Evolution of Coat Color in Snowshoe hares

Snowshoe hares (*Lepus americanus*) are found in North America and they can live for several years. Their coat alternate between brown in summer and white in winter. One of the strongest and most consistent consequences of anthropogenic greenhouse gas emissions is the decreasing duration of snow cover. Such change in snow cover might have consequences on snowshoe hares.

186 snowshoe hares were radio collared over 3 years at two sites and their coat color was measured weakly.

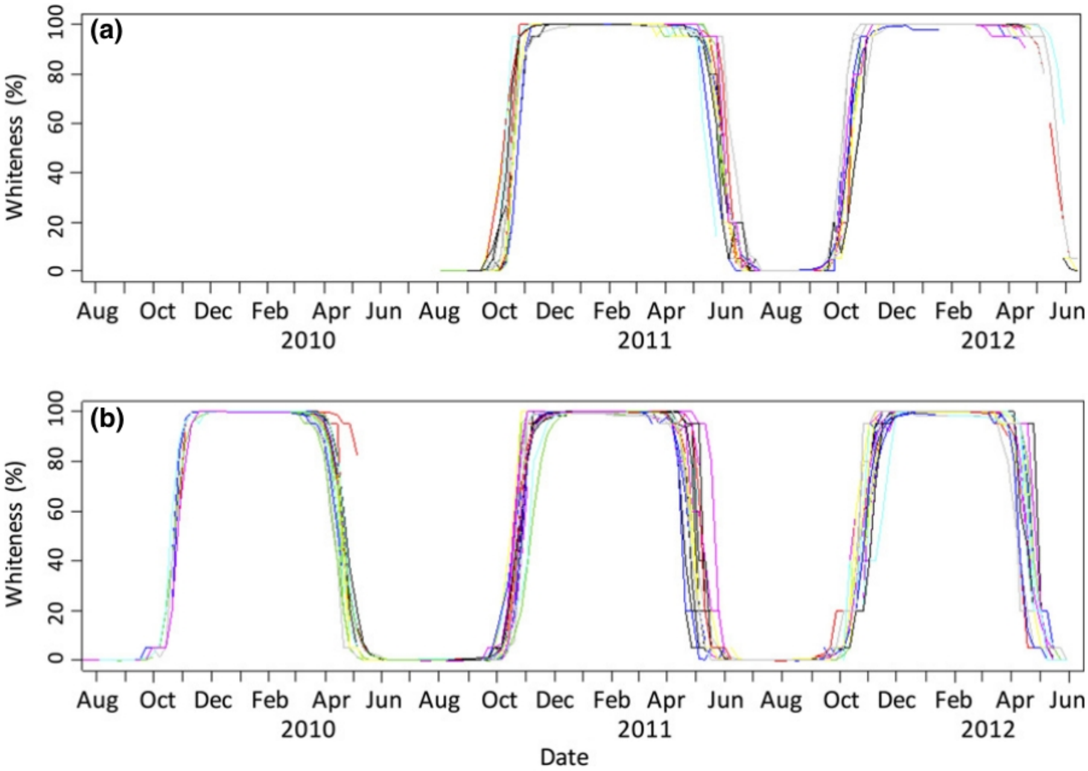

Figure 1. Individual coat color of radiocollared snowshoe hares. One continuous colored line corresponds to one individual (one color = one individual). Individuals were monitored from (a) August 2010 to July 2012 at Gardiner, MT (n = 50 individuals) and from (b) August 2009 to June 2012 at Seeley Lake, MT (n = 136 individuals).

What can you conclude from this experiment?

Color contrast was calculated as the absolute difference between the individual's estimated weekly coat color and weekly mean snow cover at its respective study site (Fig. 2).

When is color mismatch observed?
Among the 186 radiocollared individuals, how many have been observed to match their environment color during an entire year? How can you explain the observed variability between individuals and years?

