which had caused extinction of all plant species and extinction or migration of the animal species. At the same time, in the southern parts of the continent, and in the Balkan Peninsula in general, most of area was ice free, which allowed most species to survive in favourable habitats without the need to migrate latitudinally; however, elevational range shifts were detected. The three southern peninsulas (Iberian, Italian and Balkan) are considered the three main glacial refugia in Europe [3]. The terminology related to glacial refugia is rather complicated, particularly due to the new scientific achievements during the last decades. Palaeorefugia, neorefugia, macro- and microrefugia, *in situ* and *ex situ* refugia and many others have been defined [4–6], for review see [7]. The term "refugium" was originally used to describe the restricted full-glacial locations of modern mid- and high-latitude taxa, especially trees and shrubs, but gained later a wider meaning [8].

According to Kutzbach and Guetter [9], during the LGM the mean temperature in July on the Balkan Peninsula and in Bulgaria, in particular, was about 5 ºC lower than today and there was almost no ice cover [10–12]. In that period, the highest diversity did not exist at the lowest altitude (with the highest temperature), but at the middle altitude, due to the highest air humidity [13]. The peculiarities and importance of Balkan Peninsula as a glacial refugium and a high biodiversity centre have been evaluated accordingly [14, 15].

After the glacial retreat, a process of recolonization started. The recolonization of the lands that were ice-scraped until recently is a part of a more general process of postglacial migrations, including not only recolonization of the new territories, but

also moving the species into different directions as a result of the climate change and specific regional environmental conditions. The main centres in Europe where the recolonization started were the three glacial refugia in the three southern peninsulas. However, more detailed studies have shown that glacial refugia, albeit of modest size, existed also in more northern localities, mostly southward from Alps (e.g. in the present-day Slovenia) and from Northern Carpathians (Hungary and Slovakia).

FOREST TREES AS MODEL SPECIES

Large part of the studies on the postglacial migrations use the forest trees as model species. This is due to several reasons. First, many of tree species cover large areas and have a well expressed role in the formation of environmental conditions. A total of 24 taxa – genera and species – were reported for southern European refugia, based on macrofossil or reliable pollen evidence [16]. This is important also because the forests are habitat of many other living organisms. Second, many tree species (all conifers, species of genera *Quercus, Fagus, Carpinus, Ulmus, Populus* and some others) are anemophilous (wind-pollinated). They produce large quantity of pollen, which is accumulated in peat bogs, lakes and other similar places and therefore, these species are very suitable for palynological studies. Third, plant macrofossils of tree species are much more common than such of other plant life forms.

In the context of the general trend that recolonization of the northern parts of Europe started from the southern refugia and was moving northward, the hypothesis aroused that the highest intraspecific genetic diversity should be expected in the glacial refugia. However, the studies of 22 arboreal species have shown that the picture is much more complicated and the genetically most diverse populations were not located in the south, but at intermediate latitudes [3]. This can be explained first, as a consequence of the admixture of divergent lineages colonizing the continent from separate refugia, and second, with admixture of the main recolonization routes with more northern ones, even coming from smaller refugia [3, 9]. The last studies showed that glacial refugia for tree species existed northward from the Alps, although patchily distributed at low densities due to low atmospheric CO_2 concentrations and high wind-speeds [17, 18].

Both now and during the glaciations the climatic conditions in the large glacial refugia differ dramatically both in the ecological variables (temperature, humidity, soil conditions etc.), and in some specific conditions, which were important from the aspect of survival of the species. Therefore, smaller regions could be identified in the framework of the large glacial refugia, and these regions represents "hot spots" for some species or group of species (the different "traces" of these species – genetic, paleobotanical – are the most numerous there). These "hot spots" were called "refugia within refugia". Their identification and localization in the tree species are based on the geographical distribution of genetic diversity corroborated by palynological data. Unique genetic diversity can be observed in glacial refugia through modern genetic markers, based on DNA analysis, that allow comparatively fast and routine estimation of the level and distribution of genetic diversity.

Existing of "refugia within refugia" called hereafter "small refugia" shows that the glacial refugia are structured and not homogeneous. During the glaciations, different environmental factors had been combined in an optimal way for some species or group of species and thus small refugia were formed.

The structuring within the refugia has been reported for different species in different geographic regions [19–23].

Nieto Feliner stated that speaking of a single refugium to refer to each of the peninsulas, however, is an oversimplification [24]. Even speaking of multiple unconnected refugia does not adequately reflect the complexity of the processes that shaped the current genetic and specific diversity.

Even though the concept of southern peninsulas as the only glacial refugia was revised, they still provoke considerable attention, due to their size. As mentioned above, extra-Mediterranean refugia were smaller by and of smaller importance [17]. The southern refugia are presented in detail in the monograph of Weiss and Ferrand [25].

STUDIES ON THE REFUGIA OF FOREST TREES IN BALKANS

In the southern Balkans many temperate tree species survived the Quaternary climatic oscillations in isolated habitats which had particular microclimatic conditions [26]. The topographic variability of the Balkans and its long-term environmental stability may have played a major role in facilitating strong genetic differentiation on a small geographic scale [27, 28]. Growing evidence from phylogeographic studies of animal [28, 29] and plant species [30, 31] suggest that there were multiple Pleistocene microrefugia within the Balkans.

The phylogeographic studies in tree species were enhanced to a greater extent by introducing the new generation of DNA-based genetic markers. Particularly useful proved to be chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA), which are extranuclear markers and are in most cases uniparentally inherited. Usually cpDNA is maternally inherited in most Angiosperms and paternally in Gymnosperms, while mtDNA is maternally inherited in Angiosperms and most of Gymnosperms, but paternally inherited in Taxodiaceae and Cupressaceae [32]. When the mode of inheritance of cytoplasmic DNA is known, this allows tracing relatively easily the migration routes by employing the proper genetic marker. Cytoplasmic DNA markers are frequently referred to as haplotypes. There was vast amount of studies using such markers in phylogeographic studies in Europe, including tree species (see [33] for references)

The tree species on Balkan Peninsula, in particular, were studied to a lesser extent, as compared with these in Western and Central Europe. The studies, however, confirmed the structuring of this refugium. Ballian et al. [34] detected separation of the Croatian silver fir (*Abies alba* Mill.) populations from the group of the eastward situated ones, which can be explained at least partly by existence of different glacial refugia. Multiple refugia were found for the same species in other studies [35, 36] and the contemporary map of genepool distribution is complicated additionally by the existence of suture zones and introgressive hybridization [37]. Existing of multiple refugia is not unprecedented in this species, as reported also for Iberian Peninsula [38].

Similar trends were observed also in other conifer species – genetic diversity in *Pinus sylvestris* L. was the highest in Balkans suggesting existence of at least one refugium [39, 40] similarly like *Pinus nigra* J. F. Arnold [41].

European beech (*Fagus sylvatica* L.) was one of most extensively studied species due to its large distribution and economic importance. Magri et al. [42] corrected the previous concepts and proved that the species survived in multiple refugia. Even though the Balkan refugia were separated from the central European ones and did not contribute to the colonization of the land northern from the peninsula, the haplotype diversity here was the highest, with several different haplotypes, indicating existence of several micro-refugia. Micro-refugia were detected also *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl. [43, 44]

Oaks (*Quercus* spp.) received particular attention, including on Balkans. At least two lineages were identified by Bordács et al. [45] – one to the east of the Carpathian Mountains in Romania [46], and another – coming from the south. However, the southern and eastern part of the peninsula were underrepresented in this study. Recently, multiple refugia were found for most of white oaks in Balkans within a bilateral project Romania-Bulgaria [47].

Three small refugia in Balkan Peninsula were identified in Turkey oak (*Quercus cerris* L.), and even the populations from the eastern and western part of a relatively small region like Bulgaria clearly differed from each other in their genetic constitution, as determined by cpDNA haplotypes (see Figure 1 in [48]).

In a study on the European hornbeam (*Carpinus betulus* L.) Postolache et al. [49] found that its evolution, glacial and post-glacial migrations followed the scenario "refugia within refugia" (Figure 1). The results of the study revealed the significant expansion of *C. betulus*, still before the common beech (*Fagus sylvatica* L.). This major expansion is considered to be a major particularity in Holocene postglacial evolution of forests from the Romanian Carpathians and the Bulgarian Black Sea coastal zone, which was in concordance with the results of palynological studies [50, 51]. The hornbeam's postglacial evolution in the Carpathians and Balkan Peninsula was different from that in the Western Europe.

All these studies need palaeobotanical information, which is available for different periods and for different regions of Bulgaria and Balkan Peninsula [52–55].

The brief review illustrates that Balkan Peninsula is still not studied in sufficient extent, given the opportunities it provides, as harbouring one of the richest genepool of many different groups of living organisms, and particularly, tree species. Studies on the phylogeography and structure of the glacial refugia on the peninsula will be highly relevant and timely. Such studies possess an interest both from fundamental and applied point of view. Also, they are of international interest and provide good opportunity for cooperation of researchers from the different Balkan countries, and being interdisciplinary – from different research areas, too.

Acknowledgments. The author thanks Dragos Postolache and Flaviu Popescu (National Research and Development Institute "Marin Dracea" – Romania) for their cooperation and help, and to two anonymous reviewers for their suggestions for improving the final draft of the manuscript. Also, the permission of ELSEVIER to re-publish Figure 5 of the article of Postolache et al. (2017) published in the journal *Science of the Total Environment*, vol. 599–600, pp. 1492–1502, is very much appreciated.

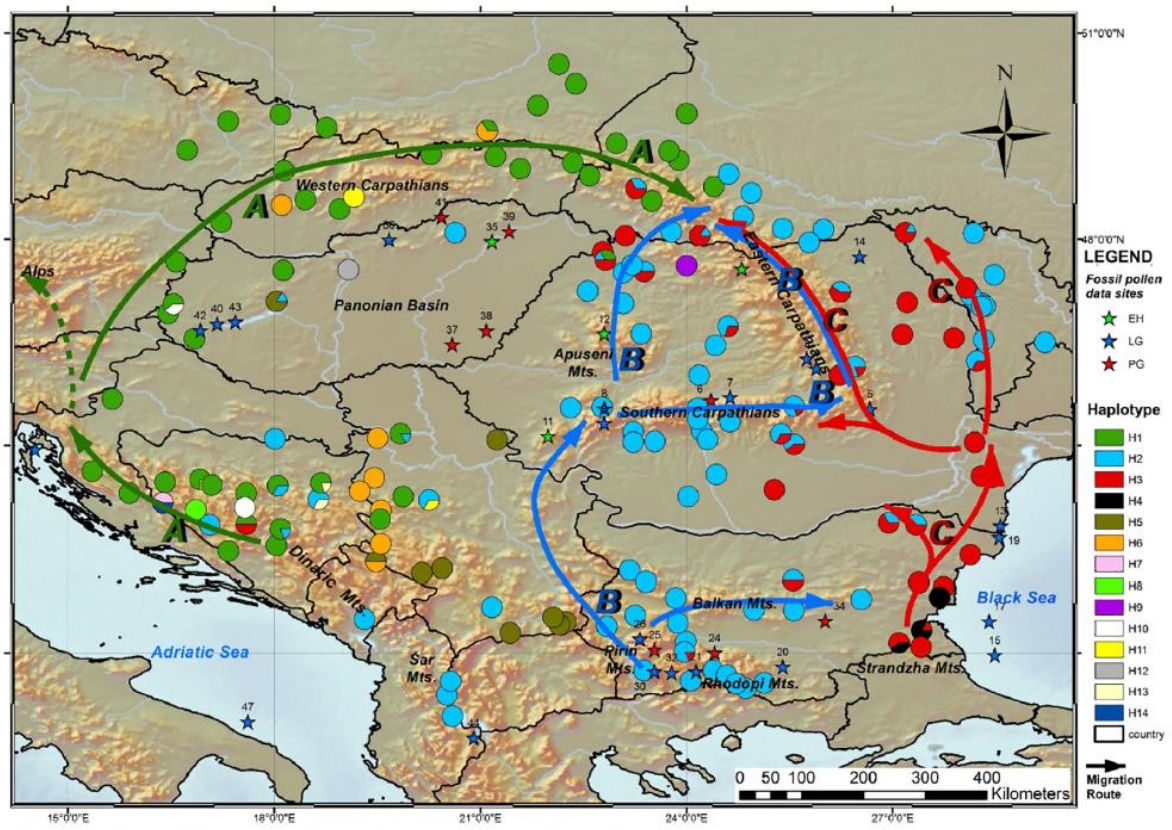

Figure 1. Post-glacial re-colonization routes of *Carpinus betulus* based on chloroplast DNA haplotypes (H) and fossil pollen data. Arrowed lines show potential post-glacial re-colonization routes (route A, route B and route C). Stars number indicates pollen sites with *C. betulus* records from Quaternary periods: EH (green star) from Early Holocene, LG (blue star) from Late Glacial, PG (red star) from Pleniglacial.

(Reprinted from: Forest Ecology and Management, Vol. 599-600. D. Postolache, F. Popescu, L. Paule, D. Ballian, P. Zhelev, S. Fărcaş, J. Paule and O. Badea. Unique postglacial evolution of the hornbeam (*Carpinus betulus* L.) in the Carpathians and the Balkan Peninsula revealed by chloroplast DNA, pages 1493–1502, Copyright (2017), with permission from Elsevier)

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СТУДИИ ЗА ГЛАЦИЈАЛНИ РЕФУГИУМИ НА ШУМСКИ ДРВЈА НА БАЛКАНСКИОТ ПОЛУОСТРОВ

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Во леденото време јужните делови на Европа, вклучувајќи го и Балканот, не биле погодени од глечерите и служеа како засолниште за многу растителни и животински видови. Сепак, дури и во рамките на секој глацијален рефугиум, условите не биле хомогени и се појавиле изолирани „потопли места“, што довело до „рефугиум во рамките на рефугиумот“. Територијата на Балканскиот Полуостров може да се подели на различни зони, кои се од различна важност од аспект на формирањето на денешната биолошка разновидност. Видовите дрвја се особено погодни за испитување на интерглацијалните и постглацијалните миграции поради нивната важна еколошка улога во обликувањето на еколошките услови. Денес проучувањето на овие прашања во голема мера е олеснето со воведувањето на генетски маркери, кои овозможуваат прецизно следење на постглацијалните миграции. На Балканскиот Полуостров темата помалку се изучува, иако регионот се смета за еден од најважните европски рефугиуми. Презентиран е краток преглед на поважните студии на оваа тема и се разгледуваат перспективата и значењето на ваквите испитувања.

Клучни зборови: глацијални рефугии; Балкан; шумски дрвја; генетски маркери; миграции

ПРИЛОЗИ, Одделение за природно-математички и биотехнички науки, МАНУ, том **38**, бр. 2, стр. 137–145 (2017)
CONTRIBUTIONS, Section of Natural, Mathematical and Biotechnical Sciences, MASA, Vol. **38,** No. 2, pp. 137–145 (2017)

Received: July 25, 2017
Accepted: October 27, 2017

ISSN 1857–9027
e-ISSN 1857–9949
UDC: 546.296:539.16.087]:69.01(497.7)
DOI: 10.20903/csnmbs.masa.2017.38.2.109

Review

RESULTS OF INDOOR RADON MEASUREMENTS IN THE REPUBLIC OF MACEDONIA: – A REVIEW –

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Radon and its short lived decay products accumulated in indoor environment are the main source of public exposure to natural radiations. The health effects as well as a great number of natural and artificial factors affecting the radon accumulation in indoor environments are some of the motives for the scientific interest in radon issue. Following this global trend, many studies of indoor radon in the Balkan region, including the Republic of Macedonia have been conducted in the last decade. This paper is an overview of the published papers regarding indoor radon concentration measurements with nuclear track detectors in the Republic of Macedonia. It gives basic information about the spatial and temporal variability of indoor radon over the territory of the country, following by a description of the some factors which affect its variations. This review attempts: to organize available indoor radon results in order to show clear picture of the so far conducted surveys; to highlight the need for continuation of more extensive radon investigation in workplaces; to motivate the building professionals to create as much as possible mitigation methods for indoor radon reduction, to motivate the health professionals for epidemiological studies etc.

Key words: indoor radon; spatial variations; temporal variations; geology; building characteristics

INTRODUCTION

Radon (^{222}Rn) is a radioactive, noble gas produced by the decay of ^{226}Ra contained in all terrestrial materials. Both of them originate from ^{238}U decay chain. Radon decays with 3.8 days half-life by alpha particle emission followed by a chain of subsequent short lived decay products: ^{218}Po ($T_{1/2}$ = 3.05 min), ^{214}Pb ($T_{1/2}$ = 26.8 min), ^{214}Bi ($T_{1/2}$ = 19.7 min) and ^{214}Po ($T_{1/2}$ = 164 μs).

Radon and its decay products accumulated in indoor environment are the main natural source of total public exposure to ionizing radiation (UNSCEAR, 2000). Some epidemiological studies have proven the association between the chronic exposure to high indoor radon concentrations (C_{Rn}) and the incidence of lung cancer (Darby et al., 2005). Considering serious health effects, the preparation and implementation of a national radon program (NRP), which main goal is the reduction of the radon risk, becomes a primary task in most countries over the world. The NRP involves a complex organizational structure that includes components for radon monitoring, prevention and mitigation of increased C_{Rn} (WHO, 2009). Its preparation requires a multidisciplinary approach conditioned by the understanding of the radon processes in indoor environment.

The source of indoor radon is the radium present in soil and building materials (UNSCEAR, 2000). After its generation, radon emanates from solid grains and transported through porous media until its exhalation from the surface into atmosphere or into indoor environment. Diffusion and advection are the radon transport mechanisms driven by the concentrations and pressure gradients, respectively. In general radon dynamics is complex and depends on many factors resulting in the high variability of radon concentration in indoor air.

The factors that influence the C_{Rn} variation could be classified into three groups: geogenic

radon potential (GRP), building characteristics and building users lifestyle (habits). GRP is a factor that relates building underlying geology and C_{Rn}. The GRP is explained through the soil gas radon concentration and soil permeability. The soil gas radon concentration depends on the ^{226}Ra specific activity in the soil, which is proven to be in relation with geology. Permeability of the soil is another key geological factor that primarily affects the dynamics of radon in the soil gas as well as its emanation from the soil surface. The density of infiltrated radon flux from the soil, as well as accumulated radon concentration in the building, depends on the characteristics of the building. In the following, different habits of building inhabitants which relate to diverse levels of room ventilation (windows opening) is in function of the inhabitant's lifestyle, but also with meteorological conditions (among other: Yarmoshenko et al., 2016; Nikolopoulos et al., 2014).

The meteorological conditions also significantly affect the radon dynamics resulting in temporal variability of C_{Rn}. Just like the meteorological parameters, the indoor radon concentrations are subject to daily, weekly, monthly and seasonal variation (Kolarž et al., 2017; Nafezi et al., 2014; Ćurguz et al., 2013; Vaupotič, 2012; Vaupotič et al., 2008; Stojanovska et al., 2011). Because of that, the requirements are to present C_{Rn} in a building as annual C_{Rn}.

The awareness of the potential health problems that could be caused by the increased C_{Rn}, led to gradual growing of the investigations of indoor radon over last decades. As in the rest of the world, a large number of scientific works the Balkans, including the Republic of Macedonia, have been done. The papers that appeared in the literature are mainly related to radon sources, radon measurements and are focused as well on factors that affected radon variability (such as: Žunić et al., 2017a, 2017b, 2014, 2013, 2010, 2007; Ivanova et al., 2016; Vucković et al. 2016; Bochicchio et al., 2014; Vaupotič at al., 2013, 2008; Carpentieri et al., 2011).

This study summarized the results of indoor radon concentrations (C_{Rn}) in the Republic of Macedonia published in the literature. These results are based on radon measurements in 520 dwellings, 74 schools and 5 kindergartens. Discussion is oriented to radon spatial variations, the effect of buildings characteristics on radon variation, as well as to seasonal variations. The measured data is also compared with reported data for some countries in the Balkan region.

MATERIAL AND METHODS

Study area

The Republic of Macedonia is situated in the central part of the Balkan Peninsula (Southeastern Europe), covering an area of 25.713 km^2. Its geographical position is shown on the left maps in Figure 1. The territory is organized into 84 municipalities contained in eight statistical regions (separated with lines in Figure 1), with a total population of 2.022.547 (State Statistical Office, 2002).

Figure 1. Left two maps: Geographical position of the Republic of Macedonia. Right map presents four Geotectonical Zones and one area within the country coloured with different colours. The territory is organized in 8 statistical regions where the names are coded with capital letters: Skopje (SKO), Polog (POL), Southwest (SW), Pelagonia (PEL), Northeast (NE), East (East).

The area is described by complex geotectonic features of diverse relief and a complex geology. It is broadly divisible into four geotectonic units: Western Macedonian zone, Pelagonian massif, Vardar zone and Serbo-Macedonian massif and a separate Kratovsko-Zletovska volcanic rock area situated between the Vardar zone and the Serbo-Macedonian massif (five different colored in Figure 1). The entire territory has a transitional climate, from Mediterranean to continental.

Radon measurements

In general, the C_{Rn} measuring methods are active or passive. There is also a consequent application depending on the goals of the measurements. So, the active methods are used to make short term measurements and they are applied in cases when quickly respond to C_{Rn} is needed in some building or to examine radon dynamics for a certain relatively short period of time. The passive techniques are time integrated methods in which C_{Rn} is measured by nuclear track detectors, exposed over longer period usually in 3, 6 or 12 months. Detector response (calibrated in Bq/m^3) presents the average C_{Rn} for a certain period. The main advantage of this technique is the possibility to perform simultaneous C_{Rn} measurements in many buildings in a particular region.

According to the goals of the four radon surveys performed in Macedonia, all measurements were made using passive nuclear track detectors. The survey code, study area, type of environment with the number of buildings considered in the survey, floor level, detector commercial name, as well as the period of detector exposure for each survey separately are given in Table 1.

The principle of detection, as well as the results processing procedures of the measured concentrations, are explained in detail in the cited references in Table 1.

Table 1. Basic information for applied methodology in four C_{Rn} surveys in the Republic of Macedonia

Survey code	Study area	Type of environment (number of buildings)	Floor level	Detector commercial name	Detector exposure	Reference
S1	Whole country territory	Dwellings (437)	Ground, First Second	RSKS, RADUET	4 successive seasons (December 2008 to November 2009)	Stojanovska, 2011a Stojanovska, 2011b
S2	Five municipalities in Vardar zone	Schools (43)	Ground	TASTRAK RADUET	3 months in spring of 2012	Stojanovska, 2014
S3	Two municipalities in Kratovsko Zletovska area and one in Vardar zone	Dwellings (40), schools (31), kindergarten (5)	Ground	RSKS, Gamma 1	Annual (from June 2013 to May 2014)	Stojanovska, 2016a Stojanovska, 2016b
S4	Whole country territory	Dwellings (43)	Ground	RSKS	3 months in winter of 2013	Stojanovska, 2017

Design of surveys

The purpose of the first survey, marked by S1 in Table 1, was to established the basic map of the annual C_{Rn} distribution across the whole country territory (Stojanovska et al., 2011a). Seasonal variations of C_{Rn} were also studied (Stojanovska et al. 2011a). To that end, the detectors were deployed in the dwellings on the whole territory of the country. The number of detectors in each municipality was determined in dependence of the population density. The C_{Rn} was measured in 4 consecutive seasons, so the annual C_{Rn} was calculated as arithmetic mean of the 4 measurements.

In order to investigate the C_{Rn} variability in primary schools, the S2 survey was conducted in 5 municipalities in the geotectonical Vardar zone. The measurements were done in the spring of 2012 and the annual concentration was assessed using seasonal correction factors (Stojanovska et al., 2011b).

