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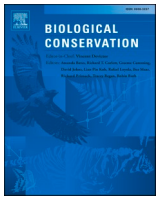

Conservation prioritisation of genomic diversity to inform management of a declining mammal species

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ABSTRACT

In our present age of extinction, conservation managers must use limited resources efficiently to conserve species and the genetic diversity within them. To conserve intraspecific variation, we must understand the geographic distribution of the variation and plan management actions that will cost-effectively maximise its retention. Here, we use a genome-wide single-nucleotide polymorphism (SNP) dataset consisting of 12,962 loci and 384 individuals to inform conservation management of the Endangered northern quoll (*Dasyurus hallucatus*), a carnivorous marsupial distributed patchily across northern Australia. Many northern quoll populations have declined or are currently declining, driven by the range-expanding cane toad (*Rhinella marina*). We (1) confirm population genomic structure, (2) investigate the contribution of each population to overall diversity, (3) conduct genomic prioritisation analyses at several spatial and hierarchical scales using popular conservation planning algorithms, and (4) investigate patterns of inbreeding. We find that the conservation of a single population, or even several populations, will not prevent the loss of substantial amounts of genomic variation and adaptive capacity. Rather, the conservation of at least eight populations from across the species distribution is necessary to retain 90 % of SNP alleles. We also show that more geographically isolated populations, such as those on islands, have very small contributions to overall diversity and show relatively high levels of inbreeding compared to mainland populations. Our study highlights the importance of conserving multiple genetically distinct populations to effectively conserve genetic diversity in species undergoing widespread declines, and demonstrates the importance of using multiple criteria to inform and prioritise conservation management.

1. Introduction

Biodiversity is declining globally. Disease, invasive species, altered fire regimes, habitat loss and climate change are driving biodiversity loss at all scales, from ecosystems to species to genetic diversity (Diamond, 1984; Díaz et al., 2019). Conservation management is crucial for halting and/or reversing these trends, but resources for conservation are limited, with a small number of species receiving a disproportionate amount of resources (Small, 2011). Although the necessity of triage approaches to conservation is still being debated (Watson et al., 2022;

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Wiedenfeld et al., 2021), the use of decision-support tools can nonetheless aid in the efficient allocation of resources to achieve conservation targets (Di Fonzo et al., 2017).

Within species, prioritisation of resources can involve difficult trade-offs between geographic regions and different populations. One approach to improve decision-making is to plan conservation actions based on a combination of relevant factors, such as anthropogenic impact, neutral and adaptive patterns of genetic diversity, and the location of climate refuges or protected areas (Nielsen et al., 2023). Populations that are genetically or geographically distinct can also be prioritised (Petit et al., 1998; von Takach et al., 2021), as these often represent unique evolutionary lineages. These can be classified into evolutionarily significant units, management units, and adaptive units, depending on various criteria (Barbosa et al., 2018).

At a broad scale, the conservation of populations that have high levels of genomic diversity is beneficial, as these populations are likely to have a greater capacity to adapt to changing environmental conditions and respond to conservation management efforts (Kardos et al., 2021). Genomic metrics such as heterozygosity, allelic richness, the number of private alleles, and the amount of adaptive variation can potentially be used as measures of intraspecific diversity (Nielsen et al., 2023; von Takach et al., 2023). It is also possible to quantify the contribution of each sampled population to the genomic diversity of the species by identifying networks of extant populations that would best represent/conserve diversity across all loci (Rick et al., 2023; von Takach et al., 2021). Having said this, other ecological and demographic factors also contribute to population viability and persistence, and should be considered when planning conservation strategies.

In Australia, one species in need of conservation management is the northern quoll (*Dasyurus hallucatus*). The northern quoll is a small (500–900 g), carnivorous marsupial that primarily feeds on insects, lizards, and small mammals, and is found across the northern third of the continent (Moore et al., 2022). Historically, the northern quoll was found in a wide variety of habitats, from savanna woodlands to monsoon rainforests, but contemporary populations persist patchily in a small subset of historical (pre-1800s) geographic locations and landscape types (Moore et al., 2022; von Takach et al., 2020).

Northern quolls are facing multiple threats and have suffered precipitous declines in their global population. Underlying threats have been present over the past 200 years, including habitat loss and fragmentation, and predation by introduced animals such as feral cats (*Felis catus*) and red foxes (*Vulpes vulpes*) (Braithwaite and Griffiths, 1994; Hernandez-Santin et al., 2016). The most significant recent declines, however, have been driven by the invasion of toxic cane toads (*Rhinella marina*). Cane toads were first introduced to Australia's east coast in 1935 to control beetle pests in the sugarcane fields of Queensland (Shine et al., 2020), and have since spread rapidly westward, now occurring across >1.5 million square kilometres of northern Australia (Urban et al., 2007). As cane toads expand into new areas, northern quolls mistake them for edible prey items, which leads to lethal poisoning, high mortality rates, and rapid population crashes of northern quoll populations (Burnett, 1997; Indigo et al., 2023; O'Donnell et al., 2010; Shine, 2010). In the last 15 years, cane toads have spread through much of the Kimberley region of northern Australia (Fig. 1); covering an area of some 270,000 km^2, and a former stronghold for northern quolls.

