Comparative genomics reveals putative evidence for high-elevation adaptation in the American pika (Ochotona princeps)

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Abstract

High-elevation environments have lower atmospheric oxygen content, reduced temperatures, and higher levels of UV radiation than found at lower elevations. As such, species living at high elevations must overcome these challenges to survive, grow, and reproduce. American pikas (Ochotona princeps) are alpine lagomorphs that are habitat specialists typically found at elevations >2,000 m. Previous research has shown putative evidence for high-elevation adaptation; however, investigations to date have been limited to a fraction of the genome. Here, we took a comparative genomics approach to identify putative regions under selection using a chromosomal reference genome assembly for the American pika relative to 8 other mammalian species targeted based on phylogenetic relatedness and (dis)similarity in ecology. We first identified orthologous gene groups across species and then extracted groups containing only American pika genes as well as unclustered pika genes to inform functional enrichment analyses; among these, we found 141 enriched terms with many related to hypoxia, metabolism, mitochondrial function/development, and DNA repair. We identified 15 significantly expanded gene families within the American pika across all orthologous gene groups that displayed functionally enriched terms associated with hypoxia adaptation. We further detected 196 positively selected genes, 41 of which have been associated with putative adaptation to hypoxia, cold tolerance, and response to UV following a literature review. In particular, OXNAD1, NRDC, and those genes critical in DNA repair represent important targets for future research to examine their functional implications in the American pika, especially as they may relate to adaptation to rapidly changing environments.

Keywords: comparative genomics; local adaptation; cold tolerance; UV radiation; hypoxia; Lagomorpha

Introduction

The environment in which a species resides can have a profound impact on its evolution (Parsons 2005; Kristensen et al. 2020). High-elevation environments offer a unique combination of challenges that can influence natural selection; these include lower atmospheric oxygen (i.e., hypoxia), reduced ambient temperatures, and increased exposure to DNA-damaging UV radiation relative to lower elevations (Sun et al. 2018). The adaptations to these abiotic factors have been shown in a multitude of species. For example, Qiu et al. (2012) compared the domestic yak (Bos grunniens) genome to that of low-altitude cattle (Bos taurus) and found evidence for functional enrichment in energy metabolism and domains related to hypoxic stress. A comparative genomic investigation coupled with functional assays of Tibetan hot-spring snakes (Thermophis spp.) identified specific amino acid substitutions for proteins involved in DNA damage repair and response to hypoxia among the high-elevation species (Li et al. 2018). Likewise, a transcriptomic analysis of lizards (Phrynocephalus erythrurus) on the Qinghai-Tibetan Plateau (QTP) revealed putative adaptations related to hypoxia, energy metabolism, and responses to UV damage (Yang et al. 2015).

Genome-enabled research into high-elevation systems continues to expand our knowledge of adaptation to extreme environments. The American pika (Ochotona princeps) is an alpine lagomorph distributed across mountain ranges in the Pacific Northwest (Smith and Weston 1990; Hafner and Smith 2010). They are considered habitat specialists and typically reside in rocky, talus slopes at elevations >2,000 m (Smith and Weston 1990; Millar and Westfall 2010; Smith and Beever 2016). Previous research has provided some evidence for American pika high-elevation adaptation on local/regional scales based on reduced representation genome sequencing (Waterhouse et al. 2018; Schmidt et al. 2021) and transcriptomics (Lemay et al. 2013); however, a comprehensive examination across the American pika genome has not been explored. Furthermore, previous work relied upon older, lower-quality reference assemblies for the American pika; for example, Wang et al. (2020) used OchPri2.0-Ens (Ensembl), which is highly fragmented (193,096 scaffolds; scaffold N50 = 53,58 kb) and poorly annotated (16,006 genes) relative to modern assemblies (Giani et al. 2020, Whibley et al. 2021). The most recent reference genome for the American pika (OchPri4.0; NCBI RefSeq
Accession: GCF_014633375.1) is significantly improved in both contiguity (9,950 scaffolds; scaffold N50 = 75.8 Mb) and annotation quality (21,186 genes), and is almost entirely (97% of total length) assembled into chromosomes (Sjodin et al. 2021). Importantly, the American pika has emerged as a sentinel species for the ecological impacts of climate change in alpine regions due to their acute environmental sensitivity (Beever et al. 2003; Galbreath et al. 2009; Beever et al. 2010, 2011; Wilkening et al. 2011; Stewart et al. 2015; Schwalm et al. 2016; Wilkening and Ray 2016); a thorough examination of climate adaptation within the American pika genome may provide important insights into potential biotic responses to changing environments while representing a valuable resource for guiding future studies.

Here, we investigated signatures of putative adaptation to high-elevation environments in the American pika. Leveraging the newest version of the *O. princeps* genome (Sjodin et al. 2021), we compared orthologous genes among the American pika and 8 other mammalian species to estimate phylogenetic relationships, examine functional enrichment in the American pika genome, and detect signatures of positive selection. We then identified putative genomic adaptations to high-elevation environments based on characterized gene functions and a targeted literature review.

**Methods and materials**

**Study design**

We leveraged the recently published and annotated American pika genome (Sjodin et al. 2021) to identify putative genomic adaptations to high-elevation environments by comparing and contrasting with 8 other paired mammalian species spanning several major taxa across Clires (i.e. lagomorphs and rodents). Each species pair included a habitat generalist and an alpine habitat specialist to also cover a broad ecological range. The habitat specialists were the American pika, long-tailed chinchilla (*Chinchilla lanigera*), alpine marmot (*Marmota marmota marmota*), and arctic ground squirrel (*Urocitellus parryii*), whereas the paired generalists were European rabbit (*Oryctolagus cuniculus*), common degu (*Octodon degus*), yellow-bellied marmot (*Marmota flaviventris*), and 13-lined ground squirrel (*Citellus tridecemlineatus*), respectively. Humans (*Homo sapiens*) were included as an outgroup. To minimize differences in annotation quality across genomes, we used only those available through the NCBI RefSeq database and annotated using the NCBI Eukaryotic Genome Annotation Pipeline (O’Leary et al. 2016). Coding domain sequences (CDSs) and protein FASTA files were downloaded for each species from NCBI and used for downstream analyses (see Supplementary Table 1 for accession information).

