

Climate and ice in the last glacial maximum explain patterns of isolation by distance inferred for alpine grasshoppers

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Abstract. 1. Cold-adapted species are likely to have had more widespread ranges and greater population connectivity during the last glacial period than is the case today. This contrasts with the trend in many species for range and population size to increase during interglacials.

2. We examined the pattern of genetic and morphological variation within an endemic, wingless, alpine grasshopper *Sigaus australis* (Orthoptera: Acrididae) in the Southern Alps of New Zealand, testing for isolation by distance using geometric morphometric and mitochondrial ND2 sequences to document variation.

3. Presence/absence data were analysed to estimate the environmental envelope (niche) of *Sigaus australis* and the resulting model used to infer the extent of available habitat for the species during the last glacial maximum. Estimates of past range size were modified using models of montane ice extent during the LGM.

4. Clinal patterns of pronotum shape variation and signatures of isolation by distance support the hypothesis of a formerly more connected species. A north/south division was observed in pronotum shape, but the phenotypic variation was not diagnostic, as one would expect within a single species.

5. Although the current habitat area occupied by *Sigaus australis* is much smaller than estimates for the LGM from our climate model, we show that realised area differed less due to the extension of valley glaciers. However, the current distribution of *S. australis* is more fragmented than in the past.

6. This and other flightless alpine species currently restricted to fragmented high elevation habitat demonstrate genetic lag but are subject to loss of diversity as anthropogenic climate warming proceeds.

Key words. Alpine species, environmental envelope, geometric morphometrics, mtDNA, niche modelling, phylogeography, range shift.

Introduction

Understanding the influence of global climate changes on the distribution and resilience of local biota has become an urgent objective in biodiversity research since the pace of the anthropogenic climate crisis became apparent (Singer, 2017; Warren *et al.*, 2018). An emphasis on predictive analysis of future

outcomes draws upon interpretation of past biological response to natural climate shifts of the Pleistocene. During the last glacial maximum (LGM), many species in temperate regions of the globe were restricted in distribution to fragmented populations in glacial refuges (Hewitt, 2001; Trewick *et al.*, 2011). Range shifting associated with past climate change generated well-recognised patterns indicating latitudinal ebb and flow through the Pleistocene across many hundreds of kilometres (e.g. ~1300 km in North American mammals; Lyons, 2003). As the earth warmed, ranges expanded to colonise the newly available habitat, leaving a genetic signature in descendent populations

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(Excoffier *et al.*, 2009). Coalescence of mtDNA haplotype diversity within these expanding populations typically indicates reduced population size during cold cycles, which is widely interpreted as the result of less habitat being available. However, some species are likely to have found their preferred habitat was shrinking during the interglacial rather than glacial phases (Lister & Stuart, 2008; Dong *et al.*, 2017). Relatively small range shifts are typically inferred for species that are perceived as tracking narrow elevational habitat zones on mountains (e.g. Schmitt, 2007; Gentili *et al.*, 2015), but it could be the case that cold-adapted species had larger populations and more continuous ranges during comparatively lengthy cold periods (Dergachev, 2015). Alpine specialists tend to be restricted in distribution to higher latitudes and/or fragmented high elevation habitats, but they might retain high genetic diversity from larger populations in the recent glacial past. Signatures of gene flow may remain from when their populations were not isolated on mountain peaks.

Our modern anthropogenic perspective is from the situation of an interglacial climate, but prevailing conditions have existed for a relatively short time (Dergachev, 2015), whereas colder ‘glacial’ climate persisted up to 10 times longer with continuous change operating in cycles. Glacial phases increased the relative extent of alpine habitats in temperate and montane areas (Birks, 2008) and also grasslands in subtropical regions (Piñeiro *et al.*, 2017). In contrast, ongoing anthropogenic global heating (Steffen *et al.*, 2018) can only attenuate an already atypical situation in terms of planetary climate in the Quaternary, and perhaps since the mid-Miocene disruption (Holbourn *et al.*, 2018). The insular nature of alpine systems, which contain a disproportionate amount of terrestrial biodiversity (Rahbek *et al.*, 2019), makes them especially vulnerable to biological erosion and significant in terms of ecosystem processes (Trisos *et al.*, 2020).

Here, we use analysis of changing conditions in the Southern Alps, New Zealand (Kā Tiritiri o te Moana, Aotearoa) to infer population level effects, focussing on the flightless alpine-adapted grasshopper *Sigaus australis* which is common above the tree line. The Southern Alps extend across almost 5° of latitude making a nice system for investigation of elevation and latitude gradients. The geology and biology of the area at the juncture of two tectonic plates (Trewick *et al.*, 2007) is well explored. Today about 50% of South Island is elevated above 500 m and more than 20% is above 1000 m providing habitat for a rich alpine biota (e.g. Halloy, & Mark, 2003; Koot *et al.*, 2020). Using niche models based on the current fragmented distribution of *S. australis*, we examined whether the environmental envelop of this species would have been more widespread and with greater connectivity during the LGM. We considered the extent of glacial ice and fragmentation of past and current habitat. Seeking evidence that *S. australis* distribution was formerly more contiguous, we surveyed the species looking for evidence of gene flow during the LGM in the form of isolation by distance, using pronotum shape and mtDNA variation. The process of divergence with gene flow would leave a signature of isolation by distance and might result in a correlation between phenotypic and genetic distance. The availability of widespread suitable habitat during the LGM would leave a

signature of a sustained large population with high genetic diversity and connectivity. We did not expect to see concordance of morphological and genetic clusters, however environmental gradients, fragmentation and the shared history of a non-recombining gene can naturally result in clusters that might have the appearance of multiple species. We did not expect the mtDNA diversity within this species to coalesce as recently as the LGM when open habitat was available to support large populations of this insect.

Methods

Sampling

We collected *S. australis* grasshoppers from 27 locations in South Island New Zealand, extending the documented range of this species (Figure 1; Supporting Information Table S1.1). Grasshoppers were collected by hand in subalpine and alpine habitat on mountains of the Southern Alps when grasshoppers were active during the New Zealand summer (December to March, 1995–2016; Trewick, 2008; Trewick & Morris, 2008; Dowle *et al.*, 2014). The majority of specimens came from habitat between 1100 and 1890 m asl, but rare low elevation (~300 m asl) populations were also sampled. Specimens were frozen before immersion in 95% ethanol, and identification followed Bigelow (Bigelow, 1967). Maturity and sex were assessed using size and shape of terminalia and tegmina, and recorded along with date, elevation and location (recorded using a portable GPS device).

Niche modelling

To estimate the environmental envelope suitable for *S. australis*, location records were acquired for 14 species of New Zealand endemic grasshoppers (Supporting Information Fig. S1.1). Including multiple species increases the accuracy of our niche models by providing reliable absence information. Records from our collection, journal articles, books and Crown Pastoral Lease Tenure Reviews (produced by Land Information New Zealand) were compiled with data from our own grasshopper collections (Supporting Information Table S1.2). All observations were included in a binary table where the presence or absence of *S. australis* was recorded for each location.

