# An assessment of population structure and genetic diversity of the mountain skink, *Liopholis montana*.

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***Summary***

Here, we investigate the genetic health of an EPBC-listed skink from southeast Australia. The mountain skink is a high-elevation species found on several mountain peaks that are isolated by lowland valleys. Upon analyses of ≥62,028 nuclear SNPs, we report that five isolated populations exist, with a significant degree of genetic-based differentiation among them—which was highly correlated with geographic distance. In addition to genetic differentiation, we found that long-term isolation has led to local declines in genetic diversity. That is, each isolated population has substantially less genetic diversity than the meta-population (60–80% of the meta-population allelic richness), and some contained a high proportion of unique genetic diversity when compared to all other populations (2–35% of the unique alleles). Therefore, local extinctions must be avoided (by ensuring population-level conservation is prioritised) as it will have a large negative impact on the species' overall fitness. Furthermore, increasing the genetic diversity of each isolated population is critical. Ultimately, the aim of conservation is to increase (breeding) population sizes and genetic diversity, while minimising inbreeding. For high-elevation species, introducing dispersal corridors is not possible, thus human-assisted gene-pool mixing (i.e., genetic rescue) can be beneficial if local adaptation can be ruled out.

We observed that recent migration had occurred between two Victorian populations—from the Wombat State Forest to the eastern Victorian population. This eastern Victorian population was characterised by the highest allelic richness among all isolated populations, possibly due to the additional diversity gained from the Wombat State Forest migrants. This rare example of natural migration between discrete *L. montana* populations gives promise for the utility of human-assisted gene-pool mixing to enhance genetic diversity of the mountain skink. In addition to their suitability for genetic rescue, the following traits also support that these populations should be prioritised for conservation and establishing breeding programs: First, we observed natural migration between these populations which substantially lowers the risk that outbreeding depression will result from mixing (reduced fitness resulting from mixing populations that are too distinct); Second, Victorian populations contain a large proportion of unique genetic diversity (20% in the Wombat State Forest and 35% in eastern Victoria) and they can therefore act as relatively large "genetic diversity banks"; Third, the Wombat State Forest population has the lowest recorded degree of inbreeding, which likely translates to a relatively high degree of offspring fitness. Once local adaptation is investigated, our findings suggest that genetic rescue trials between the two Victorian populations will have the greatest chance of success. If successful, a broarder gene-pool mixing program may be appropriate.

# 1. Introduction

## 1.1 Background

The mountain skink, *Liopholis montana*, is a rare Australian lizard with a fragmented distribution; occurring in areas of high elevation that are isolated by lowland valleys (Donnellan et al., 2002). It occurs along the Great Dividing Range, in the Southern Highlands of New South Wales (NSW) and the Australian Capital Territory (ACT), between 1400–1800 m above sea level, as well as into Victoria, to 900 m. Previously, the mountain skink's westernmost population was believed to be the upper Yarra Valley in Victoria, roughly 100 km east of Melbourne's centre. Recently, however, *L. montana* was discovered ~50 kms west of Melbourne's centre, in the Wombat State Forest (Farquhar et al., 2021). This new population occurs at the lowest known elevation for the mountain skink, 620 m above sea level, and is geographically isolated.

As *L. montana* is restricted to high elevation areas, it has been speculated that several isolated populations exist. For example, an analysis of mitochondrial DNA revealed phylogenetic structure between *L. montana* from ACT and NSW which were estimated to have become isolated 2–4 million years ago (Chapple et al., 2005). Currently, no population genetic studies have been conducted to further investigate the degree of structure among populations from ACT and NSW and to place the findings of Chapple et al. in context with the remaining populations. Therefore, the degree of migration among Victorian populations, and among all states, is currently unknown. Given the large distance that separates Victorian populations from ACT and NSW populations, and the distance between the newly discovered Wombat State Forest population and its nearest neighbour, it is likely that further population structure will be discovered. It is important to address these knowledge gaps so that conservation and management efforts can be effectively implemented.

