Convergent evolution of seasonal camouflage in response to reduced snow cover across the snowshoe hare range

Matthew R. Jones,[1,2] iD L. Scott Mills,[3,4] iD Jeffrey D. Jensen,[5] iD and Jeffrey M. Good[1,3,6] iD

[1]*Division of Biological Sciences, University of Montana, Missoula, Montana 59812*

[2]*E-mail: jonesmr9@gmail.com*

[3]*Wildlife Biology Program, University of Montana, Missoula, Montana 59812*

[4]*Office of Research and Creative Scholarship, University of Montana, Missoula, Montana 59812*

[5]*School of Life Sciences, Arizona State University, Tempe, Arizona 85281*

[6]*E-mail: jeffrey.good@umontana.edu*

Determining how different populations adapt to similar environments is fundamental to understanding the limits of adaptation under changing environments. Snowshoe hares (*Lepus americanus*) typically molt into white winter coats to remain camouflaged against snow. In some warmer climates, hares have evolved brown winter camouflage—an adaptation that may spread in response to climate change. We used extensive range-wide genomic data to (1) resolve broad patterns of population structure and gene flow and (2) investigate the factors shaping the origins and distribution of winter-brown camouflage variation. In coastal Pacific Northwest (PNW) populations, winter-brown camouflage is known to be determined by a recessive haplotype at the *Agouti* pigmentation gene. Our phylogeographic analyses revealed deep structure and limited gene flow between PNW and more northern Boreal populations, where winter-brown camouflage is rare along the range edge. Genome sequencing of a winter-brown snowshoe hare from Alaska shows that it lacks the winter-brown PNW haplotype, reflecting a history of convergent phenotypic evolution. However, the PNW haplotype does occur at low frequency in a winter-white population from Montana, consistent with the spread of a locally deleterious recessive variant that is masked from selection when rare. Simulations of this population further show that this masking effect would greatly slow the selective increase of the winter-brown *Agouti* allele should it suddenly become beneficial (e.g., owing to dramatic declines in snow cover). Our findings underscore how allelic dominance can shape the geographic extent and rate of convergent adaptation in response to rapidly changing environments.

KEY WORDS: Climate change, dominance, introgression, local adaptation, migration, parallel evolution.

In response to a shared selection pressure, populations may adapt through the migration of beneficial alleles or through independent mutations that result in the evolution of convergent phenotypes (Wright 1931; Haldane 1932; and see Ralph and Coop 2015). Distinguishing between these scenarios is crucial to understand the capacity of populations to adapt rapidly to environmental change (Bridle and Vines 2007; Hoffmann and Sgrò 2011; Ralph and Coop 2015). If gene flow between populations is sufficiently high, then beneficial variation may spread quickly (Fisher 1937) and potentially allow for rapid adaptive responses to changing environments (Bay et al. 2017). However, the spread of adaptive variation may be limited at broad geographic scales, and populations may have to rely on independent standing genetic variation or new mutations to evolve convergent traits (i.e., "convergent evolution" following Arendt and Reznick [2008]). Although there is considerable evidence for both adaptation through gene flow and independent mutations in nature (Hoekstra and Nachman 2003; Hoekstra et al. 2005;

Steiner et al. 2008; Rosenblum et al. 2010; Dobler et al. 2012; Marques et al. 2017; Kreiner et al. 2019), few empirical studies have examined the specific factors that influence these outcomes in natural populations (Ralph and Coop 2015).

When a species range encompasses a mosaic of habitats, the relative probability of adaptation through gene flow versus independent mutation is predicted to be primarily a function of the distance between habitat patches (in units of dispersal distance) and the strength of selection against locally adaptive alleles in intervening habitats (Slatkin 1973; Ralph and Coop 2010, 2015). In general, as distance between patches and the strength of purifying selection in intervening habitats increases, so does the relative probability of adaptation through independent mutations. Adaptation via independent mutations is therefore predicted to be more common in widespread populations where dispersal distance is short relative to the total range size (Ralph and Coop 2010). For example, rock pocket mice (*Chaetodipus intermedius*) have repeatedly evolved melanistic coats across patchy lava flows in the southwestern United States. Although substantial gene flow between adjacent lava flows has likely resulted in the migration of melanic alleles (Hoekstra et al. 2005), melanism is attributed to different mutations across disparate lava flows (Hoekstra and Nachman 2003; Nachman et al. 2003; Harris et al. 2020). Theoretical models further predict that the relative probability of adaptation via independent mutations increases rapidly with distance between lava flows (over the scale of tens to hundreds of kilometers; Ralph and Coop 2015), due in large part to strong selection against coat color mismatch (Hoekstra et al. 2004; Pfeifer et al. 2018; Barrett et al. 2019). Thus, there is a clear trade-off between dispersal distance and the strength of purifying selection that strongly dictates the probability of adaptation through convergent evolution or gene flow.

The effect of genetic dominance on the probability of convergent evolution has not yet been thoroughly explored (Ralph and Coop 2015). "Haldane's sieve" (Turner 1981) predicts that de novo dominant mutations enjoy a much greater probability of fixation compared to de novo recessive mutations (probability of fixation $\approx 2hs$, where h = dominance coefficient and s = selection coefficient), because rare dominant mutations are visible to selection (Haldane 1924). As de novo beneficial recessive mutations are masked to selection when rare, those that do ultimately reach fixation may spend a longer period of time drifting at low frequency (Teshima and Przeworski 2006). Likewise, rare recessive migrant alleles are expected to exhibit the same behavior, which may allow more time for mutation to generate "competing" convergent phenotypes upon which selection can act. However, Orr and Betancourt (2001) demonstrated that genetic dominance has no effect on the probability of fixation for alleles in mutation-selection balance because recessive alleles have a higher mutation-selection balance frequency. Thus, for rel-

atively high amounts of gene flow, genetic dominance may have little to no effect on the probability of adaptation via migration versus de novo mutation. In fact, the masking of low frequency recessive alleles may result in weaker purifying selection in intervening habitats and therefore facilitate the spread of adaptive variation through gene flow (Ralph and Coop 2015), although this hypothesis remains to be tested.

Snowshoe hares (*Lepus americanus*) are one of at least 21 species that have evolved seasonal molts to white winter coats to maintain camouflage in snowy winter environments. Because color molts are cued by photoperiod, and hence may become mismatched under rapidly changing environments (Mills et al. 2013; Zimova et al. 2018), seasonal camouflage provides a useful trait to understand adaptation to climate change (Mills et al. 2013, 2018; Zimova et al. 2016, 2018; Jones et al. 2018). Reduction in the extent and duration of snow cover is one of the strongest signatures of climate change in the Northern hemisphere (Knowles et al. 2006; Brown and Mote 2009), suggesting that selection should increasingly favor delayed winter-white molts in snowshoe hares to reduce the total duration of coat color mismatch. However, below some annual snow cover threshold, populations are predicted to maintain brown coloration during the winter (Mills et al. 2018). Consistent with this, snowshoe hares maintain brown winter camouflage in some temperate environments with reduced snow cover (Nagorsen 1983), a strategy that should be increasingly favored under climate change (Jones et al. 2018; Mills et al. 2018).

Winter-brown snowshoe hares are common in coastal regions of the Pacific Northwest (PNW), where this Mendelian trait is determined by a recessive variant of the *Agouti* pigmentation gene (Jones et al. 2018). The locally adaptive *Agouti* variant was introduced into snowshoe hares by hybridization with black-tailed jackrabbits nearly 7000–14,000 generations ago and subsequently experienced a local selective sweep within the last few thousand generations (Jones et al. 2020). Occasional records of winter-brown camouflage also occur in more northern regions along the Pacific coast (e.g., Canada and Alaska) and in eastern North America (Gigliotti et al. 2017; Mills et al. 2018). Although hares are expected to be more or less continuously distributed along suitable Pacific coast environments, there is a deep split and little evidence of recent gene flow between the northern "Boreal" populations and southern "PNW" populations (Cheng et al. 2014; Melo-Ferreira et al. 2014). Given this historic population structure, it remains unclear whether the distribution of winter-brown camouflage across populations from disparate parts of the range reflects independent genetic origins (i.e., trait convergence) or spread of the introgressed PNW *Agouti* allele.

