UC Berkeley
UC Berkeley Previously Published Works

Title

Multi-locus genomic signatures of local adaptation to snow across the landscape in California populations of a willow leaf beetle

Permalink

https://escholarship.org/uc/item/8dq3568k

Journal

Proceedings of the Royal Society B, 290(2005)

ISSN

0962-8452

Authors

Keller, Abigail G
Dahlhoff, Elizabeth P
Bracewell, Ryan
et al.

Publication Date

2023-08-30

DOI

10.1098/rspb.2023.0630

Supplemental Material

https://escholarship.org/uc/item/8dq3568k#supplemental

Copyright Information

Peer reviewed

1   **Multi-locus genomic signatures of local adaptation to snow across the landscape in**

2   **California populations of a willow leaf beetle**

3

4   **Authors:** Abigail G. Keller[1], Elizabeth P. Dahlhoff[2], Ryan Bracewell[3], Kamalakar Chatla[1], Doris

5   Bachtrog[1], Nathan E. Rank[4], Caroline M. Williams[1]

6

7   [1]Department of Integrative Biology, University of California, Berkeley, California, USA

8   [2]Department of Biology, Santa Clara University, Santa Clara, California, USA

9   [3]Department of Biology, Indiana University Bloomington, Bloomington, Indiana, USA

10 [4]Department of Biology, Sonoma State University, Rohnert Park, California, USA

11

12 **Corresponding Author:**

13 Abigail G. Keller, Department of Integrative Biology, University of California, Berkeley,

14 California, USA; email: agkeller@berkeley.edu

15

17   **Abstract**

18   Organisms living in mountains contend with extreme climatic conditions, including short

19   growing seasons and long winters with extensive snow cover. Anthropogenic climate change is

20   driving unprecedented, rapid warming of montane regions across the globe, resulting in reduced

21   winter snowpack. Loss of snow as a thermal buffer may have serious consequences for animals

22   overwintering in soil, yet little is known about how variability in snowpack acts as a selective

23   agent in montane ecosystems. Here we examine genomic variation in California populations of

24   the leaf beetle *Chrysomela aeneicollis*, an emerging natural model system for understanding how

25   organisms respond to climate change. We used a genotype-environment association approach to

26   identify genomic signatures of local adaptation to microclimate in populations from three

27   montane regions with variable snowpack and a coastal region with no snow. We found that both

28   winter-associated environmental variation and geographic distance contribute to overall genomic

29   variation across the landscape. We identified non-synonymous variation in novel candidate loci

30   associated with cytoskeletal function, ion transport and membrane stability, cellular processes

31   associated with cold tolerance in other insects. These findings provide intriguing evidence that

32   variation in snowpack imposes selective gradients in montane ecosystems.

Introduction

Seasonality serves as one of the strongest and most ubiquitous sources of environmental variation impacting natural systems, with distinct selective forces operating between periods of summer growth and reproduction and overwintering survival (Fretwell, 1972; Williams et al., 2017). For small montane ectotherms, elevated and variable air temperatures during summertime can cause physiological stress during critical periods of reproduction, growth, and development (Dahlhoff et al., 2008; McMillan et al., 2005; Neargarder et al. 2003). As hotter, drier summers become more common, upslope shifts in montane insect species are becoming more frequent, posing novel challenges at the limits of physiological tolerance (Larsen, 2012; Moret et al., 2016). For organisms that overwinter beneath the soil, snow cover is a key environmental factor influencing physiology and survival because snow buffers microclimate variability (Pauli et al., 2013; Slatyer et al., 2022; Zhu et al., 2019). Climate change is causing more prevalent, intense, and lengthy droughts, which in turn leads to more winters with a higher elevation snowline and lower total snowpack (Huning & AghaKouchak, 2020; Mote et al., 2018). Reductions in snowpack may expose organisms overwintering in the soil to temperature extremes that cause physiological stress, reducing their overwintering survival and reproductive success at subsequent summer emergence. Recent declines in insect populations in montane environments documented across the globe demonstrate the urgency in gaining a clear understanding of how organisms cope with greater seasonal variability in temperature and precipitation in montane ecosystems (Birrell et al., 2020; Halsch et al., 2021; Shah et al., 2020). Seasonal fluctuation can maintain genetic polymorphisms within populations (Haldane & Jayakar, 1963; Wittmann et al., 2017), and variation in the extent and magnitude of seasonal fluctuations can generate spatial

55   clines in allelic variants (Conover, 1992; Rank & Dahlhoff, 2002; Rhomberg & Singh, 1986).

56   Elucidating how past climatic conditions have structured genetic variation and corresponding

57   physiological responses for organisms in these habitats will be critical for predicting their

58   responses to future environmental change.

59   Local adaptation, which occurs when resident genotypes have a higher relative fitness in

60   their local habitat than genotypes originating from other habitats, is an important mechanism by

61   which genetic variation is maintained in heterogeneous environments (Felsenstein, 1976;

62   Hedrick et al., 1976; Kawecki & Ebert, 2004). The extent and persistence of local adaptation is

63   determined by a balance between natural selection for alleles that confer improved reproductive

64   success in a particular microclimate and the homogenizing effects of gene flow and other neutral

65   processes (Forester et al., 2016; Kawecki & Ebert, 2004; Nadeau et al., 2016; Orsini et al., 2013;

66   Slatkin, 1987). Neutral processes that influence patterns of genetic variation among populations

67   include dispersal rates, colonization history, and population expansion and contraction, which in

68   turn affects levels of genetic drift (Nadeau et al., 2016; Orsini et al., 2013). Local adaptation may

69   be detected by identifying a stronger genetic variant 'signal' from weaker, non-selective 'noise'

70   (Shafer & Wolf, 2013; Wang & Bradburd, 2014). Unfortunately, selective climatic gradients,

71   geography, and migration corridors tend to covary, which complicates quantifying the relative

72   contribution of selective and neutral evolutionary forces; thus, effects of isolation by distance and

73   population structure must be taken into account before patterns of genomic variation can be

74   associated with selective features of the environment (Forester et al., 2018a; Frichot et al., 2013;

75   Rellstab et al., 2015; Sork et al., 2013).

76   In this study, we investigated relationships between microclimatic factors and genetic

77   variation in the willow leaf beetle *Chrysomela aeneicollis*, a well-described model species for

78   understanding how climate change impacts montane ecosystems (Dahlhoff et al., 2019; Millstein,

79   2006; Rank & Dahlhoff, 2002). This insect is ideal for investigating processes of local adaptation

80   in a region of high topographic and seasonal landscape heterogeneity (Camus, 2020). During the

81   brief summer growing season, this univoltine beetle species mates, lays eggs, and undergoes one

82   generation of larval development before new adults emerge and feed before winter returns

83   (Smiley & Rank, 1986). They overwinter in the soil as freeze-tolerant adults for eight to nine

84   months before emergence of reproductively mature adults (Boychuck et al., 2015; Roberts et al.,

85   2021).

86   In western North America, *C. aeneicollis* is found living on willows in cool, moist

87   habitats separated by regions of arid or Mediterranean climates, resulting in highly fragmented

88   distribution with little connectivity among populations (Brown, 1956; Dellicour et al., 2014). In

89   California, this species inhabits regions with distinct microclimate and seasonal characteristics:

90   along high-elevation (2700 - 3400 m) streams and bogs in the Sierra Nevada, in isolated montane

91   populations on the edge of the Great Basin, and in low elevation riparian habitats along the

92   Northern California coast. Within the Sierra Nevada, populations experience stressfully warm

93   and cold temperatures throughout the year and their distribution is affected by seasonality and

94   elevation, with populations contracting upslope and declining in abundance during droughts and

95   growing in size and expanding to lower elevations after wet, snowy winters (Dahlhoff et al.,

96   2019; McMillan et al., 2005; Rank, 1994; Rank et al., 2020; Roberts et al., 2021; Smiley &

97   Rank, 1986). Despite these fluctuations in population size, Sierra Nevada populations have

98   maintained high levels of heterozygosity at protein coding genes and other loci and show no

99   deviation from Hardy Weinberg expectations with respect to expected versus observed genotype

100   frequencies (Rank 1992a, Rank et al. 2020), suggesting that they are sufficiently large to avoid

101   bottlenecks and effects of inbreeding. Montane populations show evidence of substantial, stable

102   genetic differentiation along a 60 km latitudinal gradient, from the South Fork of the Kings River

103   in the south to Rock Creek in the north, with especially high divergence at mitochondrial loci

104   and the metabolic enzyme locus *phosphoglucose isomerase*, *Pgi* (Dahlhoff et al., 2008; Rank,

105   1992a; Rank et al., 2020; Rank & Dahlhoff, 2002). Prior laboratory and field studies have also

106   shown that effects of temperature on performance and fitness components vary among

107   individuals with different nuclear and mitochondrial variants (Camus, 2020; Dahlhoff et al.,

108   2019; Dahlhoff & Rank, 2000; Dick et al., 2013; Rank et al., 2020). While extensive studies

109   support the hypothesis that variation at metabolic loci such as *Pgi* and the mitochondrion reflect

110   local adaptation (Bracewell et al., 2023; Dahlhoff et al., 2008; Dahlhoff & Rank, 2000; Rank &

111   Dahlhoff, 2002), we lack information about how variation throughout the genome reflects the

112   complex interaction of neutral and adaptive processes across the beetle's range.

113   Here, we address this gap by evaluating relationships between genomic variation and

114   environmental conditions in locations where willow beetle populations occur in four distinct

115   ecoregions of California (Griffith et al., 2016). We quantified differentiation at nuclear loci

116   among populations in three montane regions in Eastern California and populations in an isolated

117   coastal area; this sampling design covers all known regions within California where this species

118   is currently known to occur (Brown, 1956; Dellicour et al., 2014). We identified selective

119   microclimatic gradients that contribute to spatial patterns of potentially adaptive genomic

120 variation across the landscape, then used this information to predict functions of newly identified

121 genes that vary along microclimatic gradients to examine how genomic differentiation among

122 these regions may contribute to local adaptation.

