

Selection on the vascular-remodeling *BMPER* gene is associated with altitudinal adaptation in an insular lizard

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Abstract

High altitude imposes several extreme constraints on life, such as low oxygen pressure and high levels of ultraviolet radiation, which require specialized adaptations. Many studies have focused on how endothermic vertebrates respond to these challenging environments, but there is still uncertainty on how ectotherms adapt to these conditions. Here, we used whole-genome sequencing of low-altitude (100–600 m) and high-altitude (3,550 m) populations of the wide-ranging Tenerife lizard *Gallotia galloti* to uncover signatures of selection for altitudinal adaptation. The studied populations show reduced differentiation, sharing similar patterns of genetic variation. Selective sweep mapping suggests that signatures of adaptation to high altitude are not widespread across the genome, clustering in a relatively small number of genomic regions. One of these regions contains *BMPER*, a gene involved with vascular remodeling, and that has been associated with hypoxia-induced angiogenic response. By genotyping samples across 2 altitudinal transects, we show that allele frequency changes at this locus are not gradual, but rather show a well-defined shift above ca. 1,900 m. Transcript and protein structure analyses on this gene suggest that putative selection likely acts on noncoding variation. These results underline how low oxygen pressure generates the most consistent selective constraint in high-altitude environments, to which vertebrates with vastly contrasting physiological profiles need to adapt in the context of ongoing climate change.

Keywords: genomics, *Gallotia galloti*, whole-genome sequencing, high-altitude, hypoxia

Lay Summary

Animals that inhabit high-altitude areas face a variety of extreme conditions that are not felt closer to sea level, including low availability of oxygen, high levels of ultraviolet radiation, and low temperatures, to which they are adapted. For endothermic vertebrates, like mammals and birds, multiple studies have revealed how genetic changes can promote this adaptive process. A classic example is that of human populations in Tibet, which possess a high frequency of genetic variants that enable them to cope with the reduced oxygen availability in the Tibetan plateau. In contrast, ectotherms such as reptiles and amphibians have received far less research attention, so the mechanisms that mediate their responses to high altitudes are largely unknown. To fill this gap, we studied the genome of a unique species—the Tenerife lizard that is endemic to the Canary Islands. Its distribution ranges across a steep altitudinal gradient in the island of Tenerife, where it can be found from sea level all the way to the summit of the Teide volcano, at ca. 3,700 m. By comparing all the genetic information from low-altitude and high-altitude populations, we found only a reduced number of regions of the genome that show evidence of having undergone natural selection at high altitudes. Among these, the genomic region containing the gene *BMPER* (implicated in blood vessel formation and whose expression is changed by low oxygen conditions) shows a strong pattern of selection, in particular changes in the predominant genetic variants in populations living above 1,900 m. These results underline how low oxygen availability is a key factor that controls the evolution of animals living at high altitudes and that the complex and multifaceted responses to these environments may require the integration of genetic and nongenetic mechanisms.

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Introduction

High-altitude habitats are among the harshest environments for living organisms. Temperature means and extremes, high ultraviolet radiation, and low oxygen pressure are strongly divergent from those prevailing in lowland areas (Blumthaler et al., 1997; Jørgensen & Fath, 2008). These abiotic characteristics drive adaptation in highland plant and animal populations at any latitude (Monge & León-Velarde, 1991; Halbritter et al., 2018; Simonson et al., 2010; Storz & Scott, 2019; Wang et al., 2021). In addition to this natural challenge, ongoing anthropogenic environmental disturbances that include climate change (Jørgensen & Fath, 2008; Thornton et al., 2014) have the potential to interact with the constraints placed by high altitude, not only for altitudinal specialists but also for lowland species that are forced to shift their distribution upwards to remain under similar climatic conditions (Jiang et al., 2021). Coping with these multiple stressors requires a suite of behavioral and physiological adaptations, including optimization of the respiratory process, thermal or hydric regulation, or differentiation of gut microbiota as a response to a changed diet (González-Morales et al., 2021; Howald & Hoppeler, 2003; Quagliariello et al., 2019; S'khifa et al., 2022; Sannolo et al., 2020; Solano et al., 2016; Souchet et al., 2020; Storz & Scott, 2019; Žagar et al., 2022).

Multiple studies have found links between specific genes and adaptation to altitude in terrestrial vertebrates. For example, alleles of the gene Endothelial PAS Domain Protein 1 (EPAS1) have been repeatedly implicated in adaptation to altitude in mammals (e.g., Hendrickson, 2013; Huerta-Sánchez et al., 2014; Liu et al., 2019; Schweizer et al., 2019; Yang et al., 2017a). In these species, EPAS1 regulates hemoglobin concentration and angiogenesis as responses to low atmospheric oxygen pressure. Several other genes have also been associated with altitudinal adaptation, mostly hypoxia-related (e.g., Chiou et al., 2022; Witt & Huerta-Sánchez, 2019), but also with metabolism and cold resistance (Cheviron et al., 2008; Velotta et al., 2020), skeletal development (Qu et al., 2013; Wang et al., 2014) and DNA repair (Li et al., 2018; Yang et al., 2014; Zhang et al., 2016) showing the complex nature of adaptation to an environmental context consisting of multiple variables and constraints.

