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Genetic characteristics of the common dormouse (*Muscardinus avellanarius*) using microsatellites in Lithuania

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Population genetic analysis becomes more and more popular as a tool to use in animal conservation (Broquet, Petit, 2009). The common or hazel dormouse (*Muscardinus avellanarius*) is a member of the rodent family *Gliridae* (Juškaitis, 2008a). These rodents are vulnerable to natural habitat changes, and because of this monitoring of the common dormouse was carried out in many countries (Bright et al., 2006), but there is still lack of genetic data. It is well known that microsatellites are being used to investigate the genetic structure of natural populations (Balloux, Lugon-Moulin, 2002). In our study we used 5 microsatellite primers to investigate 17 common dormouse samples from different places in Lithuania and Latvia. For DNA extraction non-invasive hair samples were used. The results show that the Mav023 primer with 13 alleles is the most informative primer, while the Mav002 primer with 2 alleles is the least informative one. The expected heterozygosity (He) per locus was between 0.5 and 0.743, and the observed heterozygosity (Ho) per locus was between 0.463 and 1, except Mav005 with 0.257.

Key words: microsatellites, dormouse, non-invasive, GenAlex

INTRODUCTION

The common or hazel dormouse (*Muscardinus avellanarius*) is a member of the rodent family *Gliridae* (Juškaitis, 2008). These nocturnal animals are popular not only in Lithuania but also in the world due to their long-lasting hibernation which can last about half of the year (Juškaitis, 2008a). In Lithuania this species is common and widespread, and another three species (fat dormouse, garden dormouse and forest dormouse) are listed in the Lithuanian Red Book (Juškaitis, 2003). The common dormouse in many European countries is considered a threatened species and is included in Appendix III of the Bern Convention and Annex IV of the Habitat and Species Directive of the European Union (Juškaitis, Baltrūnaitė, 2013).

The common dormouse is dependent on the area where it lives, so human activity can change forest and damage their living conditions. Due to forest fragmentation in many countries

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active dormouse monitoring was started, which can give necessary information about their abundance, prevalence and population structure (Verbeylen, 2012). In Lithuania long-term hazel dormouse monitoring was done (Juškaitis, 2008), and it showed that forest management operations do not have negative, long-lasting effects on the abundance of *M. avellanarius*. Feeding (Juškaitis, 2013), breeding (Juškaitis, 2008b), and summer mortality (Juškaitis, 2014) were also investigated.

On purpose to make better dormice conservation ten microsatellite primers were designed (Naim, 2009). Mouton et al. (2011) performed *Muscardinus avellanarius* population studies in Belgium and noticed that monitoring with molecular analysis can be a very useful tool to understand dormouse ecological problems. The hazel dormouse population genetic study carried in the United Kingdom showed that the social structure and tendencies of dispersal are the main reasons of slight dormice population spread (Naim et al., 2012).

In this study we analyse 17 dormouse samples from various places in Latvia and Lithuania. This study aims to understand common dormouse genetic characteristics using microsatellite markers.

MATERIALS AND METHODS

Sample collection

The common dormouse was sampled at six different places in Lithuania and two places in Latvia (Figure). Dormouse hair samples were taken by plucking them with tweezers. All samples before DNA extraction were held at –20 °C.

Genetic analysis

Genomic DNA was extracted from hair using a QIAamp DNA mini kit (Qiagen Inc., Valencia, California) following the manufacturer's instructions. In this research 5 microsatellite primers – Mav002, Mav005, Mav011, Mav023 and Mav028 (Mills et al., 2013) – were used. PCR amplifications of all microsatellites were performed in a 12.5 µl volume consisting of 1 × PCR buffer (with $(NH_4)SO_4$), 3 mM $MgCl_2$, 0.8 pmol of each primer, 10 µl BSA, 0.2 mM dNTP, 1 U Taq DNA polymerase and 2.5 µl extracted template DNA. Amplification was performed using the Eppendorf Mastercycler nexus gradient with the following touch-down

