

1 **Title: Adaptive Introgression Underlies Polymorphic Seasonal Camouflage in**  
2 **Snowshoe Hares**

3  
4 **Authors:** Matthew R. Jones<sup>1\*</sup>, L. Scott Mills<sup>2,3,4</sup>, Paulo Célio Alves<sup>2,5,6</sup>, Colin M. Callahan<sup>1</sup>, Joel  
5 M. Alves<sup>5,7</sup>, Diana J. R. Lafferty<sup>2,4,8</sup>, Francis M. Jiggins<sup>7</sup>, Jeffrey D. Jensen<sup>9,10</sup>, José Melo-  
6 Ferreira<sup>5,6\*</sup>, Jeffrey M. Good<sup>1,2\*</sup>

7 **Affiliations:**

8 <sup>1</sup>Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA.

9 <sup>2</sup>Wildlife Biology Program, University of Montana, Missoula, MT 59812, USA.

10 <sup>3</sup>Office of Research and Creative Scholarship, University of Montana, Missoula, MT 59812,  
11 USA.

12 <sup>4</sup>Fisheries, Wildlife, and Conservation Biology Program, Department of Forestry and  
13 Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA.

14 <sup>5</sup>CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório  
15 Associado, Universidade do Porto, 4485-661 Vairão, Portugal.

16 <sup>6</sup>Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, 4169-007 Porto,  
17 Portugal.

18 <sup>7</sup>Department of Genetics, University of Cambridge, Cambridge CB2 3EH, UK.

19 <sup>8</sup>Department of Biology, Northern Michigan University, Marquette, MI 49855, USA.

20 <sup>9</sup>School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne,  
21 Switzerland.

22 <sup>10</sup>School of Life Sciences, Arizona State University, Tempe, AZ 85281, USA.

23  
24 \*Correspondence to: [matthew2.jones@umontana.edu](mailto:matthew2.jones@umontana.edu), [jmeloferreira@cibio.up.pt](mailto:jmeloferreira@cibio.up.pt),  
25 [jeffrey.good@umontana.edu](mailto:jeffrey.good@umontana.edu)

26 **Abstract:** Snowshoe hares (*Lepus americanus*) maintain seasonal camouflage by molting to a  
27 white winter coat, but in regions with low snow cover some hares remain brown in the winter.  
28 We show that *cis*-regulatory variation controlling seasonal expression of the *Agouti* gene  
29 underlies this adaptive winter camouflage polymorphism. Genetic variation at *Agouti* clustered  
30 by winter coat color across multiple hare and jackrabbit species, revealing a history of recurrent  
31 interspecific gene flow. Brown winter coats in snowshoe hares originated from an introgressed  
32 black-tailed jackrabbit allele that has swept to high frequency in mild winter environments.  
33 These discoveries show that exchange of genetic variants underlying key ecological traits  
34 through hybridization can seed past and ongoing adaptation to rapidly changing environments.  
35

36 **One Sentence Summary:** Introgression at the pigmentation gene *Agouti* underlies local  
37 adaptation in seasonal camouflage of snowshoe hares.

38 **Main Text:** Many species undergo reversible changes in morphology, physiology, and behavior  
39 to cope with the challenges of seasonal environments. These critical components of phenotypic  
40 plasticity often track the environment through the photoperiod-dependent release of hormones  
41 (1). However, circannual rhythms can become desynchronized when abiotic conditions change  
42 rapidly (2), leading to declines in population fitness (3). The capacity of species to adapt to  
43 rapidly changing environments will depend in part on the proximate and ultimate causes of  
44 variation underlying seasonal traits (4, 5), which remain poorly understood at the molecular level  
45 (1, 2).

46         At least 21 bird and mammal species undergo autumn molts from brown to white coats  
47 (6, 7) as part of a suite of plastic trait responses to seasonal environments. We used natural  
48 variation in seasonal camouflage of the snowshoe hare (*Lepus americanus*) to understand the  
49 genetic basis of this critical seasonal trait. Autumn molts to white winter coats are cued by  
50 photoperiod (8) and generally track seasonal snow cover. Direct estimates of hare survival have  
51 shown that mismatch between coat color and snow cover increases predation (3). White winter  
52 coats predominate across the snowshoe hare range, but some populations molt into brown winter  
53 coats (Fig. 1). In the Pacific Northwest (PNW), shifts in the probability of white coats coincide  
54 with a gradient in snow cover from warmer coastal to colder inland environments, consistent  
55 with local selection for seasonal camouflage with color morphs co-occurring across a broad  
56 polymorphic zone (Fig. 1C) (7).

57         To dissect the genetic basis of polymorphic seasonal camouflage, we used whole genome  
58 sequences for a winter-white hare from Montana (MT, 33× coverage) (9, 10) and a winter-brown  
59 hare from Washington (WA, 22× coverage) and constructed a reference through iterative  
60 mapping (11) to the rabbit genome (9, 12). We then sequenced 80 whole exomes (62 Mb, 21× ±

61 7.6 per individual) from two regions in the PNW polymorphic zone (WA:  $n=26$ , Oregon  
62 hereafter OR:  $n=26$ , each region 50% winter-white), a monomorphic winter-white locality in MT  
63 ( $n=14$ ), and a monomorphic winter-brown locality in British Columbia (BC:  $n=14$ ; table S1). If  
64 the polymorphic zone represents admixture between previously isolated populations, then  
65 genetic structure could obscure genotype-phenotype associations (13). Analysis of 38,694  
66 unlinked single nucleotide polymorphisms (SNPs) revealed geographic structure (Fig. 1C), but  
67 genome-wide genetic differentiation ( $F_{ST}$ ) between winter-brown and winter-white individuals  
68 was  $\sim 0$  within polymorphic localities (table S2). The polymorphic zone also showed no evidence  
69 of admixture based on patterns of linkage disequilibrium (fig. S1) or allele sharing with other  
70 populations (table S3) (14). Thus, geographic variation for winter coat color in the PNW likely  
71 reflects primary intergradation across a gradient in snow cover.

72 We tested 513,812 SNPs for coat color associations across polymorphic populations and  
73 identified a single outlier region on chromosome 4 in perfect association with winter coat color  
74 ( $P=4.24 \times 10^{-10}$ ; Fig. 2A, fig. S2, Additional Data table S1) (12). We then augmented exome data  
75 with low-coverage whole genome resequencing of polymorphic zone hares ( $\sim 20\times$  per color  
76 morph). Coat color associations based on genotype likelihoods (15,173,804 SNPs) (15)  
77 confirmed a single outlier region (fig. S3) localized to a  $\sim 225$  kilobase (kb) interval of elevated  
78  $F_{ST}$  between color morphs centered on the pigmentation gene *Agouti* and two flanking genes,  
79 *Ahcy* and *Eif2s2* (Fig. 2B). Winter-brown hares were homozygous ( $n=26$ ) for brown-associated  
80 alleles (hereafter, *a*), while winter-white hares were either heterozygous ( $n=24$ ) or homozygous  
81 ( $n=2$ ) for the alternative allele (hereafter, *A*; Fig. 2C). We then induced autumn molts in 18  
82 captive wild-caught hares (WA:  $n=11$ , MT:  $n=7$ ) and found perfect concordance between *Agouti*  
83 genotypes and winter coat colors (Fig. 2C, table S4). This experiment included a heterozygous

84 (*Aa*) wild-caught pregnant winter-white female from WA that gave birth in captivity to both  
85 winter-white and winter-brown offspring (Fig. 2D). Therefore, winter coat color segregates as a  
86 dominant locus in both wild and captive animals.

