

SUPPORTING INFORMATION:

Introgression drives repeated evolution of winter coat color polymorphism in hares

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SUPPLEMENTARY METHODS

Samples and whole genome sequencing

We generated whole-genome sequencing data at individual low coverage for 59 mountain hares (*Lepus timidus*) collected from the Faroe Islands (N=20 diploid individuals), Fennoscandia (N=19; Sweden, Finland, Norway) and the Alps (N=20; Austria, Italy, France, Switzerland) (Tables S1 and S2). Genomic DNA was extracted from ear or organ tissues with PureLink Genomic DNA Mini Kit (Invitrogen) and quantified with Qubit fluorometer (Invitrogen). Double-indexed libraries were prepared according to a modified version of the protocol of Meyer and Kircher (1). Briefly, genomic DNA was sheared with Bioruptor® Pico and up to 500 ng of sheared DNA was used for blunt-end repair, adapter ligation and adapter fill-in. Indexing polymerase chain reactions (PCRs) was performed in 50 µl reactions containing 1X Herculase II reaction buffer, 250 µM each dNTP, 0.2 µM P5 and P7 indexing primers, 0.5 µl Herculase II polymerase and 3.5 µl DNA template. The PCR profiles were as follows: initial denaturation $98^{\circ}C - 2$ min, 10 or 16 cycles: $98^{\circ}C - 20$ s, $60^{\circ}C - 20$ s, $72^{\circ}\text{C} - 20$ s, and final elongation $72^{\circ}\text{C} - 5$ min. The indexed libraries were quantified with quantitative PCR (qPCR), pooled and sequenced at low individual coverage in five lanes of an Illumina HiSeq 1500 sequencer in three separate runs (125 bp paired-end) at CIBIO-InBIO's New-Gen sequencing platform, Portugal.

In addition, whole-genome sequencing data at higher individual coverage from specimens from six *Lepus* species was analyzed, representing mountain hares (*L. timidus*) from the Faroe Islands, the Alps and Fennoscandia, European brown hares (*L. europaeus*) from Iberia and central Europe, two Iberian hares (*L. granatensis*) from the Iberian Peninsula, one broom hare (*L. castroviejoi*) from the Iberian Peninsula, one winter-brown and two winter-white snowshoe hares (*L. americanus*), and two black-tailed jackrabbits (*L. californicus*) (from 2, 3, 4 and this work) (Table S3).

Sequence data processing

The raw Illumina reads of whole-genome low coverage sequence data for 59 mountain hares were demultiplexed and their quality was assessed with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). We removed adapters and filtered low quality reads using Trimmomatic (5) with the following settings: TRAILING:15, SLIDINGWINDOW:4:15, MINLEN:30. The cleaned read pairs were mapped to a hare pseudo-reference (3) using bwa-mem with default settings (6). This reference was built by replacing in the European rabbit (*Oryctolagus cuniculus*) reference genome fixed differences to the group of three hare species (*L. americanus*, *L. granatensis* and *L. timidus*) (3). The European rabbit is related to hares $(-11.8 \text{ million years}$ divergence; 7) and its reference genome has high quality chromosome-level assembly and annotation (8). PCR duplicates were removed with PICARD (http://broadinstitute.github.io/picard/) and reads were locally realigned with GATK IndelRealigner (9). Then, for each individual, we calculated mean coverage and the proportion of properly mapped reads using Samtools (10). Two specimens with depth $>2X$ were randomly subsampled to $<1.5X$ to avoid overrepresentation of individual samples (Table S2). Sequencing resulted in a total coverage of 16.8X for Faroese hares, 17.1X for Alpine hares, and 18.0X for Fennoscandian hares. The resulting BAM files were used as inputs for further analyses. Taking advantage of sequencing individually barcoded DNA libraries, for all analyses using low coverage data, only sites represented by at least six different individuals from each population were used, to avoid strong biases in the representation the sequenced specimens.

The raw reads of higher coverage data were processed as described above, except the Trimmomatic settings, which were as follows: TRAILING:20, SLIDINGWINDOW:4:20, MINLEN:36. Adapters were removed with cutadapt (11). SNP calling for the whole chromosome 4 was performed with Samtools (mpileup) and Bcftools (call -m) separately for each individual. Resulting vcf files were filtered (bcftools filter) for minimum depth of 6X, maximum depth of 2 times the average depth, and a minimum QUAL of 50.

Genetic diversity

We used PoPoolation (12) to calculate genome-wide nucleotide diversity (π) and theta (θ) for each population separately (Fig. S8C). For each population, mpileup files with masked SNPs in the proximity of indels (--indel-window 5) were used as input to estimate diversity indices in 200 kb non-overlapping windows, using the following parameters: --min-count 4 --min-coverage 10 --max-coverage 51 --min-qual 20 --mincovered-fraction 0.25.

Population structure and evolutionary relationship

Principal component analysis (PCA) based on low individual coverage data was performed with ANGSD (13), using a single-read sampling approach (IBS) appropriate for low-depth data (Fig. S1A). Genotype-likelihoods were calculated using the Samtools method, as implemented in ANGSD (-GL 1). For all ANGSD analyses we used the following filtering criteria: -uniqueOnly 1 -remove_bads 1 -trim 0 -C 50 baq 2 -minMapQ 20 -minQ 20 -SNP_pval 1e-6. The PCA was based on 123,995 SNPs sampled every 20 kb with PLINK --bp-space command (14) to reduce non-independence due to linkage. The proportion of variance explained by each component was calculated with the R function *prcomp*.

A neighbor-joining tree (NJ) was constructed based on pairwise genetic distances between individuals (Fig. S1B). The distances were estimated with ngsDist (15) and ngsTools (16) from genotype posterior probabilities generated with ANGSD (-doGeno 8 -doPost 1), based on 127,413 polymorphic sites sampled at least every 20 kb. We performed 100 bootstrapped replicates by randomly sampling with replacement blocks of 100 SNPs (-n_boot_rep 100 -boot_block_size 100). Trees were generated using FastME (17).

We also constructed a population tree based on allele frequencies (Fig. 1D), as implemented in TreeMix (18). First, allele frequencies were estimated with ANGSD, using the European rabbit genome reference (OryCun2.0, Ensembl, release 80) to determine the ancestral state (-doMajorMinor 5 -doMaf 1). Then, the ANGSD output was converted into the TreeMix input (allele counts at each SNP) with custom python scripts (available at https://github.com/evochange/far), sampling sites at least every 20 kb, resulting in 136,834 SNPs. TreeMix was run 1,000 times.

For both the NJ and TreeMix analyses, the snowshoe hare (*L. americanus*) was used as outgroup. Trees were summarized with SumTrees of the DendroPy library (19) and visualized with FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

Approximate Bayesian Computation

The introduction of mountain hares into the Faroe Islands was modelled using Approximate Bayesian Computation (ABC; 20). The demographic scenario was built in accordance with the historical information about the introduction, but with wide uniform prior distributions to allow parameter inference (Table S4). Briefly, it consisted of an ancestral population of size *N_{FSC}* (effective population size of the Fennoscandia population) that split at time *Ti* generations ago (the time of the introduction into the Faroe Islands; a generation length of two years was considered; 21); while the Fennoscandian effective population size (*N_{FSC}*) was kept constant, a growth rate of *g* was set for the Faroese population, from the time of introduction (T_i) until the present (effective population size *NFAR*). Simulations were performed under a mutation rate estimated from the data, with a transition rate set to 0.33.