Here, we aim to use genomic data to inform regional conservation management of the northern quoll, with particular focus on the Kimberley region of north-western Australia, where cane toad invasion is most recent/ongoing, genetic diversity is high, and northern quoll populations are undergoing severe declines. Conservation management is crucial for reversing population trajectories in the Kimberley region, but resources for conservation are limited. We use several analyses to

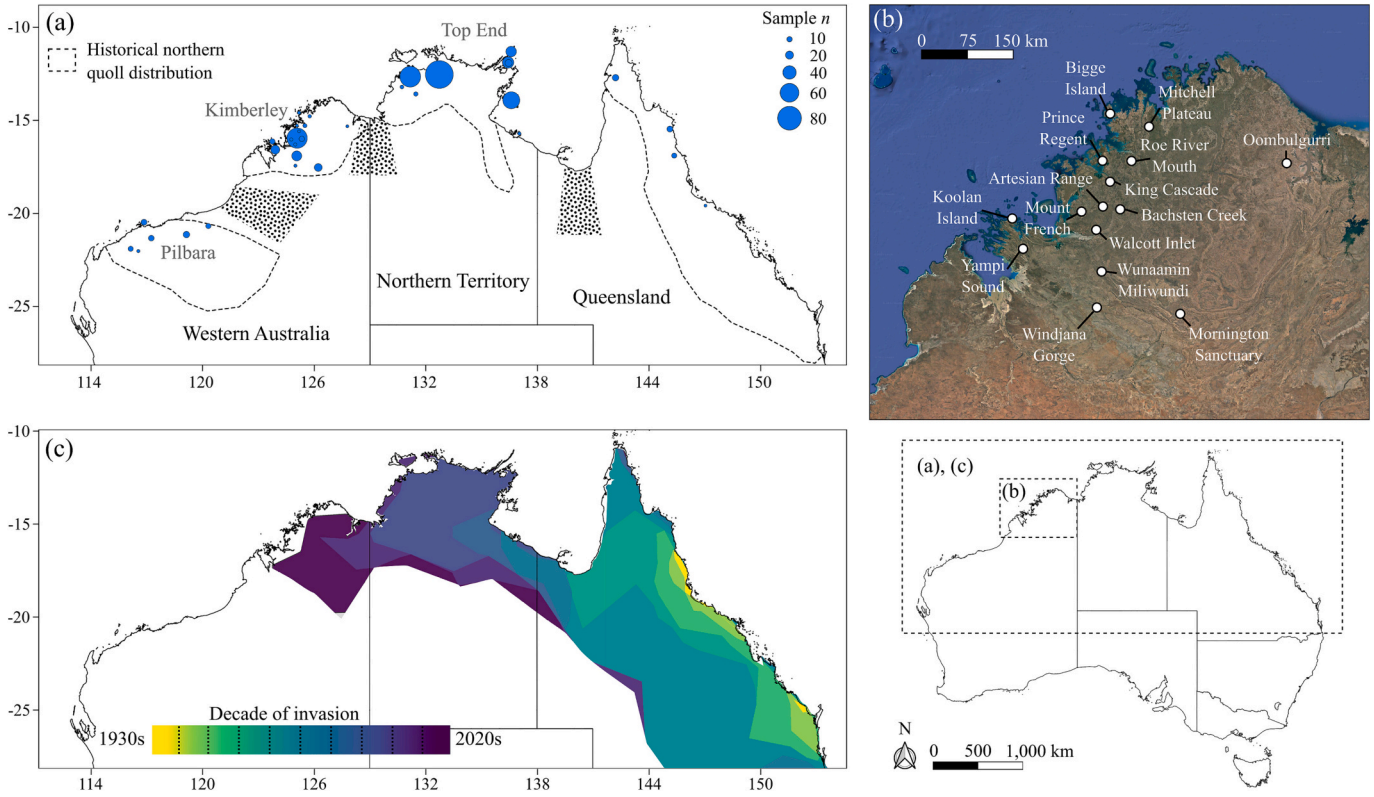

Fig. 1. Maps showing the northern quoll distribution (panel a), sampling locations across Australia (panel a), sampling locations in the Kimberley region (panel b), and spread of cane toads in Australia (panel c). Dotted zones represent locations of major known biogeographic barriers. Regional names in panel (a) are coloured grey, with state or territory names in black. The historical northern quoll distribution in panel (a) was adapted from Moore et al. (2022), and is a rough estimate of the pre-European (< 1788) distribution.

guide conservation management and prioritisation, considering population genomic structure and population level contributions to overall genomic diversity. We seek to identify pragmatic outcomes to this conservation issue by promoting management actions that maximise the retention of regional genomic diversity. We hypothesise that island populations will contribute little to overall genomic diversity of the species, and that inclusion of multiple genetically distinct populations in conservation networks will be necessary to conserve large amounts (> 90 %) of allelic diversity.

2. Materials and methods

2.1. Sample collection

In total, we processed 568 samples (including 36 technical replicates) for DNA extraction and sequencing, expanding on the work of von Takach et al. (2022b). Tissue samples were obtained from researchers who were working across the entire distribution range of the northern quoll. Tissue collection spanned nearly three decades, from 1993 to 2021, and included the regional jurisdictions of Queensland, the Northern Territory, and Western Australia (Fig. 1). To capture live animals, various trapping designs tailored to local conditions and research aims were used, but generally 10–20 cage traps were arranged in small grids (e.g. 1 ha) at intervals of about 1 km along roadsides or small vehicle tracks. In most cases, our samples consisted of small (2 mm diameter) ear tissue samples collected from live individuals. Individuals were sexed by visual inspection during processing, prior to release. Due to low capture rates of the target species in many areas, we assigned a single a priori population name (sampling locality) to all samples obtained from a given set of grids, even if the samples were collected over several years. Samples were typically stored in 70–100 % ethanol, either in a freezer or at room temperature, until being sent to us for analysis.

