Isolated on sky islands: genetic diversity and population structure of an endangered mountain lizard

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Abstract

Sky island species face climate-driven and anthropogenic habitat loss and population fragmentation, and are therefore vulnerable to genetic erosion. We conducted a genetic study of the cryptic and threatened alpine she-oak skink (*Cyclodomorphus praealtus*) throughout its range, across two regions of the mainland Australian Alps; an extensive high elevation plateau in the north ('Kosciuszko Plateau') and several smaller plateaus in the south ('southern plateaus'). We investigated whether extensive potential habitat across Kosciuszko Plateau supported larger, connected populations with better genetic health than more fragmented southern plateaus. Our analyses of genome-wide markers confirmed effective isolation of the two regions. We identified three populations from the southern plateaus, largely aligning with discrete landforms, and four populations on Kosciuszko Plateau. Only one individual, from the southern-most population, showed evidence of admixture between the two regions. Across its range, *C. praealtus* populations had low genetic diversity and small effective population sizes. In contrast to our expectations, Kosciuszko Plateau populations were smaller, with greater genetic differentiation and a higher degree of inbreeding than the southern populations. We detected admixture between populations on Kosciuszko Plateau, while the southern plateaus had limited admixture. We found no evidence of local adaptation, suggesting plateaus represent interglacial refugia. Our results suggest that *C. praealtus* has little capacity to withstand further disturbance or rapid environmental changes. Maintaining or restoring habitat quality in occupied and suitable connecting habitats across the species' range is paramount. 'Genetic rescue' should be investigated as an option to mitigate the effects of isolation and improve population resilience.

Keywords Fragmentation · Genetic diversity · Population differentiation · Single nucleotide polymorphisms (SNP) · Sub-alpine · Threatened species

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Introduction

'Sky islands' are high elevation landscapes fragmented by a 'sea' of lowlands that may be formed through palaeoclimatic and/or anthropogenic processes (Robin et al. 2015). Lowland areas can act as strong barriers to dispersal (Chala et al. 2017; Mairal et al. 2017); sky island systems therefore often support a high degree of endemism as well as ancient lineages (Chala et al. 2017; Mairal et al. 2017; He et al. 2019; Suissa et al. 2021). Globally, species are predicted to respond to climate change via adaptation or shifting their distributions to stay within their niches (Román-Palacios and Wiens 2020). If not already occupying the highest available areas, cold-adapted species may be forced to retreat to higher elevations as lowlands become unsuitably warm, which can lead to population fragmentation (Raxworthy

et al. 2008; Wiens et al. 2019; Freeman et al. 2021). Isolated populations face increased genetic drift and loss of genetic diversity, which affects long-term viability (Irwin 2002). Habitat protection and assisted admixture among otherwise isolated populations are increasingly important to prevent local extinctions and to ensure the persistence of alpine sky island species (Ralls et al. 2018).

Effective conservation requires insights from key population parameters, such as size and connectivity (Scheele et al. 2018; Woinarski 2018; Sullivan et al. 2020). While collecting such information has previously been challenging, modern genetic-based technology provides a rigorous, cost-effective means of assessing population parameters and resilience (O'Donnell et al. 2018; Atkins et al. 2019; Amor et al. 2020). For example, recent studies on mountain reptiles have used genetic data to calculate key population parameters and infer barriers to gene flow (e.g. Saijuntha et al. 2017; Agarwal et al. 2019; Esquerré et al. 2019; Wiens et al. 2019), thereby addressing knowledge gaps and informing conservation decisions.

Australia is a hotspot for reptile diversity. However, population trends for 34.2% of Australia's terrestrial reptiles remain unknown, and the proportion listed as threatened is increasing (Tingley et al. 2019). Notably, Australia's sky island species face an uncertain future (Parida et al. 2015; Sritharan et al. 2021) due to warming temperatures and relatively low mountain elevations (McGowan et al. 2018). Genetic studies in Australia's mainland Alps have identified signals of isolation by distance or landscape barriers in reptiles (Chapple et al. 2005; Koumoundouros et al. 2009; Haines et al. 2017; Atkins et al. 2019), amphibians (Banks et al. 2020), mammals (Mitrovski et al. 2007) and invertebrates (Endo et al. 2015). These studies commonly identified genetic differentiation between populations on Kosciuszko Plateau and the smaller southern plateaus, and linked past fluxes in connectivity and isolation to glacial periods, consistent with evidence of climate-driven fragmentation on other continents (Chala et al. 2017; Mastretta-Yanes et al. 2018; Wiens et al. 2019; Tonzo & Ortego 2021). One such study identified alpine she-oak skink (*Cyclodomorphus praealtus* Shea 1995; Scincidae) populations from Kosciuszko Plateau and the southern plateaus as two Evolutionary Significant Units (Koumoundouros et al. 2009). However, that study had low sample numbers from Kosciuszko Plateau ($n = 5$, from a single location) and additional occupied sites have since been identified, extending the species' known range (Clemann et al. 2016; NSW Environment Energy and Science 2021).

Here, we analyse genome-wide markers to assess genetic diversity and population differentiation of *C. praealtus* across its range in the mainland Australian Alps. We asked whether smaller plateaus support populations that are more genetically isolated and potentially more susceptible to extinction. *Cyclodomorphus praealtus* habitat is extensive across Kosciuszko Plateau but occurs in smaller patches fragmented by steep forested valleys across the southern plateaus. Due to habitat specialisation and a relatively limited dispersal capacity, we anticipate that Kosciuszko Plateau populations will be distinct from populations on the southern plateaus. Further, ecological theory predicts that larger habitats support larger populations with a lower extinction risk (Hanski 1999), therefore, we expect that Kosciuszko Plateau populations will exhibit greater gene-flow and better genetic health than populations on the southern plateaus.

Materials and methods

Study region

The high plateaus of the mainland Australian Alps (Fig. 1) are likely to be the remnants of a peneplain formed in the Mesozoic and uplifted to its current elevation approximately 8–4 Ma (Webb 2017). The Murray River Valley separates
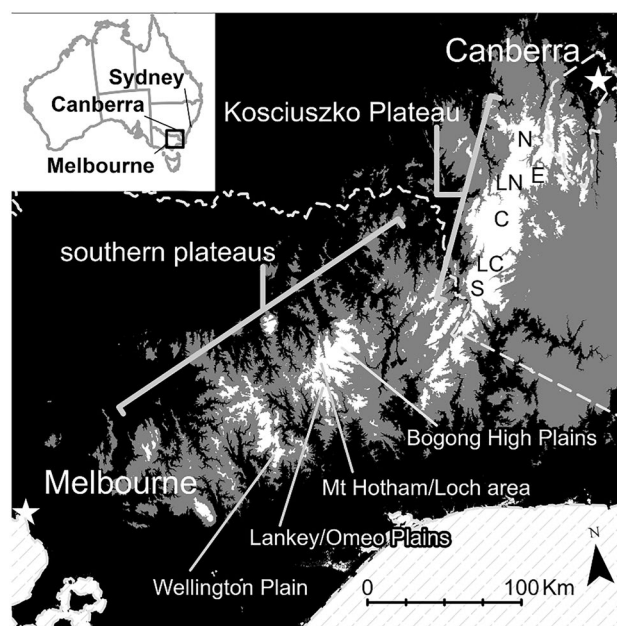
Fig. 1 Mainland Australian Alps, showing the contiguous Kosciuszko Plateau and fragmented southern plateaus, and current elevational range of *C. praealtus* (white, > 1,250 m). Shading corresponds to a 1 s digital elevation model (Gallant et al. 2011), where gray represents potential historic upland connectivity ~ 20 ka. Letters on Kosciuszko Plateau refer to nominal site groupings, where *N* northern, *E* eastern, *LN* lower-northern, *C* central, *LC* lower-central, *S* southern. Sampled southern plateaus were the Bogong High Plains, Mt Hotham/Loch area, Lankey/Omeo Plains and Wellington Plain (see Fig. 4 for specific sample locations and codes). Black represents lowlands (< 700 m). Dotted lines show jurisdiction boundaries. Stars depict capital cities. Hatched areas represent ocean. Inset shows the location of the study area within Australia