Here, we use new and previously published genetic data to investigate the roles of gene flow and mutation in shaping
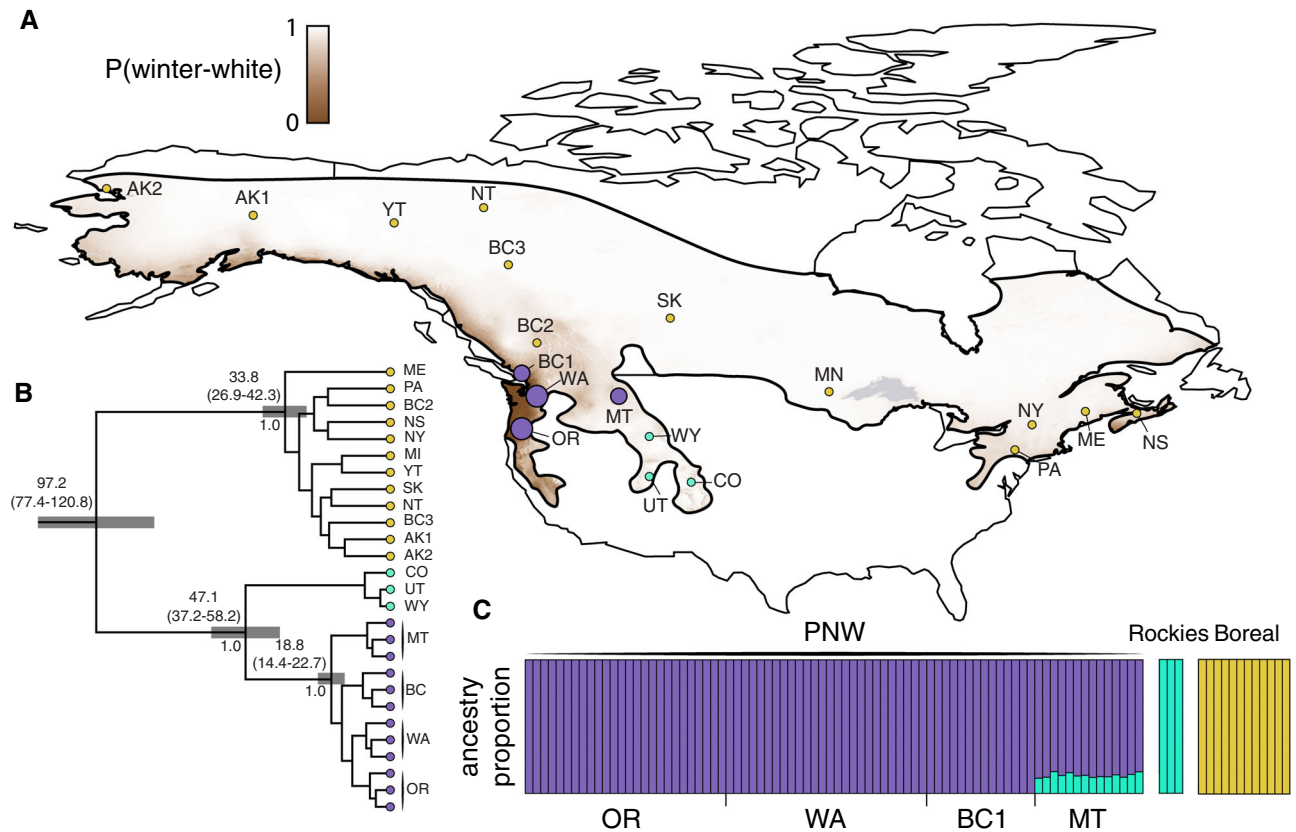
Figure 1. (A) Range-wide phylogeography of snowshoe hares based on whole exome sequences. The snowshoe hare range is colored brown to gray according to the modeled probability of winter-brown camouflage from Mills et al. (2018). Sizes of sampling locality circles are scaled to sample size and are colored according to their population assignment (see C). (B) A maximum credibility phylogenetic tree estimated with BEAST 2 (Bouckaert et al. 2014). All nodes have posterior probabilities >99%. Each major node shows the median node age in thousands of years (95% posterior density in parentheses and gray rectangles) and the ASTRAL support score. (C) The Admixture plot shows the proportion of ancestry across samples based on a $K = 3$ clustering, which had the lowest cross validation error.

the evolution of winter-brown camouflage across populations of snowshoe hares. We first combined previously published microsatellite ($n = 853$ individuals, 8 loci) with new and published whole exome data ($n = 95$ individuals) to resolve range-wide patterns of population history and gene flow in snowshoe hares. These data allow us to understand the extent to which population history and structure may limit the spread and thus adaptive potential of introgressed winter-brown camouflage to climate change. We then generated whole genome sequence (WGS) data for a winter-brown hare from Alaska (AK) to test whether winter-brown camouflage in Boreal snowshoe hares arose independently from PNW populations. Next, to understand the geographic limits of the recessive PNW *Agouti* allele, we used pooled WGS data to estimate its frequency in a winter-white population from Montana (MT), approximately 600 km from the closest winter-brown PNW population. Finally, we used both theoretical predictions and simulations to understand the factors influencing the geographic scope of the PNW *Agouti* allele and its potential to contribute to rapid adaptation in response to warming climates.

Methods

SAMPLES AND GENOMIC DATA GENERATION

To resolve patterns of range-wide population structure, we performed targeted whole exome enrichment of 12 snowshoe hares previously sampled from 12 localities across Canada, Alaska, and the eastern United States and three hares from the southern Rocky Mountains (representing the "Boreal" and "Rockies" lineages as defined by Cheng et al. [2014]; Fig. 1A). The targeted whole exome capture was designed by Jones et al. (2018) to enrich for ∼99% of genic regions annotated in the European rabbit (*Oryctolagus cuniculus*) genome (61.7-Mb spanning 213,164 intervals; ∼25-Mb protein-coding exons, ∼28-Mb untranslated region, ∼9-Mb intronic or intergenic). We followed the library preparation protocols outlined in Jones et al. (2018) and sequenced libraries on one lane of Illumina HiSeq2500 with paired-end 100 bp reads (HudsonAlpha Institute for Biotechnology, Huntsville, AL). Exome sequences for Boreal and Rockies snowshoe hares were combined with published whole exome data (Jones et al. 2018)

from 80 PNW snowshoe hares ($n = 95$ total; Table S1), including a monomorphic winter-brown population from southeast British Columbia (BC1; $n = 14$), a monomorphic winter-white population from Seeley Lake area in western MT ($n = 14$), and two polymorphic coat color populations from Oregon (OR; $n = 26$; two localities) and Washington (WA; $n = 26$; Cheng et al. 2014; Jones et al. 2018).

To resolve the historical spread of the winter-brown PNW *Agouti* allele, we performed whole genome sequencing of a winter-brown snowshoe hare museum specimen from southwest AK first noted by Link Olson and later obtained through loan from University of Alaska Museum of the North (UAM 116170, collected on 4 January 2013). We also performed whole genome sequencing on a DNA pool of 81 snowshoe hares from two localities in Glacier National Park, MT (Table S1) collected as part of a different study (Cheng et al. 2014). We extracted genomic DNA from muscle tissue of the AK hare sample following the Qiagen DNeasy Blood and Tissue kit protocol (Qiagen, Valencia, USA). For the pooled MT whole genome sequencing, we pooled previously extracted DNA samples (Cheng et al. 2014) in approximately equimolar quantities based on Qubit concentrations (Invitrogen Qubit Quantitation system LTI). We then prepared genomic libraries for all samples following the KAPA Hyper prep kit manufacturer's protocol. We sheared genomic DNA to ~300 bp using a Covaris E220evolution ultrasonicator and performed a stringent size selection using a custom-prepared carboxyl-coated magnetic bead mix (Rohland and Reich 2012). We determined indexing PCR cycle number for each library with quantitative PCR (qPCR) on a Stratagene M×3000P thermocycler (Applied Biosystems, Foster City, USA) using a DyNAmo Flash SYBR Green qPCR kit (Thermo Fisher Scientific, Waltham, USA). Final libraries were size-selected again with carboxyl-coated magnetic beads, quantified with a Qubit (Thermo Fisher Scientific, Waltham, USA), and pooled for sequencing by Novogene (Novogene Corporation Ltd., Davis, USA) on two lanes of Illumina HiSeq4000 using paired-end 150-bp reads. The WGS from the AK hare was combined with previously published whole genome sequencing of five black-tailed jackrabbits, three winter-brown snowshoe hares from OR, WA, and BC1, and three winter white snowshoe hares from MT, a hare from Utah (UT), and a hare from Pennsylvania (PA; Table S1; Jones et al. 2018).

READ PROCESSING AND VARIANT CALLING

For all raw Illumina sequence data, we trimmed adapters and low-quality bases (mean phred-scaled quality score <15 across 4 bp window) using Trimmomatic version 0.35 (Bolger et al. 2014) and merged paired-end reads overlapping more than 10 bp and with lower than 10% mismatched bases using FLASH2 (Magoč and Salzberg 2011).

