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Introgression drives repeated evolution of winter coat color polymorphism in hares

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Abstract

Changing from summer-brown to winter-white pelage or plumage is a crucial adaptation to seasonal snow in more than 20 mammal and bird species. Many of these species maintain non-white winter morphs, locally adapted to less snowy conditions, which may have evolved independently in each species. Mountain hares (*Lepus timidus*) from Fennoscandia were introduced into the Faroe Islands in 1855. While they were initially winter-white, within ~65 years all Faroese hares became winter-grey, a morph that occurs in the source population at low frequency. The documented population history makes this a valuable model for understanding the genetic basis and evolution of the seasonal trait polymorphism. Through whole-genome scans of differentiation and SNP genotyping, we associated winter coat color polymorphism to the genomic region of the pigmentation gene *Agouti*, previously linked to introgression-driven winter coat color variation in the snowshoe hare (*L. americanus*). Lower *Agouti* expression in the skin of winter-grey individuals during the autumn molt suggests that regulatory changes may underlie the color polymorphism. Variation in the associated genomic region shows signatures of a selective sweep in the Faroese population, suggesting that positive selection drove the fixation of the variant after the introduction. Whole-genome analyses of several hare species revealed that the winter-grey variant originated through introgression from a non-color changing species, in keeping with the history of ancient hybridization between the species. Our findings show the recurrent role of introgression in generating winter coat color variation by repeatedly recruiting the regulatory region of *Agouti* to modulate seasonal coat color change.

Significance

Seasonal molts to winter-white pelage or plumage are key adaptations to seasonal snow in over 20 animal species. However, winter color polymorphism within species is crucial for adaptation to environmental heterogeneity, and provides striking models to tackle the basis of repeated evolution. We show that the pigmentation gene *Agouti*, which underlies introgression-driven winter color variation in snowshoe hares, is also associated with winter-white/winter-grey polymorphism in

mountain hares, possibly due to altered expression of the gene. The winter-grey variant introgressed into the mountain hare through hybridization with a non-color changing species. These findings show that the same genomic region was repeatedly recruited to determine winter coat color in different hare species, highlighting the recurrent role of introgression in generating phenotypic variation.

Introduction

Recurrent evolution has puzzled evolutionary biologists for decades (1, 2). While controlled experiments testing the repeated nature of evolution are possible in only a few models, comparative evolutionary studies of similar traits that may have evolved independently in closely related taxa, provide powerful means to understand replicated evolution at different levels, from processes to phenotypes and their molecular bases (3-5). Seasonal pelage or plumage color change is a key adaptive phenological trait of over 20 vertebrates, such as hares, weasels and grouse, which inhabit environments with seasonal snow cover (6). Photoperiod-controlled pelage molts from summer-brown to winter-white coats allow the maintenance of camouflage year-round, reducing the fitness costs of increased predation due to coat-background color mismatch (7-9). However, genetically determined winter coat color polymorphism exists in many of these species, maintained by clinal selective pressures correlated with snow cover environmental variables (10). The occurrence of winter coat color variation across species provides valuable models to dissect pathways for recurrent phenotypic evolution. Also, such polymorphism may provide standing variation to enable rapid adaptation to snow cover reductions caused by climate change, which endangers winter-white populations (7, 10). Understanding the emergence of seasonal coat color polymorphisms is therefore crucial for quantifying the adaptive potential of seasonal coat color changing species facing climate change (10, 11).

Of the >30 species of hares (*Lepus* spp.), six undergo seasonal coat color molts, often maintaining within-species variation in winter pelage color (10). In snowshoe hares (*L. americanus*), winter-white/winter-brown polymorphism maps to the *agouti signaling protein* gene (*ASIP*; *Agouti*) and is driven by *cis*-regulatory changes influencing the expression of the gene (11). The winter-brown variant introgressed from the neighboring black-tailed jackrabbit (*L. californicus*), a winter-brown species, seeding adaptation to the warmer coastal habitat with less winter snow in the Pacific Northwest region in North America. Winter variation in coat color has also been studied in arctic

foxes (*Vulpes lagopus*), where *melanocortin 1 receptor* gene (*MC1R*) mutations induce the expression of a blue-grey winter phenotype instead of the predominant winter-white (12). These results implicate the *MC1R-Agouti* melanin pigmentation system, often involved in permanent pelage color variation in mammals (13), in the evolution of seasonal changes in coloration. However, little is known about the evolution of seasonal coat color variation across species.