**Detection of orthologous gene families and functional enrichment**

American pika genes were aligned and functionally annotated against the nonredundant NCBI and SwissProt (Boeckmann et al. 2003) protein databases using BLASTP v2.9.0 (Camacho et al. 2009) with an E-value cut-off of 1e−5. Genes with BLAST hits were then annotated with gene ontology (GO) terms using Blast2GO v5.2.5 (Götz et al. 2008). In addition, American pika genes were grouped into protein families and annotated with GO terms using InterProScan v5.50-84.0 (Cock et al. 2013; Jones et al. 2014) under default settings and merged with the BLAST GO terms in Blast2GO v5.2.5 (Götz et al. 2008).

We then identified orthologous gene families between the American pika and the other 8 mammalian species. To reduce redundancy within the dataset, we retained only the longest isoform for each protein within a species; in addition, we removed proteins with fewer than 50 amino acids to minimize false positives during ortholog detection and clustering. Orthologous protein sequences both within and among species were identified first using reciprocal best BLAST hits then clustered into gene families using a Markov Clustering algorithm as implemented in a modified version of OrthoMCL v2.0.9 (Fischer et al. 2011; www.github.com/apetkau/orthomcl-software-custom). Ortholog detection was automated with the orthomcl-pipeline using default settings (www.github.com/apetkau/orthomcl-pipeline).

We extracted gene families containing only American pika genes and grouped these with unclustered American pika genes to construct a “pika-specific” gene set. We found functionally enriched GO terms among pika-specific genes by means of a hypergeometric test using BiNGO v3.0.4 (Maere et al. 2005) as implemented in Cytoscape v3.8.2 (Smoot et al. 2011) employing the entire American pika gene set as the reference. Obtained F-values were corrected for multiple testing using the Benjamini-Hochberg false discovery rate, and significantly enriched GO terms were identified with an adjusted F ≤ 0.05. GO terms with similar functions were grouped together based on quantified information content and semantic similarities as calculated in the software GO-Figure! v1.0.1 (Reijnders and Waterhouse 2021).

**Phylogenetic reconstruction and estimation of divergence times**

To generate an ultrametric tree for downstream analysis, single-copy orthologs were extracted from the orthologous gene families, defined as those families with a single representative gene from each species. We removed gene families with any sequences shorter than 200 amino acids to minimize spurious alignments and conducted a multiple sequence alignment using MUSCLE v3.8.31 (Edgar 2004a, 2004b) and default parameters. The corresponding CDS alignments were back-translated using PAL2NAL v14 (Suyama et al. 2006). Some CDSs did not contain chromosomal locations as they were predicted based on transcriptomic or other sequencing data in the RefSeq database; gene families containing these unlocalized genes were removed from downstream analysis. We identified homologous gene blocks for the remaining orthologous groups and concatenated them into “supergenes” using Gblocks v0.91b (Talavera and Castresana 2007). We then identified and extracted 4-fold degenerate nucleotide sites (4DTV) using MEGA v10.2.5 (Tamura et al. 2021) and used these sites to reconstruct the phylogeny under the GTR+I+Γ model as implemented in MrBayes v3.2.6 (Ronquist et al. 2012). We ran 5 independent runs of the Markov chain Monte-Carlo (MCMC) process for 5 million generations with a 1 million generation burn-in each, sampling trees every 100 generations. Convergence was assessed by examining the estimated sample size (ESS) and potential scale reduction factor (PSRF) for each parameter estimate. Convergence was achieved when ESS > 100 and PSRF ≈ 1 for all parameters (see Supplementary Table 2 for summary values).

To estimate divergence times, we first separated the concatenated super genes into 3 datasets corresponding to the first, second, and third codon sites. Divergence times were then estimated under a relaxed clock model using MCMCCTREE as implemented in PAML v4.9 (Yang 2007). The tree topology was defined using the outputs from the above analysis. The mean substitution rate was estimated using BaseML in PAML v4.9 (Yang 2007). The overall substitution rate prior (r gene gamma) was set to [1, 8] and the rate drift parameter prior (sigma2 gamma) was
set to [1, 10, 1] following author recommendations (Yang 2007). We used 3 calibration points based on previous studies: the split between Primates and Glires constrained at 61.7–100.5 million years ago (mya; Benton and Donoghue 2007); the split between Rodentia and Lagomorpha constrained at 71.5–94.1 mya (Meredith et al. 2011); and the split between Leporidae and Ochotonidae constrained at 47.4–56.9 mya (Meredith et al. 2011). We ran the program using 6 million MCMC reps and a burn-in of 2 million iterations. Divergence estimates from 2 independent runs were compared to assess convergence. The topology and divergence estimates were compared with established values in the literature to ensure the appropriateness of using this dataset for downstream analyses.

Identification of expanded and contracted gene families
We identified expanded and contracted gene families along each branch and node from the above phylogeny using CAFE v2.0 (Hahn et al. 2005, 2007; De Bie et al. 2006). We compared the cluster size of each branch with the maximum likelihood cluster size of the ancestral node leading to that branch and identified expanded and contracted gene families as those with smaller or larger ancestral nodes, respectively. We calculated the family-wide P-values using a Monte Carlo resampling procedure of each branch and node and calculated the exact P-values for each significant family with P ≤ 0.01 using the Viterbi method in CAFE v2.0 (Hahn et al. 2005, 2007; De Bie et al. 2006). Significant gene family expansions/contractions were defined as those with family P-values and exact P-values ≤ 0.01. We extracted genes from significantly expanded American pika gene families and identified functionally enriched GO terms using the methods described above.

Identification of positively selected genes and putative high-elevation adaptation
We identified positively selected genes (PSGs) in the American pika from the previously identified single-copy orthologs following alignment refinement using Gblocks v0.91b (Talavera and Castresana 2007) and a branch-site model using CodeML, as implemented in PAML v4.9 (Yang 2007); these steps were automated using a custom shell script “p-codeml” (https://github.com/bsjodin/p-codeml). Refined gene alignments with length <150 bp were removed from downstream analysis to minimize spurious results. The American pika was set as the foreground branch, and all other species were set as background branches. We performed a likelihood ratio test, and resultant P-values were corrected for multiple testing using an FDR test with a Bonferroni correction. Significant PSGs were defined as those with an adjusted P ≤ 0.01 and contained at least one positively selected site with a posterior probability ≥0.99 based on Bayes Empirical Bayes (BEB) analysis. We identified functionally enriched GO terms among PSGs using the methods described above. Functional descriptions for all PSGs were automatically generated using the Alliance of Genome Resources website (Kishore et al. 2020). To identify PSGs with putative links to high-elevation adaptation, we manually searched each gene on Google Scholar using the following Boolean search term: "gene name" “cold stress” OR “cold response” OR “cold resistance” OR “hypoxia” OR “high altitude” OR “UV damage” OR “climate.” Searches were constrained to the first 10 hits, and putatively high-elevation adaptive genes were identified as those with reference support for adaptation to hypoxia, cold temperatures, and/or UV exposure in any system or organism.