WGS data were mapped to either a snowshoe hare or black-tailed jackrabbit pseudoreference (see Jones et al. (2018) for details) using default settings in BWA-MEM version 0.7.12 (Li 2013). We used *PicardTools* to remove duplicate reads with the MarkDuplicates function and assigned read group information with the AddOrReplaceReadGroups function. Using GATK version 3.4.046 (McKenna et al. 2010), we then identified poorly aligned genomic regions with RealignerTargetCreator and locally realigned sequence data in these regions with IndelRealigner. We performed population-level multi-sample variant calling using default settings with the GATK UnifiedGenotyper. Here, we called variants separately for each previously defined snowshoe hare population genetic cluster (i.e., Boreal, Rockies, BC1, MT, OR, and WA) and for black-tailed jackrabbits. We performed variant filtration in VCFtools version 0.1.14 (Danecek et al. 2011). For whole exome and whole genome data, we filtered genotypes with individual coverage <5× or >70× or with a phred-scaled quality score <30. Additionally, we removed all indel variants and filtered single nucleotide polymorphisms (SNPs) with a phred-scaled quality score <30, Hardy-Weinberg $P < 0.001$. We required that sites have no missing data across individuals.

RANGE-WIDE POPULATION GENETIC STRUCTURE AND GENE FLOW

We inferred a maximum likelihood tree with a general time reversible model in RAxML version 8.2.8 (Stamatakis 2014) using concatenated exome data of a subset of snowshoe hare individuals ($n = 12$ Boreal hares, $n = 3$ Rockies hares, $n = 12$ PNW hares; 21,167,932 total sites) with European rabbit (*Oryctolagus cuniculus*) as the outgroup. Using this maximum likelihood tree as a starting tree, we estimated a maximum clade credibility tree and node ages with a constant population size coalescent model and a calibrated Yule model in BEAST 2 (Bouckaert et al. 2014). We assumed a strict molecular clock and an HKY substitution model using empirical base frequencies. We specified default priors for the kappa parameter, gamma shape parameter, and population size parameter and used a gamma distribution (alpha = 0.0344, beta = 1) as a prior for the clock rate parameter. We ran the Markov chain Monte Carlo for 5 million steps and calibrated divergence times using a log-normal distribution for the *Oryctolagus-Lepus* node age with a median of 11.8 million years (95% prior density: 9.8–14.3; Matthee et al. 2004). To examine how divergence time estimates were affected by our assumption of the *Oryctolagus-Lepus* node age, we performed an additional analysis without data to sample the prior of the divergence time calibration parameter. Finally, we performed a species-tree analysis using gene trees in ASTRAL version 5.6.3 (Zhang et al. 2018). Gene trees were generated across 200-kb windows (excluding windows wither fewer than 500 sequenced bases) using

RAxML with a GTR+gamma model and rapid bootstrap analysis (-f a -# 10). We collapsed nodes on gene trees with low bootstrap support values ($\leq$10) and performed ASTRAL analyses on the collapsed gene trees using default settings.

To test for signatures of population structure and gene flow among lineages, we performed a population cluster analysis in ADMIXTURE version 1.3.0 (Alexander et al. 2009) using 10,388 SNPs. To reduce the effects of linkage disequilibrium, sites were selected from our exome data to be separated by at least 10 kb (median within chromosome distance = 66.6 kb). We tested K values from 1 to 10 (representing the number of population clusters) and selected the K value with the lowest cross-validation error. We also estimated range-wide effective migration and diversity surfaces with EEMS (Petkova et al. 2016) using extensive microsatellite data ($n = 853$ individuals, 8 loci) generated by Cheng et al. (2014). Varying the number of demes (50, 100, and 200) had little effect on estimates of effective rates of migration and diversity, therefore we only report results for 200 demes. We used default hyperparameter values and tuned the proposal variances such that proposals were accepted approximately 30% of the time. We ran EEMS for 2 million iterations with a burn-in of 1 million iterations and thinning iteration of 9999. Runs produced strong correlations between observed and expected genetic dissimilarity both within and between demes, indicating good model fit.

GEOGRAPHIC DISTRIBUTION OF THE PNW *AGOUTI* ALLELE

The winter-brown AK snowshoe hare was collected approximately 3000 km (via the Pacific coast) from Vancouver, BC, the northern limit of winter-brown PNW hare populations (Jones et al. 2018). To test whether the introgressed PNW *Agouti* allele has seeded winter-brown camouflage in southwest AK, we generated a tree of the PNW *Agouti* region from our WGS data of winter-brown and winter-white snowshoe hares ($n = 7$) and black-tailed jackrabbits ($n = 5$). We defined the PNW *Agouti* region (chr4: 5,340,275 – 5,633,020, oryCun2 coordinates) based on the location of the introgressed black-tailed jackrabbit tract identified with a hidden Markov model by Jones et al. (2018). We then inferred a maximum likelihood phylogeny for this interval using RAxML (Stamatakis 2014) as above. Node support values were generated from 1000 replicate bootstrap runs.

We also estimated the frequency of the PNW *Agouti* haplotype among pooled individuals from two winter white localities in MT ($n = 81$; see Mills et al. 2013) that are 575 and 627 km from the polymorphic sampling locality in WA (where winter-brown camouflage is relatively common). We used PoPoolation2 (Kofler et al. 2011) to calculate the frequency of the winter brown-associated alleles at 555 sites across the introgressed *Agouti* haplotype (chr4: 5,340,275 – 5,633,020). These positions were previously shown to be strongly associated with coat color based on a likelihood ratio test of allele frequency differences between winter-brown and winter-white individuals from low coverage WGS data (Jones et al. 2018). We summed the counts of winter-white and winter-brown alleles across all positions to estimate both the mean and standard deviation of winter-brown allele frequency. We excluded seven positions with unusually high frequencies of the winter-brown allele ($\sim$45–100%) supported by reads that did not otherwise carry brown-associated alleles (i.e., incongruent haplotype information), as these likely reflect sequencing errors.

PROBABILITY OF CONVERGENT ADAPTATION THROUGH INDEPENDENT MUTATION

To compare the observed spread of the PNW *Agouti* allele to theoretical expectations, we estimated the relative probability of adaptation via independent mutations with distance from a focal habitat patch using the model developed by Ralph and Coop (2015) (equation 12) under a range of conditions. Because the relative probability of adaptation through migration or de novo mutation depends strongly on the rate at which mutations generate convergent phenotypes (Ralph and Coop 2015), we tested a range of plausible mutation rates. A previous study using over 7 million house mice estimated a mean rate of spontaneous visible coat color mutations of 11×10^{-6} per locus/gamete (Schlager and Dickie 1971), which is $\sim$1930$\times$ higher than the average genome-wide germline per site mutation rate (5.7×10^{-9} per site/gamete; Milholland et al. 2017). Given a genome-wide mutation rate of 2.35×10^{-9} per site/generation for rabbits (Carneiro et al. 2012), we tested a high mutation rate of $\mu = 4.54 \times 10^{-6}$ (i.e., the overall expected rate of visible coat color mutations) and a low mutation rate of $\mu = 4.54 \times 10^{-8}$ (i.e., assuming only 1% of coat color mutations would lead to brown winter camouflage). We also tested how strong ($s_m = 0.01$), moderate ($s_m = 0.001$), or weak ($s_m = 0.0001$) purifying selection against the winter-brown allele in intervening (i.e., snow-covered) habitats influences the probability of adaptation through independent mutations. We assumed a relatively high mean dispersal distance of 2 km (Gillis and Krebs 1999) to produce conservative estimates of the probability of adaptation via independent mutation. We also assumed that the width of the secondary habitat patch was relatively small ($w = 1$ or $\sim$6–45 km, depending on s_m), which is consistent with observations of winter-brown camouflage at low frequency along portions of the range edge. Finally, we assumed $d = 1$ (dimension of the habitat), $C = 1$, and $\gamma = 1$.

SIMULATIONS OF SELECTION ON MIGRANT ALLELES WITH GENETIC DOMINANCE

Theory predicts that allelic dominance should not influence a mutation's probability of fixation under positive selection if

Table 1. Counts of the number of private (e.g., Boreal/Boreal) versus shared SNPs (e.g., Rockies/Boreal) for each major snowshoe hare clade. Individuals from WA represent the PNW.

