Systematic Biology Advance Access published January 30, 2012

DOI:10.1093/sysbio/syr114

Recurrent Introgression of Mitochondrial DNA Among Hares (*Lepus* spp.) Revealed by Species-Tree Inference and Coalescent Simulations

J. Melo-Ferreira[1,2,3,*], P. Boursot[3], M. Carneiro[1,2], P. J. Esteves[1,4], L. Farelo[1,2], and P. C. Alves[1,2,5]

[1]*CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485−661 Vairão, Portugal;* [2]*Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, 4099−002 Porto, Portugal;* [3]*Université Montpellier 2, CNRS UMR 5554, Institut des Sciences de l'Evolution, 34095 Montpellier cedex 5, France;* [4]*CITS—Centro de Investigação em Tecnologias da Saúde, IPSN, Gandra, CESPU, Portugal; and* [5]*University of Montana, Wildlife Biology Program, College of Forestry and Conservation, Missoula, MT 59812, USA;*
[*]*Correspondence to be sent to: CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485−661 Vairão, Portugal; E-mail: jmeloferreira@cibio.up.pt.*

Received 20 July 2010; reviews returned 9 February 2011; accepted 9 July 2011
Associate Editor: Laura Kubatko

Abstract.—Understanding recent speciation history requires merging phylogenetic and population genetics approaches, taking into account the persistence of ancestral polymorphism and possible introgression. The emergence of a clear phylogeny of hares (genus *Lepus*) has been hampered by poor genomic sampling and possible occurrence of mitochondrial DNA (mtDNA) introgression from the arctic/boreal *Lepus timidus* into several European temperate and possibly American boreal species. However, no formal test of introgression, taking also incomplete lineage sorting into account, has been done. Here, to clarify the yet poorly resolved species phylogeny of hares and test hypotheses of mtDNA introgression, we sequenced 14 nuclear DNA and 2 mtDNA fragments (8205 and 1113 bp, respectively) in 50 specimens from 11 hare species from Eurasia, North America, and Africa. By applying an isolation-with-migration model to the nuclear data on subsets of species, we find evidence for very limited gene flow from *L. timidus* into most temperate European species, and not into the American boreal ones. Using a multilocus coalescent–based method, we infer the species phylogeny, which we find highly incongruent with mtDNA phylogeny using parametric bootstrap. Simulations of mtDNA evolution under the speciation history inferred from nuclear genes did not support the hypothesis of mtDNA introgression from *L. timidus* into the American *L. townsendii* but did suggest introgression from *L. timidus* into 4 temperate European species. One such event likely resulted in the complete replacement of the aboriginal mtDNA of *L. castroviejoi* and of its sister species *L. corsicanus*. It is remarkable that mtDNA introgression in hares is frequent, extensive, and always from the same donor arctic species. We discuss possible explanations for the phenomenon in relation to the dynamics of range expansions and species replacements during the climatic oscillations of the Pleistocene. [Coalescent simulations; discordant phylogenies; introgression; *Lepus*; rapid radiation; species-tree inference.]

Population genetics and phylogenetics have historically been developed relatively independently with different methods and models. It is, however, now recognized that establishing a link between gene genealogy and population or species divergence history requires the incorporation of the variance of the coalescence process in populations, as well as the possibility of secondary exchanges after population split. Distinguishing these two major causes of conflicting signals across loci is of major importance but notoriously difficult, and several methods have been developed for identifying introgression events in a phylogenetic framework. Whereas most of these methods either do not account simultaneously for the possibility of incomplete lineage sorting (e.g., Bryant and Moulton 2004; Jin et al. 2006; Gauthier and Lapointe 2007) or do not make an assumption about the nature of the discordance (Ané et al. 2007), recent methods incorporate the coalescence of lineages while attempting to assess the possibility of gene introgression (Buckley et al. 2006; Joly et al. 2009; Kubatko 2009). Although any genomic region may be affected by introgression, literature reports of reticulate evolution induced by introgression in animals mostly concern mitochondrial DNA (mtDNA) (e.g., Ferris et al. 1983; Ruedi et al. 1997; Roca et al. 2005; Alves et al. 2006; Berthier et al. 2006). This phenomenon often generates strong conflicting phylogenetic signals between nuclear and mtDNA markers (e.g., Buckley et al. 2006; Bossu and Near 2009; Spinks and Shaffer 2009).

Introgression has been repeatedly invoked to explain the sharing of closely related mtDNA haplotypes among different species of hares (*Lepus* spp.). In fact, most suspected cases involve *Lepus timidus*, an arctic/boreal species widespread in northern Eurasia, as the mtDNA donor species (e.g., Thulin et al. 1997, 2006; Alves et al. 2003; Melo-Ferreira et al. 2005, 2007; Suchentrunk et al. 2005; Fredsted et al. 2006) (Fig. 1). These instances of apparent mtDNA introgression were reported not only in areas of present contact with the recipient species (e.g., into *L. europaeus* in Sweden or the Alps; Thulin et al. 1997, 2006; Suchentrunk et al. 2005) but also in regions from where *L. timidus* is now absent but was present until it went locally extinct at the end of the last glacial period (e.g., into *L. granatensis* and *L. europaeus* in the Iberian Peninsula; Alves et al. 2003). Interestingly, there are regions where inferred mtDNA introgression from *L. timidus* is massive and reaches quasi-fixation in some populations (as in the hares from the Iberian Peninsula; Melo-Ferreira et al. 2005). Available data also suggest that this "arctic" mtDNA lineage is shared by at least 10 other species, some inhabiting temperate regions and others also occurring in the arctic, in both

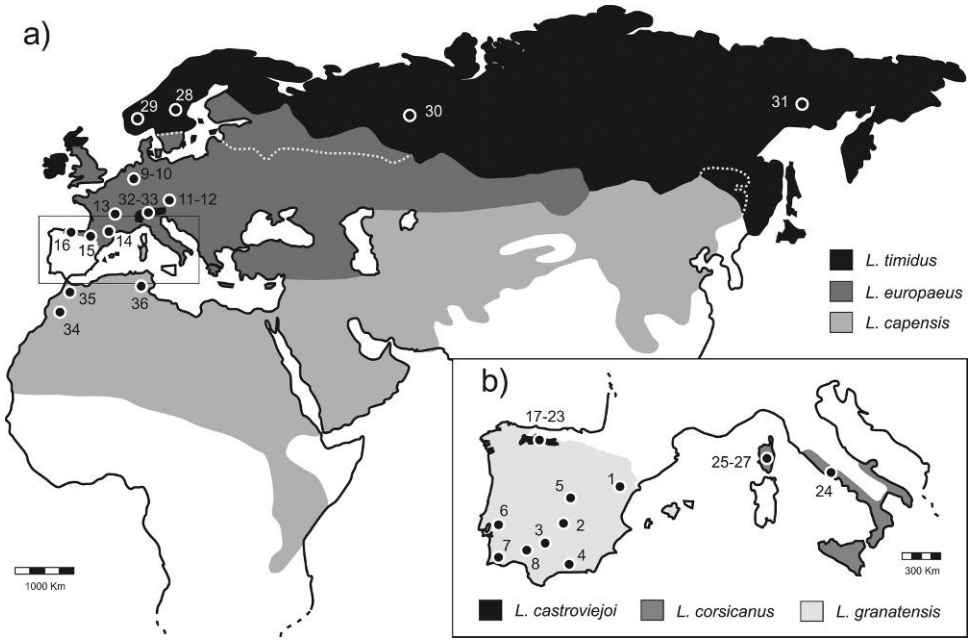

FIGURE 1. Approximate geographic distribution of the species of hares in Eurasia and Northern Africa (a), detailing South-Western Europe (b), used in this study (Flux and Angermann 1990; Mitchell-Jones et al. 1999; Angelici and Luiselli 2001). Dotted lines indicate the limits of some major distribution overlaps between species. Dots correspond to sampling locations and numbers to the specimens detailed in Table 1.

the Old and the New World (Alves et al. 2008). This lineage sharing across species suggests that either ancestral polymorphism has predominantly prevailed in the course of the speciation of hares or mtDNA introgression of *timidus* origin covers a remarkable taxonomic and geographic range (Alves et al. 2008). However, the suspicion of frequent mtDNA introgression from *L. timidus* relies on mtDNA phylogenies alone or on their qualitative comparison to poorly resolved species phylogenies based on a single or a few DNA fragments analyzed with classical phylogenetic methods (see, e.g., Alves et al. 2003). Indeed, either because gene introgression is common among species of hares or because species share characters due to recent common ancestry, the phylogeny of hares is far from established, and this genus is considered one of the most problematic within the Leporids (Robinson and Matthee 2005).

Here, using various approaches that take into account lineage sorting during speciation and gene flow, we aim at reconstructing the phylogenetic relationships of 11 of *Lepus* species, evaluating the impact of gene flow among species, and assessing the origin of the apparent ubiquity of the "arctic" mtDNA lineage: introgression or ancestral polymorphism. Our results support the conclusion that repeated introgressions of the arctic mtDNA lineage have affected 4 temperate European species, 2 of which had their mitogenome completely replaced. However, sharing of ancestral polymorphism appears to prevail among arctic taxa from America and Eurasia. Based on inferred species phylogeny and patterns of introgression, we also examine the possible biogeographic history of the genus.

MATERIALS AND METHODS

Samples and Laboratory Methods

A total of 50 specimens of hares were included in this study and represent 11 presently classified species (Figs. 1 and 2 and Table 1). This sampling corresponds to about a third of all presently accepted hare species and spans a widespread geographic range occupied by hares in Europe, Northern Asia, Africa, and North America (Figs. 1 and 2) (see also Alves and Hackländer 2008). The European wild rabbit, *Oryctolagus cuniculus*, and the Eastern cottontail, *Sylvilagus floridanus*, were included as outgroups (Table 1).