Kosciuszko Plateau in the north (~ 1,200–2,228 m elevation) from several fragmented plateaus to the southwest (~ 1,200–1,986 m elevation), creating two alpine regions on mainland Australia. The Kosciuszko Plateau is relatively large and undulating with steep slopes to the west and gradual slopes to the east (Webb 2017). It supports ~ 90,000 ha of potential *C. praealtus* habitat, compared to ~ 70,000 disjunct hectares across the plateaus and broad ridges to the south (McDougall and Walsh 2007). The Kosciuszko Plateau was the only mainland Australian region to experience Late Pleistocene glaciation; most extensively ~ 59,000 ya and least extensively ~ 17,000 ya (Barrows et al. 2001).

We sampled *C. praealtus* from Kosciuszko Plateau and the four southern plateaus where the species occurs (Fig. 1)—Bogong High Plains, Mt Hotham/Loch area, Lankey/Omeo Plains, and Wellington Plain. While the southern plateaus are largely separated by steep valleys below 1,250 m elevation, the Mt Hotham/Loch area connects to adjacent plateaus by elevations > 1,250 m (within suitable elevation for *C. praealtus*). However, these steep forest connections are unlikely to provide functional *C. praealtus* dispersal habitat.

Study species

Most reptile species endemic to the mainland Australian Alps are listed as threatened (Tingley et al. 2019). *Cyclodomorphus praealtus* is listed as Endangered by the Australian Government and IUCN due to its restricted distribution and ongoing threats, including climate change, habitat loss and fragmentation by infrastructure development, and habitat degradation caused by exotic herbivores (Australian Government 1999; Clemann et al. 2018). The species occurs in habitats that are largely treeless (Sato et al. 2014; Robertson and Coventry 2019) above 1250 m elevation (NSW Environment Energy and Science 2021). *Cyclodomorphus praealtus* has low detectability and surveys of it are resource-intensive. Consequently, genetic analyses are valuable for assessing population parameters.

Captures and tissue collection

We collected tissue samples from animals captured at 58 sites between January 2017 and April 2020. Across Kosciuszko Plateau, we collected 91 samples from 34 sites nominally grouped into six broad areas (Fig. 1). Across the four southern plateaus, we sampled 185 individuals from 24 sites. Each site was separated by over 100 m and comprised 10–25 artificial refuges (roof tiles). For each captured animal, we removed the distal 2–3 mm from its tail and stored it in ≥ 90% ethanol ($n = 1–24$ samples per site).

DNA extractions and sequencing

Diversity Arrays Technology (DArT), ACT 2617, Australia, extracted and sequenced DNA from our samples ($n = 276$, randomly allocated to wells). We replicated samples from three randomly chosen individuals for quality control. A medium density assay was sequenced via Illumina HiSeq2500, as per Atkins et al. (2019).

Data filtering

Using the pairwise Hamming distance matrix report provided by DArT, we compared pairwise distance among individuals within each site (mean = 0.097, standard deviation [sd] = 0.008) and our included replicates (mean = 0.008, sd = 0.004) to avoid accidental inclusion of recaptured individuals. There was a 9–15 times 'distance gap' between the reported distance among replicates (1.0%) and non-replicates (6.0%; Online Resource Fig. 1) across all individuals. We chose an upper distance limit of 1.5% to represent error among our replicates associated with library preparation and sequencing, after a replicated individual (BHP01G) was found to share an abnormally low pairwise distance with two independently sampled individuals (0.5%, which we considered a replicate sample; and 1.5%, which we suspect was a potential relative). Therefore, we considered any pair of samples that differed by < 1.5% as recaptured samples ($n = 17/276$; 6.2%).

We converted our raw, single-row DArTseq report into a 'genlight' object using the 'dartR' package v2.0.4 (Gruber et al. 2018) in R v4.0.5 (R Core Team 2020). Our raw dataset contained 121,039 loci from 276 individuals and 20% missing data with a mean reproducibility of 99.3% (range 86–100%). We randomly removed one sample representing each of our 17 recaptured individuals ($n = 259$; Kosciuszko Plateau = 88; southern plateaus = 171; Online Resource Fig. 1). We filtered data using a DArT-generated 'reproducibility score' threshold of ≥ 95% to ensure sufficient loci retention and quality, removed loci with a call rate of ≤ 75% (Alam et al. 2018) to balance data quality and volume, set a minimum depth of 10 reads per site, per individual. This dataset included 35,947 loci (mean depth = 17.5, missing data = 6.1%, secondary loci = 13,606). To investigate population structure, we filtered our 35,947 loci to remove those with a minor allele count of three (32,793 loci). This allowed us to retain rare alleles but remove singletons and doubletons that can confound inferences of population structure (Linck and Battey 2019).

Although missing data leads to artefactual differentiation between observed and expected single nucleotide polymorphisms (SNP)-based and genomic/autosomal heterozygosity (based on both variant and invariant sites) (Schmidt et al. 2021), only 212 of our loci had a call rate of 1 (no missing

data). Therefore, we calculated heterozygosity after retaining only loci from our 35,947 loci, that had ≤5% missing data (21,177 loci with mean missingness of 2.35%). Prior to calculating SNP-based estimates of population-level heterozygosity, we separated individuals into respective datasets, based on our genetic clustering results (see below). We then removed monomorphic loci (locally fixed, but variable between regions) to avoid lowering SNP-based calculations of heterozygosity for each inferred population via the inclusion of fixed sites.