2.2. DNA extraction, library preparation and sequencing

Tissue samples were extracted in plate format using the standard protocol of the Qiagen DNeasy 96 Blood & Tissue Kit, with an extended lysis. This involved incubation at 56 °C for 2 h, followed by a reduction in temperature to 37 °C overnight. Following extraction, double-stranded DNA concentrations were quantified and normalised to 200 ng DNA in a total volume of 25 μL. These samples were then arranged in 96-well plates for double-digest restriction-associated DNA (ddRAD) sequencing at the Australian Genome Research Facility in Melbourne, Victoria. Each plate included several within-plate and among-plate technical replicates (for a total of 36 technical replicates) and a negative control (blank).

To determine the optimal combination of two restriction enzymes for ddRAD sequencing, three establishment samples (broadly representative of the species distribution) were used. *Pst*I and *Nla*III were deemed the most suitable enzymes for achieving the best genome representation while minimising repetitive sequences. The library preparation protocol included (1) digestion using *Pst*I and *Nla*III, (2) ligation with one of 48 unique inline barcoded adapters compatible with the restriction site overhang, (3) manual sample pooling, (4) DNA purification using the QIAquick PCR Purification Kit and SPRIselect paramagnetic beads, (5) size-selection targeting fragments of 280–375 bp in size using the BluePippin from Sage Science, and (6) a PCR amplification step where one of two multiplexing index primers was added (von Takach et al., 2022b). After indexing, libraries were pooled together and loaded onto flow cells for 150 bp single-end or paired-end sequencing (with only single-end reads used for analysis). Sequencing was performed on either an Illumina NextSeq 500 (three plates) or a NovaSeq 6000 (three plates) platform.

2.3. Bioinformatics pipeline and SNP filtering

Our bioinformatics pipeline used a combination of tools and custom scripts to analyse sequencing data (Supplementary Material Table S1). Raw sequence data (2.271 billion reads) were first processed using the STACKS *process_radtags* function for demultiplexing (Catchen et al., 2013), retaining 95.8 % (2.176 billion) of reads. The demultiplexed files were then mapped to the published northern quoll chromosome-length genome assembly (https://www.dnazoo.org/assemblies/Dasyurus_hallucatus) using the BWA version 0.7.17 *mem* algorithm (Li, 2013), generating sequence alignment map (SAM) files for each sample (individuals and technical replicates), resulting in 2.25 billion alignments.

The SAM files were compressed to binary alignment map (BAM) files, and then each BAM file was filtered for unmapped alignments (retaining 98.8 % = 2.223 billion alignments), then sorted and indexed using SAMTOOLS v1.7-1 (Li et al., 2009). The filtered and sorted BAM files were then used to call single-nucleotide polymorphisms (SNPs) via the ANGSD (v0.93) software package (Korneliussen et al., 2014). The following filters were applied in ANGSD: minimum mapping quality of 20 (excluding reads that mapped poorly or mapped to repeat regions of the genome), minimum base quality of 20, minimum call rate of 0.5 (across samples), minimum depth per site of 1250, maximum depth per site of 53,200, minimum depth per individual of 6, maximum depth per individual of 100, allele balance ratio of 0.2, SNP likelihood p value $\leq 1 \times 10^{-5}$, and genotype posterior probability $\geq$0.98 (based on GATK genotype likelihood with a uniform prior). This retained 486,083 SNPs that were genotyped in $\geq$50 % of samples.

All subsequent filters and analyses were conducted in a custom R (v4.3.1) (R Core Team, 2023) script that retained loci with <5 % missing data across samples and a minor allele count $\geq$3, as well as loci with observed heterozygosity <0.6 (to exclude potential erroneously merged reads). Samples with >35 % missing data across SNPs were also excluded, to remove poorly genotyped individuals. To account for bias resulting from linkage disequilibrium (LD), we used the 'SNPRelate' package to prune SNPs in LD (Zheng et al., 2012), setting the LD threshold to 0.5 and the sliding window size to 500 kbp. The *gl.filter.sexlinked* function in the 'dartR' package (Gruber et al., 2018; Mijangos et al., 2022) was then used to check for sex-linked SNPs. We also applied the *filter.sex.linked* function of Robledo-Ruiz et al. (2022), which checks for Y-linked loci, sex-biased loci, X-linked loci, and XY gametologs. No sex-linked loci were identified.

We then checked similarity between pairs of technical replicates, finding a mean similarity of 99.97 %. Close pairing of technical replicates was also checked visually with a hierarchical clustering dendrogram (Supplementary Material Fig. S1), after which we removed one individual from each pair of technical replicates, and one individual from each pair of close relatives (relatedness $\geq$0.25), with relatedness calculated using the method-of-moments technique in the 'beta.dosage' function of the *hierfstat* package (Goudet, 2005; Goudet et al., 2018). This approach estimates kinship values between pairs of individuals relative to the average kinship values of all pairs of individuals in the sampled population (i.e., within each locality). The final dataset contained 12,962 SNPs and 384 individuals.

2.4. Population genomic structure

Strong hierarchical population genomic structure has previously been observed in northern quolls, with three broad population genomic clusters that conform to major biogeographic breaks in the species distribution (von Takach et al., 2022b). To confirm we were observing similar patterns with our larger set of samples and loci, we built an individual-level plot of the first two principal coordinate dimensions of a genetic distance matrix. Euclidean genetic distances between individuals were calculated using the *dist* function, with eigenvalues calculated using the *cmdscale* function, and the percentage of variance explained by each axis was recorded. As we intended to investigate

patterns in the Kimberley region in greater detail, we also built an equivalent principal coordinate plot using only individuals from that geographic area. Pairwise differentiation between populations and between regions was quantified using F_{ST} values, calculated via the *stamppFst* function of the "StAMPP" package (Pembleton et al., 2013; Weir and Cockerham, 1984).