	Boreal ($N = 120{,}978$ SNPs)	Rockies ($N = 158{,}207$ SNPs)	PNW ($N = 275{,}614$ SNPs)
Boreal	106,051	3760	5820
Rockies and PNW	5347	–	–
Rockies	–	117,863	31,237
PNW	–	–	233,210

it starts in mutation- or migration-selection balance (Orr and Betancourt 2001). Nonetheless, rare recessive mutations may increase in frequency more slowly relative to rare dominant mutations because they are initially invisible to selection, also allowing more opportunity for competition from convergent phenotypes arising through independent mutations. We used simulations to test how the genetic dominance of alleles at equivalent mutation-selection equilibrium frequencies influences the probability and rate of adaptation to new habitats when environmental conditions change. In SLiM 3.0 (Haller and Messer 2019), we performed 100 simulations of the MT population (estimated $N_e = 245{,}430$; Jones et al. 2020) with a positively selected recessive mutation ($s = 0.01$, $h = 0$) starting at the inferred frequency of the PNW *Agouti* allele in MT (p). We assumed that p reflects the equilibrium frequency (x) between the migration rate of the allele into the environment (m) and the selection coefficient against the allele (s), which is given as $\sqrt{\frac{m}{s}}$ for a recessive mutation. Given that x for a dominant mutation is simply $\frac{m}{s}$, we calculated x of an equivalent dominant mutation (same values of m and s) as simple p^2. We then repeated these simulations for a positively selected ($s = 0.01$), completely dominant allele ($h = 1$) starting at its expected mutation-selection balance frequency. For each simulation, we tracked the frequency of the selected allele until it was either fixed or lost. To validate these simulations, we compared the probabilities of allelic fixation from simulations to fixation probabilities analytically derived by Kimura (1957). To test for significant differences in fixation probabilities and rates of adaptation between recessive and dominant mutations, we used Student's *t*-tests in R (R Core Team 2018).

Results

SNOWSHOE HARE PHYLOGEOGRAPHY

We combined previously generated whole exome data for 80 PNW snowshoe hares (mean coverage $21\times \pm 7.6$ per individual) with new whole exomes of 15 hares across the range sequenced to a mean coverage of $20.2\times \pm 8.2$ (Fig. 1A). Exome-wide phylogenetic analyses show three broad phylogeographic groups of snowshoe hares (Fig. 1B) with unambiguous ASTRAL support scores using 6582 gene trees, consistent with previous studies (Cheng et al. 2014; Melo-Ferreira et al. 2014). However, $\sim$12–25% of SNPs within phylogeographic groups appear to be shared across some population boundaries (Table 1), indicating some amount of shared ancestral variation or gene flow. With a constant population size coalescent model, we estimated that snowshoe hares from Canada, AK, and the eastern United States—representing the Boreal lineage—diverged approximately 97.2 thousand years ago (kya; 95% posterior density: 77.4–120.8 kya) from PNW and Rockies snowshoe hares (Fig. 1B). PNW and Rockies hare populations are estimated to have split approximately 47.1 kya (95% posterior density: 37.2–58.2 kya). Under a calibrated Yule model, we estimate very similar divergence times of 96.7 kya (95% posterior density: 77.4–120.7 kya) between Boreal hares and PNW/Rockies snowshoe hares and 46.7 kya (95% posterior density: 37.2–58.2 kya) between PNW and Rockies hares. By running BEAST with no data, we estimated a median of 11.8 million years (95% posterior density: 9.02–14.47) for the prior for the *Oryctolagus-Lepus* node age, which closely matches the prior inferred with data (11.5 million years; 95% posterior density: 9.14–14.30). However, divergence times between snowshoe hare populations were much more recent ($\sim$5–6 kya), indicating that the *Oryctolagus-Lepus* node age prior alone was not driving our divergence time estimates.

Population structure analyses in ADMIXTURE also found strongest support for three population clusters, although MT snowshoe hares (in the PNW lineage) showed 11–16% of their ancestry assigned to the Rockies lineage, indicating ongoing gene flow or continuous genetic structure (Fig. 1C). The ADMIXTURE analysis also indicated little apparent gene flow between the Boreal lineage and PNW or Rockies lineages. Likewise, microsatellite-based estimates of effective migration surfaces revealed that effective migration rates are approximately 100-fold lower than the range-wide average near the apparent zone of contact between PNW and Boreal lineages in western North America (Fig. 2A). We also estimated relatively low effective migration rates across the southwestern edge of the snowshoe hare range (western United States; $\log(m) \approx -1$, or 10-fold lower than the mean). In contrast, effective migration rates in the northwest part of the range (AK and western Canada) appeared
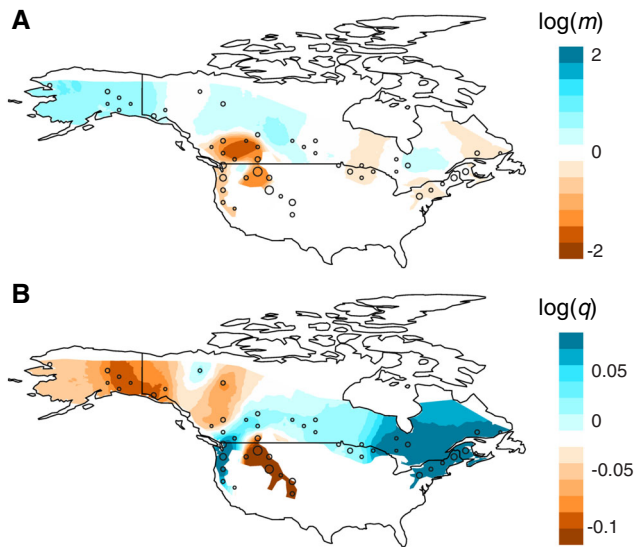
Figure 2. (A) Effective migration rates for snowshoe hares inferred from a range-wide microsatellite dataset (853 individuals, eight loci) from Cheng et al. (2014). The sizes of circles are scaled to the number of samples collected at that location. Effective migration rate is measured as the rate of decay in genetic similarity of individuals across space. Regions that are colored white are characterized by isolation-by-distance, whereas regions that are colored blue or red have higher or lower effective migration, respectively. (B) Effective diversity rates based on the same microsatellite dataset. Here, effective diversity rates measure the genetic dissimilarity between individuals in the same deme, where blue regions have higher than average diversity and red regions have lower than average diversity.

relatively high (∼10-fold higher than the mean). Effective diversity surfaces show that the highest relative genetic diversity occurs in the eastern extent (Boreal lineage) and southwestern extent (PNW lineage) of the snowshoe hare range (Fig. 2B), consistent with previous studies suggesting that these regions were likely glacial refugia (Cheng et al. 2014). Relative genetic diversity gradually decreased moving from east to west across the Boreal range and the lowest genetic diversity was observed in the Rocky Mountain lineage, distributed across the western United States.

CONVERGENT EVOLUTION AND THE DISTRIBUTION OF WINTER-BROWN CAMOUFLAGE VARIATION

Under a single origin of winter brown camouflage, we would expect the winter-brown AK individual to nest within the black-tailed jackrabbit clade at *Agouti* with the other winter-brown snowshoe hares from the PNW. However, our phylogenetic analysis indicates that the winter-brown AK individual unambiguously groups with a winter-white Boreal hare from PA (100% bootstrap support) and more broadly with other winter-white hares across the range (Fig. 3). These results demonstrate that the

winter-brown phenotype of this AK hare is caused by mutations that are independent from the introgressed *Agouti* haplotype that encodes winter-brown camouflage in the PNW, approximately 3000 km away. Determining the genetic basis of this independent origin of winter brown camouflage will require an independent genetic association study in these populations.

Pooled whole genome sequencing detected that the recessive winter-brown *Agouti* allele occurs at an estimated frequency of 1.24% (±0.01%) across predominately winter-white MT localities ∼600 km from the polymorphic zone. Interestingly, long-term live-trapping and radiotelemetry-based field work from this region (e.g., Mills et al. 2013) has resulted in a single observation of a winter-brown hare in approximately 300 winter hare observations (0.33%), although this observed frequency is slightly higher than the Hardy-Weinberg expectation under the estimated frequency of the winter-brown *Agouti* allele ($p^2 = 0.015\%$, binomial test P-value = 0.044).