The data was characterized by the following summary statistics: number of segregating sites (S), nucleotide diversity (θ_{π}) , Watterson's theta ($\theta_{\text{Watterson}}$), and Tajima's D per population, and F_{ST} between populations, including their means, standard deviations and medians (27 summary statistics). The summary statistics were calculated using PoPoolation2 (22) and npstat (23), pooling the sequencing data for each population. For the empirical dataset, we removed sites overlapping coding regions and repeats, sites with a base quality <30, and reads with a mapping quality <30. Only sites represented by at least 10 individuals in each population (determined by the individual barcodes) and with coverage between 10X and 51X per population were retained. The summary statistics were calculated from independent windows (at least 100 kb apart) of 2 kb with at least 85% of the window covered, which resulted in 501 windows. Demographic inference is sensible to the inclusion of sequencing errors as rare variants (24, 25), and we thus filtered empirical and simulated datasets to include SNPs with at least three observations of the minor allele. This approach has been suggested to be preferable to the potential bias introduced by sequencing errors (26).

Simulations of the demographic model (Fig. 1E) were performed using fastsimcoal26 (27) with 100,000 replicates. We simulated 501 windows of 2 kb under a finite-site model, considering 20 diploid individuals (40 haploid) from FAR and 19 diploids (38 haploid) from FSC, as in our dataset. We generated Arlequin files in diploid formats (-g), outputting whole DNA sequences (-S). These files were converted to population bam files, that were used to produce mpileup and sync files, inputs for npstat and PoPoolation2. Summary statistics were calculated as for the empirical dataset, in 501 2 kb non-overlapping windows for each simulation.

Parameter estimation was performed using ABCtoolbox package (28). Summary statistics were transformed via Partial Least Squares (PLS), using the *PLS* R package (29), and the root mean squared error plots (RMSEP) were inspected to determine the number of PLS components. The marginal density P-value and the Tukey P-value, both based on 1,000 retained simulations (1% closest to the empirical data), were used to evaluate whether the model was able to reproduce the empirical summary statistics. While our approach did

not explicitly model the occurrence of sequencing errors, filtering the empirical and simulated datasets for minimum allele counts allowed recovering well the empirical statistics under our demographic scenario (marginal density P-value $= 0.73$; Tukey P-value $= 0.74$). We used the PLS transformed statistics to estimate demographic parameters by applying the ABC-GLM algorithm (30) and retaining 1% of the simulations closest to the observed data (Fig. S2, Table S4). Finally, the accuracy of parameter estimates was assessed by cross-validation, based on 1,000 random simulations for which the true parameter values were known (pseudoobserved datasets) (Fig. S3).

Detection of differentiation and association outliers

Scans of differentiation were performed using an array of tests based either on pooled sequence data per population/phenotype (ensuring a minimum representation of six individuals) or from genotype likelihoods, as implemented in PoPoolation2 (22) and ANGSD (13), respectively.

A genome scan of Population Branch Statistic (PBS; 31) was performed in 20 kb non-overlapping windows (Fig. 2A). Given three structured populations (Fig. S1A) and the closer relationship of Faroese and Fennoscandian populations (Fig. 1D, Fig. S1B), this statistic is suited to identify localized strong allele frequency changes in the specific branch of the Faroese hares, which deviate from the genome-wide norm, and may result from recent selective sweeps (31). PBS was calculated in 20 kb non-overlapping windows, based on allele frequencies estimated from individual genotype likelihoods, as implemented in ANGSD (13). The European rabbit reference was used to determine the ancestral state (-anc). PBS values were plotted against chromosome position using the *qqman* R package.

Association tests based on allele frequency differences between cases and controls using genotype likelihoods were performed in ANGSD (-doAsso 1), setting the Faroese individuals (winter-grey phenotype) as cases and the remaining individuals (from Alps and Fennoscandia) as controls (Fig. S4E). For this analysis, additional filters for minimum (-setMinDepth 30) and maximum (setMaxDepth 150) depth were used. The resulting likelihood ratio statistic (LRT) was converted to $-log_{10}(P)$ values assuming a chi-square distribution with one degree of freedom. The mean $-log_{10}(P)$ values for windows of 100 SNPs were plotted against chromosome positions with a custom R script (available at https://github.com/evochange/far). The Bonferroni correction for multiple tests was used to determine the significance threshold, which was set to $p < 6.15x10^{-7}$.

In addition, the scan was performed for single pairwise comparisons, i.e. Faroese hares vs. Alpine hares and Faroese hares vs. Fennoscandian hares, adjusting coverage and significance thresholds (Fig. S4).

For PoPoolation2 analyses, concatenated bam files were used to produce mpileup files with Samtools, removing reads with mapping quality lower than 20. The mpileup files were then converted to synchronized files requiring base qualities of at least 20 and masking 5 bp on either side of inferred insertion-deletions (- indel-window 5). The synchronized file was then used as an input to calculate F_{ST} (--karlsson-fst) and Fisher Exact Tests (FET) in non-overlapping 20 kb windows, containing at least 30 SNPs (Fig. S4A and B). Analyses contrasting Faroese hares with Alpine hares and Fennoscandian hares pooled together were performed using the following parameters: --min-count 4, --min-coverage 10, --max-coverage 51,105 --window-size 20000 - step-size 20000 --min-covered-fraction 0.25 --pool-size 40:78. The maximum coverage was chosen to be 3 times the mean coverage of each concatenated bam. For FET, a Bonferroni-corrected significance threshold was used and set at $p < 3.7x10^{-7}$. Analyses contrasting Faroese hares and Alpine hares, and Faroese hares and Fennoscandian hares were done adjusting coverage and significance thresholds and pool sizes (Table S4).

Discovery of structural variants

The Faroese mountain hare genome sequenced at 6.9X was used to inspect the longest candidate region (mapped to chromosome 4) for structural variation, including deletions, insertions, inversions, duplications and translocations. Lumpy (32), which uses read-pair, split-read and read-depth information to detect structural variation, Delly (33), which relies on read-pair and split-read information, and BreakDancer, which uses readpair information (34), were used with default settings. In addition, candidate structural variants were confirmed through visual inspection of the bam files in IGV (Integrative Genomics Viewer; 35, 36). A candidate insertion-deletion was detected and validated using a three-primer PCR assay, which resulted in distinguishable PCR products: one forward primer anchored close to the insertion-deletion (*LTM_indel_F1*), one forward primer within the insertion (*LTM_indel_F01*), and a reverse primer immediately after the insertion-deletion (*LTM* indel R) (Table S5). The sequences of the amplified fragments were confirmed by Sanger sequencing.