Total genomic DNA was extracted from liver or ear tissue using standard high-salt methods similar to those described by Sambrook et al. (1989). Fourteen nuclear and 2 mtDNA fragments were amplified by polymerase chain reaction (PCR) (Table 2; primers, references, and conditions in online Appendix 1 available from http://www.sysbio.oxfordjournals.org). Of the nuclear loci used here, 13 are autosomal and 1 lies on the X chromosome (AIFM1; Table 2) in humans, mice, and rabbit, and we assumed that such was the case in hares. All PCR products were sequenced using the forward and/or reverse primers, following the ABI PRISM BigDye Terminator Cycle Sequencing 3.1 (Applied Biosystems) standard protocol. Sequences for some of the specimens had already been obtained in the previous work (Gissi et al. 1998; Alves et al. 2003; Matthee et al. 2004; Melo-Ferreira et al. 2007, 2009, 2011; Alves et al. 2008; Pietri et al. 2011) and were retrieved from GenBank (see accession numbers in online Appendix 2). The sex of each specimen was assessed

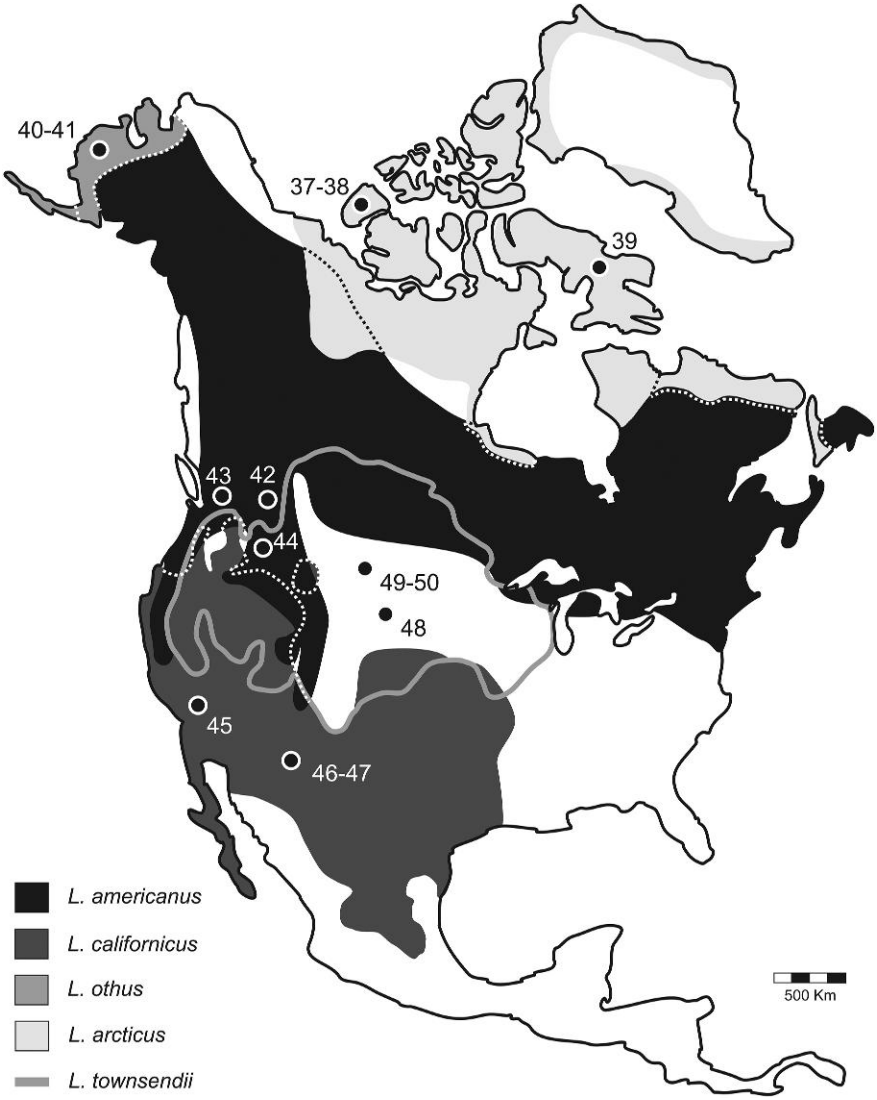

FIGURE 2. Approximate geographic distribution of the species of hares in North America studied here (Flux and Angermann 1990). The gray line delimits the distribution of *Lepus townsendii* and dotted lines indicate limits of some major distribution overlaps between species. Dots correspond to sampling locations and numbers to the specimens detailed in Table 1.

using the PCR approach outlined by Wallner et al. (2001).

Sequence Alignment and Phylogenetic Analyses

Sequences were visually inspected and aligned using ClustalW (Thompson et al. 1994). We used Phase 2.1.1 (Stephens et al. 2001; Stephens and Donnelly 2003) to reconstruct haplotypes for each nuclear data set. Five replicate runs of 1000 generations after 1000 generations of burn-in were performed. Information for known haplotypes, reconstructed from sequences with heterozygous insertion–deletions, following Flot et al. (2006), was incorporated in the analyses to facilitate phase determination. The Phase software has been shown to generate a very low rate of false positives, the great majority of nonresolved genotypes corresponding to those with an allele occurring only once in the data set (Garrick et al. 2010). Since omitting the unresolved genotypes would lead to strong biases in the estimated levels of diversity and thus on the estimates of effective population sizes (Garrick et al. 2010), we opted to keep the complete data set, including some low-probability phase calls.

The fit of each single-locus alignment to 88 models of sequence evolution was assessed using computer program jModelTest v0.1 (Posada 2008) and the Akaike information criterion. Maximum likelihood (ML) phylogenetic inferences were performed for each locus using software Garli v1.0 (Zwickl 2006) by specifying the optimal mutation model, although not fixing the model parameters. No starting topology was defined, and the program was set to run until no significantly better scoring topology (as defined by the default settings) was encountered after 5,000,000 generations. For each data set, 5 independent runs were performed to check the

TABLE 1. Latin and common names of the species included in this study and geographic location of the sampled specimens (see also Figs. 1 and 2)

Species	Spp. code	Common name	Number	Code	Locality	Sex[a]
Lepus granatensis	Lgr	Iberian hare	1	Lgr1	Spain	F
			2	Lgr2	Spain	M
			3	Lgr3	Spain	M
			4	Lgr4	Spain	M
			5	Lgr5	Spain	M
			6	Lgr6	Portugal	M
			7	Lgr7	Portugal	M
			8	Lgr8	Spain	M
Lepus europaeus	Ler	Brown hare	9	Ler1	Germany	M
			10	Ler2	Germany	M
			11	Ler3	Austria	F
			12	Ler4	Austria	M
			13	Ler5	France	M
			14	Ler6	France	M
			15	Ler7	Spain	F
			16	Ler8	Spain	M
Lepus castroviejoi	Lcs	Broom hare	17	Lcs1	Spain	F
			18	Lcs2	Spain	M
			19	Lcs3	Spain	M
			20	Lcs4	Spain	F
			21	Lcs5	Spain	M
			22	Lcs6	Spain	M
			23	Lcs7	Spain	M
Lepus corsicanus	Lcr	Italian hare	24	Lcr1	Italy	M
			25	Lcr2	France	M
			26	Lcr3	France	F
			27	Lcr4	France	M
Lepus timidus	Ltm	Mountain hare	28	Ltm1	Sweden	M
			29	Ltm2	Norway	M
			30	Ltm3	Russia	M
			31	Ltm4	Russia	M
			32	Ltm5	France	F
			33	Ltm6	Italy	M
Lepus capensis	Lcp	Cape hare	34	Lcp1	Morocco	M
			35	Lcp2	Morocco	M
			36	Lcp3	Tunisia	M
Lepus arcticus	Lar	Arctic hare	37	Lar1	Canada	F
			38	Lar2	Canada	M
			39	Lar3	Canada	M
Lepus othus	Lot	Alaskan hare	40	Lot1	USA	M
			41	Lot2	USA	M
Lepus americanus	Lam	Snowshoe hare	42	Lam1	USA	M
			43	Lam2	USA	M
			44	Lam3	USA	F
Lepus californicus	Lcf	Black-tailed jackrabbit	45	Lcf1	USA	M
			46	Lcf2	USA	M
			47	Lcf3	USA	M
Lepus townsendii	Ltw	White-tailed jackrabbit	48	Ltw1	USA	M
			49	Ltw2	USA	M
			50	Ltw3	USA	M
Oryctolagus cuniculus	Ocn	European rabbit	51	Ocn1	Spain	M
Sylvilagus floridanus	Sfl	Eastern cottontail	52	Sfl1	USA	M

Notes: [a]F: female; M: male.

consistency of the estimates. For the mtDNA data set, support of the resulting nodes was estimated using 500 bootstrap replicates as implemented in Garli. We tested whether the resulting best trees were statistically significantly different by applying the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa 1999) using PAUP* (Swofford 2001).

Bayesian inference (BI) was performed for the same data sets using software BEAST v1.6.1 (Drummond and Rambaut 2007). The best-fit model for each unlinked nuclear fragment was used, as determined with jModelTest. Regarding mtDNA, 2 partitions corresponding to cytochrome *b* and control region were considered and ascribed to a different model, although concatenating them as a single marker. The posterior probabilities were determined after runs of 10 million generations for the individual nuclear genes and of 250 million generations for mtDNA, without fixing the value of the model parameters and using the Yule tree prior. The stability of the runs and convergence of the Markov chain Monte Carlo (MCMC) were assessed using Tracer v1.5 (Rambaut and Drummond 2007). The initial 10% of each run were excluded as burn-in.

Phylogenetic methods that explicitly take into account the possibility of differential lineage sorting across individual loci are expected to perform better in multilocus data sets (see, e.g., Edwards et al. 2007; Kubatko and Degnan 2007). Thus, phylogenetic analyses based on the 14 nuclear genes were performed using the species-tree inference methodology *BEAST (Heled and Drummond

2010), as implemented in BEAST v1.6.1 (Drummond and Rambaut 2007). To avoid violating the assumption of no recombination within each locus, we reduced the sequence data sets to the largest nonrecombining blocks using the software IMgc (Woerner et al. 2007). In *BEAST, the assignment of specimens to taxa must be given as a prior for the analysis. The input file for *BEAST was created using the application BEAUti, part of the package, and partitions and models were edited by hand to fit the models determined by jModeltest. Posterior phylogenies were determined in *BEAST using an uncorrelated lognormal relaxed clock (Drummond et al. 2006) and the Yule tree prior. The prior of the relaxed clock standard deviation was set to an exponential distribution with a mean of 0.5 as recommended by the authors. All remaining priors were set to the defaults. Three replicate runs of 500 million generations were performed, sampling trees and parameter estimates every 50,000 generations. Convergence was checked using Tracer v1.5 (Rambaut and Drummond 2007), and summary trees were generated with TreeAnnotator v1.6.1, part of the BEAST package.