In order to define and project the potential niche of *S. australis*, 19 Bioclimatic variables were obtained from the Worldclim website (Hijmans *et al.*, 2005) for two different time periods – the LGM(c. 30–8 ka), and ‘current’ (data averaged from 1960 to 1990; Supporting Information Table S1.3). Climate layers were produced using the MIROC-ESM global climate model (Watanabe *et al.*, 2011), at a resolution of 2.5 arc minutes for the LGM and 30 arc-seconds for the current layer. The Worldclim files were cropped to the extent of New Zealand (Latitudes: –49, –32; Longitudes: 165, 180) using QGIS (QGIS Development Team, 2016). A variance inflation factor (VIF) (Lin *et al.*, 2011) analysis was used in a stepwise selection process to identify and remove collinear variables and reduced

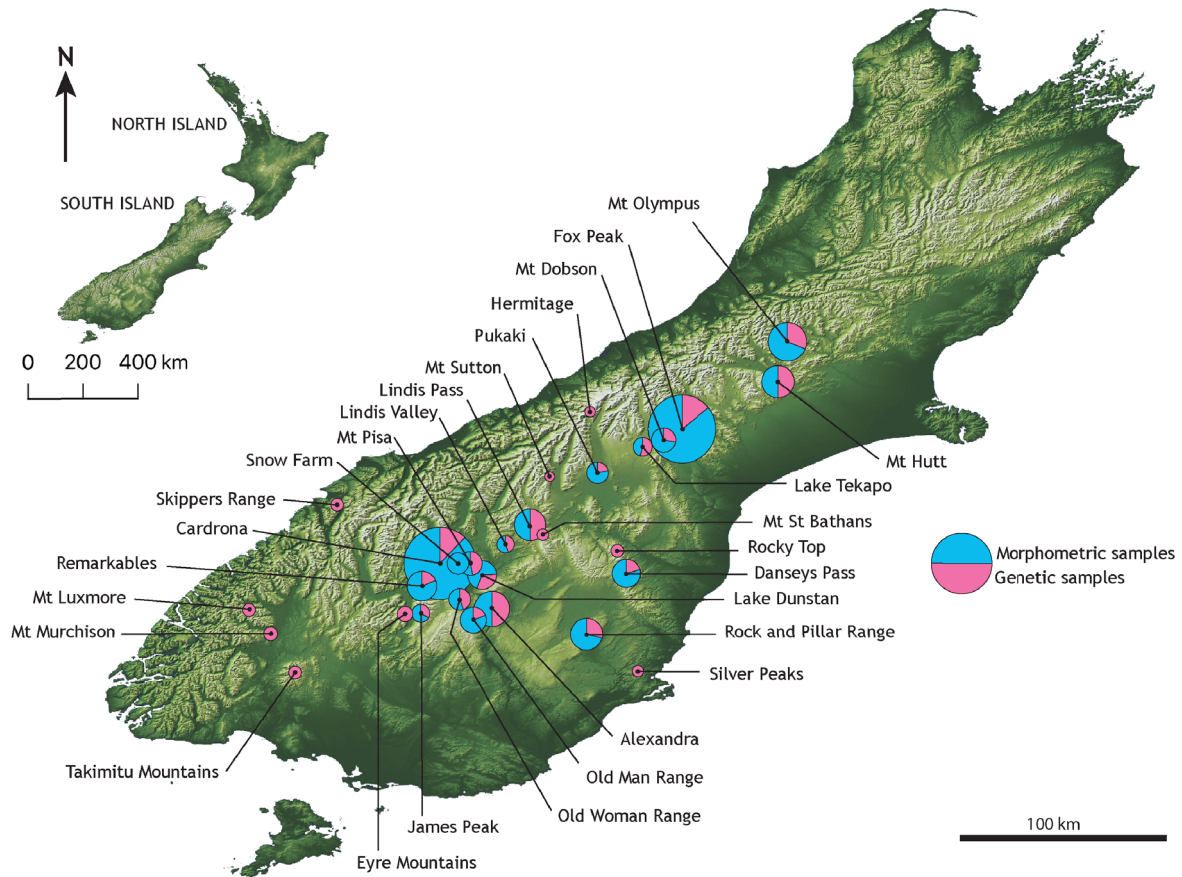


Figure 1. Sample locations for specimens of the alpine grasshopper *Sigaus australis* in South Island, New Zealand, used in morphometric and genetic analysis. Pie segments represent the proportion of each type of sample and their size represents relative sample size. [Color figure can be viewed at wileyonlinelibrary.com]

the list of 19 climate variables down to seven using the R package *usdm* (Naimi *et al.*, 2014) and a threshold of 10 (Supporting information Table S1.3). By removing variables with strong correlation we minimised over-fitting and increase parsimony during the modelling process (Fletcher *et al.*, 2016).

Soil and vegetation layers were included in the analysis because these two environmental variables are known to influence the distribution of some grasshopper species (Nattier *et al.*, 2013; Weiss *et al.*, 2013). Soil and vegetation data layers were rasterised, clipped and re-scaled to a resolution of 30 arc-seconds from their original files Fundamental Soil Layers New Zealand Soil Classification and Vegetative Cover Map of New Zealand, obtained through the Land Resource Information System (LRIS) portal (Leathwick *et al.*, 2012). These layers represent the current approximate state of vegetation and soil in New Zealand and were therefore used as static layers throughout the modelling process. In the absence of a verified model of New Zealand soil and vegetation cover during LGM, Ecological Niche Models that include static layers of this type are known to perform as well as, or better than, models where only dynamic variables are included (Stanton *et al.*, 2012). Inclusion of these static layers enabled exploration of their potential contribution

to the niche model under current conditions and were therefore retained for model prediction with LGM climate estimates. As soils associated with the Southern Alps form quickly and are dominated by weathering of rapidly uplifting rock strata (Larsen *et al.*, 2014), it is probable that climate cycling influences the rate of formation but not characteristics of the soil.

To estimate the environmental niche of *S. australis*, we used the R package *biomod2* v. 3.3-7 to apply 10 different modelling methods to the presence and absence data of the grasshoppers in the context of 'current' predictor variables (Thuiller *et al.*, 2016). Default modelling parameters were used with 80% of the data to calibrate the models and then tested with remaining 20%. Each model was repeated three times resulting in a total of 30 models. Output values of variable importance were calculated as 1 minus the mean correlation score of each variable, with scores closest to 1 indicating a variable of high importance. Investigation of model accuracy was carried out using two different evaluation methods: receiver operating characteristic (ROC) [i.e. area under the curve (AUC)] and true skill statistic (TSS). Models with ROC values of 0.9–1 and TSS values of 0.8–1 are considered to have 'excellent' predictive power (accuracy) (Thuiller *et al.*, 2009). An ensemble model was then generated from a

subset of these models based on their ROC values (>0.8). Using the ensemble model, ensemble forecasts were projected for the LGM. Plots were produced for each time period using the Ensemble Model mean weights model (EMmw). EMmw variable importance was calculated by applying the weights produced in the ensemble model to the associated models in the 30 model data set. Models were run three times for each predictor variable, these were then averaged and EMmw variable importance was calculated by summing the total of the averages for each predictor variable, and dividing by the number of modelling methods used (i.e. 10) (Fletcher *et al.*, 2016). Final scores of variable importance were converted into percentages of total variable importance for each modelling method.