87         The agouti signaling protein (ASIP) antagonizes the melanocortin-1 receptor (MC1R) in  
88 follicular melanocytes, shifting melanogenesis towards lighter pheomelanin pigments or  
89 inhibiting pigment production (16). MC1R mutations suppress expression of winter-white coats  
90 in dark or blue color morphs of arctic foxes, suggesting that ASIP-MC1R interactions are  
91 involved in the development of seasonal color molts (17). *Agouti* is typically expressed as ventral  
92 or hair cycle-specific isoforms distinguished by alternative 5' untranslated regions (UTRs, Fig.  
93 2B) (18). Both isoforms have been associated with lighter dorsal pelage (19, 20). We  
94 hypothesized that the development of winter-white coats, which mostly lack pigments (8), is  
95 controlled by isoform-specific upregulation of *Agouti* during the autumn molt. To test this, we  
96 quantified allele-specific expression of both isoforms and the closely linked *Ahcy* locus in dorsal  
97 skin biopsies from three captive heterozygous hares (*Aa*) undergoing brown-to-white molts.  
98 Quantitative PCR verified expression of *Ahcy* and the *Agouti* hair-cycle isoform while expression  
99 of the ventral isoform was negligible (Fig. 3A, table S5, table S6). Targeted pyrosequencing  
100 revealed highly skewed expression toward the white (*A*) allele of the hair-cycle *Agouti* isoform  
101 ( $P < 0.0001$ , Student's t-test), indicative of *cis*-regulatory variation, while *Ahcy* showed equal  
102 allelic expression (Fig. 3B, table S7). These data suggest that winter-white coats develop as a  
103 consequence of increased expression of *Agouti* during the autumn molt, which fits with our  
104 observed dominance relationships and previous studies on the evolution of lighter pelage in deer  
105 mice (19, 20). Our findings directly link *Agouti* expression and the evolution of seasonal

106 camouflage in snowshoe hares and suggest that *cis*-regulatory evolution plays an important role  
107 in the origin of novel seasonal traits.

108         Comparison of winter-white (MT) and winter-brown genomes (WA) revealed  
109 extraordinarily elevated levels of absolute genetic divergence across *Agouti* (*Agouti*  $d_{XY}$ = 1.6%;  
110 genome-wide  $d_{XY}$ =0.41%; bootstrapped  $P<0.0001$ ; Fig. 4A, fig. S4), indicating that the color  
111 polymorphism did not arise from a recent *de novo* mutation. Alternatively, elevated divergence  
112 could reflect either the long-term maintenance of polymorphism or introgression from another  
113 species (21, 22). Six of the 32 species of hares and jackrabbits (genus *Lepus*) have winter-white  
114 molts but evolutionary relationships within this rapid radiation are poorly resolved (23). To  
115 examine the origins of winter coat color variants, we combined whole genome sequences of two  
116 additional winter-white snowshoe hares from Pennsylvania (PA) and Utah (UT), two winter-  
117 brown black-tailed jackrabbits (*L. californicus*) from Nevada, and a previously sequenced  
118 winter-white mountain hare (*L. timidus*) from Europe (10). Phylogenetic analyses (24) predicted  
119 an exceptionally rare topology at *Agouti* that clustered individuals by winter coat color (Fig. 4B,  
120 fig. S5B). Pairwise divergence between all winter-brown and white individuals was significantly  
121 elevated across a known *cis*-regulatory region of *Agouti* (25, 26) ~40 kb upstream of the  
122 transcription start site of the hair-cycle isoform ( $P<0.001$ ; Fig. 4A, fig. S4). Divergence peaked  
123 across a ~20 kb interval ( $d_{XY}$ = 2.2-2.4%) that included a 1,033 base pair insertion on the winter-  
124 white haplotype and a ~4.3 kb deletion on the winter-brown haplotype (fig. S4). Additional  
125 functional data are needed to determine if either of these candidate mutations underlie the  
126 observed *cis*-regulatory differences in *Agouti* expression (Fig. 3B).

127         The elevated interspecific divergence between color groups suggests that the winter-  
128 white and brown *Agouti* alleles may have arisen relatively early in *Lepus* (21). In contrast,

129 divergence within color groups was strongly reduced across a larger interval encompassing  
130 *Agouti* (Fig. 4A, fig. S6), indicating that winter coat color alleles may have been shared through  
131 hybridization. In support of this hypothesis, we found low but significant levels of genome-wide  
132 introgression (27) between snowshoe hares and both black-tailed jackrabbits and mountain hares  
133 (table S8). Window-based analyses of absolute divergence and derived allele sharing (28)  
134 identified *Agouti* among the strongest genome-wide signatures of introgression in both winter-  
135 brown and winter-white clusters (fig. S7).

136 Previous studies demonstrated mitochondrial DNA introgression from black-tailed  
137 jackrabbits, a western North American prairie-scrub species, into PNW snowshoe hares and  
138 speculated that hybridization may have contributed to the evolution of brown winter coats in  
139 snowshoe hares (29, 30). Consistent with this, winter-brown snowshoe hares unambiguously  
140 nested within black-tailed jackrabbit variation at *Agouti* (Fig. 4B, fig. S5B) resulting in a 174 kb  
141 interval of significantly reduced divergence between species ( $d_{XY}=0.42\%$  versus 1.2% genome-  
142 wide) embedded within a 236 kb interval of significantly elevated admixture proportions  
143 ( $\hat{f}_{hom}=0.71$ ; Fig. 4A). Strong selection at a locus in the ancestral population can reduce  
144 divergence between species (31), resulting in false inferences of admixture (28); however,  
145 coalescent simulations of shared polymorphism with and without selection in the ancestral  
146 population indicate that such a long interval of shallow divergence is highly unlikely in the  
147 absence of interspecific gene flow (Fig. 4C, fig. S7, fig. S8). We also detected introgression  
148 within the winter-white *Agouti* group (fig. S7, fig. S8). Resolving the origin and functional  
149 relevance of these signatures awaits further investigation given that three other North American  
150 *Lepus* species undergo some degree of seasonal coat color change (7).

151 To link introgression with local adaptation we tested for selective sweeps based on allele  
152 frequency skews (32) while controlling for demographic history (fig. S9, table S9). We detected  
153 a hard sweep overlapping *Agouti* in winter-brown individuals from the polymorphic zone, but no  
154 evidence for a sweep in winter-white individuals (fig. S10, fig. S11). We estimate that the sweep  
155 of the winter-brown allele in the PNW occurred 3-15 kya, following the retreat of the Cordilleran  
156 ice sheet (33). High inferred selection coefficients ( $s$ ) on the introgressed winter-brown *Agouti*  
157 background ( $\bar{s}_{WA}=0.024$ ,  $\bar{s}_{OR}=0.015$ ; fig. S11C) and fixation of alternative *Agouti* alleles between  
158 monomorphic winter-brown (BC) and winter-white (MT) localities (Fig. 4D), despite high gene  
159 flow (table S9), indicate that seasonal camouflage is maintained under strong local selection.