The third S3 survey, was conducted in 3 municipalities, one in the geotectonical Vardar zone and the other two in the Kratovo-Zletovska area. The detectors were deployed in the dwellings, primary schools and kindergartens and exposed for a period of one year.

The fourth research (S4) deals with the seasonal variability of C_{Rn} and the effects of

seasonal correction on the spatial variability of annual C_{Rn} and its uncertainty. The detectors were exposed during the winter period in 2013, in the dwellings throughout the whole territory of the country. The annual C_{Rn} was estimated according to the linear model developed in this study.

In all surveys and for each building, the measurements were made in one of the mostly occupied rooms: a living room or bedroom in the dwellings, classroom in the schools, and a playroom or bedroom in kindergartens.

RESULTS

The maximum value (Max), the arithmetic mean (AM), the standard deviation (SD), variation coefficient (CV = SD/AM), the geometric mean (GM) and geometric standard deviation (GSD) of the annual mean radon concentrations, as well as the number (percent) of buildings where indoor radon concentrations exceed national action level of 400 Bq/m^3 for existing buildings, are given in Table 2.

Table 2. Basic descriptive statisticc of annual radon concentrations measured in 4 radon surveys in the Republic of Macedonia

Survey code	C_{Rn}						N (%)>400 Bq/m^3
	Max (Bq/m^3)	AM (Bq/m^3)	SD (Bq/m^3)	CV (%)	GM (Bq/m^3)	GSD	
S1	720	105	84	80	84	1.9	(1.8%)
S2	260	94	54	57	82	1.7	–
S3	990	186	178	95	131	2.34	7 (9%)
S4	460	120	85	69	98	1.9	1 (2%)

The mean values in the S1, S2, S3 and S4 studies expressed through AM and GM were in intervals from 94 Bq/m^3 to 186 Bq/m^3 and from 82 Bq/m^3 to 131 Bq/m^3, respectively. The C_{Rn} variations in each of them indicated by CV and GSD covered intervals: from 57% to 95% and from 1.7 to 2.34.

Building factors affecting radon variability

The GMs of annual C_{Rn} measured on the different floors in S1 survey in comparison with GM of annual C_{Rn} measured on ground floors in other three surveys are presented in Figure 2.

The GMs of C_{Rn} (with GSD in brackets) measured on ground, first and second floor in S1 were: 98 (1.9) Bq/m^3, 66 (1.7) Bq/m^3, 57 (1.6) Bq/m^3, respectively. The GM values which represented S2, S3 and S4 surveys in Figure 2 are the same values given in the Table 2, respectively, since all measurements in that surveys were performed on the ground floors.

Figure 3 shows the GMs of annual C_{Rn} grouped according to the presence or absence of basement in buildings where measurements were done. The GMs of annual C_{Rn} (with GSD in brackets) in buildings with basement: 73 (1.8) Bq/m^3, 83 (2.10) Bq/m^3, 71 (1.65) Bq/m^3, and without basement: 106 (1.8) Bq/m^3, 161 (2.27) Bq/m^3, 143 (1.7) Bq/m^3 were obtained in S1, S3 and S4 surveys, respectively.

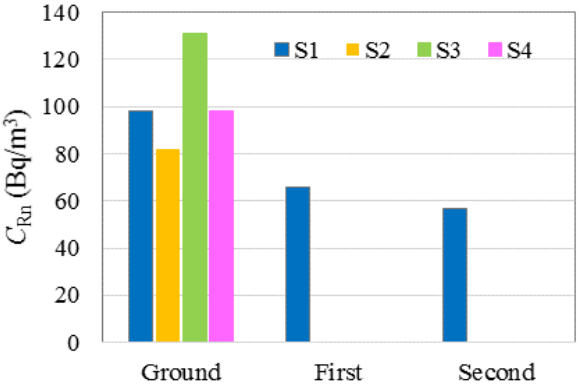

Figure 2. GM of annual C_{Rn} measured in different building floors

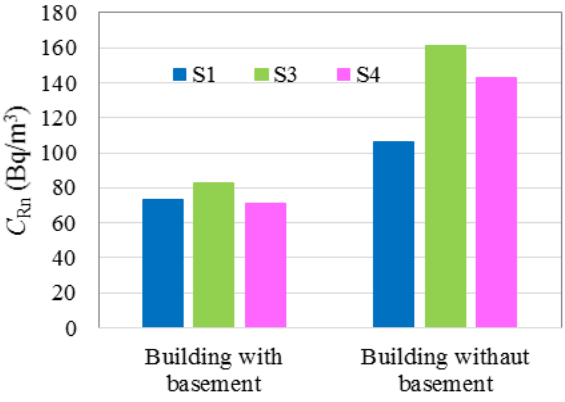

Figure 3. GMs of annual C_{Rn} measured in buildings with and without floor

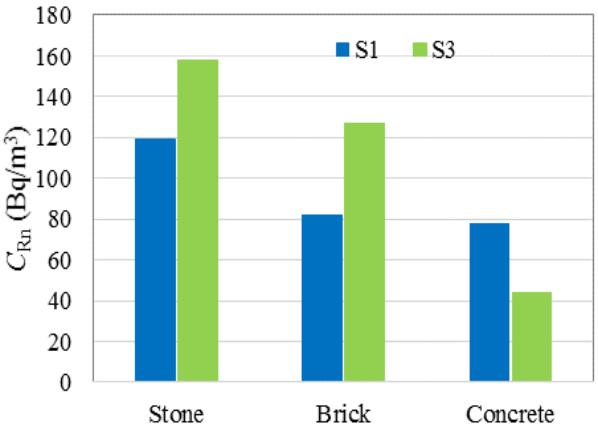

Figure 4. GMs of C_{Rn} measured in buildings with different dominant building materials

The effects of the type of dominant building materials on C_{Rn} variations were investigated in S1 and S3 surveys. Respectively for S1 and S3, the GM of C_{Rn} (with GSD in brackets) for buildings built out of stone: 119 (2.0) Bq/m^3 and 158 (2.41) Bq/m^3; brick 82 (1.9) Bq/m^3 and 127 (2.2) Bq/m^3; concrete 78 (1.8) Bq/m^3 and 44 (1.37) Bq/m^3 are presented in Figure 4.

Radon seasonal variation

The GMs of C_{Rn} (with GSD in brackets) measured in winter, spring summer and autumn were: 115 (2.02) Bq/m^3, 72 (1.97) Bq/m^3, 46 (1.95) Bq/m^3, 92 (2.02) Bq/m^3, respectively for S1 survey, while for S2 and S4 surveys these values were: 76 (1.7) Bq/m^3 and 114 (1.9) Bq/m^3 and corresponded for the measurements performed in spring and winter.

Comparison with literature

For comparison, the results from radon surveys performed in some European countries situated on the Balkan Peninsula are given in Table 3.

Table 3. Results from radon surveys performed in some countries on Balkan Peninsula

Country	Indoor (N)	Period of detector exposure (months)	C_{Rn} (Bq/m^3)			Reference
			Max	AM (SD)	GM (GSD)	
Serbia/Sokobanja	Dwellings (40)	12	189	55	43	Žunić et al. 2017
Serbia/Sokobanja municipality	Dwellings (43)	12	230	60 (44)	49 (1.8)	Mishra et al. 2014
Serbia/Niska Banja	Dwellings (65)		>6000	1163	529 (3.9)	Žunić et al. 2007
Serbia/Krusevac, Brus, Blace and Kursumlija	Dwellings (60)	3	358	82.3 (60.6)	65.6 (2.1)	Vucković et al. 2016
Serbia/ Southern part	Schools (207)	2 × 6	428	118 (78)	97 (1.9)	Žunić et al. 2013
Serbia /Kragujevac City	Kindergartens (14) Schools (28)	3	145	59.6 (25.6)	55.1 (1.18)	Stajić et al. 2015
Kosovo and Metohija/ South-Easter and Central part)	Dwellings (25)	2 × 6	378	163 (84)	143 (1.7)	Gulan et al. 2013
Kosovo and Metohija/ whole territory	Dwellings (48)	2 × 6	1016	122 (167)	71 (2.7)	Gulan et al. 2012
Republic of Srpska/ Banja Luka city	Schools (25) Schools (207)	12	549	128 (111)	99 (1.94)	Ćurguz et al. 2015
Bulgaria/Sofia city	Kindergartens (296)	3	1415	132 (118)	101 (2.08)	Ivanova et al. 2014
Bulgaria/ Sofia city, Sofia district, Plovdiv and Varna	Dwellings (373)	6	3560	158 (304)	99 (2.25)	Ivanova et al. 2013
Bulgaria/ Kremikovtsi municipality	Schools and kindergarten (9)	8	1761	694 (504)	542 (2.06)	Vuchkov et al. 2013
Greece/Xanthi prefecture	Schools (77)	8/10	958	231 (150)		Clouvas et al. 2009

DISCUSSION

Table 2 summarizes the basic statistics of the C_{Rn} measurements results obtained in the four surveys implemented in the Republic of Macedonia. The measured C_{Rn} values were observed to be normally distributed. The main conclusions from S1 were that C_{Rn} values have shown spatial variability throughout the country territory. The variabilities of C_{Rn} between country regions (Figure1), as well as within them, were significant. In general, they were mainly affected by regional and local geology features, as well as by the building characteristics. The maximum value of 720 Bq/m^3 reported for S1 survey was measured in Pelagonia statistical region (Stojanovska et al., 2011a;). In order to clarify this high value, a small measuring champagn in Kruševo (a small town in the Pelagonia statistical region) was conducted after S1 survey. The elevated values for C_{Rn} and natural radionuclides content in top soil were related to Amphibole-biotite granodiorite lithostratigraphy (Stojanovska et al. 2012).

Certain differences between results in the surveys given in the Table 2 are noticeable. They originated from the differences in each study design. For example, the S1 survey was conducted in dwellings distributed throughout the whole country territory, just like S4, but in the S4 measurements were organized only on the ground floor rooms. For these reasons, the C_{Rn} values in S4 were higher than in S1 survey. It is interesting to note that in these two studies the percentage of dwellings that exceeds the National action level of 400 Bq/m^3 is the same and it is 2% in both cases. Subsequently, the S2 survey was done on the ground floor in the Vardar zone, where from the previous knowledge elevated C_{Rn} in that area was not expected. The results confirmed the same, the obtained C_{Rn} results in S2 were lower than those corresponding to S1, also the maximum measured value was < 400 Bq/m^3. The higher C_{Rn} in Table 2 corresponds to S3 survey. In this case, the larger part of the investigated region lies down in Kratovska-Zletovska area, where bed rocks are from the volcanic origin. Detailed investigations of the C_{Rn} have been shown that the elevated C_{Rn} was in relation with tuff, tuffite of andesite and latite (Stojanovska et al., 2016). The existence of a connection between natural radioactivity and geology on the basis of broader research has been confirmed, not only due to indoor radon, but also to indoor thoron (Stojanovska et al. 2013) and ^{226}Ra (Bossew et al. 2013) in spatial variability analysis of surface soils.

The effect of the building floor on annual C_{Rn} variations can be seen in Figure 2. The GMs values of C_{Rn} measured in the ground floor in all surveys were higher than this in the first and second floors. On the other hand, the difference between the first and second floor concentrations is not significant (Stojanovska et al., 2011a;). Also the GMs values that correspond to the measurements in ground floors over the entire territory of the country (S1 and S4) are practically the same (Figure 2). Opposite to this, the results from S2 survey are lower and from S3 survey are higher than the GMs in S1 and S4 surveys. The GMs of the C_{Rn} measurements performed in buildings without basements are lower than in buildings with basements (Figure 3). Furthermore, the GMs of C_{Rn} in buildings with basements cover wider range than the GMs of C_{Rn} in buildings without basement. So, the spatial distribution of C_{Rn} in the buildings with basements is practically uniform, while in those without basements where the inhabitants are in direct contact with the ground the C_{Rn} varied considerably. These variations are associated with different influences of the geogenic radon over the different parts of the country.

From Figure 4, it can be concluded that the higher values of C_{Rn} refer to the stone buildings. On the same figure, the differences between the C_{Rn} measured in buildings made of brick and concrete are not noticeable for the S1 survey. On the contrary, the higher C_{Rn} in buildings built from bricks than these from concrete in the Kratovo-Zletovska area implicate the use of local building materials.

The fact that the global trend to improve energy efficiency in the buildings increase the C_{Rn} was confirmed in the S3 survey where the impact of new windows in buildings on C_{Rn} variations was studied. The analysis showed that the C_{Rn} in buildings with new windows was twice higher in comparison to C_{Rn} in buildings with wooden windows (GM = 110 (2.13) Bq/m^3). In the same survey, the environmental impact on C_{Rn} was examined, but it turned out that there was no difference between urban and rural areas (Stojanovska et al. 2016a).

That long term measurements of C_{Rn} in schools can be representative for a given region as well as for dwellings, even the different occupation time in schools was founded in the S3 survey. The results of annual C_{Rn} measurements in schools, dwellings and kindergartens were not different (Stojanovska et al., 2016a). There are also no differences between the results of C_{Rn} in schools when they are based on the annual measurements (12 months) and when they are in the period exempt from the summer holiday (9 months) (Stojanovska et al., 2016b).

The C_{Rn} seasonal variations are presented in Figure 5. The results clearly showed higher C_{Rn} for

the winter and autumn periods compared to spring and summer. The latter could be ascribed to the fact that during the colder months the buildings are heated, which creates a higher difference in pressure between the soil and the building. On the other hand, in order to save energy in cold periods, the inhabitants keep windows closed. The seasonal measurements in S1 survey were used to developed seasonal correction factors for annual C_{Rn} assessment due to C_{Rn} measurements in one season. The regional variability of the correction factor was confirmed only for the measurements in autumn (Stojanovska et al., 2011b). Applying the seasonal correction on measurements in winter season: the relative uncertainty budget of the annual C_{Rn} increased only for 3% as well as the regional variability over the country is not affected significantly (Stojanovska et al., 2017).

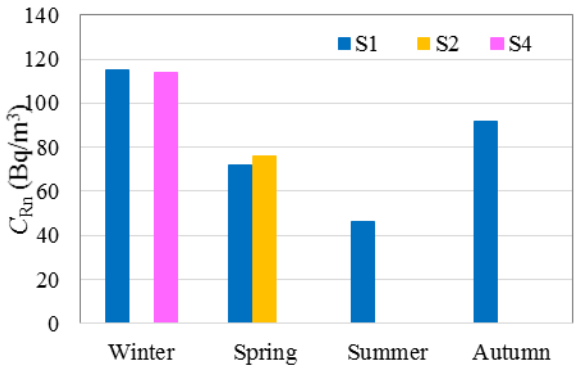

Figure 5. GM of C_{Rn} measured in different season

Finally we compared the obtained annual C_{Rn} in our research with that published in the literature. In general, the obtained GMs of C_{Rn} in all surveys (Table 2) were higher than the worldwide GM of 30 Bq/m^3 reported in the UNSCER 2000 report. On the other hand, the both mean radon concentrations expressed with AM and GM, together with its deviations, are more or less typical as the radon levels reported for some other countries in the Balkan Peninsula (Table 3).

CONCLUSION

Spatial variations of C_{Rn} have been proven in all radon studies conducted in the Republic of Macedonia. In general, in the regions where bed rocks are from volcanic origin the C_{Rn} is higher in comparison with the other parts of the country. Also, the characteristics of the buildings have a significant effect on C_{Rn} variations. The highest concentrations were measured in buildings on the ground floor without basements built with stone. When new windows are installed in such a building, the C_{Rn} increases additionally. The results show that in the few percents of buildings in Macedonia the C_{Rn} exceeds the permissible level and they have to be remediated. Further research in this field should be include health and building professionals in order to create an effective radon protection program.

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РЕЗУЛАТИ ОД МЕРЕЊАТА НА РАДОН ВО ЗАТВОРЕН ПРОСТОР НИЗ РЕПУБЛИКА МАКЕДОНИЈА: ПРЕГЛЕД

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Акумулираниот радон и неговите продукти со кусо време на распаѓање во затворен простор се главен природен извор на озрачување на населението. Ефектите врз здравјето, како и бројните природни и вештачки фактори кои влијаат врз акумулацијата на радон во затворен простор, се еден од мотивите за научниот интерес за радонот. Следејќи го овој глобален тренд на Балконот, вклучувајќи ја и Република Македонија, во последната деценија беа спроведени многу истражувања на радонот во затворен простор. Овој труд дава преглед на веќе објавените резултати од спроведените мерења на концентрацииите на радон во простории низ Република Македонија користејќи нуклеарни трагови детектори. Тој дава основни информации за просторната и временската варијабилност на радонот во затворен простор низ територијата на земјата, проследено со опис на ефектите од некои фактори кои влијаат врз тие варијации. Намерата на прегледот е: да ги организира и да ги искоментира достапните резултати за радонот во затворен простор, со цел да се даде јасна слика за досега спроведените истражувања, да се истакне потребата од продолжување на пообемно истражување на радонот во просториите на работните места, мотивирање на градежните професионалци за креирање колку што е можно повеќе мерки за ублажување и превенција од зголемини концентрации на радон во затворен простор, мотивирање на здравствените работници за епидемиолошки студии итн.

Клучни зборови*:* радон во затворен простор; просторни варијации; временски варијации; геологија; карактеристики на зграда

ПРИЛОЗИ, Одделение за природно-математички и биотехнички науки, МАНУ, том **38**, бр. 2, стр. 147-152 (2017)
CONTRIBUTIONS, Section of Natural, Mathematical and Biotechnical Sciences, MASA, Vol. **38**, No. 2, pp. 147-152 (2017)

Received: June 4, 2016
Accepted: September 20, 2016

ISSN 1857-9027
e-ISSN 1857-9949
UDC: 515.142.2:512.54
DOI: 10.20903/csnmbs.masa.2017.38.2.110

Original scientific paper

CURVATURE AND GROUP THEORY

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INTRODUCTION

This informal article covers the same material as was in my talk to the Macedonian Academy of Sciences and Arts in May 2016 on the occasion of my becoming a foreign member of that academy. I am honored by being elected to this, and I thank my Macedonian hosts, particularly my former doctoral student and longtime friend Acadamician Donco Dimovski.

It was suggested that the talk should be of a rather general nature, and that the write-up should be readable by students. So what follows should be regarded as an invitation to go deeper, not a full expository paper. A general reference for the material on $CAT(0)$ geometry is [1]. All the other material discussed here is dealt with in full detail in my book [5], where original sources are also given.

The goal of the talk is to describe some equivalences between algebraic and topological statements — statements which superficially look entirely different. Most of these equivalences are known theorems, though the last one — the most intriguing — involves an open question.

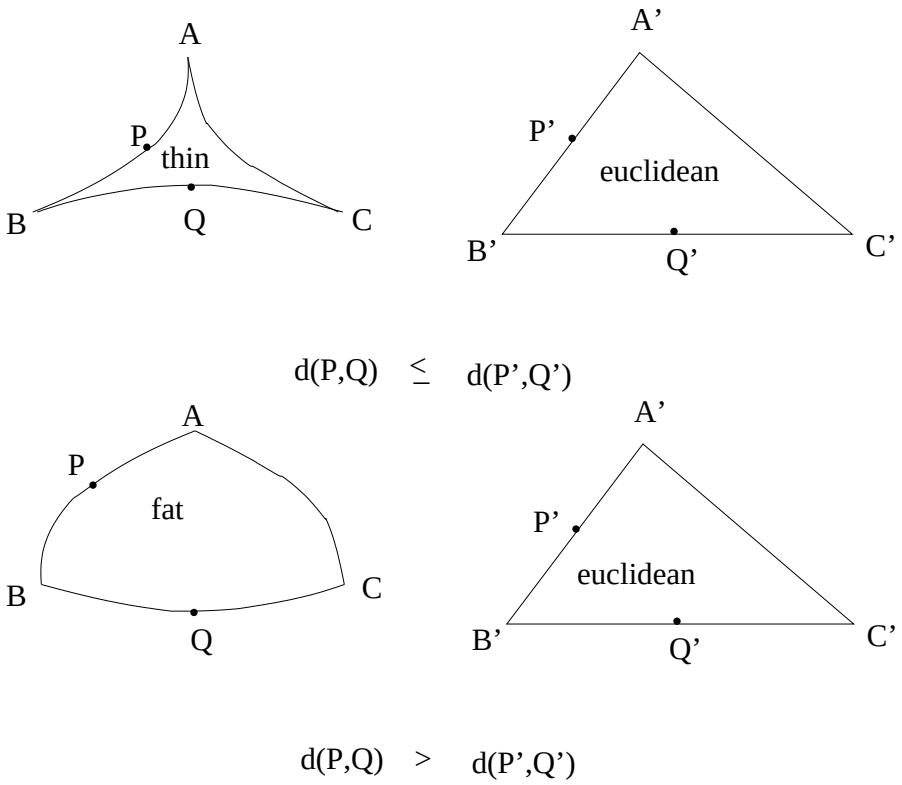
While I do things here in the elegant world of $CAT(0)$ geometry, I should say that some of the statements can be generalized to arbitrary finitely presented groups, though in that generality they are not as pretty.

CAT(0) GEOMETRY

Throughout this article M denotes a proper $CAT(0)$ metric space. More precisely, this means

- M is a metric space, with metric d;
- M is *proper*, i.e. closed balls are compact;
- M is *a geodesic space*, i.e. for any two points x and y there is an isometric embedding of the closed interval $[0, d(x, y)]$ into M with 0 going to x and the number $d(x, y)$ going to y. Such a path is called a *geodesic*.
- M satisfies the $CAT(0)$ comparison axiom, which I will now explain.

The $CAT(0)$ *axiom* compares geodesic triangles in M with corresponding triangles in the euclidean plane, and requires that the triangle in M be "no fatter" than its counterpart in the plane. More precisely, for any three points A, B and C in M, choose geodesics joining each pair, thus creating a geodesic triangle in M. In the plane $\mathbb{E}^2$, draw a corresponding triangle with vertices A', B' and C'. The axiom requires that for any choice of points P and Q on different sides of the triangle in M the distance $d(P, Q)$ be less than or equal to the distance in $\mathbb{E}^2$ between the corresponding points P' and Q'.



Remark. CAT stands for Cartan-Aleksandrov-Toponogov.

Examples of $CAT(0)$ spaces

- Euclidean n-space $\mathbb{E}^n$.
- The hyperbolic plane $\mathbb{H}^2$.
- Any locally finite tree.
- Any Euclidean building.
- Any simply connected Riemannian manifold of non-positive sectional curvature
- e.g. universal cover of a compact manifold of non-positive sectional curvature.

The last of these deserves discussion. In the classical differential geometry of Riemannian manifolds there is the notion of sectional curvature at a point. This is a real number. $CAT(0)$ captures the cruder notion of "non-positive curvature everywhere", without carrying the precise information about curvature at each point. There is the more general notion of $CAT(\kappa)$ which captures the notion of "curvature $\leq \kappa$ everywhere" for a given real number κ. The definition is similar to the $CAT(0)$ case except that a canonical space of constant curvature κ is used for the comparison instead of the euclidean plane $\mathbb{E}^2$. For more on this and on all aspects of $CAT(0)$ geometry, see [1].

Remark. It follows from the Comparison Axiom that there is exactly one geodesic joining two points of M; for if there were two different geodesics, they would make a "fat" degenerate geodesic triangle.

Remark. $CAT(0)$ spaces are contractible. To see this, choose a point $b \in M$ as base point. The (unique) geodesic from any point x to b varies continuously with x and thus provides a canonical contraction of all of M to the singleton subspace $\{b\}$.