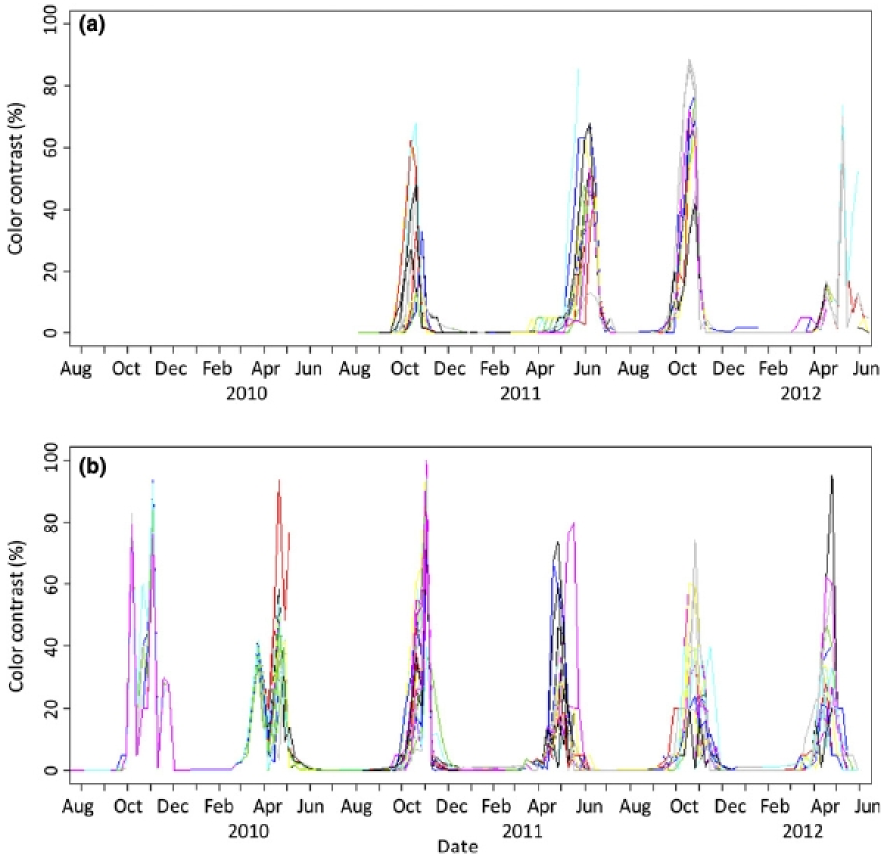

Figure 2. Individual color contrast of radiocollared snowshoe hares monitored (a) from August 2010 to July 2012 at Gardiner, MT (n = 50 individuals) and (b) from August 2009 to June 2012 at Seeley Lake, MT (n = 136 individuals).

Radio collars were equipped with mortality sensors and were used to monitor survival status weekly. When a mortality signal was detected, the individual was located to determine the cause of death. Among the 186 individuals, 124 (67%) died by predation, 24 died for other reasons (e.g. hare body found intact), 17 had radiocollar failures or permanent emigration out of the study area and 21 individuals survived until the end of the study.

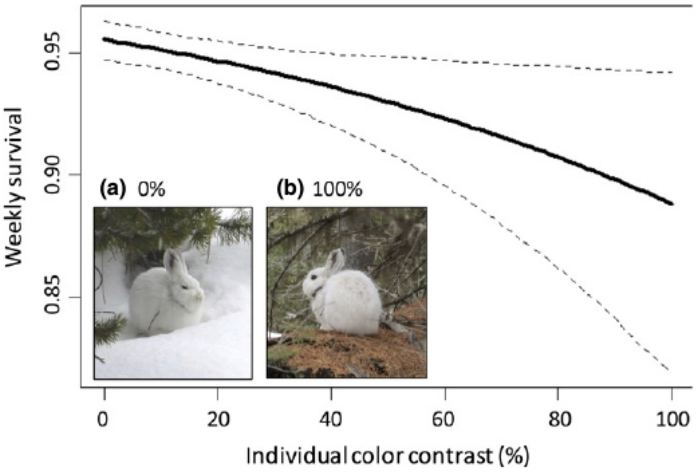

Figure 3. Effect of individual color contrast on weekly snowshoe hare survival. Dashed lines indicate 95% credible intervals, while the solid line depicts mean survival at a given level of color contrast between an individual hare and its background. Photographs depict radiocollared hares from our study showing (a) 0% and (b) 100% contrast.