123

Results

Sequencing and marker filtering

124

125

126 Illumina sequencing generated 5.06 billion paired-end reads from 175 individuals in 12

127 populations (Table 1), of which 4.05 billion total reads (80.1%) passed initial quality filters (per

128 sample: mean = 23.1 million, sd = 7.9 million). The joint genotype calling workflow identified

129 12 million hard-filtered biallelic SNPs (Tables S1, S2). We then used a conservative SNP

130 filtering approach based on minor allele frequency (MAF), heterozygosity, and inbreeding

131 coefficient, resulting in 22,323 SNPs across all individuals and 12 populations. Filtering

132 thresholds that contributed substantially to the reduced set of analyzed SNPs were those that

133 removed SNPs with a MAF < 0.01 (Table S2B) and that removed loci with low quality reads

134 within populations (Table S2C). These SNPs were distributed evenly across the nuclear genome

135 (Table S1).

Microclimate simulation

136

137 The NicheMapR microclimate model simulated 24 variables for the 12 beetle populations that

138 represent air, soil, and snow conditions beetles experience throughout their lifecycle (Table S3).

139 Simulated environmental variables demonstrated high sensitivity to the shade input parameter in

140 the model (Fig S1), but relative multivariate environmental distances between populations were

141 consistent between minimum and maximum shade conditions (Fig S2). Simulated microclimatic

142 data under minimum shade conditions were more concordant with available empirical

143 measurement based on RMSE (Table S4), so downstream analyses were therefore conducted

144 using simulated environmental variables under 10% shade.

145 *Population genomic differentiation across California landscape*

146 The first two principal components on population-level minor allele frequencies explained 55.8%

147 of total genomic variation (Fig 1B). Eastern Cascade and Coast Range ecoregions exhibited the

148 greatest genomic divergence among populations, and population genomic variation in the Sierra

149 Nevada and Central Basin ecoregions followed a latitudinal gradient (Fig 1; Table S5). SNP

150 filtering thresholds used in analyses did not meaningfully influence estimates of population

151 structure compared to more relaxed filter thresholds (Fig S3-S5).

152   A population genetic structure analysis was used to estimate proportions of individual

153 genomes originating from ancestral gene pools based on the five populations determined by

154 selecting a value of K that minimized cross entropy (Fig S6). Individuals in the Sierra Nevada

155 ecoregion show a strong pattern of genetic differentiation with latitude (Fig 1). Based on

156 proportions of estimated ancestral coefficients, individuals in the southern drainage Tuttle Creek

157 (TC) are genetically distinct and belong to one ancestral population. Individuals in Taboose Pass

158 (TP) are mixed, sharing ancestry with neighbors in Tuttle Creek to the south, Big Pine (BP) and

159 Baker (BK) Creek to the north. Individuals in South Bishop Creek (BC) and Tyee Lakes (TL)

160 share ancestry with both southern (BP, BK) and northern (NF, PC, RC) populations, which in

161 turn share ancestry with those from the Great Basin (DC). Individuals collected in Eastern

162 Cascades and Coast Range ecoregions were genetically distinct from each other and from the

163 Sierra Nevada-Great Basin complex (Fig 1).

164   Analysis of pairwise F_{st} values among population pairs revealed that populations in the

165 montane Eastern Cascades region were more similar to montane populations in the Sierra

166 Nevada and Great Basin than they were to Coast Range populations, despite similar geographic

167 distances separating each region (Fig 2). When populations were classified by habitat type

168 (coastal or mountain), F_{st} values for "coast vs. mountain" population pairs were four-fold greater

169 (LSM = 0.43 $\pm$ 0.02) than those for "mountain vs. mountain" population pairs (LSM = 0.11 $\pm$

170 0.01, $F_{1,63}$ = 197.4, P < 0.001; Fig 2). The overall relationship between geographic distance and

171 F_{st} was similar within the two types of population pairs and was consistent with 'isolation by

172 distance' genetic differentiation ($F_{1,63}$ = 24.5, P < 0.001; Fig 2). Together, these results suggest

173 that isolation by distance and isolation by environment (coastal versus montane) both shape

174 genomic differentiation, and differences in environmental conditions appear to strongly influence

175 genetic composition of *C. aeneicollis* populations.

176 *Associations between environmental and genomic variation*

177 *Partial redundancy analysis (pRDA)-* The pRDA made it possible to identify specific genetic

178 polymorphisms that were associated with environmental differences among populations. Among

179 all California populations, the pRDA was globally significant ($F_{2,7}$ = 2.18, P = 0.001; Fig 3), and

180 the constraining environmental matrix explained 17.1% of variation in genomic data, while the

181 conditioning spatial matrix explained 19.0% of genomic variation. The forward selection

182 procedure identified a significant positive spatial variable (MEM1), which was retained as

183 conditioning variable in pRDA. The forward selection procedure identified annual air

184 temperature range at 1.75 m above ground level (annual T_{max} –T_{min}) and maximum daily snowfall

185 as significant environmental predictors of genomic variation (Fig 3; annual air temperature range

186 $F_{1,7}$ = 2.28, P = 0.001; maximum daily snowfall $F_{1,7}$ = 2.08, P = 0.001). The first and second

187 RDA axes also explained substantial proportions of genomic variation (RDA1 = 18.7%, $F_{1,7}$ =

188 2.32, P = 0.014; RDA2 = 16.6% $F_{1,7}$ = 2.05, P = 0.022). Candidate single nucleotide

189 polymorphisms (SNPs) were identified based on high correlation with temperature- and snow-

190 related environmental variables (r > |0.65|) and z-score values of loadings of loci in ordination

191 space (z scores ± 2.1, two-tailed P = 0.036). Based on these criteria, 107 SNPs were identified as

192 candidate loci (Fig 3). Sixty-eight SNPs were related to annual air temperature range, thirty-

193 seven to maximum daily snowfall, and two were related to both temperature and snowfall (Table

194 S6). When the coastal Gualala River population was excluded, the pRDA was globally

195 significant ($F_{1,8}$ = 2.04, P = 0.021; Fig S7), and with MEM1 as the conditioning variable, the

196 forward selection procedure identified only maximum daily snowfall as a significant predictor of

197 genomic variation. Using the above candidate loci criteria, 116 SNPs were related to maximum

198 snowfall (Table S9).

199 *Latent factor mixed model (LFMM)-* The LFMM represents a second approach to identify SNPs

200 related to environmental variability while accounting for overall population genetic structure.

201 With all California populations, a LFMM was run with five estimated ancestry coefficients as

202 latent factors to test single-locus relationships with annual air temperature range and maximum

203 daily snowfall (ancestry coefficients shown in Fig 1). A large proportion of identified

204 polymorphisms (19.2%; 4,289 SNPs) were associated with annual air temperature range, and

205 7.2% (1,603 SNPs) were associated with maximum daily snowfall (Fig 4). Using the LFMM

206 excluding the coastal Gualala River population and four ancestral populations, 1,471 SNPs

207 (6.6% total) were associated with maximum snowfall (Table S9, Fig S8).

208 *SNP and protein functional annotations*

209 To reduce probability of false positive associations and narrow the search for candidate

210 polymorphisms, we focused on SNPs identified by both pRDA and LFMM, and we assessed

211   associations across coastal and montane populations and then again using only montane

212   populations (Tables 2, S6, S9). In analyses with all populations, most SNPs correlated with

213   annual air temperature range in pRDA were also identified using LFMM (67 of 70). A slightly

214   lower proportion of SNPs associated with maximum daily snowfall based on pRDA were also

215   identified by LFMM (26 of 39; Table S6). In analyses with only montane populations, most

216   SNPs correlated with maximum daily snowfall based on pRDA were also identified by LFMM

217   (79 of 116), and 18 SNPs identified in the pRDA with only montane populations were also

218   identified in analyses with coastal and montane populations (Table S9).

219   Analyses of all populations and only montane populations identified three non-

220   synonymous SNPs associated with maximum daily snowfall found in genes coding for proteins

221   involved in cell structure and movement (inverted formin-2 and microtubule-actin cross-linking

222   factor). Analyses including all populations or only montane populations identified five non-

223   synonymous SNPs associated daily snowfall that are found in genes coding for proteins involved

224   in ion transport or cellular membrane activity (Table 3, S8A, S11A). Three non-synonymous

225   SNPs associated with air temperature range across all populations were found in genes coding

226   for proteins involved in intracellular signaling and energetics [cytochrome p450, phospholipid

227   transfer protein, (Table 3)].

228

Discussion

Detecting accurate signals of local adaptation in the genome requires linking observed genetic patterns to underlying selective features of the environment while accounting for associations imposed by neutral processes. Here we demonstrate that populations of the willow leaf beetle *Chrysomela aeneicollis* across California are differentiated across the nuclear genome, and we provide strong evidence that snow serves as a prominent selective gradient and driver of local adaptation across their geographic range. We show that both large-scale variation in snowfall across the California landscape and small-scale variation in snowfall within montane locations are associated with adaptive genetic variation. Specifically, we provide evidence that variation in maximum daily snowfall is linked to non-synonymous polymorphisms in genes associated with cytoskeletal motility, ion transport, and membrane structure and function, highlighting the potential role of adaptive protein modifications that could enhance insect cold tolerance in cold snowy regions.

Spatial patterns of genetic divergence in Chrysomela aeneicollis

Results of this study reveal that populations of the willow leaf beetle *Chrysomela aeneicollis* living in different regions of California are genetically differentiated across loci in the nuclear genome. Three of four ecoregions sampled show substantial levels of genetic divergence among them, such that North Coast populations are distinct from those in the Eastern Cascades ecoregion, and both of those populations are distinct from populations in the Sierra Nevada and Great Basin (Fig 1). Within Eastern California, populations from northern drainages of the Eastern Sierra Nevada are less genetically isolated from populations sampled in the neighboring White Mountains than those found in southern drainages of the Sierra Nevada. Furthermore,

251 consistent with previous studies, populations within the Sierra Nevada show relatively high

252 levels of genetic divergence given their relatively close geographic proximity (Fig 1) (Dahlhoff

253 et al., 2008; Rank, 1992a; Rank et al., 2020). Patterns of genetic differentiation separating

254 populations in different ecoregions suggest that geographic and seasonal environmental variation

255 present a major selective pressure on alleles in the nuclear genome and that genes related to

256 thriving under different local environmental conditions contribute to local adaptation among

257 populations of *C. aeneicollis* (Fig 2).