Detection of non-neutral markers

We investigated putative non-neutral loci via 'BayeScan' v2.1 (Foll and Gaggiotti 2008), 'OutFLANK' v0.2 (Whitlock and Lotterhos 2014) and 'pcadapt' v4.3.3 (Luu et al. 2017). We discarded loci with a minor allele frequency (MAF) of ≤5% (retaining 15,957 loci) to reduce detection of false positives in subsequent outlier analyses and applied a false discovery rate of 10%. We performed 20 independent pilot BayeScan runs of 5,000 iterations each. Our final analysis comprised 50,000 initial iterations discarded as burn-in, and a final 100,000 retained samples. Our OutFLANK analyses were performed via the dartR package, using default 5% false discovery, as well as upper and lower flanking rates. The default of 10% heterozygosity was required before any locus was included in calculations. To run pcadapt, we converted our genlight object to a SNP matrix. We set an initial K-value of 20, then reduced this to the value prior to plateau using PCA scree plots. Outlier discovery via pcadapt (Jombart 2008) was based on q-value and Benjamini–Hochberg Procedure, which estimated 2,265 putative outliers. Only putative loci identified using multiple approaches were considered true outliers and were isolated from our dataset. BayeScan and OutFLANK failed to identify any outlier. Therefore, we did not filter any outliers from our dataset. This resulted in 15,957 loci for all individuals (6.8% missing); 17,452 loci for Kosciuszko Plateau (9.5% missing); and 11,219 loci for the southern plateaus (5.7% missing), which retained between 31 and 49% of our loci across the three datasets.

Genetic clustering

We applied three methods of genetic clustering to investigate population structure across the geographic range of *C. praealtus*, using 32,793 loci with a minor allele count of three. For Discriminant Analysis of Principal Components (DAPC), we estimated the number of clusters (K) best explained by our data via a *k*-means algorithm. We then performed *a*-score optimization to determine the number of principal components that best described our data, while avoiding over-fitting the model. With this information, we ran DAPCs without a priori locality information to determine the genetic structure among our pre-identified genetic clusters using the 'adegenet' v2.1.7 (Jombart 2008) package in R.

Secondly, we used 'fastSTRUCTURE' v1.0 (Raj et al. 2014) to determine a suitable number of genetic clusters in our dataset. We investigated the optimal K-value among (i) all; (ii) Kosciuszko Plateau; and (iii) the southern plateau individuals, comprising ten replicate runs of each K-value, from K = 1 to K = (n sites − 1). We loaded the resulting Marginal Likelihood outputs into the Cluster Markov Packager Across K (CLUMPAK) (Kopelman et al. 2015) online server 'bestK' algorithm. Optimal K-values were determined via log-likelihood probability (Pritchard et al. 2000) analysed and plotted using 'pophelper' v2.3.1 (Francis 2017).

Finally, we investigated population structure on Kosciuszko Plateau and the southern plateaus using 'geneland' v4.9.2 (Guillot et al. 2005)—a Bayesian algorithm that incorporates geography with genetic data and delineates populations in context with their spatial arrangements. We input geographic coordinates for each genetic sample and set the 'geneland' frequency model to 'correlated' for these analyses because DAPC and fastSTRUCTURE showed correlation between genetic signal and geography. Each run comprised a chain length of 40,000 generations, and we set the minimum and maximum number of populations to one and ten, respectively. We performed three independent runs per region, each with a different seed for starting population number ('npopinit = 1, 4, 8') to address potential bias introduced by our chain starting point. We discarded the first 10% of each chain as burn-in.

Genetic diversity and demography

We calculated genetic diversity measures across all individuals, individuals within regions, and among our identified genetic clusters. Observed heterozygosity (Ho) and unbiased expected heterozygosity (He) and the degree of inbreeding were calculated across all variant sites (SNP heterozygosity) and across variant and invariant sites (autosomal/genomic heterozygosity). Autosomal/genomic heterozygosity is more robust to missing data, small and uneven sample sizes (Schmidt et al. 2021) and is considered more accurate and comparable across studies/organisms (Westbury et al. 2018, 2019). We calculated the number of invariant sites ('gl.report.secondaries'), genomic/autosomal heterozygosity ('gl.report.heterozygosity'), and F_{ST} and D_{est} ('gl.basic.stats') using dartR. Allelic richness (Ar) was generated via the 'hierfstat' v0.5-7 (Goudet & Jombart 2020) package and Hedricks G"$_{ST}$ (Hedrick 2005; Meirmans & Hedrick 2011) was calculated in 'mmod' v1.3.3 (Winter 2012). We generated these statistics across all individuals and among collection sites within Kosciuszko Plateau and the southern

plateaus (Online Resource Tables 1–5). We calculated Tajima's D statistic to determine whether demographic expansion or contraction was evident. To do so, we converted our genlight object to a variant call format (VCF) file which was loaded into 'VCFtools' v0.1.16 (Danecek et al. 2011). A minor allele count of three was applied to our 35,945 loci dataset before analyses, resulting in 32,793 retained SNPs. These SNPs were binned sequentially into 22 groups of random sizes (between 560 and 2604 SNPs). Calculations were performed on binned SNPs to enable detection of variation across our dataset.

Population differentiation

To determine the degree of genetic differentiation among populations, we performed 1,000 permutations of three fixation indices; Hedrick's G''_{ST}, Jost's D statistic (Jost 2008; D_{est}) and Weir and Cockerham's F_{ST} (Weir and Cockerham 1984) among our inferred genetic clusters. We then investigated the correlation between genetic and geographic distance via Mantel's R statistic in the R package, 'vegan' v2.5-6 (Oksanen et al. 2019). We generated two independent Euclidean distance matrices among individuals using genetic and geographic data, then tested for strength and significance of their correlation using 1,000 randomized permutations of the Mantel test. We performed analysis of molecular variance (AMOVA) using the R package 'poppr' v2.9.2 (Kamvar et al. 2014), which we used to determine the degree and significance of differentiation between regions and populations. AMOVA analyses were hierarchical and included three levels of stratification: (i) between regions, (ii) among our inferred genetic clusters and (iii) among collection sites. We tested significance by comparing observed genetic variation among stratifications where samples were randomly swapped among groups (1,000 permutations). To determine the relative timing of population divergence across the species' range, we calculated the ratio between the proportion of shared alleles (D_{PS}) and F_{ST} (expected to be high in recently isolated populations and to return to equilibrium over time). First, we used the 'graph4lg' (Savary et al. 2020) package in R to calculate pairwise distances among populations based on D_{PS}, considered a contemporary measure of gene flow (Robin et al. 2015). Second, we calculated the heterozygosity-based pairwise F_{ST}, which is less sensitive to recent change.

Effective population size

We performed effective population size (Ne) calculations for each genetic cluster using the linkage disequilibrium model (Waples 2006) in 'NEestimator' v2.1 (Do et al. 2014).