Ectothermic vertebrates have limited homeostatic mechanisms and are therefore more likely to be directly exposed to temperature or precipitation fluctuations (Angilletta, 2009), which are highly dependent on altitude. This makes them important models for understanding how apparently vulnerable species may respond to the challenges of environmental change (Paaijmans et al., 2013; Sinervo et al., 2010). The genetic mechanisms associated with altitudinal adaptation in ectotherms remain, however, poorly understood. Comparative genomics (Li et al., 2018; Sun et al., 2018) and transcriptomic (Yang et al., 2016, 2017b) studies suggest varied roles for genes involved with DNA repair, energy metabolism, and response to hypoxia. Most recently, this interplay of selection on multiple functions has been suggested in an interspecific study on the Mongolian racerunner (*Eremias argus*), in which high-altitude populations showed evidence of selective sweeps around genes associated with energy metabolism, DNA damage repair, and hypoxia response pathways (Li et al., 2023).

The Tenerife lizard, *Gallotia galloti* (Figure 1A), is ideally placed to answer questions on the evolution of altitudinal adaptation in ectotherms. Populations inhabiting the southern slope of the island are found continuously from the sea level all the way to the summit of the island's volcanic cone at 3,700 m (Fariña et al., 2011). The volcanic cone itself starts at an altitude of

approximately 2,300 m and grew in the period between 170,000 and 30,000 years ago, with its last volcanic eruption occurring nearly 1,300 years ago (Carracedo et al., 2007). This eruptive history, together with recent glaciation (ca. 30,000 years ago) that likely covered high altitudes with permanent snow, suggests a relatively recent colonization of *G. galloti* at the volcano summit. The continuous distribution of these southern populations (which belong to a single evolutionary lineage, Brown et al., 2016) along an extreme altitudinal gradient offers a unique opportunity to study how ectotherms adapt to environmental variation without confounding factors such as population substructure or genetic drift.

Here, we used whole-genome sequencing to study adaptation to extreme altitudinal environments in *G. galloti*. We assembled a reference genome of this species using linked-read sequencing and re-sequenced DNA pools of high- and low-altitude populations to examine patterns of genetic variation and scan the genome for evidence of selection to high-altitude environments. Our main objective was to unravel regions of the genome with evidence of selection to high-altitude relative to low-altitude populations and assess potential links to genes with relevant functions for coping with the constraints of high-altitude conditions.

Methods

Fully detailed and referenced methods are given as [Supplementary material](#).

Field sampling

Samples of *G. galloti* were collected in two field missions held between July and August of 2017 and 2018 on the island of Tenerife spread across the altitudinal range on the southern part of the island ($n = 147$; 10 populations; Figure 1B and C; Supplementary Table S1). An additional individual from Parque Nacional del Teide (28.255, -16.621; 2,300 m elevation) was collected for reference genome assembly (Figure 1B and C). Lizards were caught either by pitfall traps using tomato and banana as bait or by noose. Tail tips were recovered as tissue for subsequent DNA extraction, and following physiological testing (Serén et al., 2023), the lizards were released in the same location of trapping.

Reference genome assembly

Chromium linked-reads (10X Genomics) were used to assemble a reference genome (Weisenfeld et al., 2017). Muscle tissue from a male was sent to the Genomics Services Laboratory of the HudsonAlpha Institute for Biotechnology for Chromium library preparation and sequencing (NovaSeq, Illumina). To assemble the genome, we used *Supernova* v2.1.1 (Weisenfeld et al., 2017) with default options (raw coverage of 69.28X). Descriptive statistics were computed using the script *assemblathon_stats.pl* (Bradnam et al., 2013), and genome completeness was assessed using BUSCO v5.3.2 (Simão et al., 2015) with the tetrapoda_odb10 database. For comparison, we extracted sequences of five other lacertid species belonging to different genera (*Podarcis muralis*, *Zootoca vivipara*, *Lacerta agilis*, *L. bilineata*, and *L. viridis*) and computed the same summary statistics.

The assembly was annotated using *GenSAS* v6.0 (Humann et al., 2019). A consensus library of repeats was built by combining *RepeatModeler* v2.0.1 (Flynn et al., 2020) and *RepeatMasker* v4.1.1 (Smit et al., 2013). Gene prediction was performed using *Augustus* v3.3.1 (Stanke et al., 2006) with default options and using the chicken (*Gallus gallus*) to model gene structure. Gene set refinement was done with *PASA* v2.11.0 (Haas et al., 2008). Functional