To test whether the best reconstructed mtDNA phylogeny significantly differed from the species tree estimated using the multispecies/multilocus coalescent method, we used a parametric bootstrap approach. Under the constraints of the species-tree topology and monophyly of species (only nodes with posterior probability >0.95 were constrained), the best ML score of such mtDNA phylogeny was determined using Garli v1.0. A full ML search was performed without constraining the mutation model parameters. The program was set to run until no significantly better scoring topology (as defined by the default settings) was encountered after 5,000,000 generations. Five replicate runs were performed to check for the consistency of the estimates. The likelihood ratio of the best unconstrained and constrained trees, D, was calculated. Using the best constrained topology and the mutation model estimated from the ML search, 500 sequence data sets were simulated using Mesquite (Maddison and Maddison 2009). The best ML phylogeny for each simulated data set was determined twice using Garli, both without constraining the topology and forcing the species-tree topology. A full ML search was performed in both cases running Garli until no better scoring topology was found after 100,000 generations. Three replicates were performed to ensure consistency of the ML estimates. For each simulated data set, D', the likelihood ratio between the best ML scores of the unconstrained and constrained run, was calculated. A distribution of D' values was built and compared with the empirical estimate.

Isolation-with-Migration Analyses and Coalescent Simulations

To test whether nuclear gene flow occurred among species showing topological discordances between nuclear and mtDNA, we used IMa2 (Hey 2010). This method co-estimates the multilocus effective population sizes (present and ancestral), divergence times, and migration rates under a model of isolation with migration (IM) (Nielsen and Wakeley 2001; Hey and Nielsen 2004). Pairs of taxa showing discordant topological positions between nuclear and mtDNA phylogenies were analyzed using this methodology: *castroviejoi* versus *timidus*, *corsicanus* versus *timidus*, *granatensis* versus *timidus*, *europaeus* versus *timidus*, *granatensis* versus *europaeus*, *timidus* versus *townsendii*, *arcticus* versus *townsendii*, *othus* versus *townsendii*, *granatensis* versus *castroviejoi*, and *granatensis* versus *corsicanus*. IMgc (Woerner et al. 2007) was used to reduce the species pairwise data sets to the largest nonrecombining block in accordance to the assumption of the IM model of no recombination within loci. This approach has been shown to reduce most of the potential biases in the final estimates (Strasburg and Rieseberg 2010). Overall, 94–100% of the characters and 97–100% of the sequences were retained. To verify the consistency of the estimates across different runs, IMa2 was run 3 times including the 14 nuclear loci with different starting seeds and parameter upper bound priors for each species pair and the infinite-sites mutation model (Kimura 1969). Locus-specific mutation rates were estimated from the *Lepus–Oryctolagus* average corrected distance, considering a split time of 11.8 myr (Matthee et al. 2004) and a generation time of 2 years (Marboutin and Peroux 1995). The geometric mean of the locus-specific mutation rates was used to calculate the effective population sizes and divergence times from IMa2 highest posterior density of each parameter, following the instructions of the IMa manual. The likelihood ratio test described by Nielsen and Wakeley (2001) was applied to assess whether migration rates were significantly different from zero. We further used likelihood ratio tests to compare models with free or constrained migration, sampling 100,000 trees, as implemented in the L-mode of IMa2. In addition, the patterns of speciation among the arctic species, *L. timidus*, *L. arcticus*, and *L. othus*, were investigated under the IM model with 3 populations (taxa) as implemented in IMa2 (Hey 2010). Given the uncertainty of the phylogenetic relationships among these species (see Results section), all three topological possibilities were tested. Both nuclear and mtDNA loci were included in this analysis with the infinite-sites and HKY (Hasegawa et al. 1985) mutation models, respectively.

In order to assess whether the conflicting phylogenetic signals found between the nuclear DNA- and the mtDNA-based phylogenies could be explained by incomplete lineage sorting alone or by gene flow, we employed a methodology similar to that developed by Joly et al. (2009). The estimates of current and ancestral population sizes and divergence times obtained under the IM model were used as input for SimCoal2 (Laval and Excoffier 2004). A total of 10,000 simulated data sets were produced for each of the pairwise species comparisons referred above, mimicking the empirical mtDNA data sets (sequence lengths and sample sizes), under a model where an ancestral haploid population

of size $N_{eA}/2$ split into 2 descendants of size $N_{e1}/2$ and $N_{e2}/2$, t generations ago, and no gene flow occurring after the split. Again, the mtDNA mutation rate was estimated considering a rabbit–hare divergence of 11.8 myr (Matthee et al. 2004) and a generation time of 2 years (Marboutin and Peroux 1995). Sequences were generated using a mutation model with unequal transition–transversion rate (the transition proportion was determined for the empirical data sets with jModeltest). For each replicate, the minimum pairwise uncorrected p-distance between the simulated descendant populations was retained and a distribution of expected minimum distances under no gene flow was produced. The empirical pairwise distance between species for mtDNA was considered to significantly reject the strict lineage sorting model if it was lower than the 5th percentile of the simulated minimum distances.

RESULTS

Sequenced Data and Phylogenetic Inferences

Sixteen DNA fragments, 14 nuclear and 2 mitochondrial (Table 2), were sequenced, yielding a total of 9318 bp in 11 *Lepus* species and 2 outgroups, the European rabbit and the Eastern cottontail (52 specimens; Table 1; newly obtained sequences were deposited in GenBank with accession numbers JN036862–JN037408—see also Appendix 2). After the removal of gaps, which are ignored in most of the analyses performed here, the nuclear DNA data set was composed of 7687 characters, which was further reduced to 6874 characters if considering only the largest nonrecombining blocks (Table 2). The final mtDNA alignment had 1061 bp in total (Table 2). Apart from loci DARC and CYB that were composed of coding sequence only, the remaining nuclear fragments were either totally intronic or were mostly intronic with a small and nearly invariant 1061 exon (see Appendix 3). The analyzed sequence matrices and the resulting phylogenetic trees were deposited in TreeBase and are available at http://purl.org/phylo/treebase/phylows/study/TB2:S11662.

The replicate runs of the ML phylogenetic reconstructions based on each individual locus yielded consistent best likelihood scores but slightly different topologies (not shown), which likely resulted from the low level of information present in each single gene. This was also reflected in the generally low posterior probabilities for most of the clades obtained in the BI of the single-gene phylogenies (Appendix 4). The ML and BI estimates of the phylogeny of the individual genes showed that the species share sequences at a high degree (Appendices 4 and 5). Also, vast discordance among loci was suggested by the frequently significant differences among the best ML topologies as assessed by the SH test (Appendix 6).

Given such discordances among nuclear loci, phylogenetic inference of the species tree based on their concatenation would be prone to errors (Edwards 2009), and thus, we used the multispecies coalescent methods implemented in *BEAST (Heled and Drummond 2010). This is a Bayesian MCMC method that uses the multispecies/multilocus coalescent to estimate the species trees from the distribution of single-gene trees, co-estimating the effective population sizes of tip and ancestral taxa. We have reduced our data set to the largest nonrecombining blocks, which eliminated 10% of the sites and 5% of the sequences. However, an analysis with the complete data set resulted in the inference of the same species tree, suggesting that recombination would have little effect in the results. The phylogeny obtained using *BEAST is shown in Figure 3a. Low posterior probabilities for some nodes were recovered, and here we only show the nodes with a support higher than 95% of the posterior trees (see Appendix 7 for full results). Finally, to test whether the inclusion of sites with low-probability phase call estimated by Phase 2.1.1 (Stephens et al. 2001; Stephens and Donnelly 2003) influenced the estimation of the species tree, we per-

TABLE 2. Variation and appropriate mutation models of the molecular markers used in this study

Marker			Number of characters				
			Total	NG[a]	LNRB[b]	Variable[c]	Mutationmodel[d]
1	ALB	Albumin	618	602	602	32	TPM2uf
2	CA2	Carbonic anhydrase II	679[e]	653	549	32	TPM1uf+Γ
3	DARC	Duffy blood group, chemokine receptor	886	877	861	35	TPM1uf+Γ
4	HPX	Hemopexin	796	650	392	33	TPM3uf
5	KITLG	KIT ligand	572	546	422	27	TPM2uf
6	PRKCI	Protein kinase C, iota	417	372	348	30	TrN
7	SPTBN1	Spectrin, beta, nonerythrocytic 1	637	574	574	36	TPM1uf+Γ
8	TF	Transferrin	429	417	417	40	TVM+Γ
9	TSHB	Thyroid-stimulating hormone, beta	357	356	356	18	TIM2
10	OXA1L	Oxidase assembly 1	684[f]	657	652	38	TIM1+Γ
11	UCP2	Uncoupling protein 2	377	301	301	25	TIM1
12	UCP4	Uncoupling protein 4	543	534	534	11	HKY
13	PPOX	Protoporphyrinogen oxidase	613	573	335	29	TPM2
14	AIFM1	Apoptosis-inducing factor 1	602	575	531	28	TPM1
Combined nuclear DNA			8205	7687	6874	414	—
15	CYTB	Cytochrome *b*	617	617	—	176	TPM1uf+Γ
16	CR	Control region	496	444	—	164	HKY+I+Γ
Combined mtDNA			1113	1061	—	340	TPM1uf+I+Γ

Notes: [a]NG: No gaps (Alignment gaps removed). [b]LNRB: Largest nonrecombining blocks. [c]Only ingroup taxa were considered. [d]See Posada (2008) for a description of models and references. [e]Insertion of 380 bp in *Sylvilagus floridanus* not considered. [f]Microsatellite not considered.