SNP and insertion-deletion genotyping

To confirm the genotype-phenotype association within the candidate regions in chromosomes 4 and 15, clarify the segregation patterns of the inferred variants and remove the confounding effect of population structure that could create spurious signals of association in our genome scans, we genotyped 59 SNPs and the inferred insertion-deletion in 97 *L. timidus* individuals from the same sampling localities included in the genome scans: FAR, $N = 35$; FSC, $N = 34$; ALP, $N = 28$ (Table S1, Fig. S5). DNA extraction for the additional samples was performed as previously described. In addition, 29 individuals from Sweden and Russia, deposited in the collection of the Swedish Museum of Natural History, originally sampled during winter and for which the winter color phenotype could be assessed – grey ($N = 15$), white ($N = 14$) – were also included in the genotyping assay (Tables S1, S6 and S7). DNA extraction of museum skin samples was performed in dedicated laboratory facilities suitable for manipulation of samples with low content of endogenous DNA, following Dabney et al. (37). All genotyped SNPs were chosen for having allele frequency differences between the Faroese and other populations between 0.75 and 1, according to the PoPoolation2 analysis (snp-frequencydiff.pl), and were located in windows showing extreme PBS. Thirty-six and five SNPs were agglomerated in the regions of *Agouti* and *USP38*, respectively, and the remaining 18 were located in other windows of extreme PBS scattered along the genome. SNPs were genotyped using the MassARRAY technology at the Genome Transcriptome Facility in the University of Bordeaux, France. The insertion-deletion was genotyped using the devised three-primer PCR approach described above with some modifications for the museum samples (indicated in parentheses). PCRs were performed in 6 µl reactions containing 2.1 µl Qiagen Multiplex PCR Master Mix (2.4 µl for museum samples), 0.2 µl primer F1, 0.1 µl primer F2, 0.3 µl primer R, 2.8 µl water (2.4 μ l) and 0.5 μ l template DNA. The PCR profile was as follows: 95 \degree C - 15 min, 35 cycles of 95 \degree C - 30 s, 62° C - 30 s (60 s) and 72^oC - 35 s (40 s), and 72^oC - 10 min. For the museum samples showing greater level of DNA degradation, an independent validation of the genotypes was performed using another three-primer PCR approach (primers *LTM_indel_*F2, *LTM_indel_F3* and *LTM_indel_R*; Table S5). A volume of 0.3 µl of each primer was used in these additional PCR reactions. Significance of the associations of SNPs and the insertiondeletion with the winter color phenotype was tested using *SNPassoc* R package (38).

Gene expression analysis

The expression of *Agouti* hair-cycle isoform (*Agouti-HC*) and reference genes *ACTB* and *SDHA* (Figs. 3 and S7) was quantified in skin biopsies sampled during the autumn molt from winter-grey (FAR; N=3) and winter-white hares (ALP; N=5). Skin was opportunistically sampled from the dorsum of mountain hares killed during regular hunting campaigns, representing three stages of the progressing molt: brown, intermediate and white/grey (nine skin samples from the three Faroese hares, and 15 skin samples from the 5 Alpine hares) (39). Total RNA was extracted from 30 mg of each skin sample with the RNeasy Mini Kit (Qiagen). Tissue was homogenized in a rotor-stator homogenizer (Mixer Mill MM400, Retsch) at 30 Hz for 10 min. The RNA integrity was checked with the Agilent Technologies TapeStation for a minimum RIN > 7 (Table S9). Firststrand cDNA synthesis was performed using oligo (dT) primers and the GRS cDNA synthesis kit (GRISP) using 400 ng of RNA from skin sample (except for two samples that had insufficient amount of RNA, resulting in no qPCR product). Relative expression was quantified using quantitative PCR (qPCR) of the gene of interest, normalized across individuals to the reference genes. Primers amplifying the *Agouti* hair cycle isoform (*Agouti-HC*) were designed to anchor in the *Agouti-HC* non-coding exon and the first *Agouti* coding exon. All primers, both for *Agouti* and reference genes, were anchored in regions showing no polymorphisms between winter color morphs. The amplification efficiencies were calculated based on the slope of a regression line fitted to C_t values of five samples from 2-fold serial dilutions ($E = 10^{(-1/slope)} - 1$), using two replicates per sample (Table S9). Then, three replicate qPCR reactions were performed per skin sample, using $1X$ iTaqTM universal SYBR[®] green supermix (Bio-Rad), 0.4 µM each primer and 1µl cDNA (2-fold dilution of stock cDNA, except for four samples marked with asterisks in Table S9), in a total volume of 10 µl. The qPCR thermal conditions were as follows: 95° C – 30 s polymerase activation and cDNA denaturation, 40 cycles of 95° C – 5 s, 62° C – 30 s, and melting curve analysis from 65 $^{\circ}$ C to 95 $^{\circ}$ C, with 0.5 $^{\circ}$ C increments. The raw qPCR results, presented as the threshold cycle (C_t) , were used to calculate expression of the *Agouti-HC* isoform, normalized by the expression of the *ACTB* and *SDHA* genes using the formula $2^{-\Delta Ct}$, where $\Delta C_t = C_t^{A \text{g} \cdot outi}}$ - C_t ref gene. We performed calculations by subtracting the mean C_t of the reference gene from each technical replicate C^t of the *Agouti* gene (Table S9), to present the relative expression as normalized individual data points. Additionally, we performed a Bayesian analysis where raw qPCR data were represented as molecule counts, and described using generalized linear mixed models under a Poisson-lognormal error distribution (40). This method is suitable also for low-abundant targets, as it uses information about no amplification status. Although reference genes may sharpen estimates of model parameters, they are not strictly required, because normalization is performed within the model. We used the naïve model implemented in *MCMC.qpcr* R package to infer expression levels of all three genes, *Agouti*, *ACTB*, *SDHA* (Fig. S7B).

Selection analyses

Tajima's D (41) for chromosome 4 (Fig. S8D) was calculated with PoPoolation1 after subsampling the mpileup file to ensure uniform coverage (--target-coverage 10), as recommended in the software's manual. Tajima's D was estimated in 200 kb non-overlapping windows with the following parameters: --min-count 1 --min-coverage 4 --max-coverage 10 --min-qual 20 --min-covered-fraction 0.25 --dissable-corrections.

To identify signals of selective sweeps along chromosome 4, we used Pool-hmm (42), which implements a hidden Markov model to detect signatures of selective sweeps in Pool-Seq data (Figs. 2D, S8 and S9). Based on the pattern of allele frequencies, this method predicts the most likely hidden state at each site from three possible states (neutral, intermediate, selection). The hidden Markov model, takes into account also the correlation of allele frequencies between sites in addition to the site frequency spectrum (SFS), thus being more robust to bottleneck scenarios than composite likelihood ratio approaches (43). We ran Pool-hmm for pooled sequence data per population with the following parameters: -n 40 -c 10 -C 51 -q 30 -e sanger - pred -k 1e-30 --theta 0.0034 (theta estimated with PoPoolation1). To take into account the confounding effect of the strong non-equilibrium situation imposed by the demographic history, the transition probability between hidden states (k) was determined based on neutral data simulated according to the inferred demographic model using ABC (Table S4). Simulations were in addition performed retaining the extremes of the 95% high posterior density intervals that maximize the strength of the bottleneck and population growth (lower 95% HPD value for *N^F* and *g* and larger 95% HPD for *NFAR*; Table S4). We simulated 1,000 replicates of 1 Mb regions using fastsimcoal26 (27), assuming recombination rate 1 cM/Mb (1x10-8 per bp per generation) (44). The conservative $k=1e-30$ was retained, which resulted in a False Discovery Rate $= 0$ under the demographic scenario and 0.008 under the extreme bottleneck and growth scenario.

We also looked for signals of selective sweeps along chromosome 4 using the composite likelihood ratio test derived from genotype likelihoods, implemented in SweeD (45) (Figs. S8 and S9). This method detects signals of recent positive selection based on local deviations in the SFS relative to the background SFS.