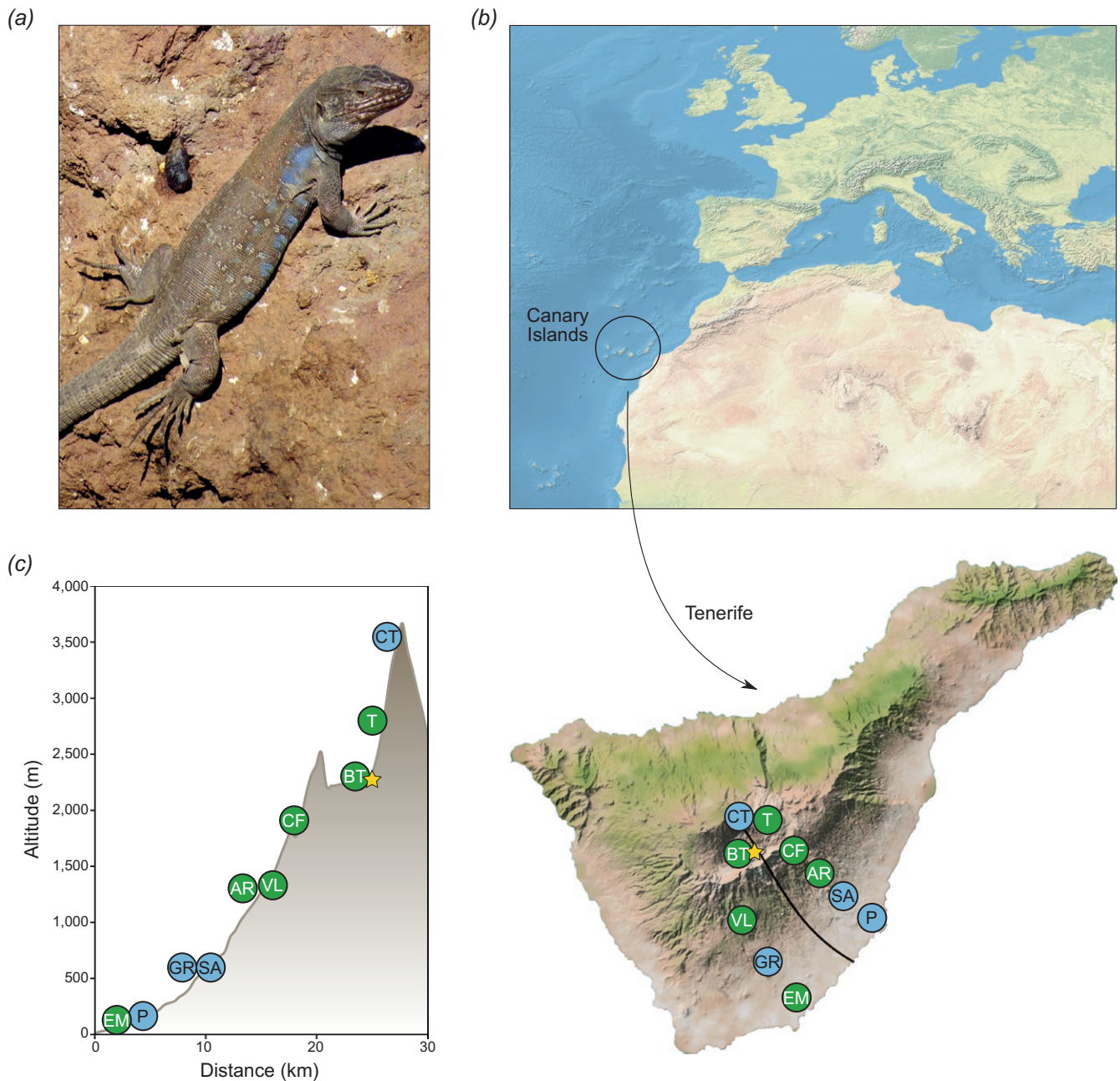


Figure 1. The continuous distribution along an extreme altitudinal gradient of the Tenerife lizard (*Gallotia galloti*). (A) The male of the Tenerife lizard (Photo by R.M.P.). (B) Geographical location of the Canary Islands archipelago and a close-up of the Tenerife Island with sampling sites. (C) Location of sampling sites along a topographic profile of the south side of the island of Tenerife. For panels (B) and (C), locations used for whole-genome pool sequencing are marked in blue (with dark characters); additional locations with samples genotyped for candidate loci are marked in green (with white characters); the location of the sample used for the genome assembly is marked with a yellow star.

annotation of the consensus gene models was done via BLAST against the SwissProt database.

Whole-genome re-sequencing, read filtering and mapping

Pool-sequencing of three low-altitude (GR, SA, P) and one high-altitude (CT) population was used for population genomics. DNA from several individuals was extracted and pooled by population (CT, $n = 14$; GR, $n = 16$; SA, $n = 12$; P, $n = 12$) in equimolar concentrations for genomic library preparation with a Nextera XT Kit (Illumina). Sequencing was performed at Novogene UK in a NovaSeq instrument (Illumina) using 2×150 bp reads.

Reads were evaluated using FastQC v0.11.8 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc>), and low-quality bases and adapters were trimmed with Trimmomatic v0.39 (Bolger et al., 2014). Reads were then mapped to our de novo assembly with BWA-MEM (Li, 2013) using default settings, and PCR duplicates were removed using Picard (<http://broadinstitute.github.io/picard>). Mapping statistics were calculated using SAMtools v0.1.19 (Danecek et al., 2021) and custom scripts.