What can you conclude from Fig. 3?

Most snowshoe hares (such as the ones studied above in Gardiner and Seedley Lake, MT) molt into white winter coats. However, a few populations molt into brown winter coats (Fig. 4).

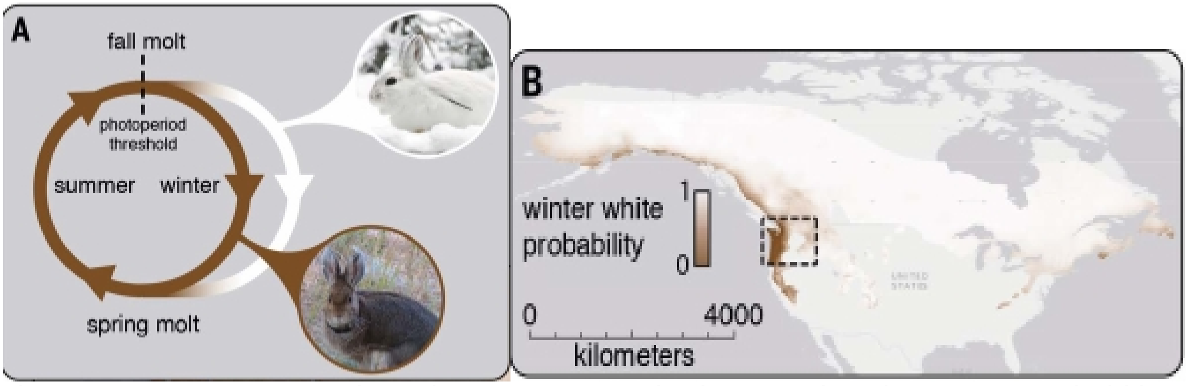

Figure 4. (A) two alternative winter color morphs in snowshoe hares. (B) Probability of winter-white coats.

How can you explain the repartition of the two winter color morphs shown in Fig. 4B? The NOAA (https://www.ncdc.noaa.gov) compiles daily measures across USA of air temperature, soil temperature, precipitations, wind speed and snow cover. How can you use NOAA data to test hypotheses?

To dissect the genetic basis of polymorphic seasonal camouflage, the genomes of a winter-white hare from Montana (MT) and of a winter-brown hare from Washington (WA) were sequenced. 80 whole exomes were also sequenced from two regions containing both forms (WA n=26, OR n=26, with 50% of winter-white individuals and 50% of winter-brown individuals for each region), a winter-white locality (MT n=14) and a winter-brown locality (BC n=14).

