1	The origin and spread of locally adaptive seasonal camouflage in snowshoe hares
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9 Abstract

10 Adaptation is central to population persistence in the face of environmental change, yet we rarely precisely understand the origin and spread of adaptive variation in natural populations. Snowshoe 11 12 hares (Lepus americanus) along the Pacific Northwest (PNW) coast have evolved brown winter 13 camouflage through positive selection on recessive variation at the Agouti pigmentation gene 14 introgressed from black-tailed jackrabbits (L. californicus). Here we combine new and published 15 whole genome and exome sequences with targeted genotyping of Agouti in order to investigate 16 the evolutionary history of local seasonal camouflage adaptation in the PNW. We find evidence 17 of significantly elevated inbreeding and mutational load in coastal winter-brown hares, consistent 18 with a recent range expansion into temperate coastal environments that incurred indirect fitness 19 costs. The genome-wide distribution of introgression tract lengths supports a pulse of 20 hybridization near the end of the last glacial maximum, which may have facilitated range 21 expansion via introgression of winter-brown camouflage variation. However, signatures of a 22 selective sweep at Agouti indicate a much more recent spread of winter-brown camouflage. 23 Through simulations we show that the temporal lag between the hybrid origin and subsequent 24 selective sweep of the recessive winter-brown allele can be largely attributed to the limits of 25 natural selection imposed by simple allelic dominance. We argue that while hybridization during 26 periods of environmental change may provide a critical reservoir of adaptive variation at range 27 edges, the probability and pace of local adaptation will strongly depend on population 28 demography and the genetic architecture of introgressed variation.

29 Introduction

30 Local adaptation is fundamental to the persistence of populations during periods of rapid 31 environmental change. In particular, local adaption to marginal habitats may increase a species' 32 niche breadth and range size (Holt and Gomulkiewicz 1997), enhancing their evolutionary 33 resilience (Sgrò et al. 2011; Slatyer et al. 2013; Forsman 2016; Mills et al. 2018). Consequently, 34 range edges where populations encounter marginal habitats and less favorable conditions may 35 harbor crucial adaptive variation that facilitates long-term persistence in the face of 36 environmental change (Hampe and Petit 2005; Hill et al. 2011; Cheng et al. 2014). Yet, range 37 boundaries may also reflect the limits of natural selection if they are defined by environments 38 where populations have failed to adapt (Antonovics 1976; Kirkpatrick and Barton 1997; Bridle 39 and Vines 2007). Revealing how adaptive variation arises and spreads along range edges is, 40 therefore, fundamental to understand the limitations of adaptation to new or changing environments (Ackerly 2003; Hampe and Petit 2005). However, we rarely possess detailed 41 42 knowledge of the genetic basis and history of local adaptation in natural populations. 43 Several decades of theoretical research have established a framework for predicting 44 demographic conditions along range margins, which are crucial in shaping population-level 45 fitness and the potential for adaptation and range expansion. Populations inhabiting marginal 46 habitats are generally predicted to be small and occur at low densities (Antonovics 1976; 47 Kirkpatrick and Barton 1997), resulting in relatively reduced rates of beneficial mutation and levels of standing genetic variation (Pfennig et al. 2016). Small range-edge populations may 48 further experience higher rates of inbreeding due to genetic drift (Wright 1931; Barton 2001) and 49 50 accumulate deleterious variation (i.e., mutational load; Lynch et al. 1995; Willi et al. 2018), which can decrease the probability of population persistence (Mills and Smouse 1994). Elevated 51

52 individual inbreeding and mutational load along range edges may also reflect past histories of adaptation and range expansion that result in non-equilibrium population dynamics. For instance, 53 54 mating between close relatives may increase in founder populations that have recently undergone 55 severe population contractions associated with range expansions (Frankham 1998). Likewise, 56 mutational load may be amplified through the colonization of new environments because 57 population contractions reduce the efficacy of selection against deleterious alleles at the 58 expansion front (i.e., expansion load; Peischl et al. 2013; Henn et al. 2016; González-Martínez et 59 al. 2017; Willi et al. 2018). Thus, when adaptation does occur along range margins it may 60 produce negative feedbacks on population fitness and evolutionary potential. 61 Patterns of migration into range edge populations are also pivotal to their fitness and 62 adaptive potential. Larger core populations are expected to produce relatively more migrants 63 than smaller edge populations, leading to asymmetric rates of gene flow between core and 64 peripheral habitats. In extreme scenarios, edge populations with low population growth rates 65 $(\lambda < 1)$ can be demographic sinks that are maintained by immigration from the core of the range (Holt and Gomulkiewicz 1997; Griffin and Mills 2009). Highly asymmetric gene flow may 66 67 further reduce fitness and hinder adaptation along the range edge by continually swamping local 68 selection (Haldane 1930; Garcia-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997; 69 Kawecki 2008). However, gene flow from core populations into edge populations may ultimately 70 promote adaptive responses when edge populations are small and ecological gradients are 71 shallow (Polechová 2018; Bontrager and Angert 2019). Hybridization between species may also 72 facilitate adaptation and range expansion if edge populations intersect with the range of closelyrelated species that are adapted to local habitats (Baker 1948; Lewontin and Birch 1966; Burke 73 and Arnold 2001; Rieseberg et al. 2007; Kawecki 2008; Pfennig et al. 2016). Introgression may 74

75	provide a crucial source of large-effect variation (Hedrick 2013), which is predicted to be scarce
76	in small populations but often necessary for range-edge adaptation and expansion (Behrman and
77	Kirkpatrick 2011; Gilbert and Whitlock 2016). Putative adaptive introgression has now been
78	shown in numerous species (e.g., Song et al. 2011; Pardo-Diaz et al. 2012; Huerta-Sánchez et al.
79	2014; Lamichhaney et al. 2015; Miao et al. 2016; Jones et al. 2018; Oziolor et al. 2019) and has
80	been specifically linked to range expansions in Australian fruit flies (Lewontin and Birch 1966),
81	sunflowers (Rieseberg et al. 2007), and mosquitoes (Besansky et al. 2003). While hybridization
82	may facilitate adaptation and range expansion via large-effect mutations (Hedrick 2013; Nelson
83	et al. 2019), the factors influencing the pace of adaptive introgression are often unclear.
84	Snowshoe hares (Lepus americanus) are broadly distributed across boreal and montane
85	forests of North America. Most populations of hares undergo seasonal molts between brown
86	(summer) and white (winter) coats to maintain crypsis in snow-covered environments. Seasonal
87	camouflage is a crucial component of fitness in this system (Mills et al. 2013) as hares that
88	become mismatched with their environment experience dramatically increased predation rates
89	(i.e., 3-7% increase in weekly survival mortality; Zimova et al. 2016). However, some hares have
90	adapted to mild winter environments by remaining brown in the winter (Mills et al. 2018).
91	Brown winter camouflage in snowshoe hares is relatively rare across the entire range (<5% of
92	the range), but predominant along portions of the southern range edge in the Pacific Northwest
93	(PNW; Nagorsen 1983) with occurrence closely tracking regions of low seasonal snow cover
94	(Mills et al. 2018). As snow cover across North America continues to decline under climate
95	change, it is predicted that winter-brown camouflage may spread from the edge to the interior of
96	the range, enhancing the evolutionary resilience of snowshoe hares (Jones et al. 2018; Mills et al.
97	2018). We previously demonstrated that brown versus white winter camouflage in PNW