Results

Genetic structure

DAPC identified seven genetic clusters when we analyzed all individuals (Fig. 2a). Each site had 100% membership assignment to their respective genetic cluster (Online
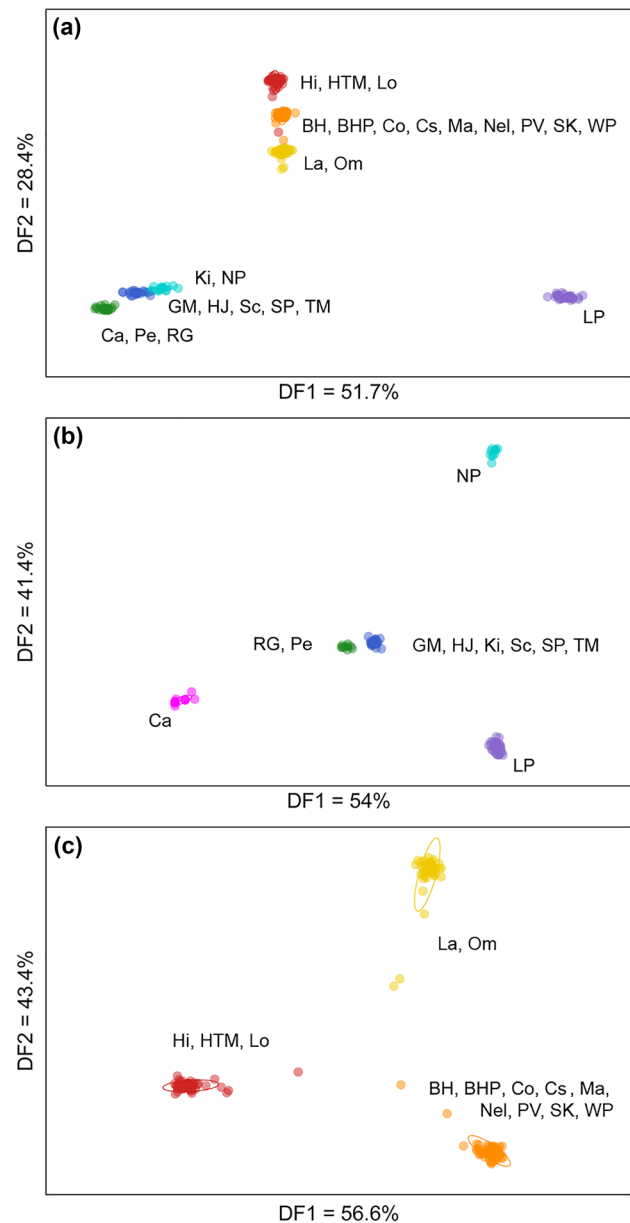

Fig. 2 Discriminant analysis of principal components (DAPC) among **a** 259 *C. praealtus* individuals from 58 sites across both regions; **b** 88 individuals from 34 sites on Kosciuszko Plateau; and **c** 171 individuals from 24 sites on the southern plateaus. Analyses are based on **a** 32,739 SNPs and **b** 13,905 SNPs for Kosciuszko Plateau; and **c** 10,202 SNPs for the southern plateaus (minor allele count = 3). Clear genetic structure is evident between regions and among sites

Resource Fig. 2). Clear genetic differentiation was evident between the two regions. Individuals from the southern plateaus grouped into three distinct clusters, largely reflecting distinct plateaus; all individuals from (i) the Bogong High Plains and Wellington Plain, (ii) Mt Hotham/Loch area, and (iii) Lankey/Omeo Plains, concordant with results from our DAPC that included only the southern plateaus individuals (Fig. 2c). The geographically isolated individual from Wellington Plain (WP) was genetically similar to those from the Bogong High Plains.

Four genetic clusters were associated with Kosciuszko Plateau when our analysis included individuals from both regions (Fig. 2a). Individuals from northern sites (LP) (Fig. 1.) formed a single genetic cluster (purple) that was notably distinct from all other Kosciuszko Plateau and southern plateaus clusters along discriminant factor 1—the primary explanatory variable (DF1 = 51.7% of variance explained). The remaining three Kosciuszko Plateau genetic clusters were tightly grouped: (i) lower-northern and eastern (KI and NP; cyan); (ii) central (GM, HJ, Sc, SP, TM; blue); and (iii) lower-central and southern (Ca, Pe, RG; green) sites.

Our DAPC analysis of Kosciuszko Plateau individuals only (Fig. 2b) showed that K-values of 4 and 5 were equally likely (BIC = 645.94; Online Resource Fig. 3b). Analysis of K = 4 resulted in four genetic clusters, similar to our DAPC-based investigation combining both regions (see Online Resource Figs. 4 and 5). However, independent analyses of Kosciuszko Plateau individuals split the lower-northern (KI) from the eastern (NP) sites, placing individuals from the eastern sites into a distinct genetic cluster (cyan) and the lower-northern individuals into the central cluster (GM, HJ, Sc, SP, TM; blue) (Fig. 2b). Analysis of K = 5 mirrored that of K = 4, except that individuals
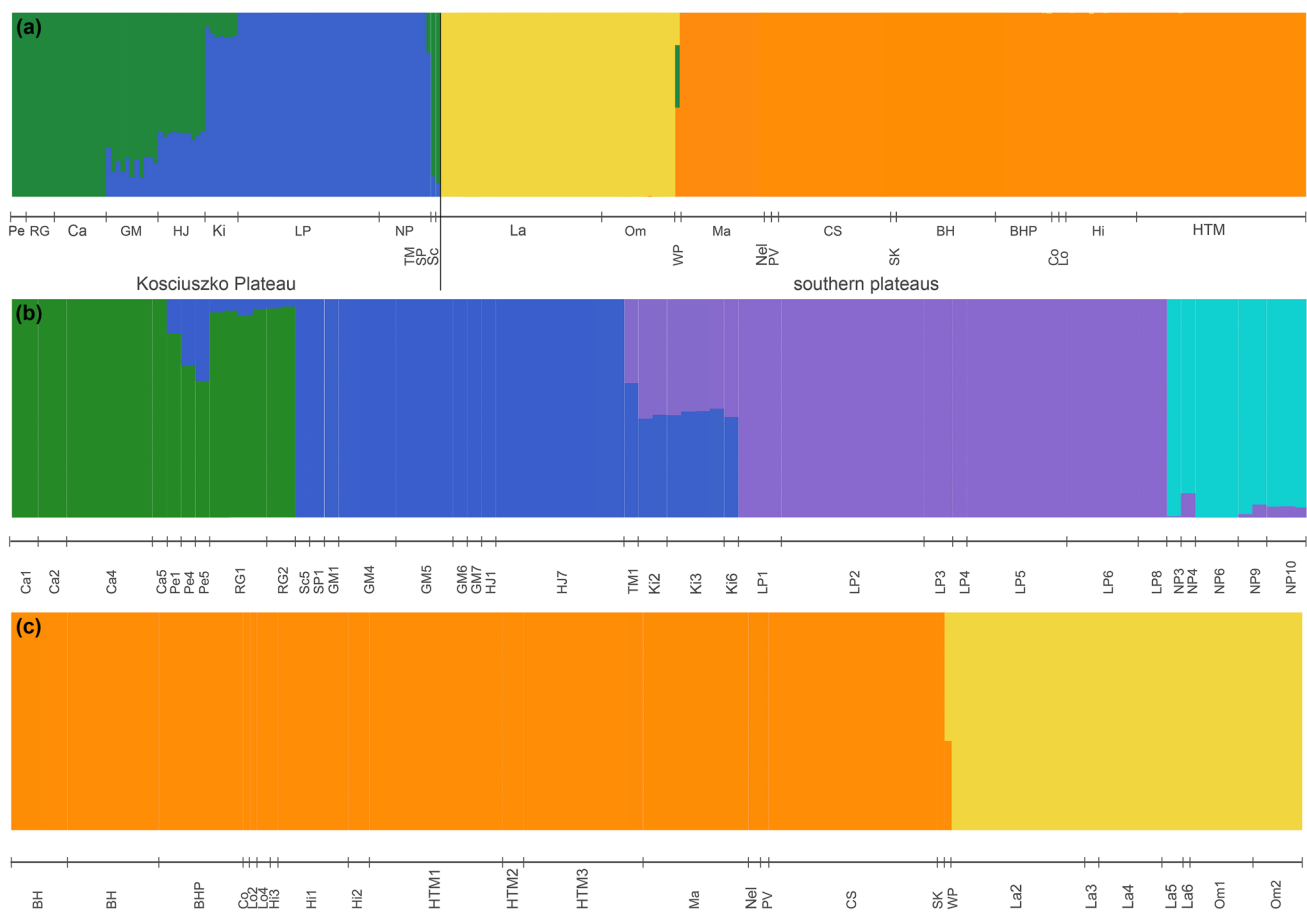
Fig. 3 Bayesian genetic clustering of *C. praealtus* individuals from Kosciuszko Plateau and the southern plateaus, using fastSTRUCTURE. Analyses are based on **a** 32,739 SNPs and **b** 13,905 SNPs for Kosciuszko Plateau; and **c** 10,202 SNPs for the southern plateaus (minor allele count = 3). When analysis included Kosciuszko Plateau and the southern plateaus (**a**) each region comprised two distinct genetic clusters with minimal sharing of genetic signal between regions. Admixture is evident among Kosciuszko Plateau populations. Analysis of Kosciuszko Plateau samples (**b**) provided greater resolution of genetic structure and fine-scale admixture. A key difference was noted within the southern plateaus; when all individuals were included, signal from Kosciuszko Plateau is seen in the individual from Wellington Plain (WP) (**a**). However, this signal is lost when only the southern plateaus individuals were analyzed (**c**)