Results
Functional enrichment of pika-specific genes
We retained a mean of 20,098 genes from each species after filtering for downstream analysis, including 18,854 genes from the American pika (see Supplementary Table 3). We found 17,127 orthologous gene families across all species; 25 gene families contained only American pika genes (n = 55 genes), and these were grouped with 881 unclustered American pika genes to construct our pika-specific dataset (total n = 936 genes; see Supplementary Table 3). Of these, 857 genes could be annotated with GO terms. We found 141 functionally enriched GO terms (see Supplementary Table 4), which were grouped into 53 parent terms (Fig. 1). Of these parent terms, we identified 10 with putative links to high-elevation adaptation, including: 2 groups with 4 total terms associated with metabolism [cellular metabolic process (GO:0044237) and fatty acid biosynthetic process (GO:0006633)]; 4 groups containing 8 terms enriched in mitochondrial function/structure [mitochondrial envelope (GO:0005740), mitochondrial respiratory complex (GO:0005746), mitochondrial membrane (GO:0031966), and mitochondrial outer translocase complex (GO:0005742)]; one group with 5 terms related to cytochrome-c oxidase activity (GO:004129); and 3 groups with a total of 17 terms associated with DNA repair [positive regulation of DNA repair (GO:0045739), error-free post replication DNA repair (GO:0042275), and DNA double-strand break (DSB) processing (GO:0000729)].

Phylogeny and divergence times from single-copy orthologs
We identified 9,170 single-copy orthologous gene families among all 9 species. Of these, 2,081 groups were removed due to a protein length <50 amino acids and an additional 2,312 groups were removed due to inconsistencies between the protein and CDSs, resulting in a total of 4,777 groups. Gene alignments were refined and concatenated, and 4DTV sites were extracted resulting in 739,038 base “supergenes.”

Our recovered topology was consistent with the recently recognized phylogenetic relationships (Gupta and Suggett 2022); each recovered node was supported with a posterior probability of 1.0 (Supplementary Fig. 1). In addition, divergence times for all nodes were largely consistent with previous estimates (Fig. 2). Our divergence estimates indicated the split between Primates and Glires occurred ~87.7 mya (Benton and Donoghue 2007; Meredith et al. 2011) with the split between Rodentia and Lagomorpha occurring shortly after at ~85.7 mya (Benton and Donoghue 2007; Meredith et al. 2011). We estimated the split between Sciurimorpha (marmots and ground squirrels) and Hysterichomorpha (chinchilla, degu) to have occurred ~73.0 mya (Montgelard et al. 2008). Chinchillidae and Octodontidae diverged next within Rodentia at ~35.4 mya (Voloch et al. 2013). Ground squirrels and marmots diverged much more recently at ~8.1 mya (Giboulet et al. 1997), with within-family divergences occurring at ~5.3 and ~3.6 mya, respectively; these latter values were somewhat earlier than previously estimated divergence times using complete cytochrome b sequences (Harrison et al. 2003). Finally, we estimated that Leporidae and Ochotonidae diverged ~52.3 mya consistent with previous findings (Wang et al. 2020).
Significantly expanded gene families in the American pika

We found 15 significantly expanded gene families in the American pika genome encompassing 88 genes, of which 83 could be annotated with GO terms (Fig. 2; see Supplementary Table 5). The 15 expanded gene families had functions related to immune response (1 family), transcription/translation (4 families), cell proliferation (1 family), catalytic activity (4 families), olfactory/pheromone receptor activity (3 families), oxidoreductase activity (1 family), and nuclear structural components (1 family). We found 18 significantly enriched GO terms (see Supplementary Table 6 in Supplementary) across these gene families related to translation (3 terms), cellular proliferation (5 terms), immune response (2 terms), membrane receptor activity (2 terms), olfactory/pheromone receptor activity (2 terms), oxidoreductase activity (1 term), catalytic activity (2 terms), and biosynthetic activity (1 term).

PSGs with putative links to high-elevation adaptation

We removed 7 single-copy orthologs from the previously identified 4,777 due to short length prior to PSG detection. Of the remaining 4,770 genes, we found 196 PSGs within the American pika genome with at least one positively selected site (BEB ≥ 0.99; corrected P ≤ 0.01; see Supplementary Table 7). One hundred and ninety PSGs were annotated with GO terms; however, we found no significantly enriched GO terms across these genes (see Supplementary Table 8). We identified 41 PSGs with putative implications for adaptation to high-elevation environments, including 16 with putative links to cold tolerance, 23 with links to hypoxia, 7 with links to UV exposure, and another 6 with associations with high-elevation populations in other species (Table 1; n.b., some genes were classified into multiple categories).
Table 1. Positively selected genes (PSGs) in the American pika genome with putative links to high-elevation adaptation.