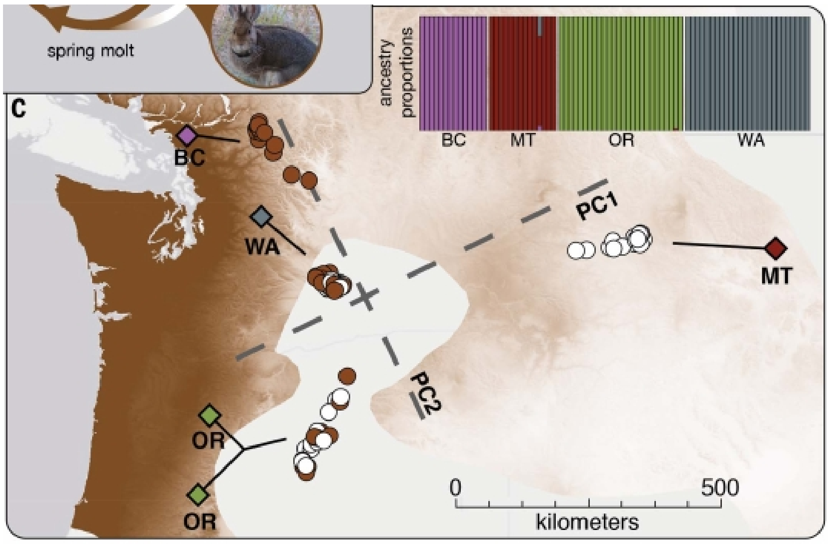

Figure 5. Magnification of the region outlined in Fig. 4B. Each dot represents one individual whose exome was sequenced (brown dot = brown winter coat, white dot = white wintercoat). Top right is the population ancestry plots of the 38,694 unlinked SNPs derived from 80 exomes (one vertical bar = one individual, individuals were grouped into 4 genetic clusters (purple, red, green and blue)).

What does the analysis of the 38,694 SNPs from the 80 exomes reveal?

Fixation index (F_{st}) is close to 0 between winter-white and winter-brown individuals within polymorphic localities. The polymorphic localities (WA and OR) show no evidence of admixture on the basis of linkage disequilibrium patterns or allele sharing with other populations.

What does the F_{st} tells you? What is admixture? What is linkage disequilibrium?

513,812 SNPs were tested for coat color association across polymorphic populations.

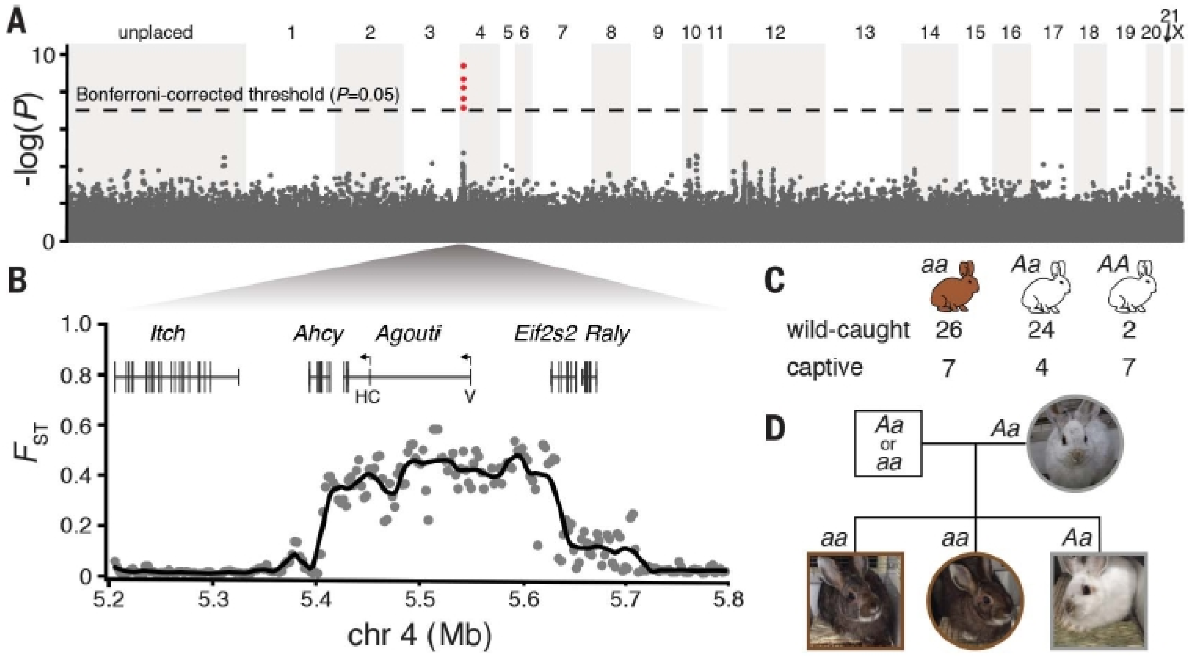

Figure 6. A: Exome SNPs association for polymorphic zone individuals. Red points above the dashed line exceed the Bonferroni-corrected threshold of P=0.05. B: Gene structures of *Itch*, *Ahcy*, *Agouti*, *Eif2s2* and *Raly* across the associated interval on chromosome 4 and alternative *Agouti* transcription start site (arrows) corresponding to hair-cycle (HC) and ventral (V) 5'UTR. Sliding window averages of Fst (5kb with 2.5kb step) between winter-white and winter-brown individuals. C: dominance of winter coat color inferred from *Agouti* genotypes in wild and captive hares. D: Pedigree and genotype of a mixed-phenotype family (paternal allele is unknown but inferred to carry the *a* allele).