Binary vectors of each ensemble model were generated for range change and fragmentation statistical analyses. These binary vectors were generated from the EMmw model dataset, where each pixel that scored greater than the predetermined cut-off value was ranked as a 1, and all other pixels as 0's. In order to compare the binary vectors, LGM binary vector was disaggregated to correspond with the resolution of current binary vector using the R package *raster* (Hijmans & Eten, 2012). When comparing binary vectors between current and past models, Biomod2 ranked pixels as: 'Never occupied' (i.e. pixels were unoccupied and remain unoccupied between models), 'Always occupied', 'Lost' (i.e. pixels were occupied but become unoccupied in the current model) or 'Gained' (i.e. pixels that were unoccupied in the LGM model become occupied in the Current model), from which range change statistics were then calculated.

As the LGM was characterised in New Zealand by the extension of valley glaciers that would limit the occupation of potential habitat predicted by the climate modelling we examined its extent using the results of a geomorphological analysis (James *et al.*, 2019). Predicted ice cover of ~6800 km³ during LGM was implemented on the binary vectors excluding the pixels overlain by the ice cover layer from James *et al.* (2019). We improvised uncertainty about glacier margins and seasonal fluctuations by iterating the analysis with the exclusion of the pixels of the binary vector where the ice layer was predicted to be over 50 m and 100 m. Fragstats, implemented in the R package *SDM Tools v 1.1–221* (VanDerWal *et al.*, 2012), also used these binary files to estimate fragmentation statistics (e.g. patch size, number of patches), which were compared between LGM and current niche models. For each scenario pixels that were connected within each of the binary files were given unique patch identities, and the area these patches calculated. The total area and the number of patches into which that area was distributed were computed excluding the smaller patches. We used a range of exclusion thresholds (from 250 km² to 3000 km²) allowing us to investigate habitat connectivity indicated by different scenarios.

Phenotypic variation

To explore morphological variation within and between populations, we used a geometric morphometric approach. Pronotum shape variation was used as a proxy for overall variation as this

structure is not susceptible to arbitrary changes during preservation (Friedrich *et al.*, 2014). The shape of the posterior margin of the pronotum provides diagnostic differences that distinguish many of the New Zealand grasshopper species and have previously been shown to be amenable to geometric morphometric approaches (Dowle *et al.*, 2014; Ober & Connolly, 2015). Shape was analysed using two-dimensional landmark-based geometric morphometrics (Webster & Sheets 2010). Grasshoppers were arranged with pronotum perpendicular (horizontal) to the camera lens axis (Figure 2). Images were taken using a Canon EOS Kiss X5 (600D) with EF100 mm f2.8 USM macro lens 1:2 mounted on a vertical stand (Kaiser). Digital images were organised into thin plate spline (TPS) files using tpsUtil (Klingenberg, 2013; Rohlf, 2015) and landmarks were digitised and scale-calibrated using tpsDig2 on a Wacom Cintiq 22HD Pen Display tablet. All photography and landmark positioning was done by one person to minimise operator error.

Analysis of shape variation used 14 landmarks identified around the perimeter and the dorsal surface of the pronotum on each image of 503 grasshoppers. Landmarks 1, 2, 6, 8 and 12 relate to the main angles of the pronotum dorsal perimeter, 13 and 14 are at the intersection of sulci (surface grooves) with the dorsal midline, 3, 4, 5, 9, 10 and 11 on the lateral carinae (Fig. 2). X and Y coordinates are then assigned to each landmark. Generalised Procrustes analysis (Goodall, 1991; Bookstein, 1992) was run using the R package *geomorph v3.0.5* (Adams *et al.*, 2017) in the R statistics environment (R Core Team, 2019). Non-shape variation was therefore mathematically removed as position, orientation and size superimposes landmark configuration using least-squares estimates for translation and rotation (Adams *et al.*, 2013; Webster & Sheets, 2010). We examined error associated with image capture using Procrustes ANOVA with 1000 permutations (Anderson & Braak, 2003). This analysis allowed estimation of repeatability using the interclass correlation coefficient (Fruciano, 2016) and revealed that the error arising from image capture and landmark positioning variation was biologically irrelevant: the repeatability is above 90% (93.7%) using 30 individuals for landmark positioning and 18 individuals for image capture.

Size-corrected shape was produced from residuals of the Procrustes ANOVA against centroid size after revealing that pronotum size have an impact on shape despite the Procrustes superimposition (Gould, 1966; Klingenberg, 2016). The package *geomorph* also implements MANCOVA, an analysis of variance of several dependent variable (landmarks coordinates) by level of an independent factor variable, such as the population or the sex of each individual, and covariation of linear independent variables such as latitude (Collyer *et al.*, 2015).

Principal components analysis (PCA) of the pronotum shape and size-corrected pronotum shape variation from the covariance matrix of the X-Y coordinates were performed using the *geomorph* package. The principal components generated by PCA reflect (mathematically) independent variation in the shape of objects, and centroid size acts as a proxy for size variation (independent of shape). Statistically significant principal components (PCs) were identified using the broken-stick test on eigenvalues to identify PCs that explain more variance in the data than