| Gene code | Adaptation/evidence                                                                 | References                          |
|-----------|-------------------------------------------------------------------------------------|-------------------------------------|
| **Cold**  |                                                                                     |                                     |
| AASS      | Differentially expressed under cold-stress conditions; may affect amino acid content (in winter turnip rape) due to cold stress | Fang et al. (2021)                  |
| CAPSL     | Putatively under a selective sweep in mosquitoes in Russia, linked to cold tolerance | Konorov et al. (2021)               |
| DNAJA2    | Upregulated due to cold stress in common carp                                       | Cossins et al. (2006)               |
| DNAJC13   | Involved in cold resistance in Chinese white wax scale insect                        | Zhang et al. (2021)                 |
| EIF4B     | Upregulated due to cold stress in Colorado potato beetle                            | Govaere et al. (2019)               |
| LOC101526896 (UQCRC2) | Over-expressed in yaks relative to cattle, improved energy metabolism due to high-altitude adaptation | Wen et al. (2019)                   |
| LOC101527142 (CRHBP) | Increased expression after exposure to cold in Chinese honeybees                  | Liu et al. (2011)                   |
| NRDC      | Critical for thermogenesis and temperature homeostasis in mice/mammals             | Hiraoka et al. (2014)               |
| NUP205    | Increased expression following cold stress in large yellow croaker                  | Qian and Xue (2016)                 |
| PHKB      | Involved in cold acclimation in fish                                               | Healy and Schulte (2019)            |
| PLA1A     | Linked to SNP outlier in cold-resilient cattle breeds                               | Igoshin et al. (2021); Passamonti et al. (2021) |
| PSMA6     | Under-expressed following chronic cold stress in gilthead sea bream                 | Sanahuja et al. (2019)              |
| SUGT1     | Linked to temperature stress in black rockfish                                     | Lyu et al. (2018)                   |
| TECD      | Associated with outlier SNP linked to temperature stress-response in red mullet     | Boulanger et al. (2022)             |
| TREH      | Plays a key role in cold resistance across numerous species                         | Shi et al. (2016); Bao et al. (2021) |
| ZNF330    | Upregulated in response to cold stress in rainbow trout kidney                      | Verleih et al. (2015)               |
| **Hypoxia** |                                                                                     |                                     |
| AASS      | Upregulated in mice exposed to oxidative stress                                     | Bertolotto et al. (2012)            |
| ACTR2     | Downregulated in human macrophages under chronic hypoxia                            | Fuhrmann et al. (2013)              |
| ADAL      | Upregulated with chronic hypoxia                                                    | Van Linden and Eltzschig (2007)     |
| BCKDHA    | Upregulated in hypoxic conditions (in bacteria)                                     | Oliveira et al. (2021)              |
| CUL1      | May be linked to hypoxia                                                            | Mikus and Zundel (2005)             |
| EIF4B     | Increased phosphorylation in liver of naked mole rats following hypoxia             | Al-attar et al. (2020)              |
| EIF5B     | Upregulated in response to hypoxia in primary endothelial cells                     | Bartoszewski et al. (2019)          |
| GBE1      | Upregulated in hypoxic conditions                                                   | Pescador et al. (2010); Goodin et al. (2013); Leveelahti et al. (2011) |
| HAUS3     | Downregulated under hypoxia in threespine stickleback                              |                                     |
| LOC101527142 (CRHBP) | Upregulated in hypoxic conditions for developing rainbow trout | Fuzzten et al. (2011) |
| MAT2B     | Downregulated under hypoxia in common sole                                         | Mazurais et al. (2014)              |
| MRPL19    | Downregulated in hypoxic conditions (in kilifish)                                  | Flight et al. (2011)                |
| OXNAD1    | Involved in hypoxia response; necessary for hypoxia cell survival                   | Jensen et al. (2011)                |
| PHKB      | Upregulated in response to hypoxia in rainbow trout                                 | Léger et al. (2021)                 |
| FNFT1     | Downregulated following oxidative stress in obscure pufferfish                     | Wen et al. (2019)                   |
| PSMA6     | Functionally enriched in American alligator cardiac tissue following hypoxia, downregulated under hypoxic conditions in threespine stickleback | Leveelahti et al. (2011); Alderman et al. (2019) |
| RNFT1     | ER-associated degradation pathway linked to hypoxia and heat stress in hard clams   | Hu et al. (2022)                    |
| RUVBL1    | Linked to CNVs in Chinese indigenous cattle, associated with lower copy numbers in high-altitude populations, linked to hypoxic stress | Zhang et al. (2020)                 |

(continued)
Life at high elevations

High-elevation environments are characterized by several extreme conditions including lower atmospheric oxygen content (hypoxia), reduced temperatures, and higher levels of UV radiation relative to what is found at lower elevations (Sun et al. 2018). Each of these present significant challenges to the occupying fauna. Hypoxia reduces oxygen supply to tissues, which can limit aerobic metabolism (Cheviron and Brumfield 2012). A lowered metabolic rate can also lead to a reduction in thermogenesis in endotherms, making these species more susceptible to colder temperatures (Cheviron and Brumfield 2012). High levels of UV radiation pose a different challenge, particularly UV-B radiation, as this radiation can cause increased DNA damage (Wang et al. 2014). Species must adapt to these unique challenges in order to survive life at high elevations, often at a genomic level (Cheviron and Brumfield 2012; Qu et al. 2020). These genomic adaptations can affect genes responsible for oxygen transport (e.g. heme binding), energy metabolism, and DNA repair (Li et al. 2018; Sun et al. 2018). Putative adaptations to hypoxia have been found in several species of pika, including the American pika (Lemay et al. 2013; Waterhouse et al. 2018), Daurian pika (Solari and Hadly 2020), and plateau pika (Zhao et al. 2004; Li et al. 2009). Wang et al. (2020) identified putative adaptations associated with cold-tolerance across all extant pikas (Ochotona spp.) and hypothesized these occurred early in their evolutionary history (Wang et al. 2020). While these studies provide some insights into adaptation to high-elevation environments for some members of Ochotonidae, our study is the first to examine these questions on a whole-genome scale for the American pika, specifically.

High-elevation adaptation in the American pika

As a first step for our comparative genomic investigation of American pika adaption to high-elevation environments, we...
reconstructed a phylogeny and estimated divergence times between our focal species and 8 other mammalian species strategically targeted based on the availability of an annotated reference genome, phylogenetic relatedness inferred from previous studies, and (dis)similarity in ecology. Our recovered topology had high nodal support and was consistent with previously estimated relationships (Gupta and Suggett 2022). In addition, our divergence time estimates largely matched previous fossil and molecular estimates for all nodes in the tree (Giboulet et al. 1997; Harrison et al. 2003; Benton and Donoghue 2007; Montgelard et al. 2008; Meredith et al. 2011; Volokh et al. 2013; Wang et al. 2020). Not only are these results consistent with prior hypotheses regarding phylogenetic relationships, but they also provide validation that the orthologs used in these analyses were appropriate for estimating evolutionary relationships among these species.