How many genomic regions are detected in the association study?
What does the plot of Fst indicate in Figure 6B?
Which allele of Agouti is dominant?

Agouti is known to be expressed as two isoforms, ventral- (V) or hair cycle (HC)–specific, which can be distinguished by alternative 5′ untranslated regions (5′UTRs; Fig. 6B). Analysis of isoform gene expression by qRT-PCR and of allele-specific gene expression levels by pyrosequencing was performed in three captive heterozygous hares.

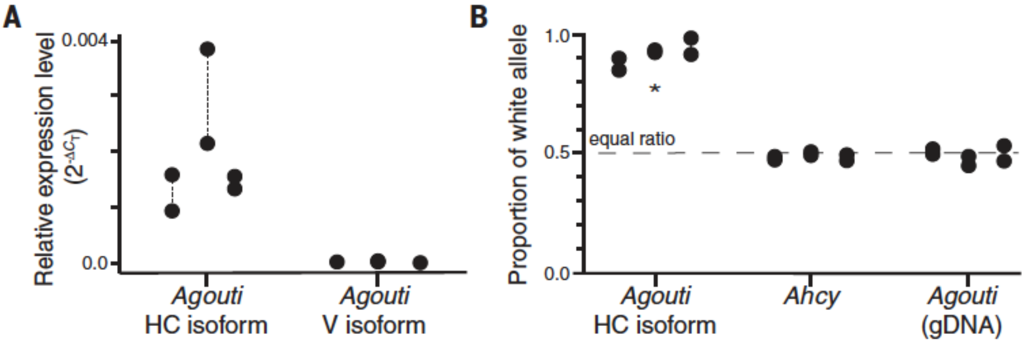

Figure 7. Relative expression measured by qRT-PCR (normalized to *Gapdh*, a constitutively

expressed gene) of *Agouti* HC and V isoforms in molting skin of winter-white (Aa) snowshoe hare. B : Relative abundance of the winter-white allele A mRNA relative to the winter-brown allele a mRNA in the same skin sample for Agouti hair cycle transcripts, Ahcy transcripts and Agouti genomic DNA (gDNA) measured by pyrosequencing ($P<0.00001$). Pairs of points represent technical replicates.

What can you infer from the results presented in Fig. 7?
How can you link these results to the dominance relation of Agouti alleles?

Comparison of winter-white and winter-brown genomes reveal elevated levels of absolute genetic divergence across Agouti : Agouti d_{XY}=1,6%, whereas genome wide d_{XY}=0,41% ($P<0.0001$ randomization test).

What is genetic divergence (d_{XY})? Is it possible that color polymorphism appeared via a recent de novo mutation?
How could this elevated divergence be explained?

The mitochondrial gene *cytb* was sequenced from many wild-caught individuals of snowshoe hares (975 individuals), and from two western North American prairie-scrub species, black-tailed jackrabbits (7 individuals) and white-tailed jackrabbit (1 individual). Results of the phylogenetic analysis of *cytb* sequences is shown in Fig. 8.
Black-tailed jackrabbits have grey-brown coats, they moult once a year and do not acquire a white coat in winter.

