New tools and methods

DEERplex: New genotyping-by-sequencing STR markers for non-invasive genetic monitoring of deer populations and application in Romanian Carpathians

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Over the last couple of decades, genetic tools have become indispensable for providing monitoring data for research and management of wildlife. However, practical applications were often limited by the considerable drawbacks of the traditionally used microsatellites (STR) analysed using capillary electrophoresis. A degree of subjectivity in allele calling, difficulties in data sharing between laboratories and slow, labour intensive, expensive analyses have particularly hampered their use in large, managed populations of large herbivores. This is particularly true for red deer (*Cervus elaphus*): despite the considerable management interest, population-level studies using noninvasive genetic sampling are still rare. Typically, thousands of samples would need to be analysed, making such studies slow and expensive with the traditional approaches. We aimed to overcome this by developing and testing a new genotyping-by-sequencing marker system. Building on successful applications of the method for large carnivores, we developed a new set of high throughput sequencing (HTS) markers for red deer genotyping.

Using a bioinformatic pipeline and existing red deer genomes, we designed 181 STR markers and five sex-ID markers. By dividing them into several multiplexes, we tested the performance of primers and their polymorphism directly in noninvasive samples. In the next step we selected 46 best performing STR markers and kept 4 sex-ID markers. By optimizing the protocol for practical application, we produced a final single multiplex of 15 STR markers and 2 sex-ID markers. This protocol can be readily scaled-up and automated in the laboratory. As allele calling is done through bioinformatic analysis and requires only limited manual checking, the approach allows cost-effective, rapid genotyping of thousands of samples. Since all data is at the level of nucleotide sequence, the genotypes are perfectly compatible between different laboratories and fully future proof. We tested the approach on 63 red deer faeces samples collected in 2023 in Romanian Carpathians and achieved an 88% genotyping success rate with reliable individual identification. Encouraged by these results we expanded sampling efforts in 2024, analysing 506 additional samples. Our method provides a robust, reliable foundation for population size, density estimates and parentage analyses in red deer, opening the door for large-scale genetic monitoring of this large herbivore. The approach that allowed us to rapidly develop and optimize an HTS genotyping system can be easily applied to other species, providing new possibilities for transborder monitoring and management of large herbivore populations.