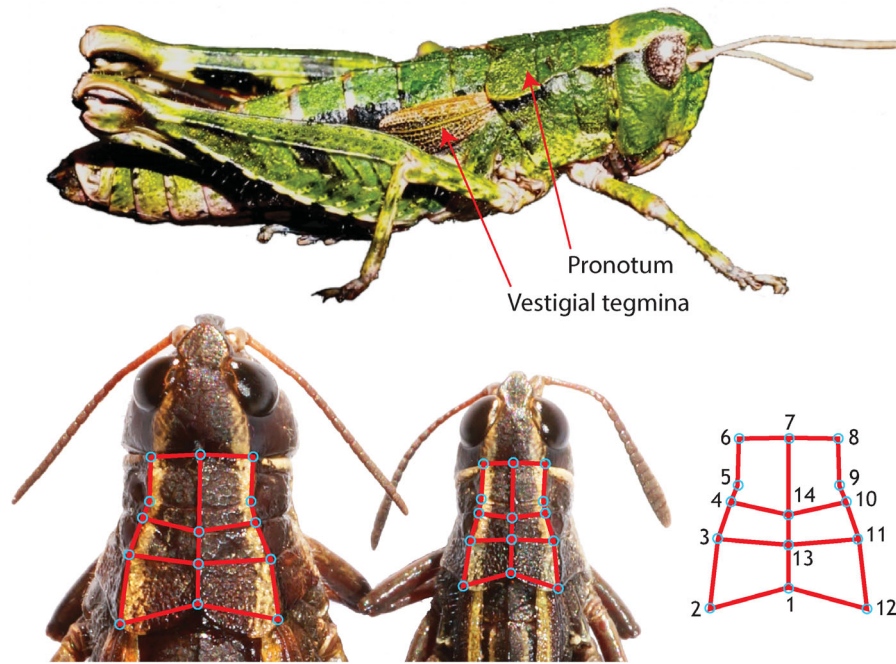


Figure 2. Phenotypic variation of the New Zealand alpine grasshopper *Sigaus australis* was quantified using geometric morphometric shape analysis with 14 landmarks arranged on the dorsal surface of the pronotum. Adult female green form (top), and anterior dorsal view of female (left) and male (centre) to the same scale with landmark array (right). [Color figure can be viewed at wileyonlinelibrary.com]

expected by chance alone, implemented in the R package *vegan* 2.2 (Oksanen *et al.*, 2018). Geographic correlation of shape between populations was tested using the significant principal components from the morphometric analysis to compute morphometric distance between individuals (Euclidian distance). Then the inter-population morphometric distance between two populations was computed by summing the distances for pairs of individuals from two different population and dividing this value by the number of sum computed (Dellicour *et al.*, 2017). The resulting distance matrix was then compare to the geographical distance of population using a Mantel test (Mantel, 1967), with 1000 bootstrap replicates.

Model-based assignment analyses were computed using significant principal components from the PCA on the pronotum shape + centroid size with the R package *Mclust* 5.4 (Scrucca *et al.*, 2016). This uses an iterative expectation–maximisation (EM) method with Gaussian mixture modelling (Fraley & Raftery, 2002) with model selection using Bayesian information criterion (BIC) scores. Variables are scaled because size is used as a variable and the Mclust algorithm assumes the same variance across all variables. To evaluate whether clustering results can be related to an actual grouping an adjusted Rand index is run. The adjusted Rand index compares the two partitions and has an expected value of 0 in the case of random partition, and it is bounded above by 1 in the case of perfect agreement between two partitions (Hubert & Arabie, 1985; Scrucca *et al.*, 2016). Canonical variate analysis (CVA) with cross-validation score was performed on shape data (503 sets of landmark coordinates) with the R package *Morpho* (Schlager, 2016). This analysis statistically tests the separation between defined

groups (sex and geographic location) using the Mahalanobis distance (Campbell & Atchley, 1981; Klingenberg, 2013).

Phylogeography

The mitochondrion remains the locus of choice for intraspecific animal phylogeography because it is single copy, uniparental and nonrecombining; this allows robust inferences about genealogy (Avice, 1986; DeSalle *et al.*, 2017). Here, we utilised the ND2 (NADH dehydrogenase 2) gene to estimate *S. australis* genealogical relationships as it has a higher capacity to accumulate haplotype diversity than the commonly analysed COI locus (Koot *et al.*, 2020).

Whole genomic DNA was extracted from muscle tissue from hind femora of 195 grasshopper specimens using a solvent-free Proteinase K and salting-out method (Sunnucks & Hales, 1996) as previously described (Sivyer *et al.*, 2018). Polymerase chain reaction used standard conditions with ND2 primers HopND2_147F (5' TGACCAACAACCTCTACAAAATTCT 3') and HopND2_1286R (5' TCAATAATGATTCTAGACTGCAATTCT 3') (Koot *et al.*, 2020). Sequencing used BigDye v3.1 chemistry and an ABI3730 DNA analyser with results edited and aligned in Geneious R10 (Kearse *et al.*, 2012). Population genetic analysis was conducted using statistical language R (R Core Team, 2019) and various packages. Matrilineal genetic diversity within each population sample ($n \geq 5$) was estimated by computing haplotype diversity (H) that represents the probability that two randomly sampled haplotypes are different, and nucleotide diversity (π), which is the average number of nucleotide differences per site in pairwise comparisons among DNA

sequences (Nei, 1987) using R package *pegas* (Paradis, 2010). Matrilinial relationships were inferred using median-joining haplotype networks (Bandelt *et al.*, 1999) generated using PopART (Leigh & Bryant, 2015) and the R package *igraph* (Csardi & Nepusz, 2006), to represent the relationships between haplotypes among populations. The haplotype network was computed under haplotype pairwise differences, giving the number of substitution steps between haplotypes.

Pairwise Φ_{st} values were computed to infer variation within and among populations with significance deviations from zero estimated by comparison with 1000 random permutations of the data (Excoffier *et al.*, 1992). Isolation by distance was assessed using the Mantel test (Mantel, 1967; Tamura & Nei, 1993) comparing pairwise population Φ_{st} and Euclidian distances between geographic coordinates with 1000 bootstrap replicates. To investigate the correlation between genetic and morphometric variation we used a Mantel test between pairwise genetic distance and a morphometric PCA distance matrix with 144 individuals specimens for which we had both data. We visualised the correlation using spatial principal components analysis (sPCA) with the genetic data using the R package *adegenet* (Jombart, 2008) and mapped an interpolation of genetic sPCA and morphometric PCA first two axis values.

Results

Niche modelling

S. australis was present at 278 of the 963 locations where grasshoppers were recorded and used to estimate the environmental envelope for the species. Most models had high receiver operating characteristics (ROC > 0.8; Supporting Information Fig. S1.2), and the EMmw ensemble model had an improved ROC (0.917) compared to individual models. Sensitivity (percentage of presences correctly predicted) and specificity (proportion of absences predicted; Allouche *et al.*, 2006) of the EMmw model were 75.9% and 88.3%, respectively. The cut-off threshold that maximised the proportion of presences and absences correctly predicted by the model used to produce binary vector layers was 536.5. Temperature attributes had the largest (~70%) influence on the environmental envelope determined for *S. australis*. Annual mean temperature was the most important predictor variable (29.1% of importance), but mean diurnal range (22.4%) and mean temperature of driest quarter (17%) were also important in explaining presence of *S. australis* (Supporting Information Fig. S1.3).

The environmental envelope was used to estimate total available habitat for *S. australis* at two time points (Fig. 3). The Potential Niche Space model for current conditions (Fig. 3a) corresponded to the known distribution (Realised Niche Space, Fig. 3d), suggesting that the distribution of *S. australis* is likely constrained by the climate variables used in our models or that these variables are close proxies for other important environmental traits. Temperature appears to be a factor limiting the distribution of *S. australis* (Supporting Information Fig. S1.4).