160 Despite widespread evidence of hybridization between animal species, introgression has  
161 rarely been directly linked to ecological adaptation (34–36). We have shown that introgression  
162 has shaped locally adaptive seasonal camouflage in snowshoe hares. Recurrent introgression of  
163 coat color variants could facilitate evolutionary responses to environmental change within  
164 populations as well as the long-term maintenance of adaptive variation among species, similar to  
165 adaptive polymorphisms in beak morphology across the radiation of Darwin’s finches (22, 34).  
166 Introgression of winter-brown coats into snowshoe hares may have enabled their persistence in  
167 environments with more ephemeral seasonal snow following the end of the last glacial  
168 maximum. Temperate snow cover duration is predicted to dramatically decrease over the next  
169 century under most models of climate change (37), which may further intensify directional  
170 selection for winter-brown camouflage (3, 6). Thus, the recent establishment of this dynamic  
171 color polymorphism through introgression is likely to be a critical component of ongoing  
172 adaptation to rapidly changing seasonal environments (7) in this iconic ecological model.



173 **References and Notes:**

- 174 1. M. E. Visser, S. P. Caro, K. van Oers, S. V. Schaper, B. Helm, Phenology, seasonal timing  
175 and circannual rhythms: towards a unified framework. *Philos. Trans. Biol. Sci.* **365**, 3113–  
176 3127 (2010).
- 177 2. B. Helm, R. Ben-Shlomo, M. J. Sheriff, R. A. Hut., R. Foster, B. M. Barnes, D.  
178 Dominoni, Annual rhythms that underlie phenology: biological time-keeping meets  
179 environmental change. *Proc. R. Soc. B Biol. Sci.* **280**, 20130016 (2013).
- 180 3. M. Zimova, L. S. Mills, J. J. Nowak, High fitness costs of climate change-induced  
181 camouflage mismatch. *Ecol. Lett.* **19**, 299–307 (2016).
- 182 4. W. E. Bradshaw, C. M. Holzapfel, Genetic response to rapid climate change: it's seasonal  
183 timing that matters. *Mol. Ecol.* **17**, 157–166 (2008).
- 184 5. A. A. Hoffmann, C. M. Sgrò, Climate change and evolutionary adaptation. *Nature.* **470**,  
185 479–485 (2011).
- 186 6. L. S. Mills, M. Zimova, J. Oyler, S. Running, J. T. Abatzoglou, P. M. Lukacs, Camouflage  
187 mismatch in seasonal coat color due to decreased snow duration. *Proc. Natl. Acad. Sci. U.*  
188 *S. A.* **110**, 7360–7365 (2013).
- 189 7. L. S. Mills, E. V Bragina, A. V Kumar, M. Zimova, D. J. R. Lafferty, J. Feltner, B. M.  
190 Davis, K. Hackländer, P. C. Alves, J. M. Good, J. Melo-Ferreira, A. Dietz, A. V Abramov,  
191 N. Lopatina, K. Fay, Winter color polymorphisms identify global hot spots for  
192 evolutionary rescue from climate change. *Science.* **359**, 1033–1036 (2018).
- 193 8. C. P. Lyman, Control of coat color in the varying hare *Lepus americanus* Erxleben. *Bull.*  
194 *Museum Comp. Zool. Harvard Coll.* **93**, 391–461 (1943).
- 195 9. M. Carneiro, C.-J. Rubin, F. Di Palma, F. W. Albert, J. Alföldi, A. Martinez Barrio, G.

196 Pielberg, N. Rafati, S. Sayyab, J. Turner-Maier, S. Younis, S. Afonso, B. Aken, J. M.  
197 Alves, D. Barrell, G. Bolet, S. Boucher, H. A. Burbano, R. Campos, J. L. Chang, V.  
198 Duranthon, L. Fontanesi, H. Garreau, D. Heiman, J. Johnson, R. G. Mage, Z. Peng, G.  
199 Queney, C. Rogel-Gaillard, M. Ruffier, S. Searle, R. Villafuerte, A. Xiong, S. Young, K.  
200 Forsberg-Nilsson, J. M. Good, E. S. Lander, N. Ferrand, K. Lindblad-Toh, L. Andersson,  
201 Rabbit genome analysis reveals a polygenic basis for phenotypic change during  
202 domestication. *Science*. **345**, 1074–1079 (2014).

203 10. F. Seixas, P. Boursot, J. Melo-Ferreira, The genomic impact of historical hybridization  
204 with massive mitochondrial DNA introgression. *In review*.

205 11. B. A. J. Sarver, S. Keeble, T. Cosart, P. K. Tucker, M. D. Dean, J. M. Good,  
206 Phylogenomic insights into mouse evolution using a pseudoreference approach. *Genome*  
207 *Biol. Evol.* **9**, 726–739 (2017).

208 12. Materials and methods are available as supplementary materials on Science Online.

209 13. J. K. Pritchard, M. Stephens, N. A. Rosenberg, P. Donnelly, Association mapping in  
210 structured populations. *Am. J. Hum. Genet.* **67**, 170–181 (2000).

211 14. D. Reich, N. Patterson, D. Campbell, A. Tandon, S. Mazieres, N. Ray, M. V Parra, W.  
212 Rojas, C. Duque, N. Mesa, L. F. García, O. Triana, S. Blair, A. Maestre, J. C. Dib, C. M.  
213 Bravi, G. Bailliet, D. Corach, T. Hünemeier, M. C. Bortolini, F. M. Salzano, M. L. Petzl-  
214 Erler, V. Acuña-Alonzo, C. Aguilar-Salinas, S. Canizales-Quinteros, T. Tusié-Luna, L.  
215 Riba, M. Rodríguez-Cruz, M. Lopez-Alarcón, R. Coral-Vazquez, T. Canto-Cetina, I.  
216 Silva-Zolezzi, J. C. Fernandez-Lopez, A. V Contreras, G. Jimenez-Sanchez, M. J. Gómez-  
217 Vázquez, J. Molina, A. Carracedo, A. Salas, C. Gallo, G. Poletti, D. B. Witonsky, G.  
218 Alkorta-Aranburu, R. I. Sukernik, L. Osipova, S. A. Fedorova, R. Vasquez, M. Villena, C.