from southern sites (*Ca*; magenta) were considered to be distinct from the genetic cluster containing lower-central sites (*RG*, *Pe*; green) (Fig. 2b).

Our fastSTRUCTURE analyses largely mirrored the DAPC results—although analyses of all individuals potentially underestimated genetic structure within Kosciuszko Plateau. Of the four Kosciuszko Plateau genetic clusters, admixture was noted between neighbouring populations (Fig. 3a, b). DAPC results were inconclusive as to whether southern (*Ca*) and lower-central (*RG* and *Pe*) were grouped (Fig. 2a, b). Our fastSTRUCTURE results revealed that these sites differed in their proportion of shared admixture with the central population and should thus be grouped. We identified two genetic clusters from the southern plateaus, separating Lankey/Omeo Plains from a cluster containing the Bogong High Plains and the Mt Hotham/Loch area, with almost no admixture between them (Fig. 3a, c). Results were mostly consistent for analyses of individuals from the southern plateaus, regardless of whether those from Kosciuszko Plateau were included, except that the individual from Wellington Plain (*WP*) displayed a genetic

signal from Kosciuszko Plateau only in the analyses that included both regions (Fig. 3a).

We used the DAPC and fastSTRUCTURE results to inform a geo-referenced model investigating population structure within each region. Analyses of Kosciuszko Plateau individuals identified six genetic clusters, which closely reflected the populations identified via DAPC and fastSTRUCTURE. However, this algorithm further delimited sites with a high degree of admixture from multiple clusters (Fig. 4a). Within the southern plateaus, geo-referenced analyses identified the same three genetic clusters as DAPC, while also distinguishing Wellington Plain; a result congruent with the fastSTRUCTURE analyses.

Based on these findings, we grouped sites into seven genetic clusters (populations): Kosciuszko Plateau: (1) green = southern and lower-central (*Ca*, *Pe*, *RG*), (2) blue = central and lower-northern (*GM*, *HJ*, *KI*, *Sc*, *SP*, *TM*), (3) purple = northern (*LP*), (4) cyan = eastern (*NP*); southern plateaus: (5) red = Mt Hotham/Loch area (*Hi*, *HTM*, *Lo*), (6) orange = Bogong High Plains and Wellington Plain (*BH*, *BHP*, *Co*, *CS*, *Ma*, *Ne*, *PV*, *SK*, *WP*), (7) yellow = Lankey/Omeo Plains (*La*, *Om*). We grouped the southern and
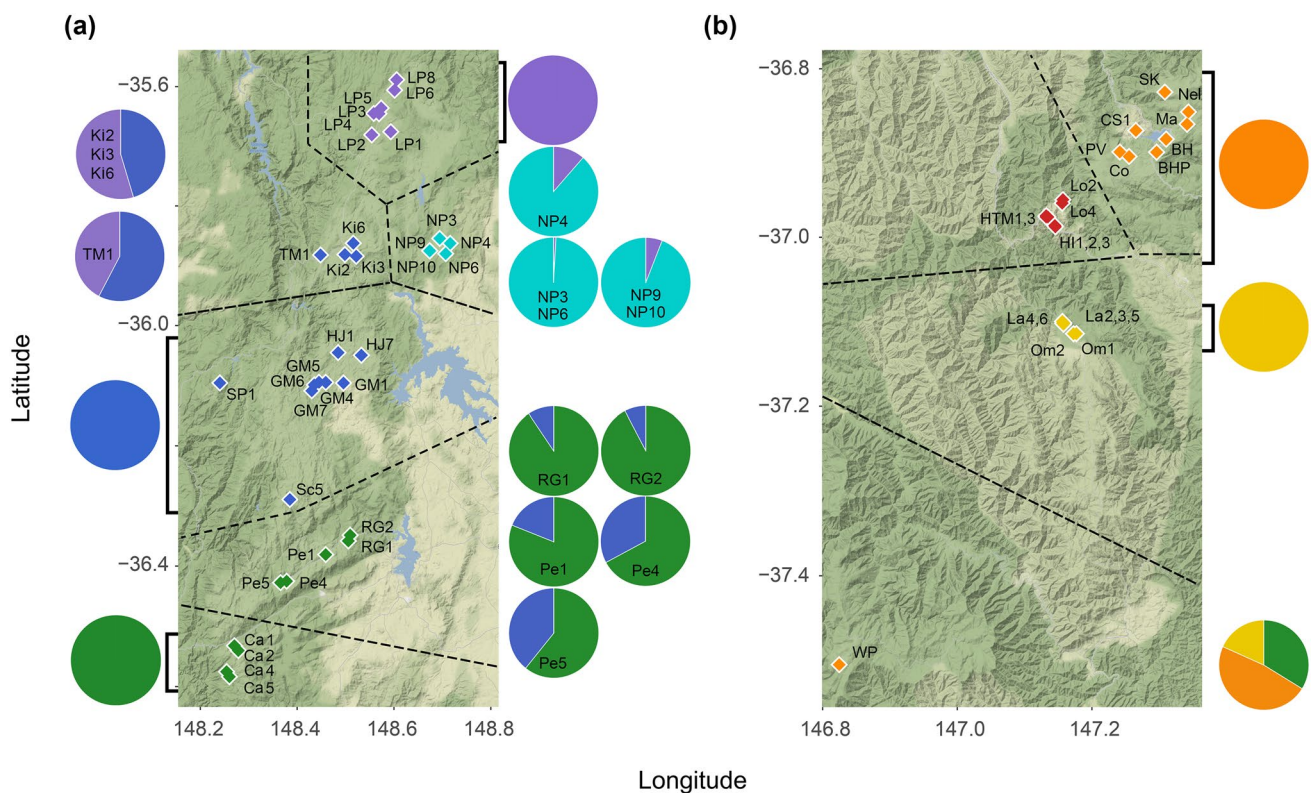
Fig. 4 Summary from the three genetic clustering algorithms used to identify populations of *C. praealtus* from **a** Kosciuszko Plateau and **b** the southern plateaus. Analyses are based on **a** 13,905 SNPs for Kosciuszko Plateau; and **b** 10,202 SNPs for the southern plateaus (minor allele count = 3). Diamonds on each map show site locations, and are coloured as per DAPC results. Pie charts reflect the results

