Taxonomy and genetics

Developing a new panel of genomic loci for mammalian taxonomic identification: MIPs (Multi-locus Intron Polymorphisms)

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Rapid species identification is a fundamental tool for wildlife management and conservation plans. Many molecular methods have been proposed to solve taxonomic issues, such microsatellites (STR), or SNP (Single Nucleotide Polymorphisms) sets generated from RAD-seq and GBS (Genotyping-by-Sequencing). However, these markers tend to be species specific (STR) or cannot be efficiently applied to samples with scarce DNA quality/quantity (RAD-seq and GBS). Here we developed a new panel of Multi-locus Intron Polymorphisms ('MIPs') for mammalian genomic surveys, species and individual identification, and monitoring of interspecific hybridization. Specific subsets for rodents, lagomorphs, canids and their hybrids were designed. To generate a panel maximizing levels of cross-amplification, 121 available genomes from across Mammalia were aligned to identify the most conserved intronic regions; 192 introns randomly distributed across the genomes were then selected for the panel. Primer pairs suitable for multiplex PCR amplifications followed by Illumina amplicon sequencing were designed on conserved exonic flanking regions. The MIPs panel was tested on 71 laboratory mouse tissue samples to assess its potential to identify two parental lineages (*Mus musculus musculus* and *M. m. domesticus*) and different hybrid classes obtained from controlled laboratory crosses. The panel was then tested on invasive samples from several pairs of species known to hybridize under natural conditions: tissue samples from 250 mountain hares (*Lepus timidus*) and 70 brown hares (*L. europaeus*) bagged in the Province of Sondrio (Italy), from three hunting areas with different intensities of *L. europaeus* restocking, two altitudinal ranges (below and above 2000 m a.s.l.) and two collection periods (2001–2008 and 2016–2023) as proxies for climate change scenarios. Further tests were also conducted on other mammalian species belonging to the families Bovidae, Canidae, Felidae, and Suidae. Amplicon sequencing data were analysed with dedicated bioinformatic pipelines to assess inter- and intraspecific genetic diversity and level of hybridization of the populations under study. We discuss the usefulness of MIPs in addressing taxonomic problems and defining hybridization classes between different mammalian species.