

ORIGINAL PAPER

THE USE OF MOLECULAR METHODS ON WILD BOARS (*Sus scrofa* L.)
PRIMJENA MOLEKULARNIH METODA NA DIVLJIM SVINJAMA (*SUS SCROFA* L.)**Šprem Nikica, Safner Roman, Tomljanović Tea, Piria Marina, Treer Tomislav, Aničić Ivica**

Faculty of Agriculture University of Zagreb, Dept. for Fisheries, Beekeeping and Special zoology, Svetošimunska 25, 10 000 Zagreb

Corresponding author: Šprem Nikica, dipl. ing., Faculty of Agriculture, Dept. for Fisheries, Beekeeping and Special zoology, Svetošimunska 25, 10 000 Zagreb, e-mail: nsprem@agr.hr, tel. 01/2393 874, fax. 2315 300

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ABSTRACT

The existence of high diversity between animal populations and species, negative human influence through overexploitation, as well as changes of natural habitats, both ensured the fact that animal entered into the focus of many molecular biological investigations. The goal of this paper has been directed on the review of the most used techniques in the differentiation of animal populations, including their advantages and weaknesses.

KEY WORDS: molecular methods; genetic diversity, wild boars**SAŽETAK**

Postojanje velike raznolikosti među životinjskim populacijama i vrstama, negativni ljudski utjecaj kroz prekomjerno iskorištavanje i promjene prirodnih staništa, činjenice su da su životinje postale središtem mnogih molekularno-bioloških istraživanja. Cilj ovog rada usmjeren je na pregled pisane dokumentacije najkorištenijih biotehnoloških metoda za razlikovanje životinjskih populacija, uključujući i njihove prednosti i nedostatke.

KLJUČNE RIJEČI: molekularne metode; genetska raznolikost, divlje svinje

DETALJAN SAŽETAK

Postojanje velike raznolikosti među životinjskim populacijama i vrstama, negativni ljudski utjecaj kroz prekomjerno iskorištavanje i promjene prirodnih staništa, činjenice su da su životinje postale središtem mnogih molekularno-bioloških istraživanja. Promjena genetske strukture divljih svinja (*Sus scrofa* L.) jedan je od najvećih problema lovstva u Hrvatskoj. Taj se problem u najvećoj mjeri javio kao posljedica Domovinskog rata, kada su domaće pasmine svinja u većem broju ušle u većinu naših lovišta, te su se križale sa divljim svinjama. Posljedica tog neželjenog križanja vidljiva je u pojavi fenotipski potpuno različitih «divljih» svinja, promjeni vremena prasenja i smanjenja trofejne vrijednosti. Za očuvanje izvorne divlje svinje nije dovoljna selekcija divljih svinja samo po fenotipu, već je za potpunu revitalizaciju izvornog genotipa divlje svinje potrebno primijeniti i molekularne metode [19]. Razvoj DNK-baziranih genskih markera uzrokovao je revolucionaran utjecaj na animalnu genetiku, od prve upotrebe alozima u 1970-ima. Sa DNK markerima moguće je teoretski promatrati i iskorištavati genetsku raznolikost u cjelokupnom genomu [15]. Primjena DNK markera omogućila je brz napredak u lovno gospodarskim istraživanjima genetske raznolikosti i inbridinga, prijenosa porijekla, određivanja vrsta i pasmina i stvaranja genskih mapa visoke točnosti za divljač [17]. Molekularne tehnike se uveliko koriste u analizi filogenetskih odnosa između različitih životinjskih grupa. Cilj ovog rada usmjeren je na pregled pisane dokumentacije najkorištenijih biotehnoških metoda za razlikovanje životinjskih populacija, uključujući i njihove prednosti i nedostatke.

INTRODUCTION

The change of genetic structure of wild boars (*Sus scrofa* L.) is one of the biggest problems in game in Croatia. The problem has occurred after the war in the 1990's when great number of pigs escaped from farms to majority of Croatian hunting-grounds, and then cross-bred with wild boars. Consequence of that unwanted cross-breeding can be seen in completely different phenotype of "wild boars", different time of littering, because sows don't litter in February and March like before, but they do that almost whole year. And sows now don't litter four or five piglets, but their number has greatly increased [10]. Except for the phenotypic changes mentioned before, the crossed populations of wild boars also have lower trophy value, so it is usually that trophy boars are mainly from mountain areas, were had not been opportunities for cross-breeding with pig. The authentic wild boar could sustain only in big forest habitats like Gorski kotar

and Lika, while the original genotype was lost in plain areas because of acorn feeding in the woods [2]. The phenotypic selection alone is not enough for preserving authentic wild boar, but for complete revitalization of the original genotype it is necessary to apply molecular methods too [20].

