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UNIVERSITY OF KAPOSVÁR
FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES
Department of Animal Breeding Technology and Management

Head of Doctorate School:
PROF. DR. MELINDA KOVÁCS
Corresponding Member of the Hungarian Academy of Sciences

Supervisors:
PROF. DR. PÉTER HORN
Regular Member of the Hungarian Academy of Sciences

PROF. DR. LÁSZLÓ OROSZ
Regular Member of the Hungarian Academy of Sciences

SURVEYING PATERNAL LINEAGES OF RED DEER POPULATIONS IN THE CARPATHIAN BASIN

Written by:
KRISZTIÁN FRANK

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1. BACKGROUND, OBJECTIVES

The red deer (*Cervus elaphus*) is the prime member of the megafauna in Hungary. It is one of the most widespread cervid species occurring in most of Europe and Asia, and some parts of Northern Africa. In most of its range the species is abundant, but increasing fragmentation of populations can be seen in Central and Southern Europe. And the species disappeared from some areas due to overhunting, habitat destruction and inadequate management. Populations of red deer have experienced anthropogenic influence for decades or centuries, in the form of selective hunting, extensive management, restocking and habitat modification/destruction. These have influenced and are still influencing the composition, dynamics and genetics of populations.

The red deer has an increasing economic, cultural and ecological importance; it is an important species for trophy hunting as well as for venison production; game management and hunting provide jobs and income from tourism. The systematic genetic research of the European populations can make a significant contribution to conservation biological and wildlife management knowledge. Genetic identification of deer has become very important in Hungary, as Hungarian populations of red deer represent a remarkable value. Game farming enclosures are spreading in the country, and a DNA genotyping system, that is capable not only for individual identification but also for parental analysis of breeding animals could help the deer industry. At the same time, the possibility of individual identification can be one of the most important tools against wildlife poaching.

The connection of animal husbandry and fundamental molecular research has created the basis of the current work, which aimed to develop a genetic marker set for the DNA-based identification of red deer individuals, and a protocol for practical application. The primary aim of my work was to develop sex chromosome linked microsatellite markers, which would allow

the mapping and monitoring of red deer paternal lines. These surveys would be completed with surveys of autosomal microsatellites and the analysis of mitochondrial D-loop sequences, to map paternal lines and to estimate the genetic diversity of populations. During this work, my objectives were the following:

1) Bioinformatic mapping the microsatellite markers of the red deer genome using genome sequencing data, and designing PCR primer for suitable loci;

2) Selecting, testing and optimizing sex chromosome linked markers from the designed primer pairs for a PCR-based genotyping protocol;

3) Genotyping red deer specimens using the developed markers to measure genetic diversity, as well as to map paternal lines;

4) Comparing the diversity indices of the new markers with those of autosomal microsatellites and mitochondrial control region analyses.

2. MATERIALS AND METHODS

The basis of marker development was the *de novo* assembly of genome sequences of a red deer stag originating from the Game Management Landscape Center of Kaposvár University (Bőszénfa, Somogy County). For marker testing, blood samples were collected from deer stags farmed at the same place. Furthermore, hair samples were also collected from the mothers of the stags for testing the sex chromosome linked markers. For population genetic surveys, muscle tissue was collected from wild red deer legally hunted in five regions of Hungary.

Bioinformatic marker development was done on the assembled scaffolds; the Perl script package QDD was used to detect microsatellite markers. Primers for each locus with at least 80 bp flanking sequence on both sides were designed using Primer3. The microsatellite database was searched to select X and Y chromosome linked microsatellite loci.

Eighteen sex chromosome linked STRs and designed primer pairs were selected for the genetic study. Primer pairs with detectable PCR amplicons during individual reactions were divided into two eight-plex systems, and these multiplexes were optimized to achieve efficient amplification and fidelity.

For the population comparisons, samples of 130 red deer stags were processed, from five regions of the Carpathian Basin (Bőszénfa, Lábod, Vajszló, Gemenc, Zemplén). Allele frequencies and, in the case of sex chromosome microsatellites, haplotype frequencies as well as allelic richness, heterozygosity values and diversity indices were calculated based on individual genotypes with the help of the Cervus and GenAlEx softwares. Individual genotypes were also processed with principal component analysis (PCA) and cluster analysis using the PAST and Structure software, respectively.