While differences in genomic diversity metrics between sampling periods within populations have been analysed previously (von Takach et al., 2022b), we calculated observed heterozygosity (H_O), expected heterozygosity (H_E), unbiased expected heterozygosity (corrected for sample sizes, uH_E), and allelic richness (*AR*) for populations sampled across multiple years to ensure bias due to sampling times was minimised.

2.5. Prioritising populations for conservation of genomic diversity

We conducted a set of analyses to inform strategies for the conservation of genetic diversity, building on the approaches used for brush-tailed rabbit-rats (*Conilurus penicillatus*) and black-footed tree-rats (*Mesembriomys gouldii*) (von Takach et al., 2023; von Takach et al., 2021). We conducted the analyses at three hierarchical levels: (1) high-level population structure where individuals were pooled into five geographic regions across the entire species distribution (equivalent to $k = 5$ in von Takach et al., 2022b), (2) a priori populations (sampling localities) across the entire species distribution, and (3) a priori populations within the Kimberley region only. While the last of these hierarchical levels could theoretically be conducted on any geographic sub-region, we chose to use the Kimberley due to both the amount of sampling that has occurred and the fact that it has experienced the most recent impact from cane toads (i.e. populations in this region require urgent conservation action).

At each of the three hierarchical levels, we conducted two primary analyses. First, we quantified the contribution of each a priori population to gene diversity and allelic diversity by partitioning these metrics into total, within population, and between population components (López-Cortegano et al., 2019; Pérez-Figueroa et al., 2009). Second, we used a quantitative prioritisation analysis to identify the most efficient network(s) of populations that best represents the amount of genetic diversity in each hierarchy.

For the analysis of allelic diversity, we assessed the contribution of each population to global (i.e. all individuals for species wide hierarchies, and all Kimberley samples for the Kimberley-specific analysis) allelic and gene diversity (expected heterozygosity). This was done using the software program METAPOP2 (López-Cortegano et al., 2019; Pérez-Figueroa et al., 2009), which provides a robust method of quantifying which populations contribute most to the overall genetic diversity of the species based on both local variation and genetic differentiation. The contribution of each population was estimated by removing that population and re-calculating the changes in within-population diversity (A_S, H_S), among-population diversity (D_A, D_G) and total allelic (A_T) and gene (H_T) diversity. The allelic diversity calculation relies on both allelic richness (i.e., the number of segregating alleles in the population) and the dissimilarity of alleles across populations, meaning that a population can have a positive or negative contribution to total diversity.

To identify the most effective networks of extant populations that best represent the amount of genetic diversity in each set of individuals, we used a MARXAN method of analysis (Ball et al., 2009; Watts et al., 2009). Our strategy included allocating an equal unit cost of 1 for the conservation of each population (given a lack of specifically costed conservation options). Using the R package 'prioritizr' (Hanson et al., 2021; Hanson et al., 2020) and the SYMPHONY integer linear programming solver (Vladislav, 2018), we examined the optimal network of populations to maximise allelic richness in the species, scrutinising the optimal solutions for scenarios ranging from one to either five (regions within species), 20 (populations within species), or 7 (populations within the Kimberley) 'protected populations'.

Using this method, each allele is considered a feature to be conserved, and each region/population is considered a planning unit. For each of 100 iterations, we randomly sampled five or 10 individuals per population or region, respectively, and calculated the total number of alleles across all populations combined to identify a conservation solution for a maximum coverage (of alleles) objective for budgets of one to the maximum number of populations/regions being analysed. We tallied the number of configurations across the 100 replicates for each budget, as well as the total allele count for each solution (using equal sample sizes).

To investigate change in heterozygosity with an increasing number of populations in the hypothetical conservation network, we calculated H_E across all conserved individuals (i.e. the total H_T), for all unique subsets/combinations of every population where $n \geq 8$ (totalling 65,535 subsets). For any population with >8 individuals, we randomly selected eight individuals. All individuals were pooled into a single population and the expected heterozygosity calculated using the *Hs* function of the 'adegenet' package (Jombart, 2008).

2.6. Extent of inbreeding

To investigate the extent of inbreeding at the population level, we plotted means and standard errors of individual inbreeding coefficients at each sampled locality. To calculate individual inbreeding coefficients from the SNP dataset, we used the modified Visscher's estimator method (method = "mom.weir") of the *snpgdsIndInbCoef* function in the 'SNPRelate' package (Yang et al., 2010; Zheng et al., 2012). To avoid bias due to differences in allele frequencies among geographic areas, we estimated allele frequencies within regions (as above, equivalent to $k = 5$ in von Takach et al., 2022b), and calculated individual inbreeding coefficients independently for each region. To minimise bias due to differing sample sizes among a priori populations, a maximum of eight individuals from any one sampling locality was used in the calculation of allele frequencies within regions. Using this approach, the estimates of inbreeding coefficients are relative to the broader allele frequencies found in the region. Because Groote Eylandt is biogeographically part of the Top End region, but is also worthy of interpretation as a separate region (von Takach et al., 2022b), we calculated the mean inbreeding coefficient relative to both its own allele frequencies as well as relative to the broader Top End set of samples.