As of August 2022, *L. montana* is listed as Endangered under the Environment Protection and Biodiversity Conservation (EPBC) Act 1999. The most immediate threats to the mountain skink include logging, habitat loss, climate change (e.g., restricted suitable habitat range; increased fire frequency and severity) and invasive predators (Clemann et al., 2018). The EPBC conservation advice fails to consider the isolation of populations as a threat, perhaps because this appears to be natural—the mountain skink is a high-elevation specialist with much of its suitable habitat fragmented by lowland valleys. However, isolation and thus reduced population size are known to continually decrease genetic diversity and population fitness (O'Grady et al. 2006; Reed 2005; Willi & Hoffmann 2009). The EPBC conservation advice details priority conservation actions for *L. montana*, including the prevention of logging (loss/modification of habitat), developing a fire management strategy and feral predator eradication. The following primary conservation outcomes were also documented as achievable within 10-years: establish stable breeding colonies across all states; increased colony numbers; increased extent of habitat protection; and improved understanding of the species' population genetics.

Successful conservation relies on a sound understanding of dispersal barriers and major threats to an organism or system. From a genetic perspective, the goal for threatened populations is to minimise genetic drift and inbreeding while maximising effective population sizes (Frankham 1995; Wang et al. 2016). Population genetics thus provides a powerful means of informing conservation strategies for threatened taxa by focusing management efforts on priority populations as well as activities that maximise heterozygosity, population size, and genetic variation—each of which is positively correlated with fitness (Reed & Frankham 2003). For the mountain skink, it is important to quantify genetic diversity, and understand the degree of population structure, throughout the known distribution, as small, isolated populations can display lower genetic diversity and higher levels of inbreeding—that is, they have reduced fitness. This information will underpin appropriate management strategies for *L. montana*, including whether translocations among populations will be of benefit as well as identifying candidate populations for the establishment of future breeding programs.

### 1.2 Aims

Following the discovery of mountain skink individuals in the Wombat State Forest, Victoria, we conducted a preliminary investigation into the population genetic health of the mountain skink from this area and placed the findings in context with individuals collected throughout southeast Australia. Here, we investigate population structure throughout the sampled distribution as well as patterns of migration and gene-flow (quantifying the degree of genetic differentiation among populations). We will then assess overall, and relative, population fitness via the calculation of genetic diversity metrics, with the aim of identifying the overall genetic health of the species and any isolated populations that are identified. We will highlight the least fit populations (lowest genetic diversity, smallest population size and greatest degree of inbreeding), as well as relatively fit populations that may support future breeding programs and translocation efforts.

## 2. Methods

### 2.1 Sample collection

*Liopholis montana* tail tips were taken from individuals sampled from 26 localities throughout southeast Australia. Nine areas within the Wombat State Forest were sampled—additional sites were located to the east and west of our sampled WSF area after our sequencing as performed. Full site details and sample coordinates have been reported to the Australian Department of Agriculture, Fisheries and Forestry and are available to land managers upon request to the authors.