98 snowshoe hares is determined by a simple *cis*-regulatory polymorphism of the *Agouti* 99 pigmentation gene that influences its expression during the autumn molt (Jones et al. 2018). The 100 locally adaptive winter-brown allele is fully recessive and derived from introgressive 101 hybridization with black-tailed jackrabbits (Lepus californicus), a closely-related scrub-grassland 102 species that remains brown in the winter (Jones et al. 2018). Thus, the evolution of brown winter 103 coats along coastal environments in the PNW represents one of the few verified cases of 104 introgression underlying an adaptive trait of known ecological relevance in mammals (Taylor 105 and Larson 2019).

106 The establishment of this genotype-to-phenotype link provides a powerful opportunity to 107 examine how population history and hybridization shape local adaptation and expansion along 108 the range edge. Here we seek to deepen our understanding of 1) the population history of PNW 109 range edge snowshoe hares and 2) the origin and spread of winter-brown camouflage across coastal PNW environments. We first use previously published targeted exome data (61.7 Mb for 110 111 80 individuals; Jones et al. 2018) to estimate historical changes in population size (N), individual 112 inbreeding coefficients, and mutational load in PNW hares. We then combine 11 whole genome 113 sequences (WGS; six new and five previously published) with 61 newly assembled complete 114 mitochondrial genomes and targeted genotyping of the introgressed Agouti region across 106 115 hares to resolve the timing of hybridization with black-tailed jackrabbits and the subsequent 116 spread of winter-brown coat color variation. We use these data to test theoretical predictions for 117 the maintenance and spread of adaptive variation in peripheral environments. Our study provides 118 rare empirical insight into the dynamic interplay of environmental change, hybridization, and 119 selection along range-edge environments and its evolutionary consequences.

120

121 Methods

122 Genomic data generation

123 All sample collection with live animals was performed under approved state permits and

associated Animal Use Protocols approved through the University of Montana Institutional

125 Animal Care and Use Committee (IACUC).

126 For some analyses, we used previously generated targeted whole exome data (61.7-Mb 127 spanning 213,164 intervals; ~25-Mb protein-coding exons, ~28-Mb untranslated region, ~9-Mb 128 intronic or intergenic) for 80 snowshoe hares (21× mean coverage) collected from Washington 129 (WA; n=13 winter-brown, n=13 winter-white), Oregon (OR; n=13 winter-brown, n=13 winter-130 white), Montana (MT; n=14 winter-white), and southwest British Columbia (BC; n=14 winter-131 brown; Jones et al. 2018). Hares from OR and WA were collected in the Cascade Range where 132 populations are polymorphic for winter coat color (Fig. 1A). Hares from Seeley Lake in western 133 MT are winter-white individuals, while those from BC were collected in low-lying regions near 134 the Pacific coast where snowshoe hares are all winter-brown (Fig. 1A). To infer the history of 135 hybridization, we performed whole genome resequencing of three black-tailed jackrabbits from 136 OR and California (CA) and two winter-brown snowshoe hares from OR and BC. These samples 137 complement WGS data previously generated for two black-tailed jackrabbits from Nevada (NV; 138 one of which was sequenced to higher coverage in this study) and snowshoe hares from MT, WA, Utah (UT), and Pennsylvania (PA; Jones et al. 2018). We extracted genomic DNA 139 140 following the Qiagen DNeasy Blood and Tissue kit protocol (Qiagen, Valencia, CA) and 141 prepared genomic libraries following the KAPA Hyper prep kit manufacturer's protocol. For all libraries, we sheared genomic DNA using a Covaris E220evolution ultrasonicator and performed 142 143 a stringent size selection using a custom-prepared carboxyl-coated magnetic bead mix (Rohland

and Reich 2012) to obtain average genomic fragment sizes of 400-500 bp. We determined
indexing PCR cycle number for each library with quantitative PCR (qPCR) on a Stratagene
Mx3000P thermocycler (Applied Biosystems) using a DyNAmo Flash SYBR Green qPCR kit
(Thermo Fisher Scientific). Final libraries were size-selected again with carboxyl-coated
magnetic beads, quantified with a Qubit (Thermo Fisher Scientific), and pooled for sequencing
by Novogene (Novogene Corporation Ltd.; Davis, CA) on two lanes of Illumina HiSeq4000
using paired-end 150 bp reads.

To resolve the history of selection on the winter-brown Agouti allele, we performed 151 152 targeted enrichment and sequencing to genotype 106 hares at the Agouti locus (n=37 WA, n=64153 OR, *n*=5 MT). We developed a custom set of IDT xGen Lockdown probes spanning a 596.4 kb 154 interval centered on the Agouti gene and extending to flanking regions (chromosome 155 4:5,250,800-5,847,200; coordinates based on the European rabbit (*Oryctolagus cuniculus*) 156 oryCun2 genome build). The probe sequences were based on a snowshoe hare pseudoreference 157 genome (~33×; Jones et al. 2018) derived from iterative mapping to the rabbit genome (Carneiro 158 et al. 2014). We targeted 96 uniquely-mapped 100 bp regions (based on low coverage WGS data 159 from Jones et al. (2018)) that contained one or more diagnostic SNPs for winter coat color, 160 allowing us to infer winter coat color for samples based on their Agouti genotype. We prepared 161 genomic libraries for targeted Agouti sequencing following a modified version of Meyer and 162 Kircher (2010), as described in Jones et al. (2018). We performed hybridization reactions on 500 163 ng of pooled libraries (10-16 individual libraries per pool), 5 µg of custom prepared snowshoe 164 hare C₀t-1 DNA, and 2 nM of blocking oligos. Washing and recovery of captured DNA was performed following the IDT xGen Lockdown probe hybridization capture protocol (version 2). 165 166 Each capture library was then amplified in 50 µl reactions with 1X Herculase II reaction buffer,

