Testing of microsatellite markers for individual identification of fallow deer

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Abstract

The fallow deer (*Dama dama*) of Hungary has excellent value to our country due to its game meat and antler trophies. As an attempt to aid law enforcement against illegal activities, such as poaching, illegal trading, and in other cases like traffic accidents, we aimed to develop a genetic marker set suitable for individual identification. During our research, 28 microsatellite markers on 15 fallow deer samples from two different populations were tested. Four microsatellites were found to be polymorphic, each with two or three alleles. Based on our current results, Hungarian fallow deer populations show low genetic diversity. This is in agreement with previous studies conducted on the species and is probably a direct result of the species' past extinction from the most of Europe during the Pleistocene and later its human-mediated reintroduction to most of its current range. The low number of polymorphic markers presents the need to include additional markers.

Keywords: fallow deer, microsatellite, individual identification

Introduction

Fallow deer (*Dama dama*) has an estimated population of approximately 40 000 individuals in Hungary. The game meat and antler trophy make the animal valuable for the hunters and the country. The hunting of the species is regulated by strict laws regarding hunting season, hunting permits and weapons usage. If any of those regulations are not met, then it is considered illegal hunting which depending on the

severity can come with a penalty ranging from the confiscation of equipment and a fine to prison sentence.

Unfortunately, illegal hunting poses an increasing problem to wildlife in Hungary (ELEK 2019), together with traffic accidents threatening the fallow deer population. Because of these reasons, the development of genetic identification methods is in demand to aid law enforcement in solving the cases that arise. If there was a method suitable for this purpose it would serve as a dissuasive to potential poachers as well. The development of methods like this is the goal of forensic animal genetics which has a more than 20 years long history in Hungary (PÁDÁR et al. 2019, PÁDÁR et al. 2020) but there is an increasing need to further expand the list of species which marker sets are available for (ZENKE et al. 2015, ZENKE et al. 2017, PÁDÁR et al. 2022, PÁDÁR et al. 2022). For several wild and domesticated animals, like deer species, big cats, wild boars, pigs, bears, and canines, microsatellite marker sets constructed for individual identification are already available (SIM et al. 2021, SINGH et al 2004, LORENZINI et al. 2020, MEREDITH et al. 2019, MEREDITH et al. 2005, ZENKE et al. 2011). Fallow deer, however, is not among those species as a marker set has not yet been tested and validated either in Hungary or in other countries. Our aim is to develop such a method by selecting and testing tetrameric microsatellite markers from closely related species.

Material and methods

We received samples from 15 fallow deer legally shot between 2019 and 2022 during the hunting season from two populations (Pilis=7, Isaszeg=8). DNA was extracted by using FavorPrep™ Tissue Genomic DNA Extraction Mini Kit. Quality and quantity control was done by agarose gel electrophoresis and Qubit® 2.0.

Table 1. Data of the tested 28 tetranucleotide microsatellite markers: source species (name of the marker groups marked with bold), locus name, and references.

Species	Microsatellite marker	Publication
red deer (*Cervus elaphus*) **DeerPlex**	C01, C229, T26, T108, T123, T156, T172, T193, T501, T507	SZABOLCSI et al. 2014
mule deer (*Odocoileus hemionus*) **Ohe**	OheB, OheC, OheD, OheE, OheF, OheG, OheH, OheI, OheJ, OheK, OheM, OheN, OheO, OheP, OheQ, OheR, OheS, OheV	JONES et al. 2000

The tetrameric microsatellites were selected from two publications (JONES et al. 2000, SZABOLCSI et al. 2014) because without a whole genome sequence marker designing was not possible. Twenty-eight markers were chosen, ten tested on red deer (*Cervus elaphus*; DeerPlex markers) (SZABOLCSI et al. 2014) and 18 designed

to mule deer (*Odocoileus hemionus*; Ohe markers) (JONES et al. 2000) (Table 1). The selection was based on the knowledge that markers developed for a species tend to work in closely related species as well, furthermore, from cross-species testing of the DeerPlex markers we already had confirmation that most of them give products in fallow deer.

PCR protocols were available for both marker sets, thus the first tests were concluded using those parameters. If the PCR did not result in an adequate quantity and quality of product the original protocols were changed accordingly.

The separation of the alleles was done using capillary electrophoresis (ABI Prism3500 GeneticAnalyzer) after that allele sizes were visualized with the help of OSIRIS software. The probability of identity (PI), observed (Ho), and expected heterozygosity (He) were calculated based on the results.