- 219 Moreau, R. Barrantes, D. Pauls, L. Excoffier, G. Bedoya, F. Rothhammer, J.-M.  
220 Dugoujon, G. Larrouy, W. Klitz, D. Labuda, J. Kidd, K. Kidd, A. Di Rienzo, N. B.  
221 Freimer, A. L. Price, A. Ruiz-Linares, Reconstructing Native American population  
222 history. *Nature*. **488**, 370–374 (2012).
- 223 15. T. S. Korneliussen, A. Albrechtsen, R. Nielsen, ANGSD: Analysis of Next Generation  
224 Sequencing Data. *BMC Bioinformatics*. **15**, 356 (2014).
- 225 16. E. Le Pape, T. Passeron, A. Giubellino, J. C. Valencia, R. Wolber, V. J. Hearing,  
226 Microarray analysis sheds light on the dedifferentiating role of agouti signal protein in  
227 murine melanocytes via the Mc1r. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 1802–1807 (2009).
- 228 17. D. I. Våge, E. Fuglei, K. Snipstad, J. Beheim, V. M. Landsem, H. Klungland, Two  
229 cysteine substitutions in the MC1R generate the blue variant of the arctic fox (*Alopex*  
230 *lagopus*) and prevent expression of the white winter coat. *Peptides*. **26**, 1814–1817 (2005).
- 231 18. H. Vrieling, D. M. Duhl, S. E. Millar, K. A. Miller, G. S. Barsh, Differences in dorsal and  
232 ventral pigmentation result from regional expression of the mouse *Agouti* gene. *Proc.*  
233 *Natl. Acad. Sci. U. S. A.* **91**, 5667–5671 (1994).
- 234 19. C. R. Linnen, E. P. Kingsley, J. D. Jensen, H. E. Hoekstra, On the origin and spread of an  
235 adaptive allele in deer mice. *Science*. **325**, 1095–1098 (2009).
- 236 20. M. Manceau, V. S. Domingues, R. Mallarino, H. E. Hoekstra, The developmental role of  
237 *Agouti* in color pattern evolution. *Science*. **331**, 1062–1065 (2011).
- 238 21. R. F. Guerrero, M. W. Hahn, Speciation as a sieve for ancestral polymorphism. *Mol. Ecol.*  
239 **26**, 5362–5368 (2017).
- 240 22. F. Han, S. Lamichhaney, B. R. Grant, P. R. Grant, L. Andersson, M. T. Webster, Gene  
241 flow, ancient polymorphism, and ecological adaptation shape the genomic landscape of

- 242 divergence among Darwin's finches. *Genome Res.* **27**, 1004–1015 (2017).
- 243 23. J. Melo-Ferreira, P. Boursot, M. Carneiro, P. J. Esteves, L. Farelo, P. C. Alves, Recurrent  
244 introgression of mitochondrial DNA among hares (*Lepus* spp.) revealed by species-tree  
245 inference and coalescent simulations. *Syst. Biol.* **61**, 367–381 (2012).
- 246 24. N. Zamani, P. Russell, H. Lantz, M. P. Hoepfner, J. R. S. Meadows, N. Vijay, E. Mauceli,  
247 F. di Palma, K. Lindblad-Toh, P. Jern, M. G. Grabherr, Unsupervised genome-wide  
248 recognition of local relationship patterns. *BMC Genomics.* **14**, 347 (2013).
- 249 25. D. M. J. Duhl, H. Vrieling, K. A. Miller, G. L. Wolff, G. S. Barsh, Neomorphic *Agouti*  
250 mutations in obese yellow mice. *Nat. Genet.* **8**, 59–65 (1994).
- 251 26. C. R. Linnen, Y.-P. Poh, B. K. Peterson, R. D. H. Barrett, J. G. Larson, J. D. Jensen, H. E.  
252 Hoekstra, Adaptive evolution of multiple traits through multiple mutations at a single  
253 gene. *Science.* **339**, 1312–1316 (2013).
- 254 27. E. Y. Durand, N. Patterson, D. Reich, M. Slatkin, Testing for ancient admixture between  
255 closely related populations. *Mol. Biol. Evol.* **28**, 2239–2252 (2011).
- 256 28. S. H. Martin, J. W. Davey, C. D. Jiggins, Evaluating the use of ABBA-BABA statistics to  
257 locate introgressed loci. *Mol. Biol. Evol.* **32**, 244–257 (2015).
- 258 29. E. Cheng, K. E. Hodges, J. Melo-Ferreira, P. C. Alves, L. S. Mills, Conservation  
259 implications of the evolutionary history and genetic diversity hotspots of the snowshoe  
260 hare. *Mol. Ecol.* **23**, 2929–2942 (2014).
- 261 30. J. Melo-Ferreira, F. A. Seixas, E. Cheng, L. S. Mills, P. C. Alves, The hidden history of  
262 the snowshoe hare, *Lepus americanus*: extensive mitochondrial DNA introgression  
263 inferred from multilocus genetic variation. *Mol. Ecol.* **23**, 4617–4630 (2014).
- 264 31. T. E. Cruickshank, M. W. Hahn, Reanalysis suggests that genomic islands of speciation

- 265 are due to reduced diversity, not reduced gene flow. *Mol. Ecol.* **23**, 3133–3157 (2014).
- 266 32. P. Pavlidis, D. Živkovic, A. Stamatakis, N. Alachiotis, SweeD: likelihood-based detection  
267 of selective sweeps in thousands of genomes. *Mol. Biol. Evol.* **30**, 2224–2234 (2013).
- 268 33. J. J. Clague, T. S. James, History and isostatic effects of the last ice sheet in southern  
269 British Columbia. *Quat. Sci. Rev.* **21**, 71–87 (2002).
- 270 34. S. Lamichhaney, F. Han, J. Berglund, C. Wang, M. S. Almen, M. T. Webster, B. R. Grant,  
271 P. R. Grant, L. Andersson, A beak size locus in Darwin’s finches facilitated character  
272 displacement during a drought. *Science.* **352**, 470–474 (2016).
- 273 35. Y. Song, S. Endepols, N. Klemann, D. Richter, F.-R. Matuschka, C.-H. Shih, M. W.  
274 Nachman, M. H. Kohn, Adaptive introgression of anticoagulant rodent poison resistance  
275 by hybridization between old world mice. *Curr. Biol.* **21**, 1296–1301 (2011).
- 276 36. C. Pardo-Diaz, C. Salazar, S. W. Baxter, C. Merot, W. Figueiredo-Ready, M. Joron, W. O.  
277 McMillan, C. D. Jiggins, Adaptive introgression across species boundaries in *Heliconius*  
278 butterflies. *PLoS Genet.* **8**, e1002752 (2012).
- 279 37. G. T. Pederson, S. T. Gray, C. A. Woodhouse, J. L. Betancourt, D. B. Fagre, J. S. Littell,  
280 E. Watson, B. H. Luckman, L. J. Graumlich, The unusual nature of recent snowpack  
281 declines in the North American Cordillera. *Science.* **333**, 332–335 (2011).
- 282 38. C. A. Matthee, B. J. Van Vuuren, D. Bell, T. J. Robinson, A molecular supermatrix of the  
283 rabbits and hares (Leporidae) allows for the identification of five intercontinental  
284 exchanges during the Miocene. *Syst. Biol.* **53**, 433–447 (2004).
- 285 39. K. Katoh, D. M. Standley, MAFFT multiple sequence alignment software version 7:  
286 improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).
- 287 40. M. R. Jones, J. M. Good, Targeted capture in evolutionary and ecological genomics. *Mol.*