To understand the factors that have shaped the geographic distribution of the PNW *Agouti* allele, we modeled the relative probability of adaptation through independent mutation with distance from a focal habitat using the theoretical framework of Ralph and Coop (2015). The change in relative probability of adaptation through independent mutation depended strongly on both the mutation rate and selection coefficient against the allele in intervening habitats (Fig. 4). Stronger selection coefficients produced a more rapid shift in the convergence probability with distance relative to weaker selection (Fig. 4). For instance, under a high mutation rate to the winter-brown phenotype ($\mu = 4.54 \times 10^{-6}$) and strong purifying selection ($s_m = 0.01$), the probability of independent adaptation transitioned sharply from $P = 0.1$ at 88 km to $P = 0.9$ at 150 km from a focal habitat. Under a low mutation rate to the winter-brown phenotype ($\mu = 4.54 \times 10^{-8}$) and weak selection ($s_m = 0.0001$), independent adaptation was much less likely at close distances, as expected, and the probability increased more gradually ($P = 0.1$ at 876 km to $P = 0.9$ at 1498 km).

MIGRATION-SELECTION BALANCE

Assuming that the recessive (winter-brown) *Agouti* allele suddenly became favorable in MT due to decreased snow cover, simulations indicate that strong positive selection ($s = 0.01$) on existing standing variation would result in a 81% probability of fixation (95% confidence intervals: 72.2–87.5%; Fig. 5A), which is consistent with an analytically derived probability of 78.1%. For an equivalent dominant mutation starting in mutation-selection balance frequency (0.015%), the simulated probability of fixation was approximately 77% using simulations (95% confidence intervals: 67.8–84.2%) or 77.9% using the Kimura (1957) equation. Neither estimate was significantly different from the fixation probability of a recessive mutation (P
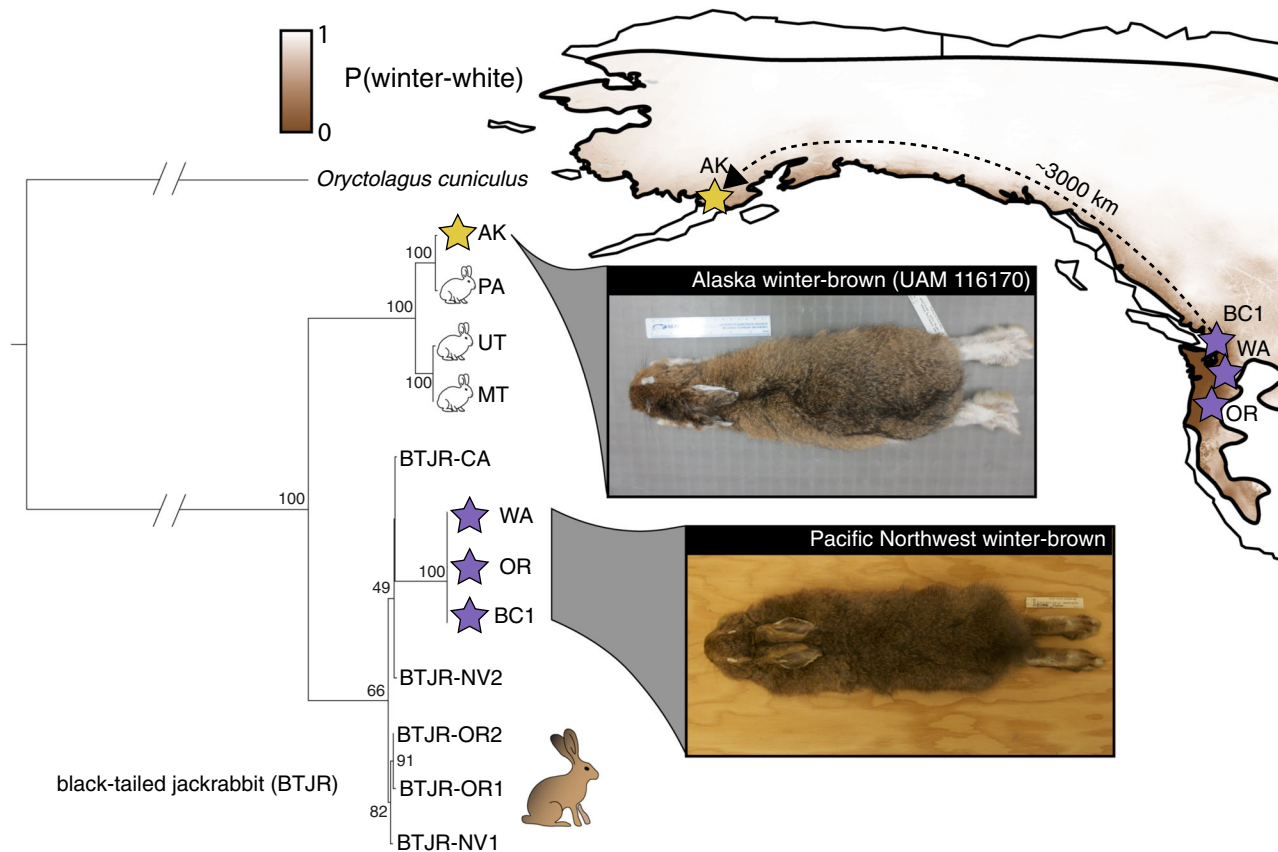
Figure 3. A maximum clade credibility tree of the introgressed *Agouti* locus (∼293 kb) based on whole genome sequencing of black-tailed jackrabbits (BTJR) and snowshoe hares. Values indicate node support based on bootstrapping. The yellow star represents the winter-brown snowshoe hare from Alaska (AK; specimen UAM 116170 pictured top) and the purple stars represent winter-brown snowshoe hares from the Pacific Northwest.

= 0.48, two-tailed test of equality of proportions). However, we observed striking differences in the rates of increase, conditional on fixation, of dominant and recessive mutations following a sudden environmental change (Fig. 5B). The mean time to fixation was significantly faster ($P < 2.2 \times 10^{-16}$, Student's *t*-test) for a recessive mutation (9645 generations, SD = 3609 generations) compared to a dominant mutation (19,445 generations, SD = 7273 generations, $P < 2.2 \times 10^{-16}$). However, as expected, we see that initial rates of allele frequency change and phenotypic adaptation are considerably faster for a positively selected dominant mutation (Fig. 5B). For instance, the mean time for the selected phenotype determined by the recessive mutation to reach a frequency of 75% ($P = 0.866$) was 8837 generations (SD = 3630 generations), compared to 1007 generations (SD = 116 generations) if determined by the dominant mutation. The striking difference in the rate at which beneficial dominant versus recessive alleles contribute to adaptation is maintained even up to the point at which the selected phenotype reaches a frequency of 99%, which takes approximately 9262 generations

(SD = 3624 generations) for a recessive mutation and only 2030 generations for dominant mutation (SD = 141 generations).

Discussion

A growing number of studies have found evidence for convergent adaptation within and between species (Hoekstra and Nachman 2003; Steiner et al. 2008; Rosenblum et al. 2010; Dobler et al. 2012; Marques et al. 2017; Giska et al. 2019; Nelson et al. 2019; Harris et al. 2020), although we often lack an understanding of the forces that determine whether local adaptation occurs through independent de novo mutations or migration of pre-existing alleles from other populations (Ralph and Coop 2015). Our study provides rare empirical insights into how gene flow, mutation, allelic dominance, and selection interact to shape the spatial scale and pace of local adaptation to new or changing environments.

Understanding the potential for adaptive variation to spread between populations across large spatial scales requires basic insights into range-wide patterns of population structure and gene flow. Winter-brown camouflage in snowshoe hares occurs across
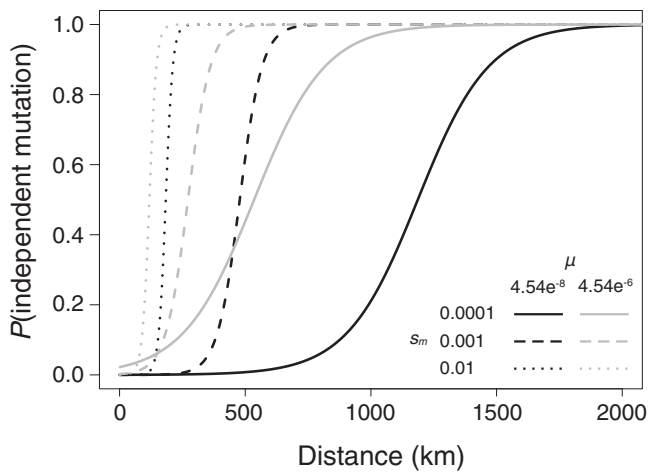
Figure 4. The probability of adaptation through independent mutations in snowshoe hares as a function of distance in kilometers from a focal habitat patch harboring a locally adaptive variant. The probability of independent mutation is calculated according to equation (12) in Ralph and Coop (2015). Here, we varied the mutation rate to the winter-brown phenotype (μ; black $= 4.54 \times 10^{-8}$, gray $= 4.54 \times 10^{-6}$) and the negative selection coefficient in intervening habitats (s_m; solid line $= 0.0001$, dashed line $= 0.001$, dotted line $= 0.01$).