We predicted where suitable niche space would have likely been available for this grasshopper lineage during the LGM using results from our initial current ENMs (Fig. 3b). The inferred wider

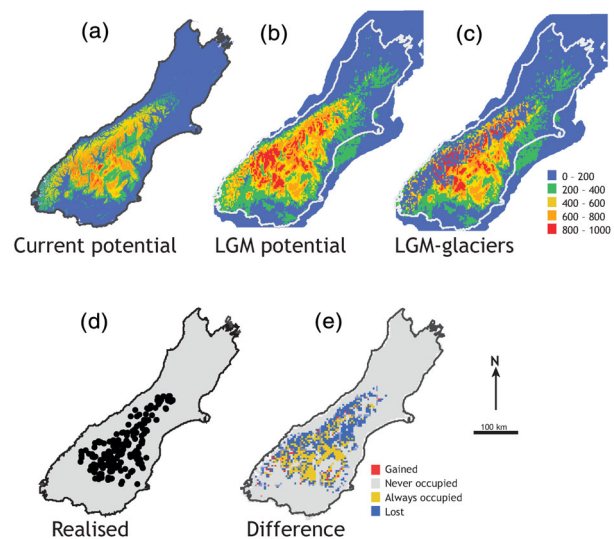


Figure 3. The realised niche space of the alpine grasshopper *Sigaus australis* in South Island, New Zealand alongside their potential niche spaces predicted using Ensemble Model Mean Weights models. (a) Predicted suitable niche space for current climate. (b) Predicted potential niche space during the Last Glacial Maximum (LGM) including inferred land extension. (c) As in B subtracting areas covered by valley glaciers. (d) Known current occurrence of *S. australis*. (e) Difference in potential habitat between LGM (no ice) and current climate models generated by comparing binary vector layers of LGM and current ENMs for pixels with score above threshold of 536.5. [Color figure can be viewed at wileyonlinelibrary.com]

extent of suitable environment during the LGM suggests *S. australis* could have had a wider range (36 359 km²) in the past than today (16 661 km²) (Fig. 3; Table 1). However, when the estimated extent of valley glaciers was considered the estimated potential habitat during LGM was reduced substantially (Fig. 3c). At maximum estimated extent, valley glaciers eliminate habitat area gains (15,178 km²), although this impact is lessened with increasing allowance of edge ice thickness to accommodate uncertainty of glacier limits, seasonal fluctuation and ice reduction post-LGM. Nevertheless, we inferred fewer large habitat fragments in the current model (43% fragments >3000 km²) than during the LGM (85% fragments >3000 km²), even if the full extent of glaciers was considered (57% fragments >3000 km²). This predicts a higher level of gene flow was possible across the species range during the LGM and that loss of genetic diversity due to drift would have been minimal. We sought evidence of this past connectivity by examining the extent and distribution of phenotypic and genetic variation displayed by extant *S. australis* populations that are isolated from one another today.

Phenotype variation

Pronotum shape of 490 adult *S. australis* specimens spanning the current range was examined. A strong significant effect of sex on pronotum shape was detected (MANCOVA on Procrustes

Table 1. Available habitat for *Sigaus australis* predicted with EMmw niche model for current climate and conditions during the last glacial maximum (LGM).

Min. patch size	Current		LGM		LGM < 100 m ice		LGM < 50 m ice		LGM < 0 m ice	
	Total	Ratio	Total	Ratio	Total	Ratio	Total	Ratio	Total	Ratio
>0	16661	1.00	36359	1.00	25825	1.00	22646	1.00	15178	1.00
>250	15258	0.92	36070	0.99	25458	0.99	22079	0.97	14932	0.98
>500	14875	0.89	35962	0.99	24931	0.97	21771	0.96	14652	0.97
>1000	14252	0.86	35591	0.98	24569	0.95	21480	0.95	14252	0.94
>3000	7097	0.43	30777	0.85	21186	0.82	15749	0.70	8653	0.57

Extension of valley glaciers during the LGM is accommodated by excluding potential habitat overlain by ice up to 100 m, 50 m and 0 m thick, respectively. We sampled at habitat patch size intervals from 0 to 3000 km² and calculated the proportion of total for each model contained within fragments of each size range.

analysis of pronotum shape), which is consistent with visible sexual dimorphism including size (Table 2(A)). Difference in pronotum shape between males and females decreased when we used a size-corrected shape analysis (Table 2(B)) revealing an allometric effect (Gould, 1966; Klingenberg, 2016). A significant effect of latitude on shape variation was detected with pronota being narrower in northern versus southern individuals. A less pronounced narrowing of pronota with increasing elevation was also apparent. Despite the significance of sex, latitude and elevation as explanatory variables, most shape variance (92%) was still unexplained (Table 2(B)).

There were three significant principal components in the pronotum shape data explaining 40.4% (PC1), 14.0% (PC2) and 8.3% (PC3) of variation. A Mantel test revealed a significant positive spatial correlation between pairwise morphological distance and geographic distance (Females, $Z = 0.4515$, $P = 0.000999$; Males, $Z = 0.2308$, $P = 0.02298$; Supporting Information Fig. S1.5).

Naive clustering (Gaussian mixture modelling) of pronotum variation using three significant PCA components and size

resolved four clusters of specimens with the best fitting model (EEE4: ellipsoidal, equal volume, shape and orientation model with four components). These clusters largely partition the permutations of male, female, northern and southern grasshoppers (Figure 4). The northern phenotype was common at Mt Olympus, Mt Hutt, Fox Peak, Danseys Pass, Pukaki, Lake Tekapo, Mt Dobson, Rock & Pillar Range, while the southern phenotype was common in Cardrona, Remarkables, Lake Dunstan, Snow Farm, Old Man Range, Lindis Pass, Old Woman Range, Lindis Valley, Alexandra, James Peak and Mt Pisa (Fig. 4(B)).

Using the four clusters resolved by Gaussian mixture modelling, specimens were in general grouped with other specimens of the same sex and geographic location (north/south), but there were 81 misassignments out of 490 individuals (adjusted Rand index = 0.6474, 83.5% correct assignment). Most specimens were correctly assigned to sex on the basis of pronotum shape (19 misassignments), but some grasshoppers from northern sites did not cluster with geographically adjacent individuals. In particular, we found several misassignments among male grasshoppers collected from Mt Olympus and Cardrona (approximately 40% of misassignments). Other geographically misassigned grasshoppers were from sites between northern and southern locations and from isolated population samples including Rock & Pillar Range, Danseys Pass and Mt Hutt.

Canonical variate analysis (CVA) on pronotum shape data showed separation between sex and between North and South individuals (classification accuracy: 88.98%) and allowed definition of the shape differences by a single component (Fig. 4 (C)). As each CVA axis mostly represents one factor in this case, we can infer that CVA1 (59%) primarily separates the sexes with females having a more triangular pronotum while males are more rectangular. CVA2 (39.1%) indicates that northern individuals tend to be narrower than those in the south.