167	250 μ M each dNTP, 0.5 μ M each primer, 1 μ l Herculase II fusion polymerase, and 20 μ l library
168	template. The PCR temperature profile consisted of a 45 second 98°C denaturation step,
169	followed by 24 cycles of 98°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds,
170	with a final 72°C elongation step for 1 minute. We cleaned and size-selected final libraries with
171	1.2X carboxyl-coated magnetic beads and verified target enrichment with qPCR. Agouti capture
172	libraries were then pooled and sequenced with other libraries across two lanes of Illumina
173	HiSeq4000 at the University of Oregon Core (Eugene, OR) and Novogene.
174	
175	Read processing and variant calling
176	For all raw sequence data, we trimmed adapters and low-quality bases (mean phred-scaled
177	quality score <15 across 4 bp window) and removed reads shorter than 50 base pairs (bp) using
178	Trimmomatic v0.35 (Bolger et al. 2014). We then merged paired-end reads overlapping more
179	than 10 bp and with less than 10% mismatched bases using FLASH2 (Magoč and Salzberg
180	2011). Cleaned exome and Agouti capture reads were mapped using default settings in BWA-
181	MEM v0.7.12 (Li 2013) to the snowshoe hare pseudoreference genome. WGS data were mapped
182	to either the snowshoe hare or a black-tailed jackrabbit pseudoreference, which was also created
183	by iteratively mapping to the rabbit genome (Jones et al. 2018). We used <i>PicardTools</i> to remove
184	duplicate reads with the MarkDuplicates function and assigned read group information with the
185	AddOrReplaceReadGroups function. Using GATK v3.4.046 (McKenna et al. 2010), we
186	identified poorly aligned genomic regions with RealignerTargetCreator and performed local
187	realignments with IndelRealigner. We performed population-level multi-sample variant calling
188	using default settings with the GATK UnifiedGenotyper and filtered variants in VCFtools
189	v0.1.14 (Danecek et al. 2011). For whole exome and whole genome data, we filtered genotypes

190	with individual coverage $<5\times$ or $>70\times$ or with a phred-scaled quality score <30 . Additionally, we
191	removed all indel variants and filtered single nucleotide polymorphisms (SNPs) with a phred-
192	scaled quality score <30 and Hardy-Weinberg $P<0.001$. We required that sites have no missing
193	data across individuals. For targeted Agouti SNP data, we additionally filtered heterozygous
194	genotypes with allelic depth ratios >3 and sites with $> 50\%$ missing data across individuals. We
195	phased haplotypes and imputed missing data with Beagle v4.1 (Browning and Browning 2007)
196	and used Haplostrips (Marnetto and Huerta-Sánchez 2017) to visualize haplotype structure.
197	
198	Population size history and inbreeding coefficients of PNW hares
199	We used the program $\partial a \partial i$ (Gutenkunst et al. 2009) to infer historical population size (N)
200	changes in PNW snowshoe hare populations (BC, MT, OR, and WA) using the folded site
201	frequency spectrum (SFS) of synonymous variants from our extensive whole exome data set. We
202	used the folded SFS to be consistent with statistical inferences of the distribution of fitness
203	effects (see below). For each population, we tested a standard neutral equilibrium model, a two-
204	epoch model (single instantaneous N change), a three-epoch model (two instantaneous N
205	changes), an exponential N growth model, and an instantaneous N change + exponential N
206	growth model. We inferred values for parameters v , the population size relative to ancestral N
207	(N_{anc} ; e.g., $v=1$ if $N=N_{anc}$) and t , the time of population size changes in units of $2N_{anc}$
208	generations. We performed 100 independent runs under each model starting with parameter
209	values sampled randomly across a uniform distribution ($0.001 < \nu < 100, 0 < 2N_et < 2$). For each
210	model, we selected parameters with the highest log-likelihood value and chose the overall best
211	model using a composite-likelihood ratio test with the Godambe Information Matrix (Coffman et
212	al. 2016). We further checked the validity of maximum likelihood models by comparing the

predicted SFS to the observed SFS for each population (Fig. S2). We determined 95%

confidence intervals for parameter estimates using the Godambe Information Matrix with 100

bootstrap data sets comprised of one randomly selected synonymous SNP per 10 kb.

216 SFS-based approaches are often underpowered or inappropriate for inferring recent 217 population size changes (Robinson et al. 2014; Beichman et al. 2018). For instance, even with a 218 sufficient sample size, a historically large population that has very recently contracted in size (i.e., not in equilibrium) may nonetheless have a large variance N_e . However, individuals in such 219 populations may exhibit elevated individual inbreeding coefficients (F_{IS}), calculated as 1- H_0/H_e 220 221 where H_o is the observed heterozygosity and H_e is expected heterozygosity assuming random 222 mating. To examine evidence for recent population contractions, we calculated the mean of the 223 individual inbreeding coefficient (F_{IS}) for each population using VCFtools and tested for 224 significant differences between populations with a two-tailed Student's t-Tests in R (R Core Team 2018). 225

226

227 <u>Mutational load and the distribution of fitness effects</u>

228 For each PNW population, we measured the proportion of homozygosity across SNPs with 229 predicted phenotypic effects (nonsynonymous and nonsense) as an indicator for relative 230 differences in mutational load under a recessive deleterious mutation model (González-Martínez 231 et al. 2017). We tested for significant differences in the proportion of homozygosity across 232 populations using two-sided Z-tests for proportions in R (R Core Team 2018). Additionally, we 233 used whole exome data to infer the distribution of selection coefficients of segregating variation, 234 more commonly referred to as the distribution of fitness effects (DFE). In principle, we can infer 235 the DFE from the SFS of selected sites because neutral, weakly deleterious, and strongly

236 deleterious variation should segregate at different frequencies in populations (Keightley and 237 Eyre-Walker 2010). The DFE of segregating variation is commonly inferred by first fitting a 238 population history model to the SFS of neutral sites (often synonymous SNPs) and then fitting a 239 mutational model to the SFS of selected sites (often nonsynonymous SNPs), while controlling 240 for the effect of population history (i.e., changes in N) on the SFS of selected sites (Keightley 241 and Eyre-Walker 2010). Here we implement this approach using the $Fit\partial a\partial i$ module (Kim et al. 242 2017). We used the maximum likelihood parameter values from our inferred demographic model to control for population history and fit a simple DFE to the folded SFS of nonsynonymous 243 244 variants (identified with SNPeff; Cingolani et al. 2012) described by a gamma distribution of 245 selective effects with a shape (α) and scale (β) parameter. To estimate variance in shape and 246 scale parameters, we used 100 bootstrap datasets randomly sampling 50% of nonsynonymous 247 sites and performed 10 independent runs on each dataset. We used random starting values 248 between 0.001 and 1 for the shape parameter and values between 0.01 and 200,000 for the scale 249 parameter. To scale the DFE from relative selection coefficients $(2N_{anc}s)$ to absolute selection 250 coefficients (s), we divided the scale parameter by $2N_{anc}$ (Kim et al. 2017).

