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Lack of geographic variation in Y-chromosomal introns of red deer (*Cervus elaphus*)

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Intra-specific Y-chromosomal sequence variation is useful for analysing the male contribution to a species' spatial genetic structure. In red deer (*Cervus elaphus*) this is especially relevant, because geographic dispersal and game translocations occur mainly through the males. However, Y-chromosomal markers for wild organisms are scarce and frequently non-polymorphic within species. We assessed the intra-specific variation of two Y-chromosomal introns in red deer, one in the *DBY* (or *DDX3Y*) gene and the other in the *UBE1Y* gene. The introns were amplified using previously published exonic primers and directly sequenced in individuals of five red deer subspecies from across Eurasia. However, no nucleotide polymorphism was observed, which rebuts the usefulness of these introns for studies of red deer phylogeography and on illegal transport of red deer within this region. Male-based phylogeographic studies should thus be focused on other Y-chromosomal markers for this species.

Introduction

As the only exclusively male-transmitted part of the mammalian genome, the non-recombining portion of the Y chromosome is an invaluable tool for studying the male contribution to a species' genetic structure. However, the mammalian Y chromosome generally has low nucleotide diversity (Hellborg & Ellegren 2004), hampering male-based phylogeographic studies. Nonetheless, Hurles and Jobling (2001) and Petit *et al*. (2002) recommended using male-specific genetic markers, which can be informative for several species (e.g. Cathey *et al*. 1998, Underhill *et al*. 2000, Geraldes *et al*. 2005).

In a species such as the red deer (*Cervus elaphus*, Cetartiodactyla: Cervidae), male-based phylogeographic studies can be especially relevant, because geographic dispersal occurs mainly through males (Clutton-Brock *et al*. 1982, 2002). Furthermore, the red deer is an economically important game species which is hunted mainly for antler trophies, hence illegal transport of male animals for game purposes also occur (Carranza *et al*. 2003).

Hellborg and Ellegren (2003) designed exonic primers for a series of mammalian Y-chromosomal introns. We analysed two primer pairs that achieved amplification in the two cervid species tested by these authors, reindeer

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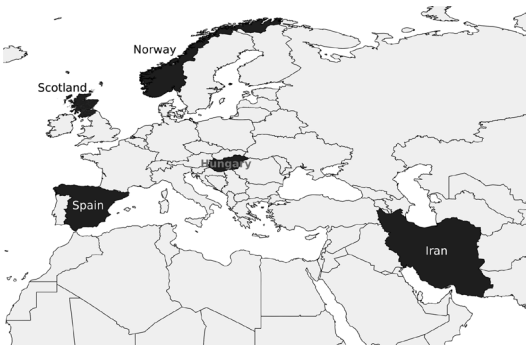

Fig. 1. Geographical origins of the analysed red deer samples.

(*Rangifer tarandus*) and roe deer (*Capreolus capreolus*). The target introns have not shown polymorphism in every species analysed so far (e.g. Hellborg & Ellegren 2004). However, red deer show considerable intraspecific diversity at other regions of their genome, such as the mitochondrial DNA control region (D-loop), cytochrome *b*, and nuclear microsatellites (e.g., Feulner *et al*. 2004, Ludt *et al*. 2004, Skog *et al*. 2009). Several red deer subspecies have been described, and there is even support for a subdivision into two species (Ludt *et al*. 2004) within Eurasia. We therefore expected Y-chromosomal introns to exhibit variability among scattered Eurasian populations of this species. The aim of this study was, thus, to gauge the variability of these introns among several red deer populations, in order to assess their usefulness for male-based phylogeograhic studies on this species.

Material and methods

Biological samples consisted of antlers or flesh of male individuals of five red deer subspecies coming from five different countries (Table 1), altogether spanning a substantial part of the Palearctic region (Fig. 1). Only one individual from each subspecies was sampled, as the aim was to check if there could be enough variation to perform a phylogeographic study based on the analysed markers.

Table 1. Subspecies of *Cervus elaphus* analysed, geographical origin of their samples, and the GenBank accession numbers of the nucleotide sequences obtained from each of the two analysed introns.