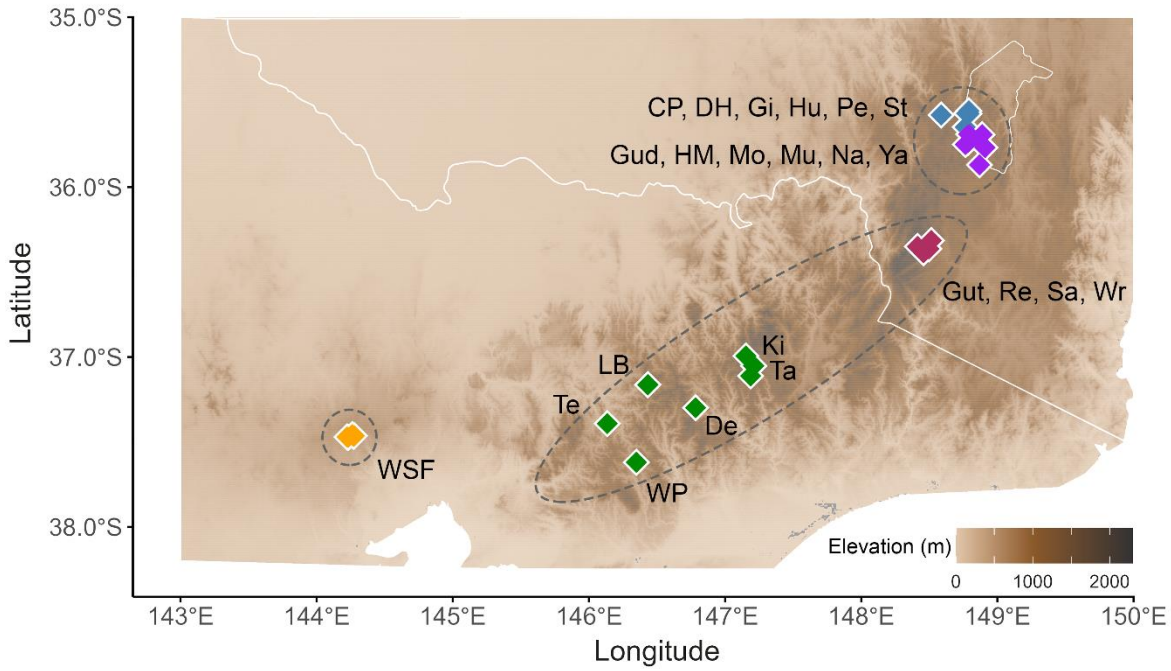


**Figure 1**. Elevation map of the mountain skink, *Liopholis montana*, in Victoria, New South Wales, and the Australian Capital Territory, Australia. Sampling localities encompass most of the known distribution and are indicated by coloured triangles, their colour reflects each individuals' inferred population via Discriminant Analysis of Principal Components (DAPC). Dashed ellipses group three populations identified by fastStructure. Both independent analyses indicate that the Wombat State Forest is a discrete population.

## 2.2 DNA extractions and sequencing

Diversity Arrays Technology (DArT), ACT 2617, Australia, extracted and sequenced DNA from our samples. A high-density assay was sequenced via Illumina HiSeq2500.

## 2.3 Data filtering

We converted our raw, single-row DArTseq report into a 'genlight' object using the 'dartR' package v2.7.2 (Gruber et al., 2018) in R v4.3.0 (R Core Team 2020). Our raw dataset contained 152,651 loci from 92 samples, 6% missing data and a mean reproducibility of 99.6% (range 86–100%). We removed all loci with <10 reads. We then removed any locus not represented by all samples (i.e., allowing no missing data). To remove physically linked sites, we randomly removed secondary SNPs via the 'gl.filter.secondaries' function. Before estimating SNP-based 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. To investigate population structure, we remove loci any that fell below a minor allele count threshold of three. This allowed us to retain rare alleles but remove singletons and doubletons that can confound inferences of population structure (Linck & Battey 2019).

## 2.4 Population structure

All genetic structure analyses were based upon 62,028 loci with a minor allele count of ≥3. We used 'fastStructure' v1.0 (Raj et al., 2014) to determine a suitable number of genetic clusters (K) in our dataset. We investigated the optimal K-value for our 92 samples, comprising 20 replicate runs of each K-value, from K=1 to K=20. 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) and were analysed and plotted using the CLUMPAK main pipeline. We also investigated population structure using Discriminant Analysis of Principal Components (DAPC) using the 'adegenet' v2.1.7 (Jombart 2008) package in R. We estimated K via a k-means algorithm and selected the optimal number based on the Bayesian Information Criterion.

## 2.5 Population-level genetic differentiation

To determine the significance and relative strength of the observed population structure, we performed analysis of molecular variance (AMOVA) using the R package 'poppr' v2.9.4 (Kamvar et al., 2014). AMOVA analyses included three levels of stratification: among states; among inferred genetic clusters (i.e., populations); and among sampling sites. We tested significance by comparing observed genetic variation among stratification where samples were randomly swapped among groups (1,000 permutations). To determine degree of genetic differentiation among mountain skink populations, we performed 1,000 permutations of pairwise fixation index calculations ($G''_{ST}$ and $D_{est}$) between our inferred genetic clusters. We also calculated pairwise allele frequency differences (AFD; Berner, 2019) between each genetic cluster, and then compared each genetic clusters' AFD to the pooled allele frequencies of the remaining four populations.

## 2.6 Genetic diversity and demography

We calculated genetic diversity measures across all individuals (see

Table  for included statistics and their definitions), not accounting for collection site as we deemed there to be no population structure present (see section 3.2). Observed heterozygosity (Ho) and unbiased expected heterozygosity (He) and the degree of inbreeding ($F_{IS}$) were calculated across all variant sites (SNP heterozygosity) as well as 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 Jost's D statistic (Jost, 2008; $D_{est}$) ('gl.basic.stats'). Allelic richness ($A_R$) 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).

Adverse genetic impacts, such as inbreeding and loss of genetic diversity, act on the breeding population, rather than census size of a species (Frankham 2018). As such, we calculated effective population size (Ne) for each inferred population. Analyses were based on the linkage disequilibrium model (Waples 2006) in 'NEestimator' v2.1 (Do et al., 2014) and were performed after removing minor alleles (threshold of ≥3).