258   The strong pattern of geographic differentiation of genomic variation across California

259 was illustrated for the first time in the present study, but it is consistent with findings of Dellicour

260 et al. (2014). The earlier study found that *C. aeneicollis* populations in Western North America

261 (Montana, coastal Oregon, Colorado, and California) were strongly differentiated at

262 mitochondrial and nuclear genetic markers, suggesting that geographic isolation among these

263 regions predates recent fluctuations in the extent of glaciation over the past 50,000 years.

264 Isolation of populations at mitochondrial loci was greater than nuclear genes, but there was

265 overall agreement among loci that differentiation among geographic regions was substantial,

266 which would contribute to conditions favoring local adaptation (Dellicour et al., 2014). To date,

267 this study provides the best picture of signatures of adaptation to seasonal variation in this wide-

268 ranging insect.

269 *Maximum daily snowfall variation contributes to adaptive genetic variation*

270 Identifying climatic variables that act as drivers of spatially varying selection will be critical for

271 predicting evolutionary responses to climate change and environmental disturbance (Bay et al.,

272 2018). Among all simulated microclimate conditions that represent air, soil, and snow conditions

273 throughout the year, we found that maximum daily snowfall explains a significant portion of

274 variation in genomic data, after controlling for spatial autocorrelation and population history (Fig

275 3, Fig S7). This association is identified both across the California landscape, where climatic

276 conditions differ greatly between coastal and montane populations, as well as within montane

277 populations, where differences in climatic conditions are more subtle. Comparisons made within

278 montane populations suggest that this snowfall gradient may characterize spatially varying

279 selective pressures related to winter cold exposure within mountain ecoregions (Fig S9). Eco-

280 physiological models for *C. aeneicollis* indicate that the relationship between elevation and cold

281 exposure in soil is strongly non-linear, with cold exposure peaking at mid-elevation montane

282 populations that are not buffered by persistent snow cover (Roberts et al., 2021). Since snow

283 decouples the relationship between air and soil temperatures, variation in snow cover reflects

284 variation in cold exposure in the soil at a given elevation. Without the thermal buffer that snow

285 provides for organisms overwintering in soil, cold microclimate temperatures can drop below a

286 species-specific cold tolerance threshold, which can result in mortality or sublethal cold injuries

287 (Bale, 1996; Sinclair et al., 2003). This result highlights the importance of snow cover variation

288 as a key factor in maintaining this variation and driving selection on genes associated with cold

289 tolerance and stress in winter.

290   Prior work in Sierra Nevada populations of *C. aeneicollis* shows that air temperature

291   varies between genetically differentiated populations and shows evidence of physiological

292   adaptation to different thermal regimes (Dahlhoff & Rank, 2000; 2007; Rank et al., 2008).

293   However, in the present study, the effect of 'annual air temperature range' (Fig 3) is largely

294   driven by climatic conditions in the Coast Range (CR). This broad thermal selective gradient

295   covaries with neutral patterns of population structure, which complicates distinctions between

296   neutral and selected loci (Nadeau et al., 2016); thus, the high detection rates observed in the

297   LFMM in the present study could also be due to residual, unaccounted population structure (Fig

298   4).

299   *Putative mechanisms of local adaptation to snow cover mirror mechanisms of cold tolerance*

300   Genes containing nonsynonymous SNPs associated with variation in snowfall encode proteins

301   with functions related to ion binding, actin and cytoskeleton binding and organization, and

302   membrane components; protein identifications were assigned with a high level of confidence, as

303   all homologous proteins are present in other beetle species (Table 3). These protein functions

304   align with previously identified mechanisms of cold tolerance and acclimation in both insects

305   and plants (Des Marteaux et al., 2018; Kim et al., 2006; Örvar et al., 2000; Pokorná et al., 2004).

306   Primary cellular challenges associated with deep and prolonged cold exposure or freezing

307   include loss of ion and water homeostasis and depolymerization of cytoskeletal components (e.g.

308   actin and tubulin), which can impair ion transport function, cause loss of cell junction integrity,

309   and exacerbate disturbances in membrane integrity caused by paracellular leaks of water and

310   ions (Cantiello, 1995; Khurana, 2000; Toxopeus & Sinclair, 2018; Turner et al., 1997). Cold-

311   acclimated insects are better able to maintain ion and water balance at low temperatures

312 compared to warm-acclimated insects (Overgaard & Macmillan, 2017), due to cellular structural

313 modifications that enhance cytoskeletal stability, thus protecting ionoregulatory tissues (e.g.

314 Malpighian tubules in insects) from chilling injury and loss of transport function (Des Marteaux

315 et al., 2018). Cold-acclimated insects also differentially regulate cytoskeletal gene expression,

316 with cold acclimation inducing upregulation of actin-associated genes or enzymes that promote

317 membrane and cytoskeletal remodeling (Des Marteaux et al., 2017; Kim et al., 2006; Toxopeus

318 et al., 2019). Because polymorphisms associated with variation in snowfall may relate to protein

319 modifications that enhance cytoskeletal and membrane stability in the cold, putative mechanisms

320 underlying local adaptation to snow are related to primary cellular mechanisms of cold

321 acclimation and tolerance. These results provide genomic evidence that variation in snowfall

322 imposes a selective gradient in exposure to cold stress, supporting the theory that snow

323 modulates cold stress and exposure for insects that overwinter in the soil (Roberts et al., 2021).

324 *Tandem genotype-environment association approach identifies signatures of local adaptation*

325 In detecting genomic signatures of local adaptation, genotype-environment associations

326 identified by various methods will depend strongly on demographic and sampling scenarios

327 (Forester et al., 2018; M. R. Jones et al., 2013; Nadeau et al., 2016; Rellstab et al., 2015).

328 Simulations conducted by Forester et al. 2016 find that multivariate ordination methods like

329 pRDA produce uniformly low false positive rates (0-2%), whereas LFMM produced high false

330 positive rates under low dispersal scenarios (Forester et al., 2016). *Chrysomela aeneicollis*

331 individuals have low levels of dispersal, with individuals often spending most of their life on a

332 single host plant (Rank, 1992b, 1994).

333   Nonetheless, correcting for population structure in pRDA can result in low power to

334   detect true associations (Lotterhos, 2023), and recent simulation modeling indicates that LFMM

335   provides the best compromise between detection power and error rates in situations with

336   complex hierarchical neutral genetic structure (De Villemereuil et al., 2014). Herbivorous insects

337   can have a subdivided population structure that reflects the distribution of their plant hosts

338   (Moraiti et al., 2014; Orrest & Thomson, 2011), and previous work found hierarchical,

339   subdivided genetic structure among patches and willows within a patch (Rank, 1992a). The

340   application of these two GEA methods highlights the trade-off between conservative and liberal

341   approaches in detecting a true adaptive signal, yet applying these methods in combination can

342   therefore yield increased confidence in true positive detections of local adaptation. Future work

343   should investigate the relationship between non-clinal allele frequency patterns and

344   environmental gradients, which can evolve under multivariate environments and can lead to

345   inaccurate inferences using GEA approaches (Lotterhos, 2023).

346   *Limitations*

347   A limitation of this study is sampling bias toward populations in the Sierra Nevada

348   ecoregion relative to the other three eco-regions included in this study (Fig 1), which may bias

349   genetic-environmental relationships and relative contributions of isolation by environment and

350   distance (Fig 2). Replicated sampling along environmental gradients increases confidence in true

351   positive detections of genotype-environmental associations (Rellstab et al., 2015), yet the

352   beetle's fragmented distribution in California limits replication across climatic conditions.

353   Another potential limitation is that temporal coverage of sampling was limited to one year in all

354   but the Sierra Nevada (SN) Ecoregions (Table 1), so that allele frequencies in these populations

355   may be influenced by environmental conditions in the collection year. Prior studies suggest that

356   genetic variation among SN, CB and CR populations has remained relatively stable since we last

357   sampled and analyzed them (Dellicour et al., 2014). We therefore expect that patterns reported

358   here reflect adaptation to long-term environmental conditions due to the geographic isolation

359   among populations.

360   Additionally, stringent SNP filter thresholds were applied to ensure quality genotypes

361   within each population, resulting in a relatively modest set of polymorphisms ($N = 22{,}323$

362   SNPs). While these thresholds did not alter overall estimates of population structure (Figs S3-

363   S5), candidate SNPs associated with environmental gradients in this study likely represent a

364   subset of loci involved in local adaptation.

365   *Conclusion*

366   Many montane species live on the periphery of both suitable habitat and physiological tolerance,

367   which contributes to the unique sensitivity of montane populations to climate change. Even small

368   environmental changes may result in large implications for survival and reproductive success

369   (Dahlhoff et al., 2019; Pepin et al., 2015; Stewart et al., 2017). The willow leaf beetle has

370   emerged as a natural model for analyzing the relationship between adaptive genetic variation and

371   environmental change (Dahlhoff et al., 2019; Dahlhoff & Rank, 2000; Rank, 1992a; Rank et al.,

372   2020b). By analyzing all known Californian *C. aeneicollis* populations across the nuclear

373   genome, this study represents the broadest investigation of adaptive genetic variation in the

374   species to date and provides a path forward for understanding the evolutionary significance of

375   variation at genes associated with response to environmental stress. Future work should identify

376   regions where genetic–environmental relationships will be most likely disrupted by climate

377   change and reduced snowfall, which will be critical for land management decisions and gene

378   conservation in vulnerable populations (Shaffer et al., 2022).

379

Methods

Study populations and sampling design

Ecoregions were identified following United States Geological Survey (USGS) designations (Griffith et al., 2016). Beetle populations from the Sierra Nevada ecoregion were surveyed at winter snowmelt (May-June) from 1996-2016, following methods detailed in Dahlhoff et al. 2019 (Appendix 1.1). In all, 175 individuals from 54 sampling locations were included and assigned *a priori* to 12 populations (Table 1, Fig 1) based on previous work (Dellicour et al., 2014a; Rank, 1992a; Rank et al., 2020a). These represent all known populations in California, and they experience a wide range of seasonality, snow cover, and air temperature variation, especially between montane and coastal regions (Table S3). Though allele frequencies can fluctuate within a beetle population across years (Rank & Dahlhoff, 2002), the magnitude of these fluctuations is relatively small compared to the magnitude of genetic divergence among regions (Dahlhoff et al., 2008; Dellicour et al, 2014; Rank et al., 2020).