For the analysis of the mitochondrial control region a 1014 bp long section containing the complete sequence of the control region (D-loop) and partial sequences of the two adjacent tRNA genes was amplified. Nucleotide sequences of the H strand were aligned using ClustalW algorithm implemented in MEGA6. The number of distinct haplotypes, haplotype and nucleotide diversities and the number of polymorphic sites were computed with DnaSP. Phylogenetic relationships between detected haplotypes were inferred using a Bayesian approach with BEAST.

3. RESULTS

A total of 978 010 repeat motifs were identified at 771 401 loci. From these 617 216 were simple STR containing sequences, and 154 158 were STRs in compound formation. Almost half (45.65%) of the microsatellites were mononucleotide repeats; the ratio of tetranucleotide repeats was also relatively high (26.06%). This is worth mentioning because tetranucleotide repeat units proved to be more feasible than other types of microsatellites because of technological issues. Primer pairs were designed on 73 870 sequences. To date this is the largest STR database for red deer that could be used for developing markers for later population genetics studies.

A total of 1779 scaffolds were mapped to the X chromosome, and 78 scaffolds to the Y chromosome. On this basis the X chromosome of red deer contains 29 454 STR loci, and its Y chromosome 714 loci.

From the 18 sex chromosome linked loci selected, 15 primer pairs successfully amplified with detectable products, and 13 of these proved to be polymorphic, i.e. they showed at least two alleles on the same locus. The number of alleles per locus ranged between 1 and 6, the average number of alleles per locus was 3.3. The average gene diversity value of the markers was 0.27, the highest gene diversity was calculated for Cel_010 (0.662), and the lowest value for the two monomorphic markers Cel_002 and Cel_015.

The gene diversity of Y chromosome microsatellites, as a measure of the information content of the markers, is equivalent to exclusion probabilities that show the reliability of the markers. The sex chromosome STR markers developed, beside of the two monomorphic loci, have a moderate informativeness. Due to the inheritance of sex chromosomes, genotypes are not defined by random combination of alleles found on sex chromosome linked loci, but by the linkage of these alleles. Thus, the combined reliability

of these moderate informative markers could be high, and the markers can be applied for population genetics.

The specific combination of linked alleles is called a haplotype; it is more useful to calculate with haplotype frequencies than with the usual allele frequencies in the case of Y chromosome based population and evolutionary genetics investigations. A total of 19 different Y chromosome lines and 76 X chromosome haplotypes were identified in the red deer stags examined. Eleven of the 19 Y chromosome haplotypes were unique to one of the populations, whereas the other eight were present in more than one of them. The most frequent Y chromosome line (Y_04) included 50 specimens: this haplotype was present in all wild populations. Beyond that, another one haplotype (Y_01) was found in 43 specimens. Other haplotypes were found only with low frequencies.

The number of Y chromosome lines per population is low, indicating that the populations can be traced back to a few stags. There were five Y chromosome lines in Lábod, and four in Vajszló, including the two most dominant haplotypes, which contained altogether more than 80% of the stags in both populations. There was more variability in Gemenc and Zemplén, with ten lines in Gemenc and eleven in Zemplén. Y_01 and Y_04 were the two most frequent haplotypes in these populations too, but less dominant than in Lábod or Vajszló. In Zemplén the most frequent line was present in only 36% of the stags. The frequency of Y chromosome lines was significantly divergent in the different populations, caused mainly by unique haplotypes occuring in only one of the populations. The value of Nei's haplotype diversity was highest in Zemplén and lowest in Bőszénfa.

The phylogenetic tree of the Y chromosome lines showed two distinct branches, suggesting the presence of two clades. Lines between Y_11 and Y_19 form clade I, whereas lines between Y_01 and Y_10 form clade II. However, there was some discrepancy between the phylogenetic placement

of haplotypes and the geographic origin of stags; both branches of the tree contained animals from Transdanubia and also from the Zemplén Mountains.

The structure and genetic connection of populations can be also investigated with the help of sex chromosome linked markers. The largest number of shared Y chromosome haplotypes were found between Gemenc and Zemplén, but the low sample numbers of other populations could result in underestimating the real number of shared haplotypes. Despite the low degree of haplotype sharing the probability of identity between populations was relatively high. Y chromosomes occurring in Lábod, Vajszló and Gemenc are rather similar, whereas the Zemplén population is somewhat different.