Using these identified orthologs, we found extensive evidence for putative high-elevation adaptation in the American pika genome. Both the pika-specific genes and expanded gene families showed functional enrichment in GO categories related to the oxidation-reduction process, including cytochrome-c oxidase activity (GO:0004129), heme-copper terminal oxidase activity (GO:0015002), hydrogen ion transmembrane transporter activity (GO:0015078), and 3 terms associated with oxidoreductase activity (GO:0016675, GO:0016676, and GO:0016491; Fig. 1; see Supplementary Tables 4 and 6). Of the genes annotated with these GO terms in the pika-specific dataset involved in DNA repair, as well as an additional term related to DNA DSB processing (Fig. 1; see Supplementary Table 4). Of the genes annotated with these terms, UBE2V2 plays a role in DNA repair, particularly with respect to DSBs, such as those caused by UV radiation (Hofmann and Pickart 1999; David et al. 2010). This enzyme forms a heterodimer with UBE2N to catalyze the synthesis of "Lys-63"-linked polyubiquitin chains, which are necessary for error-free DNA repair (Hofmann and Pickart 1999; David et al. 2010). We also detected several promising PSGs related to DNA repair and cellular resistance to UV damage (Table 1). Several of these genes, namely DNA2, MLH1, and RFC4, appear to play a critical role in DNA repair following UV-induced DNA damage (Martin et al. 2010; Pathania et al. 2011; Kciuk et al. 2020). DNA2 is involved in the 5' resolution of DNA during DSB repair (Kciuk et al. 2020; Zhao et al. 2020), while MLH1 is part of the MutL alpha complex, a key component of the DNA mismatch repair system (Martin et al. 2010). Following UV damage, RFC4 localizes to UV-stalled replication forks and contributes to checkpoint activation, leading to an increase in postreplication repair (Pathania et al. 2011). Other PSGs we found are involved in cellular resistance to UV damage. Phosphorylation of DGCR8 following UV exposure appears to be critical for cellular resistance to UV, as well as for recovery of RNA synthesis in both mice and humans (Calses et al. 2017). TEL2O acts as a regulator of the DNA damage response and is heavily involved in cellular resistance to both ionizing and UV radiation (Hurov et al. 2010; Chen et al. 2018). Collectively, these genes provide promising evidence for adaptation to increased UV exposure in the American pika.

**Other environmentally associated adaptations and considerations**

While this study focused on putative high-elevation adaptations, we did find evidence for additional adaptations related to environment. We found significant enrichment of olfactory receptor activity among both pika-specific genes as well as expanded gene families in the American pika (see Supplementary Tables 4 and 6). American pikas have 2 types of foraging behavior: grazing (direct consumption) and haying (caching plants for an over-winter food supply; Huntly et al. 1986); in addition, American pikas appear to cache higher-quality vegetation rather than the most commonly available (Smith and Erb 2013). Enhanced olfaction could potentially aid American pikas in selecting the appropriate quality of food to best survive the winter months. Related to olfaction, we also saw enrichment of pheromone receptor activity among expanded gene families (see Supplementary Table 6). Unlike other lagomorphs, both male and female American pikas...
remain territorial year-round (Boonstra et al. 2022) and are known to use cheek glands to scent mark rocks around their territory to ward off conspecifics (Meaney 1986). This association between olfactory receptor activity and territorial behavior in American pikas represents a promising avenue for future inquiry.

It is important to note that the available American pika genome was constructed from a single individual sampled within Beaverhead-Deerlodge National Forest in southwestern Montana, USA at an elevation of ~2,770 m above sea level. This site is centrally located within the Northern Rocky Mountains lineage of the American pika, which is the largest in terms of area (see Galbreath et al. 2009). The fact that the genome was constructed from a single individual from a single location could mean that observed variation may not be representative of the entire species. However, as we focused this study on coding regions, intra-specific variation should be less pronounced given evolutionary constraints including slower mutation rates. In addition, this genome should be appropriate for detecting high-elevation adaptations within the American pika given the sampling location approaches the upper extent of their contemporary elevational range (Smith and Weston 1990).

Conclusions
Here, we identified and characterized putative adaptation in the American pika genome. We found support across multiple analyses for functional enrichment in categories related to hypoxia, cold tolerance, and DNA repair, and identified numerous PSGs with links to high-elevation adaptation. Although these results do not constitute direct evidence for environmental adaptation, they provide important targets for future studies within the American pika and across other mammalian species. These investigations could include examination of gene expression along elevational gradients, molecular assays in which functional responses are measured, or even correlative approaches of genetic differentiation across varying environmental conditions. Recommended targets include OXNAD1, NRDC, and those genes which are critical in DNA repair, as we found the strongest support for these regions. Altogether, this work provides the first whole-genome examination of high-elevation adaptation in American pikas and will serve as an important reference for future studies related to environmental adaptation and climate change.

Data availability
All genome assemblies and associated data are publicly available in the NCBI database with accession information in Supplementary Table 1 in the Supplementary Tables file. All custom scripts used in this article are available at https://github.com/osjodin.

Supplemental material is available at G3 online.

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Conflicts of interest
None declared.

Literature cited
Al-attar R, Childers CL, Nguyen VC, Pamenter ME, Storey KB. Differential protein phosphorylation is responsible for hypoxia-induced regulation of the Akt/mTOR pathway in naked mole rats. Comp Biochem Physiol A Mol Integ Physiol. 2020;242:110653. doi: 10.1016/j.cbpa.2020.110653.
Alderman SL, Crossley DA, Elsey RM, Gillis TE. Hypoxia-induced reprogramming of the cardiac phenotype in American alligators (Alligator mississippiensis) revealed by quantitative proteomics. Sci Rep. 2019;9(1):8592. doi: 10.1038/s41598-019-45023-3.
Bao J, Wang X, Feng C, Li X, Jiang H. Trehalose metabolism in the Chinese mitten crab Eriocheir sinensis: molecular cloning of trehalase and its expression during temperature stress. Aquaculture Rep. 2021;20:100770. doi: 10.1016/j.aqrep.2021.100770.
Bartoszewski R, Moszyńska A, Serocki M, Cabaj A, Polen A, Ochocka R, Dell’Italia L, Bartoszewska S, Królczewska J, Dąbrowski M, et al. Primary endothelial cell–specific regulation of hypoxia-inducible factor (HIF)-1 and HIF-2 and their target gene expression profiles during hypoxia. FASEB J. 2019;33(7):7929–7941. doi: 10.1096/fj.201802650RR.
Beever EA, Brussard PF, Berger J. Patterns of apparent extirpation among isolated populations of pikas (Ochotona princeps) in the Great Basin. J Mammal. 2003;84(1):37–54. doi: 10.1644/1545-1542(2003)084<0037:POAEAt>2.0.CO;2.
Beever EA, Ray C, Mote FW, Wilkening JL. Testing alternative models of climate-mediated extirpations. Ecol Appl. 2010;20(1):164–178. doi: 10.1890/08–1011.1.
Beever EA, Ray C, Wilkening JL, Brussard PF, Mote FW. Contemporary climate change alters the pace and drivers of extinction. Global Change Biol. 2011;17(6):2054–2070. doi: 10.1111/j.1365–2486.2010.02389.x.
Benton MJ, Donoghue PCJ. Paleontological evidence to date the tree of life. Mol Biol Evol. 2007;24(1):26–53. doi: 10.1093/molbev/msl150.
Bertoletto PR, Teruya R, Chaves JC, Ikejiri AT, Somoa Neto F, Taha MO, Fagundes DJ. Oxidative stress gene expression profile in inbred mouse after small bowel ischemia/reperfusion injury. Transplantation. 2012;94(10S):737.
Boeckmann B, Bairoch A, Apweiler R, Blatter M-C, Estreicher A, Gasteiger E, Martin MJ, Michoud K, O’Donovan C, Phan I, et al. SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. Nucleic Acids Res. 2003;31(1):365–370. doi: 10.1093/nar/gk095.
Boonstra R, Gandhi N, Krauschaar A, Galbreath K. From habitat to hormones: year-around territorial behavior in rock-dwelling but not in forest and grassland lagomorphs and the role of DHEA. Horm Behav. 2022;142:105179. doi: 10.1016/j.yhbeh.2022.105179.
Boulanger E, Benestan L, Guerin P-E, Dalongeville A, Mouillot D, Manel S. Climate differentially influences the genomic patterns of two sympatric marine fish species. J Anim Ecol. 2022;91(6):1180–1195. doi: 10.1111/1365–2656.13623.
insights from transcriptome analysis. Aquaculture. 2022;549:737792. doi: 10.1016/j.aquaculture.2021.737792.