DNA Library preparation and processing of genomic sequencing data

Genomic DNA was extracted from individual beetles using NucleoMag Bacteria DNA Isolation kit (Macherey-Nagel, Düren, Germany), and whole genome libraries were prepared following the plexWell library preparation protocol by the CCGP MiniCore. Paired-end sequencing (2 x 150 bp) was performed on an Illumina HiSeq4000 platform at UC Berkeley's QB3 Genomics Core Facility (Berkeley, CA, USA). Nextera adapter sequences and low-quality bases (base quality < 15, sliding window 4 bp) were removed from each read using Trimmomatic v0.39 (Bolger et al., 2014). Reads were aligned to a *Chrysomela aeneicollis* reference genome (Bracewell et al., 2023) using the Burrows-Wheeler Aligner (BWA-MEM) algorithm (Li &

402   Durbin, 2009). Joint genotyping was performed on all samples using Genome Analysis Toolkit

403   (GATK) v4.2.6.0 functions HaplotypeCaller and GenotypeGVCFs (Poplin et al., 2017). Variant

404   data were filtered to include only biallelic SNPs, and SNPs were hard-filtered using GATK best-

405   practice recommendations (Van der Auwera & O'Conner, 2020) (Table S2A). SNPs were

406   removed if minor allele frequency (MAF) across all individuals was less than 0.01 or if

407   heterozygote frequencies deviated greatly from Hardy Weinberg expectations (e.g., excess

408   heterozygosity or inbreeding coefficient greater than ±0.5) (Table S2B). Finally, SNPs were

409   retained if 70% of all samples and 70% of samples within each population showed a read depth

410   between three and 30 and a genotype quality greater than 20 [Table S2C; (Xuereb et al., 2018)].

411   After filtering, principal components analysis (PCA) was performed on Hellinger-transformed

412   population-level minor allele frequencies (Legendre & Gallagher, 2001; Xuereb et al., 2018).

413   Because variant filter thresholds influence estimates of population structure (Linck & Battey,

414   2019; Pearman et al., 2022), we assessed sensitivity of genetic differentiation to filter threshold

415   levels.

416   *Microclimate variable simulation*

417   To obtain spatially explicit environmental variables representing local microclimate conditions

418   across the life cycle, microclimate simulations were conducted for the 12 beetle populations

419   using the biophysical modeling package *NicheMapR* (Kearney & Porter, 2017). The model

420   computes microclimatic conditions at a defined distance above ground, given local habitat

421   properties and weather conditions. The microclimate model was run using historical gridded

422   weather data from the GRIDMET daily weather database with 5 x 5 km resolution (Abatzoglou,

423   2013). The mid-latitude, -longitude, and -elevation of all demes within each population were

424 used as input in the model (Table 1, S2). The microclimate model was run in soil moisture and

425 snow modes under both minimum (10%) and maximum shade (90%) conditions for 1989-2020.

426 Simulated variables included air temperature and humidity at 1.75 m above the ground, snow-

427 related variables, and soil-related variables. To characterize mean environmental conditions,

428 daily microclimate variables were averaged over 30 simulated years (Table S3). We evaluated

429 sensitivity of simulated microclimate variables to input microclimate model parameters by

430 calculating RMSE between simulated outputs and empirically derived microclimate data from

431 available weather stations (California Department of Water Resources, CDEC). Air temperature

432 and snow depth data from CDEC were available for weather stations within 1 km of mid-

433 elevation sites in Rock Creek, Big Pine Creek, South Bishop Creek, and North Bishop Creek.

434 *Population genomic differentiation across the California landscape*

435 Population structure from SNP genotypic data was assessed by estimating proportions of

436 individual genomes originating from ancestral gene pools. A range of estimated ancestral gene

437 pools (K = 1-10) were tested using a sparse non-negative matrix factorization algorithm using the

438 function 'snmf' in the R package *LEA* v.3.6.0 [(Frichot et al., 2014; Frichot & François, 2015),

439 K= 3-7 shown in Fig S11]. The value of K that minimized cross-entropy and best explained

440 genotypic data was five [(Alexander & Lange, 2011), Fig S6] and this value was used for

441 subsequent analysis. The 'snmf' function was also used to estimate individual ancestry

442 coefficients. Five replicates were run using the best estimate of K, and individual ancestry

443 coefficients were extracted from the replicate with the lowest cross-entropy.

444   To quantify contributions of geographic and environmental distances to patterns of

445 genetic differentiation, we assessed isolation by distance (IBD) and isolation by environment

446 (IBE) for all population pairs using an analysis of covariance (ANCOVA). The ANCOVA tested

447 whether the means of pairwise F_{st} between populations were equal across habitat type, while

448 controlling geographic distance. Unbiased pairwise F_{st} using minor allele frequencies of all

449 populations were calculated using the R package *BEDASSLE* v.1.6 (Bradburd, 2022; Weir &

450 Hill, 2002). Pairwise geographic distance in kilometers was calculated using the R package *fields*

451 v.13.3 (Nychka & Furrer, 2021). Population pairs were identified as "coast vs. mountain" and

452 "mountain vs. mountain" to describe habitat type of populations, as this categorical descriptor

453 represents most environmental variation among populations (Table S3). Using the R package

454 rstatix v.0.7.1, ANCOVA was conducted with pair-wise F_{st} values as dependent variable, binary

455 environmental descriptor as categorical independent variable, and geographic distance as a

456 covariate. Least squares means were calculated for habitat types using the R package *emmeans*

457 v.1.8.3.

458 *Genotype-environment association tests to identify signatures of local adaptation*

459 Signatures of local adaptation to climate were investigated using two genotype-environment

460 association (GEA) methods, partial redundancy analysis [pRDA, (Borcard et al., 1992; Forester

461 et al., 2018; Peres-Neto et al., 2006)] and latent factor mixed modeling [LFMM, (Frichot et al.,

462 2013)], which control for signals generated by neutral processes through separate mechanisms.

463 Both GEA analyses were performed on two sets of populations: 1) all populations and 2) all

464 montane populations excluding the coastal (Gualala River) population. Partial redundancy

465 analysis (pRDA) was conducted at the population level since the resolution of environmental

466 data did not include environmental variation within a population. To account for isolation by

467 distance, we conducted a spatial eigenfunction analysis that produced a conditioning matrix in

468 the pRDA using distance-based Moran's eigenvector maps [Appendix 1.2, (Forester et al.,

469 2018b)]. All simulated environmental variables were scaled and centered to produce the

470 environmental matrix, and forward selection was used to select significant environmental

471 predictors, with significant dbMEMs as explanatory conditioning matrix and Hellinger-

472 transformed SNP minor allele frequencies as response matrix. The final pRDA was run with

473 significant (alpha < 0.05) environmental predictors using the R package *vegan* v2.6-2 (Oksanen

474 et al., 2022). Outlier loci on constrained ordination axes were determined based on loadings of

475 each locus in ordination space (Forester et al., 2018; Xuereb et al., 2018).

476 We then conducted a latent factor mixed model (LFMM) and documented overlap of

477 detections with results from pRDA (Bay et al., 2018; De Villemereuil et al., 2014; Forester et al.,

478 2018; Nadeau et al., 2016). Neutral population structure due to shared demographic history or

479 background genetic variation is introduced through unobserved, latent factors (Frichot et al.,

480 2013). This method used individual-based genotypic data, which assessed the effect of *a priori*

481 designated populations used previously in pRDA. The 'lfmm' function in the *LEA* package was

482 implemented using individual-level genotypic data (22,323 SNPs) as response matrix, forward-

483 selected environmental variables used in pRDA as environmental predictors, and the best

484 estimate of K (estimated ancestral gene pools) as number of latent factors. More detailed GEA

485 methods are provided in Appendix 1.3.

486 *SNP and protein functional annotations*

487 We identified genes containing candidate SNPs and predicted SNP's coding effects with an

488 interval forest approach using the program SnpEff v.5.1 (Cingolani et al., 2012) and Caen 1.0

489 annotated genome (Bracewell et al., 2023). SNPs were annotated based on genomic location, and

490   coding effects were predicted (Appendix 1.4). To assign a putative protein name, protein

491   sequences were aligned to NCBI's protein database using BlastP (Table 3, S7, S10). Gene

492   Ontology (GO) terms were assigned to candidate genes using the functional annotation web

493   server database Protein ANNotation with Z-scoRE [PANNZER2, (Törönen et al., 2018)].

494

References

508 **References**

509 Abatzoglou, J. T. (2013). Development of gridded surface meteorological data for ecological
510   applications and modelling. *International Journal of Climatology*, *33*(1), 121–131.
511   https://doi.org/10.1002/joc.3413

512 Alexander, D. H., & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for
513   individual ancestry estimation. *BMC Bioinformatics*, *12*, 246.

514 Bale, J. S. (1996). Insect cold hardiness: A matter of life and death. *European Journal of*
515   *Entomology*, *93*, 369–382.

516 Bay, R., Harrigan, R., Underwood, V., Gibbs, H. L., Smith, T. B., & Ruegg, K. (2018). Genomic
517   signals of selection predict climate-driven population declines in a migratory bird.
518   *Science*, *359*, 83–86.

519 Birrell, J. H., Shah, A. A., Hotaling, S., Giersch, J. J., Williamson, C. E., Jacobsen, D., & Woods,
520   H. A. (2020). Insects in high-elevation streams: Life in extreme environments imperiled
521   by climate change. *Global Change Biology*, *26*(12), 6667–6684.
522   https://doi.org/10.1111/gcb.15356

523 Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina
524   Sequence Data. *Bioinformatics*, *btu170*.

525 Borcard, D., Legendre, P., & Drapeau, P. (1992). Partialling out the spatial component of
526   ecological variation. *Ecology*, *73*(3), 1045–1055.

527 Boychuk, E. C., Smiley, J. T., Dahlhoff, E. P., Bernards, M. A., Rank, N. E., & Sinclair, B. J.
528   (2015). Cold tolerance of the montane Sierra leaf beetle, *Chrysomela aeneicollis*. *Journal*
529   *of Insect Physiology*, *81*, 157–166.

530 Bracewell, R.R., Stillman, J.H., Dahlhoff, E.P., Smeds, E., Chatla, K., Bachtrog, D., Williams,
531   C., Rank, N.E. (2023). A chromosome-scale genome assembly and evaluation of mtDNA
532   variation in the willow leaf beetle *Chrysomela aeneicollis. G3 Genes|Genomes|Genetics*.
533   jkad106. https://doi.org/10.1093/g3journal/jkad106

534 Bradburd, G. (2022). *BEDASSLE: Quantifies Effects of Geo/Eco Distance on Genetic*
535   *Differentiation* (1.6). CRAN.R-project.org/package=BEDASSLE

536 Brown, W. J. (1956). The New World species of Chrysomela L. (Coleoptera: Chrysomelidae).
537   *The Canadian Entomologist*, *88*, 1–54.