It is not hard to guess what it means to say that a proper geodesic metric space is "locally $CAT(0)$". Roughly, one asserts that the "no-fat triangles" condition holds in a neighborhood of each point. It is often important to know when such a locally $CAT(0)$ space is $CAT(0)$. Classical differential geometry suggests the answer. In the case of Riemannian manifolds the Cartan-Hademard Theorem says that when the manifold has sectional curvature everywhere non-positive, and is simply connected, then it is contractible. This suggests a version in the $CAT(0)$ world:

Theorem 2.1. *If the proper geodesic metric space M is simply connected and is locally $CAT(0)$ then M is a proper $CAT(0)$ space.*

For a proof of this "Cartan-Hadamard" theorem see [1].

The importance for topologists is that if a proper geodesic metric space M_0 is locally $CAT(0)$ and has fundamental group G then M_0 is a $K(G,1)$ space. This is because

its universal cover is $CAT(0)$ and hence is contractible.

This last statement is more subtle than it looks, because $CAT(0)$ is a metric condition, so it implies a connection between the metrics on two spaces. Let M_0 be a (connected proper) locally $CAT(0)$ geodesic metric space whose universal cover, with respect to a chosen base point, is M. Then elementary covering space theory says that the fundamental group G of M_0 acts on M by homeomorphisms. In this paper it will always be understood that G acts on M by isometries. This implies a strong connection between the metrics on M and M_0. In particular the covering projection is a local isometry, not just a local homeomorphism. The details of this are dealt with in [1] but the reader can profit by thinking out the (quite elementary) details on his/her own. Note that M is a proper metric space if and only if M_0 is. In this article I will always assume that the metrics on M_0 and M are compatible in this sense.

Recognition of the local $CAT(0)$ property is important. The most famous and useful recognition theorem was proved by Gromov. First, I must define the necessary terms.

(1) Consider the standard n-cube $I^n := [-1,1]^n$. The *link* of the vertex v in I^n is the convex simplex whose vertices are the mid points of the n edges containing v.

(2) A *cubical complex* is a regular CW complex, call it K, obtained from a disjoint set of standard cubes whose edges have length 1 by gluing faces of cubes together using isometries. Regularity implies that no two faces of a cube get glued together, so the cells are genuine cubes, and any two intersect in a common face.

(3) The *link* of a vertex $v \in K$ is the simplicial complex which is the union of the links of v in each cube which contains v.

(4) A simplicial complex is *flag* if it is entirely determined by its 1-skeleton; i.e. a collection of vertices spans a simplex if and only if each pair in that collection spans an edge.

(5) A *metric cubical complex* is a locally finite cubical complex metrized as follows: each cube gets the standard euclidean metric; each piecewise linear path thus gets a well-defined length; the distance between any two points is defined to be the *infimum* of the lengths of piecewise linear paths joining those points.

Theorem 2.2. *(Gromov) A metric cubical complex is locally $CAT(0)$ if and only if the link of each vertex is a flag complex.*

Remark. I should add a word about the point-set topology and homotopy properties. If M_0 is a proper locally $CAT(0)$ metric space then M_0 is an absolute neighborhood retract (ANR). This follows from well-established criteria found, for example, in [8]. Thus, by West's Theorem, if such a space is compact then it is homotopy equivalent to a finite CW complex. Note that in general such a space might have infinite Lebesgue covering dimension. A particularly easy example of this is the Hilbert Cube, which, with the product metric, is both compact and $CAT(0)$.

In this article the $CAT(0)$ spaces of interest will always be non-compact, but they will often be universal covers of compact spaces which are locally $CAT(0)$. It need hardly be said that $CAT(0)$ spaces are locally $CAT(0)$.

Examples of non-proper and non-$CAT(0)$ metric spaces

- A contractible space which somewhere has a region of positive curvature (e.g. a "bubble") does not satisfy the "no-fat triangles" condition.
- Banach spaces ℓ^p satisfy the "no-fat triangles" condition if and only if $p = 2$.
- Infinite dimensional Hilbert spaces are $CAT(0)$ but they are not proper metric spaces.

$CAT(0)$ GROUPS

The most immediate examples of what are called $CAT(0)$ groups occur as follows. One has a compact connected locally $CAT(0)$ space M_0 whose fundamental group is G. Then G acts by isometries freely, properly discontinuously and cocompactly on the $CAT(0)$ universal cover space M. Such a group G must be torsion free for well-known topological reasons. To allow for torsion one generalizes things slightly as follows.

A group G is a $CAT(0)$ *group* if G acts by isometries properly discontinuously and cocompactly on some proper $CAT(0)$ space. Notice that by dropping the word "free" we allow stabilizers of points to be finite subgroups of G rather than just the

trivial subgroup.

Examples of $CAT(0)$ groups

- Finitely generated abelian groups
- Finitely generated free groups
- Finitely generated Coxeter groups
- Uniform lattices in semi-simple Lie groups.
- The fundamental group of a closed Riemannian manifold of non-positive sectional curvature.

Not every finitely generated group can be a $CAT(0)$ group. Here are some necessary conditions:

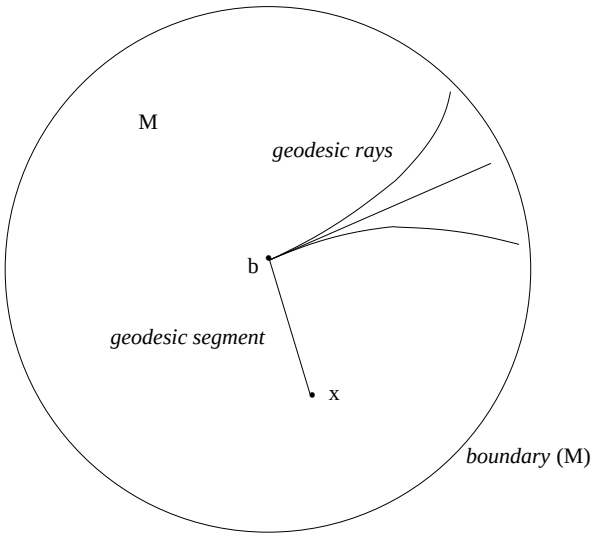
- G is finitely presented.
- The number of conjugacy classes of finite subgroups of G is finite.
- Every solvable subgroup of G is virtually abelian.
- Every abelian subgroup of G is finitely generated.

For details, see Theorem III(1.1) of [1].

THE BOUNDARY ∂M

Fix a base point $b \in M$.

A *geodesic ray* in M is an isometric embedding $[0, \infty) \to M$ such that 0 goes to b. With the compact-open topology the geodesic rays form a compact metrizable space denoted by ∂M. This space ∂M is called the *boundary of* M (*at infinity.*). This definition appears to depend on the choice of base point, but not really: another choice will give a canonically homeomorphic boundary. Or one can define the boundary in a base-point-free way, as is done in [1].



Remark. For a while people thought that perhaps the boundary might be a topological invariant of G. In other words, if G also acts properly discontinously and cocompactly on another proper $CAT(0)$ space then the two boundaries would be homeomorphic. This turned out not to be true; counterexamples can be found in [2] and [10]. However, the two boundaries are shape equivalent, as I observed long ago in [4].

There is an important sense in which the boundary ∂M compactifies M. For each $x \in M$ there is a geodesic $[0, d(b, x)] \to M$ from b to x We call this a *geodesic segment* just as we called the infinite version a geodesic ray. The latter have domain $[0, \infty)$. By a simple trick we can represent the geodesic segment from b to x also to have domain $[0, \infty)$; namely use the previous map on $[0, d(b, x)]$ and send all of $[d(b, x), \infty)$ to the point x. Then all the geodesic segments and all the geodesic rays (starting at b) can be discussed together as a function space $\widehat{M}$ with the compact-open topology. The ones of infinite length form ∂M as before, and the ones of finite length form a copy of M (which we will not distinguish from the previous M). The Arzela-Ascoli Theorem shows that $\widehat{M}$ is compact and metrizable. It is easy to see that M is open and dense in $\widehat{M}$.

Here are a few examples:

- If M = Euclidean n-space $\mathbb{E}^n$ then $\partial M \cong S^{n-1}$ and $\widehat{M}$ is an n-ball.
- If M is the hyperbolic plane $\mathbb{H}^2$ then $\partial M \cong S^1$ and $\widehat{M}$ is a disk.
- If M is a homogeneous locally finite tree of constant valence > 1 then ∂M is a Cantor set and $\widehat{M}$ compactifies M by adding an end point to every ray.

We note that every isometry of M extends to a homeomorphsism of $\widehat{M}$ which of course maps ∂M to itself homeomorphically. Thus our $CAT(0)$ group acts by homeomorphisms on ∂M.

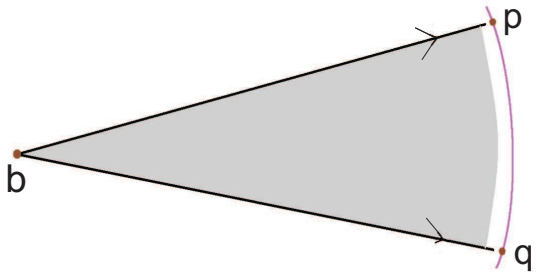
NOTIONS OF CONNECTEDNESS FOR ∂M

Every mathematician is familiar with the notion of *connected component* and the finer notion of *path component* of ∂M. In between these two extremes lie other forms of "component" that are less familiar. I will define two of these here.

A *proper ray* in M is a map $\rho : [0, \infty) \to M$ having the property that for every compact subset C of M the pre-image $\rho^{-1}(C)$ is a compact subset of $[0, \infty)$. Examples are the geodesic rays. A *proper homotopy* between two proper rays ρ and σ is a

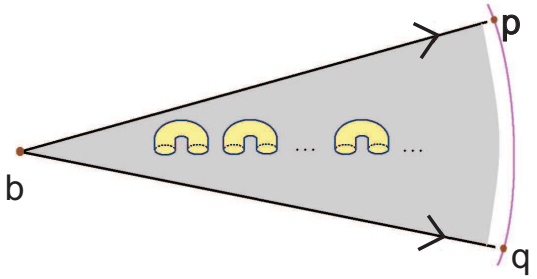
map $\Sigma : [0,\infty) \times [0,1] \to M$ which is proper (i.e. pre-images of compact sets under Σ are compact) and which agrees with ρ [resp. with σ] on $[0,\infty) \times \{0\}$ [resp. $[0,\infty) \times \{1\}$]. The given rays are *properly homotopic* if there exists such a map Σ.

Two points p and q of ∂M are in the same *strong homotopy component* of ∂M if, when considered as geodesic rays starting at b, they are properly homotopic rays.



A *proper homology* between two proper rays ρ and σ is a proper map $T : S \times [0,1] \to M$, where S is an oriented 2-manifold whose boundary consists of the two rays ρ and σ[1] The given rays are *properly homologous* if there exists such a map T.

Two points of ∂M are in the same *strong homology component* of ∂M if, when considered as geodesic rays starting at b, they are properly homologous rays.



Remark. There is a related issue as to whether every proper ray in M is properly homotopic (or properly homologous) to *some* geodesic ray. See [7] for more on this topic.

GROUP-RING COHOMOLOGY OF $CAT(0)$ GROUPS

Let G be a $CAT(0)$ group acting properly and cocompactly on the proper $CAT(0)$ space M. The integral group-ring $\mathbb{Z}G$ becomes a $\mathbb{Z}G$-module under left translation by elements of G, The cohomology groups $H^*(G, \mathbb{Z}G)$ are derived in the usual way from any free $\mathbb{Z}G$ resolution of the trivial $\mathbb{Z}G$-module $\mathbb{Z}$. If there exists a finite $K(G,1)$-complex K for G then the cellular chains in the universal cover $\widetilde{K}$ form such a resolution, and it follows that $H^*(G, \mathbb{Z}G)$ is functorially isomorphic to $H_c^*(\widetilde{K})$, the cohomology of $\widetilde{K}$ with compact supports. Now if G acts freely on M and if M_0 admits such a cellulation then the same can be said of $H_c^*(M)$ since M is the universal cover of M_0. With a little more technique this statement can be shown to remain true even if the G-action is not free, but merely proper, and if M_0 is merely homotopy equivalent (as is the case) to a finite complex. See Sections 17.5 and 17.6 of [5] for more details. Summarizing:

Proposition 6.3. *There is a functorial isomorphism between $H^*(G, \mathbb{Z}G)$ and $H_c^*(M)$.*

A straightforward exercise in algebraic topology gives

Proposition 6.4. *There is a functorial isomorphism between $H_c^*(M)$ and the Čech cohomology $\check{H}^{*-1}(\partial M)$.*

These two propositions link group-ring cohomology $H^*(G, \mathbb{Z}G)$ with the Čech cohomology of ∂M and brings us to our goal.

COMPARISON OF ALGEBRAIC AND TOPOLOGICAL PROBLEMS

The theme of this talk is that algebraic problems are sometimes equivalent to topological ones, and in unexpected ways. The particular algebra involved here is the cohomology of the group G with $\mathbb{Z}G$ coefficients. I will always assume that G is a $CAT(0)$ group, acting cocompactly and properly discontinuously by isometries on the proper $CAT(0)$ space M.

Cohomology has a highest non-zero dimension:

Theorem 7.5. *(Swenson [9]) When a group acts cocompactly by isometries on a proper*

[1]Under the orientation inherited from S one of the rays is oriented towards infinity and the other is oriented away from infinity.

$CAT(0)$ space M then ∂M has finite dimension.

A consequence for $CAT(0)$ groups G is:

Corollary 7.6. *$H^n(G, \mathbb{Z}G) = 0$ for sufficiently large n.*

Non-zero cohomology in the top dimension.

Theorem 7.7. *(Geoghegan and Ontaneda [6]): When a group acts cocompactly by isometries on a proper $CAT(0)$ space M and m is the dimension of ∂M then $H^{m+1}(G, \mathbb{Z}G) \neq 0$ (and all higher cohomology groups are zero).*

A consequence for $CAT(0)$ groups G is:

Corollary 7.8. *The cohomological dimension of G is precisely (dimension of ∂M) $+1$.*

Cohomology in dimension 0***:*** The following is elementary:

Proposition 7.9. *For a $CAT(0)$ group G the following are equivalent;*

(1) $H^0(G, \mathbb{Z}G) = 0$;
(2) M is non-compact;
(3) G is infinite.

From now on I assume M is non-compact and hence G is infinite.

Cohomology in dimension 1***:***

Theorem 7.10. *(Hopf) When a $CAT(0)$ group acts cocompactly and properly on M then the number of connected components of ∂M is 1 or 2 or is uncountably infinite.*

A consequence for $CAT(0)$ groups G is:

Corollary 7.11. *$H^1(G, \mathbb{Z}G) = 0$ or $\mathbb{Z}$ or $\bigoplus_1^\infty \mathbb{Z}$.*

i.e. The number of "ends of G" is 1 or 2 or ∞. The last part uses a famous theorem of Stallings.

Cohomology in dimension 2***:*** What can be said about $H^2(G, \mathbb{Z}G)$? A theorem of Farrell (see [5] for a proof) says:

Theorem 7.12. *Farell* [3] *$H^2(G, \mathbb{Z}G)$ is 0 or is isomorphic to $\mathbb{Z}$ or is an infinitely generated torsion free abelian group.*

The question of whether that infinitely generated abelian group is free abelian has been open for 40 years, though it is known to have a positive answer for many classes of groups. Here is the conjecture — a strengthened form of Theorem 7.12.

Conjecture 7.13. For a $CAT(0)$ group G, $H^2(G, \mathbb{Z}G) = 0$ or $\mathbb{Z}$ or $\bigoplus_1^\infty \mathbb{Z}$.

THE EQUIVALENT TOPOLOGICAL CONJECTURE

In the case of one-ended $CAT(0)$ groups Conjecture 7.13 has an equivalent (and perhaps surprising) topological statement. Recall that by Proposition 7.9 and Theorem 7.10, the assumption of one-endedness implies that ∂M is non-empty and connected, and that $H^0(G, \mathbb{Z}G) = H^1(G, \mathbb{Z}G) = 0$

Theorem 8.14. *Under these assumptions ∂M has just one strong homology component if and only if $H^2(G, \mathbb{Z}G)$ is free abelian.*

Thus we have the equivalent Conjecture

Conjecture 8.15. Under these assumptions ∂M has only one strong homology component.

Remark. Indeed, there is a stronger conjecture - namely that ∂M has only one strong homtopy component. This is also open and is known as the "Semistability Conjecture". Unlike the homology version, it does not have a familiar algebraic restatement. However, in almost all cases where Conjecture 8.15 has been proved, it is this stronger statement which is proved. This is normally done using fundamental group methods.

Remark. Conjecture 8.15 is in fact a conjecture in Steenrod homology, a notion closely related to shape theory.

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ПРИЛОЗИ, Одделение за природно-математички и биотехнички науки, МАНУ, том **38**, бр. 2, стр. 153-164 (2017)
CONTRIBUTIONS, Section of Natural, Mathematical and Biotechnical Sciences, MASA, Vol. **38**, No. 2, pp. 153-164 (2017)

Received: October 17, 2016
Accepted: July 12, 2017

ISSN 1857-9027
e-ISSN 1857-9949
UDC: 511.331:517.581
DOI: 10.20903/csnmbs.masa.2017.38.2.111

Original scientific paper

CONVERGENCE OF DIRICHLET SERIES AND EULER PRODUCTS

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The first part of this paper deals with Dirichlet series, and convergence theorems are proved that strengthen the classical convergence theorem as found e.g. in Serre's "A Course in Arithmetic." The second part deals with Euler-type products. A convergence theorem is proved giving sufficient conditions for such products to converge in the half-plane having real part greater than 1/2. Numerical evidence is also presented that suggests that the Euler products corresponding to Dirichlet L-functions $L(s,\chi)$, where χ is a primitive Dirichlet character, converge in this half-plane.

Keywords: Dirichlet series, Euler products, L-functions, primitive Dirichlet character

1. INTRODUCTION

The general theme of this note is convergence. In Section 2 this is studied for Dirichlet series and in Sections 3-5 for infinite products, in particular for Euler products. For the Dirichlet series we more or less follow [11] and give the proof (cf. Theorem 2.7) that a series such as the "Euler-Dedekind" or "alternating" zeta function $\eta(s) = \Sigma_{n=1}^{\infty}(-1)^{n-1}n^{-s}$, in addition to converging absolutely for $\sigma > 1$ (here as usual $s = \sigma + it$), converges conditionally for $\sigma > 0$. Theorem 2.9 is a refinement of Theorem 2.7, and Theorem 2.12 a further refinement which gives sufficient conditions for convergence for $\sigma > .5$.

Section 3 gives numerical data which suggests that the Euler product for the Dirichlet L-function $L(s,\chi)$ associated to a primitive mod q character χ ($q \geq 3$), which is well known to converge absolutely for $\sigma > 1$, also converges for $\sigma > .5$. Section 4 then presents some theory which gives sufficient conditions for Euler-type products to converge for $\sigma > .5$. Section 5 gives further numerical evidence which, combined with Theorem 4.3, strongly suggests such convergence for the L-functions being considered. Of course, since (as we shall see in Section 4) convergent infinite products cannot equal 0, this would imply the Generalized Riemann Hypothesis for all these L-functions, namely each such L-function cannot have a zero if $\sigma > .5$ (cf. [6], [10]).

Section 6 gives further examples and concludes with a few questions.

2. DIRICHLET SERIES

We commence with a little "review" material to establish some conventions and notation. By a Dirichlet series we mean an infinite series

$$f(s) = \sum_{n=1}^{\infty} \frac{a_n}{n^s} = \sum_{n=1}^{\infty} a_n n^{-s}, \quad a_n \in \mathbb{C}.$$

A very familiar example is the case $a_n = 1$, f is then the Riemann zeta function ζ. For $t = 0$, i.e. for $s = \sigma \in \mathbb{R}$, it is proved in elementary calculus that $\zeta(\sigma)$ diverges for $\sigma = 1$ and is absolutely convergent for $\sigma > 1$. This is called the "p-test" (where $p = \sigma$) but should really be called the "ζ-test." Another familiar example (again when $s = \sigma \in \mathbb{R}$) is the Euler-Dedekind function $\eta(\sigma)$ defined in Section

1. It is proved in elementary calculus that this series converges for $\sigma > 0$, where the convergence is conditional for $0 < \sigma \leq 1$ and absolute for $1 < \sigma$. In this section we shall prove that very similar results hold, with appropriate hypotheses on the coefficients a_n, for $s \in \mathbb{C}$, i.e. dropping the condition $t = 0$.

From elementary complex analysis, for any $x \in \mathbb{R}^+$, one has $|x^s| = x^\sigma$. In particular $|n^{-s}| = n^{-\sigma}$. Using this together with the ζ-test gives the next result immediately.

2.1 Proposition : If $|a_n|$ is bounded then the Dirichlet series $\Sigma a_n n^{-s}$ is absolutely convergent for $\sigma > 1$.

In particular this holds for $\zeta(s), \eta(s)$ and all L-functions $L(s,\chi) := \Sigma_{n=1}^{\infty} \chi(n) n^{-s}$ for any Dirichlet character χ, indeed $|a_n| \in \{0, 1\}$ for these functions.

2.2 Examples : The mod 3 character χ_2^3 is defined by $\chi_2^3(n) = 0, 1, -1$ for n congruent respectively to $0, 1, 2$ modulo 3. One has $L(s, \chi_2^3) = 1 - 2^{-s} + 4^{-s} - 5^{-s} + 7^{-s} - 8^{-s} \ldots$. By the Leibniz alternating series test we see that both $\eta(s), L(s, \chi_2^3)$ converge (conditionally) along the real line $s = \sigma$ for $0 < \sigma \leq 1$. The first objective of this section is to show that this remains true for all t, i.e. Dirichlet series such as in these two examples are convergent for $\sigma > 0$, for all t. The treatment is very close to that of [11].

2.3 Lemma : Let $\alpha, \beta, \sigma \in \mathbb{R},\ 0 < \sigma,\ 0 < \alpha < \beta$. Then $|e^{-\alpha s} - e^{-\beta s}| \leq \dfrac{|s|}{\sigma}(e^{-\alpha\sigma} - e^{-\beta\sigma})$.

Proof: We have

$$e^{-\alpha s} - e^{-\beta s} = s \int_\alpha^\beta e^{-us} du,$$

$$\text{hence } |e^{-\alpha s} - e^{-\beta s}| \leq |s| \int_\alpha^\beta |e^{-us}| du$$

$$= |s| \int_\alpha^\beta e^{-u\sigma} du = \frac{|s|}{\sigma}(e^{-\alpha\sigma} - e^{-\beta\sigma}). \qquad \square$$

2.4 Corollary : Set $\alpha = \log(m),\ \beta = \log(n)$, $0 < m < n,\ \sigma > 0$, then

$$|m^{-s} - n^{-s}| \leq \frac{|s|}{\sigma}(m^{-\sigma} - n^{-\sigma}) .$$

2.5 Lemma (Abel's summation formula) : Let $a_k, b_k \in \mathbb{C},\ n \geq 1,$ and set $A_n = a_1 + \cdots + a_n$. Then

$$\sum_{k=1}^{n} a_k b_k = A_n b_{n+1} - \sum_{k=1}^{n} A_k (b_{k+1} - b_k) .$$

Proof: Let $A_0 = 0$. Then

$$\sum_{k=1}^{n} a_k b_k = \sum_{k=1}^{n} (A_k - A_{k-1}) b_k =$$

$$= \sum_{k=1}^{n} A_k b_k - \sum_{k=1}^{n} A_k b_{k+1} + A_n b_{n+1}$$

which is the same as the right hand side of Abel's formula. $\square$

2.6 Corollary : The sum $\Sigma_{k=1}^{\infty} a_k b_k$ converges if both $\Sigma_{k=1}^{\infty} A_k (b_{k+1} - b_k)$ and $\{A_n b_{n+1}\}$ are convergent.