Huntly NJ, Smith AT, Ivins BL. Foraging behavior of the pika (Ochotona princeps), with comparisons of grazing versus haying. J Mammal. 1986;67(1):139–148. doi: 10.2307/1381010.

Hurov KE, Cotta-Ramusino C, Elledge SJ. A genetic screen identifies the Triple T complex required for DNA damage signaling and ATM and ATR stability. Genes Dev. 2010;24(17):1939–1950. doi: 10.1101/gad.1934210.

Igoshin A, Yudin N, Atinazarov R, Yurchenko AA, Larkin DM. Whole-genome resequencing points to candidate DNA loci affecting body temperature under cold stress in Siberian cattle populations. Life. 2021;11(9):959. doi: 10.3390/life11090959.

Jensen KS, Binderup T, Jensen KT, Therkelsen I, Borup R, Nilsson E, Multhaupt H, Bouchard C, Quistorff B, Kjaer A, et al. FoxO3A promotes metabolic adaptation to hypoxia by antagonizing Myc function: FoxO3A inhibits Myc to control hypoxic metabolism. EMBO J. 2011;30(22):4545–4570. doi: 10.1038/emboj.2011.323.

Jones P, Binns D, Chang H-Y, Fraser M, Li W, McAnulla C, McWilliam et al. Automated generation of gene summaries at the Alliance of Bioversity. Bioinformatics. 2014;30(9):1236–1240. doi:10.1093/bioinformatics/btu031.

Kciuk M, Marciniak B, Mojzych M, Kontek R. Focus on UV-induced DNA damage and repair: disease relevance and protective strategies. Int J Mol Sci. 2020;21(19):7264. doi: 10.3390/ijms21197264.

Kishore R, Arnaboldi V, Van Slyke CE, Chan J, Nash RS, Urbano JM, Dolan ME, Engel SR, Shimoyama M, Sternberg FW, et al. Automated generation of gene summaries at the Alliance of Genome Resources. Database. 2020;2020:baaa037. doi: 10.1093/database/baaa037.

Konorov EA, Yurchenko V, Patraman I, Lukashev A, Oyun N. The effects of genetic drift and genomic selection on differentiation and local adaptation of the introduced populations of Aedes albopictus in southern Russia. PeerJ. 2021;9:e11776. doi:10.7717/peerj.11776.

Krishnan S, Stearman RS, Zeng L, Fisher A, Mickler EA, Rodriguez BH, Simpson ER, Cook T, Slaven JE, Ivan M, et al. Transcriptomic modifications in developmental cardiopulmonary adaptations to chronic hypoxia using a murine model of simulated high-altitude exposure. Am J Physiol Lung Cell Mol Physiol. 2020;319(3):L456–L470. doi: 10.1152/ajplung.00487.2019.

Kristensen TN, Keltola T, Kronholm I. Adaptation to environmental stress at different timescales. Ann N Y Acad Sci. 2020;1476(1):5–22. doi:10.1111/nyas.13974.

Lee BY, Lee J-H, Ryun J-H, Woo DK. Whole-genome transcriptional responses to hypoxia in respiration-proficient and respiration-deficient yeasts: implication of the mitochondrial respiratory chain in oxygen-regulated gene expression. J Life Sci. 2016;26(10):1137–1152. doi:10.5352/jls.2016.26.10.1137.

Lee P, Chandel NS, Simon MC. Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. Nat Rev Mol Cell Biol. 2020;21(5):268–283. doi: 10.1038/s41580-020-0227-y.

Léger JAD, Athanasio CG, Zhora A, Chauhan MF, Simmons DBD. Hypoxic responses in Oncorhynchus mykiss involve angiogenesis, lipid, and lactate metabolism, which may be triggered by the cortisol stress response and epigenetic methylation. Comp Biochem Physiol Part D Genomics Proteomics. 2021;39:100860. doi: 10.1016/j.cbd.2021.100860.

Lemay MA, Henry P, Lamb CT, Robson KM, Russello MA. Novel genomic resources for a climate change sensitive mammal: characterization of the American pika transcriptome. BMC Genomics. 2013;14(1):311. doi:10.1186/1471-2164-14-311.

Leveelahti L, Leskinen P, Leder EH, Waser N, Nikinmaa M. Responses of threespine stickleback (Gasterosteus aculeatus, L) transcriptome to hypoxia. Comp Biochem Physiol Part D Genomics Proteomics. 2011;6(4):370–381. doi:10.1016/j.cbd.2011.08.001.

Li H-G, Ren Y-M, Guo S-C, Cheng L, Wang D-P, Yang J, Chang Z-J, Zhao X-Q. The protein level of hypoxia-inducible factor-1α is increased in the plateau pika (Ochotona curzoniae) inhabiting high altitudes. J Exp Zool A Ecol Genet Physiol. 2009;311(2):134–141. doi:10.1002/jez.510.

Li JT, Gao Y-D, Xie L, Deng C, Shi P, Guan M-L, Huang S, Ren J-L, Wu D-D, Ding L, et al. Comparative genomic investigation of high-elevation adaptation in ecotothermal snakes. Proc Natl Acad Sci USA. 2018;115(33):8406–8411. doi: 10.1073/pnas.1805348115.