3. Results

3.1. Population genomic diversity and structure

Across the species entire range, we observed population genomic structural patterns similar to those described by von Takach et al., 2022b, with the PCoA splitting sampled individuals into three distinct biogeographic clusters with some sub-structuring present in each cluster (Supplementary Material Fig. S2a). Coordinate 1 (24.8 %), which split the Western Australian populations from the Northern Territory and Queensland populations, explained substantially more of the variation than coordinate 2 (8.7 %), which split the Queensland populations from the Western Australian and Northern Territory populations.

Within the Kimberley region, coordinates 1 and 2 explained 7.3 % and 4.3 % of the overall variation, respectively (Supplementary Material Fig. S2b). Populations on Koolan Island and Mornington Sanctuary were separated from the mainland cluster of individuals in the Kimberley. The Koolan Island individuals clustered together tightly, while the Mornington Sanctuary population showed more within-population variation in genetic distances, spreading across coordinate 2.

For populations where samples were pooled across multiple years, there were no substantial differences in heterozygosity or allelic richness values between time periods (Supplementary Material Table S2).

3.2. Prioritising populations for conservation of genomic diversity

By region, the Kimberley made the largest positive contribution to both within- and among-population allelic diversity of the species (Fig. 2a). Queensland and Groote Eylandt both made strongly negative contributions to within-population allelic diversity. While Queensland made a positive contribution to among-population allelic diversity, Groote Eylandt made a slightly negative contribution. With respect to heterozygosity, Queensland and the Pilbara made large positive contributions to among-population gene diversity, but the Kimberley and Top End regions made the largest positive contributions to within-population gene diversity.

By sampling locality, several Kimberley and Top End populations (e.g. Artesian Range, Wunaamin Miliwundi, Darwin, and Kakadu) made large positive contributions to allelic diversity (Fig. 2b). Natural island populations, including Dolphin Island, Koolan Island, Marchinbar Island