The input for SweeD was prepared by converting the ANGSD allele frequencies output (mafs file) into the SweepFinder format, using a custom R script (available at https://github.com/evochange/far). The European rabbit allele (ancestral state) was used to determine the major allele (-doMajorMinor 5). We ran SweeD in ~20 kb windows (-grid 4570) using polymorphic sites and fixed derived sites. The significance P-value threshold for CLR was defined based on the CLR distribution from data simulated according to the demographic model inferred using ABC, using the parameters described above. We simulated 1,000 windows of 10 Mb, assuming recombination rate 1 cM/Mb (44), using *msms* (46) and used P<0.01 as the CLR significance threshold.

Finally, we estimated the probability of fixation of a rare allele $(1/2N_e)$ after ~65 years of evolution under drift alone with three sets of parameters using the forward simulations implemented in SLiM3 (47). The probability of fixation at 33 and 65 generations (considering putative generation times of two and one year, respectively) was determined using 10,000 replicates of the drift process under non-equilibrium scenarios (Table S10). The three tested scenarios used 1) the modes of ABC parameter estimates for size of initial population and growth rate; 2) the low 95% HPD interval of ABC parameter estimates for size of initial population and growth rate (implying the strongest bottleneck and population growth); and 3) the historical record of four founder individuals and the growth rate needed to achieve the low 95% HPD interval value of the ABC inference for current effective population size of the Faroese hare (20,000 diploid individuals) (Table S10). Negative growth backward-in-time inferred from the ABC analysis was converted into positive growth for the forward-in-time simulations. The probability of fixation was determined by the proportion of simulations where fixation was achieved at each time point.

Topology weighting

To understand the evolutionary origin of the variants associated with winter coloration in mountain hares, we first performed a topology weighting analysis along chromosome 4, as implemented in Twisst (48), using whole-genome sequencing data from *Lepus* species obtained at higher coverage (6.1 - 29.3X; Table S3). We used genomics general scripts available at https://github.com/simonhmartin/genomics general to convert the vcf file to "geno" format (script: parseVCF.py), and then to calculate neighbor-joining trees in windows of 50 SNPs (script: phyml_sliding_windows.py). Topology weighting was performed considering three different sets of species/populations: 1) one winter-grey Faroese mountain hare (*L. timidus*; FAR), two winter-white

mountain hares, from the Alps and Fennoscandia (MTH), two Iberian hares (IBH), and two European brown hares - one from the Iberian Peninsula (EBH_IB) and one from central Europe (EBH_CE) (Fig. S10A); 2) one winter-grey Faroese mountain hare (FAR), two winter-white mountain hares, one from the Alps and one from Fennoscandia (MTH), two Iberian hares (IBH), one winter-white snowshoe hare (SSH_WW), and one blacktailed jackrabbit (BTJ) (Fig. S10B); 3) one winter-grey Faroese mountain hare (FAR), two winter-white mountain hares, one from the Alps and one from Fennoscandia (MTH), two Iberian hares (IBH) and one blacktailed jackrabbit (BTJ) (Fig. 4A).

f_d **statistic and** d_{XY}

To explore the close relationship between Faroese mountain hare and the Iberian hares at the *Agouti* region, the fraction of introgression (*f^d* statistic) (49) was estimated in 20 kb overlapping windows (step size 2 kb) along chromosome 4, with at least 100 genotyped SNPs per window (Fig. 4B). We used genomics_general scripts available at https://github.com/simonhmartin/genomics general, and the following phylogenetic conformation: $PI = FSC$, $P2 = FAR$, $P3 = IBH$, $Q = RAB$. Genomics general scripts were also used to calculate the absolute genetic distance (d_{XY}) between Faroese *L. timidus* and *L. granatensis*, in 20 kb overlapping windows (step size 2 kb) along chromosome 4, with at least 25% sites covered per window. From d_{XY} , the Relative Node Depth (RND; 50) was calculated by dividing d_{XY} by the distance to the European rabbit (RAB). This statistic corrects the estimated d_{XY} using the distance to an outgroup to control for local variation in mutation rate. The *f^d* statistic was also calculated using the European brown hare (*L. europaeus*) instead of the Iberian hare (*L. granatensis*) as *P3* (Fig. S11).

RAxML local phylogeny

An alignment of six *Lepus* species (Table S3) was generated for the genomic region showing a sharp increase in *fd* values (4:5,400,000-5,760,000) (Fig. 4B) and used to construct a local maximum-likelihood phylogeny using RAxML (51) (Fig. 4F). A phylogeny for the whole chromosome 4 was also built using the same specimens (Fig. 4E). RAxML was run with the following settings: -m GTRGAMMA -f a -x 2408 -N 100 -p 2330 and the sequence from the European rabbit reference genome was used as the outgroup.

Simulations of d_{XY}

To further test whether the low d_{XY} between the winter-grey mountain hares and the Iberian hares resulted from introgression or was instead compatible with an incomplete lineage sorting scenario, we performed coalescent simulations of species divergence. Species divergence history parameters, including effective population sizes of current and ancestral populations, time of divergence and migration rates, were estimated with G-PhoCS (52), based on high coverage data from two mountain hare individuals (Alpine and Fennoscandian; Table S3) (from 3) and two Iberian hare individuals (Table S3) (from 3). Fragments of 1 kb distancing at least 50 kb were selected from intergenic regions (at least 1 kb from the nearest gene). Repeats, identified in the rabbit reference genome with RepeatMasker and downloaded as a bed file from UCSC Genome Browser (https://genome-euro.ucsc.edu), were masked in the selected 1 kb fragments. Finally, fragments with > 40% missing data were excluded. This resulted in a dataset of 11,480 independent 1 kb loci. Model parameters were inferred assuming possible bidirectional migration. The model was run three times and the convergence of the combined run was checked with Tracer v1.7 (53) by examining the effective sample size (ESS) of each parameter. For all runs, 100,000 generations were discarded as burn-in, and 1,000,000 MCMC iterations were run, sampling every 10 iterations. To convert scaled parameter estimates, we used a mutation rate (μ) of 2.8 x 10⁻⁹ substitutions/site/generation (3), considering a divergence of 11.8 million years divergence between rabbits and hares (7) and a presumable generation length of two years (21).

The parameter values from the inferred model (Table S11) were then used in *msms* (46) to simulate 1,000 fragments of 20 kb, and test if the variance of the coalescent and lineage sorting process could retain closely related variants in the species, as seen in the empirical data. Given that the estimated local mutation rate of the *Agouti* region (using $\mu = d_{XY} / (2T_D + 4N_e)$ (54), where d_{XY} is the sequence distance between *L*. *timidus* and the rabbit averaged along the region, T_D is the time of divergence between hares and rabbits of 11.8 million years (7), and N_e is the ancestral effective population size ($N_e = 1,000,000$ assumed)) is larger than the genome-wide estimate $(3.2 \times 10^{-9} \text{ vs } 2.8 \times 10^{-9} \text{ substitutions/site/generation})$ we opted to perform the simulations using the latter, to increase the conservative nature of the test.