Population genomics

Population genetic statistics were calculated based on allele count data. Differentiation between populations was assessed

through the fixation index (F_{ST}) using *PoPoolation2* v.1.201 (Kofler et al., 2011a) in nonoverlapping windows of 100 kb, restricting to positions with a minimum coverage of seven, maximum coverage of 100, minimum count of the minor allele of three to consider a position as a SNP, and excluding windows for which less than 20% of the window met the coverage criteria. Nucleotide diversity (π , Nei, 1987) and Tajima's D (Tajima, 1989) were also calculated using *PoPoolation2* v1.1.2 (Kofler et al., 2011b). For π and D , calculations were performed in nonoverlapping windows of 100 kb, restricting to positions with a minimum coverage of seven, maximum coverage of 100, and minimum count of the minor allele of two to consider a position as a SNP and excluding windows for which less than 20% of the window met the coverage criteria.

Selective sweep mapping

To detect regions of the genome under selection in high-altitude populations, the reads from the three low-altitude pools were merged to increase the overall depth of coverage. Then, several statistics were calculated to explicitly compare patterns of variation in high-altitude and the merged low-altitude pools: differentiation (F_{ST}), reductions in nucleotide diversity (π -ratio: $\pi_{\text{low-altitude}}/\pi_{\text{high-altitude}}$), and deviations from neutrality ΔD ($D_{\text{low-altitude}} - D_{\text{high-altitude}}$) (following Gazda et al., 2018). By setting the comparisons this way, the results were polarized to highlight recent selective sweeps in high-altitude populations. The window size was set to 50 kb and ran in steps of 12.5 kb.

These three statistics capture different properties of sequence data under selection, so combining them should increase the robustness of inference on selection. To do this, a de-correlated composite of multiple signals was implemented (DCMS; Ma et al., 2015), following the procedure in Gazda et al. (2018). The top 0.1% of DCMS were considered to hold stronger evidence of selection. For each of the two most conspicuous DCMS outliers (on scaffold12 and scaffold17, see Results section), we identified one single-nucleotide polymorphism (SNP) with high allele frequency change and designed primers to genotype the full dataset of samples (two altitudinal transects) using Sanger sequencing.

Functional analyses of variants and protein structure

We annotated individual variants in regions of interest using *SnPEff* v5.1 (Cingolani et al., 2012) to check for mutations with known functional impact (missense, frameshift, splice-site, nonsense), and manually inspected alignments using *IGV* v2.12.3 (Thorvaldsdóttir et al., 2013). Since three missense variants of interest were identified in the gene *BMPER*, coinciding with the genomic region with the strongest evidence for selection (see Results section), this locus was characterized in more detail. We extracted *BMPER* transcript sequences from 27 squamate species and chicken from NCBI's "Gene" portal and aligned these with *MUSCLE* v3.8.425 (Edgar, 2004), available through *AliView* v1.28 (Larsson, 2014). Protein homology modeling was performed using the *SWISS-MODEL* web server (Guex et al., 2009; Waterhouse et al., 2018). To assess the functional impact of missense mutations we calculated *GRAVY* scores (grand average of hydropathy) for the "reference" and "alternative" amino acid sequences using *Sequence Manipulation Suite* v2 (Stothard, 2000), and modeled changes in protein stability ($\Delta\Delta G$) using *DDMut* (Zhou et al., 2023).

Results

Assembly and annotation of the *Gallotia galloti* genome

The genome assembly yielded a 1.18 Gb reference with a scaffold N50 of 58.15 Mb and contig N50 of 698.5 kb (Supplementary Table S2). This represents a near-chromosome assembly as the karyotype of this species has 20 chromosome pairs (Cano et al., 1984), and the 20 largest scaffolds in our assembly were all larger than 14 Mb each and corresponded to 85.4% of the total genome size (Supplementary Figure S1). The assembly was highly complete (93.7% of complete single-copy orthologs), and the annotation identified 18,713 protein-coding sequences (78.3% of complete single-copy orthologs in the annotation). When compared to a selection of other published lacertid genomes (Supplementary Figure S1), assembly scaffolding compares well to chromosome-level assemblies of *P. muralis*, *Z. vivipara*, and *L. agilis*, and contiguity was comparable to assemblies based on long-read sequencing like *P. muralis*. The genome completeness of our de novo assembly was comparable to all other genomes tested.