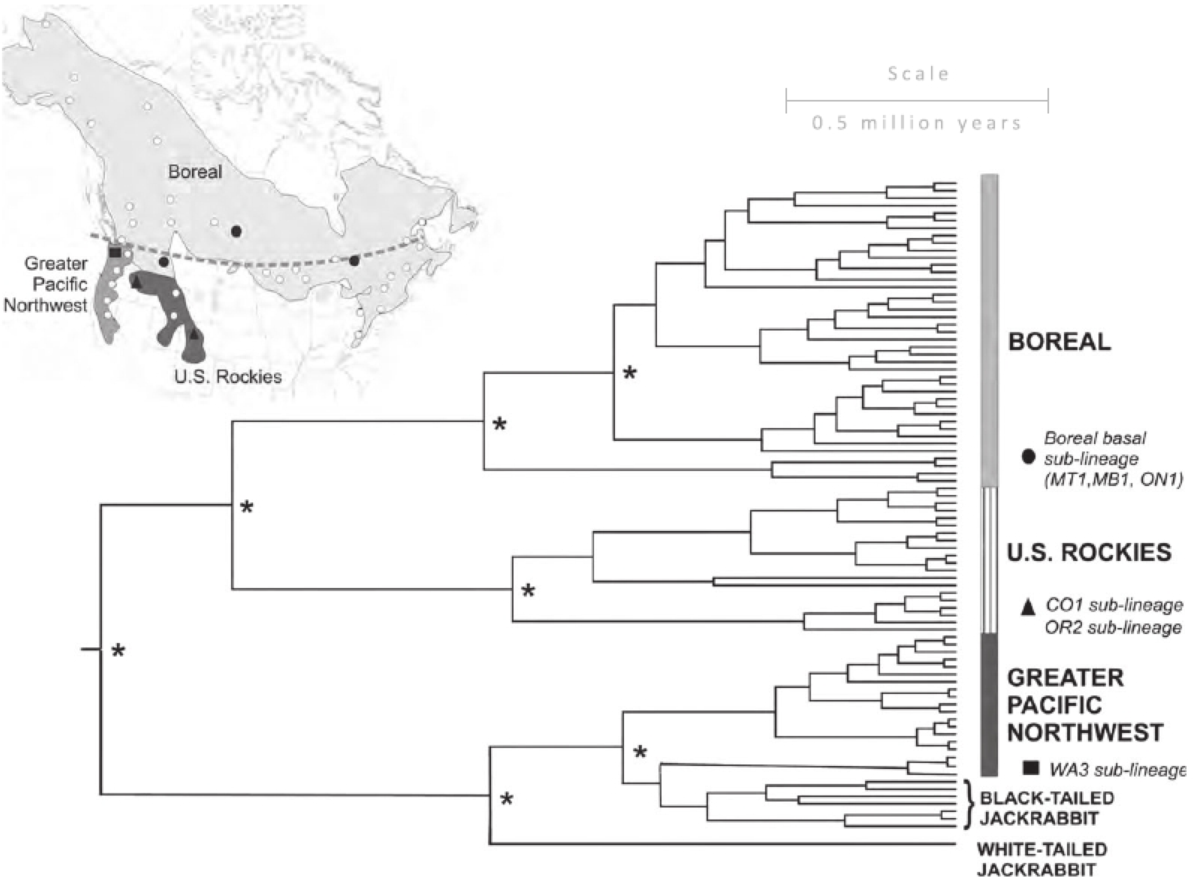

Figure 8. Snowshoe hare phylogenetic relationships, as constructed in BEAST version 1.7.4 , based on the *Cytb* gene. Asterisks (*) indicate lineages and sublineage divisions with ≥95% posterior probability support. Map shows the geographic distribution of the sampled populations of snowshoe hares. Filled symbols identify sublineages.

How do you explain that there are fewer than 975 branches for snowshoe hares in the phylogenetic tree? Can you distinguish several genetic groups of snowshoe hares

coincident with geographic distribution? If so, how many major genetic groups of snowshoe hares can be distinguished? Can you reconcile these results with the data presented in Fig. 5C?
Based on Fig. 8, can you infer for the Greater Pacific Northwestern population of snowshoe hares? Remember that mitochondrial DNA is transmitted through the mother.

Sequences across the *Agouti* locus were compared between snowshoe hares, black tailed jackrabbits (2 individuals), mountain hares (1 individual) and European rabbits (1 individual).