We remark that Abel's summation formula can be thought of as a discrete version of the familiar integration by parts formula from calculus. This should be clear by writing them side by side as

$$\begin{array}{rcl} \displaystyle\sum_{k=1}^{n} b_k a_k & = & A_n b_{n+1} - \sum_{k=1}^{n} A_k (b_{k+1} - b_k) \\ \int u dv & = & vu - \int v du \ . \end{array}$$

Before turning to the first main theorem of this section, we recall some standard facts about convergence of an infinite series of complex numbers z_n. The partial sums are written $S_n := \Sigma_{k=1}^{n} z_k$, and one says that $\Sigma_{k=1}^{\infty} z_k = S$ if and only if $\lim_{n\to\infty} S_n$ exists and equals S. In this case the series is said to be convergent. A necessary condition for convergence is $z_n \to 0$ as $n \to \infty$. A necessary and sufficient condition, the Cauchy convergence criterion, is that for any given real number $\varepsilon > 0$ there exists $N \in \mathbb{N}$ such that for all $m, n \geq N,\ |S_n - S_m| < \varepsilon$.

We now turn our attention to $z_n = a_n n^{-s}$, i.e. the Dirichlet series $\Sigma_{n=1}^{\infty} a_n n^{-s},\ a_n, s \in \mathbb{C}$. The notation $A_n = a_1 + \ldots + a_n$ for such a Dirichlet series will be used henceforth.

2.7 Theorem : Consider $\Sigma_{n=1}^{\infty} a_n n^{-s},\ a_n \in \mathbb{C}$. If $\{|A_n|\}$ is bounded then the series converges for $\sigma > 0$.

Proof: We have $|A_n| \leq C$, for some $C > 0$ and for all n. We shall use Corollary 2.6, with $a_n = a_n$ and $b_n = n^{-s}$. Then $|A_n b_{n+1}| = |A_n| \cdot |b_{n+1}| \leq C \cdot (n+1)^{-\sigma} \to 0$ as $n \to \infty$. Hence the second condition of Corollary 2.6, $\{A_n b_{n+1}\}$ converges (in this case to 0), is satisfied.

For the first condition, we apply the Cauchy convergence criterion to $\Sigma_{k=1}^{\infty} A_k((k+1)^{-s} - k^{-s})$. Given $\varepsilon > 0$ and using Corollary 2.4 we have

$$\begin{aligned} |S_n - S_m| &= |\textstyle\sum_{k=m+1}^{n} A_k((k+1)^{-s} - k^{-s})| \\ &\leq C \cdot \sum_{k=m+1}^{n} |(k+1)^{-s} - k^{-s}| \\ &\leq \frac{C|s|}{\sigma} \sum_{k=m+1}^{n} (\frac{1}{k^\sigma} - \frac{1}{(k+1)^\sigma}) \\ &= \frac{C|s|}{\sigma} (\frac{1}{(m+1)^\sigma} - \frac{1}{(n+1)^\sigma}) \\ &\leq \frac{C|s|}{\sigma(m+1)^\sigma} < \varepsilon \end{aligned}$$

for m sufficiently large. $\square$

The first objective of this section is thus accomplished. We give a corollary. Recall that the trivial (also called principal) Dirichlet character modulo q is given by $\chi(n) = 0$ for all n such that $\gcd(q, n) > 1$, and $\chi(n) = 1$ when $\gcd(q, n) = 1$.

2.8 Corollary : For $\eta(s)$ or for $L(s, \chi)$ with χ any non-trivial Dirichlet character χ modulo q, the Dirichlet series converges for $\sigma > 0$.

Proof: For $\eta,\ A_n \in \{0, 1\}$ is bounded. For any non-trivial character χ modulo q one has $A_q = \chi(1) + \ldots + \chi(q) = 0$ (cf. [10] Ex. 2.2.8) so $\{|A_n|\}$ is periodic modulo q, hence finite and bounded. $\square$

We remark that Theorem 2.7 is proved in [11], but the proof is a little less direct than the one given above, and is restricted to the case $a_n \in \mathbb{R}$ (for no apparent reason). We also remark that Corollary 2.8 is identical to [10] Ex. 2.3.4.

The second objective of this section is to consider possible strengthening of the above results, in particular 2.3, 2.7, and their corollaries. It will be seen in Sections 4-5 that such strengthening could be very useful. First consider Corollary 2.4. Another obvious (second) upper bound is $|m^{-s}-n^{-s}| \leq |m^{-s}| + |n^{-s}| = m^{-\sigma} + n^{-\sigma}$. It can be seen that for each fixed values for m, n, σ there is a t_* such that the first upper bound (from 2.4) is better for $t < t_*$ whereas the second, which is simply a constant, is better for $t > t_*$. Indeed the second becomes better and better as t increases. Whether this can be used in some way to strengthen Theorem 2.7 is presently not known. It may also be possible to find a third upper bound that improves both the first and second (of course their minimum will be one such) and also can be used to strengthen 2.7.

It is in fact possible to strengthen Theorem 2.7 using Corollary 2.4 as it stands, and the next two theorems are examples.

2.9 Theorem : Consider $\Sigma_{n=1}^{\infty} a_n n^{-s}$, $a_n \in \mathbb{C}$. If there exists a constant $C > 0$ such that $|A_n| < C \cdot \log(n)$, $n \geq 2$, then the series converges for $\sigma > 0$.
Proof: As in the proof of Theorem 2.7, the second convergence condition follows since $C \cdot \log(n) \cdot (n+1)^{-\sigma} \to 0$ as $n \to \infty$. For the first convergence condition, proceeding as in 2.7, we have

$$|S_n - S_m| = |\sum_{k=m+1}^{n} A_k \cdot ((k+1)^{-s} - k^{-s})| \leq \sum_{k=m+1}^{n} |A_k| \cdot |k^{-s} - (k+1)^{-s}| \ .$$

Here $m \geq 1$, $k \geq 2$, hence from both the hypothesis and Corollary 2.4

$$\sum_{k=m+1}^{n} |A_k| \cdot |k^{-s} - (k+1)^{-s}| \leq \frac{C|s|}{\sigma} \sum_{k=m+1}^{n} \log(k) \cdot (k^{-\sigma} - (k+1)^{-\sigma}) \ .$$

For convenience write $C|s|/\sigma = K$ henceforth, then the last expression, after a small rearrangement of the terms, equals

$$K[\log(m+1) \cdot (m+1)^{-\sigma} + \sum_{k=m+1}^{n-1} (\log(k+1) - \log(k)) \cdot (k+1)^{-\sigma} - \log(n) \cdot (n+1)^{-\sigma})]$$
$$= K[\log(m+1) \cdot (m+1)^{-\sigma} - \log(n) \cdot (n+1)^{-\sigma} + \sum_{k=m+1}^{n-1} \log(1+\frac{1}{k}) \cdot (k+1)^{-\sigma}] \ .$$

Next note that for $0 \leq u \leq 1$, $\log(1+u) = u - u^2/2 + u^3/3 + \ldots = u + \beta_u$, where $|\beta_u| \leq u^2/2$, as in the Leibniz convergence test for series with alternating signs (in fact, using the mean value theorem from elementary calculus, one sees that this remains true for $0 \leq u$). The previous sum thus equals

$$K \cdot \left[\frac{\log(m+1)}{(m+1)^{\sigma}} - \frac{\log(n)}{(n+1)^{\sigma}} + \sum_{k=m+1}^{n-1} (\frac{1}{k} + \beta_{1/k}) \cdot (k+1)^{-\sigma} \right]$$
$$\leq K \cdot \left[\frac{\log(m+1)}{(m+1)^{\sigma}} + \sum_{k=m+1}^{\infty} k^{-1-\sigma} + \frac{1}{2} \sum_{k=m+1}^{\infty} k^{-2-\sigma} \right] \ .$$

For $\sigma > 0$ the two summations are absolutely convergent, so given $\varepsilon > 0$, taking m sufficiently large will clearly guarantee that each of the three terms in the above formula will be smaller than $\varepsilon/(3K)$, completing the proof. □

Since the derivative of n^s is $\log(n) \cdot n^s$, we can use Theorem 2.9 to obtain a corollary similar to 2.8.
2.10 Corollary : For $f(s) = \eta(s)$ or for $f(s) = L(s, \chi)$ with χ any non-trivial Dirichlet character modulo q, the Dirichlet series for $f'(s)$ converges for $\sigma > 0$.

We next prove a convergence theorem even stronger than Theorem 2.11, but this time with the additional hypothesis that $\sigma > .5$. One easy lemma is first needed.
2.11 Lemma : For $k > 0$, $(k+1)^{1/2} - k^{1/2} = \frac{1}{2k^{1/2}} + \beta_k$, where $|\beta_k| < \frac{1}{8k^{3/2}}$.
Proof : This is almost immediate from the binomial theorem, which gives the convergent series

$$\beta_k = -\frac{1}{8k^{3/2}} + \frac{3}{2^3 \cdot 3! \cdot k^{5/2}} - \frac{3 \cdot 5}{2^4 \cdot 4! \cdot k^{7/2}} + \ldots \ .$$

The Leibniz test for alternating series completes the proof. □
2.12 Theorem : Consider $\Sigma_{n=1}^{\infty} a_n n^{-s}$, $a_n \in \mathbb{C}$. If there exists a constant $C > 0$ such that $|A_n| < C \cdot n^{1/2}$, $n \geq 1$, then the series converges for $\sigma > 1/2$.

Proof : The proof is similar to the proofs of both 2.7 and 2.9, using the Lemma 2.11. The second convergence condition of Corollary 2.6 follows since $C \cdot n^{1/2} \cdot (n+1)^{-\sigma} \to 0$ as $n \to \infty$.

For the first convergence condition, proceeding as in 2.7, 2.9, and omitting the first few steps (which are identical), we have

$$|S_n - S_m| \leq \frac{C|s|}{\sigma} \sum_{k=m+1}^{n} k^{1/2} \cdot (k^{-\sigma} - (k+1)^{-\sigma}) \ .$$

For convenience write $C|s|/\sigma = K$ henceforth, then the last expression, after a small rearrangement of the terms, equals

$$K[(m+1)^{1/2} \cdot (m+1)^{-\sigma} + \sum_{k=m+1}^{n-1} ((k+1)^{1/2} - k^{1/2}) \cdot (k+1)^{-\sigma} - n^{1/2} \cdot (n+1)^{-\sigma})] \ .$$

Now using Lemma 2.11, this equals

$$K \cdot \left[\frac{(m+1)^{1/2}}{(m+1)^{\sigma}} - \frac{n^{1/2}}{(n+1)^{\sigma}} + \sum_{k=m+1}^{n-1} (\frac{1}{2 \cdot k^{1/2}} + \beta_k) \cdot (k+1)^{-\sigma} \right] \ , \text{where } |\beta_k| < \frac{1}{8k^{3/2}} \ ,$$

$$\leq K \cdot \left[\frac{(m+1)^{1/2}}{(m+1)^{\sigma}} + \frac{1}{2} \sum_{k=m+1}^{\infty} k^{-1/2-\sigma} + \frac{1}{8} \sum_{k=m+1}^{\infty} k^{-3/2-\sigma} \right] \ .$$

For $\sigma > 1/2$ the two summations are absolutely convergent, so given $\varepsilon > 0$, taking m sufficiently large will clearly guarantee that each of the three terms in the above formula will be smaller than $\varepsilon/(3K)$, completing the proof. □

3. CONVERGENCE OF EULER PRODUCTS FOR $\sigma > .5$

In this section we simply present some numerical evidence for convergence of certain Euler products in the half plane $\sigma > .5$. The Euler products for any Dirichlet L-function and the Riemann zeta function are well known to converge absolutely for $\sigma > 1$. We now give some numerical evidence here that for an L-function coming from a primitive character χ mod q, $q \geq 3$, the Euler product

$$L(s, \chi) = \prod_p \frac{1}{1 - \chi(p) \cdot p^{-s}} \ ,$$

where the product is taken over all primes p, converges for $\sigma > .5$ and diverges for smaller σ. Three primitive characters are considered, χ_2^3 which takes values $0, 1, -1$ as n is respectively congruent to $0, 1, 2$ modulo 3, χ_2^4 which takes values $0, 1, 0, -1$ as n is respectively congruent to $0, 1, 2, 3$ modulo 4, and χ_2^5 which takes values $0, 1, i, -i, -1$ as n is respectively congruent to $0, 1, 2, 3, 4$ modulo 5. We consider $s = \sigma + 30i$, for $\sigma = .4$ (showing divergence) and for $\sigma = .55, .6, .7, .8, .9, 1.0, 1.1, 1.5$, which show stronger and stronger convergence as σ increases. Of course for $\sigma = 1.1, 1.5$ convergence is known and is absolute. We choose $t = 30$ as a fairly typical t value, similar results can be seen for other t values.

The figures below show the absolute value of the truncation error Δ for three Dirichlet L-functions as a function of the number of factors taken in their Euler product representations. Curves are shown for selected values of σ. To aid in extracting numerical values from the graphs, below each figure is a corresponding table giving Δ when the number of factors is a power of 10.

Preliminary calculations were performed with Maple [9], which also provided reference values for the L-functions used in calculating the truncation errors. To overcome performance limitations of Maple when extending the results to large numbers of factors in the Euler products, a Fortran program based on MPFUN2015 libraries [2] was used. Mathematica [12] was used to generate a file of the first 10^9 primes, which was used as input to the Fortran calculation. Thanks go to Information Technologies at the University of Calgary for providing access to the large-memory nodes of the Helix cluster for the bulk of the computations.

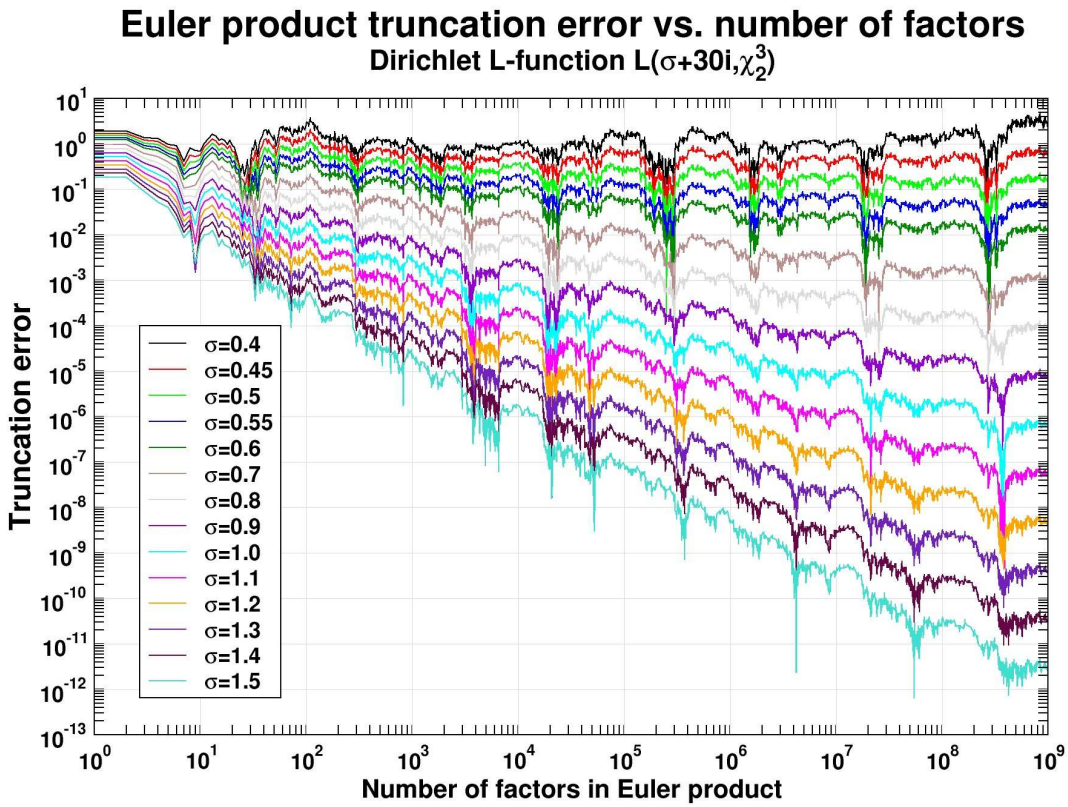

FIGURE 1. Graph of error terms for $L(\sigma + 30i, \chi_2^3)$

σ	$\Delta(10^2)$	$\Delta(10^3)$	$\Delta(10^4)$	$\Delta(10^5)$	$\Delta(10^6)$	$\Delta(10^7)$	$\Delta(10^8)$	$\Delta(10^9)$
.4	2.64	.948	1.04	1.53	1.28	.929	1.49	3.16
.55	.596	.217	.196	.135	.0809	.0595	.0513	.0497
.6	.384	.131	.107	.0628	.0335	.0222	.0166	.0141
.7	.167	.0478	.0313	.0139	.00582	.00304	.00175	.00116
.8	.0757	.0175	.00909	.00311	.00102	4.14×10^{-4}	1.85×10^{-4}	9.57×10^{-5}
.9	.0352	.00646	.00265	7.02×10^{-4}	1.80×10^{-4}	5.66×10^{-5}	1.97×10^{-5}	7.98×10^{-6}
1.0	.0167	.00239	7.74×10^{-4}	1.59×10^{-4}	3.18×10^{-5}	7.73×10^{-6}	2.10×10^{-6}	6.69×10^{-7}
1.1	.00801	8.89×10^{-4}	2.28×10^{-4}	3.63×10^{-5}	5.65×10^{-6}	1.06×10^{-6}	2.24×10^{-7}	5.65×10^{-8}
1.5	4.63×10^{-4}	1.76×10^{-5}	1.76×10^{-6}	1.02×10^{-7}	5.84×10^{-9}	3.70×10^{-10}	2.99×10^{-11}	3.00×10^{-12}

TABLE 1. Error terms for $L(\sigma + 30i, \chi_2^3)$

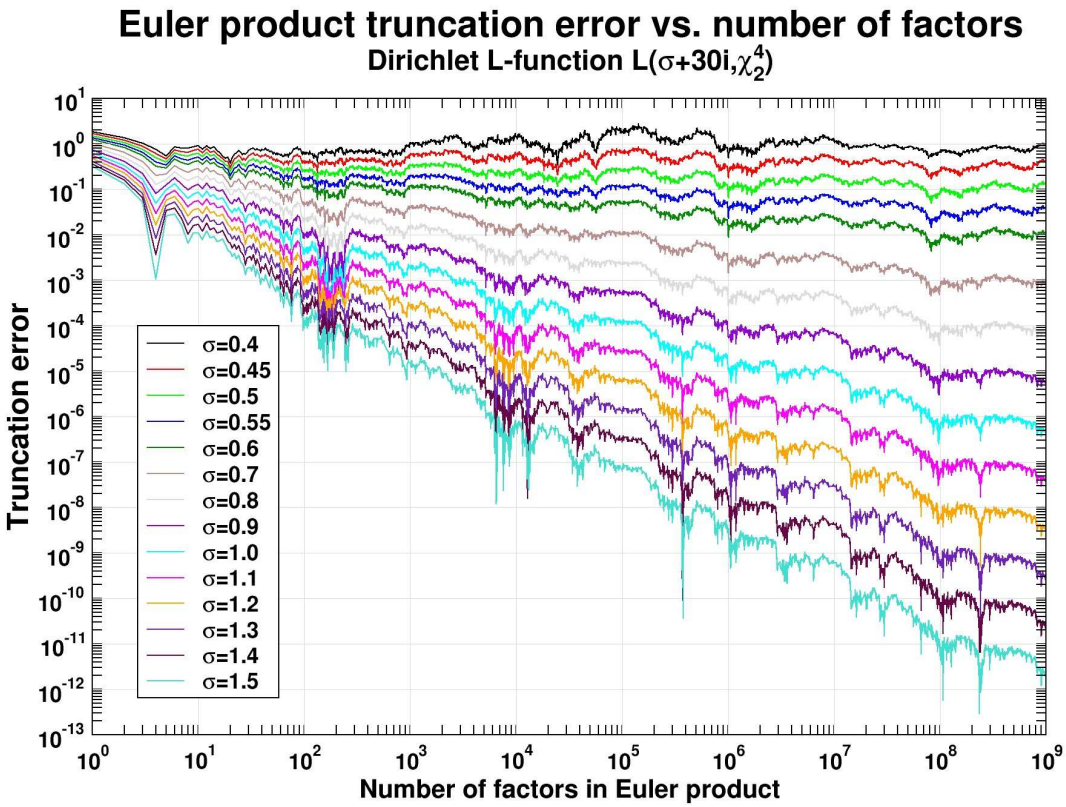

FIGURE 2. Graph of error terms for $L(\sigma + 30i, \chi_2^4)$

σ	$\Delta(10^2)$	$\Delta(10^3)$	$\Delta(10^4)$	$\Delta(10^5)$	$\Delta(10^6)$	$\Delta(10^7)$	$\Delta(10^8)$	$\Delta(10^9)$
.4	.599	.970	1.43	1.84	.573	1.21	.673	.979
.55	.154	.194	.118	.109	.0315	.0642	.0253	.0391
.6	.0998	.115	.0569	.0487	.0122	.0236	.00756	.0111
.7	.0427	.0414	.0140	.0104	.00197	.00327	6.75×10^{-4}	8.84×10^{-4}
.8	.0187	.0151	.00353	.00229	3.40×10^{-4}	4.62×10^{-4}	6.08×10^{-5}	7.19×10^{-5}
.9	.00835	.00557	.000915	.000515	6.15×10^{-5}	6.62×10^{-5}	5.56×10^{-6}	5.96×10^{-6}
1.0	.00379	.00207	.000243	.000117	1.14×10^{-5}	9.53×10^{-6}	5.19×10^{-7}	4.99×10^{-7}
1.1	.00175	.000774	6.63×10^{-5}	2.66×10^{-5}	2.14×10^{-6}	1.38×10^{-6}	5.03×10^{-8}	4.22×10^{-8}
1.5	8.87×10^{-5}	1.57×10^{-5}	5.22×10^{-7}	7.32×10^{-8}	2.66×10^{-9}	6.10×10^{-10}	7.12×10^{-12}	2.31×10^{-12}