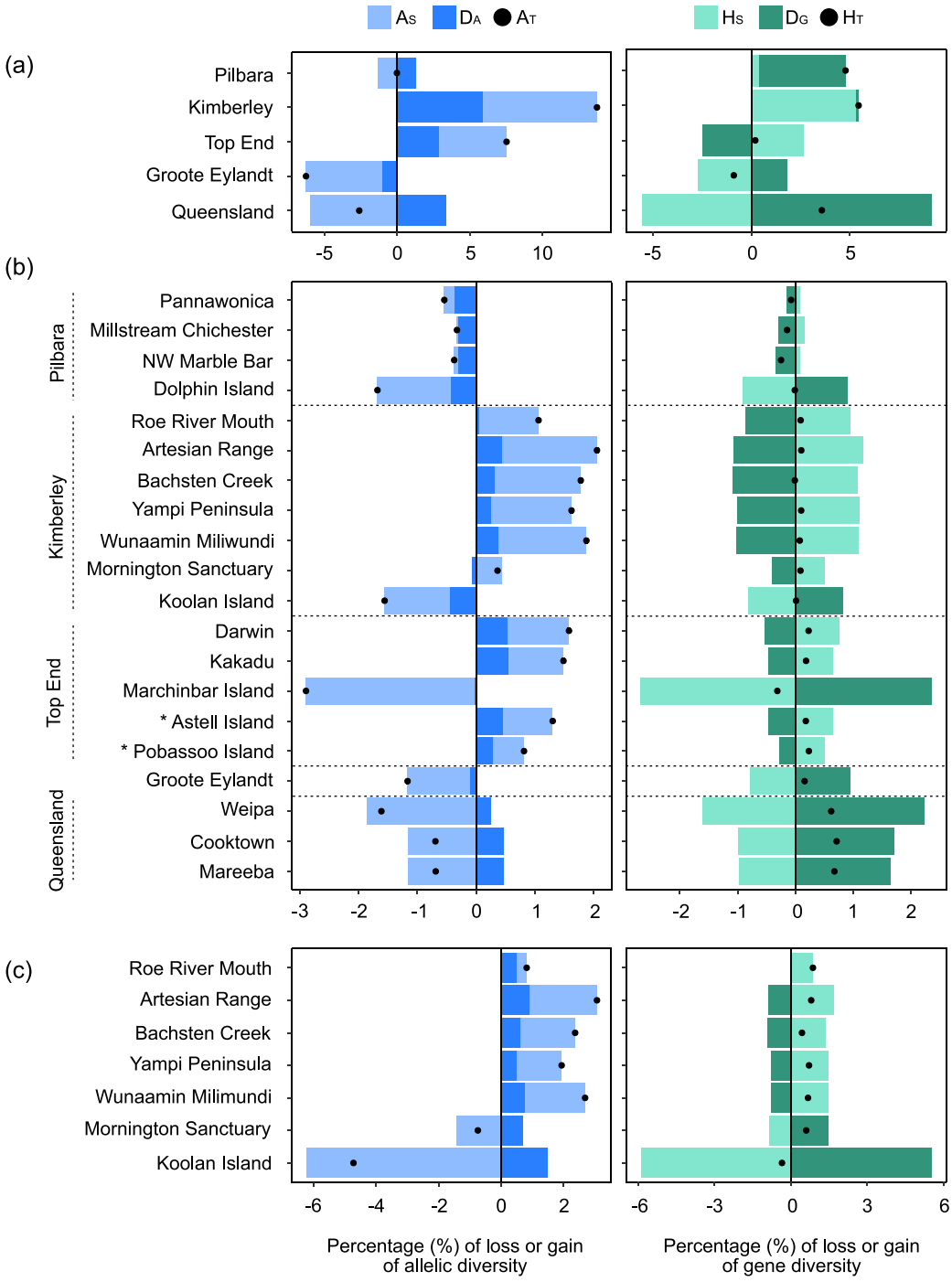

Fig. 2. Contribution of northern quoll populations to genomic diversity. Analyses are shown for (a) regions, (b) all sampling localities, and (c) the Kimberley region. Total (A_T, H_T; black circle) contributions to diversity are partitioned into components of within- (A_S, H_S; lighter colours) and between- (D_A, D_G; darker colours) allelic (blue) and gene diversity (green), respectively. A_S = within-subpopulation allelic diversity, H_S = average gene diversity within subpopulations, D_A = allelic distance between pairs of subpopulations, and D_G = average gene diversity between subpopulations. Populations denoted with an asterisk (*) are artificially established populations sourced from several mainland localities in the Northern Territory. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and Groote Eylandt, all showed negative contributions to both within- and among-population allelic diversity. With respect to heterozygosity, many populations showed overall contributions close to zero, with their contributions to among-population gene diversity being roughly equal but opposite to their contributions to within population gene diversity (Fig. 2b). Natural island populations, and populations in Queensland, showed negative contributions to within population gene diversity and positive contributions to among-population gene diversity, whereas mainland populations in the Pilbara, Kimberley and Top End regions showed the opposite trend.

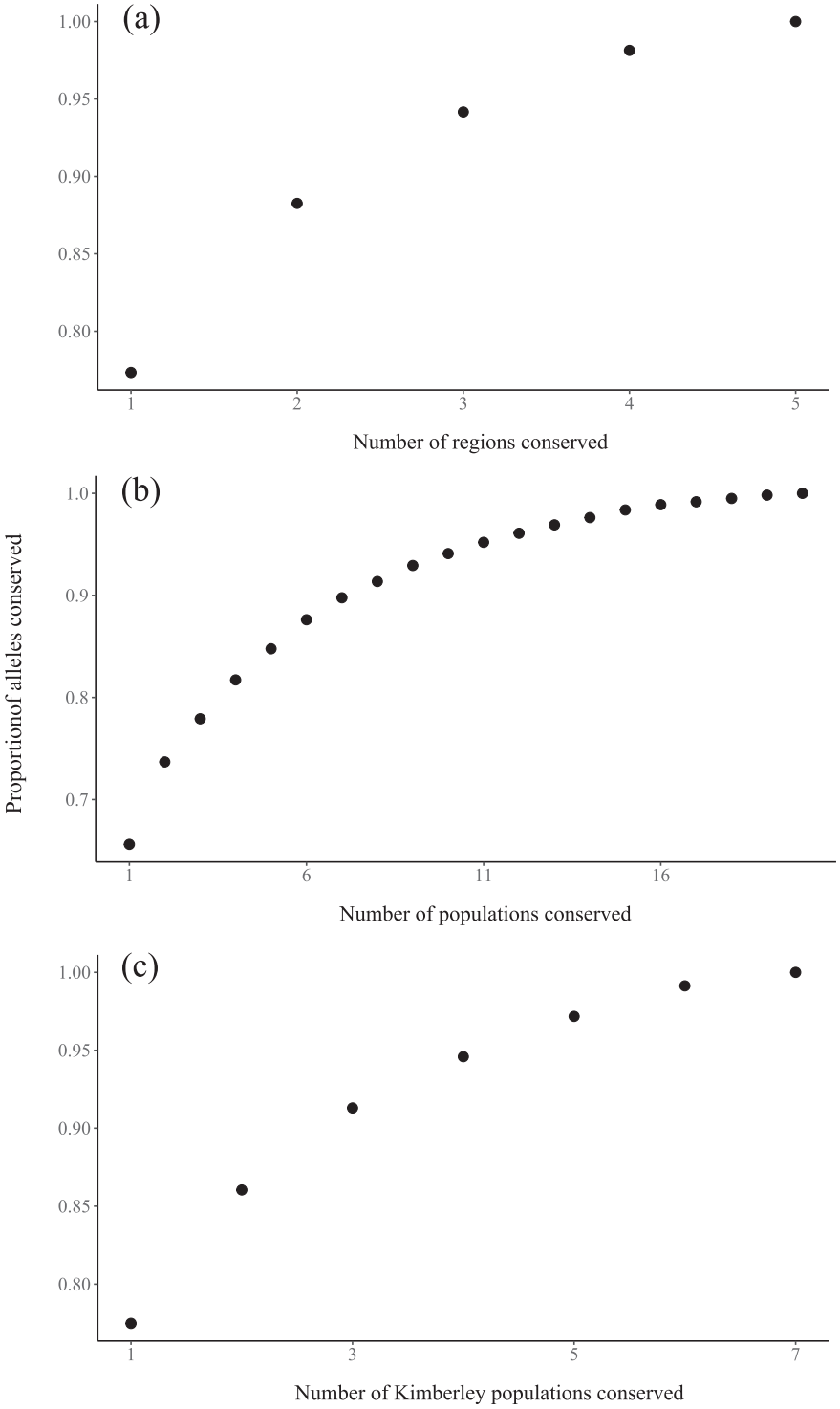

Fig. 3. The impact that conserving differing numbers of populations of the northern quoll has on the proportion of SNP alleles conserved. Panel (a) represents the conservation of alleles across the species distribution when differing numbers of geographic regions are conserved, where each region represents an ancestral population cluster at $k = 5$. Panel (b) represents the conservation of alleles across the species distribution when differing numbers of populations (sampling localities) are conserved. Panel (c) represents the conservation of alleles within the Kimberley region when differing numbers of populations are conserved. Only the most cost-efficient scenario is shown in each panel, and only loci that are polymorphic within the Kimberley region are considered in panel (c).