Simulations were done under four demographic models: i) the full demographic model, to assess the reliability of the demographic inference to replicate genome-wide empirical data; ii) inferred demographic model but without inter-species migration, to assess d_{XY} expectations under a strict lineage sorting model; iii) model with strong selection in the ancestral population, leading to fixation of one variant before the split and reducing d_{XY} to a minimum, and no migration, and iv) model with selection prior to the split and with bidirectional migration. The *msms* commands were as follows:

i) full demographic model:

msms 4 1000 -t 70.8 -I 2 2 2 0 -m 1 2 0.190 -m 2 1 0.010 -n 1 0.57 -n 2 0.96 -en 0.74 2 1 -ej 0.74 1 2,

where $t = 4 \times 316277 \times 2.8 \times 10^{-9} \times 20000$;

ii) demographic model without migration:

msms 4 1000 -t 70.8 -I 2 2 2 -n 1 0.57 -n 2 0.96 -en 0.74 2 1 -ej 0.74 1 2;

iii) selection in ancestral population, model without migration:

msms 4 1000 -t 70.8 -I 2 2 2 0 -n 1 0.57 -n 2 0.96 -en 0.74 2 1 -ej 0.74 1 2 -N 316277 -SFC -SI 0.755 2 0 0.0000016 -Sc 0.74 2 63255 0 0 -Sp 0.5;

where selection coefficient $s = 0.1$, selection starts 25,000 generations ago before population split, and an initial frequency of the beneficial allele of *1/2Ne*. By using -SFC flag we kept only simulations where the beneficial allele was not lost, therefore leading to its fixation.

iv) selection in ancestral population, model with migration:

msms 4 1000 -t 70.8 -I 2 2 2 0 -m 1 2 0.190 -m 2 1 0.010 -n 1 0.57 -n 2 0.96 -en 0.74 2 1 -ej 0.74 1 2 -N 316277 -SFC -SI 0.755 2 0 0.0000016 -Sc 0.74 2 63255 0 0 -Sp 0.5;

The empirical distribution of d_{XY} along the *Agouti* association region was generated by sampling with replacement 20,000 sites from the region 1,000 times, and calculating d_{XY} for each replicate (Fig. 4D and S12A). This analysis was replicated for the divergence between the mountain hare (using the same wholegenome sequence data; Table S3) and the European brown hare (using one genome from the Iberian Peninsula and another from central Europe; Table S3). In this case, the parameters of the demographic model of divergence were inferred from 5,705 independent 1 kb loci. d_{XY} simulations were conducted as described above (Fig. S12B and C).

Fig. S1. Population structure and evolutionary relationships among mountain hare populations: grey - the Faroe Islands (FAR), blue - the Alps (ALP), orange - Fennoscandia (FSC). **(A)** Principal component analysis based on 123,995 polymorphic sites sampled every 20 kb to reduce non-independence. The proportion of variance of the first two principal components is shown. **(B)** A neighbor-joining tree based on individual pairwise distances, constructed with 127,413 polymorphic sites and node support derived from 100 bootstrap replicates; the black line depicts the outgroup, the snowshoe hare (*Lepus americanus*).

Fig. S2. Distribution of posterior probabilities of the demographic parameters inferred with ABC. *N_{FSC}* – effective population size (haploid numbers) of Fennoscandian population, *NFAR* – effective population size (haploid numbers) of Faroese population, N_F – effective population size (haploid numbers) of the Faroese hare founder population, T_i – time of the introduction in generations, g – growth rate (negative value backward in time implies expansion forward in time; calculated according to the equation $N_F = N_{FAR} e^{gT_i}$, μ – mutation rate in units of mutations per site per generation.

Fig. S3. Validation of parameter estimates with ABC for the model investigated, based on 1,000 parameter values picked from the prior distribution (randomValidation in ABCtoolbox). *N_{FSC}* – effective population size (haploid numbers) of Fennoscandian population, *NFAR* – effective population size (haploid numbers) of Faroese population, N_F – effective population size (haploid numbers) of the Faroese hare founder population, T_i – time of the introduction in generations, *g* – growth rate (negative value backward in time implies expansion forward in time; calculated according to the equation $N_F = N_{FAR} e^{gT_i}$, μ – mutation rate in units of mutations per site per generation.

Fig. S4. Manhattan plots of differentiation scans and case-control association tests. **(A)** F_{ST} of Faroese hares (FAR) vs. pooled Alpine and Fennoscandian populations (ALP + FSC) estimated with PoPoolation2, based on 14,102,734 SNPs. F_{ST} values are plotted against the position of 20 kb non-overlapping windows on each chromosome. **(B)** Fisher exact test of allele frequency differences between Faroese hares (FAR) and pooled Alpine and Fennoscandian populations (ALP + FSC) estimated with PoPoolation2, based on 14,102,734 SNPs. The $-log_{10}(P)$ values are plotted against the position of 20 kb non-overlapping windows on each chromosome. **(C)** Fisher exact test of allele frequency differences between Faroese hares (FAR) and Alpine hares (ALP). **(D)** Fisher exact test of allele frequency differences between Faroese hares (FAR) and Fennoscandian hares (FSC). **(E)** Manhattan plot of case-control association tests statistics for Faroese hares (FAR; cases) vs. Alpine and Fannoscandian hares (ALP + FSC; controls), inferred from genotype likelihoods, plotted as mean $-log_{10}(P)$ values per windows of 100 SNPs. **(F)** Manhattan plot of case-control association tests statistics for Faroese hares (FAR; cases) vs. Alpine hares (ALP; controls). **(G)** Manhattan plot of case-control association tests statistics for Faroese hares (FAR; cases) vs. Fennoscandian hares (FSC; controls). The dashed lines represent the significance thresholds corrected for multiple tests, calculated from the data.

Fig. S5. Distribution of 36 genotyped SNPs (black dots) and one insertion-deletion (orange dot) along the association region in chromosome 4, including the structure of four genes: *AHCY*, *Agouti* (*HC* – non-coding exon of the hair-cycle isoform, *V* – non-coding exon of the ventral isoform), *EIF2S2* and *RALY*.

Fig. S6. Two common types of hairs present in Faroese mountain hares. **(A)** Black hair with white band. **(B)** White hair with black tip.

Fig. S7. Expression of the *Agouti* hair-cycle isoform in mountain hares during the autumn molt. Skin tissue samples were collected from the dorsum, representing the brown, intermediate, and white/grey stages of the molt. **(A)** The expression level (2^{-ΔCt}) is shown as relative to the reference gene *SDHA* (see Fig. 3 for the analysis using *ACTB* as reference gene). Each point represents one relative measure and dashed lines connect technical replicates. **(B)** The expression level (log₂(abundance)) estimated using the naïve model of *MCMC.qpcr* R package. The points are posterior means and the whiskers denote 95% credible intervals. See Table S10 for sequences of primers and raw C_t values.

Fig. S8. Signatures of selective sweeps in Faroese mountain hares. **(A)** Nucleotide diversity (π) along the first 10 Mb of chromosome 4. **(B)** Tajima's D along the first 10 Mb of chromosome 4. **(C)** Selective sweeps detected by Pool-hmm along the whole chromosome 4. **(D)** Selective sweep detected by Pool-hmm along the first 10 Mb of chromosome 4 **(E)** SweeD Composite Likelihood Ratio (CLR) along the first 10 Mb of chromosome 4. The red line represents the CLR threshold based on demographic simulations ($p < 0.01$). Vertical dashed grey lines delimit the association region: 4:5,400,000-5,760,000 bp in A, B, E.