Results and discussion

Out of the 28 markers, two failed to amplify during PCR (T26 and OheD) while one had shown too many non-target PCR products (OheS) leaving 25 markers for further testing.

Altogether four markers were proven to be polymorphic (13%), out of which C229 and T156 had two alleles each (C_1/C_2; T_1/T_2) while OheF and OheQ had three alleles respectively ($F_1/F_2/F_3$; $Q_1/Q_2/Q_3$) within the 15 samples (table 2). In the case of OheQ and T156, only one population had heterozygotes, the first was monomorphic in the Isaszeg population samples while the latter was monomorphic in the Pilis population samples. Observed heterozygosity of all 15 samples was 0.133 (OheQ and T156) and 0.267 (OheF and C229). Expected heterozygosity varied from 0.115 (OheQ) to 0.232 (C229). The calculated PI was 0.22. The interpretation of this is that out of 100 fallow deer on average 22 would have the same genetic profile.

All these statistics indicate low genetic diversity among Hungarian fallow deer. For comparison, the DeerPlex markers in Hungarian red deer were all polymorphic (sample size=303). The marker with the lowest number of alleles had seven alleles (C229) and the marker with the highest number of alleles had 27 alleles (T156) (FRANK et al. 2022). Other studies regarding fallow deer showed similar results, suggesting the potential causes to be the Pleistocene extinction, bottleneck effect, human-mediated reintroduction, and other anthropogenic effects (BAKER et al. 2017). While this reasoning seems sound and well-established, we cannot completely discard the possibility that the markers we tested are not suitable for fallow deer and thus we cannot draw conclusions regarding the Hungarian population solely based on these markers, especially with the small sample size (n=15) and only two sampled populations. However, the low genetic diversity in all other European populations indicates that the former explanation is more likely.

Table 2. Statistical data of the four polymorphic microsatellite markers: locus name, number of alleles, observed heterozygosity (Ho), allele frequencies, expected heterozygosity (He) examined fallow deer samples, sample size (n), and locations: Pilis (P), Isaszeg (I), total sample size (Σ).

Locus	Number of alleles			Ho		
	P (n=7)	I (n=8)	Σ (n=15)	P (n=7)	I (n=8)	Σ (n=15)
C229	2	2	2	0.286	0.250	0.267
T156	1	2	2	0	0.250	0.133
OheF	3	2	3	0.286	0.250	0.267
OheQ	3	1	3	0.286	0	0.133
Locus	Allele frequencies			He		
	P (n=7)	I (n=8)	Σ (n=15)	P (n=7)	I (n=8)	Σ (n=15)
C229	C_1=0.142 C_2=0.856	C_1=0.125 C_2=0.875	C_1=0.133 C_2=0.877	0.243	0.219	0.226
T156	T_1=1.000	T_1=0.125 T_2=0.875	T_1=0.067 T_2=0.933	0	0.219	0.125
OheF	F_1=0.070 F_2=0.070 F_3=0.860	F_1=0.125 F_3=0.875	F_1=0.003 F_2=0.010 F_3=0.087	0.251	0.219	0.232
OheQ	Q_1=0.070 Q_2=0.070 Q_3=0.860	Q_1=1.000	Q_1=0.030 Q_2=0.030 Q_3=0.940	0.251	0	0.115

Similarly, low genetic diversity was observed in other wild animals, such as marsh deer (*Blastocerus dichotomus*). Several genetic markers were tested in this species as well (proteins (OLIVEIRA et al. 2005), mitochondrial DNA (MÁRQUEZ et al. 2006) and dimeric microsatellites (OLIVEIRA et al. 2008)), and they all showed low genetic polymorphism just like in fallow deer. The causes in this species are mainly inbreeding and their polygamous mating system (OLIVEIRA et al. 2005).

Conclusion and recommendation

In conclusion, Hungarian fallow deer showed to have a low allele polymorphism and genetic diversity. Out of 25 markers, only four were polymorphic and even those only had two or three alleles. Both the expected and observed heterozygosity were low and based on the PI value the marker set in its current state does not have the required power of discrimination for forensic investigations.
The inclusion of further markers is necessary as well as the increase of sample size and number of sampled populations. We are planning to test all available markers with tetrameric structure from closely related species in the future.

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