**Table 1**. Population diversity metrics investigated.

| Abbrev. | Statistic | Description |
|---|---|---|
| $A_R$ | Allelic richness | The average number of alleles per locus, standardized by sample size. As a measure of variability of genetic material within a population, $A_R$ is a key measure of diversity for conservation and management and is used to infer a population's long-term evolutionary potential, adaptability, and persistence. |
| $D_{est}$ | Jost's *D* statistic | A measure of heterozygosity-weighted allelic differentiation. Unity is reached when each deme consists entirely of private alleles (i.e., unique alleles absent in other demes) and equals zero when all demes have equal alleles at equal frequencies. |
| $F_{IS}$ | Inbreeding coefficient | The proportion of the variance in the sub-population contained in an individual, from zero to one. High $F_{IS}$ is considered to represent a high degree of inbreeding—inbreeding depression is considered to be the most immediate and harmful of the genetic-based extinction factors. |
| $G''_{ST}$ | Hedrick's GST | A standardised measure of heterozygosity-based genetic differentiation among demes. Calculated as the difference between the expected heterozygosity of the whole population, relative to the mean expected heterozygosity of the individual demes. Relative to other genetic differentiation statistics, $G''_{ST}$ allows comparisons across markers with different mutation rates (e.g., allozymes, microsatellites, mitochondrial DNA) as well as organisms with very different effective population sizes. |
| He | Expected heterozygosity | The probability that two randomly chosen gametes are of different alleles (1.0 minus the sum of the squared gene/allele frequencies). High values typically indicate healthy genetic variation and thus population sizes. |
| Ho | Observed heterozygosity | The frequency of heterozygous individuals in a population, averaged over loci, from zero to one. Correlated with the overall fitness of individuals. High values typically indicate healthy population sizes. |
| Ne | Effective population size | An estimation of the number of breeding individuals effectively contributing to the next generation—calculated as the size of a random mating population that has the same rate of genetic drift (increased inbreeding and/or decreased genetic diversity) as the study population. Generally, much lower than population census size. |

# 3. Results

## 3.1 Population structure

We investigated population structure to determine whether any breaks in connectivity were evident among individuals or sampling localities, or whether individuals were free to migrate among sites. Visualisation of our DAPC plot revealed that the sampled mountain skinks formed five discrete genetic clusters (i.e., populations) (Figure 2). The observed pattern among genetic clusters closely reflects the geographic isolation of individuals. That is, meaningful genetic differences occur when a large distance separate populations (60–150 kms; see Figure 1).

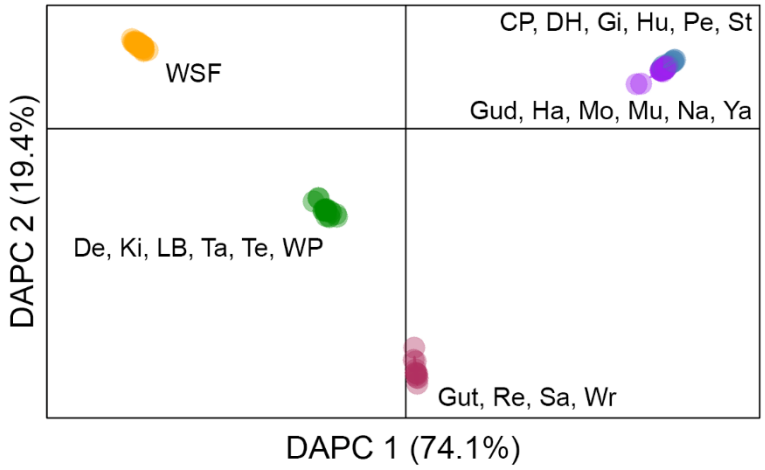


**Figure 2**. Discriminant Analysis of Principal Components (DAPC) plot of 92 mountain skinks collected from 25 southeast Australian sites. Analysis was based on 62,028 loci with no missing data and a minor allele count of ≥3. Five distinct genetic clusters (i.e., populations) were supported via DAPC, which corresponded to discrete geographic areas (shown by coloured points). The distinction of these five genetic clusters closely resembles their geography (see Figure 1), which indicates that a strong correlation between genetic and geographic distance exists.