538 Camus, M. F. (2020). Digest: Mitonuclear interactions modulate life history phenotypes in the
539   wild. *Evolution*, *74*(8), 1877–1878. https://doi.org/10.1111/evo.13988

540 Cantiello, F. (1995). Actin filaments stimulate the Na+-K+-ATPase. *American Journal of*
541   *Physiology - Renal Physiology*, *269*(5), 637–643.

542 Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., Land, S. J., Lu, X., &
543   Ruden, D. M. (2012). A program for annotating and predicting the effects of single
544   nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster*
545   strain w1118; iso-2; iso-3. *Fly (Austin)*, *6*(2), 80–92.

546 Conover, D. O. (1992). Seasonality and the scheduling of life history at different latitudes.
547   *Journal of Fish Biology*, *41*, 161–178. https://doi.org/10.1111/j.1095-
548   8649.1992.tb03876.x

549 Dahlhoff, E. P., Dahlhoff, V. C., Grainger, C. A., Zavala, N. A., Otepola-Bello, D., Sargent, B.
550   A., Roberts, K. T., Heidl, S. J., Smiley, J. T., & Rank, N. E. (2019). Getting chased up the
551   mountain: High elevation may limit performance and fitness characters in a montane
552   insect. *Functional Ecology*, *33*(5), 809–818. https://doi.org/10.1111/1365-2435.13286

553 Dahlhoff, E. P., Fearnley, S. L., Bruce, D. A., Gibbs, A. G., Stoneking, R., McMillan, D. M.,
554   Deiner, K., Smiley, J. T., & Rank, N. E. (2008). Effects of temperature on physiology and
555   reproductive success of a montane leaf beetle: Implications for persistence of native
556   populations enduring climate change. *Physiological and Biochemical Zoology*, *81*(6),
557   718–732. https://doi.org/10.1086/590165

558 Dahlhoff, E. P., & Rank, N. E. (2000). Functional and physiological consequences of genetic
559   variation at phosphoglucose isomerase: Heat shock protein expression is related to
560   enzyme genotype in a montane beetle. *Proceedings of the National Academy of Sciences*
561   *of the United States of America*, *97*(18), 10056–10061.
562   https://doi.org/10.1073/pnas.160277697

563 De Villemereuil, P., Frichot, E., Bazin, E., François, O., & Gaggiotti, O. E. (2014). Genome scan
564   methods against more complex models: When and how much should we trust them?
565   *Molecular Ecology*, *23*(8), 2006–2019.

566 Dellicour, S., Fearnley, S. L., Lombal, A., Heidl, S. J., Dahlhoff, E. P., Rank, N. E., & Mardulyn,
567   P. (2014). Inferring the past and present connectivity across the range of a North
568   American leaf beetle: Combining ecological-niche modeling and a geographically
569   explicit model of coalescence. *Evolution*, *68*, 2371–2385.

570 Des Marteaux, L. E., McKinnon, A. H., Udaka, H., Toxopeus, J., & Sinclair, B. J. (2017). Effects
571   of cold-acclimation on gene expression in Fall field cricket (*Gryllus pennsylvanicus*)
572   ionoregulatory tissues. *BMC Genomics*, *18*(1), 1–17. https://doi.org/10.1186/s12864-017-
573   3711-9

574 Des Marteaux, L. E., Stinziano, J. R., & Sinclair, B. J. (2018). Effects of cold acclimation on
575   rectal macromorphology, ultrastructure, and cytoskeletal stability in *Gryllus*
576   *pennsylvanicus* crickets. *Journal of Insect Physiology*, *104*, 15–24.
577   https://doi.org/10.1016/j.jinsphys.2017.11.004

578 Dick, C. A., Rank, N. E., McCarthy, M., McWeeney, S., Hollis, D., & Dahlhoff, E. P. (2013).
579   Effects of temperature variation on male behavior and mating success in a montane
580   beetle. *Physiological and Biochemical Zoology*, *86*(4), 432–440.

581 Felsenstein, J. (1976). The theoretical population genetics of variable selection and migration.
582   *Annual Review of Genetics*, *10*, 253–280.

583 Forester, B. R., Jones, M. R., Joost, S., Landguth, E. L., & Lasky, J. R. (2016). Detecting spatial
584   genetic signatures of local adaptation in heterogeneous landscapes. *Molecular Ecology*,
585   *25*(1), 104–120. https://doi.org/10.1111/mec.13476

586   Forester, B. R., Lasky, J., Wagner, H., & Urban, D. L. (2018). Comparing methods for detecting
587   multilocus adaptation with multivariate genotype–environment associations. *Molecular*
588   *Ecology*, *27*(9), 2215–2233.

589   Fretwell, S. D. (1972). Populations in a seasonal environment. *Monographs in Population*
590   *Biology*, *5*, 1–217.

591   Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association
592   studies. *Methods in Ecology and Evolution*, *6*(8), 925–929.

593   Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G., & François, O. (2014). Fast and efficient
594   estimation of individual ancestry coefficients. *Genetics*, *196*, 973–983.

595   Frichot, E., Schoville, S. D., Bouchard, G., & François, O. (2013). Testing for associations
596   between loci and environmental gradients using latent factor mixed models. *Molecular*
597   *Biology and Evolution*, *30*, 1687–1699.

598   Griffith, G. E., Omernik, J. M., Smith, D. W., Cook, T. D., Tallyn, E., Moseley, K., & Johnson,
599   C. B. (2016). *Ecoregions of California: U.S. Geological Survey Open-File Report 2016–*
600   *1021, with map, scale 1:1,100,000.*

601   Groffman, P. M., Driscoll, C. T., Fahey, T. J., Hardy, J. P., Fitzhugh, R. D., & Tierney, G. L.
602   (2001). Colder soils in a warmer world: A snow manipulation study in a northern
603   hardwood forest ecosystem. *Biogeochemistry*, *56*(2), 135–150.
604   https://doi.org/10.1023/A:1013039830323

605   Halsch, C. A., Shapiro, A. M., Fordyce, J. A., Nice, C. C., Thorne, J. H., Waetjen, D. P., &
606   Forister, M. L. (2021). Insects and recent climate change. *Proceedings of the National*
607   *Academy of Sciences of the United States of America*, *118*(2), 1–9.
608   https://doi.org/10.1073/PNAS.2002543117

609   Haldane, J.B.S., Jayakar, S.D. (1963). Polymorphism due to selection in varying directions.
610   *Journal of Genetics*. 58: 237-242.

611   Hedrick, P. W., Ginevan, M. E., & Ewing, E. P. (1976). Genetic polymorphism in heterogeneous
612   environments. *Annual Review of Ecology, Evolution, and Systematics*, *7*, 1–32.

Huning, L. S., & AghaKouchak, A. (2020). Global snow drought hot spots and characteristics. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(33), 19753–19759. https://doi.org/10.1073/PNAS.1915921117

Jones, M. R., Forester, B. R., Teufel, A. I., Adams, R. V., Anstett, D. N., Goodrich, B. A., Landguth, E. L., Joost, S., & Manel, S. (2013). Integrating landscape genomics and spatially explicit approaches to detect loci under selection in clinal populations. *Evolution*, *67*(12), 3455–3468. https://doi.org/10.1111/evo.12237

Kawecki, T., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241.

Kearney, M. R., & Porter, W. P. (2017). NicheMapR – an R package for biophysical modelling: The microclimate model. *Ecography*, *40*(5), 664–674. https://doi.org/10.1111/ecog.02360

Khurana, S. (2000). Role of actin cytoskeleton in regulation of ion transport: Examples from epithelial cells. *Journal of Membrane Biology*, *178*(2), 73–87. https://doi.org/10.1007/s002320010016

Kim, M., Robich, R. M., Rinehart, J. P., & Denlinger, D. L. (2006). Upregulation of two actin genes and redistribution of actin during diapause and cold stress in the northern house mosquito, *Culex pipiens*. *Journal of Insect Physiology*, *52*(11–12), 1226–1233. https://doi.org/10.1016/j.jinsphys.2006.09.007

Larsen, T.H. (2012). Upslope range shifts of Andean dung beetles in response to deforestation: Compounding and confounding effects of microclimatic change. *Biotropica*. 44: 82-89.

Legendre, P., & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia*, *129*, 271–280.

Lotterhos, K.E. (2023). The paradox of adaptive trait clines with nonclinal patterns in the underlying genes. *Proceedings of the National Academy of Sciences of the United States of America,* 120(12): e2220313120.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760.

640   Linck, E., & Battey, C. J. (2019). Minor allele frequency thresholds strongly affect population
641   structure inference with genomic data sets. *Molecular Ecology Resources*, *19*(3), 639–
642   647. https://doi.org/10.1111/1755-0998.12995McMillan, D. M., Fearnley, S. L., Rank, N.
643   E., & Dahlhoff, E. P. (2005). Natural temperature variation affects larval survival,
644   development and Hsp70 expression in a leaf beetle. *Functional Ecology*, *19*(5), 844–852.

645   Millstein, R. (2006). Natural Selection as a Population-Level Causal Process. *The British Journal*
646   *for the Philosophy of Science*, *57*(4), 627–653.

647   Moraiti, C. A., Nakas, C. T., & Papadopoulos, N. T. (2014). Diapause termination of *Rhagoletis*
648   *cerasi* pupae is regulated by local adaptation and phenotypic plasticity: Escape in time
649   through bet-hedging strategies. *Journal of Evolutionary Biology*, *27*(1), 43–54.
650   https://doi.org/10.1111/jeb.12273

651   Moret, P., Arauz, M.D., Gobbi, M., Barragan, A. (2016). Climate warning effects in the tropical
652   Andes: First evidence for upslope shifts of Carabidae (Coleoptera) in Ecuador. I*nsect*
653   *Conservation and Diversity*. 9: 342-350.