251

252 <u>The timing of hybridization</u>

If hybridization between snowshoe hares and black-tailed jackrabbits is rare, then the age of
hybridization may also reflect the age of *Agouti* introgression. We used two complementary
approaches to estimate the timing of hybridization between PNW snowshoe hares and blacktailed jackrabbits. Previous phylogenetic analysis of partial cytochrome *b* sequences revealed
that some PNW snowshoe hares carry introgressed mitochondrial DNA (mtDNA) genomes
derived from hybridization with black-tailed jackrabbits (Cheng et al. 2014; Melo-Ferreira et al.

259	2014). We estimated the age of mtDNA introgression using complete mtDNA genomes for
260	snowshoe hares ($n=56$) and black-tailed jackrabbits ($n=5$) that we assembled <i>de novo</i> from newly
261	and previously generated WGS data (Jones et al. 2018) with the program NOVOPlasty
262	(Dierckxsens et al. 2017). We aligned individual mtDNA assemblies, including the rabbit
263	mtDNA reference as an outgroup (total assembled length= 16,251 bp), using default settings in
264	Clustal W v2.1 (Larkin et al. 2007) and visually verified alignment quality. We then estimated a
265	maximum clade credibility tree and node ages with a Calibrated Yule model in BEAST 2
266	(Bouckaert et al. 2014), assuming a strict molecular clock and an HKY substitution model using
267	empirical base frequencies. We specified default priors for the kappa and gamma shape
268	parameters and used a gamma distribution (alpha=0.001, beta=1000) as a prior for the clock rate
269	and birth rate parameter. We ran the MCMC for 5 million steps and calibrated divergence times
270	using a log-normal distribution for the rabbit-Lepus node age with a median of 11.8 million
271	generations (95% prior density: 9.8–14.3; Matthee et al. 2004).
272	We also examined patterns of autosomal introgression tracts to infer the age of nuclear
273	admixture. Given that mtDNA admixture may have been relatively ancient (Melo-Ferreira et al.
274	2014), admixture dating approaches based on linkage disequilibrium (LD) may have low power
275	due to erosion of LD through ongoing recombination (Loh et al. 2013). Therefore, we developed
276	an approach to fit the distribution of empirically inferred introgression tract lengths to tract
277	lengths simulated under various models of admixture. We first identified genome-wide tracts of
278	introgression using the program PhylonetHMM (Liu et al. 2014), which assigns one of two
279	parent trees (species tree or hybridization tree) to each variable position using a hidden Markov
280	model. PhylonetHMM robustly distinguishes between incomplete lineage sorting (ILS) and
281	introgression by allowing for switches between gene trees within each parent tree (Liu et al.

282	2014; Schumer et al. 2016). Alignments of WGS data for the phylogenetic analysis included two
283	black-tailed jackrabbits sampled from CA (BTJR1) and NV (BTJR2), a UT snowshoe hare
284	(previously shown as non-admixed; Jones et al. 2018), and a winter-brown WA snowshoe hare to
285	represent the admixed PNW snowshoe hare population. Here, the species tree is defined as
286	((WA,UT),(BTJR1, BTJR2)) and the hybridization tree is defined as (UT,(WA,BTJR1/BTJR2)).
287	We specified base frequencies and transmission/transversion rates based on analysis with
288	RAxML. We identified introgression tracts as contiguous regions of the genome with an average
289	hybridization tree probability >95% across 25 variant windows (1 variant step) and excluded
290	introgression tracts shorter than 10-kb (Schumer et al. 2016). We then used the program SELAM
291	(Corbett-Detig and Jones 2016) to simulate a single pulse of admixture (lasting either 1
292	generation or 100 generations) occurring at a frequency of 0.01%, 0.1%, or 1% and recorded
293	introgression tracts >10kb every 1000 generations for 50,000 generations across 21 autosomes.
294	We performed a goodness of fit test between empirical and simulated tract length distributions
295	using Kolmogorov-Smirnov tests (K-S tests), which measure differences in the cumulative
296	fraction of data across the range of observed values (Massey 1951). To estimate the variance in
297	hybridization timing, we performed K-S tests on 100 bootstrap data sets generated by
298	subsampling 30% of the genome-wide introgression tracts.
299	
300	The time to most recent common ancestor of the winter-brown haplotype
301	To understand the history of the spread of winter brown camouflage, we used targeted Agouti

- 302 SNPs to estimate the time to most recent common ancestor (TMRCA) for the winter-brown
- 303 Agouti haplotype in OR (n=47 individuals) and WA (n=35 individuals). We estimated the
- 304 TMRCA using a Markov chain Monte Carlo approach implemented in *startmrca* (Smith et al.

305 2018), which leverages information on the length distribution of the fixed selected haplotype and 306 the accumulation of derived mutations. We assumed a constant recombination rate of 1 cM/Mb (Carneiro et al. 2011) and tested an upper and lower estimate for mutation rate in European 307 rabbit (2.02 x 10⁻⁹ and 2.35 x 10⁻⁹ mutations/site/generation; Carneiro et al. 2012). We also 308 309 explored the influence of using a divergent population (homozygous winter-white individuals 310 from MT; n=5 individuals) or a local population (homozygous winter-white individuals from OR 311 and WA; *n*=19 individuals) to represent the ancestral winter-white haplotype (Smith et al. 2018). We assumed chr4:5480355 (in oryCun2 coordinates) as the site of the "selected allele", which 312 313 lies in the center of the association interval between two strong candidate insertion-deletion 314 mutations in the 5' cis-regulatory region of Agouti and is perfectly correlated with winter coat 315 color (Jones et al. 2018). We performed 100,000 iterations with a standard deviation of 20 for the 316 proposal distribution and used the final 10,000 iterations to generate posterior TMRCA 317 distributions (Smith et al. 2018).

318

319 <u>Simulations of selection on a recessive beneficial allele</u>

320 Assuming fixation of a single haplotype, the above framework for inferring the TMRCA should 321 reflect the age at which the beneficial haplotype began to increase rapidly in frequency (Smith et 322 al. 2018), which under some conditions may be much more recent than the age of the beneficial 323 mutation itself (Teshima and Przeworski 2006; Kelley 2012). For instance, the masking of 324 recessive alleles to selection at low frequency is expected to decrease the rate at which they 325 begin to increase in frequency, conditional on fixation (Teshima and Przeworski 2006), 326 potentially resulting in a temporal lag between a fixed allele's origin and TMRCA. However, 327 such a scenario may be unlikely as the masking of rare recessive alleles is also expected to