TABLE 2. Error terms for $L(\sigma + 30i, \chi_2^4)$

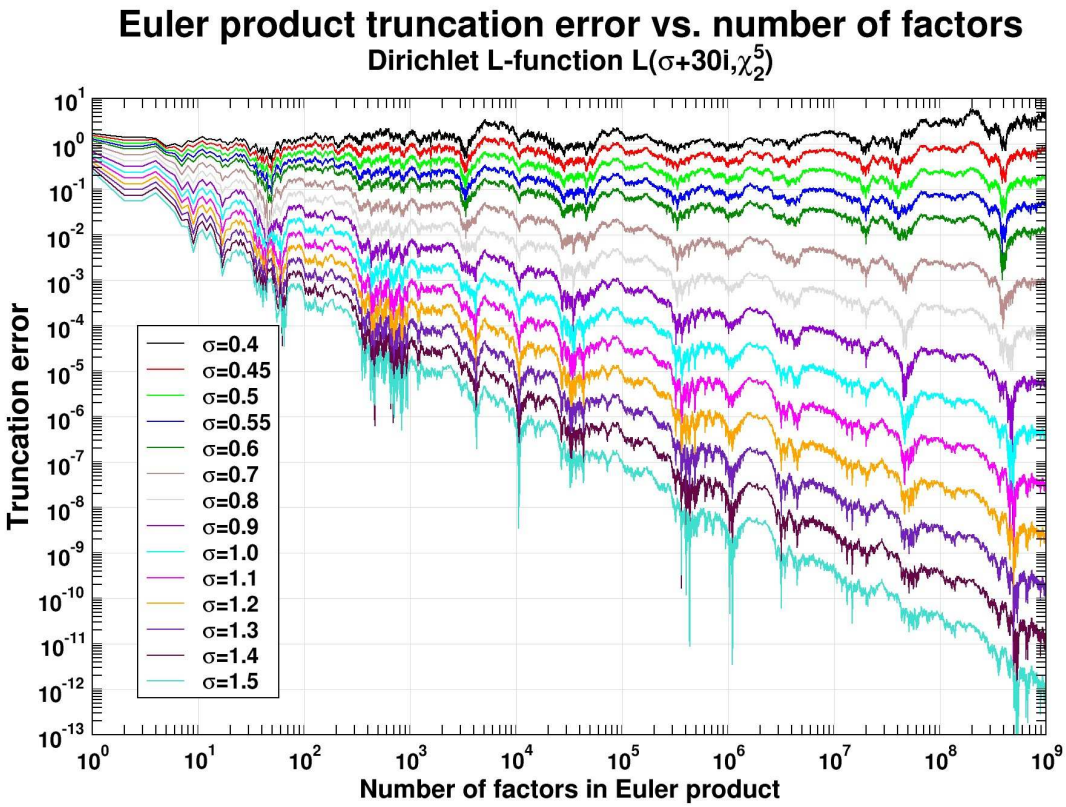

FIGURE 3. Graph of error terms for $L(\sigma + 30i, \chi_2^5)$

σ	$\Delta(10^2)$	$\Delta(10^3)$	$\Delta(10^4)$	$\Delta(10^5)$	$\Delta(10^6)$	$\Delta(10^7)$	$\Delta(10^8)$	$\Delta(10^9)$
.4	1.40	1.75	1.49	1.80	.767	1.86	2.80	3.95
.55	.452	.283	.161	.190	.0577	.0977	.0756	.0476
.6	.310	.163	.0784	.0871	.0231	.0347	.0241	.0130
.7	.145	.0556	.0191	.0185	.00367	.00439	.00248	9.94×10^{-4}
.8	.0686	.0195	.00473	.00398	5.86×10^{-4}	5.64×10^{-4}	2.58×10^{-4}	7.66×10^{-5}
.9	.0325	.00693	.00120	8.74×10^{-4}	9.45×10^{-5}	7.38×10^{-5}	2.73×10^{-5}	5.94×10^{-6}
1.0	.0155	.00249	3.08×10^{-4}	1.94×10^{-4}	1.54×10^{-5}	9.82×10^{-6}	2.90×10^{-6}	4.62×10^{-7}
1.1	.00741	.000901	8.13×10^{-5}	4.34×10^{-5}	2.55×10^{-6}	1.33×10^{-6}	3.11×10^{-7}	3.60×10^{-8}
1.5	4.05×10^{-4}	1.63×10^{-5}	5.50×10^{-7}	1.16×10^{-7}	2.41×10^{-9}	5.01×10^{-10}	4.33×10^{-11}	1.34×10^{-12}

TABLE 3. Error terms for $L(\sigma + 30i, \chi_2^5)$

4. THEORY OF EULER PRODUCT CONVERGENCE

We start this section with a brief discussion of infinite products and the related convergence issues, and conclude with a theorem that seems to give an approach to proving that Euler products of the type considered in Section 3 converge, for $\sigma > 1/2$. Intuitively one would say that $\Pi_{n=1}^{\infty} u_n$, $u_n \in \mathbb{C}$, converges when $\lim_{N\to\infty} \Pi_{n=1}^{N} u_n = L$ exists, and then define $\Pi_{n=1}^{\infty} u_n = L$. But this has complications, especially if any $u_n = 0$. For the most general definition see Apostol's text [1], p. 207. This definition has quite a few cases and even a few surprises, e.g. if $u_n = 1/n$ we say $\Pi_{n=1}^{\infty} u_n$ *diverges* to 0. For our purposes it suffices to avoid these complications by using a subset of the Apostol definition and requiring:

(a) $u_n \neq 0$ for all n, and
(b) $\lim_{n\to\infty} u_n = 1$.

Then we may now follow Lang [8] pp. 372-373 and define $\Pi_{n=1}^{\infty} u_n$ to be convergent (resp. absolutely convergent) if the infinite series $\Sigma_{n=1}^{\infty} \log(u_n)$ is convergent (resp. absolutely convergent), provided

we are a little careful with the multivalued logarithmic function, as follows. From (a) $\log(u_n)$ is defined for all n, and from (b), discarding a finite number of terms if so required (which has no effect on convergence issues), we can suppose $|u_n - 1| < 1/2$ for all sufficiently large n. We then choose the branch of the logarithm for which $\log(1) = 0$. This also implies $\lim_{n\to\infty}(\log(u_n)) = 0$. Now write $w := \Sigma_{n=1}^{\infty} \log(u_n) \in \mathbb{C}$, and using the continuity of the exponential function we see that

$$\prod_{n=1}^{\infty} u_n = \lim_{N\to\infty} \prod_{n=1}^{N} u_n = \lim_{N\to\infty} e^{(\sum_{n=1}^{N} \log(u_n))}$$
$$= e^{\lim_{N\to\infty}(\sum_{n=1}^{N} \log(u_n))} = e^w$$

exists and further is non-zero. It is also clear that $\Pi_{n=1}^{\infty}(u_n)^{-1} = 1/(e^w)$.

We shall henceforth write $u_n = 1 - \alpha_n$, and next give two results that connect the convergence of $\Pi_{n=1}^{\infty} u_n$ with the convergence of $\Sigma_{n=1}^{\infty}\alpha_n$. The first concerns absolute convergence and is found in many texts, cf. [8]. The second concerns convergence and can be found in [3], p.405, at least for the case $u_n \in \mathbb{R}$.

4.1 Theorem : Let $\alpha_n \in \mathbb{C} \setminus \{1\}$ and suppose $\Sigma_{n=1}^{\infty}\alpha_n$ is absolutely convergent. Then $\Pi_{n=1}^{\infty} u_n$ is absolutely convergent.

Sketch of proof (following [8]): The hypotheses on α_n imply conditions (a), (b) for u_n hold, so we consider $\Sigma_{n=1}^{\infty} \log(u_n) = \Sigma_{n=1}^{\infty} \log(1-\alpha_n)$. Discarding a finite number of α_n if necessary we have $|\alpha_n| < 1/2$, whence

$$\log(1-\alpha_n) = -\alpha_n - \frac{\alpha_n^2}{2} - \frac{\alpha_n^3}{3} - \ldots =$$
$$-\alpha_n(1 + \frac{\alpha_n}{2} + \frac{\alpha_n^2}{3} + \ldots) \ .$$

It is then easily seen that $|\log(1-\alpha_n)| \leq (3/2)\cdot|\alpha_n|$ and the convergence of $\Sigma_{n=1}^{\infty}|\alpha_n|$ thus implies convergence of $\Sigma_{n=1}^{\infty}|\log(u_n)|$. □

The proofs of the next lemma and theorem follow Bartle's proofs for the case $\alpha_n \in \mathbb{R}$ (cf. [3], given as a "Project"), with a couple of changes that are discussed in Remark 4.5 below.

4.2 Lemma : Let $z \in \mathbb{C}$, $|z| < 1/2$. Then $(1/6)|z|^2 < |z + \log(1-z)| < (5/6)|z|^2$.

Proof: We have

$$z + \log(1-z) = z - z - \frac{z^2}{2} - \frac{z^3}{3} - \ldots = -\frac{z^2}{2}(1+R),$$

where $R = \sum_{n=1}^{\infty} \frac{2z^n}{n+2}$. Now $|R| \leq (2/3)|z| + (2/4)|z|^2 + (2/5)|z|^3 + \ldots \leq (2/3)|z|(1+|z|+|z|^2+\ldots) = \frac{2|z|}{3}\frac{1}{1-|z|} < \frac{2}{3}\cdot\frac{1}{2}\cdot\frac{1}{1-1/2} = \frac{2}{3}$, whence $1/3 < |1+R| < 5/3$. Multiplying by $|z|^2/2$ completes the proof. □

4.3 Theorem : Let $\alpha_n \in \mathbb{C} \setminus \{1\}$ and suppose $\Sigma_{n=1}^{\infty}\alpha_n$ is convergent. Then $\Pi_{n=1}^{\infty} u_n$ is convergent if $\Sigma_{n=1}^{\infty}|\alpha_n|^2$ is convergent.

Proof: We start as in the proof of 4.1 and have (again n is assumed sufficiently large so $|\alpha_n| < 1/2$) $\log(1-\alpha_n) = -\alpha_n + \beta_n$, where $\beta_n = \alpha_n + \log(1-\alpha_n)$, so by Lemma 4.2 $(1/6)|\alpha_n|^2 < |\beta_n| < (5/6)|\alpha_n|^2$. By hypothesis $\Sigma_{n=1}^{\infty}\alpha_n$ converges, thus $\Sigma_{n=1}^{\infty}\log(1-\alpha_n)$ converges if and only if $\Sigma_{n=1}^{\infty}\beta_n$ converges. But by the above inequality (right-hand side) this will follow from the convergence of $\Sigma_{n=1}^{\infty}|\alpha_n|^2$, indeed $\Sigma_{n=1}^{\infty}\beta_n$ is absolutely convergent here. □

4.4 Corollary: Let $\alpha_n \in \mathbb{C} \setminus \{1\}$ and suppose $\Sigma_{n=1}^{\infty}\alpha_n$ is convergent. Then $\Pi_{n=1}^{\infty} u_n$ is convergent if $\alpha_n = O(n^{-r})$, $r > 1/2$.

4.5 Remark: In the real case $\alpha_n \in \mathbb{R}$, as in [3], one actually obtains the following stronger result: Let $\alpha_n \in \mathbb{R}$, $\alpha_n < 1$, and suppose $\Sigma_{n=1}^{\infty}\alpha_n$ is convergent. Then $\Pi_{n=1}^{\infty} u_n$ is convergent if and only if $\Sigma_{n=1}^{\infty}|\alpha_n|^2$ is convergent. To see this one simply observes, for n sufficiently large, that $\beta_n = -\frac{\alpha_n^2}{2} - \frac{\alpha_n^3}{3} - \ldots = -\frac{\alpha_n^2}{2}\left(1 + \frac{2\alpha_n}{3} + \frac{2\alpha_n^2}{4} + \ldots\right) < 0$. Hence $\Sigma\beta_n$ converges if and only if $\Sigma|\beta_n|$ converges, and then the left hand side of the inequality mentioned in the above proof of Theorem 4.3 can be used, showing that $\Sigma|\beta_n|$ converges implies $\Sigma|\alpha_n|^2$ converges.

5. MORE ON CONVERGENCE OF EULER PRODUCTS FOR $\sigma > .5$

Based on the theorems in Section 4, we present further numerical evidence for the convergence of the Euler products for $\sigma > .5$, for the three Dirichlet characters considered in Section 3. Indeed, this evidence is stronger in the sense that it will apply to any $s = \sigma + it$, $\sigma > .5$, whereas in Section 3 we took just one typical value $t = 30$. Furthermore, it also appears to be at least as or even more convincing than the evidence in Section 3.

As seen in Section 4, the convergence of an infinite product is equivalent to that of its reciprocal, so we consider the reciprocal Euler product $L^{-1}(s,\chi) = \Pi_p(1-\chi(p)\cdot p^{-s})$, taken over the primes p, for any Dirichlet character χ. It will be convenient to write p_n for the n'th prime, so this formula becomes

$$L^{-1}(s,\chi) = \prod_{n=1}^{\infty}(1 - \chi(p_n)\cdot p_n^{-s}) = \prod_{n=1}^{\infty}(1-\alpha_n) \ ,$$

where $\alpha_n = \chi(p_n)\cdot p_n^{-s}$. Note that $\Sigma\alpha_n$ is a Dirichlet series $\Sigma a_n \cdot n^{-s}$ with $a_n = 0$ at all composite numbers n, and $a_p = \chi(p)$ at a prime p. To show convergence of the above infinite product we seek to apply Corollary 4.4 above. Since $|\chi(p_n)| \in \{0,1\}$ and $\sigma > 1/2$, the second condition of this corollary

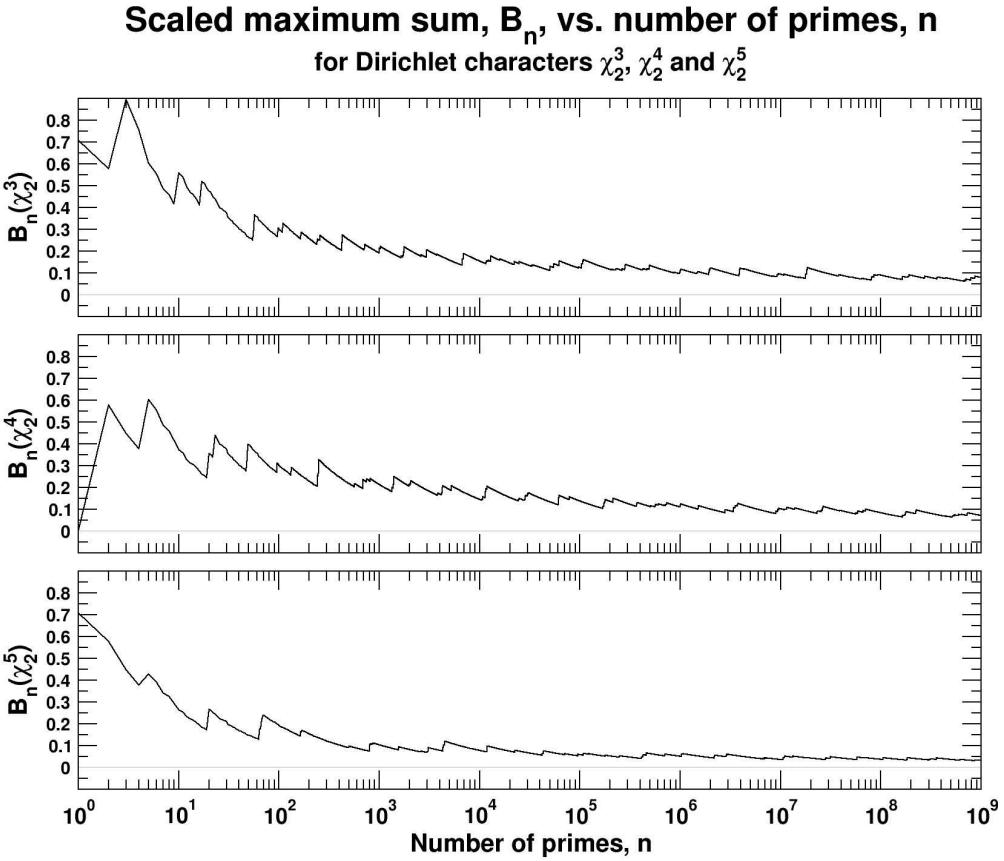

FIGURE 4. Graphs of B_n for $\chi_2^3, \chi_2^4, and \chi_2^5$

is fulfilled for any Dirichlet character χ. As far as the first condition $\Sigma_{n=1}^{\infty}\alpha_n$ convergent, we must now assume χ to be a primitive character and attempt to apply Theorem 2.12, where we now have $A_n = \chi(p_1) + \ldots + \chi(p_n)$. Figure 4 above and the corresponding Table 4 give strong evidence that $|A_n| < C \cdot p_n^{1/2}$, indeed we define $B_n = \max\{|A_j| : j \leq p_n\}/\sqrt{p_n}$, and observe from the figure and table that B_n is not only bounded but appears to be converging to 0. Furthermore, the following Figure 5 suggests that $B_n = O((\log(n))^{-1})$. The intuition behind Figure 4 and Table 4 is that for a primitive mod q character, density theorems imply that the primes in the various congruence classes mod q occur with approximately equal frequency. This causes much cancellation in the sum for $|A_j|$ and therefore, even though these sums will be unbounded, they cannot grow too rapidly.

This is also related to the "prime number races," cf. [5]. Indeed, the description in Section 1 of that paper, related to the "mod 3 and mod 4 races," implies that for infinitely many primes the mod 3 race, which is between primes congruent to 1 modulo 3 and primes congruent to -1 modulo 3, is tied. And for such primes p_n one will have $A_{p_n} = 0$ (for χ_2^3). It follows that $\liminf_{n\to\infty} A_{p_n} = 0$ for χ_2^3. The situation for χ_2^4 is the same (but not for χ_2^5).

n	10	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9
$B_n(\chi_2^3)$	.557	.301	.214	.155	.125	.111	.0938	.0896	.0788
$B_n(\chi_2^4)$	.371	.301	.214	.145	.138	.110	.102	.0838	.0712
$B_n(\chi_2^5)$	.263	.192	.104	.0780	.0587	.0509	.0363	.0370	.0319

TABLE 4. Values of B_n

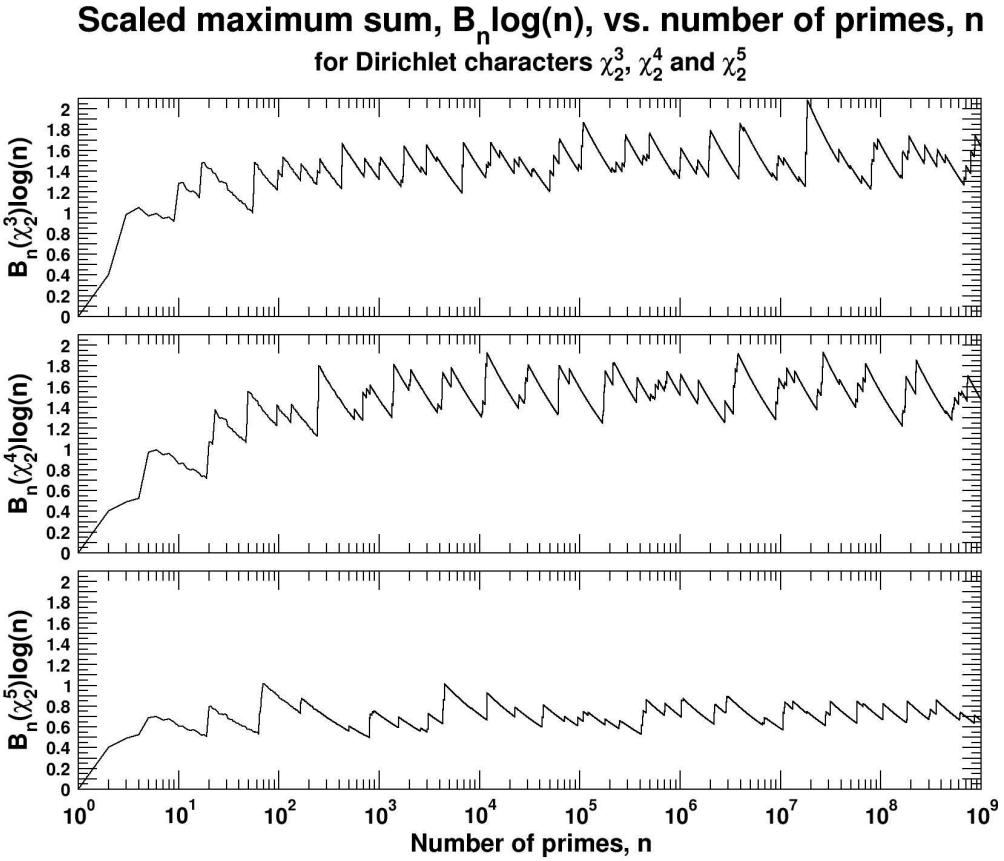

FIGURE 5. Graphs of $B_n \cdot \log(n)$

6. EXAMPLES AND QUESTIONS

Our first example is standard. Here (as before) Π_p denotes a product and Σ_p denotes a sum, both over the prime numbers, and p_n is the n'th prime.

6.1 Example : The Euler product for $\zeta(s)$, or for any Dirichlet L-function $L(s,\chi)$, is absolutely convergent for $\sigma > 1$.

To see this, e.g. for $\zeta(s)$, consider the Euler product $\zeta^{-1}(s) = \Pi_p(1-p^{-s})$. Then, using the notation of Section 4, $\alpha_n = p_n^{-s}$ so $\Sigma_n \alpha_n$ is clearly absolutely convergent for $\sigma > 1$, and Theorem 4.1 then implies that $\zeta^{-1}(s) = \Pi_n(1-\alpha_n)$ is absolutely convergent. As seen in Section 4 this is equivalent to absolute convergence of the Euler product for ζ. The proof for the L- functions is similar.

The following examples will involve Theorem 4.3 (or its Corollary 4.4) and be less straightforward. As in Example 6.1 we will generally look at the inverse of the function in question for convergence, without specific mention.

6.2 Example : Let

$$f(s) = \prod_{n\geq 2} \frac{1}{1-(-1)^n n^{-s}} = \frac{1}{1-2^{-s}} \cdot \frac{1}{1+3^{-s}} \cdots \quad .$$

Then the infinite product $f(s)$ converges for $\sigma > 1/2$.

Proof: Here $\alpha_n = (-1)^n n^{-s}$, $n \geq 2$. Using Theorem 2.7 shows $\Sigma_{n\geq 2}\alpha_n$ is convergent, $\sigma > 0$. The convergence of $f(s)$ follows by Corollary 4.4. □

The next example is very similar to Example 6.2 but n is replaced by p_n so that it is an Euler-type product.

6.3 Example : Let

$$g(s) = \prod_{n\geq 1} \frac{1}{1+(-1)^n p_n^{-s}} =$$

$$\frac{1}{1-2^{-s}} \cdot \frac{1}{1+3^{-s}} \cdot \frac{1}{1-5^{-s}} \cdot \frac{1}{1+7^{-s}} \cdot \frac{1}{1-11^{-s}} \cdots \quad .$$

Then the infinite product $g(s)$ converges for $\sigma > 1/2$.

Proof: Similar to that of Example 5.2.

6.4 Examples : Euler products that arise from Dirichlet L-functions are further examples, and as we have seen in Section 3 and Section 5, for primitive characters, they appear to also converge for $\sigma > 1/2$. We close this section with some potentially interesting questions.

6.5 Questions : Do the functions f, g in Examples 6.2, 6.3 (particularly 6.3), also satisfy a functional equation relating the function values at z and $1-z$. Are they in the Selberg class? If so they may give

an example of a function in the Selberg class that satisfies the Riemann Hypothesis. Is there another example of this type? Thanks to theorems of Kaczoworski and Petrelli [7], and of Conrey and Ghosh [4], it is known that the Selberg class in degree $d = 0$ consists of the single constant function 1, is empty for $0 < d < 1$, and in degree $d = 1$ consists of the Riemann zeta function, together with all L-functions coming from primitive characters and their vertical translates. So f, g, if in the Selberg class, would have to have $d > 1$.

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КОНВЕРГЕНЦИЈА НА РЕДОВИ НА ДИРИХЛЕ И ОЈЛЕРОВИ ПРОИЗВОДИ

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Во првиот дел на овој труд се докажани теореми за конвергенција на редови на Дирихле, кои се подобрување на класичните теореми за конвергенција, како на пример во книгата „Курс по аритметика“ од Сер. Вториот дел се однесува на производи од Ојлеров тип. Докажана е теорема за конвергенција која дава доволни услови за такви производи да конвергираат во полурамнината што има реален дел поголем од ½. Исто така изнесени се нумерички согледувања, кои сугерираат дека Ојлеровите производи кои што соодветствуваат на L – функцијата на Дирихле, L (s, χ), конвергираат во таа полурамнина, каде што χ е примитивниот карактер на Дирихле.