Genetic variation

We generated ND2 DNA sequences of 673 bp for individuals from 17 *S. australis* population samples. The aligned ND2 sequences ($n = 194$) had 178 polymorphic sites and 95 haplotypes were identified (GenBank accession MT712921 - MT713114). Haplotype diversity was high in 14 of the 17 sampled populations ($H > 0.700-1$; Supporting

Table 2. Morphological variation within *Sigaus australis* using landmark coordinates considering effect of sex, latitude and elevation on shape variation using Procrustes MANCOVA with 1000 permutations. (A) Procrustes-aligned coordinates of pronotum landmarks and (B) size-corrected Procrustes-aligned coordinates of pronotum landmarks

(A)	d.f.	SS	MS	F	P
Sex	1	0.20022	0.200221	105.227	<0.001
Latitude	1	0.12792	0.127921	67.229	<0.001
Elevation	1	0.02591	0.025909	13.616	<0.001
Residuals	484	0.92094	0.001903		
Total	487	1.27499			
(B)	d.f.	SS	MS	F	P
Sex	1	0.00960	0.009601	5.0727	<0.001
Latitude	1	0.13363	0.133628	70.6060	<0.001
Elevation	1	0.02197	0.021970	11.6083	<0.001
Residuals	484	0.91601	0.001893		
Total	487	1.08121			

d.f., degree of freedom; SS, sum of square; MS, mean square; F, F-score; P, P-value.

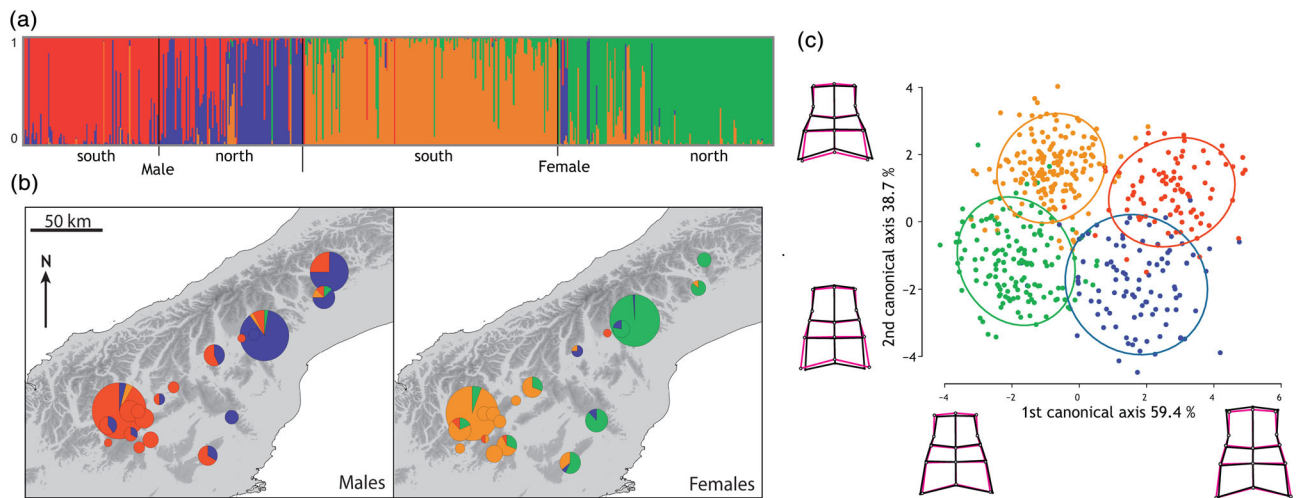


Figure 4. Phenotypic variation within *Sigaus australis* based on geometric morphometric analysis of pronotum shape. (a) Assignment probabilities of grasshopper individuals to each of four clusters (represented by different colours) inferred naively by *mclust* with the optimal EEE4 model, arranged by sex and region. Each bar represents assignment probability of one individual. (b) Spatial distribution of predominant cluster assignment of males and females, where each pie represents a population sample location scaled by the number of individuals and each colour represent the proportion of each cluster. (c) The first two axes of a canonical variates analysis on pronotum landmark coordinates with sex and region of origin defined a priori. Results indicate that these categories are relevant to cluster shape and provide an explanation for the clustering result (88.98% of classification accuracy). Shape differences gathered by each canonical axis are represented by the estimated pronotum shape for a high and low value of the canonical component. The black landmarks are the estimated landmarks and pink is the consensus shape of the sample. [Color figure can be viewed at wileyonlinelibrary.com]

Information Table S1.4) and low in the smallest samples (e.g. Lake Dunstan, Lake Tekapo and Remarkables). Nucleotide site variation differed considerably among population samples, ranging from 0 to 0.0347.

Few ND2 mitochondrial DNA haplotypes were shared among population samples, and the overall Φ_{ST} was significantly greater than zero (0.8829, $P < 0.001$). In general, geographically adjacent population samples had similar haplotypes and the lowest pairwise Φ_{ST} estimates were observed when these neighbours were compared. Over the entire species range IBD was apparent with significant correlation between pairwise Φ_{ST} and geographic distance (Mantel test; $Z = 0.2299$, $P = 0.01898$, Supporting Information Fig. S1.6). Major haplotype clusters within *S. australis* were apparent between the most northern population samples (Mt Olympus, Mt Hutt, Lake Tekapo, Mt Dobson and Fox Peak) and all others and were separated by more than 50 nucleotide substitutions (Fig. 5).

Concordance of genetic and phenotypic clusters

We found a significant correlation between morphometric PCA distance and genetic distance using a Mantel test among individuals common to these datasets ($n = 144$, $Z = 0.1589$, $P = 0.001$; Supporting Information Fig. S1.7). The broad correlation of shape and lineage is apparent from the interpolation of genetic sPCA and the first two morphometric PCA axes (Fig. 6), especially between the first axis of PCA and the first axis of sPCA.

Discussion

Of all the climatic and environmental variables included in our models, we found temperature to be the most important factor determining the presence or absence of the alpine grasshopper *S. australis* in New Zealand. The key variables in our model are likely to be proxies for a set of correlated variables that include temperature. Temperate grasshoppers need to bask in the sun (Koot *et al.*, 2020), so their ranges in New Zealand are limited to open habitats (Bigelow, 1967). In a landscape naturally dominated by wet forest (Trewick & Morgan-Richards, 2009), the elevational tree line is the major constraint on the distribution of such habitat and is itself primarily controlled by temperature (Körner, 1998). As a result, climate cycling during the Pleistocene was characterised by substantial shifts in both the latitudinal and elevational extent of forest and open habitat. The cooling and attendant aridification experienced globally during Pleistocene cold phases was locally emphasised in South Island, New Zealand where the Southern Alps cast an eastern rain-shadow that extended available open habitat (Ausseil *et al.*, 2011).