Reduced genetic differentiation between high and low-altitude populations

Re-sequencing of the four populations yielded a total of 632,659,131 short reads (Supplementary Table S3). After mapping and duplicate removal, an average of 14.6X coverage per pool was obtained, and these reads mapped to the reference genome with high mapping rates (~99%). We started analyses of these data by examining global patterns of genetic variation. Pairwise values of F_{ST} between the four populations, based on a total of 7,944,720 SNP, showed moderate differentiation between populations (F_{ST} between 0.072 and 0.100; Figure 2A). Importantly, no geographical pattern of stronger differentiation associated with altitude was recovered. Nucleotide diversity was similar across all populations (π between 0.69% and 0.66%; Figure 2B). Patterns of D showed a common tendency towards negative values in all populations, albeit showing some variability between them (Figure 2C). Additionally, the high-altitude population displayed a tendency towards more negative D values (possibly an effect of a recent population expansion after a bottleneck).

Signatures of selection for high-altitude adaptation

Genetic differentiation across the genome between low and high-altitude populations was reduced for most regions of the genome, as suggested in the global analyses (Supplementary Figure S2). As some of the few exceptions, two genomic regions with moderate differentiation were identified on scaffold12 ($F_{ST} = 0.137$) and scaffold17 ($F_{ST} = 0.175$) (Figure 3A). Results for π -ratio and ΔD also suggested overall reduced selection in high-altitude populations (Supplementary Figure S3). Outliers were few and had little overlap with the relative outliers of the F_{ST} scan, with the notable exceptions of the same two regions in scaffold12 (π -ratio = 1.944; $\Delta D = 0.347$) and scaffold17 (π -ratio = 1.858; $\Delta D = 0.823$). The correlation between the three statistics was weak (Spearman's correlation ρ ; F_{ST} - $\pi = 0.174$; F_{ST} - $D = 0.042$; π - $D = 0.142$; $p < 0.001$ for the three comparisons). The integration of these three statistics into DCMS data gave further support for a scenario of reduced overall selection, with few outliers relative to the genomic background (Figure 3B). Considering the top 0.1% windows ($n = 91$) as the genomic regions with the best evidence of selection, these were grouped into 13 intervals (Supplementary Table S4). The two intervals in scaffold12 (18 windows, 20% of the

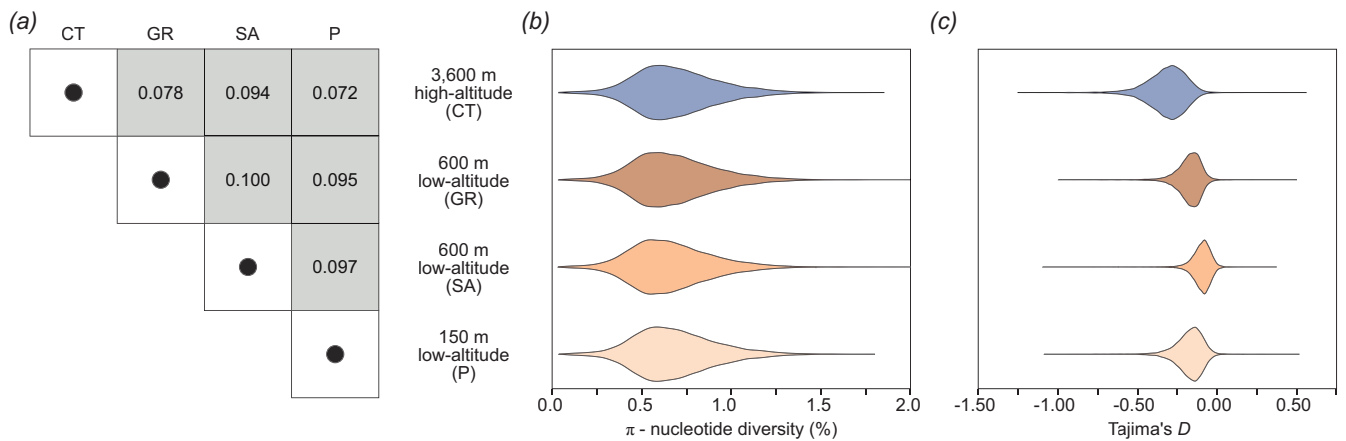


Figure 2. Population genomics of low and high-altitude populations of *Gallotia galloti*. (A) Pairwise genetic differentiation (F_{ST}) between pools of samples from the four populations used for genome re-sequencing. (B) Violin plots with the genomic distribution of values of nucleotide diversity (π , %) for the four populations (a small fraction of windows with values higher than 2% were discarded); and (C) Same as the previous panel, for Tajima's D . F_{ST} , π , and D were calculated in nonoverlapping windows of 100 kb.