Subspecies	Origin	*UBE1Y6*	*DBY8*
C. e. hispanicus	Spain	EU219368	EU219373
C. e. atlanticus	Norway	EU219369	EU219374
C. e. scoticus	Scotland	EU219370	EU219375
C. e. hippelaphus	Hungary	EU219371	EU219376
C. e. maral	Iran	EU219372	EU219377

Total DNA was extracted using a salt-out procedure (Miller *et al*. 1988) on flesh pieces or antler shavings. The target introns were then amplified by polymerase chain reaction (PCR) using the primer pairs *DBY*8 (forward: CCCCAACAAGAGAATTGGCT, reverse: CAGCACCACCATAKACTACA) and *UBE1Y*6 (forward: CCCCTGCAGACCKRCAT, reverse: AAGGCCAAGTTGATRAARCT) designed by Hellborg and Ellegren (2003). PCR products were electrophoresed on 1.6% agarose gels stained with SYBR safe (Invitrogen, Barcelona) and visualized under ultraviolet light to confirm amplification and assess the size of the fragments.

Amplicons were purified using ExoSAP-IT (GE Healthcare) and directly sequenced in both directions using the PCR primers and a BigDye Terminator v1.1 Cycle Sequencing kit (Applied BioSystems). Sequences were recorded with an ABI3130 automated sequencer and aligned with BioEdit (Hall 1999) for comparison. A FASTA search (Pearson & Lipman 1988) was performed to confirm correspondence with the target genes.

Results and discussion

The approximate sizes of the amplified *UBE1Y6* and *DBY8* introns were 300 and 200 bp, respectively. The sequences were uploaded to GenBank and their accession numbers are provided (Table 1). Sequence alignment and comparison revealed no polymorphisms among the analysed individuals, in neither the *DBY*8 nor the *UBE1Y*6 intron.

Although the sample size was small, the analysed individuals represent five distinct nominal subspecies (Table 1) and encompass a wide geographical coverage (Fig. 1). The results thus strongly suggest reduced variation at these loci in red deer, at least within Eurasia. Variation might still exist among populations, but the value of these markers for phylogeographic studies of this

species is visibly limited. This lack of variation, however, is not generalised in the genome of the analysed red deer: the same individuals studied here show nucleotide polymorphisms at mitochondrial loci (J. L. Fernández-García unpubl. data).

Hellborg and Ellegren (2004) showed that intraspecific Y-chromosome variability differs significantly between species. The same introns we analysed have shown intra-specific variability in wolf *Canis lupus* and field vole *Microtus agrestis* (Hellborg & Ellegren 2004, Hellborg *et al*. 2005), for example. Conversely, Brändli *et al*. (2005) found significant intraspecific variation in the Y chromosome of greater white-toothed shrew *Crocidura russula*, but not in the *DBY*8 intron, which they also analysed. Our introns have also shown absence of variability within reindeer *Rangifer tarandus*, cattle *Bos taurus* (Hellborg & Ellegren 2004) and the horse *Equus caballus* (Lindgren *et al*. 2005).

Other studies have revealed low general Y-chromosomal variability, both within wild species such as the hamadryas baboon *Papio hamadryas* (Lawson Handley *et al*. 2006) and within domestic species such as the horse (Lindgren *et al*. 2005). This uniformity was attributed to biased reproduction caused by polygyny in the former case, and by sex-biased domestication in the latter, with few males contributing genetically to the subsequent generations. The fact that red deer also have a polygynous mating system (Clutton-Brock *et al*. 1982) may, therefore, help to explain the low variability observed in the analysed Y-chromosomal sequences.

Despite the high intraspecific genetic diversity observed elsewhere in the red deer genome (e.g., Ludt *et al*. 2004, Skog *et al*. 2009, J. L. Fernández-García unpubl. data) and the fact that red deer females appear to mate with genetically dissimilar males (Carranza *et al*. 2009), neither of the two analysed Y-chromosomal introns exhibited nucleotide differences in any of the five subspecies analysed. Male-based phylogeographic studies on red deer should thus turn to other Y-chromosomal markers.

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