328 decrease their fixation probability (i.e., 'Haldane's sieve', Haldane 1924; Turner 1981). 329 Alternatively, an environmental change could favor a previously neutral or deleterious variant, 330 resulting in a delayed spread of a segregating mutation. Indeed, Orr and Betancourt (2001) 331 demonstrated that the bias against fixation of recessive alleles disappears when positive selection 332 acts on pre-existing variation in mutation-selection balance. We used simulations to test whether 333 different estimates of the timing of hybridization (i.e., the origin of the winter-brown haplotype) 334 and TMRCA of the winter-brown allele could be due to the masking of recessive alleles at low frequency. Using SLiM 3.1 (Haller and Messer 2019), we simulated an equilibrium population 335 336 $(N_e=257219$ for OR population; Table 1) experiencing positive selection on a recessive allele 337 (s=0.026, which reflects our updated median estimate of s for winter-brown haplotype in OR; 338 Jones et al. 2018). At the beginning of the simulations, the recessive allele was introduced at a 339 rate of 0.01% or 0.1% per generation for 1 or 100 generations, which reflects various rates and durations of hybridization. Under each hybridization scenario, we performed 100 simulations 340 341 and tracked the frequency of the recessive allele every generation, conditioning on fixation. We 342 saved tree sequences (Haller et al. 2019) and analyzed them using *msprime* (Kelleher et al. 2016) 343 to identify the TMCRA for fixed beneficial alleles and determine whether selection resulted in 344 fixation of a single copy (hard sweep) or multiple copies (soft sweep) of the beneficial allele.

345

346 **Results**

347 <u>Range-edge population history and mutational load</u>

348 We found support for a single relatively strong N contraction (i.e., a two-epoch model) occurring

349 ~24-100 kya in OR, WA, and BC hares (Table 1, Fig. S2). In contrast, the history of the MT

350 population was characterized by an instantaneous + exponential N change model, in which the

population experienced a sudden $17 \times \text{expansion} \sim 129$ kya followed by a gradual reduction to $\sim 53\%$ of N_{anc} . Despite population contractions, estimates of contemporary N_e across all populations were relatively large (161654-257219; Table 1).

354 Using the same exome data set, we previously estimated the joint SFS for pairs of 355 snowshoe hare populations to infer histories of population split times, migration rates, and 356 effective population size in $\partial a \partial i$ (Jones et al. 2018). These pairwise models supported histories 357 of high symmetrical migration rates between populations and N contractions following 358 population splits, but generated significantly smaller estimates of contemporary N_e compared to 359 the new estimates that we report in Table 1. However, we made a scaling error while estimating 360 θ (= 4N_e μ) under these previous models. This error affected our previously reported 361 demographic parameter estimates for snowshoe hares (Table S9 in Jones et al. 2018) and 362 associated selection coefficient parameter estimates (e.g., previous mean s_{WA} =0.024, s_{OR} =0.015; 363 updated mean s_{WA} =0.049, s_{OR} =0.027; Durrett and Schweinsberg 2005; Jones et al. 2018), but not 364 the main inference of introgression at Agouti underlying the genetic basis of polymorphic coat 365 color in snowshoe hares. After scaling parameter values to the correct value of θ and excluding 366 models beyond *a priori* divergence time parameter bounds (>500 thousand years), we found that 367 our maximum likelihood demographic model (reported here in Table S1) still includes high 368 migration rates between populations (\sim 1-2.63 migrants per generation), but with appreciably larger N_e estimates than we previously reported and that are comparable to our new estimates 369 370 (Table 1).

We found significantly elevated F_{IS} in the coastal BC population compared to the other three PNW populations (p<0.01; Fig. 1B), which combined with our previous inference of elevated LD in this population (Jones et al. 2018) could suggest recent inbreeding and population

374	size reduction. We further found a significantly higher proportion of homozygosity for
375	synonymous, nonsynonymous, and nonsense SNPs in BC relative to other populations (Fig. 1C),
376	which suggests elevated mutational load in BC under a recessive deleterious mutation model. BC
377	individuals also have a significantly higher proportion of strongly deleterious nonsynonymous
378	variants in (27.7%; $ s \ge 10^{-3}$) relative to other populations (0.8-3.2%; Fig. 1D, Table S2). Because
379	we have the same sample size for MT and BC ($n=14$ individuals) this striking difference in the
380	DFE is likely not the result of the small BC sample size, which can lead to overestimation of the
381	proportion of strongly deleterious variation (Kim et al. 2017). Notably, if synonymous SNPs
382	used for demographic inference experience direct or linked selection (e.g., Akashi 1994;
383	Stoletzki and Eyre-Walker 2006; Resch et al. 2007; Pouyet et al. 2018), then our demographic
384	model could be misinferred (Ewing and Jensen 2016) in such a way that we will underestimate
385	the strength of purifying selection on non-synonymous SNPs. Regardless, assuming levels of
386	linked selection are similar across populations, the relative differences we observe in the DFE
387	are unlikely driven by weak or linked selection on synonymous variants.

389 The history of hybridization and introgression

From our complete mtDNA assemblies, we estimated a divergence time of 3.299 million

391 generations ago (95% HPD interval: 2.555-4.255 million generations ago; Fig. 2) between black-

tailed jackrabbit and non-introgressed snowshoe hares, which is consistent with previous

estimates of species' split times (Matthee et al. 2004; Melo-Ferreira et al. 2014; Jones et al.

- 2018). Within the non-introgressed snowshoe hare mtDNA clade, we found a relatively deep
- split between the UT snowshoe hare (representing the 'Rockies' cluster identified by Cheng et al.

2014) and all other snowshoe hares (641 thousand generations ago, 95% HPD interval: 476-834
thousand generations ago; Fig. 2).

398 A significant portion of snowshoe hares from the PNW (100% of OR hares and 50% of 399 WA hares) formed a reciprocally monophyletic clade relative to black-tailed jackrabbits (100% 400 posterior node support; Fig. 2). As previously demonstrated through coalescent simulations 401 (Melo-Ferreira et al. 2014), this phylogenetic pattern cannot be plausibly explained by ILS and is 402 consistent with asymmetric introgression of black-tailed jackrabbit mtDNA into snowshoe hares. 403 As expected, mtDNA was not associated with winter coat color in the PNW polymorphic zone 404 (chi-squared P=1). However, if we assume that hybridization is rare then mtDNA may track the 405 same hybridization event that introduced winter-brown Agouti variation into PNW hares. The 406 estimated split time between black-tailed jackrabbit and introgressed PNW hare mtDNA 407 sequences was 516 thousand generations ago (95% HPD interval: 381-668 thousand generations ago, Fig. 2). However, this split time does not account for segregating ancestral polymorphism 408 409 (Arbogast et al. 2002) or unsampled mtDNA variation within black-tailed jackrabbits. If we 410 assume extant variation in snowshoe hares represents a single mtDNA introgression event, then 411 the TMRCA of introgressed PNW snowshoe hare variation suggests a more recent date of 412 mtDNA introgression of ~228 thousand generations ago (95% HPD interval: 168-301 thousand 413 generations ago).