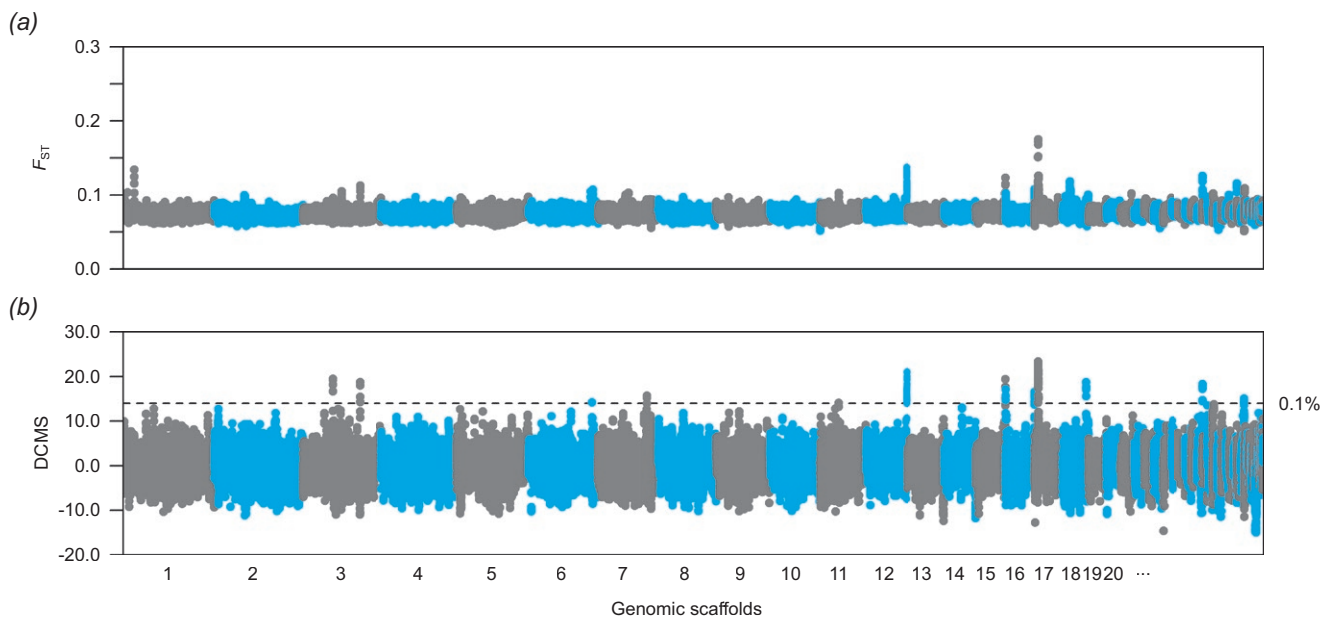


Figure 3. Signature of selection for high-altitude adaptation in *Gallotia galloti*. (A) Genetic differentiation (F_{ST}) between a high-altitude population and the combined data from three low-altitude populations. (B) De-correlated composite of multiple statistics (DCMS), calculated with values of F_{ST} , π -ratio, and ΔD . The dashed line corresponds to the threshold indicating the top 0.1% of windows in the distribution (windows above it were considered as representing strong evidence of selection). Each dot on the Manhattan plots represents one 50 kb window (windows were calculated with an overlap of 12.5 kb). Colors represent alternating genomic scaffolds; the first 20 scaffolds are numbered below the x-axis.

outliers) and scaffold17 (31 windows, 34% of the outliers) were the two most marked outliers in this analysis.

Individual genotyping along two altitudinal transects did not confirm an association for scaffold17; allele frequencies were variable between populations but with no altitudinal pattern (Spearman's correlation: $\rho = -0.024$, $P = 0.947$; [Supplementary Figure S4](#)). The genomic association could be an artifact driven by the particular populations that were used in those analyses. For scaffold12, we confirmed a significant correlation between allele frequencies and altitude (Spearman's correlation: $\rho = 0.841$, $P = 0.002$; [Figure 4A](#)). The pattern of association was not linear but rather driven by a steep cline in allele frequency occurring between populations at 1,350 m and 1,900 m (below and above the interval, populations showed similar patterns of allele frequency). The outlier at the terminal end of scaffold12 is likely to be in synteny

with the outlier at the terminal end of scaffold40, based on the *P. muralis* assembly ([Andrade et al., 2019](#)). These regions contained the genes *BMPER*, *FANCD2OS*, *NSPR1* (scaffold12), and *COP1* (scaffold40), with no other genes within a 100 kb range ([Figure 4B](#)). Differentiation was particularly important within the open reading frame of *BMPER*. *BMPER* is a regulator of bone morphogenetic proteins (BMPs) in endothelial tissues, fine-tuning the activity of BMPs in angiogenesis ([Heinke et al., 2008](#)), likely including the known role of BMPs in vascular remodeling as a response to pulmonary hypertension typical of chronic hypoxia ([Frank et al., 2005](#)).

Coding variation at the *BMPER* locus is likely not implicated in adaptation

Within the scaffold12 outlier, we identified 15 highly differentiated ($\Delta AF \geq 0.5$) intronic and intergenic variants ($\Delta AF = 0.500\text{--}0.624$);