We also performed 20 replicate fastStructure analyses to determine the number of genetic clusters supported by our data and to quantify the probability that each individual belongs to a given group. Summaries of median log-normal probabilities indicated that K=3 was the optimal number of genetic clusters. All 20 replicate runs supported the presence of three genetic clusters (Figure ), which included individuals from (i) the Wombat State Forest; (ii) green and maroon DAPC clusters; and (iii) purple and blue DAPC clusters. We see evidence of genetic material from the Wombat State Forest in the eastern Victorian localities (Q=<0.12). No further admixture is evident.

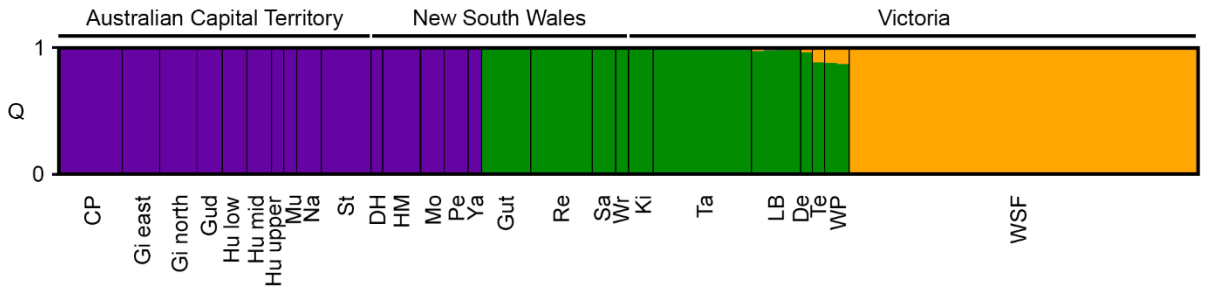


**Figure 3**. Summary of 20 fastStructure runs for 92 mountain skinks collected from southeast Australia. Analysis was based on 62,028 loci with no missing data and a minor allele count of ≥3. All 20 runs resulted in the same output. Y-axis represents membership probability (Q) for each individual to three colour-coded groups. A Q-score between >0 and <1 may indicate admixture among/between groups.

### 3.2 Population-level genetic differentiation

We found evidence for genetic differentiation among our inferred populations, relative to the metapopulation ($D_{est}$=0.03; $G''_{ST}$=0.3; Table 5). To determine the strength and significance of the variance among our samples, we performed an AMOVA. Our AMOVA results indicate that population-level management will yield the greatest conservation of genetic diversity. That is, we found significant differentiation among our inferred populations, which accounted for the greatest variance in the AMOVA model (17.9%; p=<0.001; Table 2). We also found that the variation among collection sites was significant (p=<0.001), however, this difference was less than among-population variance (5.7%). State-level as well as within site-level differences were not significant (p=>0.1) and accounted for 10.9% and 2.2% of the overall AMOVA model variation, respectively. An AMOVA model based on the three inferred populations identified by fastStructure showed no meaningful differences in the variation or significance of each data stratification (data not shown).

Our pairwise comparisons of genetic difference among inferred DAPC populations revealed that greater geographic distance resulted in larger genetic variation (Tables 3, 4). Indeed, we found a strong and significant correlation between the geographic and genetic distances of individuals (Mantel's R=0.83; p=0.001). For example, our most distant groups (orange [Wombat State Forest] and purple) were 3–10× more different than the two sites with the closest proximity (blue and purple). Mean differentiation was estimated at 0.08 (AFD; SD=0.01), 0.32 ($G''_{ST}$; SD=0.08), and 0.03 ($D_{est}$; SD=0.01). We also investigated the number of private alleles found in each of the five inferred populations and compared them to the remaining four populations combined. Here, we found that both Victorian populations contained the greatest levels of unique genetic variation (green=34.9% and orange [Wombat State Forest] =20%). The remaining three populations, blue, maroon, and purple contained 11.7%, 8.9% and 2.1% of the unique alleles sampled, respectively.