Here, we study the genetic basis and evolution of winter coat color polymorphism, using the winter-white/winter-grey coat color variation in mountain hares (*L. timidus*). In most of the species' range, mountain hares molt to winter-white coats, but alternative winter color morphs occur in the species. For example, in Ireland mountain hares remain brown year-round, and in Fennoscandia a winter-grey morph exists (i.e. the heath-hare; also called blue-grey) in addition to the predominant winter-white morph (Fig. 1*A* and *B*) (14, 15). Winter-grey mountain hares were common in southern Sweden but have become progressively rarer since the early 20th century, likely due to a combination of predation by the red fox (*V. vulpes*), habitat degradation, and competition with the introduced European brown hare (*L. europaeus*) (16). In 1855, four mountain hare leverets were translocated from the Kragerø region in southern Norway to Tórshavn in the Faroe Islands, where no hares were present, and they multiplied rapidly (14, 17). Initially only winter-white hares were observed in the Faroe Islands, but years later winter-grey hares began to be seen, and by the 1870s hunting bags were composed by an equal number of winter-white and winter-grey hares. The percentage of winter-grey hares increased to 75% in 1882 and 95% in 1890, and the last winter-white hare was observed in 1916-1917 (18). Breeding experiments suggest that the winter-grey coat is inherited as a recessive trait (14), implying that the individuals that founded the Faroese hare population carried the causal allele. The rapid fixation of winter-grey morphs may have resulted from adaptation to the milder snow conditions of the Faroe Islands, influenced by the warm waters of the North Atlantic Current (17). Using whole-genome sequencing data, we map winter coat color polymorphism to a

single genomic region, and show that the winter-grey variant was introduced into the mountain hare through introgression.

Results and discussion

Sequencing, population structure and evolutionary history. Whole genomes of 59 mountain hare specimens were sequenced at low individual coverage from individually barcoded libraries. Three populations were sampled: hares from the Faroe Islands (winter-grey; N = 20 individuals; 16.8X total population depth), Fennoscandia (the source population of the Faroese hares; N = 19; 18.0X), and the Alps (winter-white; N = 20; 17.1X) (Fig. 1C; *SI Appendix*, Tables S1 and S2). One Faroese hare was sequenced at higher depth (6.9X; *SI Appendix*, Table S3). Reads were mapped to a hare pseudo-reference genome (97% mapped reads on average per specimen; *SI Appendix*, Table S2), built through iterative mapping to the European rabbit (*Oryctolagus cuniculus*) genome (from 19). Principal component analysis of genetic variation (123,995 single nucleotide polymorphisms, SNPs) showed three groups, corresponding to the geographic origin of specimens (*SI Appendix*, Fig. S1*A*). Phylogenies based on population allele counts (136,834 sites) (Fig. 1*D*) and on individual pairwise differences (*SI Appendix*, Fig. S1*B*) showed that the Faroese mountain hares were closely related to Fennoscandian hares and formed a single clade, in agreement with the historical information about the introduction (14). Using Approximate Bayesian Computation, we fitted a scenario compatible with the historical reports, but with wide priors to allow parameter inference (Fig. 1*E*). The historical records of the time of introduction and the size of the founding Faroese population are within the inferred 95% high posterior density intervals (Fig. 1*E* and *SI Appendix*, Figs. S2 and S3, Table S4).

Association region includes pigmentation gene *Agouti.* To identify genomic regions with exceptional differentiation in winter-grey Faroese hares, a genome scan of the Population Branch Statistic (PBS) was performed, based on genotype likelihoods after filtering sites to represent a minimum of six

individuals per population. Given three structured populations and the closer relationship of Faroese and Fennoscandian hares (Fig. 1*D* and *SI Appendix*, Fig. S1), this statistic localizes strong allele frequency changes specific to Faroese hares. The scan identified consecutive outlier 20 kb windows (top 0.01% of genome-wide values) in chromosome 4, and a few in chromosome 15 (Fig. 2*A*). These results were concordant with F_{ST} scans, Fisher exact tests of allele frequency differences (based on 14,102,734 SNPs, derived from pooled population data), and case-control association tests (8,129,498 SNPs; estimated from genotype likelihoods) (*SI Appendix*, Fig. S4). More than 700 variants with extreme allele frequency differences between Faroese and the other populations were found along a ~360 kb region in chromosome 4. In these SNPs, the allele fixed in Faroese hares segregates at low frequencies in Fennoscandian hares and is generally absent in Alpine hares, as predicted by the geographic distribution of winter-grey morphs (14). This genomic region includes the pigmentation gene *Agouti* as well as *EIF2S2*, *RALY* and *AHCY* genes, which have no known functions in pigmentation (Fig. 2*B*). An overlapping genomic interval has been implicated in winter coat color variation in the snowshoe hare (11). The second genomic region, with a few windows of increased differentiation, maps to chromosome 15 and is located ~40 kb upstream of *USP38*. This gene has no known direct function in pigmentation but belongs to the ubiquitin proteasome system, which is involved in the regulation of skin pigmentation through endogenous degradation of tyrosinase (20).