654   Mote, P. W., Li, S., Lettenmaier, D. P., Xiao, M., & Engel, R. (2018). Dramatic declines in
655   snowpack in the western US. *Npj Climate and Atmospheric Science*, *1*(1), 2.
656   https://doi.org/10.1038/s41612-018-0012-1

657   Nadeau, S., Meirmans, P., Aitken, S., Ritland, K., & Isabel, N. (2016). The challenge of
658   separating signatures of local adaptation from those of isolation by distance and
659   colonization history: The case of two white pines. *Ecology and Evolution*, *6*(24), 8649–
660   8664.

661   Neargarder, G., Dahlhoff, E. P., & Rank, N. E. (2003). Variation in thermal tolerance is linked to
662   phosphoglucose isomerase genotype in a montane leaf beetle. *Functional Ecology*, *17*(2),
663   213–221. https://doi.org/10.1046/j.1365-2435.2003.00722.x

664   Nychka, D., & Furrer, R. (2021). *fields: Tools for spatial data* (13.3). University Corporation for
665   Atmospheric Research. https://github.com/dnychka/fieldsRPackage

666 Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R.
667   B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M.,
668   Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Durand, S., Evangelista, H. B. A., …
669   Cajo, J. F. (2022). *vegan: Community Ecology Package* (2.6-2). https://CRAN.R-
670   project.org/package=vegan

671 Orrest, J. R. K., & Thomson, J. D. (2011). An examination of synchrony between insect
672   emergence and flowering in Rocky Mountain meadows. *Ecological Monographs*, *81*(3),
673   469–491.

674 Orsini, L., Vanoverbeke, J., Swillen, I., Mergeay, J., & De Meester, L. (2013). Drivers of
675   population genetic differentiation in the wild: Isolation by dispersal limitation, isolation
676   by adaptation and isolation by colonization. *Molecular Ecology*, *22*(24), 5983–5999.
677   https://doi.org/10.1111/mec.12561

678 Örvar, B. L., Sangwan, V., Omann, F., & Dhindsa, R. S. (2000). Early steps in cold sensing by
679   plant cells: The role of actin cytoskeleton and membrane fluidity. *Plant Journal*, *23*(6),
680   785–794. https://doi.org/10.1046/j.1365-313X.2000.00845.x

681 Otto, S. B., Berlow, E. L., Rank, N. E., Smiley, J., & Brose, U. (2008). Predator diversity and
682   identity drive interaction strength and trophic cascades in a food web. *Ecology*, *89*(1),
683   134–144.

684 Overgaard, J., & Macmillan, H. A. (2017). The integrative physiology of insect chill tolerance.
685   *Annual Review of Physiology*, *79*, 187–208. https://doi.org/10.1146/annurev-physiol-
686   022516-034142

687 Pauli, J. N., Zuckerberg, B., Whiteman, J. P., & Porter, W. (2013). The subnivium: A
688   deteriorating seasonal refugium. *Frontiers in Ecology and the Environment*, *11*(5), 260–
689   267. https://doi.org/10.1890/120222

690 Pearman, W. S., Urban, L., & Alexander, A. (2022). Commonly used Hardy–Weinberg
691   equilibrium filtering schemes impact population structure inferences using RADseq data.
692   *Molecular Ecology Resources*, *22*, 2599–2613. https://doi.org/10.1111/1755-0998.13646

693 Pepin, N., Bradley, R. S., Diaz, H. F., Baraer, M., Caceres, E. B., Forsythe, N., Fowler, H.,
694   Greenwood, G., Hashmi, M. Z., Liu, X. D., Miller, J. R., Ning, L., Ohmura, A., Palazzi,
695   E., Rangwala, I., Schöner, W., Severskiy, I., Shahgedanova, M., Wang, M. B., … Yang,
696   D. Q. (2015). Elevation-dependent warming in mountain regions of the world. *Nature*
697   *Climate Change*, *5*(5), 424–430. https://doi.org/10.1038/nclimate2563

698 Peres-Neto, P. R., Legendre, P., Dray, S., & Borcard, D. (2006). Variation partitioning of species
699   data matrices: Estimation and comparison of fractions. *Ecology*, *87*, 2614–2625.

700 Pokorná, J., Schwarzerová, K., Zelenková, S., Petrášek, J., Janotová, I., Čapková, V., & Opatrný,
701   Z. (2004). Sites of actin filament initiation and reorganization in cold-treated tobacco
702   cells. *Plant, Cell and Environment*, *27*(5), 641–653. https://doi.org/10.1111/j.1365-
703   3040.2004.01186.x

704 Poplin, R., Ruano-Rubio, V., DePristo, M. A., Fennell, T. J., Carneiro, M. O., Van der Auwera,
705   G. A., Kling, D. E., Gauthier, L. D., Levy-Moonshine, A., Roazen, D., Shakir, K.,
706   Thibault, J., Chandran, S., Whelan, C., Lek, M., Gabriel, S., Daly, M. J., Neale, B.,
707   MacArthur, D. G., & Banks, E. (2017). Scaling accurate genetic variant discovery to tens
708   of thousands of samples. *BioRxiv*, *201178*.

709 Rank, N. E. (1992a). A hierarchical analysis of genetic differentiation in a montane leaf beetle
710   (*Chrysomela aeneicollis*). *Evolution*, *46*, 1097–1111.

711 Rank, N. E. (1992b). Host plant preference based on salicylate chemistry in a willow leaf beetle
712   (*Chrysomela aeneicollis*). *Oecologia*, *90*(1), 95–101.
713   https://doi.org/10.1007/BF00317814

714 Rank, N. E. (1994). Host-Plant Effects on Larval Survival of a Salicin-Using Leaf Beetle
715   *Chrysomela aeneicollis Schaeffer* (Coleoptera: Chrysomelidae). *Oecologia*, *97*(3), 342–
716   353.

717 Rank, N. E., & Dahlhoff, E. P. (2002). Allele frequency shifts in response to climate change and
718   physiological consequences of allozyme variation in a montane insect. *Evolution*, *56*(11),
719   2278–2289.

720   Rank, N. E., Mardulyn, P. M., Heidl, S. J., Roberts, K. T., Zavala, N. A., & Dahlhoff, E. P.
721   (2020). Mitonuclear mismatch alters performance and reproductive success in naturally
722   introgressed populations of a montane leaf beetle. *Evolution*, *74*(8), 1724–1740.

723   Rellstab, C., Gugerli, F., Eckert, A. J., Hancock, A. M., & Holderegger, R. (2015). A practical
724   guide to environmental association analysis in landscape genomics. *Molecular Ecology*,
725   *24*(17), 4348–4370. https://doi.org/10.1111/mec.13322

726   Rhomberg, L. R., & Singh, R. S. (1986). Evidence for a link between local and seasonal cycles
727   in gene frequencies and latitudinal gene clines in a cyclic parthenogen. *Genetica*, *78*(1),
728   73–79. https://doi.org/10.1007/BF00058677

729   Roberts, K. T., Rank, N. E., Dahlhoff, E. P., Stillman, J. H., & Williams, C. M. (2021). Snow
730   modulates winter energy use and cold exposure across an elevation gradient in a montane
731   ectotherm. *Global Change Biology*, *27*(23), 6103–6116.
732   https://doi.org/10.1111/gcb.15912

733   Shafer, A. B. A., & Wolf, J. B. W. (2013). Widespread evidence for incipient ecological
734   speciation: A meta-analysis of isolation-by-ecology. *Ecology Letters*, *16*(7), 940–950.
735   https://doi.org/10.1111/ele.12120

736   Shaffer, H. B., Toffelmier, E., Corbett-Detig, R. B., Escalona, M., Erickson, B., Fiedler, P., Gold,
737   M., Harrigan, R. J., Hodges, S., Luckau, T. K., Miller, C., Oliveira, D. R., Shaffer, K. E.,
738   Shapiro, B., Sork, V. L., & Wang, I. J. (2022). Landscape genomics to enable
739   conservation actions: The California Conservation Genomics Project. *Journal of*
740   *Heredity*, esac020. https://doi.org/10.1093/jhered/esac020

741   Shah, A. A., Dillon, M. E., Hotaling, S., & Woods, H. A. (2020). High elevation insect
742   communities face shifting ecological and evolutionary landscapes. *Current Opinion in*
743   *Insect Science*, *41*, 1–6. https://doi.org/10.1016/j.cois.2020.04.002

744   Sinclair, B. J., Vernon, P., Klok, C. J., & Chown, S. L. (2003). Insects at low temperatures: An
745   ecological perspective. *Trends in Ecology and Evolution*, *18*(5), 257–262.

746   Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science*, *236*,
747   787–792.

748 Slatyer, R. A., Umbers, K. D. L., & Arnold, P. A. (2022). Ecological responses to variation in
749   seasonal snow cover. *Conservation Biology*, *36*(1). https://doi.org/10.1111/cobi.13727

750 Smiley, J. T., & Rank, N. E. (1986). Predator protection versus rapid growth in a montane leaf
751   beetle. *Oecologia*, *70*, 106–112.

752 Sork, V. L., Aitken, S. N., Dyer, R. J., Eckert, A. J., Legendre, P., & Neale, D. B. (2013). Putting
753   the landscape into the genomics of trees: Approaches for understanding local adaptation
754   and population responses to changing climate. *Tree Genetics and Genomes*, *9*(4), 901–
755   911. https://doi.org/10.1007/s11295-013-0596-x

756 Stewart, J. A. E., Wright, D. H., & Heckman, K. A. (2017). Apparent climate-mediated loss and
757   fragmentation of core habitat of the American pika in the Northern Sierra Nevada,
758   California, USA. *PLoS ONE*, *12*(8), 1–18. https://doi.org/10.1371/journal.pone.0181834

759 Törönen, P., Medlar, A., & Holm, L. (2018). PANNZER2: A rapid functional annotation web
760   server. *Nucleic Acids Research*, *46*(W1), W84–W88.

761 Toxopeus, J., Des Marteaux, L. E., & Sinclair, B. J. (2019). How crickets become freeze tolerant:
762   The transcriptomic underpinnings of acclimation in *Gryllus veletis*. *Comparative*
763   *Biochemistry and Physiology - Part D*, *29*, 55–66.
764   https://doi.org/10.1016/j.cbd.2018.10.007

765 Toxopeus, J., & Sinclair, B. J. (2018). Mechanisms underlying insect freeze tolerance. *Biological*
766   *Reviews*, *93*(4), 1891–1914.