**Table 2**. Analysis of Molecular Variance (AMOVA) investigating the genetic variation of mountain skinks, *Liopholis montana*, collected from 21 sites in southeast Australia. Analysis is based on 62,028 loci with no missing data and a minor allele count of ≥3. Five inferred populations were investigated based on the output of Discriminant Analysis of Principal Components (see Figures 1, 2).

| | *df* | Sum Sq | Mean Sq | Variation (%) | Significance |
|---|---|---|---|---|---|
| Among states | 2 | 109,349 | 54,675 | 10.9 | p=0.1 |
| Among inferred populations | 4 | 81,051 | 20,263 | 17.9 | p=<0.001* |
| Among collection sites | 18 | 70,681 | 43,927 | 5.7 | p=<0.001* |
| Among samples within sites | 67 | 184,557 | 2,754 | 2.2 | p=0.3 |
| Within samples | 92 | 237,129 | 2,577 | 63.4 | p=<0.001* |

**Table 3**. Pairwise absolute Allele Frequency Difference (AFD; lower left) between inferred *Liopholis montana* populations from southeast Australia (lower left). The number of total private alleles differentiating populations is also presented (upper right), with those recorded in each population in parentheses—bold populations are reported first (columns) and those in regular font (rows) are reported second. The far-right column represents the proportion of private alleles contained within a population, relative to all remaining populations (pooled). Analysis is based on 96,385 SNPs. Minor alleles were not removed.

| | **Orange** | **Green** | **Maroon** | **Purple** | **Blue** | **Private alleles contained within population (%)** |
|---|---|---|---|---|---|---|
| Orange | - | 55687 (**35512**:20166) | 42620 (**15457**:27163**)** | 43600 (**14386:**29214) | 49734 (**23719**:26015) | 20 |
| Green | 0.073 | - | 48964 (**10956**:38008) | 54074 (**11950**:42124) | 58876 (**20617**:38259) | 34.9 |
| Maroon | 0.086 | 0.069 | - | 31514 (**14196**:17318) | 39126 (**24268**:14858) | 8.9 |
| Purple | 0.09 | 0.081 | 0.081 | - | 20536 (**16534**:4002) | 2.1 |
| Blue | 0.089 | 0.078 | 0.079 | 0.032 | - | 11.7 |

**Table 4**. Pairwise $G''_{ST}$ (lower) and $D_{est}$ (upper) comparisons among *Liopholis montana* genetic clusters identified by Discriminant Analysis of Principal Components. Analysis is based on 96,385 SNPs.

| | **Orange** | **Green** | **Maroon** | **Purple** | **Blue** |
|---|---|---|---|---|---|
| Orange | - | 0.018 | 0.031 | 0.040 | 0.037 |
| Green | 0.202 | - | 0.016 | 0.027 | 0.024 |
| Maroon | 0.330 | 0.186 | - | 0.033 | 0.030 |
| Purple | 0.419 | 0.307 | 0.377 | - | 0.004 |
| Blue | 0.388 | 0.271 | 0.342 | 0.075 | - |

### 3.3 Genetic diversity and demography

Genetic diversity and population size are positively correlated with fitness (Reed & Frankham 2003). That is, a species likelihood of persistence and capacity to adapt to environmental change increases with diversity. We found that the mountain skink had higher levels of genetic diversity (2–13×) than several threatened skinks from southeast Australia (Ho=0.0016; He=0.0023; $A_R$=2; Table 5), although we observed a 1.8–5.9× higher mean level of inbreeding ($F_{IS}$=0.296) (see Table 6 for comparisons with other threatened species from southeast Australia). Although the mountain skink metapopulation has a high $A_R$, our inferred populations have a much lower number of alleles. The five mountain skink populations inferred by DAPC have mean $A_R$ values of between 1.2–1.4 (i.e., each have 60–70% of the number of alleles found in the meta-population). Furthermore, mean estimates of Ne suggest that approximately 217 individuals comprise the breeding population within our study area (not accounting for unsampled populations).

**Table 5**. Genetic diversity and population differentiation statistics for five mountain skink (*Liopholis montana*) populations from southeast Australia. The entire sampling effort is represented (*L. montana*), as are the inferred populations obtained via Discriminant Analysis of Principal Components and fastStructure.