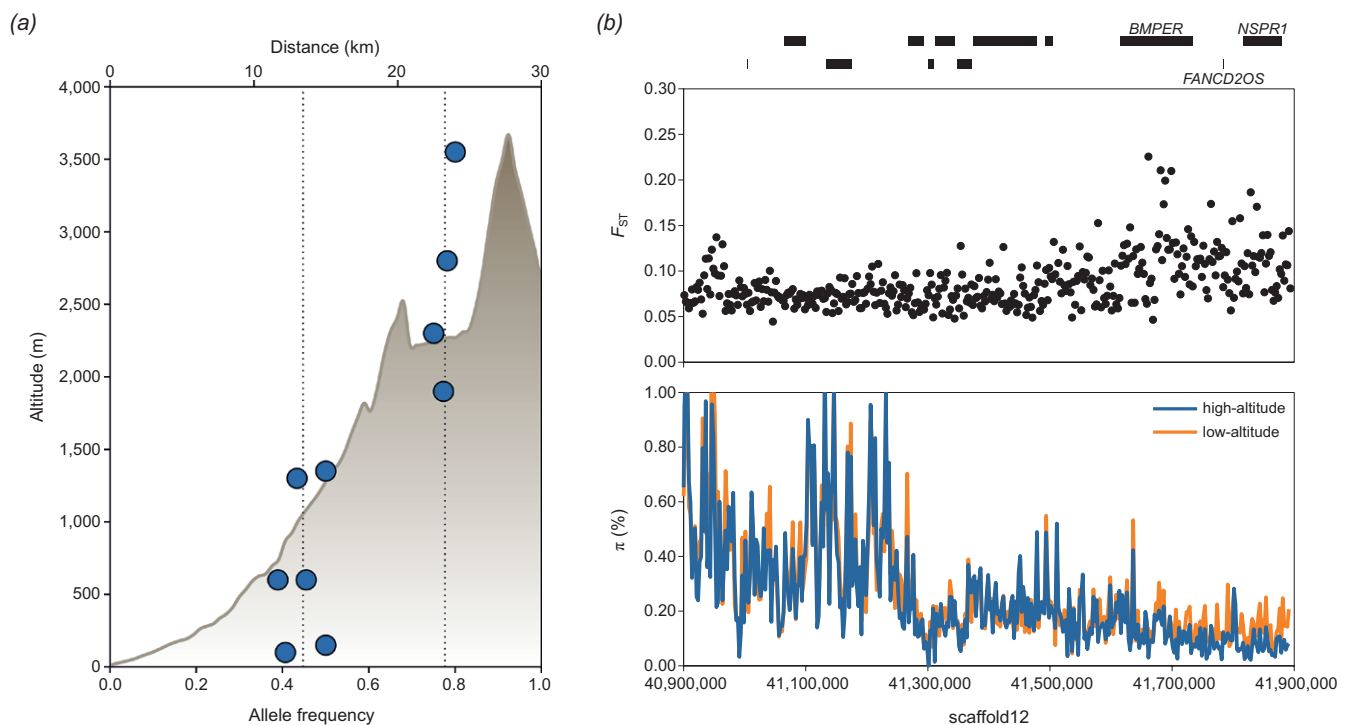


Figure 4. Selection for high-altitude adaptation overlapping *BMPER* in *Gallotia galloti*. (A) Allele frequencies in 10 populations for a single-nucleotide polymorphism in the candidate region, based on individuals sampled along the altitudinal gradient (topographic profile based on Figure 1). Each dot corresponds to the average allele frequency of one population. The two vertical dashed lines correspond to the average allele frequency values for each of the two main groups (below 1,350 m and above 1,900 m). (B) Patterns of F_{ST} and π for the outlier region in scaffold12, calculated in nonoverlapping 2.5 kb windows. Annotated genes in these regions are shown on top (gene names adapted from the human orthologs).

four other highly differentiated noncoding variants were found in the syntenic scaffold40 outlier ($\Delta AF = 0.520\text{--}0.600$). Additionally, we identified three missense variants overlapping *BMPER* with moderate allele frequency differences between high and low-altitude populations ($\Delta AF = 0.102\text{--}0.215$), which raises the possibility of a role for protein-coding variation in adaptation. An alignment of our annotated *G. galloti* *BMPER* coding sequence (2,538 bp, 14 exons) to *BMPER* transcripts from 27 other squamate genomes (plus a chicken transcript) suggests that two of these three missense variants reside within an exon, which is a potential annotation artifact (Supplementary Figure S5). Specifically, this exon did not have a homologous exon in any other species, and a protein structural model of the corresponding amino acid sequence had lower support (best model GMQE = 0.67; MolProbity Score = 2.18; template A0A6P9B210.1.A, *BMPER* of *Pantherophis guttatus*; Supplementary Figure S5) when compared to a second model of a translated coding sequence without this exon (best model GMQE = 0.82; MolProbity Score = 1.10; template A0A6P9B210.1.A, *BMPER* of *P. guttatus*; Supplementary Figure S5).

The remaining missense mutation ($\Delta AF = 0.102$) was located in the predicted final exon of the *G. galloti* *BMPER*. GRAVY scores between the reference (threonine) and alternative (alanine) alleles indicated that the alternative amino acid sequence is slightly more hydrophilic (GRAVY_{ref} = -0.36715 ; GRAVY_{alt} = -0.36733). Modeling of protein stability change between the two alleles indicated a trend for destabilization in the alternative allele ($\Delta\Delta G^{\text{Stability}}_{\text{ref}\rightarrow\text{alt}} = -0.03$ kcal/mol). However, for both statistics, the magnitude of the differences between reference and alternate alleles is very small. Supporting this, a model of the protein structure with the alternative allele had identical GMQE and MolProbity scores as the model based on the reference. The reduced protein structural

differences between alleles, together with the modest ΔAF of this mutation, argue against coding variation as a mechanism promoting adaptation through this locus.