414 Our previous work revealed elevated signatures of genome-wide nuclear admixture

415 presumably coincident with introgression of seasonal camouflage variation (Jones et al. 2018).

416 Here we identified 1878 individual introgression tracts (median length = 28,940 bp),

417 encompassing ~1.99% of the genome (Fig. 3). Across various simulated hybridization scenarios,

418 the most strongly supported age of hybridization was 7-9 thousand generations ago with ranges

of 95% confidence intervals spanning 6-10.5 thousand generations ago (Fig. S4). Different rates
of admixture or admixture pulse lengths appeared to have little effect on the inferred
hybridization age or the overall fit to empirical data (Fig. S4). Furthermore, we observed poor

422 model fitting for very recent hybridization (< 5 thousand generations ago).

423

424 <u>Positive selection for winter-brown camouflage</u>

We identified the Agouti region as one of the longest (209,012 bp) and most highly supported 425 426 introgression tracts (mean introgression probability=0.99) in the WA winter-brown hare (Fig. 4). 427 To understand the history of positive selection on brown winter camouflage, we estimated the 428 TMRCA of the selected winter-brown Agouti haplotype in snowshoe hares using targeted 429 sequencing across the *Agouti* region (mean coverage per interval $34 \times \pm 17 \times$). Using a divergent population, a local population, or both to represent the ancestral haplotype had little effect on 430 431 TMRCA estimates (Table S3), so here we present estimates using both populations. Under a low 432 or high estimate of the rabbit mutation rate, we inferred a TMRCA of approximately 1278 433 generations (95% CI: 1135-1441 generations) or 1226 generations (95% CI: 1054-1408 434 generations) for the winter-brown OR haplotype and approximately 1392 generations (95% CI: 435 1153-1607 generations) or 972 generations (95% CI: 766-1169 generations) for the WA 436 haplotype, respectively (Table S3). We observed no consistent allelic differences between the 437 fixed haplotypes in WA and OR (Fig. 4), consistent with a hard selective sweep. 438 Haplotype-based methods are known to underestimate the TMRCA and accounting for 439 this systematic error produces TMRCA estimates of approximately 2-4 thousand generations (for 440 a fully recessive allele, $\log_2(\text{estimate/true}) \approx -1.5$; Kelley 2012) for the winter-brown haplotype in 441 OR and WA. If our estimates are accurate, then there appears to be a ~3-8 thousand generation

442 lag between the origin of the winter-brown haplotype in snowshoe hares (i.e., the inferred hybridization date ~ 7-9 thousand generations ago) and the increase in frequency of the winter-443 444 brown haplotype in the PNW from a single copy. Simulations show that such temporal lags are 445 expected for selection on recessive variation, however the duration of this lag (and the total 446 sojourn time) is negatively associated with the hybridization rate and fixation probability, as 447 expected (Table 2). For instance, under the lowest hybridization rate (0.01% for 1 generation) the 448 mean lag time was 2140 generations (95% CI: 101-8322 generation) with only a 0.8% fixation probability and under the highest hybridization rate (0.1% for 100 generations) the mean lag time 449 450 was only 625 generations but with 100% fixation probability. Conditional on fixation, increased 451 hybridization rates also tended to be more often associated with soft rather than hard sweeps 452 (e.g., 38% hard sweeps for 0.1% hybridization rate for 100 generations versus 98% hard sweeps 453 for 0.01% hybridization rate for 1 generation). However, under intermediate hybridization 454 scenarios (0.1% for 1 generation or 0.01% for 100 generations), we observed relatively long 455 mean lag times (2514 and 1587 generations, respectively) associated with high probabilities of 456 fixation (12% and 79%, respectively), often through hard selective sweeps (81% and 96%; Table 457 2).

458

459 **Discussion**

460 Range-edge adaptation may enhance a species' evolutionary resilience to environmental change 461 (Hampe and Petit 2005; Hill et al. 2011), however rigorous population genetic evaluations of 462 predictions for range-edge demography and adaptation are limited (Bridle and Vines 2007). In 463 snowshoe hares, the evolution of brown winter coats in temperate climates along the PNW coast 464 represents the clearest example of local phenotypic adaptation in this wide-ranging species.

Given its direct link to reduced snow cover, the evolution of brown winter camouflage may
further foster persistence of snowshoe hares in the face of climate change (Mills et al. 2018).
Here we leveraged our understanding of the genetic basis of brown winter camouflage to
examine the history of range-edge adaptation, lending insights into the potential for rapid
adaptation following environmental change.

470

471 <u>Population history and mutational load at the range edge</u>

Populations along range margins are predicted to be small, limiting their ability to adapt to local 472 473 conditions (although see Moeller et al. 2011; Graignic et al. 2018). Although we cannot assess relative differences in N_e across the entire hare range, we uncovered high N_e estimates across 474 475 PNW populations (161654-257219; Table 1), despite evidence for strong ancient population size 476 reductions. However, our N_e estimates derive from predictions of genetic drift (i.e., variance N_e) 477 over long evolutionary time scales and may be a weak reflection of current census sizes, 478 especially if local populations experience migration (Wang and Whitlock 2003) or have 479 undergone recent size changes that are undetectable with the SFS (Beichman et al. 2018). We 480 found evidence of significantly higher inbreeding coefficients and mutational load in coastal 481 (BC) populations relative to the inland and montane populations (Fig. 1), signatures that are 482 indicative of a recent population size reduction (Peischl et al. 2013, 2015; Bosshard et al. 2017; 483 Gilbert et al. 2018). Elevated F_{IS} and LD (Fig. S1 in Jones et al. 2018) could instead be related to 484 cryptic population substructure (i.e., the Wahlund effect; Waples 2015). However, we have 485 found no evidence for substructure or admixture in BC that could produce this effect (Jones et al. 486 in prep). Similar signatures of elevated mutational load (e.g., homozygosity for deleterious 487 alleles) have been found in other range-front populations, including the plant Mercurialis annua

488 (González-Martínez et al. 2017) and in human populations that migrated out of Africa (Henn et al. 2016, although see Simons and Sella 2016). Thus, an intriguing potential explanation for 489 490 these patterns is that they reflect signatures of a founder event associated with a recent range 491 expansion. Moreover, given that we observe these signatures in the coastal winter-brown 492 population, it is possible that this expansion was enabled by the evolution of locally adaptive 493 brown winter camouflage. Winter-white hares experience heavy predation when mismatched 494 (Zimova et al. 2016) and are not known to occur in low-lying coastal regional west of the Cascade Range (Nagorsen 1983; Mills et al. 2018), suggesting that coastal environments with 495 496 ephemeral snow cover were likely unoccupied prior to local camouflage adaptation.