Biological invasions by non-native species constitute one of the leading threats to agriculture, human health and ecosystem sustainability [13]. The effects of invasive species are numerous and often irreversible [7]. Understanding the spatial structure, dispersal and population genetics of the species of concern is important if the impact of the species is to be reduced or reversed. This is particularly so if effective control programmes for feral or invasive species are to be developed, and if risk analyses to be informative and reliable [7]. New approaches, using contemporary molecular techniques in conjunction with demographic data, can be extremely useful for improving the understanding of the dynamics, population structure and social biology of many invasive species [22]. All these parameters are important to quantify because the effective management of any species requires at least some basic understanding of their dynamics [8].

The wild boars are distributed in northern Africa and throughout Euroasia, with about 25 classified subspecies [5]. In the light of the similarity in osteological characteristics, and high levels of variability (polymorphism) of microsatellites, subspecies of the European wild-boar group and the south-east Asian pig group have made a common choice of genetic markers for such analyses [20].

The development of DNA-based genetic markers has had a revolutionary impact on animal genetics, since the first widespread use of allozymes in the 1970s. With DNA markers, it is theoretically possible to observe and exploit genetic variation in the entire genome [15]. The application of DNA markers has allowed rapid progress in game management investigations of genetic variability and inbreeding, parentage assignments, species and strain identification, and the construction of high-resolution genetic linkage maps for game species [17]. Molecular techniques have been widely used to analyse phylogenetic relationships among various animal groups. The popularity of these techniques, especially DNA sequence analysis, is mainly because of the evolutionary information that can be drawn from sequence data [14]. By comparing DNA sequences, one can derive evolutionary relationships, levels of variability and geographical substructuring within and between groups of animals [12].

MOLECULAR TECHNIQUES OF DIFFERENTIATION OF ANIMAL POPULATION

Polymorphism on DNA level is created as result of spotted mutations and replication errors. Studies of these variations became possible due to development of DNA analysis, first of all with discovering restriction enzymes which cut DNA molecule in fragments and with development polymerases chain reaction (PCR) which allows amplification of DNA fragments from a minimal tissue sample.

Animal mitochondrial DNA is highly polymorphic, and without genetic recombination. The clonal transmission of mtDNA haplotypes allows the discrimination of maternal lineages within species and the analysis of sequences of their most variable regions can be used to investigate the genetic origin of animal populations and breeds [2].

Mitochondrial DNA (mtDNA) has been widely used for phylogenetic studies for several reasons. First, evolution of mammalian mtDNA occurs primarily at single base pair substitutions, with only infrequent major sequence rearrangements [24]. Secondly, the rate of mtDNA evolution appears to be as much as 10 times faster than that of nuclear DNA [3]. Thirdly, mtDNA is maternally inherited, haploid and non-recombining [1]. These features facilitate the use of mtDNA as a tool for determining relationships among individuals within species and among closely related species with recent times of divergence [3]. The D-loop region of mtDNA is known to be more variable in sequence than other regions and thus has been frequently used for phylogenetic analyses of closely related groups [4]. A few genetic studies on pigs have been carried out using mtDNA D-loop sequence variations [9] as well as restriction fragment length polymorphism (RFLP) of mtDNA [23]. Cytochrome b polymorphism were used for evolutionary analysis of the suiformes and also to determine relationship among some *Sus scrofa* populations [18]. Nuclear sequences such as the glucosephosphate isomerase pseudogene (GPIP) gene have also been used to analyse European and Asian wild boar [9]. In the area of nuclear mtDNA because of great polymorphism many micro satellites are successfully used in analysis of animal populations, and enable successful identification of individuals and relations within families [16]. The micro satellites are combined from simple repeating nucleotide flows, size from 2 to 6 base pairs and the most numerous are in eukaryotic cells but they are in prokaryotic cells too. Analysis of these markers enables identification of a tool and relation not only in the first but in next generations too, and that enables the analysis of the reproduction success and "fitness" of the particular individual [6]. Micro satellites are very useful in studies of genetic variation in genetic of population,

because it is easily revealed with PCR technique and the difference in alleles of only 1bp can be noted on high resolution gels [11]. The chain reaction of polymerases could be well optimized so it is possible to amplify several different locuses in the same process. Numerous new studies in game often include all these methods, classical and molecular too, and thus increase their accuracy [13]. Although DNA analyses can be long lasting, exhausting and expensive, amount of differences spotted in this way is much bigger then with other studies and that is why the DNA technology is progressively develop. At this moment main methodological trend in this field is development of faster and easier data gathering which firstly affects automation. Computer bases of nuclear sequences of many genes, DNA markers, primers and similar information are made and are available for scientists. Special statistical analyses and similar computer programs are made, so these conditions allow wide use of DNA polymorphism analysis of different animal populations.

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