767 Turner, J. R., Rill, B. K., Carlson, S. L., Carnes, D., Kerner, R., Mrsny, R. J., & Madara, J. L.
768   (1997). Physiological regulation of epithelial tight junctions is associated with myosin
769   light-chain phosphorylation. *American Journal of Physiology - Cell Physiology*, *273*(4),
770   C1378-1385. https://doi.org/10.1152/ajpcell.1997.273.4.c1378

771 Van der Auwera, G. A., & O'Conner, B. D. (2020). *Genomics in the Cloud: Using Docker,*
772   *GATK, and WDL in Terra* (1st Edition). O'Reilly Media.

773 Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. *Molecular Ecology*, *23*(23),
774   5649–5662. https://doi.org/10.1111/mec.12938

775 Weir, B. S., & Hill, W. G. (2002). Estimating F-statistics. *Ann. Rev. Gen.*, *36*, 949–952.

776 Williams, C. M., Henry, H. A. L., & Sinclair, B. J. (2015). Cold truths: How winter drives
777   responses of terrestrial organisms to climate change. *Biological Reviews*, *90*(1), 214–235.
778   https://doi.org/10.1111/brv.12105

779 Williams, C. M., Ragland, G. J., Betini, G., Buckley, L. B., Cheviron, Z. A., Donohue, K.,
780   Hereford, J., Humphries, M. M., Lisovski, S., Marshall, K. E., Schmidt, P. S., Sheldon,
781   K. S., Varpe, Ø., & Visser, M. E. (2017). Understanding evolutionary impacts of
782   seasonality: An introduction to the symposium. *Integrative and Comparative Biology*,
783   *57*(5), 921–933. https://doi.org/10.1093/icb/icx122

784 Wittman, M.J., Bergland, A.O., Feldman, M.W., Schmidt, P.S., Petrov, D.A. (2017). Seasonally
785   fluctuating selection can maintain polymorphism at many loci via segregation lift.
786   *Proceedings of the National Academy of Sciences of the United States of America*,
787   *114*(46): e9932-e9941.

788 Xuereb, A., Kimber, C., Curtis, J., Bernatchez, L., & Fortin, M. J. (2018). Putatively adaptive
789   genetic variation in the giant California sea cucumber (*Parastichopus californicus*) as
790   revealed by environmental association analysis of restriction-site associated DNA
791   sequencing data. *Molecular Ecology*, *27*(24), 5035–5018.

792

793

794 **Tables**

795

796 **Table 1. Localities and sample sizes for population genomic studies.**

Ecoregion Population name	Latitude	Longitude	Elevation (m)	*N* sites	*N* beetles (total)	Year(s)[1]
Sierra Nevada						
Tuttle Creek (TC)	36.53779	-118.21530	3012	1	10	2019
Taboose Pass (TP)	36.96824	-118.43419	3321	3	18	2009
Big Pine Creek (BP)	37.12863	-118.48704	3142	11	28	1998-2014
Baker Creek (BK)	37.16780	-118.47143	3120	3	18	1999
S Bishop Creek (BC)	37.16601	-118.55171	3098	14	38	2004-2014
Tyee Lakes (TL)	37.18567	-118.57565	3191	4	9	2014
N Bishop Creek (NF)	37.21760	-118.64757	3131	6	12	2003-2014
Pine Creek (PC)	37.34442	-118.72861	3057	2	4	2013
Rock Creek (RC)	37.45561	-118.74034	3030	5	10	2013-2014
Central Basin						
Davis Creek (DC)	37.78392	-118.23650	2895	1	12	2003
Eastern Cascades						
Fitzhugh Creek (FC)	41.35091	-120.29662	1968	3	11	2020
Coast Range						
Gualala River (GR)	38.74906	-123.51919	12	1	8	2016

797 [1]We sampled newly emerged overwintered adults, either from the most recent population

798 expansion (2013-14), or the most recent observation of overwintered beetles at that site. Further

799 details of sampling design are described in Appendix 1.1.

800 **Table 2. Candidate SNPs correlated with microclimate variables.** Total number of SNPs are

801 shown. For genic SNPs, the number of unique genes containing at least one non-synonymous

802 (NSY) or synonymous (SYN) substitution, as well as genes where the SNP is located in an

803 intron, are also indicated. Candidate SNPs and genes are delineated by identification through

804 analyses with montane and coastal populations or analyses with only montane populations.

Microclimate variable	Populations	Detection Method	Number of SNPs		Number of genes		
			Total	Genic	NonSyn	Syn	Intron
Air temperature range	Montane & coastal	pRDA & LFMM	67	33	4	11	18
		pRDA only	70	33	4	11	18
Maximum daily snowfall	Montane & coastal	pRDA & LFMM	26	16	4	4	4
		pRDA only	39	21	5	4	7
Maximum daily snowfall	Montane only	pRDA & LFMM	79	40	4	10	11
		pRDA only	116	62	8	13	15

805
806

807 **Table 3. Candidate proteins that vary with microclimate**. Proteins were identified using

808 BlastP alignment using predicted amino acid sequence; associated NCBI accession number is

809 noted for sequence with highest homology to reference taxa; populations included in analysis

810 (montane and coastal, montane only, or both) are indicated.

| Gene ID	Amino acid variants	Protein	Reference sequence (taxa[A], % identity) Accession number	Gene Ontology (GO) terms[B]
Air temperature range				
Montane & Coastal				
05_00.257	Val/Ile	nuclear valosin-containing protein-like	*Ld*: 99, 71 XP_023023254.1	ATP binding and hydrolysis [2] ribosome binding[2], biogenesis[3] telomerase activity[3]
06_05.960	Asn/His	cytochrome P450 315a1, mitochondrial	*Ld*: 100, 58 XP_023020072.1	Monooxygenase, oxidoreductase activity[2]; ecdysone biosynthesis[3]
15_02.330	Leu/Phe	phospholipid transfer protein	*Ag*: 100, 75 XP_018561647.1	membrane[1] nucleotidyl trans. activity[2] phosphorylation, signal transduction[3]
Maximum daily snowfall				
Montane & Coastal				
02_12.369	Ser/Gly	transmembrane protein 131	*Ld*: 94, 66 XP_023015832.1	membrane[1]
14_11.590	Val/Ala	testin[C]	*Tg*: 100, 63 XP_008194458.1	zinc ion binding[2]
Montane only				
02_16.309	Ser/Pro	long-chain-fatty-acid CoA ligase	*Ld*: 99, 76 XP_023012248.1	ligase activity[2], lipid metabolic process[3], neuron cellular homeostasis[3]
03_01.420	Val/Leu	protein pigeon (PION)[C]	*Ld*: 100, 73 XP_023019414.1	regulation of membrane protein (amyloid-beta) formation[3]
03_04.184	Asn/Asp	ankyrin repeat; IBR domain-containing protein[C]	*Ag*: 98, 81 XP_018576173.1	metal ion binding[2], ubiquitin-protein transferase activity[2], protein ubiquitination[3]
04_00.113	Ile/Val	zinc transporter ZIP-1 like isoform[C]	*Ld*: 100, 67 XP_023015018.1	metal ion transmembrane transporter activity[2,3]
18_04.151	Asn/Thr	ribosomal protein	*Ld*: 100, 68 XP_023030475.1	ribosome[1], translation[3]
Montane & Coastal + Montane only				
10_13.168	Glu/Asp[y] Pro/Ala[y]	inverted formin-2	*Ag*: 98, 63 XP_023310886.1	actin cytoskeleton organization[2]
20_03.324	Gln/His	microtubule-actin cross-linking factor	*Dv*: 45, 69 XP_028131989.1	membrane[1], cytoskeleton[1] Ca^{++} ion binding[2], microtubule binding[2]; cytoskeleton organization[3]

811 [A]Reference taxa: *Leptinotarsa decemlineata (Ld:* Colorado potato beetle), *Anoplophora glabripennis* (*Ag:* Asian
812 long-horned beetle)*, Triboleum castaneum (Tc:* Red flour beetle), *Diabrotica virgifera* (*Dv:* Corn rootworm beetle).
813 [B]GOTerm Categories: cellular component[1], molecular function[2], biological process[3]
814 [C]SNP only detected with pRDA;[y]SNP locations directly adjacent (Table S6)

815

Figures

818
819
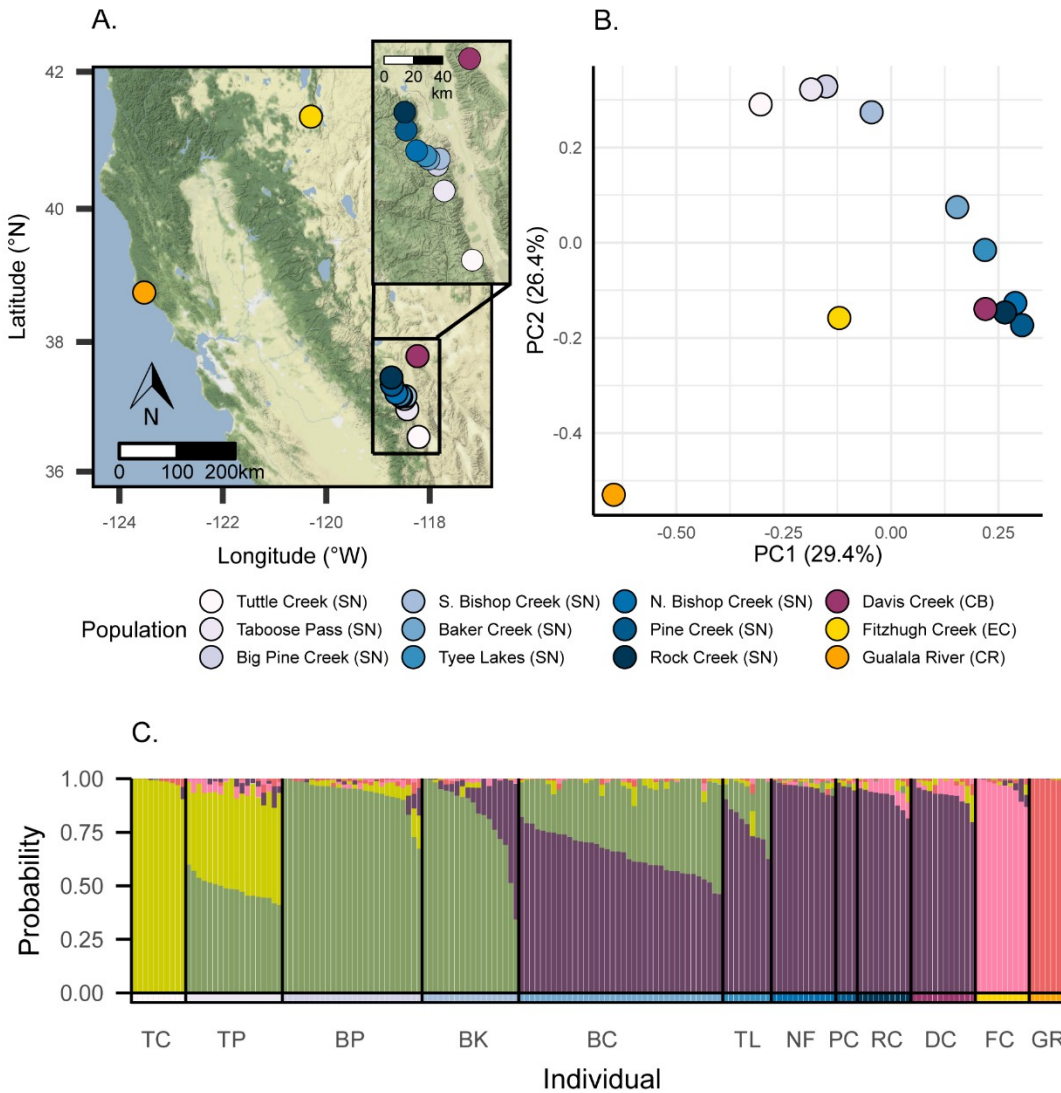
Figure 1. Genetic differentiation and structure of *Chrysomela aeneicollis* populations across