Fig. S9. Selection scans at the *Agouti* association region in Alpine and Fennoscandian populations of the mountain hare. **(A)** Selective sweeps detected in chromosome 4 of Alpine hares using Pool-hmm. **(B)** Selective sweep detected in chromosome 4 of Fennoscandian hares using Pool-hmm. **(C)** SweeD Composite Likelihood Ratio (CLR) in the first 10 Mb of chromosome 4 in the Alpine hares. **D)** SweeD CLR in the first 10 Mb of chromosome 4 in the Fennoscandian hares. Grey vertical dashed lines represent the *Agouti* association region. The horizontal dashed line represents the 99% CLR cutoff (chromosome 4 empirical distribution).

Fig. S10. Twisst topology weightings along the *Agouti* association region in chromosome 4 (delimited by dashed lines). All topologies include winter-grey (FAR) and winter-white (MTH_WW) mountain hares (*Lepus timidus*) and the Iberian hares (IBH; *L. granatensis*). Topology support is plotted with loess smoothing (span $= 0.075$). Only the most common topologies are shown in detail (the remaining ones are represented in grey). **(A)** Analyses include two European brown hares (*L. europaeus*), from the Iberian Peninsula (EBH_IB) and central Europe (EBH_CE). **(B)** Analyses include the black-tailed jackrabbit (BTJ; *L. californicus*) and a winterwhite snowshoe hare (SSH_WW; *L. americanus*).

Fig. S11. Signatures of variant sharing between Faroese mountain hare (*Lepus timidus*) and the European brown hare (*L. europaeus*) from the Iberian Peninsula (EBH_IB) and central Europe (EBH_CE). **(A)** Relative node depth (RND) between the Faroese mountain hare and the European brown hare from the Iberian Peninsula (EBH_IB). **(B)** Fraction of introgression **(***f^d* statistic) estimated with the following phylogenetic conformation: *P1 = FSC, P2 = FAR , P3 = EBH_IB, O = RAB*. **(C)** RND between the Faroese mountain hare and the brown hare from central Europe (EBH_CE). **(D)** f_d statistic estimated with the following phylogenetic conformation: $P1 = FSC$, $P2 = FAR$, $P3 = EBH_CE$, $O = RAB$. In all plots, blue dots represent 1% lowest (RND) or highest (*fd*) values along the chromosome 4 empirical distribution. FSC - Fennoscandian mountain hare; RAB – European rabbit.

Fig. S12. Distributions of *dxy*. **(A)** *dxy* between the Faroese mountain hare *(Lepus timidus)* and the Iberian hare (*L. granatensis*; IBH); simulated data derived from a model assuming bidirectional migration. **(B)** d_{XY} between the Faroese mountain hare (*L. timidus*) and the European brown hare (*L. europaeus)* from the Iberian Peninsula (EBH_IB); simulated data derived from a model assuming bidirectional migration. with simulations including migration. **(C)** d_{XY} between the Faroese mountain hare (*L. timidus*) and the European brown hare (*L. europaeus*) from the Iberian Peninsula (EBH_IB); simulated data derived from a model assuming no migration.

Table S1. Mountain hare specimens used in this study: samples' code, populations of origin (FAR - the Faroe Islands, FSC - Fennoscandia, RUS - Russia, ALP - the Alps), localities, and data collected (WGS – low individual coverage whole-genome sequencing; SNP Genotyping; qPCR – quantitative PCR). Specimens from the Swedish Museum of Natural History are indicated as MG – museum winter-grey and MW – museum winter-white in the "Population" column. NCBI Sequence read archive (SRA) BioSample accession numbers for the sequence data are indicated.

Table S2. Sequencing statistics for low individual coverage data: samples' codes, populations (FAR – the Faroe Islands, FSC – Fennoscandia, ALP – the Alps), number of raw paired reads, percentage of mapped reads and final mean coverage per site. Values in parentheses represent final mean coverage after subsampling.

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Table S3. Information on higher depth individual whole-genome resequencing data used in this study: species, samples' codes, localities of origin (FAR – Faroe Islands; FSC – Fennoscandia; ALP – Alps), coverage, NCBI Sequence Read Archive (SRA) BioSample accession number and reference.

Species	Sample code	Origin	Coverage [X]	SRA BioSample	Reference		
	LTM.3978	FAR/Signabøur	6.9	SAMN12710256	this study		
L. timidus	LTM.2012	FSC/Finland	17.9	SAMN07526960	(3)		
	LTM.3109	ALP/France	22.0	SAMN07526962	(3)		
L. europaeus	LER.1515	Spain/Navarra	15.7	SAMN12618118	(2)		
	LER.1639	Austria/Vienna	10.8	SAMN12618122	(2)		
L. granatensis	LGR.2553	Spain/Ciudad Real	20.8	SAMN07526967	(3)		
	LGR.2544	Spain/Navarra	21.8	SAMN07526972	(3)		
	Allab3	USA/Pennsylvania	6.8	SAMN08146528	(4)		
L. americanus	DOlsen1	USA/Utah	6.8	SAMN08146529	(4)		
	A0961	USA/Montana	29.3	SAMN08146494	(4)		
L. californicus	MacKay2	USA/Nevada	6.1	SAMN08146530	(4)		
	MacKay3	USA/Nevada	7.1	SAMN08146531	(4)		
L. castroviejoi	LCS.1991	Spain/León	10.0	SAMN12640762	this study		

Table S4. Approximate Bayesian Computation demographic modelling. Prior and posterior distributions of parameters' estimate (marginal density P-value: 0.73, Tukey depth P-value: 0.74), based on PLS transformed data. *N_{FSC}* – effective population size of the Fennoscandian population; *N_{FAR}* – effective population size of the Faroese population; N_F – effective population size of the Faroese hare founder population; T_i – time of the introduction in generations; μ – mutation rate in units of mutations per site per generation; g – growth rate (negative value backward in time implies expansion forward in time; calculated according to the equation *N^F* $=N_{FAR} e^{gT_i}$). Effective population sizes are reported as haploid numbers.

Table S5. Sequences of PCR primers used to confirm and genotype the insertion-deletion detected in the *Agouti* association region. The coordinates on the European rabbit reference genome are shown (*OryCun2.0*). The reverse primer (*LTM_indel_R*) is common to all three PCRs.

Table S6. Genotypes at selected SNPs and insertion-deletion along the Agouti region (chromosome 4). Rows depict individuals and columns represent site coordinates according to the rabbit ge 2C, i.e. black: homozygous Faroese variant, light grey: homozygous alternative variant, dark grey: heterozygous, and white: missing data.

Table S7. Genotypes at selected SNPs in the USP38 gene region (chromosome 15) and genome-wide. Rows depict individuals and columns represent site coordinates according to the rabbit genome (OryCun2.0; chromosome: site). Co concordant with Fig. 2C, i.e. black: homozygous Faroese variant, light grey: homozygous alternative variant, dark grey: heterozygous, and white: missing data.