Because of the high number of X chromosome haplotypes, the majority of haplotypes is found only in single animals, which resulted in at least one order of magnitude greater probability of identity values. Surprisingly, most shared X chromosome haplotypes were found between Lábod and Zemplén populations, and the fewest between Lábod and Vajszló. This is in contrast to the geographical distance of the populations, but low sample numbers from Lábod and Vajszló can cause a bias in this regard. It seems that maternal lines are more different between populations than their paternal lines. That could be expected because red deer stags tend to disperse more than females.

Analysis of molecular variance (AMOVA) of Y chromosome lines showed that the majority (87%) of the genetic variance can be found among individuals, and only 13% of the variance was found among populations. The paternal lines of populations are significantly different ($\Phi st = 0.131$, $p < 0.001$); based on pairwise comparisons, the paternal lines of all wild populations in this study were significantly different (each $p \leq 0.04$). Thus, the haplotypes based on these four new Y chromosome linked markers are capable of differentiating adjacent populations of red deer.

Autosomal STR markers showed a high genetic diversity in red deer: the number of alleles per locus varied between 6 and 18, with an average number of 13.8 alleles per locus. Heterozygosity values were also high; mean expected and observed heterozygosities were 0.833 and 0.759, respectively. Other diversity indices (PIC and Shannon-Weaver index) also showed high values for all loci. PIC per locus was between 0.456 and 0.904 with an average value of 0.815. Shannon-Weaver Index values per locus were between 1.004 and 2.552 with an average value of 2.131. The markers used were highly informative and showed high diversity.

Heterozygosity and allelic richness values per population were in the same range as for the whole sample set. The lowest allelic richness was found in deer stags from Bőszénfa (N_A = 5.7), which is not surprising considering the low number of samples; and this low value is not necessarily representative for the entire deer stock. The highest allelic richness was found in the Zemplén population (N_A = 10.7), but other wild populations also showed nearly this high diversity. Of other diversity indices, average PIC values per population were between 0.640 and 0.805, being lowest in stags from Bőszénfa, and highest in animals from Zemplén. Shannon-Weaver Index values per population were between 1.415 and 2.031, with a similar tendency as PIC values. Based on these values, Hungarian red deer has an outstanding genetic diversity, comparable to genetic diversity found in adjacent Central Europe.

In the case of the autosomal STRs, the probability of identity values per population were remarkable; the smaller this probability, the more is the reliability of the individual recognition. The lowest probability of identity value was found in Bőszénfa, which is not surprising given the low number and high relatedness of samples. Thus, individual identification would be less certain, but even in this population misidentification would only occur in about every ten billionth individual, which is a very good reliability. In wild

populations at least one order of magnitude greater probability of identity values were present, which indicate thet these markers are also appropriate and reliable for forensic use.

Allele frequencies of autosomal loci can differ heavily among populations. This could indicate that populations are not connected genetically, the gene flow between them is limited. Genetic structure was investigated with AMOVA. Based on autosomal STRs, the majority (90%) of the genetic variance was found within individuals, only 6% of the total variance was found among individuals and 4% among populations. Despite this, populations are significantly different ($Fst = 0.040$, $p < 0.001$). Based on pairwise comparisons, the Vajszló and Lábod populations did not differ significantly ($Fst = 0.005$, $p = 0.185$), whereas all other populations were significantly different (each $p \leq 0.002$). Thus, the haplotypes based on these four new Y chromosome linked markers are capable of differentiating adjacent populations of red deer.

Further analysis of genetic structure was done by principal component analysis (PCA). Based on the PCA populations were not separated completely, but a slight separation can be seen. The Gemenc, Lábod and Vajszló populations largely overlap, whereas the Zemplén population is slightly separated. Based on the size of PCA polygons, genetic diversity was highest in Gemenc and lowest in Bőszénfa. This is similar to the picture shown by diversity indices, and indicates genetically diverse populations with some gene flow between them; in other words, there are genetically not independent.

The software Structure assigned the highest average likelihood scores to the cases of two and three genetic units ($K = 2$ and $K = 3$), whereas the second order rate of change in log Pr indicates the presence of five clusters ($K = 5$). The clustering of individual genotypes did not show any clear groups, only mixed genotypes. Based on autosomal STRs the Zemplén population differs

from the Transdanubian populations, but in cases for more than three clusters, this also is a mixed stock.