497 Long-term persistence of populations under environmental change ultimately requires 498 adaptive evolution and the ability to colonize novel environments. If the colonization of coastal 499 PNW environments by snowshoe hares was enabled by the evolution of brown winter coats, our 500 results underscore that local adaptation to new environments can act as a negative feedback on 501 fitness through the accumulation of deleterious mutations (Pujol and Pannell 2008; Gilbert et al. 502 2017; González-Martínez et al. 2017; Stewart et al. 2017; Willi et al. 2018). Although the 503 consequences of mutational load for the persistence of PNW hare populations is unclear, high 504 recessive mutational load may compromise the adaptive potential of populations (Assaf et al. 505 2015; González-Martínez et al. 2017) and increase the probability of extinction in small 506 populations (Mills and Smouse 1994; Frankham 1998). In experiments of isolated Tribolium 507 populations, short-term fitness gains via adaptive evolution were entirely lost over longer time 508 periods as a consequence of increasing mutational load, although fitness could be readily 509 restored through admixture (Stewart et al. 2017). In snowshoe hares, the potential fitness costs 510 linked to mutational load may be mitigated by high gene flow between populations (Table S1) or

511 superseded by the enhanced species-level evolutionary resilience afforded by brown-winter 512 camouflage during periods of declining snow cover. Regardless, we suggest that any 513 conservation efforts to promote adaptation to climate change should weigh the potential for 514 enhanced long-term population persistence against the potential short-term fitness costs that may 515 arise through mutational load.

516

517 <u>Hybridization and the origin of the winter-brown allele</u>

Hybridization may play an important role shaping adaptation and expansion of range-edge 518 519 populations (Pfennig et al. 2016), but evidence for this mode of adaptation stems from only a 520 handful of examples (e.g., flies, Lewontin and Birch 1966; mosquitoes, Besansky et al. 2003; 521 sunflowers, Rieseberg et al. 2007). In snowshoe hares, range and niche expansion into mild 522 PNW coastal environments appears to have been enabled by adaptive introgression, although the 523 history of hybridization has remained unclear. We estimated that mtDNA introgression in PNW 524 snowshoe hares occurred ~228 thousand generations ago, which could be interpreted as a 525 conservative upper-bound for the timing of hybridization with black-tailed jackrabbits. 526 Meanwhile, the genome-wide distribution of introgression tract lengths, which should be less 527 sensitive to ILS and population structure within hares (Liu et al. 2014), suggest a much more 528 recent pulse of hybridization ~7-9 thousand generations ago (Fig. 3, Fig. S5). The different 529 genome-wide and mtDNA estimates may also reflect independent pulses of ancient 530 hybridization. Severe systematic overestimation of divergence dates may be common with 531 mtDNA genomes calibrated with a relatively divergent outgroup because of high mutation rates 532 and substitution saturation (Zheng et al. 2011). The divergence dates among major snowshoe 533 hare mtDNA lineages also appear much deeper than our best estimates derived from population

(nuclear) genomic data (~2-3 fold deeper, unpublished data), which suggests that our analyses based on mtDNA likely overestimate the timing of introgression. We assumed a relatively simple molecular clock model and more complicated models (e.g., relaxed clocks) might better account for mutational processes observed in mtDNA genomes. However, it would seem that there would be little insight to be gained by additional modeling here given the myriad of limitations associated with extrapolating population history from a single stochastic realization of the coalescent process (Hudson and Turelli 2003).

541 Several recent studies have also noted that introgression is positively correlated with local recombination rate (Nachman and Payseur 2012; Janoušek et al. 2015; Schumer et al. 2018; 542 543 Edelman et al. 2019; Li et al. 2019; Martin et al. 2019), presumable due to the effects of linked 544 selection against deleterious mutations in hybrids. If this relationship generally holds, then it is 545 possible that our dating approach based on the distribution of introgression tract lengths is also 546 upwardly biased. However, contemporary range overlap between snowshoe hares and black-547 tailed jackrabbits appears restricted to relatively sharp ecological transitions between sage-scrub 548 and montane forests in OR and CA (Fig. 2) and no records exist of putative hybrids, suggesting 549 that contemporary hybridization is likely exceedingly rare or absent and has not resulted in 550 discernable gene flow. Thus, the collective evidence suggests that historical hybridization 551 between snowshoe hares and black-tailed jackrabbits was not more recent than 5 thousand 552 generations ago. Notably, the timing of genome-wide admixture, assuming 1-2 generations per 553 year in hares (Marboutin and Peroux 1995), appears coincident with the retreat of the Cordilleran 554 ice sheet from low-lying coastal habitats in southern BC and northern WA at the end of the last 555 glacial maximum (~18 thousand years ago; Darvill et al. 2018) and thus the opening of suitable 556 habitat for winter-brown snowshoe hares. This period of rapid climatic change resulted in

individualistic range shifts for many North American mammal species (Graham 1986),

558 potentially leading to novel community assemblages and thus promoting hybridization events

559 (Swenson and Howard 2005), which could have created conditions favorable to adaptive

560 introgression.

561

562 The spread of winter-brown camouflage and the tempo of local adaptation

563 Although theory predicts adaptation in small range-edge populations may be slow and mutation-limited, hybridization may alleviate the lack of beneficial variation along range 564 565 margins (Pfennig et al. 2016). Revealing how introgressed alleles adaptively spread through 566 populations is therefore a critical component of understanding the limitations of range-edge 567 adaptation. Here, we identified Agouti as one of the largest (>200 kb) and most strongly 568 supported introgression tracts genome-wide (Fig. 4), consistent with our previous study showing exceptionally low genomic divergence in this region between black-tailed jackrabbits and 569 570 winter-brown snowshoe hares (Jones et al. 2018). Assuming our genome-wide estimates of 571 hybridization age reflect the origination of the Agouti allele through introgression (~9 thousand 572 generations ago), our findings suggest a ~3-8 thousand generation delay until the selective sweep 573 of the winter-brown haplotype in the PNW.

One potential biological explanation for this temporal lag is that winter-brown
camouflage was not immediately beneficial in snowshoe hares. Rather, the winter-brown variant
may have initially segregated as a neutral or deleterious allele for a period of time until an
environmental shift allowed positive selection to act quickly on standing variation (e.g.,
Colosimo et al. 2005). However, our simulations suggest that beneficial recessive alleles
segregating at frequencies as high as ~10% (equivalent to simulations of 0.1% hybridization rate

580 for 100 generations) take on average ~5612 generations (95% CI: 4319-6757 generations) to 581 reach fixation (Table 2). Thus, under an environmental shift scenario, the starting frequency of 582 the winter-brown variant would likely have to be quite large (>10%) in order for selection to 583 quickly drive it to fixation. Although allelic fixation under this model would be virtually 584 guaranteed (Table 2), we suspect that such a high level of hybridization between black-tailed 585 jackrabbits and snowshoe hares is unlikely given their ecological distinctiveness and our lower 586 estimate of the genome-wide proportion of introgression (~1.99%). Furthermore, the high starting allele frequency needed to result in rapid fixation is at odds with the evidence that 587 588 selection fixed a single haplotype, as higher hybridization rates tended to result in softer sweeps 589 (Table 2).