- 288 *Ecol.* **25**, 185–202 (2016).
- 289 41. M. S. Ferreira, P. C. Alves, C. M. Callahan, J. P. Marques, L. S. Mills, J. M. Good, J.  
290 Melo-Ferreira, The transcriptional landscape of seasonal coat colour moult in the  
291 snowshoe hare. *Mol. Ecol.* **26**, 4173–4185 (2017).
- 292 42. D. W. Nagorsen, Winter pelage colour in snowshoe hares (*Lepus americanus*) from the  
293 Pacific Northwest. *Can. J. Zool.* **61**, 2313–2318 (1983).
- 294 43. W. B. Grange, The pelages and color changes of the snowshoe hare, *Lepus americanus*  
295 *phaeonotus*, Allen. *J. Mammal.* **13**, 99–116 (1932).
- 296 44. M. Meyer, M. Kircher, Illumina sequencing library preparation for highly multiplexed  
297 target capture and sequencing. *Cold Spring Harb. Protoc.* (2010),  
298 doi:10.1101/pdb.prot5448.
- 299 45. N. Rohland, D. Reich, Cost-effective, high-throughput DNA sequencing libraries for  
300 multiplexed target capture. *Genome Res.* **22**, 939–946 (2012).
- 301 46. V. A. Trifonov, N. N. Vorobieva, W. Rens, in *Fluorescence In Situ Hybridization (FISH)*  
302 — *Application Guide* (Springer, Berlin, Heidelberg, 2009), pp. 99–109.
- 303 47. A. M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina  
304 sequence data. *Bioinformatics.* **30**, 2114–2120 (2014).
- 305 48. T. Magoč, S. L. Salzberg, FLASH: fast length adjustment of short reads to improve  
306 genome assemblies. *Bioinformatics.* **27**, 2957–2963 (2011).
- 307 49. H. Li, Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM  
308 (2013) (available at <http://arxiv.org/abs/1303.3997>).
- 309 50. A. McKenna, M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytzky, K.  
310 Garimella, D. Altshuler, S. Gabriel, M. Daly, M. A. DePristo, The Genome Analysis

- 311 Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data.  
312 *Genome Res.* **20**, 1297–1303 (2010).
- 313 51. P. Danecek, A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E.  
314 Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, R. Durbin, The variant call  
315 format and VCFtools. *Bioinformatics.* **27**, 2156–2158 (2011).
- 316 52. E. Han, J. S. Sinsheimer, J. Novembre, Characterizing bias in population genetic  
317 inferences from low-coverage sequencing data. *Mol. Biol. Evol.* **31**, 723–735 (2014).
- 318 53. H. Li, B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis,  
319 R. Durbin, 1000 Genome Project Data Processing Subgroup, The Sequence  
320 Alignment/Map format and SAMtools. *Bioinformatics.* **25**, 2078–2079 (2009).
- 321 54. S. Y. Kim, K. E. Lohmueller, A. Albrechtsen, Y. Li, T. Korneliussen, G. Tian, N. Grarup,  
322 T. Jiang, G. Andersen, D. Witte, T. Jorgensen, T. Hansen, O. Pedersen, J. Wang, R.  
323 Nielsen, Estimation of allele frequency and association mapping using next-generation  
324 sequencing data. *BMC Bioinformatics.* **12**, 231 (2011).
- 325 55. D. H. Alexander, J. Novembre, K. Lange, Fast model-based estimation of ancestry in  
326 unrelated individuals. *Genome Res.* **19**, 1655–1664 (2009).
- 327 56. N. Patterson, A. L. Price, D. Reich, Population structure and eigenanalysis. *PLoS Genet.*  
328 **2**, e190 (2006).
- 329 57. S. R. Browning, B. L. Browning, Rapid and accurate haplotype phasing and missing-data  
330 inference for whole-genome association studies by use of localized haplotype clustering.  
331 *Am. J. Hum. Genet.* **81**, 1084–1097 (2007).
- 332 58. J. K. Pickrell, J. K. Pritchard, Inference of population splits and mixtures from genome-  
333 wide allele frequency data. *PLoS Genet.* **8**, e1002967 (2012).

- 334 59. S. Purcell, B. Neale, K. Todd-Brown, L. Thomas, M. A. R. Ferreira, D. Bender, J. Maller,  
335 P. Sklar, P. I. W. de Bakker, M. J. Daly, P. C. Sham, PLINK: a tool set for whole-genome  
336 association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575  
337 (2007).
- 338 60. S. Purcell, S. S. Cherny, P. C. Sham, Genetic Power Calculator: design of linkage and  
339 association genetic mapping studies of complex traits. *Bioinformatics.* **19**, 149–150  
340 (2003).
- 341 61. L. Skotte, T. S. Korneliusen, A. Albrechtsen, Association testing for next-generation  
342 sequencing data using score statistics. *Genet. Epidemiol.* **36**, 430–437 (2012).
- 343 62. R. Kofler, P. Orozco-terWengel, N. De Maio, R. V. Pandey, V. Nolte, A. Futschik, C.  
344 Kosiol, C. Schlötterer, PoPoolation: a toolbox for population genetic analysis of next  
345 generation sequencing data from pooled individuals. *PLoS One.* **6**, e15925 (2011).
- 346 63. T. D. Schmittgen, K. J. Livak, Analyzing real-time PCR data by the comparative CT  
347 method. *Nat. Protoc.* **3**, 1101–1108 (2008).
- 348 64. J. L. Feder, X. Xie, J. Rull, S. Velez, A. Forbes, B. Leung, H. Dambroski, K. E. Filchak,  
349 M. Aluja, Mayr, Dobzhansky, and Bush and the complexities of sympatric speciation in  
350 *Rhagoletis*. *Proc. Natl. Acad. Sci. U. S. A.* **102 Suppl**, 6573–6580 (2005).
- 351 65. J. W. Poelstra, N. Vijay, C. M. Bossu, H. Lantz, B. Ryll, I. Muller, V. Baglione, P.  
352 Unneberg, M. Wikelski, M. G. Grabherr, J. B. W. Wolf, The genomic landscape  
353 underlying phenotypic integrity in the face of gene flow in crows. *Science.* **344**, 1410–  
354 1414 (2014).
- 355 66. A. Stamatakis, RAxML version 8: a tool for phylogenetic analysis and post-analysis of  
356 large phylogenies. *Bioinformatics.* **30**, 1312–1313 (2014).