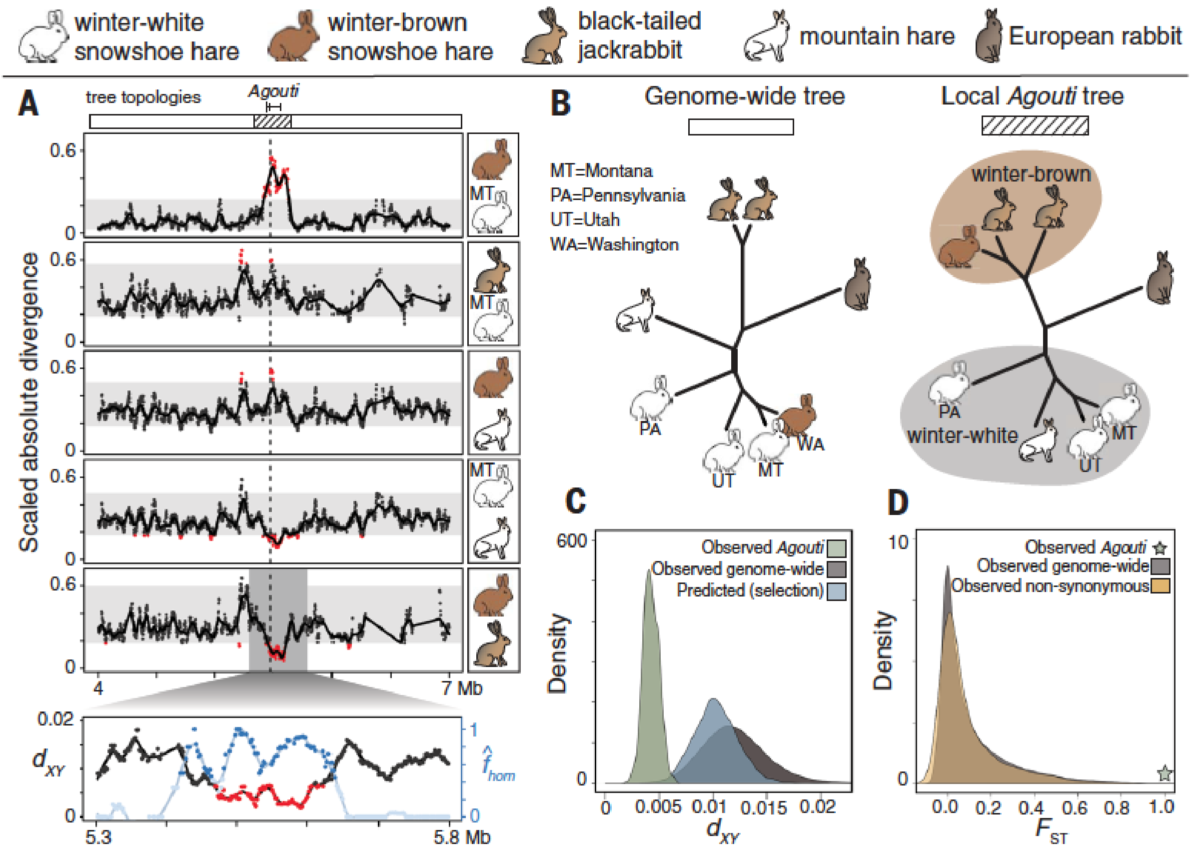

Figure 9. (A) Pairwise comparisons of genetic divergence in 20-kb sliding windows (dashed line indicates location of candidate insertion-deletion mutations in *Agouti*). Gray rectangles represent 99.8% bootstrap quantiles, and red points are windows with one-tailed P < 0.001 based on randomization tests. Bottom plot shows a finer scale of absolute divergence in black (d_{XY}, red points with one-tailed P < 0.001) and the fraction of introgression in blue (f hom, dark blue points with z score > 4) between black-tailed jackrabbits and the WA winter-brown snowshoe hare. (B) The most common genome-wide topology (white) and the local *Agouti* topology (hatched; rabbit outgroup). Brown and gray-shaded regions indicate winter-brown and winter-white groups, respectively. (C) Distributions of d_{XY} between the winter-brown snowshoe hare and black-tailed jackrabbits genome-wide (gray), at *Agouti* (green), and under simulations of strong ancestral selection (blue). (D) Distributions of SNP F_{ST} values between BC (monomorphic winter-brown) and MT (monomorphic winter-white) hares genome-wide (gray) and for nonsynonymous SNPs (yellow). The green star indicates F_{ST} = 1 at a diagnostic *Agouti* SNP.