Клучни зборови: Редови на Дирихле, Ојлерови производи, L-функции, примитивен карактер на Дирихле

ПРИЛОЗИ, Одделение за природно-математички и биотехнички науки, МАНУ, том **38**, бр.2, стр. 165–184 (2017)
CONTRIBUTIONS, Section of Natural, Mathematical and Biotechnical Sciences, MASA, Vol. **38,** No. 2, pp. 165–184 (2017)

Received: September 12, 2017
Accepted: November 3, 2017

ISSN 1857–9027
e-ISSN 1857–9949
UDC: 631.528.6
636.033/.034.082.22:602.6
DOI:10.20903/csnmbs.masa.2017.38.2.112

Review

APPLICATION OF MOLECULAR TOOLS IN ANIMAL BREEDING, CROP SCIENCE, FOOD CONTROL AND AGROBIODIVERSITY IN THE REPUBLIC OF MACEDONIA

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Molecular methods have become non-replaceable for the improvement of food production and its control. The Department for Biochemistry and Genetic Engineering (DBGE) as a part of the Faculty of Agriculture Sciences and Food is a crucial entity using molecular techniques in different areas of agriculture and livestock production. DBGE is mainly dealing with marker assisted selection (MAS), evolutionary studies; determine the origin of food products; enzyme analysis in food processing; transcriptional and translational analyses; molecular detection of plant viruses; and GMO analysis. The target molecules were proteins, RNA and DNA. MAS is systematically applied in animal breeding in Macedonia using RYR-1, κ and αS1-casein genes. Evolutionary studies were performed using DNA microsatellites to estimate genetic distance among autochthonic strains of sheep and tomato varieties. The identification of food products origin and gender identification in fish were done using protein profiling. Gene expression was studied analysing different growth factors and inhibitors at RNA level. More than 3.000 plant and animal samples were analysed in the Laboratory for biochemistry and molecular biology. GMO laboratory as a part of the DBGE is the first authorized facility for GMO testing of food and feed samples. In more than 100 samples RT PCR methods were used.

Key words: livestock; crops; food; molecular tools; Macedonia

INTRODUCTION

The premise on which the recombinant DNA technology is based is that genetic information is a resource that can be manipulated in different ways to achieve certain goals in basic and applied science [1]. Application of modern molecular methods in selection of crops and livestock has become a crucial factor in the improvement of food production. It is now possible to identify, characterize and manipulate individual genes [2].

At certain sites along the genomes the sequences vary between individuals. These sites, where differences in DNA sequence occur, are known as molecular markers. When differences in DNA occur within genes, these differences have the

potential to affect the function of the gene and hence the phenotype of the individual. Molecular markers are wide and effective tools for efficient selection of desired qualitative and quantitative agronomical traits because the markers are based on the genotypes and not influenced by the environmental factors [3]. However, most molecular markers are not associated with a visible phenotype. Based on the knowledge about molecular markers, specific approaches in selection called **marker assisted selection (MAS)** have been developed. In countries where the economic environment supports high input agriculture, there has been a dramatic increase in the level of productivity from the selective improvement of livestock using MAS. The major challenge that molecular geneticists face is the identification of markers for genes that control the phenotypic variation in the target traits. The most effective markers are the functional mutations within the trait genes. Once the functional polymorphism is known it is possible to predict the effect of particular alleles in a population. Therefore, so called 'direct' markers are more useful than 'linked' markers for predicting the phenotypic variation of target traits within a population [4].

Studies on genetic polymorphisms in animal breeding are closely related to production traits and for this purpose, techniques capable of detecting small variations in the DNA sequence are used. Djedović et al. used these molecular techniques to obtain more data about the individual genome structure [5].

Marker assisted selection in crop production may facilitate agronomical and biochemical traits [3, 6]. The main types of "linked" molecular markers are DNA microsatellites or simple sequence repeats (SSR), and single nucleotide polymorphisms (SNP) [3]. The reproducibility, co-dominance, relative abundance and complete genome coverage of SSR markers have made them one of the most useful tools for detecting genetic diversity, genetic linkage mapping, association mapping and evolution analysis [7].

Genetic modification is a method of introducing new genes into an organism. It is a method of altering an organism without copying (cloning) it. A new genetically modified organism (GMO) or transgenic organism contains newly introduced DNA (a recombinant DNA construct) that imparts a new trait to an organism. Beside the continuous controversies and dilemmas about the possible risks of their application, there are substantial achievements provided by a variety of transgenic plants. On the other hand "biopharming" refers to the production of biopharmaceuticals, based on heterologous genes inserted into different genomes. Various recombinant products from transgenic organisms have been produced, including: human insulin, human growth factor, antibodies, protein antigens, and several enzymes [1]. The accelerated development of GMOs during the past twenty years has raised a new set of questions about the release and traceabilityof such GMOs and their possible adverse effects on the safety of both the environment and the consumer [8]. Different methods (qualitative or quantitative) linked to GMO detection, which are divided in protein and DNA based methods should be registered in GMO Detection Method Database (GMDD). All these different methods used by GMO detection laboratories need to be validated and accredited according to ISO 17025 standard [9]. There is a clear need for global harmonization and standardization of the methods used for sampling and for different methods used to perform GMO detection analysis [10].

In addition, the molecular tools have an irreplaceable role in detection and characterization of causative agents for crop diseases, especially viruses. These agents can be detected using the Double Antibody Sandwich – Enzyme Linked Immune Sorbent Assay (DAS-ELISA) method at the protein level, while PCR based techniques are used for detection at the RNA/DNA level.

Protein and DNA based methods are widely used in food control especially in terms of determination of its quality and origin. Authentication of milk and meat, as well as their derived products, is important because fraudulent incorporation of non-declared raw materials, during technological processing, can cause problems related to intolerance or allergy, ethical objections and legal requirements [11].

Also, molecular methods are suitable tools for the analysis of the purity of the enzymes used in food processing industries. Reproducible and standardized methods need to be developed and implemented in order for data from different sources to be reliably interpreted.

The aim of this article is to make a review on the application of molecular methods in agricultural sector in the Republic of Macedonia in the past twenty years.

EXPERIMENTAL SECTION

Applications of molecular tools in livestock and crop production, agro-biodiversity and food control in the Republic of Macedonia were implemented by the Department of Biochemistry and Genetic Engineering as a part of Faculty of Agricultural Sciences and Food in Skopje. In the past

almost 20 years more than 3,000 animal, plant, and food samples were analyzed in the Biochemistry and Molecular Biology lab and GMO lab. The analyses were performed on both the protein and nucleic acid levels.

Targeted protein molecules were analyzed using electrophoretic and immunological techniques. Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS – PAGE) was the electrophoretic technique of choice for the detection and identification of proteins for the investigation of gender specific milk and muscle specific proteins, seed proteins and in the estimation of chymosin purity. Starch Gel Electrophoresis (SGE) was used in characterizing polymorphisms in sheep hemoglobin. ELISA as an immunochemical based technique was employed in the detection of the CP4 EPSPS protein which is the result of the expression of an inserted gene for the genetic modification for glyphosate herbicide tolerance of different crops.

Nucleic acid molecules of interest in these studies were analyzed using PCR based methods. The methods include reverse transcription, digestion, hybridization, fragment analysis, sequencing and Real Time PCR. Firstly, the extraction of RNA and or DNA was done using appropriate kits for isolation of nucleic acids or by "in house" developed techniques. RNA was usually extracted using the TRIzol® method, plant DNA using Cetyl trimethylammonium bromide (CTAB) method, animal DNA using Promega kit, while DNA from processed food samples was done using so called PREP Plant X kit from SureFood, for delicate samples. Isolated RNA was turned into complementary DNA using a commercial kit for reverse transcription. The crucial step, after the extraction of nucleic acids, was the Polymerase Chain Reaction (PCR), which was performed using appropriate sets of primers under previously optimized conditions [12]. Polymorphism in marker genes was usually determined using Restriction Fragment Length Polymorphism (RFLP) for Ryanodine Receptor 1 (RYR-1) gene in swine genome, κ-casein in cattle genome and αS1 casein in the sheep genome. Hybridization of amplified DNA was performed for confirmation of mutation in the RYR-1 gene. Endpoint PCR was used for the detection of "housekeeping or reference" and inserted genes during the GMO analyses. Quantification of GMO was done by Real Time PCR using commercial kits specifically designed for such purposes. Evolutionary studies on autochthonic strains of Pramenka sheep breed and local tomato varieties were based on analysis of microsatellite DNA. Determination of genetic distance was done using PCR, followed by fragment analysis using LiCor gel system for sheep strains which is based on capillary electrophoresis for tomato varieties. RNA plant viruses were analyzed using reverse transcription, followed by PCR and ending with DNA sequencing. The potential for muscle growth in Koi carp was determined using simultaneous extraction of RNA and DNA from the same sample and calculation of the RNA/DNA ratio.

During the studies, presented in the previous section, a few innovations and modifications of the existing methods were made. On a protein level simple, efficient techniques were developed for the identification of specific milk and meat proteins from different origin, the determination of chymosin purity and a reliable technique to determine the gender in Koi carp using a female specific protein. At the nucleic acid level, the polymorphism of RYR-1 in the swine genome was confirmed for the first time using dot-blot hybridization, while the detection of housekeeping and inserted genes in GMO detection was done using duplex PCR, instead of individual PCR reactions.

RESULTS AND DISCUSSION

The achievements of these studies are separated into the following three sections: animal breeding, crop science and food control.

Animal breeding

In the past two decades, molecular tools in animal science have been applied to study polymorphisms in hemoglobin and αs1-casein in sheep breeding, κ-casein in cattle breeding and the presence of porcine stress syndrome in swine breeding. In the field of sheep breeding, microsatellite DNA as a tool for the determination of genetic distance between domestic strains of Pramenka sheep was analyzed. Growing potential in fisheries was analyzed comparing RNA/DNA and the expression level in white muscles in Koi carp breeding. A female specific protein was investigated as a tool for gender determination. In all these mentioned cases, markers were analyzed on protein or DNA level promoting MAS in livestock production and protection of endangered breeds of domestic animals in the Republic of Macedonia.

Molecular characterization of porcine stress syndrome in the Republic of Macedonia

Porcine stress syndrome (PSS) is a serious problem in swine breeding that has negative implications on the pork production. One of the causes for PSS is a point mutation in RYR-1 gene that is

responsible for the transport of Ca^{++} in the sarcoplasmic reticulum of the muscle cells. The molecular basis of PSS was determined in the first decade of twentieth century [13] and it enabled an application of molecular methods in the detection of PSS. The first step in all those methods is amplification of the DNA region around nucleotide 1843 of the RYR-1 gene where a mutation C→T is occurred. After that, the amplified fragment is analyzed using RFLP [14] or dot-blot hybridization based on our innovation. [15]. The aim of this study was to determine the presence of PSS in the swine population in the Republic of Macedonia in the different categories and breeds, to find out the correlation between the genotypes for PSS with some biochemical parameters and to determine the influence of PSS on production and reproductive attributes in the studied animals. Specifically, 278 animals were included in this study. Genotyping of PSS was performed using PCR based techniques (Figure 1).

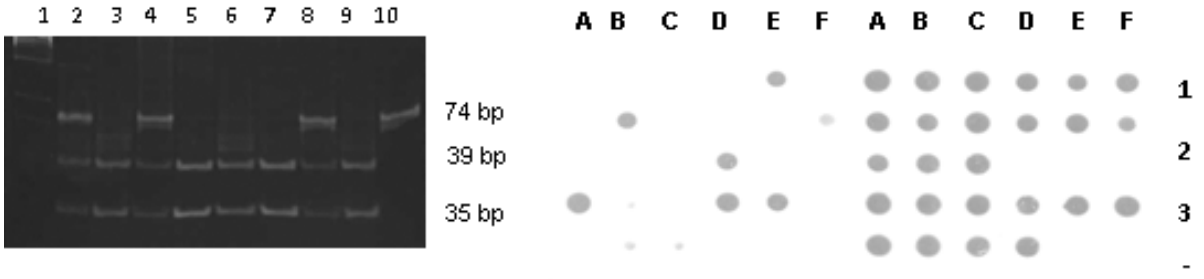


Figure 1. Genotypes of PSS
A) 13% polyacrylamide gel electrophoresis of the digestions of PCR products from a RYR-1 gene using restriction enzyme Hha I,.ln 1 DNK ladder, lns 2, 4, 8 heterozygous with a genotype Nn; lns 3,5,6,7,9 normal animals with a genotype NN; ln 10 homozygous with genotype nn. B) Dot-blot hybridization. A4, B2, D4, E1 I E4 heterozygous with a genotype Nn, E3 homozygous with genotype nn, all others are normal with a genotype NN.

PSS is the most frequent in Landrace breed with allele frequency (AF) of mutant allele of 0.211. From the biochemical perspective, the highest correlation with the genotype for PSS showed the enzymes creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) which were initially reported in our study [16]. The results related to the influence of the genotype for PSS on some productive and reproductive traits in boars showed that the stress sensitive animals with genotype nn have less food conversion, better daily gain, thinner back fat and higher percentage of lean meat, compared with stress free animals. But the level of mortality and appearance of so called pale, soft and exudative meat is higher in stress susceptible animals. Analysis of some reproductive traits showed that stress sensitive sows have smaller average of live born and successfully refused piglets compared with heterozygous animals (stress careers). It is obvious that the stress syndrome has serious implications on the quality of the pork meat mainly because of the rapid drop in pH immediately postmortem resulting in a higher percentage of pale, soft and exudative (PSE) meat in carcasses of stress susceptible animals. Thus, it will be necessary in the future to undertake measures for the systematic exclusion of stress sensitive and stress carrying animals in the further breeding strategies [17].

Determination of milk protein polymorphisms in cow milk using SDS PAGE electrophoresis

Milk proteins are divided into two fractions: milk serum and caseins. The major protein components of milk serum are α-lactalbumin (α-La) and β-lactoglobulin (β-Lg). In the casein fraction, five proteins have been intensively studied: α_{S1}-casein (α_{S1}-Cn), α_{S2}-casein (α_{S2}-Cn), β-casein (β-Cn), γ-casein (γ-Cn) and κ-casein (κ-Cn). Electrophoretic analyses of milk proteins demonstrated the existence of different variants for these proteins [18], all of them determined by co-dominant alleles of the closely linked autosomal genes. In this case our task was to develop an appropriate technique for the discrimination of different types of κ-casein in cow milk. As a starting material for such analysis we used serum and lyophilized milk. We developed a modified approach in the separation of polymorphic types of those proteins based on molecular weight and isoelectric point [19]. Using this method we found six different haplotypes of milk proteins (Figure 2).

Due to the low resolution of polymorphic fractions and impossibility of determining the polymorphism in each milk protein, we continued with the molecular characterization of separate milk genes using DNA methods.

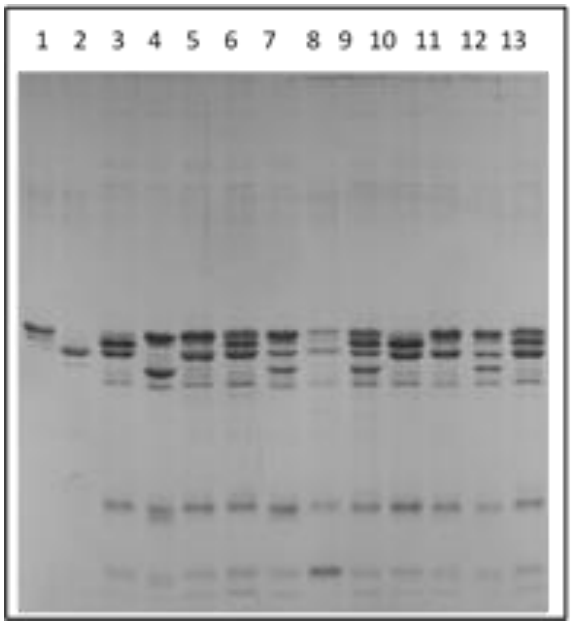


Figure 2.12.5% SDS-PAGE of analyzed milk samples. ln 1 α casein, ln 2 β casein, ln 8 mix milk standards, lns 3,4,5,6,7,9,10,11,12 and 13 milk samples from different animals

Kappa κ-casein polymorphisms in Holstein-Friesian cattle in the Republic of Macedonia

Kappa κ-casein is one of the five milk casein proteins and is the product of the gene positioned on the 6th bovine chromosome. It plays an essential role in stabilizing the casein micelles and therefore has a significant influence on milk manufacturing properties. Genetic variants of κ-casein have been extensively studied in cattle at the protein and DNA levels. Numerous alleles have been revealed [20]. Two of 14 allele variants, A and B, are particularly frequent in the cattle population, in Macedonia. According to their allele frequency, the A allele is considered to be the initial type of allele [21]. The polymorphisms that appear in the κ-casein gene are associated with the milk yield and its technological properties [22]. Identification of these polymorphisms opens possibilities of further improvements in the dairy industry.κ–casein is used as an important marker in the selection of dairy cows. A recent study was undertaken to determine the presence of κ-casein genotypes in Holstein-Friesian cows in Macedonia and to check if any correlation exists between the genotype and some of the biochemical properties of their milk. Technological properties of milk obtained from dairy cows with different genotypes were also checked to confirm any link among them. The analyses were done on DNA isolated from blood or semen of 227 black-white Holstein-Friesian cattle. Genotyping of κ-casein polymorphisms was done by PCR-RFLP using three different restriction enzymes. According to the electrophoretic analysis we found three different genotypes for κ-casein AA, AB and BB (Figure 3).

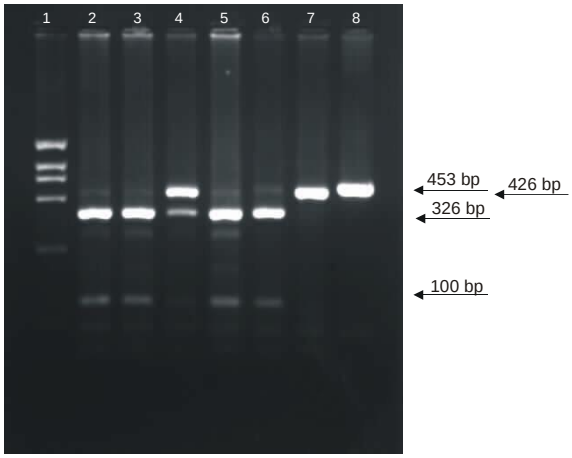


Figure 3. 1.5% agarose gel electrophoresis of digested PCR products using *Hinf* I enzyme. ln 1 DNA ladder, lns 2, 3, 5 and 6 AA genotype, ln 4 AB genotype; ln 7 BB genotype, ln 8 undigested amplification.

The frequencies were 0.747:0.253 of the alleles A and B respectively, indicating dominancy of the A allele. Statistical analyses show that dairy cows with BB and AB genotype of κ-casein have 10% higher yield of milk than those with AA genotype. It was also shown that the duration of lactation was 12% longer in cows with the BB genotype compared with AA and AB genotypes. Milk yielded by cows with the BB genotype exhibited the shortest time for the initial and final coagulation in the process of cheese making which is 30% shorter in contrast with that of AA genotype. Milk yielded by cows with AB genotype showed an intermediate effect. During the cheese making process it was shown that syneresis was 14% higher in the AB and BB genotypes in contrast to the AA genotype. Based on these data, in the future, dairy cows with the BB genotypes should be favored in the reproduction in order to gain higher yields of milk and cheese [23].

Polymorphisms in ovine αs1-casein gene among autochthon strains of Pramenka breed sheep in the Balkans

Alpha S_1 ($\alpha S_{1)}$ casein represents the main milk protein fraction which has an important role in

the transport calcium phosphate in milk. There are four alleles (A, B, C and D) present in the αS_1 casein gene. In domesticated ruminants αS_1 casein is polymorphic and the variants are related to the milk yield and quality [24]. Polymorphisms were detected within ovine α_{s1}-casein gene using PCR – RFLP [25]. One hundred seventeen DNA samples were isolated from 6 different strains of Pramenka sheep from different parts of the Balkan Peninsula: Karakachanka (Macedonia), Svrljiska (Serbia), Bardoka (Kosovo), Istarska (Croatia), Dubska (Bosnia and Herzegovina) and Pivska (Montenegro). Three distinct patterns (designated nonA/nonA, nonA/A and A/A) were observed (Figure 4).

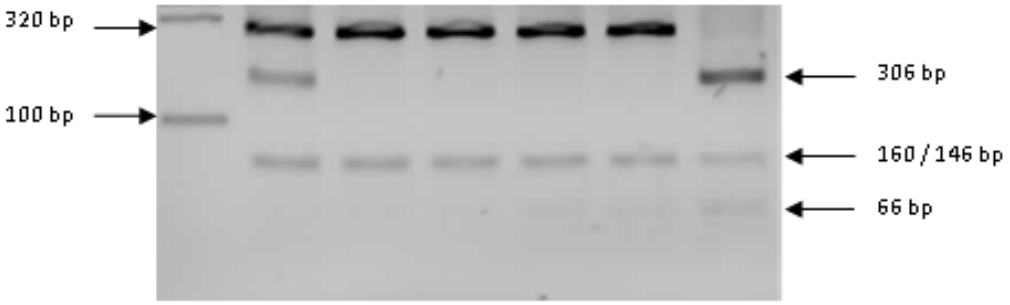


Figure 4. 3% agarose gel electrophoresis of *Mbo II* digested PCR products:
ln 1 – DNA ladder; ln2 – A/nonA, lns 3–6,– nonA/nonA; ln 7– A/A.

Allelic frequencies were 0.08/0.92 A/nonA. No polymorphisms were observed in Bardoka and Istarska strains and all animals carried the nonA/nonA genotype. The highest diversity, related to αS_1 casein gene, were in the Karakachanka strain where 7 animals had nonA/A genotype, while 2 carried genotype A/A [26].

Polymorphisms of hemoglobin in conserved nucleus of Karakachan sheep in Macedonia

Karakachan sheep is an autochthonic and endangered strain of Pramenka breed in the southern part of the Balkan Peninsula was part of the program for the conservation of autochthonic breeds and strains of domestic animals. In the Republic of Macedonia, 60 Karakachan sheep were identified and conserved in situ. In 1966, Acad. Efremov discovered a new hemoglobin type in sheep [27]. The aim of this study was to determine the polymorphism of hemoglobin among those 60 animals using the established techniques by Acad. Efremov. 11% starch gel electrophoresis in TRIS-HCl buffer pH 8.3 was used for the separation of different types of hemoglobin. Using this type of horizontal electrophoresis we found two different types of hemoglobin A and N (Figure 5).

The frequency of the allele A in this sheep population is 0.884, while the frequency of the N allele of hemoglobin is 0.116 [28]. There are confirmed associations of the different types of hemoglobin with sheep milk quantity.

