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Y DNA and mitochondrial lineages in European and Asian populations of the brown hare (*Lepus europaeus*)

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Abstract

Both the Cyt*b* gene of mtDNA and Y chromosome markers were studied in a relatively large sample of brown hares (*L. europaeus*) from Europe and Anatolia (Turkey and Israel), together with other seven *Lepus* species, in order to enable comparative analysis of possible sex-specific gene flow. Furthermore, Y chromosome markers were compared with data from biparentally inherited markers in an attempt to understand whether or not their pattern of distribution was congruent with that of allozymes or whether they rather matched mtDNA phylogenies, with which they share uniparental inheritance. Consistent with the general observation, levels of interspecific genetic variability were very low for the Y chromosome markers compared with mtDNA. Moreover, lack of interspecific variation for the Y-DNA studied within *Lepus* genus rendered these markers improper for any further phylogenetic analysis. With the highest nucleotide diversity in Anatolia compared with Europe, both marker systems confirmed an unbroken species history in Anatolia, corroborated the hypothesis of continuous gene flow from Anatolia's neighbouring regions, and supported the idea of a quick postglacial colonization followed by expansion of the species in large parts of Europe. Phylogenetic analysis under mtDNA revealed the existence of four different haplogroups with a well defined distribution across Europe and Anatolia. Both genetic systems supported the deep separation of Anatolian and European lineages of *L. europaeus*. Nevertheless, Anatolian Y-DNA lineages extended across a longer geographic distance in south-eastern Europe than Anatolian mtDNA haplotypes, probably as a result of higher female philopatry that makes mtDNA introgression more difficult in brown hares.

Keywords: Mitochondrial DNA; SRY; DBY; Brown hare; *Lepus europaeus*

Introduction

Many biological aspects of a species, such as dispersal rate and pattern, and the mating systems, combined with environmental and landscape characteristics (e.g. geographical barriers) in the recent and remote past (e.g. glaciations) seriously affect levels and patterns of genetic

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variation. However, several other factors, particularly anthropogenic, could have additional impacts in shaping species' population genetic status, especially when game species, such as brown hare (*Lepus europaeus* Pallas, 1778) are studied. The quaternary climatic oscillations, and in particular the last glacial maximum (LGM, ca. 20.000 ybp) and the subsequent warmer period, produced remarkable consequences on the population genetic variation in several species (e.g., Taberlet et al. 1998; Hewitt 2000; Hofreiter et al. 2004). Nevertheless, concerning the brown hare, several other parameters, mostly related to human activities, such as changes in agricultural management, heavy hunting pressure, and more or less uncontrolled introductions of individuals, could have had a particular influence on the presently observed pattern of genetic variation. It is likely that European populations of brown hare have been affected by uncontrolled and rarely documented restocking operations over the last decades that could have modified the genetic status both by contributing to the recent demographic expansion and by mixing different genetic pools (Flux and Angermann 1990; Suchentrunk et al. 2006; Stamatis et al. 2007).

During the past decade, several studies, using exclusively mtDNA data have attempted to portray phylogenetic relationships among diverse populations within *Lepus europaeus* species. Under mtDNA analysis (Thulin et al. 1997; Pierpaoli et al. 1999; Mamuris et al. 2001; Kasapidis et al. 2005; Ben Slimen et al. 2007; Fickel et al. 2008; Stamatis et al. 2007, 2008, in press; Sert et al. in press), brown hare populations from Europe, Asia Minor, and other parts of the Middle East, showed a phylogeographic pattern, which likely corresponds to late-Pleistocene refugia in the central/southern Balkans and in Anatolia and reflects the post-glacial colonization history of brown hares in Europe. Furthermore, a high degree of spatial partitioning of mtDNA was observed since four haplogroups predominately or exclusively occurred in well-defined geographical areas (Kasapidis et al. 2005; Stamatis et al. 2008, in press).

On the other hand, all available population genetic data on brown hares from several regions in Europe indicate fairly high gene flow at the nuclear DNA level across large ranges (e.g. Hartl et al. 1993; Fickel et al. 1999, 2005; Suchentrunk et al. 2000, 2001, 2003; Mamuris et al. 2002; Ben Slimen et al. 2005; Sert et al. 2005). Moreover, different classifications based on morphological parameters such as fur coloration and patterns, body size, external body measurements, as well as skull and tooth characteristics (overview in De Beaufort 1991), have defined various subspecies, namely *Lepus europaeus carpathous*, *L. e. creticus*, *L. e. cyrensis*, *L. e. ghigii*, *L. e. meridiei*, *L. e. niethammeri*, *L. e. parnassius*, *L. e. rhodius*, *L. e. transsylvanicus*. However, genetic analyses do not support all these classifications, which are perhaps largely due to the large intra- and interspecific morphological variation in the genus *Lepus* (e.g. Flux and Angermann 1990), but possibly also to discordance of ecogenetic and phylogenetic causes of morphological variation.

Obviously, significant discrepancy exists between mtDNA-based evolutionary hypotheses and those derived from proteins and morphology, which can be considered indirect reflections of the nuclear genome (Suchentrunk et al. in press). Differences in transmission between the two genomes (Hoelzer 1997; Seielstad et al. 1998) combined with the fact that natal dispersal might be sex biased, i.e. males disperse whilst females tend to remain within their breeding group (Hulbert et al. 1996; Reitz and Leonard 1994), are the likely causes of their incongruent topologies. Given these contradictions and in order to obtain a more accurate picture of the species' evolutionary history, comparative investigation of other genomic regions characterized by different inheritance patterns and mutation rates is necessary.

To gain further insight into this issue, we examined sequences of the mtDNA Cytochrome *b* (Cyt*b*) gene in comparison with exonic sequence of SRY (sex determination region) and intronic sequence of DBY (DEAD box Y-linked) genes of the Y chromosome, two male-specific molecular markers, showing no recombination (Gubbay et al. 1990; Sinclair et al. 1990; Hellborg and Ellegren 2004). Since the early 1990s mtDNA has been extensively used in phylogeography due to its transmission without recombination (but see Rokas et al. 2003), high mutation rate, and the availability of universal primers for PCR amplification (Kocher et al. 1989). However, mtDNA provides information only about the female germ line and its rapid evolution makes it prone to mutational saturation (homoplasy) over long evolutionary timescales. Furthermore, it is well known that evolutionary patterns of single genes or sequences are not necessarily paralleled by organism evolution (e.g. Avise 2004). On the other hand, the mammalian Y chromosome has strict paternal inheritance and a slow mutation rate relative to mtDNA (Schaffner 2004). Although mtDNA and Y chromosome loci are both uniparentally inherited haploid systems, the sex-biased dispersal patterns of brown hare could result in significant geographic structure of mtDNA haplotypes, but a single species-wide gene pool of Y chromosome types (Melnick and Hoelzer 1992).

Therefore, studying both mtDNA and the Y chromosome should enable comparative analysis (a) to follow populations' gene flow across species' distribution in Europe and Anatolia; and (b) to understand whether or not Y chromosome markers, having a dispersal pattern linked to male-mediated nuclear gene flow, yield topologies congruent with allozymes and morphology or they match mtDNA phylogenies, with which they share uniparental inheritance.