What can you conclude about the winter-brown snowshoe hares from the topologies of the trees in Fig. 9B and the pair-wise comparisons of genetic divergence of Fig. 9A,C?
What can you conclude about the PA-winter-white snowshoe hares and the mountain hares? Propose at least two scenarios.
What would you sequence to distinguish between these alternatives?

To test for local adaptation, composite likelihood ratio was computed in 50-kb windows along chromosome 4 for each population of snowshoe hares. The higher the CLR, the more likely the region experienced positive selection. The CLR P-value threshold was established using a posterior predictive simulation approach.

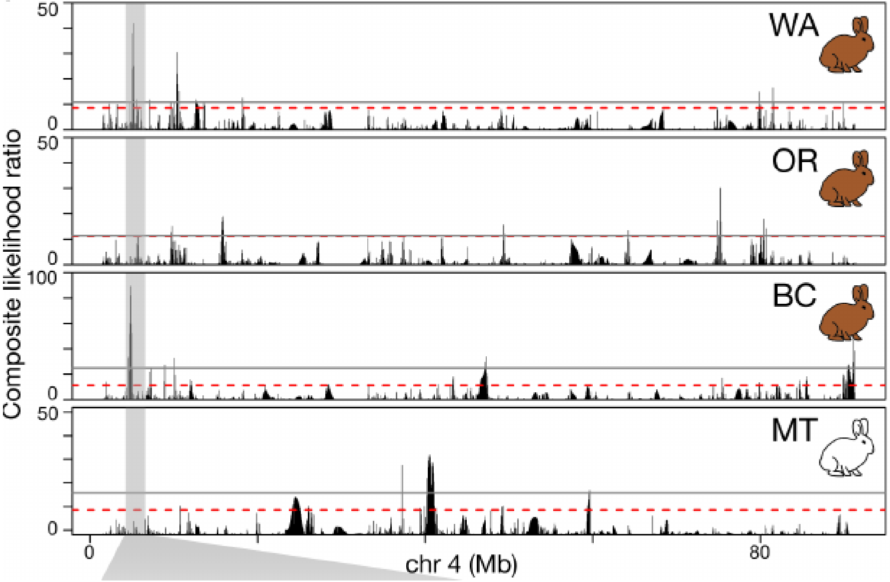

Figure 10. Composite likelihood ratio (CLR) across chromosome 4 for winter-brown individuals from WA, OR, and BC and winter-white individuals from MT. The gray line is the 99% CLR quantile and the dashed red line is P=0.01 threshold based on inferred population history. The *Agouti* locus is highlighted in gray.

What can you detect at the Agouti locus?

Based on all data, reconstruct the evolution of winter-brown snowshoe hares.

Further reading

Cheng, E., Hodges, K. E., Melo-Ferreira, J., Alves, P. C., & Mills, L. S. (2014). Conservation implications of the evolutionary history and genetic diversity hotspots of the snowshoe hare. *Molecular ecology*, *23*(12), 2929-2942.
Zimova, M., Mills, L. S., & Nowak, J. J. (2016). High fitness costs of climate change-induced camouflage mismatch. *Ecology letters*, *19*(3), 299-307.
Matthew R. Jones, L. Scott Mills, Paulo Célio Alves, Colin M. Callahan, Joel M. Alves, Diana J. R. Lafferty, Francis M. Jiggins, Jeffrey D. Jensen, José Melo-Ferreira, Jeffrey M. Good, 2018. Adaptive introgression underlies polymorphic seasonal camouflage in snowshoe hares. Science, 360, 1355-1358.