only ∼5% of the total range but is broadly distributed from the western edge of the range along the Pacific coast to the eastern extent of the range in Pennsylvania (Nagorsen 1983; Gigliotti et al. 2017; Mills et al. 2018). Previous phylogeographic studies based on limited genetic data suggest that this distribution spans a deep phylogenetic split between Boreal and PNW lineages (∼2 million years divergence; Cheng et al. 2014; Melo-Ferreira et al. 2014) with little evidence for gene flow. Our phylogenetic analysis based on whole exomes ($n = 95$) also supports a deep split but suggests a substantially more recent divergence time between Boreal and Rockies/PNW lineages than previously estimated (∼97 kya; Fig. 1B). Our more recent estimates may be attributable to the increased power of whole exome data or the heavy reliance on mitochondrial DNA in previous studies (Melo-Ferreira et al. 2014), which can lead to overestimation of divergence times (Zheng et al. 2011). Consistent with previous studies, our ADMIXTURE analyses and range-wide effective migration surfaces suggest low gene flow between the PNW and Boreal lineages (Figs. 1C and 2A). The absence of observable gene flow is perhaps striking given their close proximity to each other in western North America. Melo-Ferreira et al. (2014) hypothesized that these lineages may exhibit incipient reproductive isolation. The evolution of reproductive isolation between these populations is possible, but, given our more recent divergence time estimates, we suggest that reduced gene flow may reflect quite recent secondary contact between these populations in western North

America. Genetic and fossil evidence suggests the common ancestors of PNW and Boreal populations occupied separate refugia in southern and eastern North America, respectively, during the last glacial maximum (Cheng et al. 2014). The east-to-west phylogenetic clustering (Fig. 1B) and genetic diversity gradient (Fig. 2B) of the Boreal population are consistent with a recent range expansion from this eastern refugia (Cheng et al. 2014), which implies that the PNW and Boreal hares have only recently experienced secondary contact. However, more detailed population history modeling and sampling of the putative contact zone is required to test these hypotheses.

Consistent with low historical gene flow, the AK winter-brown hare lacks the PNW winter-brown *Agouti* haplotype (Fig. 3), indicating a role for independent mutation leading to the convergent evolution of brown winter coats. This likely reflects constraints on the migration of adaptive variation between populations. Even in the absence of intrinsic or extrinsic reproductive isolation, the rate at which a positively selected variant spreads across a uniform environment is constrained by dispersal distance and the strength of positive selection (Fisher 1937). Across spatially or temporally heterogenous landscapes, the spread of an adaptive allele between patches is further constrained by the strength or frequency of purifying selection in maladaptive habitats or climatic periods (Ralph and Coop 2015). Given that previous models suggest a low probability of winter-brown camouflage along the majority of the Pacific coast (Mills et al. 2018), we suspect that the winter-brown PNW variant would have to traverse snowy habitats where it is strongly disfavored to reach AK, ∼3000 km away via the coast. At this distance, the probability of adaptation through gene flow is virtually zero under a range of model assumptions (Fig. 4). In fact, dispersal limitations and patchy range-edge habitats should generally favor independent evolution of winter-brown camouflage at scales beyond 100–1000 km (depending on the assumed values of μ and s_m; Fig. 4). A limitation of our study is the use of a single individual to infer convergent evolution of winter-brown camouflage. Although our data are sufficient to verify the role of independent mutations, additional research based on more sampling is needed to dissect the genetic basis of winter-brown camouflage in the northwestern and eastern edges of the range (e.g., AK and PA). Subtle phenotypic similarities between observed winter-brown hares in AK and PA could imply a shared genetic basis (e.g., white feet vs. brown feet in the PNW, Fig. 3, unpubl. data). However, our theoretical modeling would suggest that they likely reflect independent mutations as well (i.e., ∼5900 km between AK and PA sampling sites).

Convergent evolution is thought to be more common for "loss-of-function" traits because they may have a larger mutational target size relative to "gain-of-function" traits (Rosenblum et al. 2010; Manceau et al. 2011). Convergent color adaptation
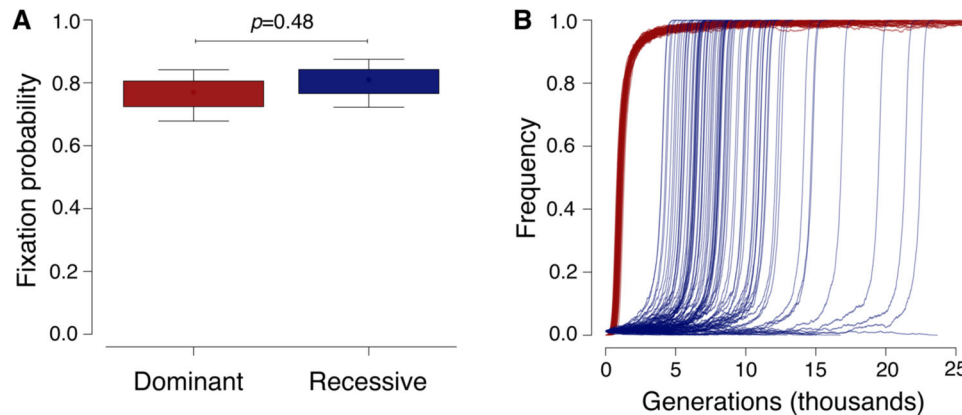
Figure 5. (A) The simulated probability of fixation of a completely dominant (red, mean = 77%, *N* = 100) or recessive (blue, mean = 81%, *N* = 100) mutation experiencing positive selection and starting in migration-selection balance frequency (0.015% for dominant, 1.24% for recessive). (B) The simulated allele frequency trajectories of the same dominant (red) and recessive (blue) mutations starting in migration-selection balance.

involving loss-of-function mutations has been shown between different species of lizard (Rosenblum et al. 2010; Laurent et al. 2016) and cavefish (Protas et al. 2006) and the repeated evolution of melanism across populations of deer mice involves convergent loss-of-function *Agouti* mutations (Kingsley et al. 2009). In PNW hares, adaptive introgression of *Agouti* variation from black-tailed jackrabbits appears to have caused a reversion to the ancestral winter-brown condition in *Lepus* (i.e., the likely ancestral state before the common ancestor of winter-white *Lepus* species). As the ancestral winter-brown variant is recessive, this implies that derived winter-white camouflage in snowshoe hares evolved through dominant gain-of-function mutations, consistent with the seasonal upregulation of *Agouti* during the development of white coats (Ferreira et al. 2017; Jones et al. 2018). Independent origins of winter-brown camouflage across the snowshoe hare range could similarly involve relatively simple loss-of-function mutations that break the molecular pathways contributing to the development of white winter coats. Indeed, the evolution of darkened winter coats in some populations of mountain hares (*Lepus timidus*) appears to have also involved introgression of a recessive, loss-of-function *Agouti* variant (Giska et al. 2019). Collectively, our results suggest that geographic distance and mutational target size, in addition to population structure and history, should play a crucial role in generating hypotheses about the relative roles of independent mutation and gene flow in adaptation.

Despite the evidence for independent winter-brown mutations at broad spatial scales, we also find that the PNW *Agouti* allele has likely traversed a substantial distance (∼600 km) to western Montana—a predominately winter-white locality—where it occurs at an estimated frequency of 1.24%. However, we cannot rule out the possibility that the winter-brown allele was introduced into Montana independently via hybridization with black-tailed jackrabbits. In either case, both theory and simulations

show that a shift in environmental conditions toward favoring the winter-brown allele in Montana results in a ∼80% probability of fixation when starting at the observed frequency (Fig. 5). The high probability of adaptation through gene flow ∼600 km from a focal patch is consistent with a scenario of very weak purifying selection ($s_m = 0.0001$) against the winter-brown allele in snowy environments under the Ralph and Coop (2015) model (P (independent mutation) = ∼1.5% ($\mu = 4.54 \times 10^{-8}$) or ∼61% ($\mu = 4.54 \times 10^{-6}$); Fig. 4). Although winter-brown camouflage is likely more strongly deleterious in winter-white environments given the known fitness consequences of mismatch (Zimova et al. 2016), purifying selection against the winter-brown *Agouti* allele in winter-white environments may be weak or absent given that it is recessive and thus hidden to selection at low frequency. In agreement with Ralph and Coop (2015), we therefore suggest that adaptation between distant populations via gene flow may be relatively more common through recessive variation compared to dominant variation when purifying selection is strong in intervening habitats.