Examination of the mitochondrial control region was performed in 87 animals originating from two populations (Gemenc and Zemplén). The complete assembly of the red deer mitochondrial control region was 916 bp long. Within the Hungarian sequences, 68 positions were variable and 62 were parsimony-informative, resulting in 39 different haplotypes. Both the haplotype and nucleotide diversities of Hungarian samples were high; in the combined Hungarian sample set $D = 0.929$, and $\pi = 0.015$. Mitochondrial analyses confirmed, similarly to the Y chromosome and autosomal STR surveys, that the genetic diversity of Hungarian red deer populations is high as compared with other European populations.

The phylogenetic tree of the mitochondrial haplotypes detected showed two distinct clades. Only two haplotypes, Hap1 and Hap3, were shared between the Gemenc and Zemplén populations; other haplotypes were present only in one of them. There was some discrepancy between the geographic origin of haplotypes and their phylogenetic placement, because both branches of the phylogenetic tree contained haplotypes originating from both the Gemenc and the Zemplén population. This also indicates that these two populations, despite the geographic distance between them, are or previously were in contact. The extended data set of European red deer haplotypes confirmed the presence of both the Western European haplogroup A and the Eastern European haplogroup C in the Carpathian Basin, but no haplotypes belonging to the Mediterranean haplogroup B were found. Haplogroup C was dominant in the Gemenc population and haplogroup A in the Zemplén population, but haplotypes belonging to both of the haplogroups were found in each region. The haplotypes Hap1 and Hap3 from the western lineage were shared between the Gemenc and Zemplén populations, moreover 15 individuals (41.7%) belonging to the Balkan lineage were detected in Zemplén and 4

stags (7.8%)belonging to the Iberian lineage were detected in Gemenc. These haplotypes had discrepancies between their phylogenetic placement and geographic origin. In total 47 of the 51 stags from Gemenc (92.9%) belonged to the eastern haplogroup C, and 21 of the 36 stags from Zemplén (58.3%) belonged to the western haplogroup A. These results are in good agreement with the concept of postglacial expansion of the range of the eastern red deer lineages from the Balkans northwards into Eastern Europe, and, concurrently, the recolonization of Central Europe by red deer lineages originating from the Iberian refuge.

The phylogenetic tree of Y chromosomal haplotypes also showed two distinct clades. In total 15 of the 19 Hungarian Y chromosome lines were detected in the Gemenc and Zemplén red deer populations. Similarly to the mitochondrial haplogroups, there are two distinct lineages of Y chromosome lines present in the red deer of Gemenc and Zemplén; but there was some discrepancy between the geographic origin of haplotypes and their phylogenetic placement. Unlike mitochondrial haplotypes, more of the Y chromosome lines, 6 of them, were shared between the two regions, 4 of them were present only in Gemenc, whereas 5 of them were present only in Zemplén.

4. CONCLUSIONS

Beyond general biological, population genetic and evolutionary aspects, the topic of the ddissertation also has wildlife biological and management implications, since the practical application of the results can improve our understanding of the life history of a major big game, and can thus help the long-term maintenance and management of wild and farmed stocks.

Previous microsatellite markers for red deer have been developed mostly by adopting markers designed for other species, and included lengthy laboratory work. By comparison bioinformatic marker screening resulted in mapping more than 978 000 microsatellite repeats and almost 74 000 loci convenient for primer design in the red deer genome. Based on the reference sex chromosomes, the red deer X chromosome contained 29 454 STRs, and the Y chromosome contained 714 STRs. The bioinformatic protocol made the development of new STR markers remarkably easy, and resulted in numerous potential markers. Because of the cross-amplification seen in related species, the STR and primer database can be used for searching new markers in cervid species, and can thereby have practical importance in the examination of the origin and relationship of populations. It should be noted that the described bioinformatics pipeline can be easily used in other species for screening genome sequences to develop new microsatellite markers.

From the sex chromosome linked markers, 18 primer pairs were selected for the genetic study. Three of these did not amplify any detectable products, thus 83% of the markers developed are suitable for genetic studies. The bioinformatics pipeline is feasible for the development of chromosome-specific STR markers, as demonstrated for the sex chromosomes. Using this approach a large number of STR markers can be developed for QTL mapping, map-based gene cloning, and the integration of the genetic and physical maps, and even for marker-assisted selection.

Thirteen of the selected markers showed polymorphisms, at least two alleles of different sizes, in the examined deer samples. Since single alleles on sex chromosome loci are linked and do not define DNA profiles in random combination, these moderately informative markers can be applied for population genetics.