To validate the association and examine allelic segregation patterns, we genotyped 59 SNPs showing large allele frequency differences between Faroese and other hare populations, located in windows of extreme PBS. These SNPs spanned the candidate regions in chromosomes 4 (36 SNPs; including *Agouti* and neighboring genes) and 15 (5 SNPs in the *USP*38 region), and some were scattered along the genome (18 SNPs). In addition, a ~1.2 kb deletion, detected in the *Agouti* region of the Faroese hare genome using reads from the specimen sequenced at higher depth, was genotyped (*SI Appendix*, Fig. S5, Tables S3, S5 and S6). Sampling was extended from 59 to 126 specimens (*SI Appendix*, Tables S6 and S7) and included 29 museum specimens sampled in Sweden and Russia (15

white and 14 grey; Swedish Natural History Museum) for which the winter coat color had been recorded (Fig. 1*A* and *B*). The genome scans were performed in highly structured populations, which could result in the inference of spurious associations, so genotyping of white and grey museum specimens originating from overlapping localities (*SI Appendix*, Table S1) was carried out in order to provide an independent test of association, devoid of population structure. Thirty-six loci genotyped in the *Agouti* region were significantly associated with winter coat color in the museum specimens (recessive model, $p < 0.05$ and codominant model, $p < 0.001$). A ~180 kb block (chromosome 4, 5,419,007-5,599,413), encompassing part of *AHCY* and the whole *Agouti,* showed the strongest association. In contrast, the remaining genotyped SNPs, including those from the *USP38* region, showed no association with winter coat color in the museum specimens, though they did confirm the strong allele frequency differences between Faroese and other mountain hares (Fig. 2*C* and *SI Appendix*, Table S7). Two SNPs in the *Agouti* region (chromosome 4, positions 5,457,584 and 5,460,551) were perfectly associated with winter coat color in the museum specimens (Fig. 2*C*). In both, winter-grey hares were homozygous for the derived allele and the ancestral state was conserved at deep phylogenetic levels (*SI Appendix*, Table S8). This suggests that the winter-grey coat is inherited as a recessive trait, in agreement with breeding experiments (14). In the strongest association block, perfect association across genotyped loci was only disrupted in one specimen that was classified as winter-grey and heterozygous for most SNPs (Fig. 2C and *SI Appendix*, Table S6). While this suggests incomplete dominance of white over grey (14), it is also possible that the specimen was collected while in the process of molting to white.

Downregulation of *Agouti* hair-cycle isoform in Faroese hares. Our results show an association between the *Agouti* genomic region and white/grey winter color variation. The agouti signaling protein antagonizes the melanocortin 1 receptor, shifting melanogenesis to the production of lighter phaeomelanin or inhibiting pigment production (21), and has been implicated in lighter permanent pigmentation in other organisms (13, 22). Moreover, *cis*-regulatory variation in *Agouti* has been

associated with the determination of white or brown winter coats in snowshoe hares, where the winter-white allele is dominant and causes increased expression of *Agouti* relative to winter-brown during the autumn molt (11). In Faroese hares, the winter-grey coat is formed by a mixture of white hairs with short black tips and dark hairs with short white bands (*SI Appendix*, Fig. S6). No associated variants were found in the *Agouti* coding exons, and we hypothesized that the grey coat could be caused by an overall downregulation of the *Agouti* hair cycle isoform (expressed on the dorsum; 23) during the autumn molt.

We quantified the expression of *Agouti* hair-cycle isoform in the skin of mountain hares undergoing the autumn molt, sampled in the Faroe Islands (N = 3 individuals) and the Alps (N = 5), normalized by the expression of reference genes (*SI Appendix*, Table S9). Three skin types were sampled in each specimen, representing three stages – early molt (brown coat patch), ongoing molt (intermediate), and late molt (white or grey coat patch) – to capture the expression changes during the molt (following 24). Notwithstanding the expected variance in quantitative measures of gene expression in a small number of animals sampled in the wild, and batch effects that could not be controlled for, our results suggest lower expression of *Agouti* in winter-grey hares ($p = 0.028$, Aligned Rank Transform) (Fig. 3 and *SI Appendix*, Fig. S7), most notably at the early stage of the molt when melanogenesis is active (24). These results indicate that, as in snowshoe hares (11), *cis*-regulatory changes downregulating *Agouti* expression during the molt may cause non-white winter coats in mountain hares. These regulatory modifications should be confirmed with controlled essays, either by using a larger number of captive winter-grey and winter-white hares sampled at one time, or by estimating allele-specific gene expression in heterozygous individuals (as in 11). *Cis*-regulatory evolution at single genes emerges as a mechanism responsible for repeated phenotype modifications (25, 26), which may result from fewer pleiotropic effects of such mutations (27).