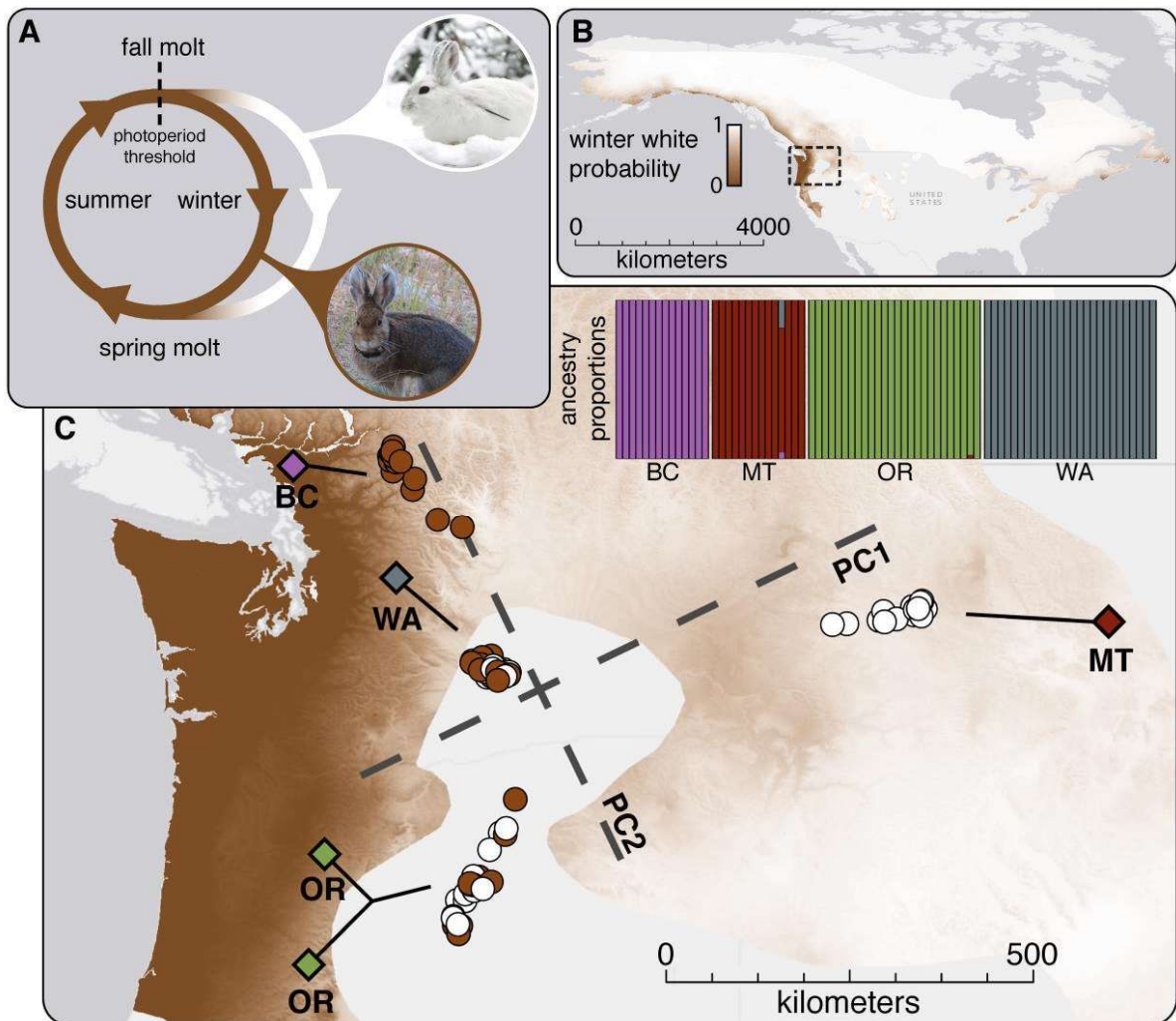
- 357 67. K. Chen, J. W. Wallis, M. D. McLellan, D. E. Larson, J. M. Kalicki, C. S. Pohl, S. D.  
358 McGrath, M. C. Wendl, Q. Zhang, D. P. Locke, X. Shi, R. S. Fulton, T. J. Ley, R. K.  
359 Wilson, L. Ding, E. R. Mardis, BreakDancer: an algorithm for high-resolution mapping of  
360 genomic structural variation. *Nat. Methods*. **6**, 677–681 (2009).
- 361 68. X. Huang, A. Madan, CAP3: A DNA sequence assembly program. *Genome Res.* **9**, 868–  
362 877 (1999).
- 363 69. R. E. Green, J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H.  
364 Li, W. Zhai, M. H. Y. Fritz, N. F. Hansen, E. Y. Durand, A. S. Malaspina, J. D. Jensen,  
365 T. Marques-Bonet, C. Alkan, K. Prüfer, M. Meyer, H. A. Burbano, J. M. Good, R.  
366 Schultz, A. Aximu-Petri, A. Butthof, B. Höber, B. Höffner, M. Siegemund, A. Weihmann,  
367 C. Nusbaum, E. S. Lander, C. Russ, N. Novod, J. Affourtit, M. Egholm, C. Verna, P.  
368 Rudan, D. Brajkovic, Z. Kucan, I. Gušić, V. B. Doronichev, L. V Golovanova, C.  
369 Lalueza-Fox, M. de la Rasilla, J. Fortea, A. Rosas, R. W. Schmitz, P. L. F. Johnson, E. E.  
370 Eichler, D. Falush, E. Birney, J. C. Mullikin, M. Slatkin, R. Nielsen, J. Kelso, M.  
371 Lachmann, D. Reich, S. Pääbo, A draft sequence of the neandertal genome. *Science*. **328**,  
372 710–722 (2010).
- 373 70. J. B. Pease, M. W. Hahn, Detection and polarization of introgression in a five-taxon  
374 phylogeny. *Syst. Biol.* **64**, 651–662 (2015).
- 375 71. W. Zhang, K. K. Dasmahapatra, J. Mallet, G. R. P. Moreira, M. R. Kronforst, Genome-  
376 wide introgression among distantly related *Heliconius* butterfly species. *Genome Biol.* **17**,  
377 25 (2016).
- 378 72. J. H. Gillespie, C. H. Langley, Are evolutionary rates really variable? *J. Mol. Evol.* **13**,  
379 27–34 (1979).

- 380 73. R. N. Gutenkunst, R. D. Hernandez, S. H. Williamson, C. D. Bustamante, Inferring the  
381 joint demographic history of multiple populations from multidimensional SNP frequency  
382 data. *PLoS Genet.* **5**, e1000695 (2009).
- 383 74. G. Ewing, J. Hermisson, MSMS: a coalescent simulation program including  
384 recombination, demographic structure and selection at a single locus. *Bioinformatics.* **26**,  
385 2064–2065 (2010).
- 386 75. M. Carneiro, F. W. Albert, J. Melo-Ferreira, N. Galtier, P. Gayral, J. A. Blanco-Aguiar, R.  
387 Villafuerte, M. W. Nachman, N. Ferrand, Evidence for widespread positive and purifying  
388 selection across the European rabbit (*Oryctolagus cuniculus*) genome. *Mol. Biol. Evol.* **29**,  
389 1837–1849 (2012).
- 390 76. M. Carneiro, S. Afonso, A. Geraldes, H. Garreau, G. Bolet, S. Boucher, A. Tircazes, G.  
391 Queney, M. W. Nachman, N. Ferrand, The genetic structure of domestic rabbits. *Mol.*  
392 *Biol. Evol.* **28**, 1801–1816 (2011).
- 393 77. P. Cingolani, A. Platts, L. L. Wang, M. Coon, T. Nguyen, L. Wang, S. J. Land, X. Lu, D.  
394 M. Ruden, A program for annotating and predicting the effects of single nucleotide  
395 polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118;  
396 iso-2; iso-3. *Fly.* **6**, 80–92 (2012).
- 397 78. A. J. Coffman, P. H. Hsieh, S. Gravel, R. N. Gutenkunst, Computationally efficient  
398 composite likelihood statistics for demographic inference. *Mol. Biol. Evol.* **33**, 591–593  
399 (2016).
- 400 79. P. Pavlidis, J. D. Jensen, W. Stephan, Searching for footprints of positive selection in  
401 whole-genome SNP data from nonequilibrium populations. *Genetics.* **185**, 907–922  
402 (2010).