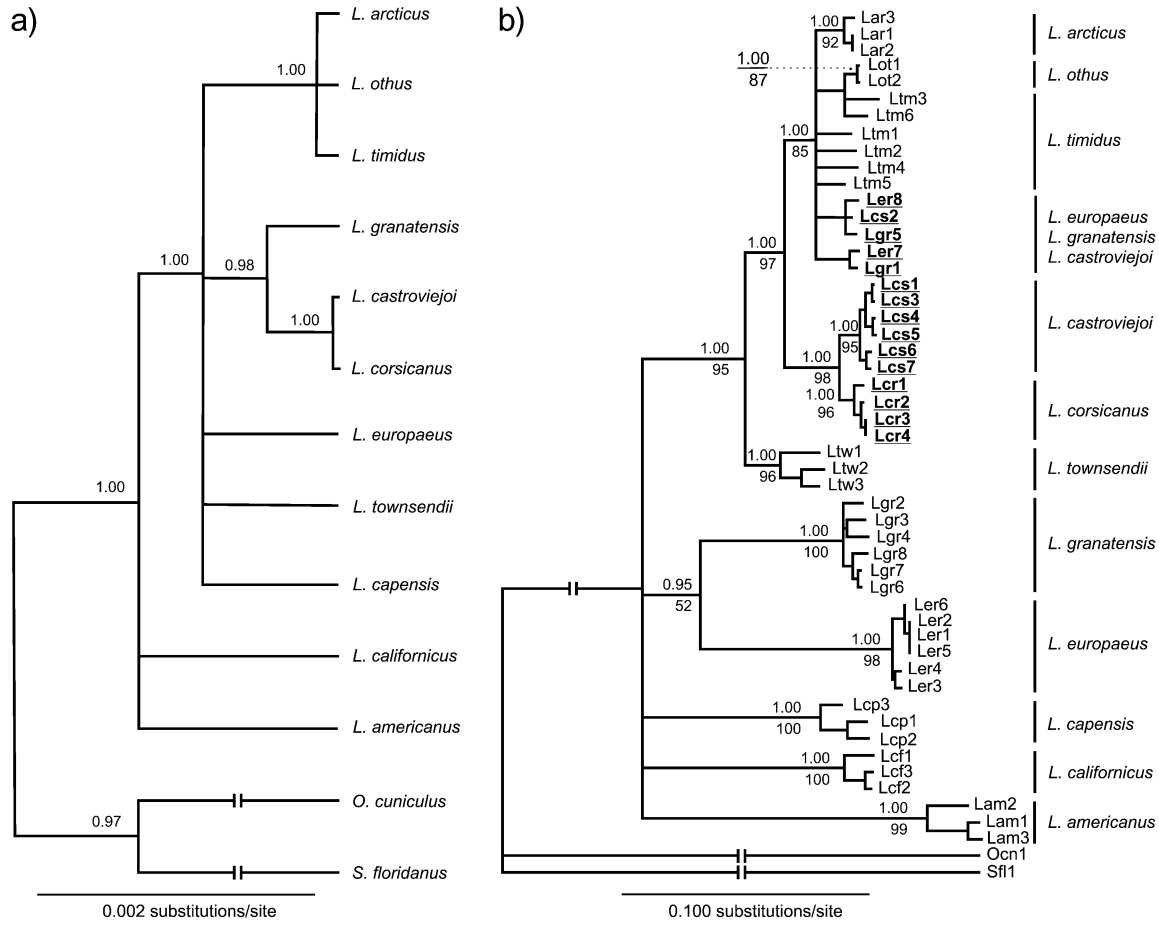

FIGURE 3. a) Nuclear DNA consensus tree generated from the *BEAST species-tree inference output for the 14 loci (the posterior probability of each clade is depicted in front of each node). b) mtDNA majority rule consensus tree generated from the BI (numbers above branches indicate the posterior probabilities of each clade and numbers below branches depict the ML bootstrap supports). Nodes with posterior probability <0.95 were collapsed. Codes are those shown in Table 1. In (b), the underlined individual codes indicate the introgressed haplotypes revealed by the coalescent simulations (see Table 4).

formed another analysis coding these sites as missing data. Again, similar results were obtained (not shown).

The first split among the species of hares separates 2 North American species, *L. californicus* and *L. americanus*. *L. castroviejoi* and *L. corsicanus*, 2 southern European species with disjoint and restricted distributions in Northern Iberia and southern Italy, respectively (Fig. 1), are sister taxa and are closely related to *L. granatensis* (Fig. 3a). A highly supported clade was recovered for the arctic hares, *L. timidus*, *L. othus*, and *L. arcticus*, that span the arctic ring, but the split order among these species is unresolved.

The phylogeny obtained from the mtDNA sequences is depicted in Figure 3b. The parametric bootstrap showed that the obtained mtDNA phylogeny is not compatible with the inferred species tree based on nuclear DNA markers ($P = 0$; see Appendix 8). A comparison between the topologies of the phylogenies based on nuclear and mtDNA (Fig. 3) points to some inconsistencies. The major discrepancy concerns the inclusion of some of the *L. granatensis*, *L. europaeus* and *L. castroviejoi* mtDNA sequences in the clade of *L. timidus*. Other discrepancies concern the relative positions of some species but with low support (Fig. 3). The causes of such discordance were further investigated using coalescent approaches to separate incomplete lineage sorting from the possibility of introgression (see below).

IM and Coalescent Simulations

Although *BEAST incorporates the uncertainty of the coalescent process in the estimate of the phylogeny, it assumes that no gene flow occurred after the initial split. To quantify the potential impact of gene flow on the evolution of nuclear genes, we fitted our data to the IM model (Hey and Nielsen 2004). The approximate posterior density curves of the model parameters that resulted from these analyses were consistent across replicate runs. However, particularly in the estimates of divergence time and ancestral effective population size, the right tail of the posterior density curves often failed to reach zero (not shown). Using the average *Lepus–Oryctolagus* divergence, we

estimated the mutation rate to be 4.1×10^{-9} substitutions/site/generation for nuclear DNA (geometric mean of 14 loci), which is similar to that estimated for mice (4×10^{-9}; Waterston et al. 2002), and 4.9×10^{-9} substitutions/site/generation when considering both the nuclear and the mtDNA loci (geometric mean of 15 loci). Estimates of effective population size (N_e) correlated well with the present day geographic range of the species (Fig. 1), with *L. corsicanus* and *L. castroviejoi* having the lowest and *L. europaeus* and *L. timidus* the highest N_e (Table 3). To test whether estimated levels of gene flow of nuclear genes among species were significantly different from zero and whether a model with equal migration rates in both directions could explain the data, we used the log likelihood ratio (LLR) tests implemented in IMa2. Among European species, a model with no gene flow was rejected in most cases, except between *L. granatensis* and *L. corsicanus*. Among European species, gene flow (migration) was found to be significantly different from zero only in one of the directions in each species pair (except between *L. granatensis* and *L. corsicanus*), and interestingly, *L. timidus* is the donor species in all pairs where it is involved (Table 3). However, only in one case was a model of equal migration in both directions rejected (*L. granatensis* and *L. castroviejoi*). No gene flow was inferred between the arctic hares and *L. townsendii* (Table 3). The analyses of the group of 3 arctic hares (*L. timidus*, *L. arcticus*, and *L. othus*) showed no significant migration in any direction, whichever topology was assumed for the species tree (Appendix 9).

We used the estimates of divergence times and N_e to simulate data sets mimicking the mtDNA sequences obtained in this work, using a mutation rate of 6.0×10^{-8} substitutions/site/generation estimated from the average corrected divergence between hares and the European rabbit. The 5th percentile of the minimum pairwise uncorrected *p*-distance among simulated species is shown in Table 4. In the analyses involving *L. castroviejoi* or *L. corsicanus* and *L. timidus*, all the observed pairwise mtDNA divergences were smaller than the 5th percentile of the simulated minimum distances, that is, in all cases, these low divergences appeared unlikely to result from the variance of the coalescent process in the absence of gene flow. In the remaining analyses, only a few of the between-species pairwise distances were significantly lower than expected under an incomplete lineage sorting scenario (Table 3). These distances involve sequences that are placed within the *L. timidus* clade in the mtDNA phylogeny while belonging to another species (Fig. 3b).

Discussion

In this work, we shed light on the poorly known phylogeny of hares and, by contrasting the inferred history to the evolutionary patterns obtained for mtDNA, test how widespread and repeated mtDNA introgression is among species, particularly that originating from the arctic *L. timidus*. Any attempt to reconstruct the speciation history must take into account both the variance of the coalescent among genomic regions and the possibility of introgression. By applying an IM model to pairs of species, we were able to infer very limited nuclear gene flow. The evolutionary history of hares was reconstructed from the nuclear genes using a multispecies coalescent method that takes into account differential lineage sorting across markers. Treating mtDNA independently, we could ask whether its pattern of divergence among species was compatible with the species history in the absence of gene flow. In several instances, we found that this was not the case, thus supporting the conclusion of mtDNA introgression.

Nuclear Gene Flow and Rapid Radiation as Sources of Phylogenetic Uncertainty

Although around 8 kb of nuclear genome of hares was sequenced here, these were split among 14 loci and therefore the length of each fragment was rather small and low variation was found (Table 2). Such low variation translated into some degree of uncertainty on the estimation of single-gene trees with frequent sequence sharing among species (see Appendices 4 and 5). However, we found significant gene-to-gene discordance of phylogenetic signals, which may indicate differential prevalence of ancestrally shared polymorphism across markers (see Appendices 4 and 5). These results highlight the importance of using a method that takes into account the information of each gene tree in a coalescent framework and that takes advantage of sampling of multiple individuals per species (McCormack et al. 2009).