Evidence of a selective sweep in Faroese hares. Our genotyping confirmed that the winter-grey variant segregates in Fennoscandian mountain hares (Fig. 2*C*), suggesting that the founder individuals of the Faroese population carried at least one copy of the causal allele, which became fixed after ~65 years (14, 18). We looked for signals of selective sweeps along chromosome 4 in Faroese hares, to understand whether fixation may have been driven by positive selection. Indeed, *Agouti* is at the edge of a long region (chromosome 4: 3.0-5.8 Mb) characterized by lower nucleotide diversity and Tajima's D in Faroese hares than in Alpine and Fennoscandian hares and the rest of the Faroese hare genome ($p<0.001$, 100,000 random samples; *SI Appendix*, Fig. S8*A* and *B*). A selective sweep in Faroese hares but not in Fennoscandian or Alpine hares was confirmed by using a window-free Hidden Markov Model approach suitable for pooled data (Fig 2*D* and *SI Appendix*, Figs. S8*C* and *D*, S9*A* and *B*), and a composite likelihood-ratio approach derived from genotype likelihoods (*SI Appendix*, Figs. S8*E*, S9*C* and *D*). This signal remained when controlling for the demographic history of the Faroese hares using simulated data (*SI Appendix*, Fig. S8*C*). Given the stronger sweep signal at *Agouti* neighboring sites (*SI Appendix*, Fig. S8*C*), we cannot fully exclude other selection targets, but in non-equilibrium situations such as the introduction bottleneck (28) and with heterogeneous landscapes of recombination (29), the inferred sweeps may not be centered around the target of selection. Together with the Population Branch Statistic (Fig. 2*A* and *B*), our results suggest that positive selection underlies the fixation of the winter-grey morph in the Faroese hares. In agreement with this hypothesis, forward demographic simulations suggested a low probability of fixation for an initially rare variant after 65 years of neutral evolution ($p < 0.05$; *SI Appendix*, Table S10). The translocation of mountain hares to an environment with less snow and with pressure for crypsis on the rocky screes imposed by intensive hunting by humans (30), may have led to the rapid adaptation from standing genetic variation determining winter color.

The winter-grey variant introgressed into the mountain hare. Our results suggest an overlapping genetic and functional basis of winter coat color polymorphism in mountain and snowshoe hares. We

236 investigated the evolutionary origin of the concerned variants by combining higher individual
237 coverage (6.1X – 29.3X) whole-genome sequencing data from six hare species from western Europe
238 (mountain hares, European brown hares, Iberian hares – *L. granatensis* – and the broom hare – *L.*
239 *castroviejoi*) and North America (snowshoe hares and black-tailed jackrabbits) (from this work and
240 11, 19, 31) (*SI Appendix*, Table S3). In snowshoe hares, the winter-brown variant introgressed from
241 the neighboring black-tailed jackrabbit, so the local *Agouti* phylogeny differs from the species tree
242 (11). We detected a topological change at the *Agouti* association region (Fig. 4*A* and *SI Appendix*, Fig.
243 S10), grouping the Faroese hares with the Iberian hares and not with other mountain hares.
244 Maximum-likelihood phylogenies contrasting the whole chromosome 4 and the association region
245 confirmed these results, and showed, as expected, grouping of the winter-brown snowshoe hares
246 and black-tailed jackrabbits at *Agouti* (Fig. 4*E* and F). The discordant local *Agouti* phylogeny suggests
247 introgression from the Iberian hare. This hypothesis was further supported by patterns of variation at
248 *Agouti*, namely i) increased sharing of derived variants between Iberian hares and Faroese mountain
249 hares (f_d statistic; $p < 0.001$, 100,000 random samples) (Fig. 4*B*), and ii) reduced scaled genetic
250 distance (RND; $p < 0.001$, 100,000 random samples) (Fig. 4*C*). Modelling the divergence between
251 mountain and Iberian hares (*SI Appendix*, Table S11) and simulating the expected absolute sequence
252 divergence (d_{XY}) showed that reduced d_{XY} in *Agouti* cannot be explained by incomplete lineage
253 sorting alone, even if ancestral polymorphism had been reduced to a minimum by an extreme
254 selective sweep before speciation (Fig. 4*D*). Collectively, these results show that the winter-grey
255 variant introgressed from the Iberian hare into the mountain hare. Though the species are not
256 currently in contact, genetic exchange during post-glacial contact in southern Europe led to vast
257 introgression into the Iberian hare genome (19). We found that part of the genomic block
258 determining the winter-grey morph is also shared with the European brown hare from the Iberian
259 Peninsula, but not with that from Central Europe (*SI Appendix*, Fig. S11). These results point to Iberia
260 as the arena of the introgressive hybridization events, and to the Iberian hare as the source of the
261 winter-grey allele. Still, we cannot fully exclude stepwise introgression pathways, for example with

the brown hare, a species that is in contact with and hybridizes with both the Iberian hare (32) and the mountain hare (33), acting as a vehicle and transmitting the variant to the mountain hare (*SI Appendix*, Fig. S12).

Conclusions

Our work reveals that the evolution of non-white winter coats was remarkably parallel in two hare species with seasonally changing coat color, suggesting that evolution found similar solutions to the same problem (34). Non-white winter coats are determined by genetic variation at the region of the *Agouti* pigmentation gene both in snowshoe hares (11) and in mountain hares, most likely through similar regulatory changes that influence the expression of the gene during the autumn molt. These inferences confirm that the prominent role of *Agouti* in animal pigmentation (13, 35) extends to seasonal color polymorphism. In both hare species, the generation of winter coat color polymorphism resulted from a replicated process of introgressive hybridization with non-color changing close relatives affecting the same genomic region. Independently of the nature of the winter-grey variant in the initially introgressed populations (neutral, advantageous or possibly mildly deleterious), it persisted in mountain hare populations before reaching fixation in the Faroese hares. Given the slow emergence of novel adaptive mutations, standing introgressed variation has the potential to accelerate local adaptation (36, 37). In this context, the recurrent introgression of genetic variation, generating phenotypes upon which selection can act (11, 38, 39), may fuel replicated adaptive responses to rapidly changing environments.