| Genetic group | N sites | *n* | SNP Ho (SD) * | SNP He (SD) * | Genomic Ho (SD) ** | Genomic He (SD) ** | $A_R$ (SD) * | $F_{IS}$ ** | $D_{est}$ * | $G''_{ST}$ * | Mantel's R (p-value) * | Ne (95% CI) * |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Liopholis montana* | 25 | 92 | 0.058 (0.67) | 0.083 (0.11) | 0.00161 (0.015) | 0.00228 (0.022) | 2 (0) | 0.296 | 0.026 | 0.300 | 0.83 (0.001) | - |
| DAPC orange / fastStructure group i | 1 | 28 | 0.063 (0.13) | 0.066 (0.13) | 0.00174 (0.023) | 0.00178 (0.023) | 1.27 (0.4) | 0.038 | - | - | - | 71.6 (41.2-202.9) |
| DAPC green, maroon / fastStructure group ii | 12 | 30 | 0.063 (0.09) | 0.079 (0.11) | 0.00173 (0.0186) | 0.00214 (0.022) | 1.61 (0.47) | 0.208 | - | - | - | - |
| DAPC green | 6 | 18 | 0.066 (0.11) | 0.077 (0.11) | 0.00181 (0.021) | 0.00207 (0.022) | 1.41 (0.42) | 0.151 | - | - | - | 34.8 (19.1-106.7) |
| DAPC maroon | 6 | 12 | 0.058 (0.14) | 0.064 (0.14) | 0.00161 (0.024) | 0.00169 (0.024) | 1.23 (0.41) | 0.088 | - | - | - | 4 (2-13.2) |
| DAPC purple, blue / fastStructure group iii | 12 | 34 | 0.051 (0.11) | 0.056 (0.11) | 0.00140 (0.019) | 0.00151 (0.020) | 1.35 (0.46) | 0.090 | - | - | - | - |
| DAPC purple | 8 | 11 | 0.047 (0.12) | 0.050 (0.12) | 0.00129 (0.021) | 0.00132 (0.021) | 1.20 (0.4) | 0.065 | - | - | - | 21.7 (7.9-∞) |
| DAPC blue | 4 | 23 | 0.053 (0.11) | 0.056 (0.11) | 0.00145 (0.020) | 0.00151 (0.020) | 1.25 (0.38) | 0.056 | - | - | - | 85.3 (41.8-928.7) |

* 43,077 SNPs, one SNP per locus with secondaries removed, minor allele count of ≥3.
** 96,385 loci including variant and non-variant sequence data, secondary SNPs retained.

**Table 6**. Mean estimates of genetic diversity and inbreeding for threatened skinks from southeast Australia.

| Organism | Species | *n* | Mean autosomal Ho | Mean autosomal He | Mean $F_{IS}$ | Reference |
|---|---|---|---|---|---|---|
| Mountain skink | *Liopholis montana* | 92 | 0.00161 | 0.00228 | 0.3 | Present study |
| Alpine bog skink | *Pseudemoia cryodroma* | 27 | 0.00016 | 0.00017 | 0.08 | Amor et al. (2021a) |
| Alpine she-oak skink | *Cyclodomorphus praealtus* | 259 | 0.00082 | 0.00105 | 0.17 | Clemann et al. (2021); Hartley et al. (2023) |
| Swamp skink | *Lissolepis coventryi* | 17 | 0.00025 | 0.00024 | 0.051 | Amor et al. (2021b) |

# 4. Discussion

## 4.1 Population structure

Two independent approaches supported the distinction of Wombat State Forest individuals from the remaining mountain skink individuals. Across the known distribution, we observed that distance and the presence of low-elevation valleys act as barriers to gene flow. FastStructure identified fewer populations than DAPC, pooling blue and purple groups, as well as green and maroon groups. Thus, gene-pool mixing within fastStructure-based groups may be a suitable first step, if local adaptation can be ruled out. Mixing these relatively genetically similar populations will still increase the genetic diversity of each and is thus worthwhile. We also found that some genetic exchange had occurred in the recent past, between the Wombat State Forest population and the westernmost sites of the eastern Victorian population. However, the low Q-score of our sampled individuals (that showed signs of admixture; Q=0.002–0.12) indicates that several generations have passed since this mating event. However, this gives promise for the potential to undertake human-assisted gene flow to enhance the genetic diversity of both Victorian populations. Future work should focus on the environmental variables that are unique to each population and their correlation with genetic differences.

Past work, based on mitochondrial DNA, found that the genetic divergence between mountain skink populations from ACT and NSW was consistent with Pliocene and Pleistocene glacial-interglacial cycles and dated their split at 4.1–2.4 Ma (Chappel et al., 2005). This potentially indicates that populations should be treated as independent lineages. Farquhar et al. (2021) highlighted this potential concern in relation to the conservation of the Wombat State Forest population. However, Hartley et al. (2023) suggested that alpine she-oak skink populations, that share an overlapping distribution with *L. montana* (although at higher elevation), were isolated as the climate warmed during past interglacial cycles, 21–17 Ka, which has been highlighted as a potential dispersal window for other mountain lizards (Weins et al., 2019). We found that mountain skink populations were 2–7× less divergent than alpine she-oak skink populations, which is a candidate for genetic rescue trials (Clemann et al., 2021; Hartley et al., 2023). Furthermore, the recent successful interstate captive breeding effort of Australia's highest elevation skink, *Liopholis guthega*, suggests that despite being isolated for similar times to *L. montana*, gene-pool mixing may be a viable option across the *L. montana* distribution.