Liu L, Yu X, Meng F, Guo X, Xu B. Identification and characterization of a novel corticotropin-releasing hormone-binding protein (CRH-BP) gene from Chinese honeybee (Apis cerana cerana). Arch Insect Biochem Physiol. 2011;78(3):161–175. doi:10.1002/arch.20451.

Lyu L, Wen H, Li Y, Li J, Zhao J, Zhang S, Song M, Wang X. Deep transcriptomic analysis of black rockfish (Sebastes schlegelii) provides new insights on responses to acute temperature stress. Sci Rep. 2018;8(1):9113. doi:10.1038/s41598-018-27013-z.

MacArthur RA, Wang LCH. Behavioral thermoregulation in the pika Ochotona princeps: a field study using radiotelemetry. Can J Zool. 2015;52(3):353–358. doi:10.1139/cjz-042.

Maere S, Heymans K, Kuiper M. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. Bioinformatics. 2005;21(16):3448–3449. doi:10.1093/bioinformatics/bti551.

Majoros H, Ujfaludi Z, Borsos BN, Hudacsek VV, Nagy Z, Coin F, Buzas K, Kovács I, Bíró T, Borsos IM, et al. SerpinB2 is involved in cellular response upon UV irradiation. Sci Rep. 2019;9(1):2753. doi:10.1038/s41598-019-39073-w.

Martin LM, Marples B, Coffey M, Lawler M, Lynch TH, Hollywood D, Marignol L. DNA mismatch repair and the DNA damage response to ionizing radiation: making sense of apparently conflicting data. Cancer Treat Rev. 2010;36(7):518–527. doi:10.1016/j.ctrv.2010.03.008.

Mazurais D, Ferrareso S, Gatta PP, Desbryères E, Severe A, Corporeau C, Claireaux G, Bargelloni L, Zambonino-Infante J-L. Identification of hypoxia-regulated genes in the liver of common sole (Solea solea) fed different dietary lipid contents. Mar Biotechnol (NY). 2014;16(3):277–288. doi:10.1007/s10126-013-9545-9.

Meaney CA. Scent-marking in pikas (Ochotona princeps): Test of a breeding-facilitation hypothesis. In: D Duvall, D Müller-Schwarze, RM Silverstein, editors. Chemical Signals in Vertebrates 4. Boston (MA): Springer US; 1986. p. 571–577 [accessed 2022 Aug 23]. http://link.springer.com/10.1007/978-1-4613-2235-1_45.

Meredith RW, Janecka JE, Gatesy J, Ryder OA, Fisher CA, Teeling EC, Good alfia A, Eizirk E, Simaö TLL, Stadler T, et al. Impacts of the Cretaceous terrestrial revolution and KPg extinction on mammal diversification. Science. 2011;334(6055):521–524.

Mikus P, Zundel W. COPing with hypoxia. Semin Cell Dev Biol. 2005;16(4–5):462–473. doi:10.1016/j.scbdb.2005.03.008.

Millar CI, Westfall RD. Distribution and climatic relationships of the American pika (Ochotona princeps) in the Sierra Nevada and Western Great Basin, U.S.A.: periglacial landforms as refugia in warming climates. Arct Antarct Alp Res. 2010;42(1):76–88. doi:10.1101/gad.1934210.
fast-evolving nucleotides in mitochondrial, exon and intron fragments. BMC Evol Biol. 2008;8:321. doi: 10.1186/1471-2148-8-321.

O’Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 2016;44(D1):D733–745. doi:10.1093/nar/gkv1189.

Oliveira LN, Lima PdB, Araújo DS, Portis IG, Santos Júnior AdCd, Coelho ASC, de Sousa MV, Ricart CAO, Fontes W, Soares CDa. iTRAQ-based proteomic analysis of Paracoccidioides brasiliensis in response to hypoxia. Microbiol Res. 2021;247-126730. doi: 10.1016/j.micres.2021.126730.

Parsons PA. Environments and evolution: interactions between stress, resource inadequacy and energetic efficiency. Biol Rev Camb Philos Soc. 2005;80(4):589–610. doi: 10.1017/S1464793105006822.

Passamonti MM, Somenzii E, Barbato M, Chihlemi G, Colli L, Joost S, Milanesi M, Negrin R, Santini M, Vajena E, et al. The quest for genes involved in adaptation to climate change in ruminant livestock. Animals. 2021;11(10):2833. doi: 10.3390/ani1102833.

Pathania S, Nguyen J, Hill SJ, Scully R, Adelmann GO, Marto JA, Feunteun J, Livingston DM. BRCA1 is required for postreplication repair after UV-induced DNA damage. Mol Cell. 2011;44(2):235–251. doi: 10.1016/j.molcel.2011.09.002.

Pétron N, Artigaud S, Infante J-LZ, Le Bayon N, Charrier G, Pichereau V, Laroche J. Proteomic responses of European flounder to temperature and hypoxia as interacting stressors: differential sensitivities of populations. Sci Total Environ. 2017;586:890–899. doi: 10.1016/j.scitotenv.2017.02.068.

Pescador N, Villar D, Cifuentes D, García-Rocha M, Ortiz-Barahona A, Vazquez S, Ordoñez A, Cuevas Y, Saez-Morales D, García-Bermejo ML, et al. Hypoxia promotes glycogen accumulation through hypoxia inducible factor (HIF)-mediated induction of glycogen synthase 1. PLoS One. 2010;5(3):e9644. doi: 10.1371/journal.pone.0009644.

Qian B, Xue L. Liver transcriptome sequencing and de novo annotation of the large yellow croaker (Larimichthys crocea) under heat and cold stress. Mar Genomics. 2016;25:95–102. doi: 10.1016/j.margen.2016.03.003.

Qi Q, Zhang G, Ma T, Qian W, Wang J, Ye Z, Cao C, Hu Q, Kim J, Sanahuja I, Fernández-Alacid L, Sánchez-Nurio S, Ordoñez-Grande R, Ibarz A. Chronic cold stress alters the skin mucus interactome in a temperate fish model. Front Physiol. 2019;9:1916–1918. doi: 10.3389/fphys.2018.01916.

Schmidt DA, Waterhouse MD, Sjödin BMF, Russello MA. Genome-wide analysis reveals associations between climate and regional patterns of adaptive divergence and dispersal in American pikas. Heredity (Edinb). 2021;127(5):443–454. doi: 10.1007/s10044-021-00472-3.

Schwalm D, Epps CW, Rodhouse TJ, Monahan WB, Castillo JA, Ray C, Jeffress MR. Habitat availability and gene flow influence diverging local population trajectories under scenarios of climate change: a place-based approach. Glob Chang Biol. 2016;22(4):1572–1584. doi: 10.1111/gcb.13189.