Despite this, our modelling indicates that total habitat area available to *S. australis* during the LGM was probably similar to the present. Although the estimated LGM environmental envelop was more than twice that available today, valley glaciers comprising about 100 times as much ice as they currently do, obscured more than half of this. Nonetheless, our models suggest that *S. australis* populations were less fragmented during the LGM than experienced by their current high elevation distribution. As *S. australis* (and all other endemic alpine grasshoppers; Koot *et al.*, 2020) have vestigial tegmina (Fig. 2) and cannot fly,

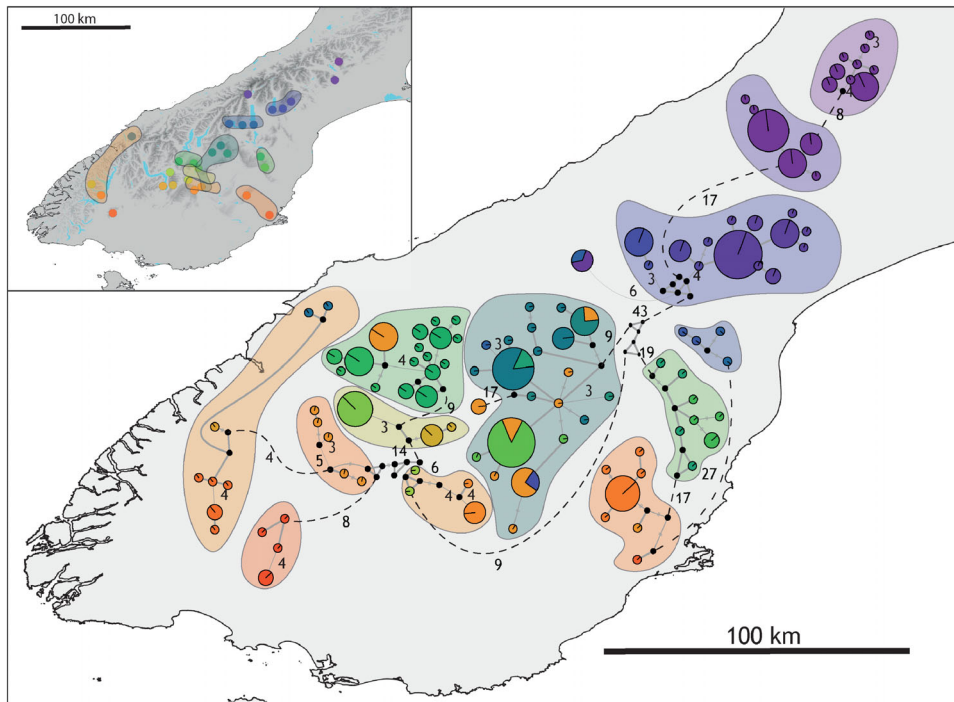


Figure 5. MtDNA ND2 haplotype phylogeography of *Sigaus australis* grasshoppers. Median-joining haplotype network overlain on population sample locations. Nodes represent haplotypes and are colour-matched with their sample membership proportional to pie size. Edges represent minimum genetic distance between most similar haplotypes with number of substitutions between linked nodes displayed. Inset shows location sample sites coloured by predominant ND2 cluster. [Color figure can be viewed at wileyonlinelibrary.com]

mountain populations are reproductively isolated from one another by the tree line that responds to mountain shape and other variables (Case & Buckley, 2015). In contrast, during the considerably longer glacial periods (each ~100 kya) gene flow for 'alpine' species was less constrained.

Interpretation of alpine phylogeography has followed from the findings made of low elevation biota that identified population refugia during the colder periods of the Pleistocene (Taberlet *et al.*, 1998; Hewitt, 2000). Indeed, population expansion from glacial refugia has also been proposed for some northern hemisphere alpine species including grasshoppers with limited intraspecific diversity (Knowles, 2000; Berger *et al.*, 2010). This suggests such species were rarer in glacial than inter-glacial phases of the Pleistocene. One explanation offers nunataks (ridges protruding between glaciers) as micro-refugia during glaciation by referencing modern conditions and does predict reduction in available habitat for alpine adapted taxa. Data, however, do not strongly favour this interpretation (e.g. Rogivue *et al.*, 2018; Zhang *et al.*, 2018).

Given inferences of range shifts in populations of low elevation taxa during Pleistocene glacial phases, it is implicit that alpine-like conditions extended at the same time. This wider availability of open habitats in the northern hemisphere is, for instance, demonstrated by phylogeographic signal from mammoth remains (Palkopoulou *et al.*, 2013). The idea that alpine taxa might have been subject to 'glacial refugia' (Holderegger & Thiel-Egenter, 2009), implying LGM range restriction and small

populations, is counter to the high intraspecific genetic diversity detected in many alpine species (e.g. Pauls *et al.*, 2006; Bettin *et al.*, 2007). Generally, high genetic diversity is considered indicative of large populations (Charlesworth, 2009), and this is borne out in alpine habitats by demographic analyses (e.g. Huang *et al.*, 2016). Therefore, reference to range change rather than 'refugia' is more appropriate as it avoids the implication that the current climatic situation represents the norm.

Among New Zealand alpine species, high intraspecific genetic diversity observed in populations with small modern ranges probably reflects large populations sustained throughout the Pleistocene (e.g. Trewick *et al.*, 2000; King *et al.*, 2003; McCulloch *et al.*, 2009; O'Neill *et al.*, 2009). A similar situation is observed in Australian mountains (e.g. Endo *et al.*, 2015). From this, we infer that any loss of potential range for cold-adapted, open-habitat specialists is most likely to have occurred during relatively short interglacials rather than glacials (Sivyer *et al.*, 2018). In *S. australis* grasshoppers, we find better support for more continuous range in the LGM than for alternative hypotheses of isolated nunatak refugia. Grasshoppers in southwestern populations (e.g. Skippers Range, Takitimu Mountains) that are today isolated from others in the Southern Alps, are most similar to grasshoppers in nearby south central habitat. In contrast, eastern central populations (e.g. Danseys Pass, Rock and Pillar Ranges) represent a more deeply diverged mtDNA lineage consistent with unglaciated habitat continuity during the LGM. High haplotype diversity within the central area (e.g. Lindis