590 An alternative explanation for the delayed rise in frequency of the winter-brown allele 591 invokes the limits of positive selection on recessive variation, which is predicted to result in an 592 extended period of drift while at low frequency until homozygous recessive genotypes become 593 more common. Consistent with this, we find significant temporal lags between the timing of 594 hybridization and the TMRCA of fixed beneficial recessive alleles under low and moderate rates 595 of hybridization. Although fixation under our lowest simulated rate of hybridization was highly 596 unlikely ($\sim 0.8\%$; Table 2), the two intermediate scenarios still resulted in relatively high fixation 597 probabilities (12-78%) and tended to produce hard sweeps (81-96%), consistent with observed 598 patterns of genetic variation at the winter-brown Agouti haplotype. These results demonstrate 599 that one does not need to invoke changing selective coefficients to explain the apparent lag 600 between the origin and the TMRCA of the winter-brown allele. Rather, our data are consistent 601 with the winter-brown variant being immediately beneficial, although predominately hidden to 602 selection, after introduced through hybridization at moderate frequency (~0.1-1%). Indeed, this

603 mutation-limited scenario is consistent with other known instances of colonization of novel 604 environments through the evolution of locally adaptive camouflage in Nebraska deer mice and 605 White Sands lizards (Laurent et al. 2016; Pfeifer et al. 2018; Harris et al. 2019). 606 Rates of adaptation at range edges are potentially an important component of species' responses to climate change (Hampe and Petit 2005). Our study highlights the key role that 607 608 hybridization can play in seeding adaptive variation and facilitating range expansion during 609 periods of environmental change. In some cases, introgression appears to facilitate rapid adaptation to environmental change (Norris et al. 2015; Oziolor et al. 2019). However, 610 611 introgression may not always be an efficient solution for rapid adaptation, as here we 612 demonstrate that the rate of adaptation to novel mild winter environments in snowshoe hares 613 appears to have been limited by the dominance coefficient of the winter-brown allele. 614 Collectively, our findings demonstrate key factors that promote and limit adaptation to changing 615 environments and, in particular, highlight the importance of characterizing genetic dominance of 616 beneficial variants for understanding rates of adaptation and range expansion under climate 617 change.

618

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- data of snowshoe hare (BioProject PRJNA420081, SAMN02782769, SAMN07526959) are also
- 631 available in the Sequence Read Archive.

А P(winter-white) 0 WA MT 500 0 kilometers B _{0.1} Proportion O 0.15-0.0 b 0.10-<u>ഗ</u>-0.1 -0.2 0.05 b b -0.3-0.00 synonymous nonsynonymous nonsense WA OR BC ΜT Population SNP category D о.з -Proportion 0.2 -0.1 -0.0 -10⁻³<*s s*<10⁻⁶ 10⁻⁶<*s*<10⁻⁵ 10-5<5<10-4 10⁻⁴<*s*<10⁻³ Absolute value of s

633

Figure 1. (A) Snowshoe hare range map colored by the probability of winter-white camouflage
(white=1, brown=0). The Pacific Northwest region is magnified and shows sampling localities

636	and coat color phenotypes for BC (purple), MT (red), OR (green), and WA (blue) populations
637	used to generate whole exome data. (B) Box-and-whisker plots representing distributions of
638	individual inbreeding coefficients (F_{IS}) within each population. (C) The proportion of
639	homozygosity across PNW populations for SNPs classified as synonymous, nonsynonymous
640	(missense), or nonsense. Different letters denote significant differences between populations
641	(p <0.01). (D) The inferred distribution of fitness effects for each population shown as the
642	proportion of mutations with given selection coefficients.



Figure 2. Snowshoe hare and black-tailed jackrabbit (BTJR) ranges with sampling localities for
whole genome sequencing. The phylogenetic tree is a maximum clade credibility tree based on
whole mitochondrial genome assemblies of snowshoe hares and black-tailed jackrabbits
(European rabbit as outgroup) with median estimated split times for crucial nodes. Sample
locality names and colors correspond to those on the map. Gray rectangles show the 95% highest
posterior density (HPD) for each node age estimate.











665 Table 1. Maximum likelihood demographic model parameter estimates.

Population	Model	Nanc	N_B	N_F	t (generations)
- MT	Inst. change +	459809	8121810	245430	129400
111	exp. growth	(450035-469582)	(5984297-10344665)	(197252-295473)	(98087-161953)
DC	Two-epoch	669265	210484		97853
BC		(662769-675760)	(184353-237086)	-	(70702-125517)
W/A	Two-epoch	509979	161654		24357
WA	-	(503464-516493)	(125444-198747)	-	(20061-28755)
OD	Two-epoch	494903	257219		52540
OK	-	(482587-507220)	(191394-326076)	-	(44017-61431)
Values in m	month agag and	the 050/ confiden	a_{n} intervals $M = a$	anulation size of	

666 Values in parentheses are the 95% confidence intervals. *Nanc* = population size of common

ancestor; N_B = population size following instantaneous change at time *t*. N_F is the population size

668 following an exponential change beginning immediately after time *t*.

669 Table 2. Results from simulations of positive selection on recessive variation.

	1 generation pulse		100 generation pulse	
	0.01%	0.1%	0.01%	0.1%
D(fivation)	0.0082	0.12	0.79	1.0
r(iixatioii)	(0.0068 - 0.010)	(0.098-0.13)	(0.71-0.85)	(0.96-1.0)
D(hard swaan)	0.98	0.81	0.96	0.38
r (naru sweep)	(0.93-0.99)	(0.72 - 0.87)	(0.90-0.98)	(0.29-0.48)
T(soioum)	8911	9448	6472	5612
<i>I</i> (sojouili)	(5179-16208)	(5626-15746)	(5088-15339)	(4319-6757)
$T(1, \alpha)$	2140	2514	1587	624
I(idg)	(101-8322)	(97-7740)	(87-7253)	(91-1899)

⁶⁷⁰ The beneficial variant was introduced through hybridization during a 1 or 100 generation pulse at

673 sojourn time (*T*(sojourn)), and the mean lag time between a fixed mutation's origin and TMRCA

674 (T(lag)). In parentheses are 95% confidence intervals.

a rate of 0.01% or 0.1%. Data are shown for the probability of fixation (P(fixation)), and,

⁶⁷² conditional on fixation, the probability of a hard selective sweep(*P*(hard sweep)), the mean

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