Materials and methods

Additional detailed information on materials and methods with associated references is provided in *SI Appendix*.

Samples and sequencing. Individually barcoded whole-genome sequence data at low individual coverage (Illumina) was generated for 59 mountain hares (*Lepus timidus*) collected from the Faroe Islands (N = 20 individuals; total population coverage 16.8X), Fennoscandia (N = 19; 21.7X) and the Alps (N = 20; 17.1X) (Fig. 1*C*). Samples were obtained opportunistically during the regular permitted hunting season or were kindly provided by researchers; no animals were killed for the purpose of this research, and all samples were collected before the Nagoya Protocol came into force. Higher individual coverage whole-genome sequence data from one Faroese hare and six *Lepus* species were also analyzed (6.1-29.3X; *SI Appendix*, Table S3). The reads were mapped to a hare pseudo-reference genome built through iterative mapping (19).

Population structure and evolutionary relationships. Principal component analysis was performed using a single-read sampling approach. The evolutionary relationship among populations was determined inferring a neighbor-joining tree based on pairwise genetic distances between individuals, and a population tree based on allele frequencies. The introduction of hares into the Faroe Islands was modelled using coalescent simulations and Approximate Bayesian Computation.

Scans of differentiation, SNP genotyping and selection analyses. The Population Branch Statistic, F_{ST} and Fisher exact tests were estimated in 20 kb non-overlapping windows, and case-control association tests were performed, averaged across non-overlapping windows of 100 SNPs. Fifty-nine SNPs (MassArray System) and one insertion-deletion (PCR) were genotyped in 127 mountain hares, including 29 museum specimens with recorded winter coat color (15 winter-grey and 14 winter-white; Swedish Museum of Natural History). Tajima's D and nucleotide diversity were estimated in 200 kb windows. Scans for selective sweeps were performed along chromosome 4 using Hidden Markov Model and composite likelihood-ratio approaches, taking into account the inferred demographic model. Forward simulations under the demographic scenario were used to estimate the probability of fixation of a rare allele.

Gene expression. The expression of *Agouti* hair-cycle isoform was quantified in skin biopsies sampled in mountain hares from the Faroe Islands (N = 3 individuals) and the Alps (N = 5) during the autumn molt. Relative expression was measured using qPCR, normalized to reference genes *ACTB* and *SDHA* using a ΔC_t approach, and estimating $\log_2$(abundance) using a Bayesian approach.

Phylogenies and introgression inferences. Topology weighting analyses were performed along chromosome 4 using higher-coverage genome data from several *Lepus* species (*SI Appendix*, Table S3). Maximum-likelihood local *Agouti* and broad chromosome 4 phylogenies were inferred. Introgression was tested by estimating the fraction of introgression (f_d) and the relative node depth (RND) in 20 kb overlapping windows of 2 kb steps along chromosome 4. The divergence between mountain hares and Iberian hares was modelled using higher-coverage whole genome data (N = 2 individuals per species), and the inferred parameters were used to perform coalescent simulations of the expected distribution of genetic distances.

Data availability

All data discussed in the paper will be made available to readers.

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Figure Legends

Fig. 1. Mountain hare winter coat color morphs, sampling and evolutionary relationships among populations. **(*A*)** Winter-white morph (specimen NRM588859, Swedish Museum of Natural History - NRM). **(*B*)** Winter-grey morph (NRM588861, NRM). Pictures reproduced with permission of NRM. **(*C*)** Distribution of the mountain hare in Europe (grey area) and approximate sampling localities of individuals used in whole-genome analyses (*SI Appendix*, Table S1): FAR - the Faroe Islands (N = 20), ALP - the Alps (N = 20), FSC - Fennoscandia (N = 19). The red dot indicates the region of origin of the hares translocated to the Faroe Islands. **(*D*)** Population tree based on allele frequencies, with bootstrap supports. **(*E*)** Parameter estimates for the demographic history of Faroese hares: *N* - effective population sizes (number of diploid individuals) of the Fennoscandian (FSC), Faroese (FAR) and founder (F) populations; T_i - time of the introduction (years; assuming two years per generation), *g* - growth rate (negative value backward-in-time implies expansion forward-in-time) according to $N_F = N_{FAR}\, e^{gT_i}$; mode of estimated parameters is shown and 95% HPD intervals are in parentheses (see *SI Appendix*, Table S4).