from fastSTRUCTURE, and show the degree of admixture among populations (squared brackets on map edges group the sites that share 100% pie charts). Dashed lines on each map show geo-referenced genetic clusters identified via 'geneland' (raw 'geneland' outputs are presented in Online Resource Fig. 6)

lower-central Kosciuszko Plateau sites into the green cluster because fastSTRUCTURE analyses showed high genetic similarity among these three sites, and DAPC k-means analysis suggested this was equally optimal to delimiting them. This had the added benefit of eliminating the occurrence of $n = 1$ for a group, which facilitated filtering out loci with a minor allele frequency $< 5\%$.

Genetic diversity and demography

Estimates of genomic/autosomal heterozygosity were low ($Ho = 0.00082$, $He = 0.00105$; Table 1). Autosomal observed and expected heterozygosity calculations were greatest on Kosciuszko Plateau (which contrasted less robust findings based only on variant sites). Allelic richness was also greatest across Kosciuszko Plateau as a whole ($A_R = 1.79$). However, each population within the Kosciuszko Plateau region contained low and unique allelic richness ($A_R \leq 1.2$). This is supported by a greater degree of population differentiation (Kosciuszko: $F_{ST} = 0.151$, $D_{est} = 0.046$, $G''_{ST} = 0.234$; southern plateaus: $F_{ST} = 0.089$, $D_{est} = 0.026$, $G''_{ST} = 0.151$), lower effective population sizes (Kosciuszko: $Ne = 11$–125; southern plateaus: $Ne = 115$–203) and greater levels of inbreeding (Kosciuszko: F_{IS} 0.224; southern plateaus: $F_{IS} = 0.116$)—greater expected, relative to observed, heterozygosity also indicates inbreeding. Estimates of Tajima's D were positive for 21/22 bins (0.020–0.468; mean $= 0.205$; sd $= 0.134$), and negative for a single bin (-0.032) indicating that *C. praealtus* has experienced population contraction (Online Resource Table 6).

Within Kosciuszko Plateau, the southern population was characterized by the greatest degree of inbreeding ($F_{IS} = 0.157$) and lowest effective population size ($Ne = 11.5$). The central population had the lowest genetic diversity ($Ho = 0.00087$, $He = 0.00097$), while the northern population had the lowest allelic richness ($Ar = 1.135$). Genetic distance was highly correlated with spatial arrangements of populations on Kosciuszko Plateau (Mantel's $R = 0.801$, $p = 0.001$). Within the southern plateaus, genetic diversity was lowest in the Bogong High Plains/Wellington Plain population ($Ho = 0.00055$, $He = 0.0006$), where allelic richness was greatest.

Population differentiation

Genetic and geographic distances among individuals were highly and significantly correlated (Table 1), particularly among individuals from Kosciuszko Plateau (Mantel's $R = 0.8$, $p = 0.001$). Pairwise estimates of genetic differentiation showed that between-region variation was ~2.5 times greater than within regions (Table 2), indicating that these two regions have been isolated for longer than populations within them. Kosciuszko Plateau populations had ~two

times greater differentiation (G''_{ST} and D_{est}) than southern plateaus populations (1.5 times greater F_{ST}). The low D_{PS}/F_{ST} ratios (0.5–0.9; Online Resource Table 7) indicate that the observed population structure and levels of isolation are historical and not driven by recent human activities. Recall the difference in DAPC (Fig. 2) and fastSTRUCTURE (Fig. 3) grouping of the Mt Hotham/Loch area and Bogong High Plains; these two putative populations are delimited by the lowest pairwise genetic differences and the second-most recent population divergence across the species' range.

The results of AMOVAs further supported high genetic differentiation between regions and among populations. We observed significant genetic variance at all levels of stratification, with 29.5% of the overall variation attributed to differences between regions (AMOVA: $p = 0.001$; Table 3), and 10.5% explained by differences among populations within each region. The small amount of variance explained by sites within genetic clusters suggests the clusters accurately reflect 'real-world' populations. Independent AMOVAs of Kosciuszko Plateau and the southern plateaus confirmed that highly structured clusters in one region did not drive our results ($p \leq 0.001$; Online Resource Tables 9 and 10).

We estimated effective population sizes of 12–204 breeding individuals among our seven populations (Table 1), and < 1,000 breeding individuals in total, suggesting the effective population size from the southern plateaus is ~2.6 times larger than Kosciuszko Plateau populations. The southern plateaus populations had relatively consistent effective population size estimates (mean $= 159$, sd $= 33$); in contrast with Kosciuszko Plateau (mean $= 61$, sd $= 48$). Central and southern Kosciuszko Plateau effective population sizes were the smallest.

Discussion

Fragmentation and low genetic diversity are evident across *C. praealtus* populations, which is consistent with other sky island species worldwide (Bálint et al. 2011; Rubidge et al. 2012; Tonzo and Ortego 2021) and is most likely driven, at least in part, by past climate and geological events. We confirmed isolation of populations on Kosciuszko Plateau and the southern plateaus. Despite apparent habitat continuity, populations on Kosciuszko Plateau had greater inbreeding and smaller effective population sizes than populations on southern plateaus. Populations on southern plateaus largely aligned with landforms. Interestingly, markers from both regions were present in the southern-most population, which was the sole representation of historic habitat connectivity. Low genetic diversity and isolated populations with small effective population sizes underscore *C. praealtus*' potential vulnerability to habitat disturbance and stochastic events. Given this species' habitat is currently being impacted

Table 1 Genetic diversity and population differentiation statistics for *C. praealtus* based on 35,947 loci