- 403 80. R. Durrett, J. Schweinsberg, A coalescent model for the effect of advantageous mutations  
404 on the genealogy of a population. *Stoch. Process. their Appl.* **115**, 1628–1657 (2005).
- 405 81. M. I. Jensen-Seaman, T. S. Furey, B. A. Payseur, Y. Lu, K. M. Roskin, C.-F. Chen, M. A.  
406 Thomas, D. Haussler, H. J. Jacob, Comparative recombination rates in the rat, mouse, and  
407 human genomes. *Genome Res.* **14**, 528–538 (2004).
- 408 82. C. D. Huber, M. DeGiorgio, I. Hellmann, R. Nielsen, Detecting recent selective sweeps  
409 while controlling for mutation rate and background selection. *Mol. Ecol.* **25**, 142–156  
410 (2016).

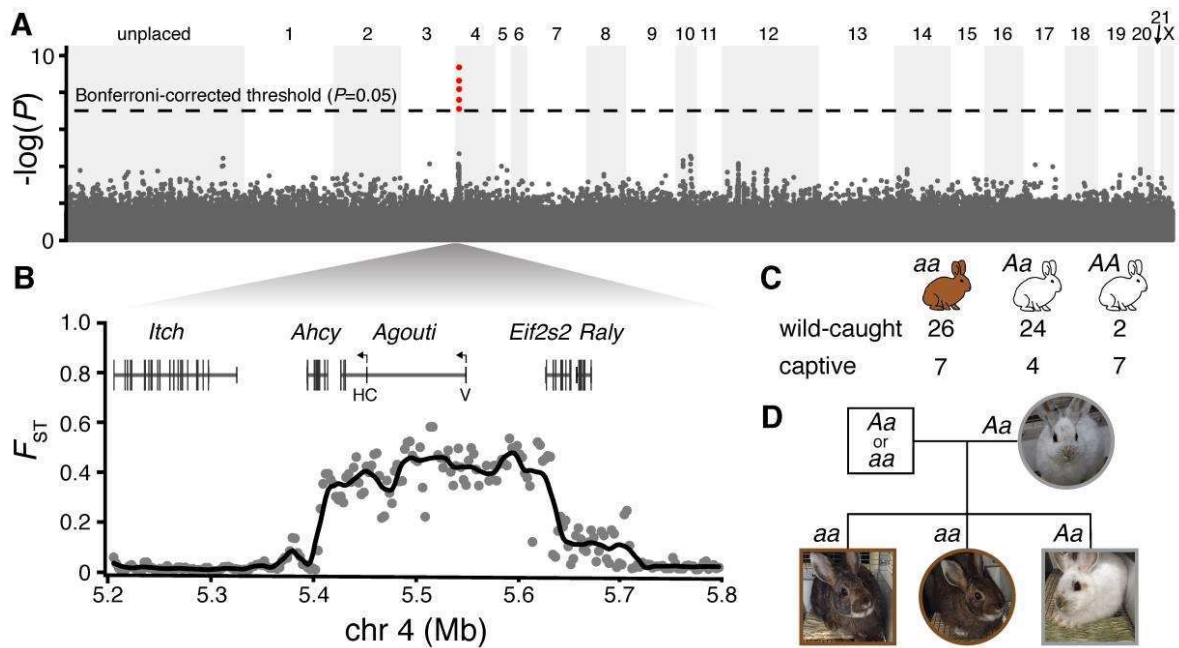
411 **Acknowledgments:** We thank E. Cheng, K. Garrison, and P. Zevit for assistance with sample  
412 collection. We thank R. Bracewell, T. Brekke, M. Carneiro, Z. Clare-Salzler, M. Dean, E.  
413 Kopania, M. S. Ferreira, N. Herrera, E. Larson, M. Nachman, B. Payseur, B. Sarver, and  
414 members of the NSF EPSCoR UNVEIL network for helpful discussion. R. Bracewell, B. Cole,  
415 T. Cosart, L. Farelo, E. Larson, S. Laurent, T. Max, S. Pfeifer, B. Sarver, and K. Zarn provided  
416 computational or laboratory support. A. Kumar assisted with the preparation of Fig. 1.  
417 Sequencing was performed at the University of Montana Genomics Core, the CIBIO-InBIO  
418 University of Porto New-Gen sequencing platform, the University of Oregon Genomics and Cell  
419 Characterization Core Facility, the HudsonAlpha Institute for Biotechnology, and Novogene  
420 Technology Co. Ltd. Computational resources were provided by the University of Montana  
421 Genomics Core and the Vital-IT Center for high-performance computing of the SIB Swiss  
422 Institute of Bioinformatics. **Funding:** National Science Foundation (NSF) Graduate Research  
423 Fellowship (DGE-1313190), NSF Doctoral Dissertation Improvement Grant (DGE-1702043),  
424 NSF Graduate Research Opportunities Worldwide, Portuguese Fundação para a Ciência e a  
425 Tecnologia (FCT) project grant CHANGE (PTDC/BIA-EVF/1624/2014, supported by National  
426 Funds), NSF EPSCoR (OIA-1736249), NSF (DEB-0841884), FCT Investigator Grant  
427 (IF/00033/2014, supported by POPH-QREN funds from ESF and Portuguese MCTES/FCT),  
428 FLAD (Luso-American Development Foundation; PORTUGAL – U.S. Research Networks  
429 Program), the Drollinger-Dial Foundation, American Society of Mammalogists Grant-in-aid of  
430 Research, Swiss Government Excellence Scholarship, and European Union’s Seventh  
431 Framework Programme (CIBIO New-Gen sequencing platform; grant agreement 286431).  
432 **Author contributions:** M.R.J., L.S.M., P.C.A., J.D.J., J.M.F., and J.M.G. designed the study.  
433 J.M.G. coordinated the study. M.R.J, C.M.C., J.A., and D.J.R.L. generated data. J.A. and F.M.J.

434 helped develop the exome capture experiments. M.R.J. performed data analyses under the  
435 guidance of J.M.G., J.M.F., and J.D.J. M.R.J. and J.M.G. wrote the paper with input from the  
436 other authors. All authors approved the manuscript before submission. **Competing interests:**  
437 None declared. **Data and materials availability:** Original sequence data are available in the  
438 Sequence Read Archive ([www.ncbi.nlm.nih.gov/sra](http://www.ncbi.nlm.nih.gov/sra)) under BioProject PRJNA420081 (accession  
439 numbers SAMN08146448- SAMN08146534). Previously generated whole genome sequence  
440 data of snowshoe hare (SAMN02782769, SAMN07526959) and mountain hare  
441 (SAMN07526960) are also available in the Sequence Read Archive.