Polymorphisms of the developed sex chromosome markers provide an opportunity to survey ancestry lineages and to examine genetic structure and relationship of populations. Based on detected X and Y chromosome haplotypes the populations of the examined regions were significantly different, but some overlap exist. This overlap could be caused by the dispersal of red deer stags, but the influence of human-engineered translocations cannot be ruled out. However, the impact of translocations on the Y chromosomal lines has not been studied. The mapping of lines may be important for wildlife management and forensic genetics purposes, because illegal translocations would become easily detectable.

Autosomal STR markers showed a high genetic diversity, with an average number of 13.8 alleles per locus. This high allelic richness is comparable to that described in adjacent Central European red deer. Heterozygosity values were also high; mean expected and observed heterozygosities were 0.833 and 0.759, respectively. These values were similar to that of previously described Hungarian deer populations, and did not differ from the heterozygosities of other examined red deer populations. The markers used were highly informative and showed high diversity, therefore they are appropriate for population genetics applications. This is not surprising, given that the markers were developed for individual identification purposes, and can help kinship investigations. The use of these markers is recommended for individual identification in forensic genetics and for parentage analysis in animal husbandry and wildlife management.

For the study of genetic diversity and structure a more extensive sampling is recommended in the Carpathian Basin, associated with genotyping and evaluation of results. The diversity values of autosomal and sex chromosomal markers were analogous in the examined populations. The lowest diversity was found in farmed animals from Bőszénfa, but this may be caused by insufficient sample numbers. The diversities of wild populations were similar to each other, with the Zemplén population having the highest values.

The sequence of the complete mitochondrial control region has been defined for the first time in red deer from the Carpathian Basin. The complete assembly of the D-loop was 916 bp long. Within the Hungarian sequences, 68 positions were variable and 62 were parsimony-informative, thus resulting in 39 different haplotypes. Both the haplotype and nucleotide diversities of Hungarian samples were high. Mitochondrial analyses confirmed, similarly to the Y chromosome and autosomal STR surveys, that the genetic diversity of Hungarian red deer populations is high compared with other European populations.

The phylogenetic tree of the mitochondrial haplotypes showed two distinct clades. The extended data set of European red deer haplotypes confirmed the presence of both the Western European haplogroup A and the Eastern European haplogroup C in the Carpathian Basin, but no haplotypes belonging to the Mediterranean B haplogroup were found. Haplogroup C was dominant in the Gemenc population and haplogroup A in the Zemplén population, but haplotypes belonging to both of the haplogroups were found in each region. These results are in good agreement with the concept of postglacial expansion of the range of the eastern red deer lineages from the Balkans northwards into Eastern Europe, and, concurrently, the recolonization of Central Europe by red deer lineages originating from the Iberian refuge. Human-engineered translocations could also – at least partially – justify the observed pattern, if red deer cows belonging to one of the lineages were

introduced to the Carpathian Basin by humans. As an important game species, red deer have been translocated for centuries, and such activities are still being carried out, often illegally. Translocations, however, had little impact on the large-scale phylogenetic pattern, at least as far as the mitochondrial genome, i.e. the maternal line is concerned. Thus, natural dispersal seems a more reasonable explanation for the described pattern.

The genetic structure of red deer populations and the connection between them were identified using autosomal and Y chromosome STR markers and mtDNA sequences. Hungarian populations have a weak genetic structure according to AMOVA, PCA and Structure analyses, which made the discrimination of the populations difficult. Thus, the populations are genetically connected, there is a gene flow present between the a priori defined groups. It is noteworthy that genetic differentiation is not correlated with the geographic distance of populations for all marker types. But low sample numbers can impose a bias on these results.

The presence of a genetic pattern is an important wildlife management issue, because the genetic diversity and health of stocks have been shown to be associated. Inbreeding enhances the occurence of health problems, and deteriorates the viability and other quality features. Gene flow, maintained by the dispersal of animals, enhances genetic diversity. It is well-known that the environmental influence of humans has a heavy impact on the connectivity of populations of many animal species, thus can hamper gene flow. The genetic survey and monitoring of genetic connections of populations would help to improve genetic diversity and avoid inbreeding. These results may be directly applied in wildlife management, therefore the genetic survey and monitoring of populations would be desirable, and assuring passages for animals between populations is highly recommended.