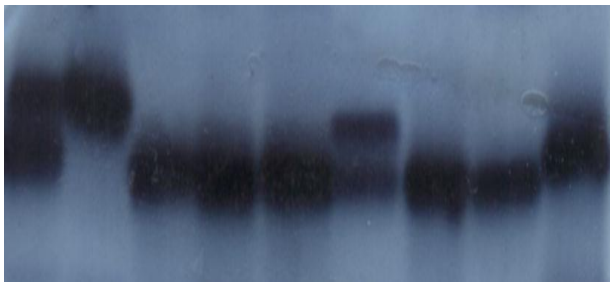
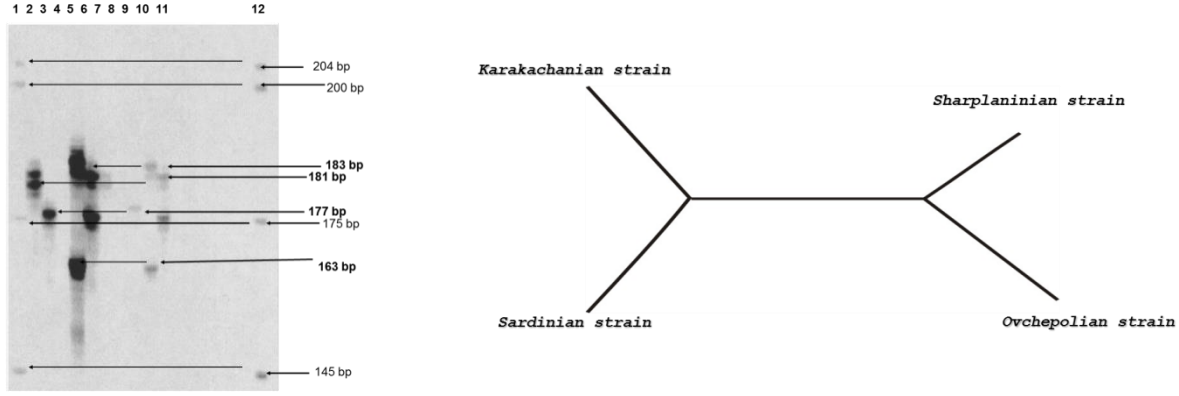


Figure 5. Starch gel electrophoresis of sheep hemoglobin:
1–genotype AX; 2–genotype XX;
3–5–genotype AA, 6–genotypy AX, 7–8 genotype AA,
9–human hemoglobin (control)

Variability of microsatellite DNA among autochthonic strains of Pramenka breed

The microsatellites known as simple sequence repeats (SSRs) are a novel class of DNA markers which are able to detect higher level of genetic variability. They are short tandem repeats (2–10 bp), middle repetitive, tandemly arranged, hypervariable DNA sequences dispersed throughout fungal, plant, animal and human genome [29]. SSRs have been used in population genetics, parentage testing, individual identification and for shortening breeding programs. Pramenka is the common name for all coarsewooled sheep breed in the Balkan mountains. There are many different phenotypical types of Pramenka, and some of them still have an important place in sheep breeding, especially in areas with rough climate conditions and poor pastures. The aims of this study were to esti-

mate the applicability and degree of polymorphism of used DNA microsatellites loci in the genome of Sharplaninian, Ovchepolian and Karakachanian strains of Pramenka sheep breed; to determine the genetic distance and genetic diversity between sheep strains of Pramenka sheep breed in the Republic of Macedonia; to determine the genetic variability of individuals from the analyzed strains of Pramenka sheep breed as well asto construct the phylogenic tree of analyzed sheep strains of Pramenka sheep breed using Unrooted Neighborhood Joint Tree method. In this study 105 adult individuals from indigenous sheep breed, Pramenka, (35 individuals per population) were analyzed. To avoid sampling of closely related animals, collection was carried out on seven different sheep herds at different locations [30]. As a comparing breed was used the Sardinian one. The determined values for genetic distance and coefficients of genetic identity (D = 0.381; I = 0.627) have shown that the closest genomes are those of Sharplaninian and Ovchepolian sheep strains. The biggest differentiation (D = 0.426; I = 0.606) is noted between genomes of Sharplaninian and Karakachanian sheep strains. The lowest similarity or highest distance (D = 0.460; I = 0.578) is present between the genomes of Karakachanian and Ovchepolian strains. Values of genetic distance and genetic identity between Macedonian sheep breed strains and Sardinian sheep breed are due to the geographical distance and different production traits. Based on the values for genetic distance and coefficient of genetic identity, the degree of divergence between analyzed indigenous sheep populations can be determined. The introduction of herd book represents necessity for further controls of productive and reproductive characteristic in indigenous sheep populations. DNA microsatellites represent just a part of the total genomic DNA but because of their very useful properties they are widely used in animal population studies. Genetic diversity between indigenous sheep populations in the Republic of Macedonia and inside the populations in this study were determined trough the analysis of 15 DNA microsatellites (4 bovine loci, 5 goat loci and 6 sheep loci)[31] (Figure 6).



A)B)

Figure 6. Genetic distance between autochthonic strains of Pramenka breed in Macedonia.
A) Fragment analysis of microsatellite DNA. **B)** Phylogenetic tree

The influence of size and living temperatures on the muscle protein profile of Koi carp (*Cyprinus carpio haematopterus*)

The fish muscle proteins can be divided into 3 groups: myofibrillar proteins – soluble in concentrated salt solutions (actin, myosin, actomyosin, tropomyosin etc.); sarcoplasmic proteins – soluble in water or soluble in diluted salt solutions (metabolic enzymes, globulin, myoglobin etc.); and connective tissue proteins – proteins insoluble in water or salt solutions like collagen [32]. Protein separation is commonly done using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) which achieves separation predominantly based on the length of their polypeptide chains [33]. The goal of this study was to show the variations in the muscle protein gene expression in Koi carp (*Cyprinus carpio haematopterus*) caused by the fish size and living temperature differences. SDS-PAGE methodology, image and statistical analyses were used [34]. Samples from 24 different fish were analyzed, separated into two groups, large and small fish, raised in three different temperatures 5, 25 and 30 ^{o}C (Figure 7).

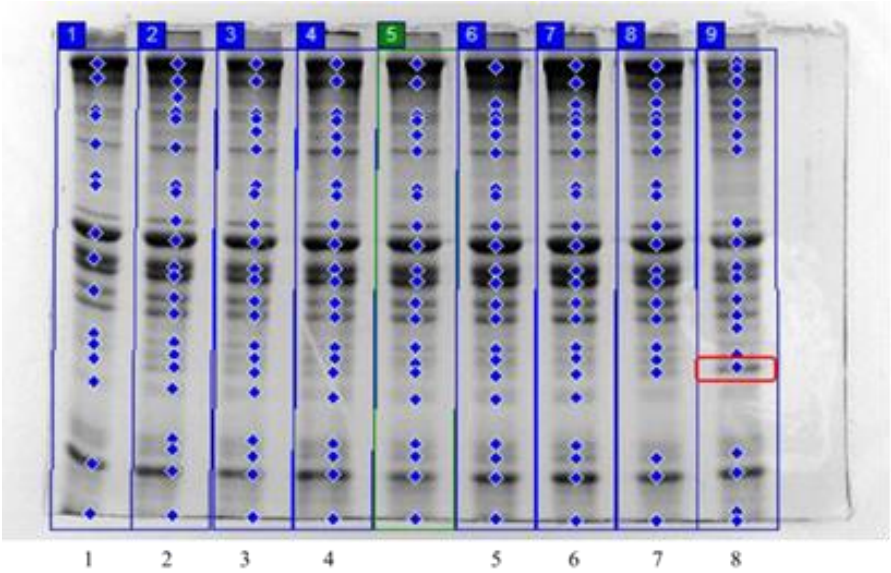


Figure 7. 10% SDS-PAGE analysis: samples 1, 2, 3 and 4 were prepared from small fish raised at 5 ^{o}C; samples 5, 6, 7 and 8 were prepared from large fish raised at 5 ^{o}C

The results showed concentration differences in proteins located at the same region in the gels, present in some of the large fish raised at temperatures of 5 ^{o}C and 25 ^{o}C, which points to the possible influence of the fish size and living temperatures on the muscle protein profile of the Koi carp. These types of variations were not recognized in the remaining analyzed samples [35].

Determination of RNA/DNA ratio in white muscle samples of Koi carp using different techniques

The amount of DNA in a cell is constant; the amount of RNA indicates how actively the cell is synthesizing proteins, and thus perhaps an indication of the metabolic status of the organism in terms of growth [36]. DNA content remains relatively constant even during periods of starvation and can serve to normalize the measured RNA. RNA is the template for the translation the genetic code into the cell's metabolic and structural machinery. The amount of RNA fluctuates in response to multiple stimuli including food availability in the natural food habitats. When properly calibrated the RNA/DNA ratio (R/D) ratio can be used to estimate instantaneous growth rates. The R/D ratio is an indicator of muscle growth capacity in fish. Some studies have advocated caution in the use of the R/D ratio because of the lack of sensitivity. In this study we estimated the R/D ratios in 24 samples of Koi carp divided in 2 groups (small and large size) and three temperature subgroups 5 ^{o}C, 25 ^{o}C and 30 ^{o}C. All fish were grown under the same feeding conditions for 6 weeks. Modified TRIzol™ method for simultaneous isolation of RNA and DNA was used according to the manufacturer's recommendation to isolate and purify RNA and DNA (Figure 8).

The results showed that the average values for the R/D ratio in large fish adapted to 25 ^{o}C was the highest (4.23), while the difference was shown in the samples from fish adapted to 5 ^{o}C and 30 ^{o}C. The results obtained using the TRIzol™ method are consistent with the literature [37] and showed that the fish adapted to 5 ^{o}C had a higher R/D ratio compared with those from the fish adapted to 30 ^{o}C ($P = 0.027$) (Figure 9).

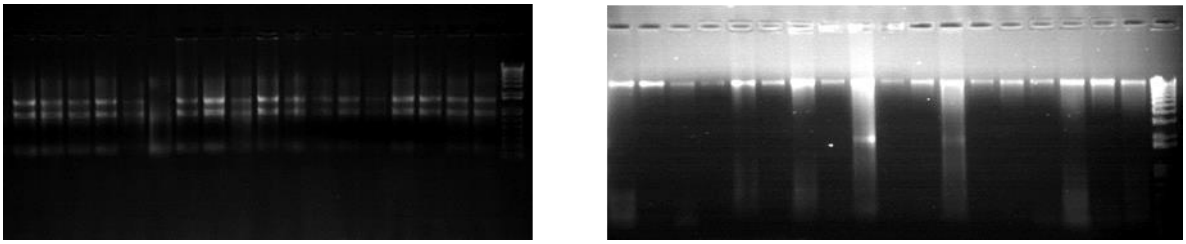


Figure 8. 0.9% agarose gel electrophoresis of RNA and DNA simultaneous isolates using the TRIzol^{TM} method

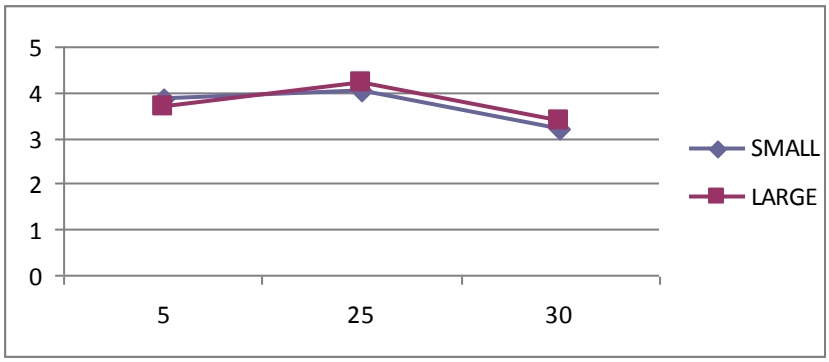


Figure 9. Average values of R/D ratios among different groups of fish grown at different temperatures determined using TRIzol™ technique

Electrophoretic determination of a female specific protein in Koi carp as a tool for gender identification

Numerous studies have demonstrated the impact of proteomics on answering key biological questions; especially those that help us understand vital functions of a living system [38]. Plasma proteins are a suitable proxy for the physiological status in the organism. Gender identification among fish species, based on morphological features, is very difficult and therefore the electrophoresis of plasma proteins could be simple and reliable tool. In this study, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), combined with image analysis, was used to analyze plasma proteins of Koi carp *Cyprinus carpio haematopterus* (Figure 10).

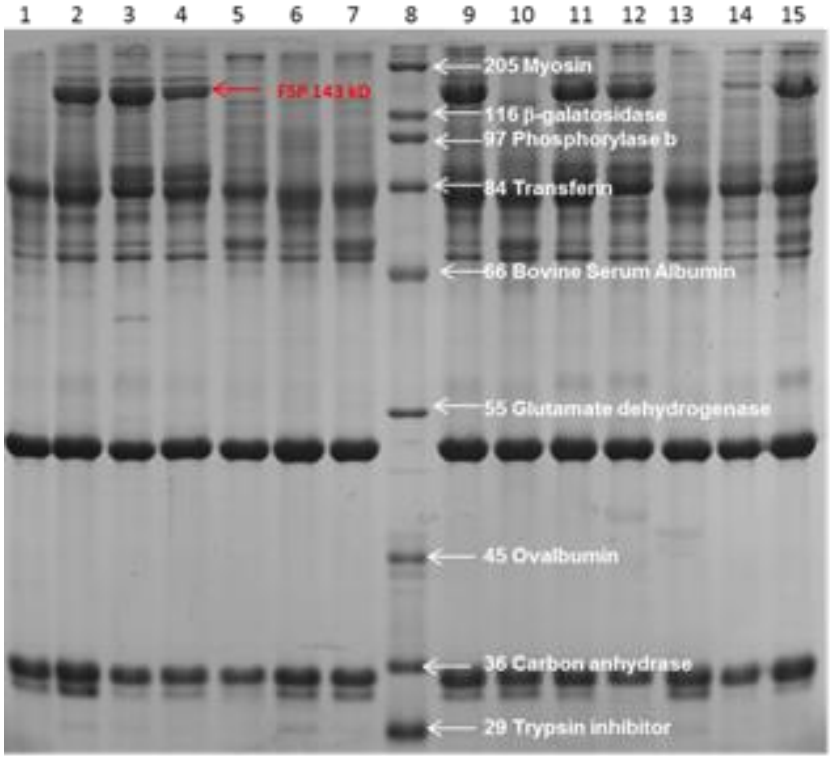


Figure 10. 12.5 % SDS-PAGE on plasma proteins from 14 samples of Koi carp.
1, 5, 6, 7, 10, 13 and 14 males / 2, 3, 4, 9, 11, 12, 15 females; 8 Broad Range Standard (myosin 205 kD, galatosidase 116 kD, phosphorilase b 97 kD, transferrin 84 kD, bovine plasma albumin 66 kD, glutamate dehydrogenase 55 kD, ovalbumin 45 kD, carbon anhydrase 36 kD and trypsin inhibitor 29 kD)

The electropherograms showed that using SDS-PAGE is a simple and reliable tool to discriminate male from female samples based on the presence of female specific protein (FSP). This protein, therefore, can be used as a suitable marker. The molecular weight of FSP is approximately 200 kDa and is consistent with pervious findings from the studies of different fish species [39].

Achievements in crop science

The targeted crops in our studies were tomato and pepper because they are the most important vegetables in production in the Republic of Macedonia. The tomato was investigated at both the protein and DNA levels in order to determine the genetic distance between six tomato varieties conserved in a National Gene Bank at the Institute of Agriculture. The pepper was analyzed from the phyto-pathological point of view to identify and characterize cucumber mosaic virus (CMV), alfa-alfa mosaic virus (AAMV) and potato virus Y (PVY) as most frequent viruses.

Differences in tomato seed protein profiles obtained by SDS-PAGE analysis

The use of proteins as markers hasbeen shown to have wide application in the identification and estimation of plant quality characteristics, disease resistance and environmental stress conditions. Seed storage protein analysis is very useful for differentiating and characterizing ecotypes. Endosperm seed protein analysis constitutes a valid and/or improved approach to cultivar identification, which is commonly based on morphological traits recorded in the field [40]. Storage proteins, as direct gene products, are currently used as genetic markers in parallel with morphological characteristics [41].The protein profiles of tomato seeds from sub-species (*subsp. cultum* Brezh., *subsp. subspontaneum* Brezh. and *subsp. spontaneum* Brezh.) were analyzed using SDS-PAGE. Electropherograms and densitograms of total, soluble and non-soluble proteins of 31 different samples showed quantitative and qualitative differences (Figure 11).

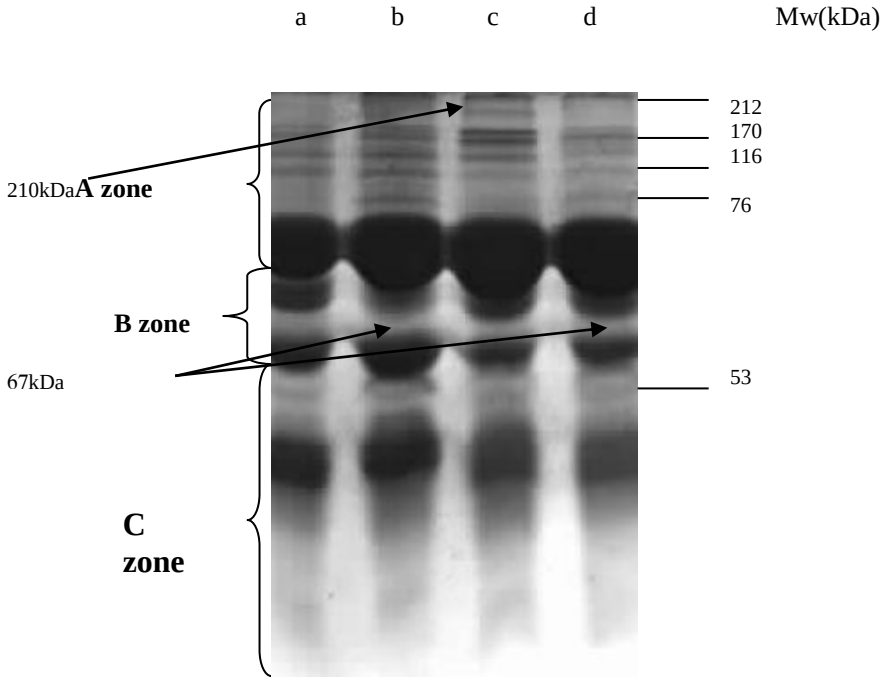


Figure 11. SDS-PAGE electropherogramsof non-soluble tomato seed proteinsa - *population Volovskosrce*; b- *line 12 mercedes*; c-*hybrid 677*; d-*hybrid 312*

Qualitative differences in electropherograms of total seed proteins refer to protein fragments in zone A (114 kDa, 83 kDa and 65 kDa) and protein fragments in zone C (17 kDa). Qualitative differences in electropherogramsof soluble seed proteins refer to protein fragment in zone A (94 kDa). Qualitative differences in electropherogramsof non-soluble seed proteins refer to protein fragments with molecular weights: 212 kDa, 17085 kDa, 116 kDa 76 and 53 kDa [42].

Study of DNA microsatellites in different tomato varieties

The microsatellites, also called simple sequence repeats (SSRs) appear as suitable molecular markers because of their highly polymorphic character. The informativeness of microsatellites, as a genetic marker has been shown to have great success in several plant species [43]. The aim of this study was research of applicability of 12 DNA microsatellites loci in genetic characterization of 6 tomato varieties of *Lycopersicon esculentum* Mill.: var. *grandifolium* from subsp. *cultum*; var. *cerasiforme* (red and yellow), var. *pruniforme* and var. *pyriforme*from subsp. *subspontaneum*; and var. *racemigerum* from subsp. *spontaneum.* The data was analyzed using the specific programs: such as POWER MARKER SOFTWARE and MEGA3. In the investigated tomato varieties, the biggest number of alleles (6) was detected at locus LEEF1Aa and locus LE20592, and only one allele was detected at locus LECHSOD by using of fragment analyses shown in Figure 12 [44, 45].

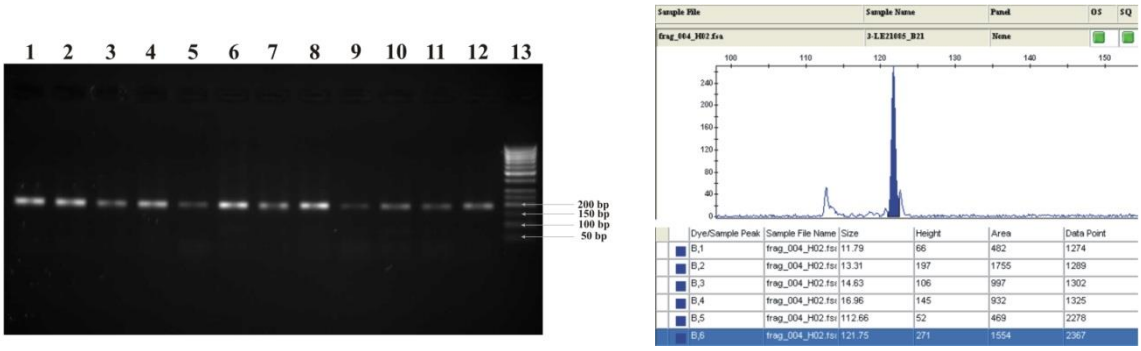


Figure 12. A) 2 % agarose gel electrophoreses of PCR products for LE21085 locus in different tomato DNA: 1 – 12 - PCR products; 13 – DNA ladder, **B)**Electropherogramsof locus LE21085

For all microsatellites loci in the investigated tomato varieties, the average number of detected alleles was (3.6250). Average PIC value for all the 8 DNA microsatellites loci was (0.3571) and they belong to the group of modest informative markers. Based on microsatellites loci research, analysis of variance (AMOVA) showed that 25% of the total variance in investigated varieties was between varieties and 74.7% of the total variance in investigated varieties was within the varieties. The results of genetic distance showed slight genetic distance (16.7415) between *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (yellow) and *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (red), and the biggest genetic distance (34.9859) was noticed between *Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme* and *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium.* The results of determination of genetic distance [46], showed that the smallest genetic distance (22.1446) was noticed between *Lycopersicon esculentum* subsp. *subspontaneum* and *Lycopersicon esculentum* subsp. *spontaneum*, and the biggest genetic distance (19.7147) was noticed between *Lycopersicon esculentum* subsp. *subspontaneum* and *Lycopersicon esculentum* subsp. *cultum* [47]. Based on the statistical data a precise dendrogram was created (Figure 13).

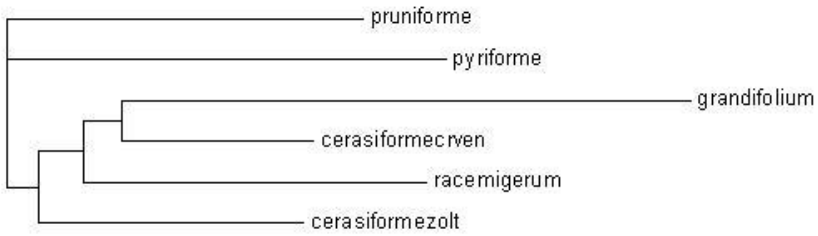


Figure 13. Dendrogram of analysed tomato varieties based on the genetic distance[47]

Molecular characterizationofCMV, AAMVandPVYon peppercultivated on open fieldsinthe Republic ofMacedonia

Pepper viruses are the main limiting factor in the pepper production on open fields in the Republic of Macedonia. By determining the nucleotide sequences of the CP-gene, the isolates have been clustered in various groups by different authors [48]. The aim of this study was to determine the presence and spread of Cucumber mosaic virus – CMV, Alfalfa mosaic virus – AAMV and Potato virus Y – PVY, the time of virus appearance, the dynamics of virus infection, the presence of mixed infections, as well as virus isolation and molecular characterization on peppers produced on open fields in the Republic of Macedonia. The data in this study are gained during the research that was conducted throughout the three vegetations in eight regions in the Republic of Macedonia on pepper plants produced on open fields. After the three year trial, it was determined that pepper plants produced on open fields are extremely susceptible to viral infections. During the analyzed years, cucumber mosaic virus was the most dominant virus, in some cases causing a 100 % infection. Molecular characterization of the cucumber mosaic virus sequences is shown in Figures 14 and 15.