Genetic cluster	N (sites)	n	SNP Ho (SD)	SNP He (SD)	Genomic Ho (SD)	Genomic He (SD)	A_R (SD)	F_{IS} (SD)	F_{ST}	D_{est}	G''_{ST}	Mantel's R (p-value)	Ne (95% CI) lowest allele frequency 0.05	Ne (95% CI) lowest allele frequency 0.01
C. praealtus	58	259	0.107 (0.134)	0.138 (0.166)	0.00082 (0.01457)	0.00105 (0.01823)	1.698 (0.416)	0.170 (0.226)	0.182	0.071	0.358	0.903 (-0.001)	≈ 607.9 [a] (11.4–11.5)	≈ 710 [a] (11.8–17.3)
All Kosciuszko Plateau (KP)	34	88	0.111 (0.126)	0.154 (0.164)	0.00088 (0.01449)	0.00121 (0.01918)	1.794 (0.391)	0.224 (0.256)	0.151	0.046	0.234	0.8 (-0.001)	≈ 161.8 [a]	≈ 204 [a]
Southern KP (green)	9	20	0.124 (0.154)	0.159 (0.179)	0.00091 (0.01692)	0.00114 (0.01995)	1.159 (0.179)	0.157 (0.315)	–	–	–	–	11.5 (11.4–11.5)	14.2 (11.8–17.3)
Central KP (blue)	13	29	0.118 (0.163)	0.135 (0.175)	0.00087 (0.01725)	0.00097 (0.01856)	1.153 (0.164)	0.085 (0.254)	–	–	–	–	55.7 (55.6–55.9)	65 (64.8–65.2)
Northern KP (purple)	7	30	0.130 (0.194)	0.143 (0.186)	0.00095 (0.01999)	0.00103 (0.01975)	1.135 (0.174)	0.054 (0.382)	–	–	–	–	94.6 (94.1–95.1)	124.8 (124.1–125.5)
Eastern KP (cyan)	5	9	0.125 (0.146)	0.159 (0.172)	0.00092 (0.01641)	0.00110 (0.01884)	1.149 (0.195)	0.134 (0.280)	–	–	–	–	–[b]	–[b]
All southern plateaus	24	171	0.102 (0.142)	0.123 (0.168)	0.00075 (0.01466)	0.00089 (0.01727)	1.602 (0.441)	0.116 (0.195)	0.089	0.026	0.151	0.503 (-0.001)	≈ 446.1 [a]	≈ 506 [a]
Mt Hotham/Loch area (red)	8	55	0.134 (0.160)	0.149 (0.172)	0.00062 (0.01415)	0.00067 (0.01520)	1.604 (0.446)	0.082 (0.218)	–	–	–	–	115.9 (115.4–116.3)	130.8 (130.3–131.3)
Bogong high and wellington plains (orange)	9	69	0.121 (0.165)	0.132 (0.174)	0.00055 (0.01381)	0.00060 (0.01463)	1.696 (0.385)	0.053 (0.187)	–	–	–	–	171.3 (170.6–172.0)	171.9 (171.3–172.6)
Lankey/Omeo Plains (yellow)	7	47	0.130 (0.168)	0.140 (0.175)	0.00060 (0.01436)	0.00063 (0.01501)	1.579 (0.463)	0.044 (0.169)	–	–	–	–	158.9 (157.9–160.0)	203.3 (201.9–204.8)

Values are: allelic richness (Ar); three commonly used fixation indices (F_{ST}, D_{est} and G''_{ST}); correlation between genetic and geographic distances (Mantel's R); and effective population size (Ne). Observed heterozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient (F_{IS}) estimates are based on 21,117 SNPs with 5% missingness—using only variant sites (SNPs) as well as both variant and invariant sites (genomic/autosomal). Analyses were performed for all individuals, Kosciuszko Plateau, the southern plateaus, and among genetic clusters identified via DAPC and fastSTRUCTURE

Ne estimates were calculated for each genetic cluster. [a]Values were summed for each region independently, and combined for the species

[b]Ne estimates were 0, however, we caution against interpretations as this estimate was based off only nine sampled individuals

Table 2 Pairwise G"$_{ST}$ (lower) and D$_{est}$ (upper) comparisons among *C. praealtus* genetic clusters identified by fastSTRUCTURE and DAPC

Region	Genetic cluster	elevational range (m)	southern KP (green)	central KP (blue)	northern KP (purple)	eastern KP (cyan)	Mt Hotham/ Loch area (red)	Bogong High and Wellington Plains (orange)	Lankey/Omeo Plains (yellow)
Kosciuszko plateau (KP)	Southern (green)	1469–1753	–	0.054	0.103	0.085	**0.172**	**0.163**	**0.179**
	Central (blue)	1363–1809	0.221	–	0.051	0.062	**0.186**	**0.179**	**0.193**
	Northern (purple)	1324–1372	0.374	0.214	–	0.082	**0.235**	**0.227**	**0.242**
	Eastern (cyan)	1331–1357	0.336	0.258	0.331	–	**0.208**	**0.201**	**0.214**
Southern plateaus	Mt Hotham/ Loch area (red)	1788–1851	**0.517**	**0.533**	**0.616**	**0.590**	–	0.032	0.048
	Bogong High Plains and Wellington Plain (orange)	1530–1859	**0.496**	**0.515**	**0.601**	**0.573**	0.142	–	0.048
	Lankey/ Omeo Plains (yellow)	1542–1625	**0.537**	**0.553**	**0.634**	**0.608**	0.212	0.209	–

Inter-region comparisons (shown in bold; mean G"$_{ST}$ = 0.564, mean D$_{est}$ = 0.200) are ~ 2.5 times greater than intra-region comparisons (mean G"$_{ST}$ = 0.255, mean D$_{est}$ = 0.063). Populations from Kosciuszko Plateau show between 1.7–3.2 times greater intra-region differentiation (mean G"$_{ST}$ = 0.289, mean D$_{est}$ = 0.073) than those from the southern plateaus (mean G"$_{ST}$ = 0.188, mean D$_{est}$ = 0.042). Pairwise F$_{ST}$ values are presented in Online Resource Table 8

Table 3 Analysis of molecular variance (AMOVA) between *C. praealtus* from Kosciuszko Plateau and the southern plateaus, as well as the seven genetic clusters

	df	Sum Sq	Mean Sq	Sigma	Variation (%)	Significance
Between regions	1	245,623.8	245,623.8	934.4	29.5	p ≤ 0.001
Among clusters within regions	5	137,298.4	27,459.7	332.5	10.5	p ≤ 0.001
Among sites within clusters	51	161,249.1	3,161.7	140.7	4.4	p ≤ 0.001
Between samples within sites	201	409,620.9	2,037.9	278.7	8.8	p ≤ 0.001
Within samples	259	383,427.7	1,480.4	1,480.4	46.7	p ≤ 0.001

To determine the significance of the genetic variation found between regions, we compared observations with 1,000 permutations whereby samples were randomly distributed

Effective population size

by reduced snow cover (Green 2016), extensive grazing by exotic species (Hartley et al. 2021), increased fire risk (Hoffman et al. 2019) and human infrastructure development (Clemann et al. 2018; Normyle and Pittock 2020), investigations into census population sizes and demographics are needed to further clarify the species' susceptibility to extinction.