		Usp38 region (chromosome:site)				Genome wide (chromosome:site)																		
Origin	Specimen	15:212267	15:212366	15:213519	15:213529	15:213732	1:34929	1:232574	2:1113582	3:688624	3:1445514	4:72246	4:207793	4:219990	4:232361	4:238188	5:329094	6:131284	7:451944	12:917999	13:768437	14:430055	17:744891	18:481934
	Code	63	41	40	56	27	82	58	24	73	71	15	95	72	52	53	37	52	36	89	55	58	91	91
FSC/MG	NRM588802	$\mathbf 0$	$\mathbf 0$	AA	GG	GG	TT	0	TT	$\mathbf 0$	cc	cc	$\mathbf 0$	Ω	TC	TT	$\mathbf 0$	$\mathbf 0$	AA	$\mathbf{0}$	$\mathbf 0$	$\mathbf 0$	GG	TT
FSC/MG	NRM588803	GG	cc	AA	GG	GG	GT	GG	Ω	0	CT	TC	GG	GG	cc	TT	CC	GG	AA	AA	GA	TC	GG	cc
FSC/MG	NRM588806	GG	cc	AA	GG	GG	TT.	0	TT	CG	cc	cc	GG	GG	TC	TT	AA	GG	AA	GG	GG	TT	GG	TT
FSC/MG	NRM588810	GG	cc	AA	GG	GG	GT	GG	TT	cc	CC	TC	GG	GG	TC	CT	cc	GG	AA	AG	GG	TT	GG	CT
FSC/MG	NRM588812	GG	cc	AG	GA	GT	TT.	GG	TT	CG	TT	cc	GG	GG	TC	TT	CA	GG	AA	GG	GG	TT	AG	CT
FSC/MG	NRM588831	GG	cc	AA	GG	GG	TT	GG	TT	0	TT	TC	GG	GA	TT	TT	CA	GG	AG	AG	GG	ТC	GG	TT
FSC/MG	NRM588832	AA	TT	GG	AA	TT	TT.	GA	TA	CG	cc	TC	GG	GG	TC	TT	cc	CG	AG	GG	0	TT	GG	TT
FSC/MG	NRM588833	GG	cc	AA	GG	GG	TT.	GG	TT	$\mathbf 0$	cc	TC	0	GG	cc	TT	CA	cc	AA	GG	GG	тс	AA	CT
FSC/MG	NRM588834	GG	cc	AA	GG	GG	TT.	GA	TT	CG	CC	TC	GG	GG	TT	TT	AA	CG	AG	GG	GA	тс	AG	TT
FSC/MG	NRM588861	GG	cc	AA	GG	GG	GT	AA	TT	cc	cc	CC	GG	GG	TT	CT	CA	CG	AA	GG	GA	ТC	GG	TT
FSC/MG	NRM588870	AG	TC.	AA	GG	GT	GT	GG	TA	0	cc	CC	0	GG	TT	TT	CC	CG	AA	GG	GG	cc	GG	TT
FSC/MG	NRM588871	GG	cc	AA	GG	GG	GT	AA	AA	cc	cc	cc	GG	GG	TT	CT	CC	CG	AA	AA	GA	TC	GG	CT
RUS/MG	NRM595175	GG	cc	AA	GG	GG	GT	GG	TT	CG	cc	cc	GG	GG	TC	TT	CA	CG	AG	AG	GA	0	GG	TT
FSC/MG	NRM588865	GG	cc	AA	GG	GG	TT	GG	TT	CG	CT	CC	GG	GG	TC	CT	CA	GG	AG	AG	GA	TT	AG	CT
FSC/MW	NRM588815	GG	cc	AA	GG	GG	TT	GA	TT	cc	CT	cc	GG	GG	TC	TT	cc	CG	AG	AG	0	TC	GG	TT
FSC/MW	NRM588809	AG	TC	AA	GG	GG	TT.	GG	TT	CG	CT	CC	GG	GG	TT	TT	cc	CG	AA	AG	GG	TC	AA	CT
FSC/MW	NRM588845	AG	TC	AG	GA	GT	TT	Ω	TT	0	cc	cc	Ω	AA	TT	TT	cc	cc	AA	AG	GG	TT	GG	TT
FSC/MW	NRM588850	GG	cc	AA	GG	GG	TT	AA	TT	CC	CT	TT	GG	GG	TT	TT	CA	CG	AA	AA	GA	тс	GG	CT
FSC/MW	NRM588852	AG	TC .	AA	GG	GG	TT	GA	Ω	cc	cc	CC	GG	GG	TC	TΤ	AA	CG	AA	GG	GG	TC	GG	CT
FSC/MW	NRM588857	GG	cc	AA	GG	GG	TT	GG	TT	cc	cc	CC	GG	GG	TT	TT	_{CC}	CG	AA	GG	GA	TC	GG	TT
FSC/MW	NRM588866	GG	cc	AA	GG	GG	TT.	GG	TT	CG	CT	CC	GG	AA	TT	CT	0	CG	AG	GG	GA	Ω CC	GG	TT
FSC/MW RUS/MW	NRM588862 NRM592555	AG GG	TC	AG AA	GA	GG GG	TT. TT	GA AA	Ω TT	cc CC	cc cc	cc CC	GG GG	GA GG	TC TT	CT TT	CA cc	CG CG	AA AA	AG GG	AA AA	TT	GG GG	TT.
RUS/MW	NRM592556	GG	cc cc	AA	GG GG	GG	TT	GA	TT	cc	cc	cc	GG	GG	TT	TT	cc	CG	AG	GG	GA	TT	GG	TT. TT
FSC/MW	NRM598844	GG	cc	AA	GG	GG	TT	AA	TT	cc	cc	cc	GG	GA	TT	TT	cc	CG	AG	AG	GG	TT	GG	TT
FSC/MW	NRM588859	AG	TC.	AA	GG	GG	GT	GA	TA	CC	CT	TC	GG	GA	TC	TT	0	CG	AG	GG	0	Ω	GG	TT
FSC/MW	NRM588860	GG	cc	AG	GA	GT	TT.	0	TT	CG	cc	CC	GG	GG	TT	CC	CA	cc	GG	AG	GA	CC	GG	CT
FSC/MW	NRM588838	GG	cc	AA	GG	GG	GT	GG	TT	cc	cc	CC	GA	GA	TT	TT	CC	GG	AG	GG	GG	TT	GG	TT
FSC/MW	NRM588851	AG	TC.	AG	GA	GT	TT	GA	TT	cc	cc	TC	GG	GG	TT	CT	CA	GG	AG	GG	AA	TT	AG	CT
FAR	LTM.3655	AA	Ω	GG	AA	TT	GG	AA	AA	GG	TT	TT	AA	AA	cc	cc	AA	CC	GG	AA	AA	0	AA	cc
FAR	LTM.3656	AA	TT	GG	AA	TT	GG	AA	AA	GG	TT	\mathbf{T}	AA	AA	cc	cc	CA	CC	GG	AA	AA	CC	AG	CC
FAR	LTM.3658	AA	$\mathbf{0}$	GG	AA	TT	GT	AA	AA	GG	TT	TT	GA	GA	TC	cc	AA	CC	GG	AG	AA	$\mathbf 0$	AG	cc
FAR	LTM.3659	AA	TT	GG	AA	T	GG	AA	AA	GG	TT	TT	AA	AA	CC	cc	AA	cc	AG	AA	AA	cc	AA	cc
FAR	LTM.3661	AA	TT	GG	AA	TT	GT	AA	AA	GG	TT	TT	GA	GA	TC	CT	AA	cc	GG	AA	AA	cc	AA	cc
FAR	LTM.3663	AA	TT	GG	AA	TT	GG	AA	AA	GG	TT	TT	GG	GG	TT	cc	CA	cc	GG	AA	AA	cc	AA	CC
FAR	LTM.3664	AG	TC	AG	GA	TT	GG	AA	AA	GG	TT	TT	GA	GA	TC	CT	AA	cc	GG	AG	AA	CC	AA	CC
FAR	LTM.3667	AG	Ω	AG	GA	TT	GT	AA	AA	GG	TT	TT	AA	AA	cc	cc	AA	cc	GG	AA	AA	cc	AA	Ω
FAR	LTM.3668	AA	Ω	GG	AA	TT	GG	Ω	AA	Ω	TT		AA	AA	cc	cc	AA	Ω	GG	AA	Ω	Ω	AA	cc
FAR	LTM.3670	AA		GG	AA	- 11	GG	AA	AA	GG	- 11	a katika s	GA	GA	TC.	CC.	AA	CC	GG	GG.	AA	cc	AA	CC.
FAR	LTM.3672	AG	TC	AG	GA	TT	GG	AA	AA	GG	TT	TT	AA	AA	CC	cc	AA	CC	GG	AA	AA	CC	AA	CC
FAR	LTM.3673	AA	0	GG	AA	TT	GG	AA	AA	GG	TT	TC	AA	AA	CC	CC	AA	CC	GG	AA	AA	CC	AA	CC
FAR	LTM.3674	AA	TT	GG	AA	TT	GT	AA	AA	GG	TT	TT	AA	AA	cc	cc	AA	cc	GG	AA	AA	CC	AA	CC
FAR	LTM.3675	AA	TT	GG	AA	TT	GG	AA	AA	GG	TT	TC	AA	AA	cc	cc	AA	CC	GG	AA	AA	CC	AA	CC
FAR	LTM.3677	AA	TT	GG	AA	T	GG	AA	AA	GG	TT	TT	AA	AA	cc	cc	AA	cc	GG	AA	AA	CC	AA	CC
FAR	LTM.3678	AG	TC	AG	GA	TT	GG	AA	AA	GG	TT	TT	AA	AA	cc	cc	AA	CC	GG	AG	AA	CC	AA	CC
FAR	LTM.3680	AG	TC	AG	GA	\mathbf{T}	GG	AA	AA	GG	TТ	TТ	GA	GA	TC	CT	CA	CC	GG	AG	AA	CC	AA	CC
FAR	LTM.3682	AA	TT	GG	AA	TT	GG	AA	AA	GG	TT	TT.	AA	AA	CC	CC	AA	CC	GG	AA	AA	$\mathbf 0$	AA	CC
FAR	LTM.3683	AA	TT	GG	AA	T	GG	AA	AA	GG	TT	TT	AA	AA	cc	cc	AA	cc	GG	AA	AA	CC	AG	CC
FAR	LTM.3688 LTM.3691	AA	$\mathbf 0$	GG GG	AA	TT TT	GG GG	AA AA	TA A	GG GG	TT TT	TT TТ	AA AA	AA AA	TC CC	CT	AA AA	cc CC	GG GG	AA	AA AA	$\mathbf{0}$ CC	AA	CC CC
FAR FAR	LTM.3692	AA AA	TT TT	GG	AA	TT				GG	CT	TТ	AA	AA	cc	CC cc	AA	cc	GG	AA AG	AA	CC	AA AA	CC
FAR	LTM.3695	AA	TT	GG	AA AA	TT	GG GG	AA AA	TA. TA	GG	TT.	TT	GG	GA .	TT	CT	AA	CC	GG	AA	AA	CC	AA	CC
FAR	LTM.3696	AA	TT	GG	AA	T	GG	AA	TA	GG	TT	\mathbf{T}	AA	AA	cc	cc	AA	cc	GG	AA	AA	CC	AA	CC
FAR	LTM.3697	AG	$\mathbf 0$	AG	GA	\mathbf{T}	GG	AA	AA	CG	CT	TT	GA	GA	TT	CT	AA	CC	GG	AA	AA	CC	AA	CC
FAR	LTM.3698	AA	TT	AG	GA	T	TT	AA	AA	GG	TT	TT	AA	AA	CC	cc	AA	CC	GG	AA	AA	CC	AA	CC
FAR	LTM.3699	AG	TC	AG	GA	TT	GT	AA	AA	CG	CT	TТ	AA	GA	TT	cc	AA	CC	GG	AG	AA	CC	AA	CC
FAR	LTM.3702	AG	0	AG	GA	GT	GG	AA	AA	$\mathbf{0}$	CT	TC.	AA	AA	cc	cc	AA	cc	GG	AA	AA	$\mathbf 0$	AA	CC
FAR	LTM.3703	AA	TT.	GG	AA	TT	GG	AA	AA	GG	TT	TT	GA	GA	TC	cc	AA	cc	GG	AA	AA	CC	AG	CC
FAR	LTM.3707	AA	TT	GG	AA	TT.	GT	AA	AA	GG	TТ	TT	AA	AA	CC	CC	AA	CC	GG	AG	AA	CC	AA	CC
FAR	LTM.3978	AA	TT	GG	AA	TT	GG	AA	AA	CC	TT	TT	AA	AA	TC	cc	AA	CC	GG	AA	AA	CC	AA	CC