Climate change is expected to reduce winter snow cover across the snowshoe hare range (Mills et al. 2013), which could potentially result in winter-brown camouflage being favored in predominately winter-white populations (Mills et al. 2018). Under this scenario, positive selection could operate on convergent de novo winter-brown mutations or on standing genetic variation for winter-brown camouflage to facilitate rapid adaptation. Using simulations of positive selection, we show that although dominant and recessive mutations in mutation/migration-selection balance share a similar fixation probability (Fig. 5A), they are likely to experience vastly different frequency change dynamics (Fig. 5B; Teshima and Przeworski 2006) and thus lead to very different rates of adaptation following environmental change. For instance, we show that in MT the initial rate of adaptation

from segregating winter-brown *Agouti* variation is likely to be slow relative to an equivalently beneficial dominant mutation. Although recessive mutations tended to fix more quickly than dominant mutations, they also took substantially longer to reach high frequencies in populations (e.g., 8837 vs. 1007 generations for a phenotype determined by recessive vs. dominant mutation to reach a population frequency of 75%, respectively; and see Fig. 5B). This pattern can be explained by the different allele frequency phases most strongly affected by genetic drift. For instance, positive selection can readily act on beneficial dominant mutations at low frequency, but at high frequency the recessive wild-type allele is hidden in heterozygotes and genetic drift governs (and generally slows) fixation dynamics. The opposite is true for beneficial recessive mutations, which are governed by genetic drift at low frequency but driven to fixation by selection. Overall, selection on low-frequency recessive variation may be a relatively ineffective way for populations to adapt rapidly to changing environments. In fact, the temporal lag for the spread of beneficial recessive variation may be sufficient enough to allow time for dominant independent mutations (e.g., gain-of-function MC1R mutations that result in melanism; Nachman et al. 2003) to appear and spread before the recessive allele increases appreciably. These findings highlight the important roles of genetic dominance in shaping both the geographic scope and rate of convergent adaptation to changing environments and underscores the need for further study.

Collectively, our study provides important insights into long-standing evolutionary questions, as well as the potential for adaptation to climate change in snowshoe hares and other species. A key to understanding the potential of adaptive variation to spread under climate change is revealing how it has spread in the past. We show that adaptive responses to reduced snow cover in snowshoe hares have involved both the local spread of winter-brown camouflage in the PNW as well as convergent evolution elsewhere in the range. In snowshoe hares and other seasonally changing species, regions of winter-camouflage polymorphism may be crucial areas to maintain population connectivity as conduits for the spread of winter-brown camouflage across broader portions of the range (Mills et al. 2018). However, facilitating gene flow alone may not be sufficient to facilitate rapid adaptive responses, given the broad range of snowshoe hares and the genetic architecture of winter-brown camouflage variation. Rather, we suspect that rapid climate change responses in winter-white populations far from winter-brown habitat will also likely involve independent origins of winter-brown camouflage or selection on variation in coat color phenology (i.e., shifts in the timing of winter-white molts). Although the genetic basis of variation in camouflage phenology remains unresolved in this system, it is likely quantitative and perhaps more responsive to selection. Regardless, this study represents an important step toward making

predictions about evolutionary responses in snowshoe hares and other species experiencing parallel changes in climate across their range.

DATA ARCHIVING
Previously generated and original whole exome and genome sequence data are available in the Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) under BioProject PRJNA420081 (original samples: SAMN13999397-13999411). Various scripts and data sets are deposited on FigShare. A SLiM script for simulating sojourns of beneficial dominant and recessive mutation is available at https://doi.org/10.6084/m9.figshare.12030759.v1. A Python script to calculate the probability of convergent adaptation is available at https://doi.org/10.6084/m9.figshare.12030756.v1. Bed files for both targeted and probed exome regions can be found at https://doi.org/10.6084/m9.figshare.12030774.v1 and https://doi.org/10.6084/m9.figshare.12030777.v1, respectively. Files containing genotypes and coordinates for SNPs used for the Admixture analysis can be found at https://doi.org/10.6084/m9.figshare.12030663.v1 and https://doi.org/10.6084/m9.figshare.12030711.v1, respectively.

LITERATURE CITED
Alexander, D. H., J. Novembre, and K. Lange. 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Res. 19:1655–1664.

Arendt, J., and D. Reznick. 2008. Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? Trends Ecol. Evol. 23:26–32.

Barrett, R. D. H., S. Laurent, R. Mallarino, S. P. Pfeifer, C. C. Y. Xu, M. Foll, K. Wakamatsu, J. S. Duke-Cohan, J. D. Jensen, and H. E. Hoekstra. 2019. Linking a mutation to survival in wild mice. Science 363:499–504.

Bay, R. A., N. Rose, R. Barrett, L. Bernatchez, C. K. Ghalambor, J. R. Lasky, R. B. Brem, S. R. Palumbi, and P. Ralph. 2017. Predicting responses to contemporary environmental change using evolutionary response architectures. Am. Nat. 189:463–473.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C.-H. Wu, D. Xie, M. A. Suchard, A. Rambaut, and A. J. Drummond. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10:e1003537.

Bridle, J. R., and T. H. Vines. 2007. Limits to evolution at range margins: when and why does adaptation fail? Trends Ecol. Evol. 22:140–147.

Brown, R. D., and P. W. Mote. 2009. The response of northern hemisphere snow cover to a changing climate. J. Clim. 22:2124–2145.

Carneiro, M., F. W. Albert, J. Melo-Ferreira, N. Galtier, P. Gayral, J. A. Blanco-Aguiar, R. Villafuerte, M. W. Nachman, and N. Ferrand. 2012. Evidence for widespread positive and purifying selection across the European rabbit (*Oryctolagus cuniculus*) genome. Mol. Biol. Evol. 29:1837–1849.

Cheng, E., K. E. Hodges, J. Melo-Ferreira, P. C. Alves, and L. S. Mills. 2014. Conservation implications of the evolutionary history and genetic diversity hotspots of the snowshoe hare. Mol. Ecol. 23:2929–2942.

Danecek, P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, et al. 2011. The variant call format and VCFtools. Bioinformatics 27:2156–2158.

Dobler, S., S. Dalla, V. Wagschal, and A. A. Agrawal. 2012. Community-wide convergent evolution in insect adaptation to toxic cardenolides by substitutions in the Na,K-ATPase. Proc. Natl. Acad. Sci. USA 109:13040–13045.

Ferreira, M. S., P. C. Alves, C. M. Callahan, J. P. Marques, L. S. Mills, J. M. Good, and J. Melo-Ferreira. 2017. The transcriptional landscape of seasonal coat colour moult in the snowshoe hare. Mol. Ecol. 26:4173–4185.

Fisher, R. A. 1937. The wave of advance of advantageous genes. Ann. Eugen. 7:355–369.

Gigliotti, L. C., D. R. Diefenbach, and M. J. Sheriff. 2017. Geographic variation in winter adaptations of snowshoe hares (*Lepus americanus*). Can. J. Zool. 95:539–545.

Gillis, E. A., and C. J. Krebs. 1999. Natal dispersal of snowshoe hares during a cyclic population increase. J. Mammal. 80:933–939.

Giska, I., L. Farelo, J. Pimenta, F. A. Seixas, M. S. Ferreira, J. P. Marques, I. Miranda, J. Letty, H. Jenny, K. Hackländer, et al. 2019. Introgression drives repeated evolution of winter coat color polymorphism in hares. Proc. Natl. Acad. Sci. USA 116:24150–24156.

Haldane, J. B. S. 1924. A mathematical theory of natural and artificial selection (Part I). Trans. Cambridge Philos. Soc. 23:19–41.

———. 1932. The causes of evolution. Longmans, Green and Co., Lond.

Haller, B. C., and P. W. Messer. 2019. SLiM 3: forward genetic simulations beyond the Wright-Fisher model. Mol. Biol. Evol. 36:632–637.

Harris, R. B., K. Irwin, M. R. Jones, S. Laurent, R. D. H. Barrett, R. D. H. Nachman, J. M. Good, C. R. Linnen, J. D. Jensen, and S. P. Pfeifer. 2020. The population genetics of crypsis in vertebrates: recent insights from mice, hares, and lizards. Heredity 124:1–14.

Hoekstra, H. E., and M. W. Nachman. 2003. Different genes underlie adaptive melanism in different populations of rock pocket mice. Mol. Ecol. 12:1185–1194.