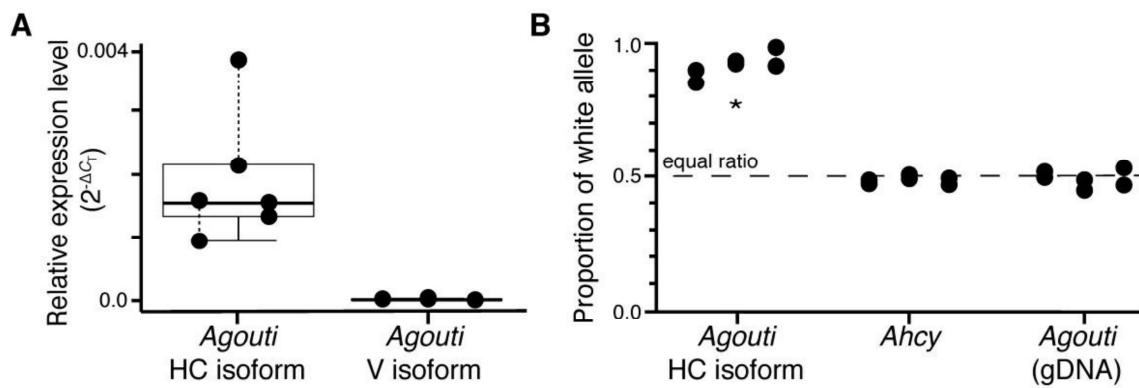
442

443 **Fig. 1. Winter coat color polymorphism and population structure in snowshoe hares.** (A)  
 444 Alternative winter color morphs in snowshoe hares. (B) The modeled range-wide probability of  
 445 winter-white coats, adapted from (7). (C) Principle components (PC1 – 7.42%, PC2 – 5.27%;  
 446 coat color represented as brown/white circles) and population ancestry plots of 38,694 unlinked  
 447 SNPs derived from 80 exomes sampled from five localities (colored diamonds) overlaid on the  
 448 probability of winter-white coats in the Pacific Northwest.



449

450 **Fig. 2. The genetic basis of winter coat color polymorphism.** (A) Exome SNP associations ( $-\log_{10}$  of  $P$ -values, assuming dominant minor allele, 513,812 SNPs) for polymorphic zone  
 451 individuals. Red points above dashed line exceed the Bonferroni-corrected threshold of  $P=0.05$ .  
 452 (B) Gene structure across the associated interval and alternative *Agouti* transcription start sites  
 453 (arrows) corresponding to hair-cycle (HC) and ventral (V) 5' UTRs. Sliding window averages of  
 454  $F_{ST}$  (5 kb with 2.5 kb step) between winter-white and winter-brown individuals with low-  
 455 coverage whole genomes (15,173,804 SNPs). (C) Dominance of winter coat color inferred from  
 456 *Agouti* genotypes of wild (OR and WA; Hardy-Weinberg  $\chi^2 = 1.6$ ,  $P=0.21$ ) and captive (WA and  
 457 MT) hares. (D) Pedigree and genotypes of a mixed phenotype family (paternal genotype is  
 458 unknown, but inferred to carry the  $a$  allele).  
 459



460

461

462

463

464

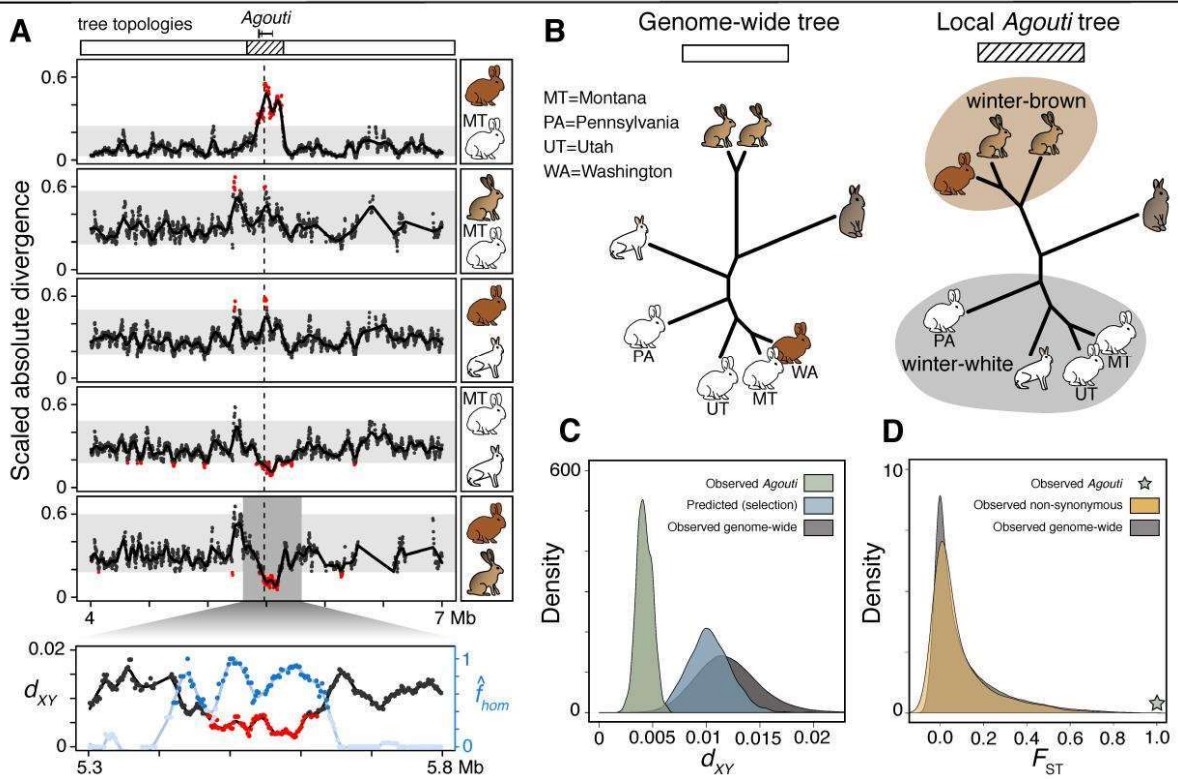
465

466

467

**Fig. 3. *Agouti* expression in snowshoe hares during autumn molts.** (A) The relative expression level ( $2^{-\Delta C_T}$ , normalized to *Gapdh*) of hair-cycle (HC) and ventral (V) *Agouti* isoforms in molting skin of winter-white (*Aa*) snowshoe hares. (B) Relative abundance of the winter-white allele in the same skin samples for *Agouti* hair-cycle transcripts, *Ahcy* transcripts, and *Agouti* genomic DNA. White allele proportions were significantly elevated in *Agouti* transcripts compared to *Ahcy* transcripts and *Agouti* genomic DNA ( $P < 0.00001$ , Student's t-test). Pairs of points represent technical replicates.





468

469

470

471

472

473

474

475

476

477

478

479

**Fig. 4. The evolution of winter coat color alleles in hares and jackrabbits. (A)** Estimated tree topologies across the *Agouti* region (top, see 4B). Mutation-scaled absolute genetic divergence in 20 kb sliding windows for pairs of individuals (dashed line indicates location of candidate insertion-deletion mutations). Gray rectangles represent 99.8% bootstrap quantiles and red points are windows with one-tailed  $P < 0.001$ . 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 ( $\hat{f}_{hom}$ , 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). **(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

480 (monomorphic winter-brown) and MT (monomorphic winter-white) hares genome-wide (gray)  
481 and for non-synonymous SNPs (yellow).  $F_{ST}=1$  at a diagnostic *Agouti* SNP, indicated with a  
482 green star.