California. A. Map of study populations. Abbreviation in parenthesis refers to population

ecoregion (SN: Sierra Nevada, CB: Central Basin, EC: Eastern Cascades, CR: Coast Range).

Inset map features the sampled populations located in the Sierra Nevada and Central Basin

ecoregions. Populations in the Sierra Nevada ecoregion are presented using a blue color gradient

and are ordered based on latitude, south to north. representing increasing latitude. ***B.*** PCA

826   ordination highlighting genomic differentiation among populations based on the minor allele

827   frequencies. *C.* Stacked barplots for each individual (N = 175 total) indicate estimated ancestry

828   coefficients, representing the posterior probability that an individual originates from K = 5

829   ancestral gene pools. Colors below the stacked barplot indicate each individual's a priori

830   population designations, as shown in parts *A* and *B*. Two-letter population designations are

831   described in Table 1.

832

833
834
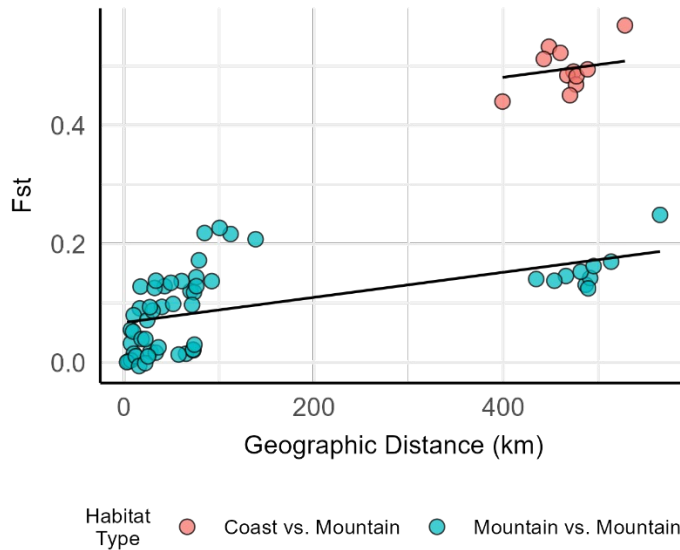
835
836 **Figure 2. Genomic differentiation as a function of geographic distance and habitat type for**

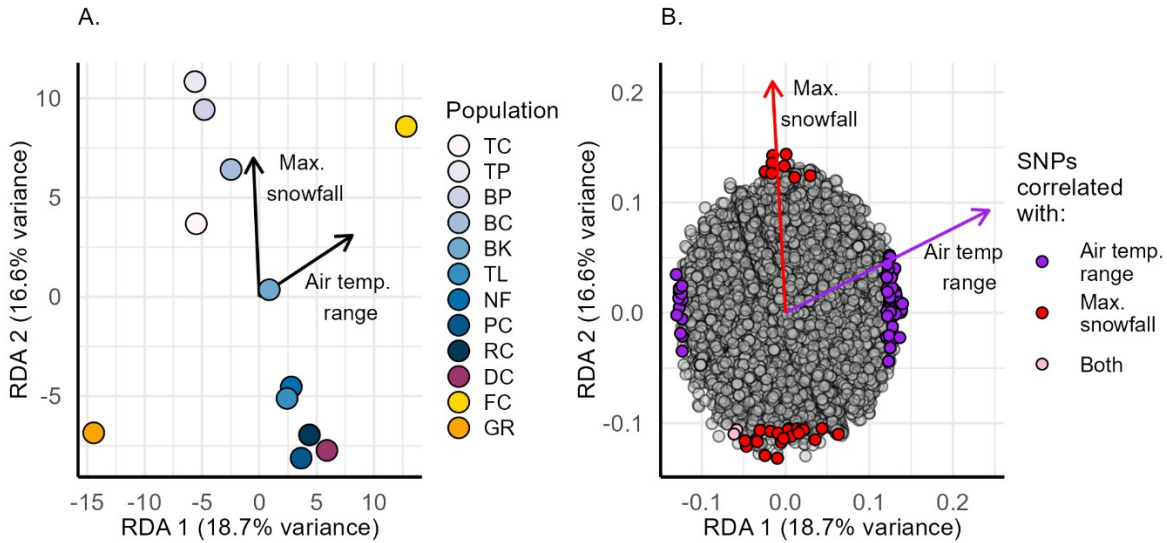837 **California willow beetle populations.** Data shown highlight the relationship between pairwise

838 geographic distance (km) and pairwise genetic distance (Fst). The black lines indicate the fitted

839 values from the ANCOVA model, and points are color coded by the categorical independent

840 variable used in the ANCOVA model.

841

Figure 3. Partial redundancy analysis (pRDA) identifies candidate loci associated with

selective climatic gradients. A. Ordination of populations and environmental variable loadings

in multivariate space. Environmental variable loadings are multiplied by 10 to improve

visualization. **B.** Ordination of SNP loci and environmental variable loadings in multivariate

space. Outlier loci are colored based on correlation with an environmental variable (Pearson's r >

|0.65|). Environmental variable loadings are multiplied by 0.4 to improve visualization. Two-

letter population designations are described in Table 3. Results of pRDA with only montane

populations are provided in Figure S7.

852

A. Air Temperature Range

B. Maximum snowfall
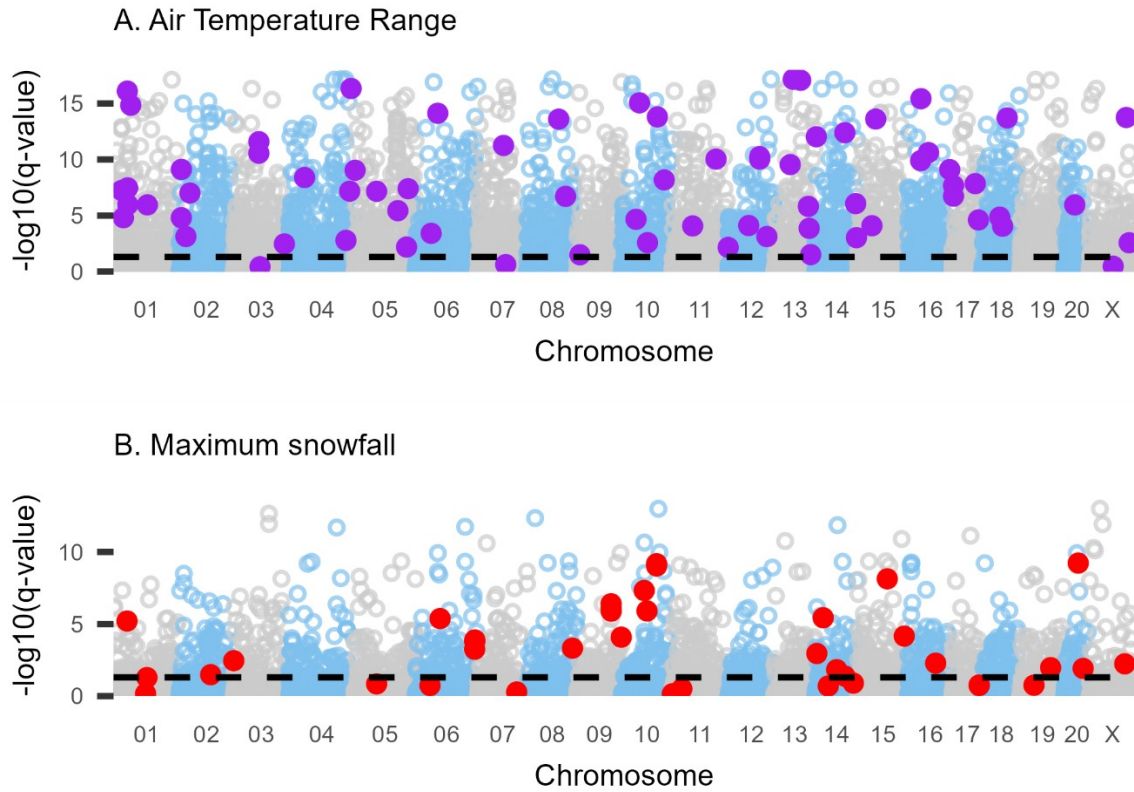
853
854 **Figure 4**. **Latent factor mixed model (LFMM) identifies candidate loci associated with**

855 **selective climatic gradients.** Points indicate the FDR-adjusted p-value (q-value) of the

856 association between a locus and an environmental gradient. The dotted black line represents a q-

857 value of 0.05, and purple and red colored loci are those detected by the pRDA. *A.* Loci

858 associations with annual air temperature range. *B.* Loci associations with maximum daily

859 snowfall. Results of LFMM with only montane populations are provided in Figure S8.

860