Hares are thought to have radiated very rapidly throughout Eurasia with the general development of temperate grasslands (Corbet 1986; Yamada et al. 2002; Matthee et al. 2004) about 4–6 Ma (Matthee et al. 2004). The low posterior probabilities we obtained as support for the internal nodes of the species tree may result from such rapid radiation and reflect extensive incomplete lineage sorting (see, e.g., Belfiore et al. 2008). However, because the species-tree inference methodology does not take introgression into account (Heled and Drummond 2010), the effects of potential nuclear gene flow were neglected. Previous population-level single nucleotide polymorphism analyses have suggested that some introgression of nuclear genes may have occurred from *L. timidus* into *L. europaeus* and *L. granatensis*, although reaching very low frequencies in the affected populations (nuclear introgression affected a maximum of 3 of 10 loci, with frequencies mostly under 10%; Melo-Ferreira et al. (2009), but see Melo-Ferreira et al. (2011) for a case of massive introgression of an X-linked fragment not included in the present study). Our analyses here using the IM model among 5 European species suggested gene flow in some directions, although a model with no migration was rarely rejected (Table 3). Also, the inferred values of the migration parameters were

TABLE 3. ML estimates (95% posterior density intervals in parentheses) of demographic parameters obtained with IMa2 between pairs of hare species (generation length of 2 years)

Sp.1	Sp.2	N_{e1}[a]	N_{e2}[a]	N_{eA}[a]	t[b]	$2Nm_1$[c]	$2Nm_2$[c]	ABCDD[d]	ABC0D[d]	ABCD0[d]	ABC00[d]
Lcs	Ltm	26,448 (11,790–52,577)	185,836 (125,229–275,057)	188,385	716,322	0.0386* (0.0041–0.0934)	0.0076 (0–0.1077)	n.s.	n.s.	n.s.	*
Lcr	Ltm	6685 (5730–32,468)	174,819 (162,341–344,418)	172,782	705,387	0.0113* (0–0.0419)	0.0004 (0–0.0932)	n.s.	*	n.s.	*
Lgr	Ltm	82,035 (50,165–126,889)	181,186 (119,807–268,532)	91,478 (0–301,583)	545,327 (266,762–1,003,308)	0.0488* (0.0041–0.1418)	0.0299 (0–0.1595)	n.s.	*	n.s.	*
Ler	Ltm	134,172 (85,026–198,920)	192,024 (123,653–290,082)	67,495 (0–197,284)	483,859 (278,161–759,682)	0.0820* (0.0134–0.2281)	0.0326 (0–0.2097)	n.s.	*	n.s.	*
Lgr	Ler	101,937 (64,092–151,991)	141,003 (93,392–206,927)	235,005	604,544	0.0003 (0–0.0884)	0.0431* (0.0043–0.1818)	n.s.	n.s.	*	*
Lgr	Lcs	92,726 (61,157–135,943)	21,784 (11,183–39,373)	372,184	892,310	0 (0–0.0251)	0.0327* (0.0109–0.0633)	*	n.s.	*	*
Lgr	Lcr	87.717 (56,614–131,866)	6290 (1631–17,823)	323,259	687,288	0 (0–0.0279)	0.0050 (0–0.0149)	n.s.	n.s.	n.s.	n.s.
Ltm	Ltw	165,488 (102,908–256,133)	156,512 (83,186–293,175)	24,273 (126–131,101)	389,383 (197,220–622,002)	0.1130 (0–0.4150)	0 (0–0.3033)	n.s.	n.s.	n.s.	n.s.
Lar	Ltw	18,844 (8388–114,903)	28,496 (13,444–175,801)	22,981 (0–203,722)	163,161	0.024 (0–0.2051)	0 (0–0.1661)	n.s.	n.s.	n.s.	n.s.
Lot	Ltw	74,681 (31,206–161,630)	140,072 (73,371–271,686)	91,594 (0–272,401)	288,243	0.097 (0–0.5345)	0.023 (0–0.3898)	n.s.	n.s.	n.s.	n.s.

Notes: Missing values correspond to cases where parameters could not be reliably estimated; see Table 2 for codes of species.

[a]Effective population size of population 1 (N_{e1}), 2 (N_{e2}), and the ancestral population (N_{eA}).

[b]Time in generations since species 1 and 2 split.

[c]Population migration rate into population 1 ($2Nm_1$) and population 2 ($2Nm_2$) (significant values indicated *$P < 0.05$; Nielsen and Wakeley 2001).

[d]Likelihood ratio test of nested models with equal gene flow between populations (ABCDD), no gene flow into population 1 (ABC0D), no gene flow into population 2 (ABCD0), and with no gene flow (ABC00). The test statistic was calculated as follows: ABCDD (2LLR against ABCDE) follows a chi-square distribution with 1 degree of freedom with critical value *$P < 0.05$ at 2LLR $\geqslant$ 3.84; ABC0D and ABCD0 (2LLR against ABCDE) and ABC00 (2LLR against ABCDD) follow a chi-square distribution that is $^1/_2\times$ chi-square(1) + $^1/_2\times$ chi-square(0) with critical value *$P < 0.05$ at 2LLR $\geqslant$ 2.70.

TABLE 4. Results of the coalescent simulations of mtDNA sequences from population parameters estimated with multiple nuclear loci

Species 1[a]	Species 2[a]	5% Lower simulated distance[b]	Estimated *Dxy*[c]	$P < 0.05$[d]
Lcs	Ltm	0.0678	0.0151–0.0547	All comparisons
Lcr	Ltm	0.0679	0.0434–0.0528	All comparisons
Lgr	Ltm	0.0490	0.0179–0.1056	Lgr1, Lgr5–Ltm
Ler	Ltm	0.0424	0.0179–0.1093	Ler7, Ler8–Ltm
Lgr	Ler	0.0566	0.0075–0.1074	Lgr1, Lgr5–Ler7, Ler8
Ltm	Ltw	0.0330	0.0490–0.0584	None
Lar	Ltw	0.0122	0.0509–0.0603	None
Lot	Ltw	0.0320	0.0518–0.0575	None

Notes: [a]Species codes in Table 2. [b]Highest bound of the 5th percentile of the lowest simulated average nucleotide divergence (*Dxy*, Nei 1987) for mtDNA between species 1 and 2. [c]Range of estimated average nucleotide divergence (Nei 1987) for mtDNA between species 1 and 2. [d]Strict lineage sorting model is rejected if observed *Dxy* is lower than 5% of simulated distances.

very low when compared with those inferred for other mammals (e.g., Won and Hey 2005; Geraldes et al. 2008; Bonhomme et al. 2009; Carneiro et al. 2009; Hey 2009; Stevison and Kohn 2009), even in cases where mtDNA introgression is known to be massive such as from *L. timidus* into *L. granatensis*. It is nevertheless important to note that by applying the IMa2 methodology to species pairs when a third species may exist in the equation and may have also exchanged genes, we are violating one assumption behind the IM model (Nielsen and Wakeley 2001; Hey and Nielsen 2004). However, for moderate to low levels of gene flow ($N_em < 0.2$, as we inferred here—Table 3), the effect of a third unsampled species exchanging genes with the focal species on the estimates of the demographic parameters appears to be minimal (Strasburg and Rieseberg 2010). Also importantly, the IM model assumes panmixia of the involved species and does not take into account possible variations of population sizes. Again, IMa seems to be fairly robust to these violations, at least for a range of realistic parameter estimates (Strasburg and Rieseberg 2010). It is hard to evaluate the impact of such levels of nuclear introgression on the inference of the species tree, but previous work has shown that methods based on the multispecies coalescent seem robust to moderate levels of gene introgression ($Nm < 0.1$ and 5 sampled loci; Eckert and Carstens 2008).

Lepus *Biogeography*

Some insights on the biogeographic history of genus *Lepus* are supported by our phylogenetic inferences (Fig. 3). Both nuclear DNA- and mtDNA-based phylogenies consistently show that the Old World species belong to a clade that seems to have diversified more recently than the split between the American *L. californicus* and *L. americanus*. Fossil records (Lopez-Martinez 2008) and a vicariance analysis of the phylogeny of the lagomorphs (e.g., Matthee et al. 2004) suggest that North America is the region of origin of the genus. However, the North American species do not form a monophyletic clade since *L. arcticus* and *L. othus* are included in a clade with the Eurasian *L. timidus*. This suggests that a colonization of species from Eurasia to North America has occurred, likely through the Beringia land bridge that intermittently connected these continents throughout the Quaternary glaciations. The close phylogenetic relationship inferred here among the arctic species *L. timidus*, *L. arcticus*, and *L. othus* (Fig. 3a) was expected since these species share extensive morphological similarities (Best and Henry 1994), which induced earlier studies to suggest they should be classified into the same species (Baker et al. 1983; Dixon et al. 1983). Previous analyses based solely on mtDNA had shown the inclusion of the mtDNA haplotypes of *L. arcticus* and *L. othus* in the clade of *L. timidus* (e.g., Waltari and Cook 2005). Since these previous molecular analyses were solely based on mtDNA, Alves et al. (2008) suggested that these two species may have also been affected by mtDNA introgression of *L. timidus* origin. To clarify this issue, we applied the IM model to these three species. Although the data contained little information to estimate the parameters of the model, it seems to support that no migration (gene flow) occurred after the split of the species (Appendix 9). This analysis thus suggests that these three species are very closely related and may have started diverging at about 270,000 years ago in a presumably strict allopatric speciation process. However, additional data will have to be collected to confirm this hypothesis.

Another highly supported clade is that made of *L. granatensis*, *L. corsicanus*, and *L. castroviejoi*, species with a distribution in southern European peninsulas, Iberia and Italy (Fig. 1). Although we have dismissed nuclear DNA introgression as a major cause of wrong phylogenetic inference, some caution may be needed in this case, as some degree of nuclear gene flow was inferred from *L. granatensis* into *L. castroviejoi* (Table 3; although not into *L. corsicanus*). We must at this stage consider that this phylogenetic proximity is due to common ancestry, but this conclusion must await confirmation from a more thorough exploration of the nuclear genome.

Repeated mtDNA Introgression from L. timidus *and Mitochondrion Replacement*

Deep phylogenetic discordances between nuclear DNA- and mtDNA-based phylogenies have often been used as an argument to demonstrate instances of mtDNA introgression (e.g., Buckley et al. 2006; Bossu and Near 2009; Spinks and Shaffer 2009). Sometimes mtDNA shows a high degree of species paraphyly or polyphyly (Funk and Omland 2003), even though given

its lower effective population size it is expected to more readily sort lineages among species than the nuclear genome (Moore 1995). These conclusions of introgression are, however, frequently presented without explicitly testing the alternative hypothesis that incomplete lineage sorting alone may explain the phylogenetic discrepancies (e.g., Buckley et al. 2006; Bossu and Near 2009; Spinks and Shaffer 2009).