Discussion

Hypoxic conditions are among the most physiologically demanding abiotic constraints in high-altitude habitats (reviewed in Pamerter et al., 2020). Our discovery of a strong selection signal overlapping a gene linked to responses to hypoxia is further evidence of the essential role these functions play in the colonization and survival of organisms at high altitudes. This is no surprise: above 2,000 m, atmospheric oxygen pressure drops to less than 80% of the normal sea level pressure and lower than 65% above 3,500 m (Chavala, 2018). When exposed to reduced O_2 availability, animals from low altitudes can alter their phenotype by increasing pulmonary ventilation, hemoglobin concentration, and heart rate (Banchemo, 1987; Storz & Scott, 2019). However, although plastic responses can short-term counteract hypoxia, prolonged hypoxic conditions (chronic hypoxia) induce serious pathologies that are often driven by some of these plastic responses themselves, such as high-altitude pulmonary edema and pulmonary hypertension (Storz & Scott, 2019).

BMPER, a regulator of vascular cell growth and proliferation, is thus an excellent candidate to mediate adaptation to evade chronic hypoxia in *G. galloti* populations living at high altitudes. This is an endothelial regulator of BMPs, which in turn are essential tissue “architects.” In mammals, *BMPER* is expressed in skin, heart, and lung tissue, where it is essential for BMP4-mediated angiogenesis (Heinke et al., 2008). *BMP4* itself has also been shown to be upregulated in lung tissue during hypoxia to

promote proliferation and remodeling of arterial smooth muscle (Frank et al., 2005; Wu et al., 2014), and BMP receptors have been shown to be implicated in pulmonary hypertension pathogenesis (Takahashi et al., 2007). Our genotyping of an SNP in this candidate region indicated that allele frequency changes in this locus show an abrupt shift for populations at altitudes between 1,350 and 1,900 m. Interestingly, Vinegar and Hillyard (1972) described that, in populations of the lizard *Sceloporus occidentalis* sampled across an altitudinal gradient between ca. 150 and 2,100 m in Arizona, oxygen-carrying capacity did not change linearly with altitude. Rather, this phenotype was similar within low-altitude and within high-altitude populations but with a significant shift in the 1,200–1,800 m range (although in other lizards, hematological variables may not vary predictably with altitude, e.g., González-Morales et al., 2017, 2023). Altogether, these results suggest that selection for increasing respiratory capacity may be common across ectotherms living in high altitudes and that in *G. galloti* this can be partially explained by selection on the vascular remodeling gene *BMPER*, likely mediated by noncoding variants that regulate the expression of this gene. Other genes within our candidate regions, such as *ENO1* (associated with resistance to hypoxia in pancreatic cancer cells; Wang et al., 2019) or *TPBG* (regulates the angiogenic activity of pericytes; Spencer et al., 2019) may play additional roles, but without further detailed physiological testing, genotyping, and functional genomic studies, their roles are less clear.

Our study provides evidence for altitudinal adaptation in an ectotherm, in line with previous findings in endotherms that hypoxia acts as the most demanding selection pressure in high-altitude environments. However, there are a variety of other abiotic stressors in these environments, such as UV, temperature, and water availability differences, for which we did not find obvious candidates. For example, increased UV exposure should (in principle) drive selection for increased pigmentation, but we did not find melanin or DNA repair genes in our outliers. This may occur because lizards actively regulate UV exposure, something that has been recently recognized (Conley & Lattanzio, 2022). It is, therefore, possible that high-altitude populations of *G. galloti* are able to respond through a combination of genetic adaptation, phenotypic plasticity in morphology (shifts in growth), behavior (behavioral thermoregulation and UV-regulation), physiology (hydroregulation, metabolic rates), or other mechanisms (Bels & Russell, 2019; Muñoz, 2021; Serén et al., 2023). This is consistent with previous findings in this species (Gilbert et al., 2024; Serén et al., 2023). Genetic adaptation and phenotypic plasticity thus likely play complex and interdependent roles in mediating responses to high abiotic stress in ectotherms.

Supplementary material

Supplementary material is available online at *Evolution Letters*.

Data and code availability

Raw 10X Genomics Chromium sequencing data and whole-genome pool-sequencing data are available in the Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) under BioProject PRJNA833592.

Author contributions

N.S., C.P., A.Ž., and M.C. designed the study. N.S., R.M.P., P. Aguilar, A.Ž., and M.C. conducted fieldwork and performed experiments.

N.S., C.P., and P. Andrade analyzed data. N.S. and P. Andrade wrote an initial draft of the manuscript. All authors reviewed and approved the final version of the manuscript.

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