Hoekstra, H. E., K. E. Drumm, and M. W. Nachman. 2004. Ecological genetics of adaptive color polymorphism in pocket mice: geographic variation in selected and neutral genes. Evolution 58:1329–1341.

Hoekstra, H. E., J. G. Krenz, and M. W. Nachman. 2005. Local adaptation in the rock pocket mouse (*Chaetodipus intermedius*): natural selection and phylogenetic history of populations. Heredity 94:217–228.

Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. Nature 470:479–485.

Jones, M. R., L. S. Mills, P. C. Alves, C. M. Callahan, J. M. Alves, D. J. R. Lafferty, F. M. Jiggins, J. D. Jensen, J. Melo-Ferreira, and J. M. Good. 2018. Adaptive introgression underlies polymorphic seasonal camouflage in snowshoe hares. Science 360:1355–1358.

Jones, M. R., L. S. Mills, J. D. Jensen, and J. M. Good. 2020. The origin and spread of locally adaptive seasonal camouflage in snowshoe hares. Am. Nat. In press. https://doi.org/10.1086/710022

Kimura, M. 1957. Some problems of stochastic processes in genetics. Ann. Math. Stat. 28:882–901.

Kingsley, E. P., M. Manceau, C. D. Wiley, and H. E. Hoekstra. 2009. Melanism in *Peromyscus* is caused by independent mutations in *Agouti*. PLoS ONE 4:e6435.

Knowles, N., M. D. Dettinger, D. R. Cayan, N. Knowles, M. D. Dettinger, and D. R. Cayan. 2006. Trends in snowfall versus rainfall in the western United States. J. Clim. 19:4545–4559.

Kofler, R., P. Orozco-terWengel, N. De Maio, R. V. Pandey, V. Nolte, A. Futschik, C. Kosiol, and C. Schlötterer. 2011. PoPoolation: a toolbox for population genetic analysis of next generation sequencing data from pooled individuals. PLoS ONE 6:e15925.

Kreiner, J. M., D. A. Giacomini, F. Bemm, B. Waithaka, J. Regalado, C. Lanz, J. Hildebrandt, P. H. Sikkema, P. J. Tranel, D. Weigel, et al. 2019. Multiple modes of convergent adaptation in the spread of glyphosate-resistant *Amaranthus tuberculatus*. Proc. Natl. Acad. Sci. 116:21076–21084.

Laurent, S., S. P. Pfeifer, M. L. Settles, S. S. Hunter, K. M. Hardwick, L. Ormond, V. C. Sousa, J. D. Jensen, and E. B. Rosenblum. 2016. The population genomics of rapid adaptation: disentangling signatures of selection and demography in white sands lizards. Mol. Ecol. 25:306–323.

Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 1303.3997v2.

Magoč, T., and S. L. Salzberg. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963.

Manceau, M., V. S. Domingues, R. Mallarino, and H. E. Hoekstra. 2011. The developmental role of Agouti in color pattern evolution. Science 331:1062–1065.

Marques, D. A., J. S. Taylor, F. C. Jones, F. Di Palma, D. M. Kingsley, and T. E. Reimchen. 2017. Convergent evolution of SWS2 opsin facilitates adaptive radiation of threespine stickleback into different light environments. PLoS Biol. 15:e2001627.

Matthee, C. A., B. J. Van Vuuren, D. Bell, and T. J. Robinson. 2004. A molecular supermatrix of the rabbits and hares (Leporidae) allows for the identification of five intercontinental exchanges during the Miocene. Syst. Biol. 53:433–447.

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, D. Altshuler, S. Gabriel, M. Daly, et al. 2010. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20:1297–1303.

Melo-Ferreira, J., F. A. Seixas, E. Cheng, L. S. Mills, and P. C. Alves. 2014. The hidden history of the snowshoe hare, *Lepus americanus*: extensive mitochondrial DNA introgression inferred from multilocus genetic variation. Mol. Ecol. 23:4617–4630.

Milholland, B., X. Dong, L. Zhang, X. Hao, Y. Suh, and J. Vijg. 2017. Differences between germline and somatic mutation rates in humans and mice. Nat. Commun. 8:15183.

Mills, L. S., M. Zimova, J. Oyler, S. Running, J. T. Abatzoglou, and P. M. Lukacs. 2013. Camouflage mismatch in seasonal coat color due to decreased snow duration. Proc. Natl. Acad. Sci. USA 110:7360–7365.

Mills, L. S., E. V. Bragina, A. V. Kumar, M. Zimova, D. J. R. Lafferty, J. Feltner, B. M. Davis, K. Hackländer, P. C. Alves, J. M. Good, et al. 2018. Winter color polymorphisms identify global hot spots for evolutionary rescue from climate change. Science 359:1033–1036.

Nachman, M. W., H. E. Hoekstra, and S. L. D'Agostino. 2003. The genetic basis of adaptive melanism in pocket mice. Proc. Natl. Acad. Sci. USA 100:5268–5273.

Nagorsen, D. W. 1983. Winter pelage colour in snowshoe hares (*Lepus americanus*) from the Pacific Northwest. Can. J. Zool. 61:2313–2318.

Nelson, T. C., M. R. Jones, J. P. Velotta, A. S. Dhawanjewar, and R. M. Schweizer. 2019. UNVEILing connections between genotype, phenotype, and fitness in natural populations. Mol. Ecol. 28:1866–1876.

Orr, H. A., and A. J. Betancourt. 2001. Haldane's sieve and adaptation from the standing genetic variation. Genetics 157:875–884.

Petkova, D., J. Novembre, and M. Stephens. 2016. Visualizing spatial population structure with estimated effective migration surfaces. Nat. Genet. 48:94–100.

Pfeifer, S. P., S. Laurent, V. C. Sousa, C. R. Linnen, M. Foll, L. Excoffier, H. E. Hoekstra, and J. D. Jensen. 2018. The evolutionary history of Nebraska deer mice: local adaptation in the face of strong gene flow. Mol. Biol. Evol. 35:792–806.

Protas, M. E., C. Hersey, D. Kochanek, Y. Zhou, H. Wilkens, W. R. Jeffery, L. I. Zon, R. Borowsky, and C. J. Tabin. 2006. Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. Nat. Genet. 38:107–111.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ralph, P. L., and G. Coop. 2010. Parallel adaptation: one or many waves of advance of an advantageous allele? Genetics 186:647–668.

———. 2015. Convergent evolution during local adaptation to patchy landscapes. PLoS Genet. 11:e1005630.

Rohland, N., and D. Reich. 2012. Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. Genome Res. 22:939–946.

Rosenblum, E. B., H. Römpler, T. Schöneberg, and H. E. Hoekstra. 2010. Molecular and functional basis of phenotypic convergence in white lizards at White Sands. Proc. Natl. Acad. Sci. USA 107:2113–2117.

Schlager, G., and M. M. Dickie. 1971. Natural mutation rates in the house mouse. Estimates for five specific loci and dominant mutations. Mutat. Res. 11:89–96.

Slatkin, M. 1973. Gene flow and selection in a cline. Genetics 75:733–756.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313.

Steiner, C. C., H. Rompler, L. M. Boettger, T. Schoneberg, and H. E. Hoekstra. 2008. The genetic basis of phenotypic convergence in beach mice: similar pigment patterns but different genes. Mol. Biol. Evol. 26:35–45.

Teshima, K. M., and M. Przeworski. 2006. Directional positive selection on an allele of arbitrary dominance. Genetics 172:713–718.

Turner, J. R. G. 1981. Adaptation and evolution in *Heliconius*: a defense of NeoDarwinism. Annu. Rev. Ecol. Syst. 12:99–121.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97–159.

Zhang, C., M. Rabiee, E. Sayyari, and S. Mirarab. 2018. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. BMC Bioinform. 19:153.

Zheng, Y., R. Peng, M. Kuro-o, and X. Zeng. 2011. Exploring patterns and extent of bias in estimating divergence time from mitochondrial DNA sequence data in a particular lineage: a case study of salamanders (Order Caudata). Mol. Biol. Evol. 28:2521–2535.

Zimova, M., L. S. Mills, and J. J. Nowak. 2016. High fitness costs of climate change-induced camouflage mismatch. Ecol. Lett. 19:299–307.

Zimova, M., K. Hackländer, J. M. Good, J. Melo-Ferreira, P. C. Alves, and L. S. Mills. 2018. Function and underlying mechanisms of seasonal colour moulting in mammals and birds: what keeps them changing in a warming world? Biol. Rev. 93:1478–1498.

Associate Editor: W. Haerty
Handling Editor: D. W. Hall

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Sample list and metadata.