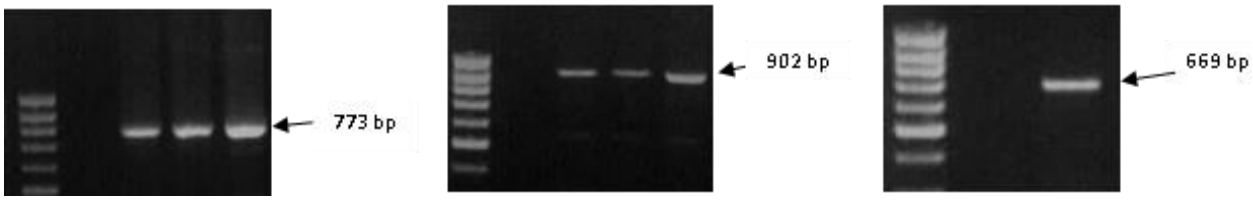


Figure 14. Agarose gel electrophoresis of amplicons from CMV, AMV, and PVY

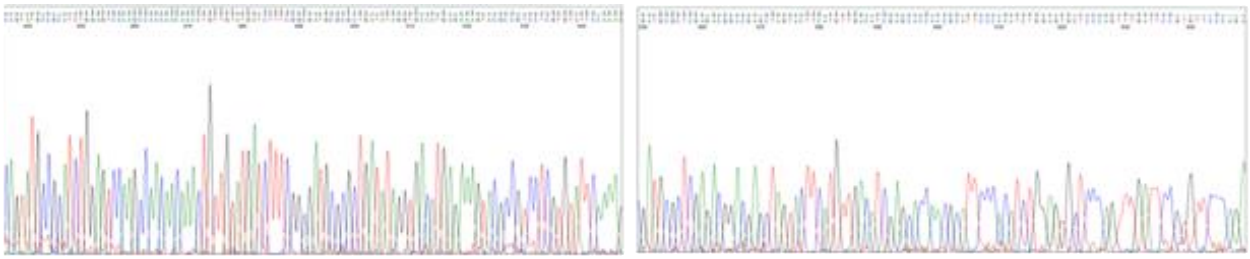


Figure 15. Nucleotide sequence of CMV2 isolate (582 bp) gained on Genetic Analyzer 3500, Applied Biosystems

Based on these data was determined that the CMV isolates from the Republic of Macedonia belong to the IA subgroup which is shown in the dendrogram (Figure16). After the analysis of potato virus Y sequences, it was established that isolates from the Republic of Macedonia are clustered in PVYpep group, together with the other European PVY pepper isolates from the Mediterranean countries. After the molecular characterization of the alfalfa mosaic virus, it was determined that the AAMV isolate from the Republic of Macedonia belongs to the II group [49].

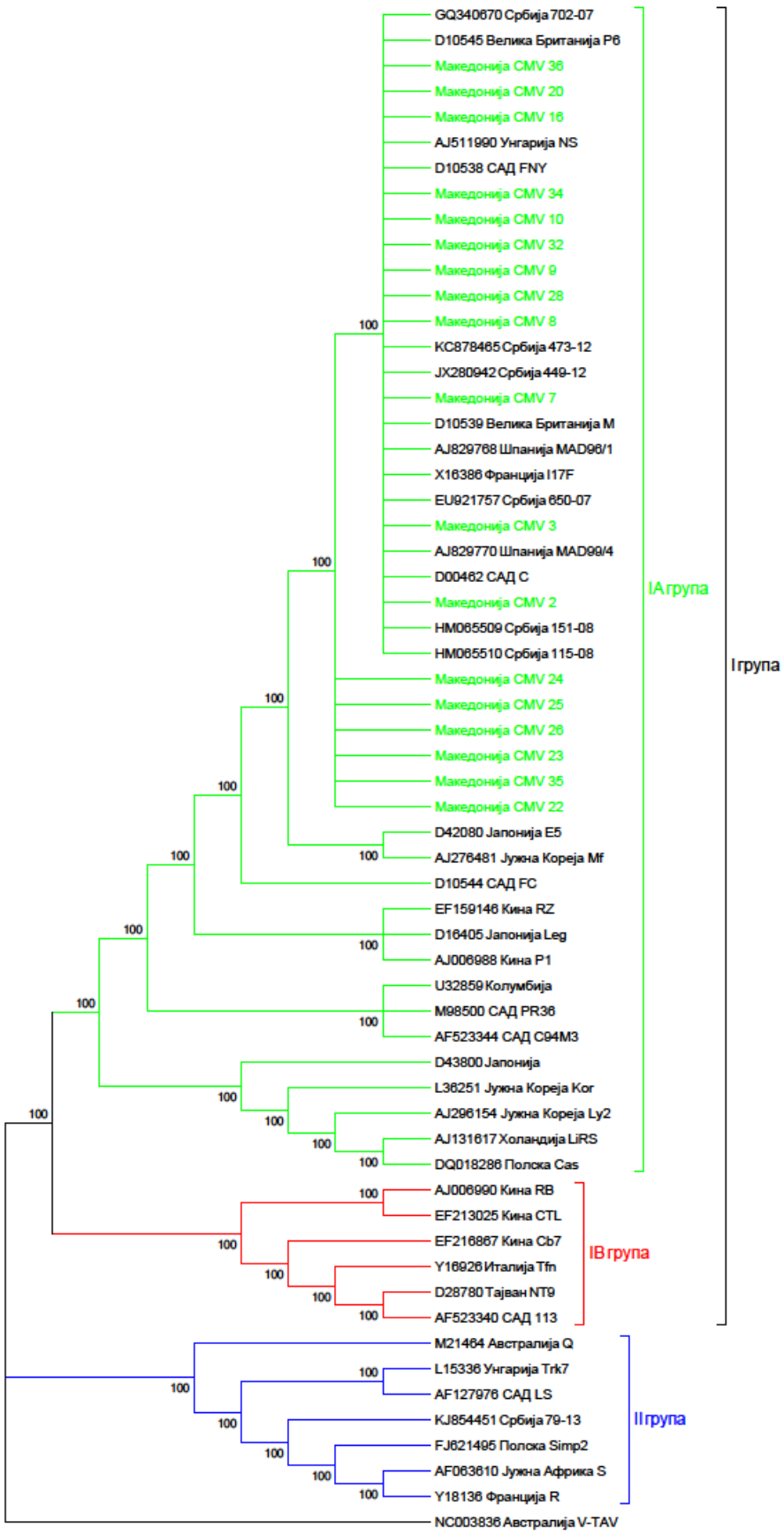


Figure 16. Dendrogram based on the differences in the sequence of core protein gene in CMV

Achievements in food control

Food control is a sector which cannot be imagined without systematic application of molecular methods. Currently, there are several commercialized kits for detection and quantification of different ingredients or contaminants in the food based on the molecular approach. Since 2006, the GMO lab has been authorized for GMO control in the country by applying available methods developed and widened by the European Network of GMO Laboratories (ENGL) and European Union Reference Laboratory for Food and Feed Analysis. Beside, the routine application of validated methods for GMO control, there were also some screening procedures innovated as well as checking the level of sensitivity and specificity of some "in-house" techniques [50]. Beside the innovations in GMO control, procedures for investigation of the origin of dairy and meat products, and purity of chymosin as a main enzyme in milk processing were developed.

Different approaches in GMO detection and semiquantification using CP4 EPSPS protein in GM corn

All testing methodologies currently available for GMOs testing are based to detect either the novel DNA or the novel protein. Both protein and DNA-based methods have been developed and applied for detection of transgenic crops, and their derivatives. Protein based techniques are referred to as immunological techniques because the detection is based on the immunological principle of conjugation between an antigen (the target) and an antibody [51].In this study are presented the findings using different approaches in GMO detection in food samples on a protein level. As a starting material we have used corn GM seed NK603 with different concentrations of GMO provided from ISTA in the frame of proficiency testing. On the protein level we use dip sticks as qualitative and ELISA as a semiquantitative method where the target molecule was CP4 EPSPS enzyme. The results showed that dip sticks could detect the presence of 0,6 % GMO (Figure 17).

Figure 17. Determination of LOD on GM corn NK603.**A**) 0.6 %, B) 0.1 % and C) 0.0 %. II. Paste prepared from 0.6 % NK603 sample and baked at: (A) 50 °C, (B) 100 °C, (C) 150 °C, (D) 200 °C and (E) 250 °C.

ELISA method could reveal GM corn with concentration of 0,1% with the restriction for exact and precise quantification.. Beside the analysis of sensitivity level in a raw material in terms of concentration, we also analyzed, at the protein level, the influence of temperature on the targeted protein for GMO detection in thermally treated corn flour (Figure 18).

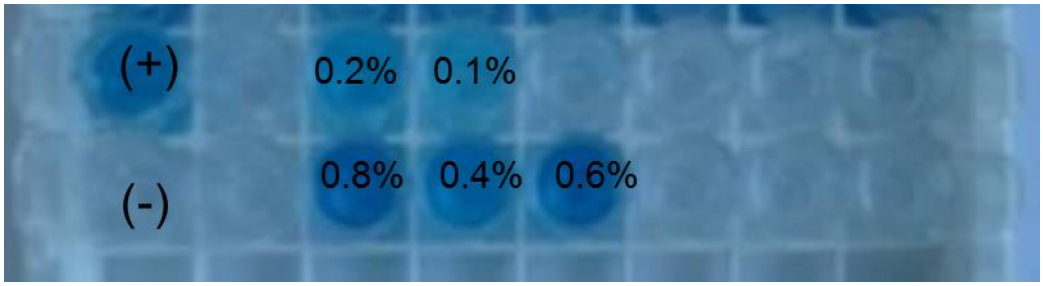


Figure 18.ELISA assay on GM corn samples NK603.with concentration of 0.1 %, 0.2 %, 0.4 %, 0.6 % and 0.8 %

In this case it was found that the last signal among the thermally treated samples using dip sticks with lowest signal was appearing at 80 ºC [52].

Detection and quantification of GMO at the DNA level

Because the nucleotide sequence of GMOs is well known, the detection is done effectively using PCR. In order to reduce the costs in this study we did the screening of raw soy and some food products that contain soy in a single step with duplex PCR [53]. This study reports the screening of raw soy and some products that contain soy in a single step using duplex PCR. In the past, the screening had to be performed in two steps, one for revealing the soy DNA, and the second for detecting the presence of the construct that is present in GM soy. Optimization of the PCR conditions was performed focusing on $MgCl_2$ concentration and primers annealing temperature. The data showed that a concentration of 3.0 mM $MgCl_2$ and temperature of 60 ºC were optimal to amplify both fragments in a single reaction. The results did not show any false positive or false negative data. They were well-matched with those from the separately performed reactions (Figure 19).

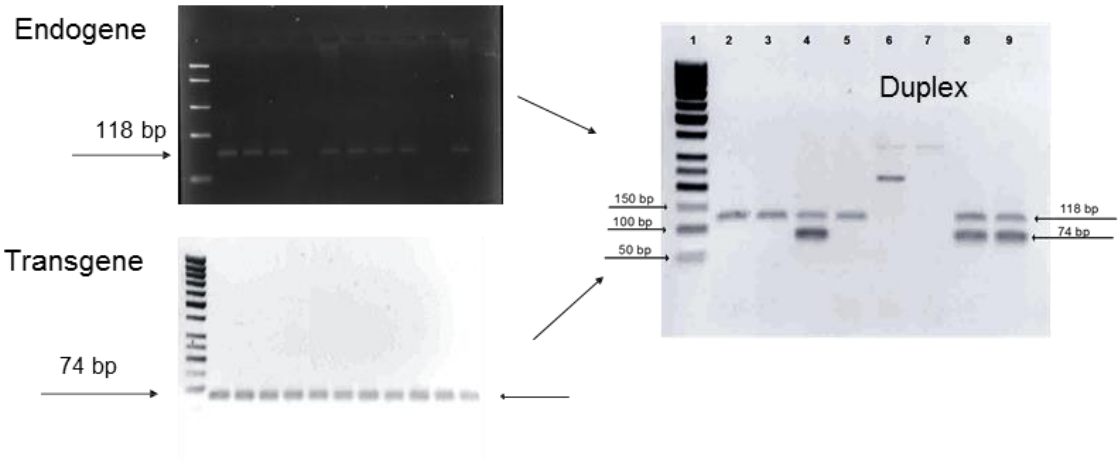


Figure 19. Electropherogram of duplex PCR: 1 - 50 bp ladder; 2, 3 soybeans, 4 positive control, 5 negative control, 6 soy milk (nonspecific amplification), 7 blank, 8, 9 chicken sausage

This kind of duplexed PCR enable faster detection of the presence of GM-soy. The method is shorter and cheaper [53]. This method eliminates so many negative samples before the quantification step with real-time PCR showed as shown in Figure 20.

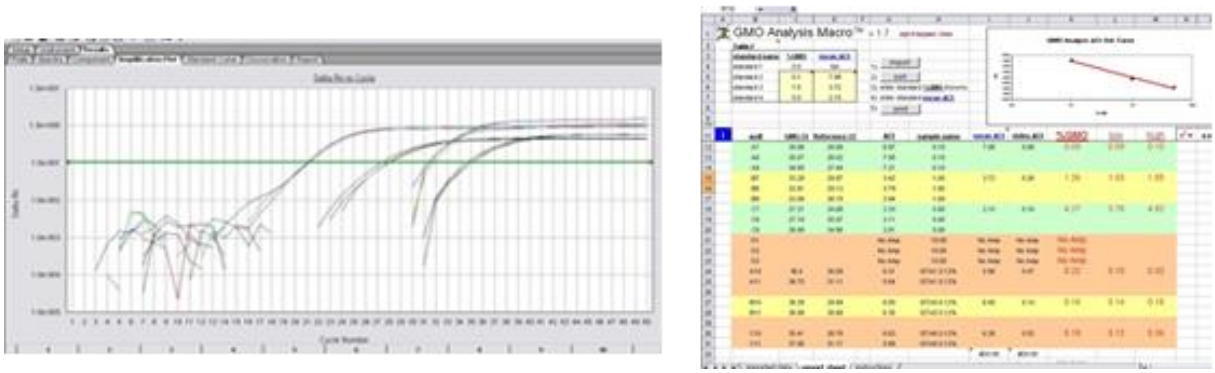


Figure 20. Real Time PCR quantification of GM corn during the proficiency testing

Rapid electrophoretic tool for origin identification of the different dairy products

The major milk proteins are caseins, lacto-albumin, and lacto-globulin. These globular proteins are significant indicators of the milk and dairy products quality. Caseins, lacto-albumins and lacto-globulins vary in molecular weight and concentration in different types of milk. These differences can be used for determination of milk origin. The aim of this study was to develop an appropriate method for discriminating proteins from different origins. Twelve samples of milk, white cheese, yellow cheese and curd from dairy cattle, sheep and goats were collected and analyzed (Figure 21).

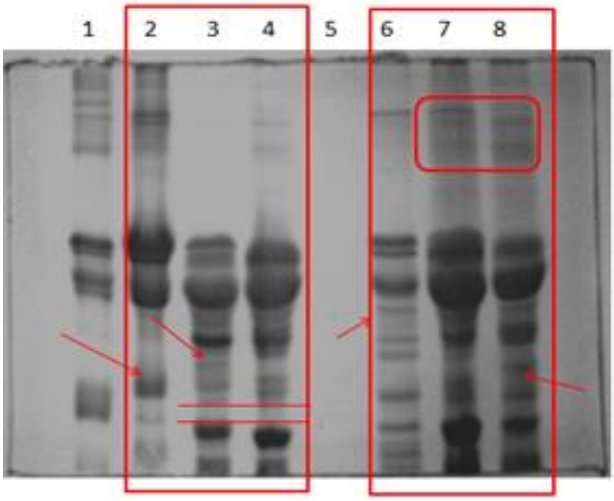


Figure 21. 15% SDS-PAGE: 1.Milk standards-mix, 2. Cow yellow cheese, 3.Goat yellow cheese, 4. Sheep yellow cheese, 5. /, 6. Cow white cheese, 7. Goat white cheese, 8. Sheep white cheese

The molecular weights of the proteins were determined using protein standards. The results showed differences, as well as the presence other fractions that can be used for identification of the origin [54].

Different approaches in identification of the meat origin based on the protein profiling

Meat origin is a fundamental factor impacting the quality and the usage of the meat products. Currently, modern kits for identification of the meat origin are available [55], however they are relatively expensive. This study developed a simple inexpensive technique that can be used in teaching purposes and in production management. We used SDS–PAGE for the identification of meat origin based on a given protein level and validated using simple PCR for amplification of part of RYR1 gene. [15]. The SDS-PAGE method previously optimized by changing the running conditions, amount of loaded sample materials and the concentration of the gel itself, has shown that different types of meat could be distinguished (Figure 22).

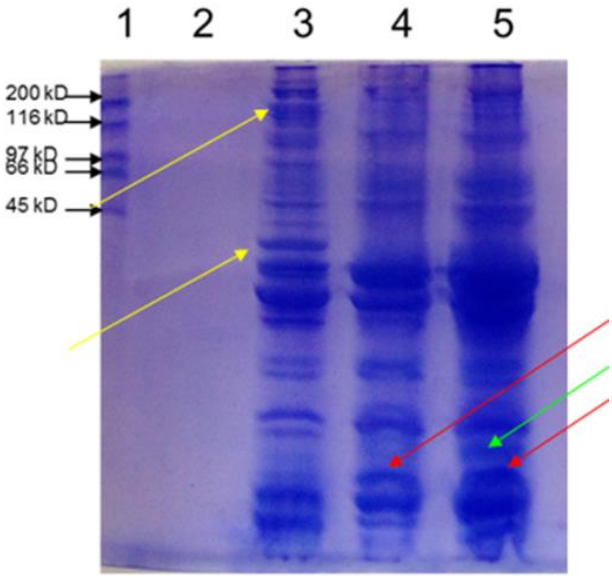


Figure 22. Differentiation of meat origin using 15% SDS PAGE of meat proteins: 1 protein standards; 2 empty; 3 chicken meat; 4 pork meat; 5 beef meat

The differences in protein profile of poultry meat were observed compared to the other samples where two specific fractions between 116 and 200 kDa are identified in the zone of myosin heavy chains and one below 45 kDa in the zone of actin. In the beef samples there is a specific fraction in the zone of tropomyosin, while in pork and beef samples a fraction appears in the zone of myosin light chains [56].

Development of a protocol for analyzing chymosin purity

Chymosin, aspartic endopeptidases (EC 3.4.23.4) is a proteolytic enzyme found in rennet, and is the key enzyme in cheese production [57]. The aim of this study was to determine the purity of different commercially available chymosins and its equivalents using electrophoretic and chromatographic techniques. Chymosins produced by the company Chr. Hansen, CHY-MAX 200 and CHY-MAX Plus, CHY-MAX PowderExtra NB as well as Maxiren 1800 Granulate from the company DSM, Sirnik from SZR – Travnik, Kraljevo and Planika from Mikroprocessing, Bileca were used in this

study. The purity level of the commercially available enzymes was analyzed using SDS-PAGE (Figure 23).

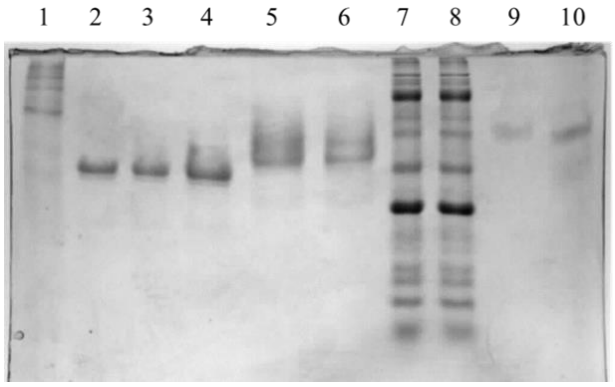


Figure 23. 12,5 % SDS-PAGE analysis: 1 – High range protein standard 2,5 µl; 2 – CHY-MAX PowderExtra NB 5 % 15 µl; 3 – Maxiren1800 Granulate 5 % 15 µl; 4 –CHY-MAX Plus 15 µl; 5-CHY-MAX M 200 15 µl ; 6 – CHY-MAX M 200 10 µl; 7 -Full range protein standard 2,5 µl; 8 –Full range protein standard 2,5 µl; 9 –Sirnik 15 µl; 10– Planika 15 µl

Results showed no presence of undeclared protein fractions in the samples except for CHY-MAX M 200 which had two protein fractions, most likely as a result of a polymorphism. All the CHY-MAX and Maxiren samples have chymosin as the active component (36 kDa), except for Planika and Sirnik which have a natural protease from *R. miehei* [58].

FUTURE PERSPECTIVES

Presented achievements in application of contemporary molecular tools in livestock, agriculture and food processing sector in the Republic of Macedonia showed that the country posses human potential and technical capacities for widening of this methods on the routine basis. It should be recognized as non-replaceable factor which can increase the productivity, the efficiency of food control and rational use of natural and climate conditions and genetic resources. Most of those outcomes are gained in the frame of international projects, so currently is important to provide national contribution from the budget of the ministries closely related to this field.

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ПРИМЕНА НА МОЛЕКУЛАРНИ АЛАТКИ ВО СТОЧАРСТВОТО, ЗЕМЈОДЕЛСТВОТО, КОНТРОЛАТА НА ХРАНА И ВО АГРОБИОДИВЕРЗИТЕТОТ ВО РЕПУБЛИКА МАКЕДОНИЈА

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Молекуларните методи претставуваат незаменливи фактори за унапредување на производството на храна, како и за нејзина контрола. Катедрата за биохемија и генетско инженесрство (КБГИ) при Факултетот за Земјоделски науки и храна има клучна улога во постојаната примена на молекуларни техники во разчни области на земјоделското и сточарското производсво. КБГИ главно се занимава со селекција со примена на маркери (СПМ), еволуциони студии, идентификација на потеклото на разни видови храна, анализа на ензими употребувани при преработка на храна, генска експресија, молекуларна детекција на растителни вируси и ГМО-анализи. Целни молекули на применуваните техники се протеините, молекулите на РНК и ДНК. СПМ системски се применува во Македонија при одгледување на животни, користејќи ги гените за: RYR-1,к и αS1-казеин. Еволуционите студии беа вршени користејќи ДНК микросателити со цел да се определи генетската сродност меѓу различни автохтони видови на овци и вариетети на домати. Идентификација на потеклото на храна и идентификација на полот на риби беше вршено преку техники за анализа на протеински профили. Генската експресија беше анализирана преку разни фактори и инхибитори за раст на нивото на РНК. Во лабораторијата за биохемија и молекуларна биологија се анализирани повеќе од 3.000 примероци од растително и анимално потекло. Лабораторијата за ГМО како дел од КБГИ е првата овластена лабораторија за анализирање на ГМО во храна и во добиточна храна. За овие анализи за повеќе од 100 примерока се користени Real Time – PCR (RT PCR) методи.

Клучни зборови: добиток; земјоделски култури; храна; молекуларни техники; Македонија

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The comments and recommendations of the referees and the Editorial Board are sent to the authors for further action. The authors are allowed 30 days to undertake revisions and return the corrected text to the Editorial Board. The final decision on acceptance or rejection is made by the Editorial Board. This decision, together with any relevant reasons, will be send to the corresponding author.

Publication process. The accepted manuscript is again checked for conformation to the Instructions to Authors and to ensure that all necessary paperwork is present. Any areas that are identified as problematic will be addressed by the Editorial Board in consultation with the corresponding author. The papers will be prepared for publication by a professional copy editor responsible for ensuring that the final printed work is consistent in form and style.

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