In the mtDNA phylogeny, *L. granatensis*, *L. europaeus*, and *L. castroviejoi* clearly harbor 2 types of haplotypes, one type grouping in a specific clade and the second type grouping within *L. timidus* (Fig. 3b). This phylogenetic pattern was previously interpreted as resulting from introgression of *L. timidus* origin into these Iberian species, which was inferred to have occurred at the end of the last glacial period (Alves et al. 2003; Melo-Ferreira et al. 2005, 2007). Here, we were able to test introgression against an alternative incomplete lineage sorting scenario, and our results deserve a deeper look into the case of *L. castroviejoi*. Haplotypes belonging to this species cluster at 2 different positions in the mtDNA tree: one group forms a clade with *L. corsicanus* (translating the recent common ancestry of these species; Alves, Melo-Ferreira, Branco, et al. 2008) that is sister to the arctic clade and one haplotype falls within the arctic clade (as identified by Melo-Ferreira et al. 2005, 2007) (Fig. 3b). A previous interpretation of this pattern considered that the former group of haplotypes could represent the aboriginal mtDNA lineage of *L. castroviejoi* (and *L. corsicanus*) and the latter to result from recent introgression from *L. timidus* (Melo-Ferreira et al. 2005, 2007). This rationale implies that *L. castroviejoi* and *L. corsicanus* are phylogenetically closely related to *L. timidus.* This relatedness is, however, contradicted by the nuclear data where *L. castroviejoi* and *L. corsicanus* do not appear related to *L. timidus* (Fig. 3a). In fact, among the collection of nuclear DNA trees resulting from the *BEAST MCMC chain, only a tiny fraction (0.9%) sustains a close relationship between *L. castroviejoi* and *L. corsicanus* and the "arctic" clade, thus suggesting it to be highly unlikely. We thus raised the hypothesis that the original *L. castroviejoi*/*L. corsicanus* mtDNA has not been sampled and has been replaced by a *timidus*-like mtDNA lineage at some point in the history of the species. To test this hypothesis, we modeled the nuclear divergence from *L. timidus* in an IM framework and used the estimated parameters to simulate mtDNA data. Given the difficulties in obtaining good quality posterior curves for the time of divergence and ancestral population sizes, we also inferred divergence times applying a simple mutation–drift expectation with the assumption of no gene flow ($k = 2\mu t + \pi$, where k is the average pairwise differences among alleles from 2 populations, μ is the mutation rate, π is the nucleotide diversity of the ancestral population averaged from the descendants, and t is the time of divergence between species). The obtained estimates are, however, remarkably similar to those inferred using IMa2 (Appendix 10).

The results of the coalescent simulations showed that all mtDNA haplotypes from *L. castroviejoi* and *L. corsicanus* present a divergence to *L. timidus* that is smaller than expected under a simple pure lineage sorting scenario (Table 4). We note that this analysis could be compromised if our estimates of mtDNA divergence were affected by saturation, but we found no evidence of such a phenomenon (Appendix 11). Previous work using a more extensive sampling of *L. castroviejoi* and *L. corsicanus* failed to identify any mtDNA haplotype unrelated to the arctic clade (Pierpaoli et al. 1999; Alves et al. 2003; Melo-Ferreira et al. 2005; Alves, Melo-Ferreira, Branco, et al. 2008), which suggests that a complete mitochondrion replacement of these species may have occurred. Despite the many uncertainties on divergence time estimates and the difficulty of finding paleontological calibration points within *Lepus*, it seems clear that mtDNA introgression occurred at 2 different epochs, first presumably into the ancestor of *L. castroviejoi* and *L. corsicanus* and then more recently into the former, in the Iberian Peninsula. This method also provided the first formal confirmation that the extensive sharing of mtDNA haplotypes of 2 Iberian species, *L. granatensis* and *L. europaeus*, with *L. timidus* is not compatible with sharing of ancestral polymorphism and thus can only be explained by introgression (specimens Lgr1, Lgr5, Ler7, and Ler8; Table 3 and Fig. 3b). In contrast, we cannot exclude that the proximity between *L. townsendii* and the arctic clade apparent in the mtDNA phylogeny is due to incomplete lineage sorting alone (Table 3).

On the Repeated mtDNA Introgression among Hares

The widespread and repeated introgression from *L. timidus* into several temperate species that we document here raises the question of whether some deterministic factor, specific to this species or to its mtDNA, may have produced this striking reticulation pattern. The most generic condition under which such extensive interspecific introgression can occur has recently been proposed and modeled (Currat and Excoffier 2004; Currat et al. 2008). When the territory of a resident species is invaded by another more successful species, even rare hybridization events at the front of this expansion can with high probability lead to introgression from the resident species into the expanding one. Drift at the front of invasion and during the following expansion is responsible for the phenomenon. The important range changes of species caused by glacial oscillations are likely to have induced repeated and transient secondary contacts among species, promoting situations of competitive replacement. The phylogeographic patterns inferred for many European species support these range displacements that often result in hybrid zones where populations that evolved in different glacial refugia meet and exchange genes (reviewed, e.g., by Hewitt 2001, 2004). Although it has been suggested that many of those hybrid zones are stable, there are many evidences of historical movement of hybrid zones (Buggs 2007; Hewitt 2011; Wang et al. 2011), which could then correspond to situations of competitive replacement. In Eurasia, some

cases of interspecific gene flow were inferred to result from situations of range replacement, as for example, among *Myotis* bats (Berthier et al. 2006) or *Myodes* voles (Tegelström 1987; Abramson et al. 2009). In the case of hares, it is known that *L. timidus* was widespread in Europe during the last glacial period, as is attested by fossil records of this species found in southern France (Lopez-Martinez 1980) and northern Iberian Peninsula (Altuna 1970). The current distribution of the species (see Fig. 1) thus results from the recent retreat to higher latitudes or altitudes (as the Alps). Introgression from *L. timidus* into the temperate species could then correspond to phases of climate warming, when the latter species would be favored and replace the former. Melo-Ferreira et al. (2007) suggested that the most recent of such events, in the Iberian Peninsula, could correspond to the last major episode of climate warming at the end of the last glaciation. We attempted to date the more ancient introgression event that affected the ancestor of *L. castroviejoi* and *L. corsicanus* using the rationale applied above (between *L. castroviejoi–L. corsicanus* and *L. timidus*; $k = 2\mu t + \pi$) and estimated it to have occurred about 550,000 years ago, during the Pleistocene glacial cycles.

It is striking that mtDNA introgression appears to always have occurred in the same direction, that is, from the cold-adapted species into the more temperate ones. Why the reverse phenomenon did not occur during cooling periods, when cold-adapted species presumably replaced temperate ones, remains to be understood. Several hypotheses can be tentatively proposed. One is that the reverse phenomenon may simply not have occurred because ad hoc ecological conditions did not take place. Temperate species may have gone extinct during cooling periods before the cold-adapted species managed to colonize the territories previously occupied by them. Or cooling of the climate may have been too slow to favor the rapid expansion of cold-adapted species that is needed for introgression to be extensive. In fact, the dynamics of cycles of climate changes is generally not symmetrical, and phases of cooling and warming can occur at very different paces (Petit et al. 1999; Cheddadi et al. 2005). Also, the types of ecological changes that occur during climate fluctuations may be favorable to the phenomenon of competitive replacement in one direction but not the other. Temperate and arctic species may differ in the broadness of their climatic envelope, which could account for some asymmetry in the replacement processes (with the possible complication that ecological requirements of these species may have changed over time). A more thorough understanding of the nature of the ecological changes, in relation to the ecology of these species, is needed. It could also simply be that traces of introgression into the arctic species at the previous cooling period might have disappeared during the Holocene with the extinction of the arctic species from presently temperate geographic regions that they previously occupied and where introgression could have occurred. In this case, the memory of past introgressions would be kept only in case of complete replacement. Other factors such as asymmetric reproductive behavior in situations of hybridization, or asymmetries in genomic incompatibilities, could also be invoked to account for the asymmetry of the phenomenon, as discussed in Melo-Ferreira et al. (2009), but would need to cause a consistent bias of introgression across different interspecific interactions of *L. timidus*, which appears less likely.

An alternative explanation to the asymmetry of introgression would be that mtDNA of *L. timidus* has some selective advantage that promotes its introgression. Arguments in favor of this hypothesis are, however, yet far from established. First of all, it should be shown that introgression of mtDNA is indeed more thorough than that of other parts of the genome. The detailed population genetics study of Melo-Ferreira et al. (2009) revealed limited introgression at 10 autosomal markers in 2 species suffering extensive mtDNA introgression (*L. granatensis* and *L. europaeus*), but a more recent study (Melo-Ferreira et al. 2011) showed very extensive introgression of an X chromosome fragment from *L. timidus* into *L. granatensis*. On the other hand, Melo-Ferreira et al. (2011) provided some population genetics evidence that the foreign mtDNA might have outcompeted the native one in the latter species. Thus, the case for selection is far from resolved, and as often in evolution, going from patterns to processes will need the integration of additional knowledge in various fields, including ecology, palaeoclimate, physiology, and genomics.

Supplementary Material

Supplementary material, including online-only appendices, can be found at http://www.sysbio.oxfordjournals.org/.

Funding

This work was partially supported by FEDER through the COMPETE program and Portuguese national funds through the FCT—Fundação para a Ciência e a Tecnologia (POCI2010/BIA-BDE/58817/2004, PTDC/BIA-BDE/72277/2006, PTDC/BIA-EVF/111931/2009, and PTDC/BIA-EVF/115069/2009 research projects) and by Programa Operacional Potencial Humano-Quadro de Referência Estratégico Nacional funds from the European Social Fund and Portuguese Ministério da Educação e Ciência (FCT, SFRH/BPD/43264/2008, SFRH/BPD/16207/2006, and SFRH/BPD/72343/2010 postdoc grants to J.M.F., P.J.E., and M.C., respectively).

Acknowledgments

We thank Christian Gortazar, Rafael Villafuerte, Alexei Kryukov, Ettore Randi, Christian Pietri, Franz Suchentrunk, and L. Scott Mills for providing some of the samples used here. The *Lepus arcticus* and *Lepus othus* tissue samples were gently yielded by the University of Alaska Museum (original codes: Lar1—UAM47253;

Lar2—UAM47257;Lar3—UAM66293;Lot1—UAM42143; Lot2—UAM45545). DNA sequencing was performed on the platform of IFR119 ("Montpellier Environnement Biodiversité") and in Centro de Testagem Molecular (CTM) of CIBIO. We thank Jack Sullivan, Ron DeBry, Laura Kubatko, Brian Carstens, and 3 anonymous reviewers for insightful and invaluable comments on an early version of the manuscript. This is contribution ISEM−2011−063 from Institut des Sciences de l'Evolution.

REFERENCES

Abramson N.I., Rodchenkova E.N., Kostygov A.Y. 2009. Genetic variation and phylogeography of the bank vole (Clethrionomys glareolus) in Russia with special reference to the introgression of the mtDNA of a closely related species, red-backed vole (C. rutilus). Russ. J. Genet. 45:533–545.

Altuna J. 1970. Hallazgo de una liebre artica (*Lepus timidus*) en el yacimiento prehistorico de Urtiga (Guipuzcoa). Munibe. 22:165–168.