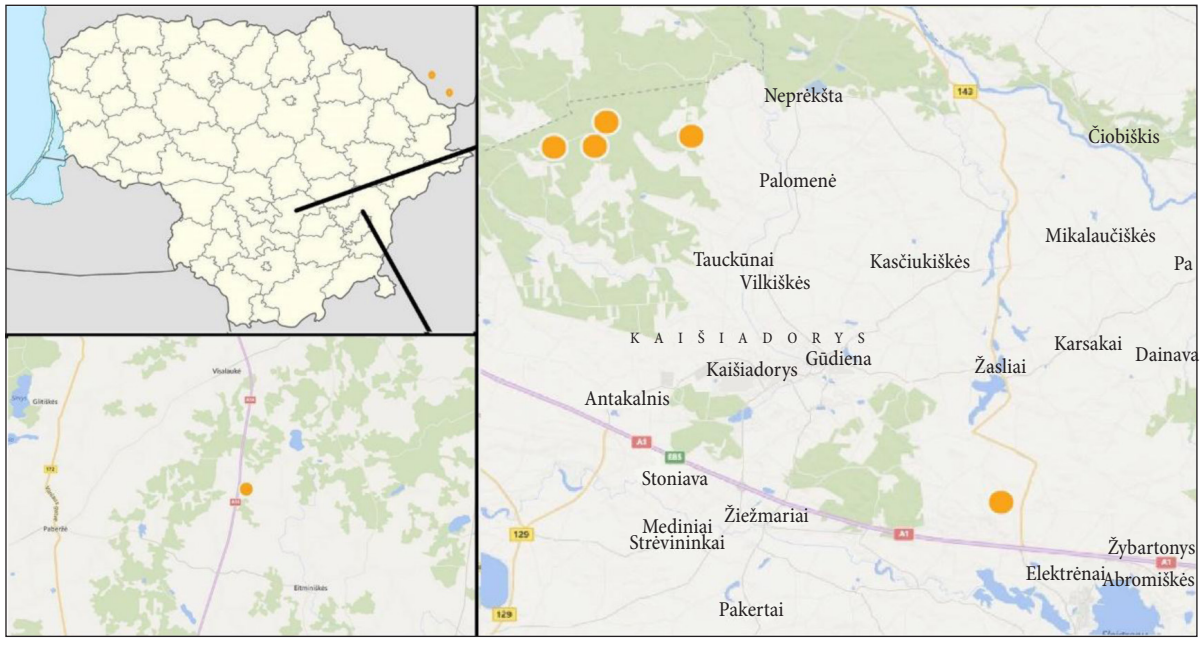

Figure. Places where samples were taken

program: 95 °C for 3 min, followed by 16 cycles of 95 °C for 30 s, primer annealing for 45 s (decreasing by 0.5 °C every cycle from 63 °C to 55°) and 72 °C for 45 s; then 22 cycles of 95 °C for 30 s, 55 °C for 45 s and 72 °C for 45 s, and the final step of 72 °C for 4 min (Mills et al., 2013). The amplified DNA products were separated according to their molecular weights using polyacrylamide gel electrophoresis. To determine the molecular weight of amplified bands the GeneRuller™ 50 bp DNA ladder and the O RangeRuller 20 bp DNA ladder were used. Gels were examined visually using a silver staining procedure (Benbouza et al., 2006).

Statistical analysis

Genetic diversity parameters, like allele frequencies, heterozygosity, F statistics and polymorphism, were calculated using GenAlex 6.1 (Peakall, Smouse, 2006).

RESULTS AND DISCUSSION

The PCR amplicons ranged between 120 bp and 350 bp in size (Table 1). Considering all loci, the number of alleles ranges from 2 to 13 in all investigated samples. The most polymorphic microsatellite was Mav023 with 13 different alleles, while the least polymorphic was Mav002 with two alleles (Table 2). The mean was 8.2 alleles per locus. The expected (He) and observed (Ho) heterozygosity was also investigated (Table 2). The observed heterozygosity per locus varied from 0.257 for Mav005 to 1 for Mav002. The expected heterozygosity per locus varied from 0.5 for Mav002 to 0.743 for Mav011.

However, the results of this study differ from those of Mills et al. (2013): PCR amplicons ranged between 103 bp and 342 bp in size, also the number of alleles ranged from 5 to 9. In the UK the common dormouse population Ho per locus varied from 0.5 for Mav002 and Mav011 to 0.77 for Mav028; He per locus varied from 0.55 for Mav011 to 0.77 for Mav023.

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Table 1. Information about 5 molecular markers analysed

Name	Primers sequences	Size, bp
Mav002	5'-CTACCATGTGCTTGGCTGAG-3'	120–124
Mav005	5'-AGGCATATGGCAGCAGAGC-3'	220–350
Mav011	5'-CCCGAGTACTGGGATTACAGG-3'	180–215
Mav023	5'-GGGAGTATAGCCCGGAGGT-3'	140–160
Mav028	5'-CCTGCTCTGGCTGTAGGC-3'	240–300

Table 2. Names of markers, numbers of alleles, observed (Ho) and expected (He) heterozygosity

Marker name	Number of alleles	Heterozygosity	
		Expected (He)	Observed (Ho)
Mav002	2	0.500	1.000
Mav005	11	0.552	0.257
Mav011	9	0.743	0.910
Mav023	13	0.627	0.547
Mav028	6	0.581	0.463
Mean	8.2	0.6006	0.6354

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LAZDYNINIŲ MIEGAPELIŲ (*Muscardinus Avellanarius*) GENETINĖS CHARAKTERISTIKOS TYRIMAI LIETUVOJE NAUDOJANT MIKROSATELITINIUS ŽYMENIS

Santrauka

Populiacijų genetinė analizė vis dažniau naudojama siekiant apsaugoti gyvūnus (Broquet, Petit, 2009). Lazdyninė miegapelė (*Muscardinus avellanarius*) yra graužikų šeimos *Gliridae* narė (Juškaitis, 2008a). Šie graužikai itin pažeidžiami dėl natūralios aplinkos pokyčių, todėl daugelyje šalių pradėtas miegapelių monitoringas (Bright et al., 2006), bet genetinės analizės duomenų vis dar stinga. Gerai žinoma, kad mikrosatelitai yra naudojami įvertinant natūralių populiacijų genetinę struktūrą (Balloux, Lugon-Moulin, 2002). Šiame tyrime pasitelkėme 5 mikrosatelitinius pradmenis ir tyrėme 17 lazdyninių miegapelių mėginių iš skirtingų Lietuvos ir Latvijos vietų. DNR tyrėme iš neinvazinių mėginių – plaukų. Gauti rezultatai rodo, kad informatyviausias yra Mav023 pradmuo, turintis 13 alelių, o mažiausia informatyvus – Mav002 su 2 aleliais. Tikėtinas heterozigotiškumas (He) lokuse svyravo tarp 0,5 ir 0,743, o stebimas heterozigotiškumas (Ho) – tarp 0,463 ir 1, išskyrus Mav005 – 0,257.

Raktažodžiai: mikrosatelitai, miegapelės, neinvaziniai mėginiai, GenAlex