Geological isolation of alpine plateaus

Genetic analyses have identified divergence in a range of species of mountain lizards likely resulting from barriers, such as inhospitable habitats (Torres-Pérez et al. 2007; Dubey and Shine 2010; Atkins et al. 2019), mountain uplift (Mendes et al. 2018), or indirect effects such as predation or competition (Tennessen and Zamudio 2008). Our results suggest *C. praealtus* retreated from lower elevations as the climate warmed during past interglacial cycles. Australian alpine habitats occurred as low as 700 m elevation 21,000–17,000 years ago (Colhoun and Barrows 2011; Barrows et al. 2021), and likely allowed for a more contiguous *C. praealtus* population, explaining the extant distribution of the species. As proposed for other mountain lizards (e.g. Atkins et al. 2019; Wiens et al. 2019), it is probable that the

genetic differentiation we report is driven by fragmentation. However, we cannot rule out adaptation to local conditions as reduced representation data, utilized here, do not provide whole genome coverage (Catchen et al. 2017; Lowry et al. 2017).

Genetic differentiation among populations

Our results suggest that forested, lower elevation valleys prevent admixture among populations from the southern plateaus, as little as 10 km apart. In the absence of such barriers on Kosciuszko Plateau, gene flow occurred among populations separated by over twice this distance. Gene flow was not ubiquitous throughout Kosciuszko Plateau, however, suggesting relatively localised dispersal. The reasons for restricted admixture on Kosciuszko Plateau are unclear and warrant investigation. Kosciuszko Plateau populations have overlapping elevational ranges (Table 2), and while potential barriers such as forested slopes and rivers occur within and between populations, they do not form comprehensive geographical breaks, as they do for the southern plateaus.

We speculate that glacial activity isolated Kosciuszko Plateau populations historically. Southern and central populations may have been divided by glaciation along the Snowy River Valley (Colhoun and Barrows 2011). Fragmentation of northern, eastern and central populations may reflect extensive periglacial activity that occurred in this area (Barrows et al. 2001), and/or glacial retreat to different sides of the mountain range. However, phylogeographic breaks can occur in the absence of environmental barriers when species have limited dispersal capacity and occur at small population sizes (Irwin 2002; Sullivan et al. 2020). Such effects may be exacerbated by variation in habitat quality or localized extinctions (Irwin 2002); thus the effect of habitat quality on *C. praealtus* should be investigated.

Species resilience

Isolation and small effective population sizes likely increased inbreeding and reduced genetic diversity in *C. praealtus* populations. Diversity was lower than recent calculations for the Endangered south-eastern Australian grasshopper (*Keyacris scurra*; Ho = 0.00158–0.00371; Schmidt et al. 2021), and our SNP-based estimates of mean observed and expected heterozygosity were lower (43–58%) than recent estimates for the Endangered Guthega skink, *Liopholis guthega* (Atkins et al. 2019). Overall genetic diversity was relatively high across the entire Kosciuszko Plateau, however we found low allele diversity and greater differentiation among populations within. In contrast, the southern plateaus were less genetically diverse, yet were characterised by larger effective population sizes that were less genetically discrete and had lower levels of inbreeding. We, therefore,

speculate that the isolated *C. praealtus* populations identified here may have a reduced capacity to withstand further habitat degradation or adapt to rapid environmental changes, relative to a hypothetical admixed metapopulation. These disturbances include infrastructure development (Sato et al. 2014; Normyle and Pittock 2020), exotic herbivores (Hartley et al. 2021), altered fire regimes (Hoffmann et al. 2019; Thomas et al. 2022) and change in vegetation composition (Camac et al. 2017) across the species' range. Further habitat disturbance will likely impact fitness, genetic diversity and effective population sizes. The degree of *C. praealtus*' resilience to environmental change requires a more representative 'genomic' approach to investigate local adaptation and further hypothesis testing.

Management implications and recommendations

Low genetic diversity, and isolated populations with small effective population sizes, mean that effective conservation of *C. praealtus* requires informed management interventions. Climate change is causing the retreat of alpine habitats globally (Hansson et al. 2021; Lu et al. 2021) and is predicted to reduce *C. praealtus* habitat as heathlands and woodlands expand into grasslands (Camac et al. 2021). Further preventable habitat loss, fragmentation, and degradation must be avoided. Populations occupying very small areas subject to habitat destruction, such as at Mt Hotham, are at immediate risk of rapid decline. We recommend regular monitoring, including further genetic tissue collection, to address key conservation questions such as estimating the population census/effective population size ratio (Palstra and Fraser 2012; Peart et al. 2020).

Given notably low effective population sizes on Kosciuszko Plateau, increasing breeding and survival through habitat protection, and potentially population augmentation, should be prioritized. When suitable dispersal corridors are present, gene flow among populations is more likely when populations are larger. Human assisted gene flow may be an important conservation action for this species because inbreeding is high and populations are relatively genetically distinct. However, in the absence of historical demography studies for this Endangered species, assisted admixture should be preceded by rigorous genetic investigations with additional markers and individuals, and experimental testing of gene pool mixing. The southern and eastern Kosciuszko Plateau populations are likely priorities for genetic rescue because they have the lowest effective population sizes and greatest degree of inbreeding. Although we sampled across the species' range, we could not comprehensively sample all populations due to the species' low detectability and extensive potential habitat. Further research that includes additional samples is needed to determine the cause of small effective

populations sizes, dispersal capacity and the effects of habitat quality on dispersal to inform management.

Although the populations on the southern plateaus will benefit from increased population sizes, lower genetic diversity and less differentiation among populations means maintenance or enhancement of genetic diversity and gene-flow among populations are conservation priorities. If a dedicated investigation with additional markers and individuals suggest genetic rescue is necessary, the Mt Hotham/Loch area and Bogong High Plains populations will likely be the most conservative first option for testing gene pool mixing because they represent the least genetically distinct populations; indeed, they are less distinct than Kosciuszko Plateau populations where admixture is evident at intermediate localities. Further surveys and genetic analysis of the Wellington Plain and its surrounds are urgent priorities. Although individuals from this site are genetically like those from the Bogong High Plains, contemporary gene-flow is improbable because they are separated by ~ 70 km of forested valleys and ridges. Wellington Plain was extensively burned in a bushfire in early 2019. Only one (gravid) survivor has been detected since then, despite ongoing monitoring. Augmentation may be necessary for this population to persist and maintain sufficient genetic diversity. Importantly, genetic rescue alone cannot prevent extinction—it must be underpinned by habitat protection (Roycroft et al. 2021) and a commitment to address the causes of decline.

Data availability Genetic data will be available for download at http://dx.doi.org/10.17632/k5np2t43y5.1 from December 2025. Detailed threatened species location information has been submitted to New South Wales and Victorian Governments but will not be made publicly available.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval Sampling was conducted under DELWP Research Permit No. 10008640, Arthur Rylah Institute for Environmental Research Animal Ethics Committee Authority (19-003), Department of Planning, Industry and Environment Scientific Licence (SL102141) and Australian National University Animal Ethics Protocol (A2018/51).

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