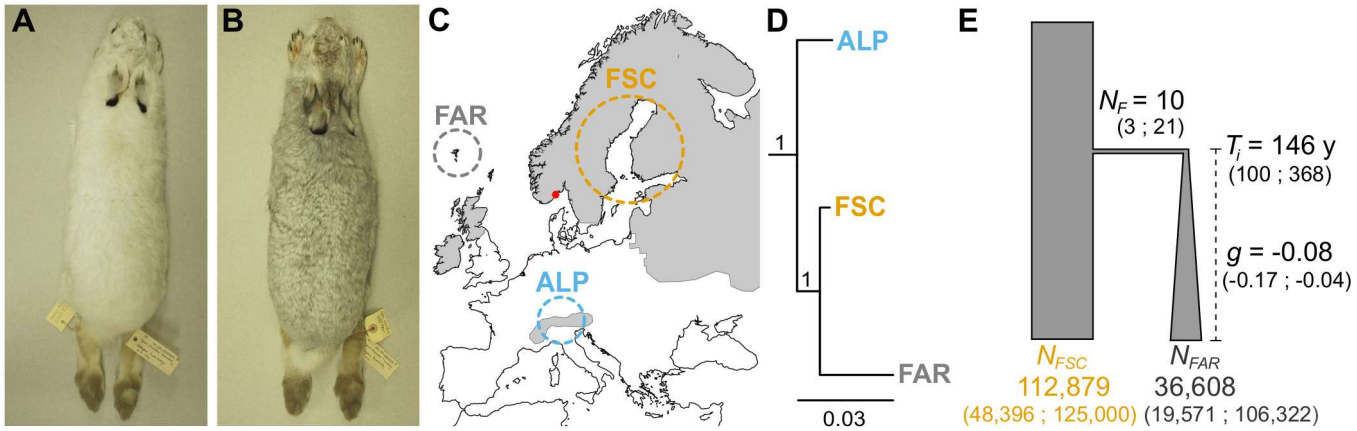
Fig. 2. The candidate association region for winter-white/winter-grey coat color polymorphism in mountain hares. **(*A*)** Genome scan of population branch statistic (PBS) for Faroese hares in 20 kb non-overlapping windows. **(*B*)** Zoomed-in view of PBS in the chromosome 4 candidate region and gene structure (non-coding exons of *Agouti* are marked as *HC* – hair-cycle specific isoform, *V* – ventral isoform). The dashed line represents the 0.01% genome-wide cutoff. **(*C*)** Genotypes at 60 loci spanning the *Agouti* (chromosome 4) and *USP38* (chromosome 15) candidate regions, and other windows of high PBS along the genome (*SI Appendix*, Tables S6 and S7 and Fig. S5). Rows depict specimens and columns indicate genotyped loci (coordinates of first and last SNPs in the *Agouti* region are indicated). Black: homozygous for the Faroese variant; light grey: homozygous for the

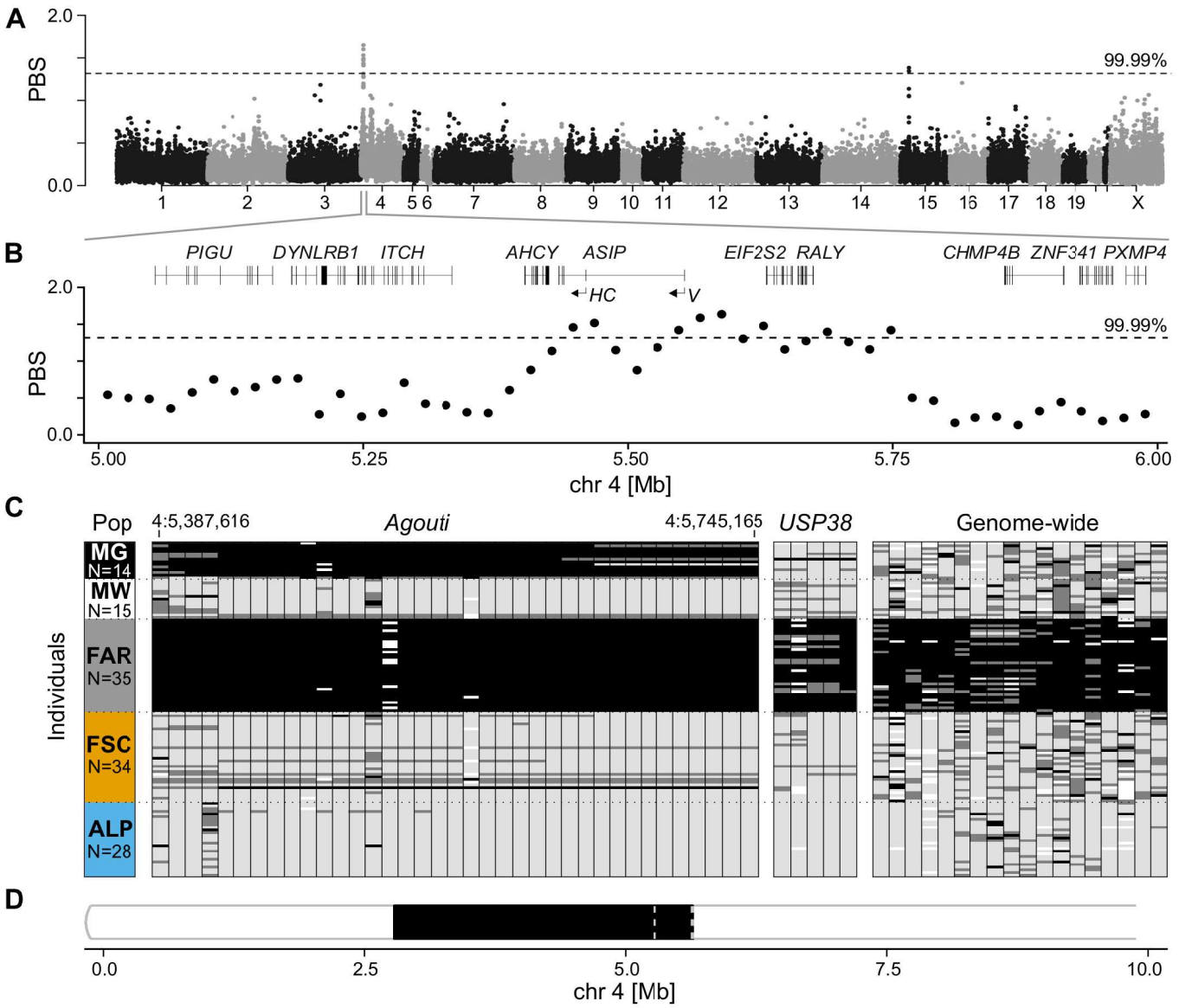
alternative variant; dark grey: heterozygous; white: missing data; MG: winter-grey museum specimens; MW: winter-white museum specimens; FAR: Faroe Islands; FSC: Fennoscandia; ALP: Alps. **(*D*)** Selective sweep detected by Pool-hmm in the Faroese hares on the first 10 Mb of chromosome 4; dashed lines delimit the association region (5.40-5.76 Mb).