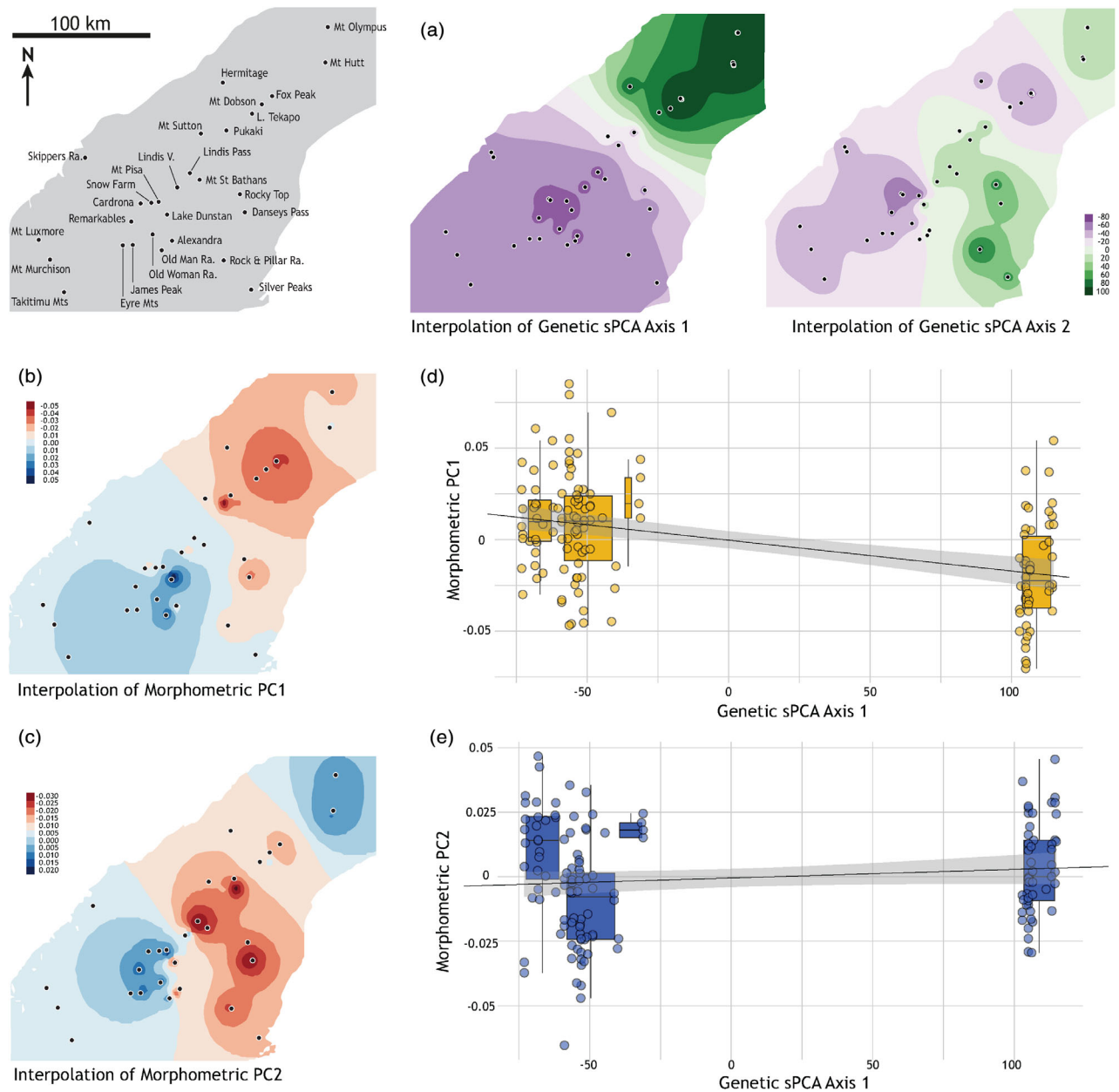


Figure 6. Comparison between geometric morphometric and genetic clustering of *Sigaus australis* alpine grasshoppers ($n = 141$). (a) Interpolation of first and second axes of spatial PCA on ND2 mtDNA haplotypes. (b) Interpolation of first axis of PCA of pronotum shape. (c) Interpolation of second axis of PCA of pronotum shape. (d) First axis of sPCA on ND2 against first axis of PCA of pronotum shape. Boxplots created with a bin width of 20, and linear regression with 95% confidence interval in grey. (e) First axis of sPCA on against second axis of PCA of pronotum shape. [Color figure can be viewed at wileyonlinelibrary.com]

Valley, Lake Dunstan) associated with low elevation semi-arid habitat suggests admixture (Dowle *et al.*, 2014; Sivyer *et al.*, 2018). We have investigated genetic divergence using a single non-recombining locus that represents the matrilineal history of the species efficiently and effectively (DeSalle *et al.* 2017) and is especially informative when accompanied by other types of information such as our morphometric analysis (Rubinoff & Holland 2005). Although high mtDNA genetic

diversity might result from biological artefacts (Hurst & Jiggins 2005), it is also the well-understood result of large population size (Morgan-Richards *et al.*, 2017).

Niche models are limited by data, and the distribution of suitable habitat needs to be considered as well as total size inferred from climate models. The inferred area of suitable habitat for *S. australis* during the LGM based on a niche model, that assumes the grasshopper's environmental requirements have

not substantially changed, was more than twice the size of our estimates that also considered the LGM distribution of montane glaciers in New Zealand. After excluding the potential habitat likely to have been covered by ice during the LGM reduced our estimate of the past *S. australis* habitat to slightly less than the current potential habitat. MtDNA diversity detected in *S. australis* was high and mostly partitioned among population samples, suggesting that *S. australis* populations have not expanded from small glacial refugia but have remained large. Our knowledge of Milankovitch cycles suggests that habitat suitable for *S. australis* would have fluctuated many times, being smallest during the warm and cold maxima, and larger as the climate cycled between. However, it appears unlikely that at any time would habitat and population size have reduced sufficiently for mtDNA coalescence, thus maintaining high intraspecific diversity at this locus. Importantly, our niche models for *S. australis* led us to infer that their past populations would have been less fragmented during the LGM compared to their current distribution. Local differentiation with gene flow will lead to an intraspecific population structure of isolation by distance if dispersal is limited by geographical distance (Slatkin, 1993). The current patterns of isolation by distance detected in presumably neutral pronotum shape and limited mtDNA variation indicate gene flow among populations in the recent past. Thus, in this flightless grasshopper, we see a signature of population connectivity from the LGM although many populations are today isolated from one another at high elevation. Connectivity of populations during cool phases has probably prevented speciation during short warm phases when populations are more fragmented. We detected some evidence of divergence between populations with a north/south distribution but half of our population samples were polymorphic, and phenotype and mtDNA was not strictly concordant. Current predictions of global warming will shrink habitat resulting in reduction of population size, further fragmentation and potential local extinction. The high genetic and phenotypic variation within *S. australis* might enhance potential for adaptive response to the challenging climatic changes, but loss of open alpine habitat to expanding forest at higher elevations will cause *S. australis* and other alpine specialists to decline. Future climate warming is likely to further fragment and reduce population sizes and connectivity of all cold-adapted species.

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Data Availability Statement

All mtDNA sequences were deposited in GenBank (MT712921 - MT713114). Geometric morphometric datasets,

sequence alignments and distribution data will be deposited on Dryad (doi:10.5061/dryad.wh70rxwk8).

Supporting information

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

Appendix S1: Supporting Information

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