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Hares on thin ice: Introgression of mitochondrial DNA in hares and its implications for recent phylogenetic analyses

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Mitochondrial DNA has long been used to infer phylogenetic relationships among taxa and help revising the taxonomy. When dealing with recently diverged and potentially hybridizing taxa however, one must bear in mind that mtDNA is essentially a single locus, with a peculiar mode of transmission. Confounding population history with that of mtDNA alone can be misleading because of erroneous amplification of mitochondrial pseudogenes inserted in the nuclear genome (*numts*; [Thalmann et al., 2005](#)), due to the stochastic nature of lineage sorting during speciation, or even more seriously because of possible secondary introgressions (see, e.g., [Ballard and Whitlock, 2004](#) or [Nichols, 2001](#)).

Hybridization and mtDNA introgression are phenomena that can leave traces of ancient history on the mitochondria, shaping and introducing errors on naive phylogenies. Hybridization and cytoplasmic genome introgression are frequent in plants (see [Arnold, 1997](#)), but reports of such cases

in animals are accumulating in the literature (see [Avice, 2004](#) for a compilation), including cases of contemporaneous or past introgression, sometimes even resulting in a complete interspecific mitochondrial replacement ([Bernatchez et al., 1995](#)). Examples in mammals include pocket gophers ([Ruedi et al., 1997](#)), deer ([Goodman et al., 1999](#)) and elephants ([Roca et al., 2005](#)). It is now clear that genomes remain porous long after speciation is completed, allowing multiple opportunities for widespread introgression and, thus, misinterpretations of single gene genealogies ([Chan and Levin, 2005](#); [Mallet, 2005](#)).

Although introgression of mtDNA from the mountain hare, *Lepus timidus*, into populations of the brown hare

L. europaeus has been described for almost a decade ([Thulin et al., 1997](#)), phylogenies of various hare species groups have been estimated based solely on mtDNA (e.g., [Pierpaoli et al., 1999](#); [Wu et al., 2000](#)) and even used to support or refute taxonomic units ([Halanych et al., 1999](#)), ignoring the possible problem of introgression when using a single marker. However, in the mtDNA phylogeny of 15 hare species presented in [Alves et al. \(2003\)](#), a lineage that appeared specific of *L. timidus* also contained representatives of several other species, and these authors warned that this could be an indication of introgression of *L. timidus* mtDNA into as many as eight species (*L. granatensis*, *L. europaeus*, *L. arcticus*, *L. castroviejo*, *L. corsicanus*, *L. oiostolus*, *L. othus* and *L. townsendii*). By comparison with the phylogeny of the nuclear gene transferrin of some of these species, they confirmed that some Iberian specimens of *L. granatensis* and *L. europaeus* harbored mtDNA of *L. timidus* origin, which was unexpected since *L. timidus* has disappeared from the Iberian Peninsula during the last deglaciation ([Altuna, 1970](#)). Further analysis of 695 hares from the Iberian Peninsula clearly demonstrated that the introgressed *L. timidus* mtDNA is widespread in the northern regions, being predominant in *L. granatensis* populations, almost fixed in *L. europaeus* across its Iberian range, and present in *L. castroviejo* as well ([Melo-Ferreira et al., 2005](#)).

Somewhat surprisingly, three recent articles have addressed arctic hare relationships based solely on mtDNA sequences, without discussing the possible impact of introgression ([Waltari et al., 2004](#); [Waltari and Cook, 2005](#); [Wu et al., 2005](#)). These articles make important taxonomic and biogeographic proposals based on this data. [Wu et al. \(2005\)](#) used mtDNA data to infer phylogenetic relationships among nine presently recognized hare species from China and include GenBank sequences to extend the analysis to 24 worldwide species. The same data set was further used to infer the evolutionary biogeography of the genus *Lepus* and to date the putative speciation events. These authors suggest two southern European species, *L. corsicanus* and *L. castroviejo*, to

be considered subspecies of *L. timidus*, neglecting the fact that these species are suggested to have introgressed mtDNA (Alves et al., 2003), have distinct morphologies and that their nuclear gene pool remains unexamined. Further, *L. coreanus*, *L. arcticus* and *L. othus* are considered *L. timidus* by Wu et al. (2005) without further evidence from nuclear markers. Since *L. capensis* from China also has *L. timidus* mtDNA, Wu et al. (2005) assume “the Cape Hare does not exist in China”. Using the same argument, we would have concluded that *L. granatensis* and *L. europaeus* do not exist in some locations in the Iberian Peninsula. Waltari et al. (2004) and Waltari and Cook (2005) infer the arctic hares phylogeny as well as historical population decreases in *L. othus* and *L. arcticus*, based exclusively on the mitochondrial control region. MtDNA monophyly of *L. arcticus* and *L. othus* is believed to support species classification although *L. timidus* is clearly paraphyletic. Again, introgression from *L. timidus* is a possibility that cannot be overlooked and, without additional nuclear evidence, the history of these other arctic hares cannot be completely understood.

To conclude, as has repeatedly been stated before, “one is not enough” (Ballard, 2000), and the evolutionary history of the genus *Lepus* is a remarkable example of this. Introgression of *L. timidus* mtDNA into other hare species appears widespread, fixed in some populations and perhaps even in some species, possibly indicating a selective advantage to the introgressed lineage, as suggested by Melo-Ferreira et al. (2005). Since this introgression must be ancient, at least in some cases such as in the Iberian Peninsula, it is unsurprising that these populations now contain unique haplotypes relative to bona fide *L. timidus* specimens.

Hares promise to be an exciting model to study mtDNA evolution, hybridization and adaptation, provided the right questions are asked and the right tools used to address them. But hare phylogeny and taxonomy based solely on mtDNA and ignoring potential introgression is treading on thin ice indeed.

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