Alves P.C., Ferrand N., Suchentrunk F., Harris D.J. 2003. Ancient introgression of *Lepus timidus* mtDNA into *L. granatensis* and *L. europaeus* in the Iberian Peninsula. Mol. Phylogenet. Evol. 27:70–80.

Alves P.C., Hackländer K. 2008. Lagomorph species: geographical distribution and conservation status. In: Alves P.C., Ferrand N., Hackländer K., editors. Lagomorph biology: evolution, ecology, and conservation. Berlin: Springer. p. 395–406.

Alves P.C., Harris D.J., Melo-Ferreira J., Branco M., Ferrand N., Suchentrunk F., Boursot P. 2006. Hares on thin ice: introgression of mitochondrial DNA in hares and its implications for recent phylogenetic analyses. Mol. Phylogenet. Evol. 40:640–641.

Alves P.C., Melo-Ferreira J., Branco M., Suchentrunk F., Ferrand N., Harris D.J. 2008. Evidence for genetic similarity of two allopatric European hares (*Lepus corsicanus* and *L. castroviejoi*) inferred from nuclear DNA sequences. Mol. Phylogenet. Evol. 46:1191–1197.

Alves P.C., Melo-Ferreira J., Freitas H., Boursot P. 2008. The ubiquitous mountain hare mitochondria: multiple introgressive hybridization in hares, genus *Lepus*. Philos. Trans. R. Soc. Lond B. 363:2831–2839.

Ané C., Larget B., Baum D.A., Smith S.D., Rokas A. 2007. Bayesian estimation of concordance among gene trees. Mol. Biol. Evol. 24:412–426.

Angelici F.M., Luiselli L. 2001. Distribution and status of the Apennine hare *Lepus corsicanus* in continental Italy and Sicily. Oryx. 35:245–249.

Baker A.J., Eger J.L., Peterson R.L., Manning T.H. 1983. Geographic variation and taxonomy of the arctic hare. Acta Zool. Fennica. 174:45–48.

Belfiore N.M., Liu L., Moritz C. 2008. Multilocus phylogenetics of a rapid radiation in the genus Thomomys (Rodentia: Geomyidae). Syst. Biol. 57:294–310.

Berthier P., Excoffier L., Ruedi M. 2006. Recurrent replacement of mtDNA and cryptic hybridization between two sibling bat species *Myotis myotis* and *Myotis blythii*. Proc. R. Soc. Lond. B.273:3101–3109.

Best T.L., Henry T.H. 1994. Lepus othus. Mamm. Species.458:1–5.

Bonhomme M., Cuartero S., Blancher A., Crouau-Roy B. 2009. Assessing natural introgression in 2 biomedical model species, the rhesus macaque (Macaca mulatta) and the long-tailed macaque (Macaca fascicularis). J. Hered. 100:158–169.

Bossu C.M., Near T.J. 2009. Gene trees reveal repeated instances of mitochondrial DNA introgression in orangethroat darters (Percidae: Etheostoma). Syst. Biol. 58:114–129.

Bryant D., Moulton V. 2004. Neighbor-net: an agglomerative method for the construction of phylogenetic networks. Mol. Biol. Evol. 21:255–265.

Buckley T.R., Cordeiro M., Marshall D.C., Simon C. 2006. Differentiating between hypotheses of lineage sorting and introgression in New Zealand alpine cicadas (Maoricicada Dugdale). Syst. Biol. 55:411–425.

Buggs R.J. 2007. Empirical study of hybrid zone movement. Heredity. 99:301–312.

Carneiro M., Ferrand N., Nachman M.W. 2009. Recombination and speciation: loci near centromeres are more differentiated than loci near telomeres between subspecies of the European Rabbit (*Oryctolagus cuniculus*). Genetics. 181:593–606.

Cheddadi R., de Beaulieu J.L., Jouzel J., Andrieu-Ponel V., Laurent J.M., Reille M., Raynaud D., Bar-Hen A. 2005. Similarity of vegetation dynamics during interglacial periods. Proc. Natl. Acad. Sci. U.S.A. 102:13939–13943.

Corbet G.B. 1986. Relationships and origins of the European lagomorphs. Mamm. Rev. 16:105–110.

Currat M., Excoffier L. 2004. Modern humans did not admix with Neanderthals during their range expansion into Europe. PLoS Biol. 2:2264–2274.

Currat M., Ruedi M., Petit R.J., Excoffier L. 2008. The hidden side of invasions: massive introgression by local genes. Evolution. 62:1908–1920.

Dixon K.R., Chapman J.A., Willner G.R., Wilson D.E., Lopez-Forment W. 1983. The New World jackrabbits and hares (genus *Lepus*). 2. Numerical taxonomic analysis. Acta Zool. Fennica. 174:53–56.

Drummond A.J., Ho S.Y., Phillips M.J., Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4:e88.

Drummond A.J., Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214.

Eckert A.J., Carstens B.C. 2008. Does gene flow destroy phylogenetic signal? The performance of three methods for estimating species phylogenies in the presence of gene flow. Mol. Phylogenet. Evol. 49:832–842.

Edwards S.V. 2009. Is a new and general theory of molecular systematics emerging? Evolution. 63:1–19.

Edwards S.V., Liu L., Pearl D.K. 2007. High-resolution species trees without concatenation. Proc. Natl. Acad. Sci. U.S.A. 104: 5936–5941.

Ferris S.D., Sage R.D., Huangj C.M., Nielsen J.T., Ritte U., Wilson A.C. 1983. Flow of mitochondrial DNA across species boundary. Proc. Natl. Acad. Sci. U.S.A. 80:2290–2294.

Flot J.F., Tillier A., Samadi S., Tillier S. 2006. Phase determination from direct sequencing of length-variable DNA regions. Mol. Ecol. Notes. 6:627–638.

Flux J.E.C., Angermann R. 1990. The hares and jackrabbits. In: Chapman J. A. and Flux J. E. C. , editors. Rabbits, hares and pikas. Status survey and conservation action plan. Gland (Switzerland): IUCN.

Fredsted T., Wincentz T., Villesen P. 2006. Introgression of mountain hare (*Lepus timidus*) mitochondrial DNA into wild brown hares (*Lepus europaeus*) in Denmark. BMC Ecol. 6:17.

Funk D.J., Omland K.E. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Syst. 34:397–423.

Garrick R.C., Sunnucks P., Dyer R.J. 2010. Nuclear gene phylogeography using PHASE: dealing with unresolved genotypes, lost alleles, and systematic bias in parameter estimation. BMC Evol. Biol. 10:118.

Gauthier O., Lapointe F.J. 2007. Seeing the trees for the network: consensus, information content, and superphylogenies. Syst. Biol. 56:345–355.

Geraldes A., Basset P., Gibson B., Smith K.L., Harr B., Yu H.T., Bulatova N., Ziv Y., Nachman M.W. 2008. Inferring the history of speciation in house mice from autosomal, X-linked, Y-linked and mitochondrial genes. Mol. Ecol. 17:5349–5363.

Gissi C., Gullberg A., Arnason U. 1998. The complete mitochondrial DNA sequence of the rabbit, Oryctolagus cuniculus. Genomics. 50:161–169.

Hasegawa M., Kishino H., Yano T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22:160–174.

Heled J., Drummond A.J. 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27:570–580.

Hewitt G.M. 2001. Speciation, hybrid zones and phylogeography—or seeing genes in space and time. Mol. Ecol. 10:537–549.

Hewitt G.M. 2004. Genetic consequences of climatic oscillations in the Quaternary. Philos. Trans. R. Soc. Lond. B. 359:183–195.

Hewitt G.M. 2011. Quaternary phylogeography: the roots of hybrid zones. Genetica. 139:617–638.