Table S8. Variants at two genotyped SNPs (4:5,457,584 and 4:5,460,551) showing perfect association with winter coat color in the mountain hare, in other *Lepus* species and several other mammals (from Ensembl).

Table S9. Gene expression analyses. **(A)** Sequences of primers used in quantitative PCR (qPCR) and amplification efficiencies. **(B)** Raw threshold cycle (C_t) values for the *Agouti-HC* isoform and the reference genes, *ACTB* and *SDHA*, in skin sampled at three stages of the autumn molt (early, intermediate, late). qPCR reactions of samples marked with the asterisks were run using undiluted cDNA (2-fold dilution was used for remaining ones).

B)

A)

Table S10. Probability of fixation of a rare allele due to genetic drift alone after 65 years of evolution (33-65 generations, generation time of two or one year, respectively) under three demographic scenarios, based on 10,000 SLIM simulations. All scenarios consider an initial allele frequency of $1/2N_F$, where N_F is the effective population size of the founder (diploid individuals; $2N_F$ report haploid numbers): 1 – mode of the HPD of the ABC inference; 2 – lower 95% HPD of the ABC inference, 3 – historical record. Three growth rate parameters were used: 1 – mode of the HPD of ABC inference; 2 – lower 95% HPD interval of the ABC inference; 3 – parameter value implying growth from *N_F* to the lowest 95% HPD interval of the current effective population size in Faroese hares, inferred with ABC. ABC inference is shown in Table S4. The P-value indicates the proportion of simulations where fixation was achieved after *n* generations.

Table S11. Demographic parameters inferred with G-PhoCS for the history of divergence between **(A)** the mountain hare (MTH) and the Iberian hare (IBH), and **(B)** the mountain hare (MTH) and the European brown hare (EBH). For conversion of raw estimates, mutation rate $\mu = 2.8 \times 10^{-9}$ substitutions/site/generation and generation time of two years were used. Mean values of estimated parameters are presented with 95% HPD intervals in parentheses.

A)

B)

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