In the Kimberley-specific analysis, the populations of Artesian Range and Wunaamin Miliwundi made the greatest contributions to the overall allelic diversity of the region (Fig. 2c). Unsurprisingly, the more isolated, low diversity, and differentiated populations of Koolan Island and Mornington Sanctuary (Supplementary Material Fig. S3) made negative contributions to within population allelic diversity and positive contributions to among-population allelic diversity. With respect to heterozygosity, similar trends were present to those found for allelic diversity, but the mainland populations made slightly negative contributions to among-population gene diversity.

Our prioritisation analysis found that, if we aimed to conserve genomic diversity in a single regional-level cluster of northern quolls, the most effective region to conserve would be the Kimberley (Supplementary Material Table S3), and this action would retain 77.3 % of all SNP alleles in the species (Fig. 3a). If we were to conserve two regional populations, we would conserve a maximum of 88.3 % of SNP alleles, including the Top End and Kimberley regions. The only way to conserve >95 % of all SNP alleles would be to conserve four regional populations, which would include the Top End, Queensland, Kimberley, and Pilbara populations (98.1 % of SNP alleles).

If we ignore the region of origin, and conserve just a single population, the highest number of SNP alleles we could conserve would be 66 %, and this would be achieved by conserving the Artesian Range population (Fig. 4a). Less optimal solutions would be to conserve other Kimberley populations, such as those at Bachsten Creek or Wunaamin Miliwundi (Supplementary Material Table S4). Based on current sampling efforts, achieving a target of 90 % of SNP alleles would require the conservation of eight populations (Fig. 3b), with the most optimal populations being Darwin, Kakadu NP, Bachsten Creek, Cooktown, Astell Island, Millstream Chichester, Wunaamin Miliwundi, and Artesian Range. This includes three populations in the Kimberley region, three populations in the Top End region, one population in the Queensland region, and one population in the Pilbara region (Fig. 4c). Similarly, if we had a target of 95 % of SNP alleles, we would require the conservation of 12 populations, with the most optimal solution requiring five in the Kimberley, three in the Top End, two in Queensland, one in the Pilbara, and Groote Eylandt (Fig. 4d). Other natural island populations that are strongly differentiated from adjacent mainland populations (e.g. Marchinbar Island, Supplementary Material Fig. S3), were not considered high priority for conservation (Supplementary Material Table S4).

Focusing only on the Kimberley region, we found that a 90 % target of SNP alleles could be met with three populations, if these included Artesian Range, Bachsten Creek, and Wunaamin Miliwundi (Supplementary Material Table S5). A target of 95 % of SNP alleles could only be met if five populations were conserved, including Artesian Range, Bachsten Creek, Wunaamin Miliwundi, Roe River Mouth and Yampi Peninsula (Fig. 3c).

With an increasing number of populations in a hypothetical conservation network, the expected heterozygosity across all conserved individuals rapidly approaches an asymptote (Supplementary Material Fig. S4). Once four or more populations are conserved, the maximum attainable value of H_E does not noticeably increase, although the minimum attainable value does increase, and there are many different networks of populations that result values of H_E close to the maximum value (Supplementary Material Fig. S4).

3.3. Inbreeding coefficients

Unsurprisingly, naturally occurring populations of northern quolls on islands, such as Marchinbar Island (NT), Koolan Island (WA) and Dolphin Island (Pilbara), showed substantially higher levels of inbreeding relative to their adjacent mainland populations (Supplementary Material Fig. S5). Despite being much larger in size than other islands, Groote Eylandt showed a high level of inbreeding relative to the Top End mainland populations. However, when treated as its own region (i.e. using local allele frequencies for reference), the Groote Eylandt population showed a slight negative coefficient. Artificially established island populations, including those on Astell Island and Pobassoo Island, did not show high levels of inbreeding, resulting from their recent establishment using a mixture of source populations.

Analogous to the results for island populations, mainland populations with a greater level of recent or longer-term geographic isolation from other northern quoll populations typically showed higher mean values of inbreeding coefficients, including Mornington Sanctuary in the Kimberley region and the Weipa population in Queensland (Supplementary Material Fig. S5).

There was a moderately strong Pearson's product-moment correlation ($r = 0.54$, $p = 0.01$) between the mean inbreeding coefficient for a population and the number of populations needed in the conservation network before that population was selected in ≥90 % of iterations, indicating that more inbred populations were lower priorities for the conservation of alleles.

4. Discussion

Population declines are impacting the loss of intraspecific genomic diversity and adaptive potential of conservation-dependent species worldwide (Des Roches et al., 2021; Exposito-Alonso et al., 2022). Using genomic data, we quantified the number and combination of populations that are required to conserve varying amounts of genomic diversity in a threatened mammal species, the northern quoll. We found that, to retain at least 90 % of the alleles at polymorphic loci in the species, the conservation of populations in at least eight localities across the species distribution is required, with less optimal solutions requiring greater effort (i.e. more localities conserved). Importantly, these sampling localities cannot be evenly spread across the distribution, with greater conservation effort required in the geographic area with the highest current genomic diversity, and a greater focus on conserving large, diverse mainland populations rather than small, isolated island populations.

Focusing on this area of highest current genomic diversity, the Kimberley region, we showed that at least three sampling localities would be needed to conserve >90 % of the alleles at locally polymorphic loci. We also found that the population with the greatest individual contribution to overall allelic diversity in the region (Artesian Range) was the most optimal population for the conservation of alleles.