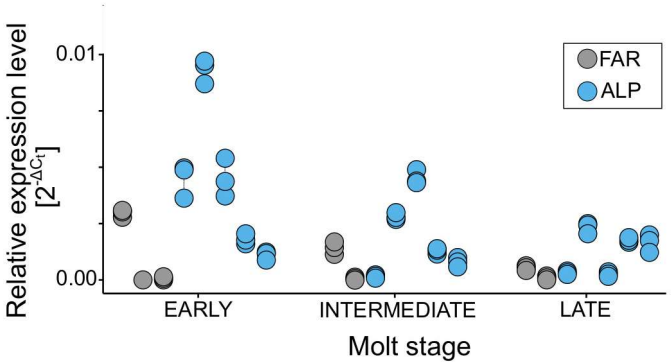
Fig. 3. *Agouti* hair-cycle isoform expression in mountain hares during the autumn molt (skin sampled at early, intermediate and late molt per specimen, following 24). The expression level ($2^{-\Delta Ct}$) is shown relative to reference gene *ACTB* (see additional analyses in *SI Appendix*, Fig. S7). Points represent relative measures and dashed lines connect technical replicates. FAR – winter-grey Faroese mountain hares, ALP – winter-white Alpine mountain hares.

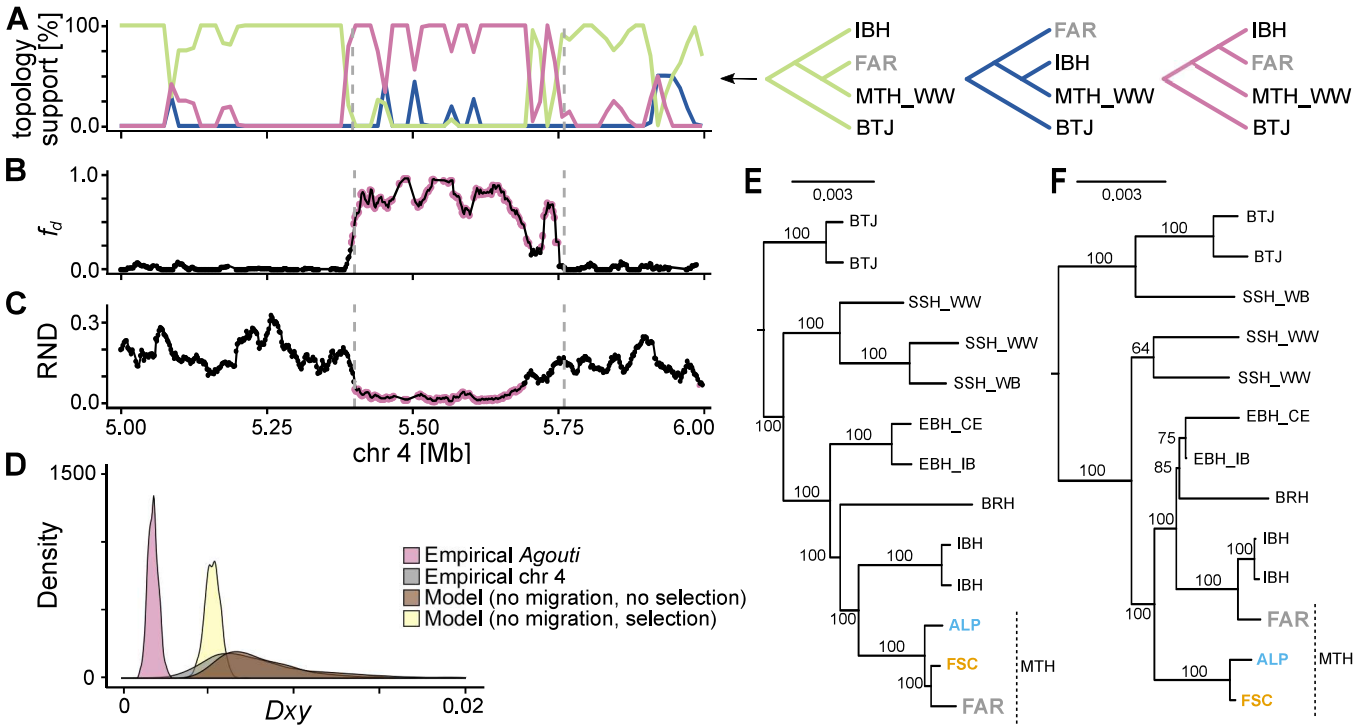
Fig. 4. The evolution of the winter-grey variant of the mountain hare: signatures of introgression from the Iberian hare. **(*A*)** Weightings for three tree topologies including the Faroese mountain hare (FAR), winter-white mountain hares (MTH_WW), the Iberian hare (IBH) and the black-tailed jackrabbit (BTJ), plotted with loess smoothing (span = 0.025). **(*B*)** Fraction of introgression (f_d) between the Iberian hare and the Faroese mountain hare (20 kb windows with 2 kb steps). Pink dots indicate 1% highest values on chromosome 4. **(*C*)** Relative node depth (RND) between the Faroese mountain hare and the Iberian hare (20 kb windows with 2 kb steps). Pink dots indicate 1% lowest values in chromosome 4. **(*D*)** Distributions of d_{XY} between the Faroese mountain hare and the Iberian hare on chromosome 4 (grey), at the *Agouti* region (pink), simulated under a model without migration (brown), and simulated with strong ancestral selection (yellow) (see *SI