Schweizer RM, Jones MR, Bradburd GS, Storz JF, Senner NR, Wolf C, Cheviron ZA. Broad concordance in the spatial distribution of adaptive and neutral genetic variation across an elevational gradient in deer mice. Mol Biol Evol. 2021;38(10):4286–4300. doi: 10.1093/molbev/msab161.

Shi Z, Liu X, Xu Q, Qin Z, Wang S, Zhang F, Wang S, Tang B. Two novel soluble trehalase genes cloned from Harmonia axyridis and regulation of the enzyme in a rapid changing temperature. Comp Biochem Physiol B Biochem Mol Biol. 2016;198:10–18. doi: 10.1016/j.cbpb.2016.03.002.

Sjödin BMF, Galbreath KE, Lanier HC, Russello MA. Chromosome-level reference genome assembly for the American pika (Ochotona princeps). J Hered. 2021;112(6):549–557. doi: 10.1093/jhered/esa031.

Smith AT, Beever E. Ochotona princeps. International Union for Conservation of Nature the IUCN Red List of Threatened Species. 2016;2016:e.T41267A45184315. doi:10.2305/IUCN.UK.2016-3.RLTS.T41267A45184315.en.

Smith AT, Weston ML. Ochotona princeps. Mammalian Species. 1990;(352):1. doi: 10.2305/3504319.

Smith JA, Erb LP. Patterns of selective caching behavior of a generalist herbivore, the American pika (Ochotona princeps). Arct Antarct Alp Res. 2013;45(3):396–403. doi:10.1657/1938-4246-45.3.396.

Smoot ME, Ono K, Ruscheinski J, Wang P-L, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics. 2011;27(3):431–432. doi: 10.1093/bioinformatics/btt675.

Solari KA, Hadly EA. Experimental study of hypoxia-induced changes in gene expression in an Asian pika, Ochotona daurica. PLoS One. 2020;15(10):e0240435. doi: 10.1371/journal.pone.0240435.

Solari KA, Ramakrishnan U, Hadly EA. Gene expression is implicated in the ability of pikas to occupy Himalayan elevational gradient. PLoS One. 2018;13(12):e0207936. doi: 10.1371/journal.pone.0207936.

Stewart JE, Perrine JD, Nichols LB, Thorne JH, Millar CI, Goehring KE, Massing CP, Wright DH. Revisiting the past to foretell the future: summer temperature and habitat area predict pika extirpations in California. J Biogeogr. 2015;42(5):880–890. doi: 10.1111/jbi.12466.

Sun Y-B, Fu T-T, Murphy RW, Hillis DM, Zhang Y-P, Che J, O’Leary NA, Wright DH. Revisiting the past to foretell the future: summer temperature and habitat area predict pika extirpations in California. J Biogeogr. 2015;42(5):880–890. doi: 10.1111/jbi.12466.

Suyama M, Torrents D, Bork P. PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments. Nucleic Acids Res. 2006;34(Web Server issue): W609–W612. doi: 10.1093/nar/gkl315.

Talavera G, Castresana J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol. 2007;56(4):564–577. doi: 10.1080/10635150701472122.
Whibley A, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022–3027. doi:10.1093/molbev/msab120.

Tian D, Zhou B, Han B, Liu S, Tian F, Qi D, Zhao K. Deep genome sequencing provides potential novel insights into plateau adaptations in domestication of goats to extreme environments. In Review. 2021 [accessed 2022 Mar 9]. https://www.researchsquare.com/article/rs-453887/v1.

Van Linden A, Eltzschig HK. Role of pulmonary adenosine during hypoxia: extracellular generation, signaling and metabolism by surface adenosine deaminase/CD26. Expert Opin Biol Ther. 2007;7(9):1437–1447. doi:10.1517/14712598.7.9.1437.

Verleih M, Borchel A, Krasnov A, Rebl A, Koryta, Goldammer T. Impact of thermal stress on kidney-specific gene expression in farmed regional and imported rainbow trout. Mar Biotechnol (NY). 2015;17(5):758–592. doi:10.1007/s11210-015-9640-1.

Voloch CM, Vilela JF, Loss-Oliveira L, Schrago CG. Phylogeny and chronology of the major lineages of New World hysticomorphous rodents: insights on the biogeography of the Eocene/Oligocene arrival of mammals in South America. BMC Res Notes. 2013;6(1):160. doi:10.1186/1755-0500-6-160.

Wang Q-W, Hidema J, Hikosaka K. Is UV-induced DNA damage greater at higher elevation? Am J Bot. 2014;101(5):796–802. doi:10.3732/ajb.1400010.

X, Tu X-L, Zhang M, et al. DNA end resection and its role in DNA replication and DSB repair choice in mammalian cells. Exp Cell Res. 2020;398:427–348. doi:10.1016/j.yexcr.2019.04.004.

X, Tu X-L, Zhang M, et al. DNA end resection and its role in DNA replication and DSB repair choice in mammalian cells. Exp Cell Res. 2020;398:427–348. doi:10.1016/j.yexcr.2019.04.004.

Waterhouse MD, Erb LP, Beever EA, Russello MA. Adaptive population divergence and directional gene flow across steep elevational gradients in a climate-sensitive mammal. Mol Ecol. 2018;27(11):2512–2528. doi:10.1111/mec.14701.

Zhang H-P, Liu W, An J-Q, Yang P, Guo L-H, Li Y-Q, Lv J, Yu S-H. Transcriptome analyses and weighted gene coexpression network analysis reveal key pathways and genes involved in the rapid cold resistance of the Chinese white wax scale insect. Arch Insect Biochem Physiol. 2021;107(1):e21781. doi:10.1002/arch.21781.

Zhang L, Yang Q, Liu J, Liu Z, Wang K, Yang H, et al. Comparative transcriptome analysis of the swimbladder reveals expression signatures in response to low oxygen stress in channel catfish, Ictalurus punctatus. Physiol Genomics. 2018;50(8):636–647. doi:10.1152/physiogenomics.00125.2017.

Zhao TB, Ning HX, Zhu SS, Sun P, Xu SX, Chang ZJ, Zhao XQ. Cloning of hypoxia-inducible factor 1a cDNA from a high hypoxia tolerant mammal—plateau pika (Ochotona curzoniae). Biochem Biophys Res Commun. 2004;316(2):565–572. doi:10.1016/j.bbrc.2004.02.087.