Hey J. 2009. The divergence of chimpanzee species and subspecies as revealed in multipopulation isolation-with-migration analyses. Mol. Biol. Evol. 27:921–933.
Hey J. 2010. Isolation with migration models for more than two populations. Mol. Biol. Evol. 27:905–920.
Hey J., Nielsen R. 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. Genetics. 167:747–760.
Jin G., Nakhleh L., Snir S., Tuller T. 2006. Maximum likelihood of phylogenetic networks. Bioinformatics. 22:2604–2611.
Joly S., McLenachan P.A., Lockhart P.J. 2009. A statistical approach for distinguishing hybridization and incomplete lineage sorting. Am. Nat. 174:E54–E70.
Kimura M. 1969. The number of heterozygous nucleotide sites maintained in a finite population due to steady flux of mutations. Genetics. 61:893–903.
Kubatko L.S. 2009. Identifying hybridization events in the presence of coalescence via model selection. Syst. Biol. 58:478–488.
Kubatko L.S., Degnan J.H. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. Syst. Biol. 56:17–24.
Laval G., Excoffier L. 2004. SIMCOAL 2.0: a program to simulate genomic diversity over large recombining regions in a subdivided population with a complex history. Bioinformatics. 20: 2485–2487.
Lopez-Martinez N. 1980. Les lagomorphs (Mammalia) du pléistocène supérieur de Jaurens. Nouv. Arch. Mus. Hist. Nat. Lyon. 18:5–16.
Lopez-Martinez N. 2008. The lagomorph fossil record and the origin of the European rabbit. In: Alves P. C., Ferrand N., Hackländer K., editors. Lagomorph biology: evolution, ecology, and conservation. Berlin: Springer-Verlag. p. 27–46.
Maddison W.P., Maddison D.R. 2009. Mesquite: a modular system for evolutionary analysis, version 2.6. Available from: http://mesquiteproject.org.
Marboutin E., Peroux R. 1995. Survival pattern of European hare in a decreasing population. J. Appl. Ecol. 32:809–816.
Matthee C.A., van Vuuren B.J., Bell D., Robinson T.J. 2004. A molecular supermatrix of the rabbits and hares (Leporidae) allows for the identification of five intercontinental exchanges during the Miocene. Syst. Biol. 53:433–447.
McCormack J.E., Huang H., Knowles L.L. 2009. Maximum likelihood estimates of species trees: how accuracy of phylogenetic inference depends upon the divergence history and sampling design. Syst. Biol. 58:501–508.
Melo-Ferreira J., Alves P.C., Freitas H., Ferrand N., Boursot P. 2009. The genomic legacy from the extinct *Lepus timidus* to the three hare species of Iberia: contrast between mtDNA, sex chromosomes and autosomes. Mol. Ecol. 18:2643–2658.
Melo-Ferreira J., Alves P.C., Rocha J., Ferrand N., Boursot P. 2011. Interspecific X-chromosome and mitochondrial DNA introgression in the Iberian hare: selection or allele surfing? Evolution. 65: 1956–1968.
Melo-Ferreira J., Boursot P., Randi E., Kryukov A., Suchentrunk F., Ferrand N., Alves P.C. 2007. The rise and fall of the mountain hare (*Lepus timidus*) during Pleistocene glaciations: expansion and retreat with hybridization in the Iberian Peninsula. Mol. Ecol. 16:605–618.
Melo-Ferreira J., Boursot P., Suchentrunk F., Ferrand N., Alves P.C. 2005. Invasion from the cold past: extensive introgression of mountain hare (*Lepus timidus*) mitochondrial DNA into three other hare species in northern Iberia. Mol. Ecol. 14:2459–2464.
Mitchell-Jones A., Amori G., Bogdanowicz W., Krystufek B., Reijnders P., Spitzenberger F., Stubbe M., Thissen J., Vohralik V., Zima J. 1999. Atlas of European mammals. London: Academic Press.
Moore W.S. 1995. Inferring phylogenies from mtDNA variation—mitochondrial-gene trees versus nuclear-gene trees. Evolution. 49:718–726.
Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.
Nielsen R., Wakeley J. 2001. Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics. 158:885–896.
Petit J.R., Jouzel J., Raynaud D., Barkov N.I., Barnola J.M., Basile I., Bender M., Chappellaz J., Davis M., Delaygue G., Delmotte M., Kotlyakov V.M., Legrand M., Lipenkov V.Y., Lorius C., Pepin L., Ritz C., Saltzman E., Stievenard M. 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature. 399:429–436.
Pierpaoli M., Riga F., Trocchi V., Randi E. 1999. Species distinction and evolutionary relationships of the Italian hare (*Lepus corsicanus*) as described by mitochondrial DNA sequencing. Mol. Ecol. 8:1805–1817.
Pietri C., Alves P.C., Melo-Ferreira J. 2011. Hares in Corsica: high prevalence of *Lepus corsicanus* and hybridization with introduced *L. europaeus* and *L. granatensis*. Eur. J. Wildl. Res. 52:313–321.
Posada D. 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25:1253–1256.
Rambaut A., Drummond A.J. 2007. Tracer v1.4. Available from: http://beast.bio.ed.ac.uk/Tracer.
Robinson T.J., Matthee C.A. 2005. Phylogeny and evolutionary origins of the Leporidae: a review of cytogenetics, molecular analyses and a supermatrix analysis. Mamm. Rev. 35:231–247.
Roca A.L., Georgiadis N., O'Brien S.J. 2005. Cytonuclear genomic dissociation in African elephant species. Nat. Genet. 37:96–100.
Ruedi M., Smith M.F., Patton J.L. 1997. Phylogenetic evidence of mitochondrial DNA introgression among pocket gophers in New Mexico (family Geomyidae). Mol. Ecol. 6:453–462.
Sambrook E., Fritsch F., Maniatis T. 1989. Molecular cloning. Cold Spring Harbour, NY: Cold Spring Harbour Press.
Shimodaira H., Hasegawa M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16:1114–1116.
Spinks P.Q., Shaffer H.B. 2009. Conflicting mitochondrial and nuclear phylogenies for the widely disjunct ewmys (Testudines: Emydidae) species complex, and what they tell us about biogeography and hybridization. Syst. Biol. 58:1–20.
Stephens M., Donnelly P. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am. J. Hum. Genet. 73:1162–1169.
Stephens M., Smith N.J., Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68:978–989.
Stevison L.S., Kohn M.H. 2009. Divergence population genetic analysis of hybridization between rhesus and cynomolgus macaques. Mol. Ecol. 18:2457–2475.
Strasburg J.L., Rieseberg L.H. 2010. How robust are "Isolation with Migration" analyses to violations of the IM model? A simulation study. Mol. Biol. Evol. 27:297–310.
Suchentrunk F., Mamuris Z., Stamatis C., Ben Slimen H., Hackländer K., Haerer G., Giacometti M. 2005. Introgressive hybridization in wild living mountain hares (*L. timidus varronis*) and brown hares (*L. europaeus*) and morphological consequences. Mamm. Biol. 70(Suppl.):39–40.
Swofford D.L. 2001. PAUP* phylogenetic analysis using parsimony (and other methods). Sunderland (MA): Sinauer Associates.
Tegelström H. 1987. Transfer of mitochondrial DNA from the northern red-backed vole (*Clethrionomys rutilus*) to the bank vole (*C. glareolus*). J. Mol. Evol. 24:218–227.
Thompson J.D., Higgins D.G., Gibson T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673–4680.
Thulin C.G., Fang M., Averianov A.O. 2006. Introgression from *Lepus europaeus* to *L. timidus* in Russia revealed by mitochondrial single nucleotide polymorphisms and nuclear microsatellites. Hereditas. 143:68–76.
Thulin C.G., Jaarola M., Tegelstrom H. 1997. The occurrence of mountain hare mitochondrial DNA in wild brown hares. Mol. Ecol. 6:463–467.
Wallner B., Huber S., Achmann R. 2001. Non-invasive PCR sexing of rabbits (*Oryctolagus cuniculus*) and hares (*Lepus europaeus*). Mamm. Biol. 66:190–192.
Waltari E., Cook J.A. 2005. Hares on ice: phylogeography and historical demographics of *Lepus arcticus, L. othus*, and *L. timidus* (Mammalia: Lagomorpha). Mol. Ecol. 14:3005–3016.
Wang L., Luzynski K., Pool J.E., Janousek V., Dufkova P., Vyskocilova M.M., Teeter K.C., Nachman M.W., Munclinger P., Macholan M.,

Pialek J., Tucker P.K. 2011. Measures of linkage disequilibrium among neighbouring SNPs indicate asymmetries across the house mouse hybrid zone. Mol. Ecol. 20:2985–3000.

Waterston R.H., Lindblad-Toh K., Birney E., Rogers J., Abril J.F., Agarwal P., Agarwala R., Ainscough R., Alexandersson M., An P., Antonarakis S.E., Attwood J., Baertsch R., Bailey J., Barlow K., Beck S., Berry E., Birren B., Bloom T., Bork P., Botcherby M., Bray N., Brent M.R., Brown D.G., Brown S.D., Bult C., Burton J., Butler J., Campbell R.D., Carninci P., Cawley S., Chiaromonte F., Chinwalla A.T., Church D.M., Clamp M., Clee C., Collins F.S., Cook L.L., Copley R.R., Coulson A., Couronne O., Cuff J., Curwen V., Cutts T., Daly M., David R., Davies J., Delehaunty K.D., Deri J., Dermitzakis E.T., Dewey C., Dickens N.J., Diekhans M., Dodge S., Dubchak I., Dunn D.M., Eddy S.R., Elnitski L., Emes R.D., Eswara P., Eyras E., Felsenfeld A., Fewell G.A., Flicek P., Foley K., Frankel W.N., Fulton L.A., Fulton R.S., Furey T.S., Gage D., Gibbs R.A., Glusman G., Gnerre S., Goldman N., Goodstadt L., Grafham D., Graves T.A., Green E.D., Gregory S., Guigo R., Guyer M., Hardison R.C., Haussler D., Hayashizaki Y., Hillier L.W., Hinrichs A., Hlavina W., Holzer T., Hsu F., Hua A., Hubbard T., Hunt A., Jackson I., Jaffe D.B., Johnson L.S., Jones M., Jones T.A., Joy A., Kamal M., Karlsson E.K., Karolchik D., Kasprzyk A., Kawai J., Keibler E., Kells C., Kent W.J., Kirby A., Kolbe D.L., Korf I., Kucherlapati R.S., Kulbokas E.J., Kulp D., Landers T., Leger J.P., Leonard S., Letunic I., Levine R., Li J., Li M., Lloyd C., Lucas S., Ma B., Maglott D.R., Mardis E.R., Matthews L., Mauceli E., Mayer J.H., McCarthy M., McCombie W.R., McLaren S., McLay K., McPherson J.D., Meldrim J., Meredith B., Mesirov J.P., Miller W., Miner T.L., Mongin E., Montgomery K.T., Morgan M., Mott R., Mullikin J.C., Muzny D.M., Nash W.E., Nelson J.O., Nhan M.N., Nicol R., Ning Z., Nusbaum C., O'Connor M.J., Okazaki Y., Oliver K., Overton-Larty E., Pachter L., Parra G., Pepin K.H., Peterson J., Pevzner P., Plumb R., Pohl C.S., Poliakov A., Ponce T.C., Ponting C.P., Potter S., Quail M., Reymond A., Roe B.A., Roskin K.M., Rubin E.M., Rust A.G., Santos R., Sapojnikov V., Schultz B., Schultz J., Schwartz M.S., Schwartz S., Scott C., Seaman S., Searle S., Sharpe T., Sheridan A., Shownkeen R., Sims S., Singer J.B., Slater G., Smit A., Smith D.R., Spencer B., Stabenau A., Stange-Thomann N., Sugnet C., Suyama M., Tesler G., Thompson J., Torrents D., Trevaskis E., Tromp J., Ucla C., Ureta-Vidal A., Vinson J.P., Von Niederhausern A.C., Wade C.M., Wall M., Weber R.J., Weiss R.B., Wendl M.C., West A.P., Wetterstrand K., Wheeler R., Whelan S., Wierzbowski J., Willey D., Williams S., Wilson R.K., Winter E., Worley K.C., Wyman D., Yang S., Yang S.P., Zdobnov E.M., Zody M.C., Lander E.S. 2002. Initial sequencing and comparative analysis of the mouse genome. Nature. 420:520–562.

Woerner A.E., Cox M.P., Hammer M.F. 2007. Recombination-filtered genomic datasets by information maximization. Bioinformatics. 23:1851–1853.

Won Y.J., Hey J. 2005. Divergence population genetics of chimpanzees. Mol. Biol. Evol. 22:297–307.

Yamada F., Takaki M., Suzuki H. 2002. Molecular phylogeny of Japanese Leporidae, the Amami rabbit *Pentalagus furnessi*, the Japanese hare *Lepus brachyurus*, and the mountain hare *Lepus timidus*, inferred from mitochondrial DNA sequences. Genes Genet. Syst. 77:107–116.

Zwickl D.J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. [PhD. dissertation]. Austin (TX): University of Texas.