Appendix*, Fig. S12*A*). **(*E*)** Maximum-likelihood tree with bootstrap supports for chromosome 4. MTH – mountain hares, from the Faroe Islands (FAR), Alps (ALP) and Fennoscandia (FSC); BRH – broom hare; BTJ – black-tailed jackrabbit; EBH_IB, EBH_CE – European brown hares from the Iberian Peninsula and Central Europe, respectively; IBH – Iberian hare; SSH_WW, SSH_WB – winter-white and winter-

brown snowshoe hares, respectively. **(*F*)** Maximum-likelihood tree for the ~360 kb *Agouti* association region.


A
B
C
FAR
FSC
ALP
D
ALP
1
FSC
1
FAR
0.03
E
N_F = 10
(3 ; 21)
T_i = 146 y
(100 ; 368)
g = -0.08
(-0.17 ; -0.04)
N_{FSC}
112,879
(48,396 ; 125,000)
N_{FAR}
36,608
(19,571 ; 106,322)


A
PBS
2.0
0.0
99.99%
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X
B
PIGU
DYNLRB1
ITCH
AHCY
ASIP
EIF2S2
RALY
CHMP4B
ZNF341
PXMP4
HC
V
PBS
2.0
0.0
99.99%
5.00
5.25
5.50
5.75
6.00
chr 4 [Mb]
C
Pop
4:5,387,616
Agouti
4:5,745,165
USP38
Genome-wide
Individuals
MG
N=14
MW
N=15
FAR
N=35
FSC
N=34
ALP
N=28
D
0.0
2.5
5.0
7.5
10.0
chr 4 [Mb]


Relative expression level
[2^-ΔCt]
0.01
0.00
FAR
ALP
EARLY
INTERMEDIATE
LATE
Molt stage


A
topology support [%]
100
0.0
IBH
FAR
MTH_WW
BTJ
FAR
IBH
MTH_WW
BTJ
IBH
FAR
MTH_WW
BTJ
B
f_d
1.0
0.0
C
RND
0.3
0.0
5.00
5.25
5.50
5.75
6.00
chr 4 [Mb]
D
Density
1500
0
0
Dxy
0.02
Empirical Agouti
Empirical chr 4
Model (no migration, no selection)
Model (no migration, selection)
E
0.003
BTJ
BTJ
SSH_WW
SSH_WW
SSH_WB
EBH_CE
EBH_IB
BRH
IBH
IBH
ALP
FSC
FAR
MTH
F
0.003
BTJ
BTJ
SSH_WB
SSH_WW
SSH_WW
EBH_CE
EBH_IB
BRH
IBH
IBH
FAR
ALP
FSC
MTH