Our observations of strong population structure highlight the genetic differences among isolated *L. montana* individuals. Indeed, the genetic differentiation among our samples was best explained at the population level. Therefore, conserving genetic diversity at the population-level will have the greatest fitness outcomes for the mountain skink. Past studies have also highlighted the importance of maintaining population-level genetic diversity (Kahilainen et al., 2014). For the mountain skink, setting conservation quotas (as per the EBPC guidance: establishing stable breeding colonies across ACT, NSW and Victoria; increased colony numbers; increased extent of habitat protection) at the population level will be the most effective way to preserve genetic diversity and increase fitness. For species- or state-level management to be successful, targeting the retention of genetic diversity of each of the five populations our data supports will have the greatest outcome.

## 4.2 Genetic diversity and demography

We found that the genetic diversity of the mountain skink was high compared to some endangered and threatened skinks from southeast Australia. However, the isolation of the five studied populations has resulted in each having less genetic diversity than the metapopulation. We found that gene flow had occurred from Wombat State Forest to the eastern Victorian population, and, therefore, this population had the greatest allelic diversity. Indeed, the two Victorian populations had the greatest levels of genetic diversity and unique genetic material (private alleles). When compared to the pool of all four remaining populations, the Wombat State Forest population contained 20% of the private alleles in the metapopulation. The eastern Victorian population accounted for 35% of the private alleles in the metapopulation, while the remaining populations from ACT and NSW contained between 2–12%. Erosion of genetic material at either Victorian population will have

meaningful negative implications for the species genetic diversity, and thus the Wombat State Forest and eastern Victoria should be prioritised for independent protection. Furthermore, we found that the Wombat State Forest has the second greatest breeding population (effective population size) and the lowest degree of inbreeding. As inbreeding is known to negatively impact the fitness of the species, population, and offspring (Charlesworth & Charlesworth, 1987), the relative fitness of the Wombat State Forest population makes it an important genetic diversity 'bank' and an ideal candidate for supporting breeding and translocation programs.

### 4.3 Management implications

Gene-pool mixing will have the greatest positive impact on the genetic diversity and fitness of *L. montana*, and thus it will substantially decrease the mountain skink's extinction risk. Investigating whether populations are locally adapted should be considered as the highest research-based conservation priority for *L. montana*. Given the relatively high genetic diversity of the Wombat State Forest and eastern Victorian populations, their independent protection will have the most benefit to the species. Protecting both populations and maintaining their independent genetic integrity will have a meaningful positive impact to species fitness. Furthermore, as we see evidence that gene flow has occurred between these sites, with a measurable increase in allelic diversity in eastern Victoria, human assisted gene flow (i.e., genetic rescue) should be investigated as an opportunity to promote enhanced genetic diversity and fitness of both populations. Although, the protection of the remaining populations is still essential as they still contain a meaningful level of unique genetic diversity.

We found that the populations sampled here were composed of between (mean estimates) 4–85 breeding individuals, with the census size being larger and not estimated here. Several studies have shown that population size is linked to persistence and the capacity to adapt to environmental change (O'Grady et al. 2006; Reed 2005; Willi & Hoffmann 2009). These studies show that a population census size of 20 individuals can result in a ~50% fitness loss, relative to populations comprising 1000 individuals and that populations of 100 can display a ~40% reduction in fitness when inbreeding is high (O'Grady et al. 2006). Increasing population sizes via breeding programs and decreasing inbreeding by facilitating admixture will have a meaningful impact to the mountain skinks fitness. Given the high population size and relatively low degree of inbreeding in the Wombat State Forest, this population can play a large role in the above-mentioned programs.

We recommend the following two main conservation goals. First, the avoidance of further population decline, and thus fitness loss, is critical. Maintenance of population-level diversity is essential, as facilitating increased population sizes and admixture among the five identified populations. Ideally, conservation measures will include actions that lead to improved population numbers (e.g., improved habitat condition and protection; removal of invasive predators; breeding programs). Second, we provide strong evidence that the mountain skink will benefit greatly from greater levels of admixture. A study investigating local adaptation and the potential for genetic rescue is a priority.

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