5. NEW SCIENTIFIC RESULTS

1. The work is based on a red deer genom project, in which the first red deer reference genome (CerEla1.0) is produced. This assemply is available at the NCBI databank under the accession number MKHE00000000.1. I used a bioinformatics pipeline to develop microsatellite markers from the genome sequencing data of red deer, whereby a database of several hundreds of thousands of repeats and tens of thousands of microsatellite primers were created.

2. From the primers designed, I selected, tested and optimized sex chromosome linked markers for the individual genotyping of red deer. So 15 markers were described, 13 of which showed polymorphism in red deer. Two multiplex systems, each containing eight primer pairs, were developed from the markers. Primer sequences, fluorescent labels and the size range of PCR products are evaluated.

3. I genotyped red deer from the Carpathian Basin with the markers developed, in this way the Y chromosomal diversity of Hungarian red deer was described, and the distribution of paternal lines was mapped for the first time. The genetic structuring and genetic connection of populations was showed.

4. I compared diversity values of Y chromosome markers with diversity values of autosomal microsatellite markers, and found that red deer in the Carpathian Basin have an outstanding genetic diversity in Europe.

Furthermore a weak genetic structure with some gene flow was found.

5. The sequence of the complete mitochondrial control region was defined for the first time in red deer from the Carpathian Basin. I used these sequences to elucidate the phylogenetic pattern of red deer, and to the presumption that the Carpathian Basin was recolonized after the last ice age by red deer migrating using two paths. Migration probably took place in a natural manner, and human translocations had little impact on the large-scale phylogenetic pattern. The phylogenetic pattern of Y chromosome lines strengthens the presence of two recolonization paths, but in this case there are no European data to compare.

6. RECOMMENDATIONS

The bioinformatics pipeline used for microsatellite marker development from genome sequencing data of red deer made the generation of new STR markers remarkably easy, and resulted in a large quantity of putative markers. Because of the cross-amplification seen in related species, the STR and primer database can be used for searching new markers in many cervid species, and may therefore have practical importance in the examination of the origin and relationship of populations. It should be noted that the bioinformatics pipeline described here can be easily used in other species for screening genome sequences to develop new microsatellite markers. Using the bioinformatic approach, a large number of STR markers can be developed at the chromosome level, as demonstrated for the sex chromosomes. These chromosome-specific markers can facilitate QTL mapping, map-based gene cloning, and the integration of the genetic and physical maps for red deer chromosomes of interest.

The sex chromosome STR markers developed, beside the two monomorphic loci, have a moderate informativeness. Due to the inheritance of sex chromosomes, genotypes are not defined by random combination of alleles found on sex chromosome linked loci, but by the linkage of these alleles. Thus, the combined reliability of these moderately informative markers could be high, and the markers can be applied for population genetics. Polymorphisms of the developed sex chromosome markers provides an opportunity to survey ancestry lineages and to examine the genetic structure and relationship of populations.

Based on detected X and Y chromosome haplotypes the populations of the examined regions were significantly different, but some overlap exists. This overlap could be caused by the dispersal of red deer stags, but the influence of human-engineered translocations cannot be ruled out. However, the impact

of translocations on the Y chromosomal lines has not been studied, the mapping of lines may be important for wildlife management and forensic genetics purposes, because illegal translocations could become easily detectable. Therefore, genotyping of Hungarian and, in a long run, European red deer is desirable with the use of the developed Y chromosome linked loci. The genetic structure of red deer populations and the connection between them were identified using autosomal and Y chromosome STR markers and mtDNA sequences. Hungarian populations have a weak genetic structure, which made the discrimination of the populations difficult. The populations are genetically connected. Because of this, a more detailed genetic survey of Transdanubian populations would be recommended, after a stronger sampling in the region. In addition, the maintenance of genetic diversity would be important and required, for conservation biological and management purposes.

Genetic survey and monitoring of genetic connections of populations would help to improve genetic diversity and avoid inbreeding. These results may be directly applied in wildlife management, therefore the genetic survey and monitoring of populations would be required, and assuring passages for animals between populations is recommended.

7. PUBLICATIONS ABOUT THE SUBJECT OF THE DISSERTATION

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Frank K., Barta E., Bana Á.N., Nagy J., Horn P., Orosz L., Stéger V. (2016) Complete mitochondrial genome sequence of a Hungarian red deer (*Cervus elaphus hippelaphus*) from high-throughput sequencing data and its phylogenetic position within the family Cervidae. Acta Biologica Hungarica 67(2): 133-147. doi: 10.1556/018.67.2016.2.2

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