4.1. Implications for conservation management

Conservation of multiple genetically distinct populations is vital for the effective long-term conservation of species (Allendorf et al., 2022; Des Roches et al., 2021; Luck et al., 2003). Our results build on this concept by showing that even conserving populations at several localities may not be enough to conserve substantial amounts of genomic variation, and thus is likely insufficient to conserve the adaptive capacity of a species. As populations of vertebrates decline around the world (Ceballos et al., 2017), intraspecific genomic diversity is being eroded (Des Roches et al., 2021; Mimura et al., 2017; von Takach et al., 2022b), which can accelerate the loss of populations and species (James, 1970; Lynch et al., 1995; Robertson, 1997).

Genomically diverse northern quoll populations in Western Australia's Kimberley region are experiencing rapid cane toad-induced declines (Doody et al., 2023; Indigo et al., 2023), and we can expect substantial loss of intraspecific diversity in this region through time (Exposito-Alonso et al., 2022). To avoid this loss, we need to carefully enact and evaluate management actions that will help mitigate northern quoll population declines or otherwise safeguard genomic diversity. The invasion of cane toads has proceeded unchecked for >80 years, and to date, there is little evidence that there are practical solutions to mitigate their impact on northern quoll populations when they invade an area (Indigo et al., 2023; Indigo et al., 2021). Other management strategies

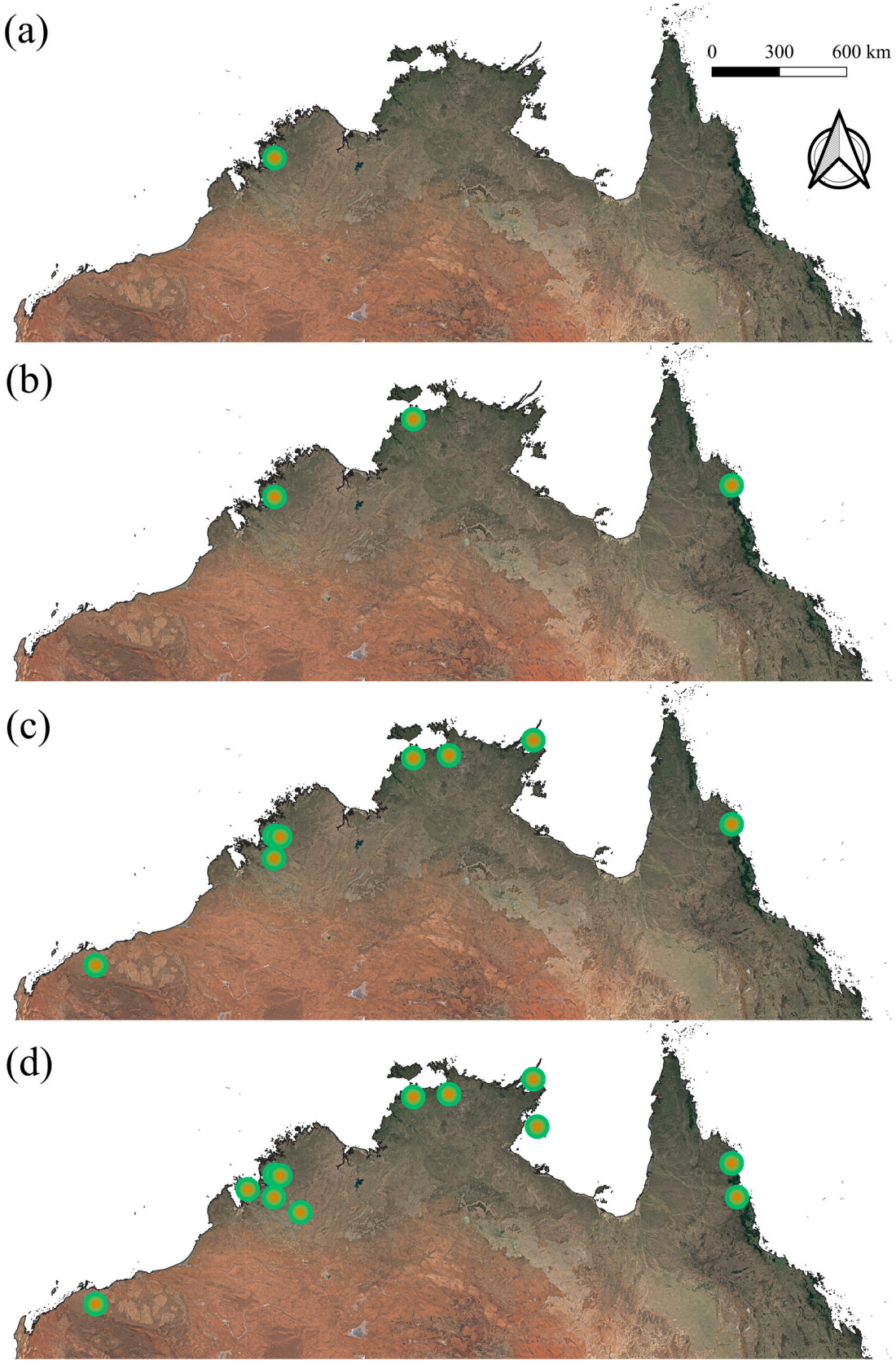

Fig. 4. Potential scenarios for the most efficient conservation of allelic diversity in northern quolls. Each coloured circle represents an a priori population (sampling locality). Panels (a), (b), (c) and (d) respectively represent the best scenario for conservation of ≥66 %, ≥ 75 %, ≥ 90 % or ≥ 95 % of total alleles in the species, which requires at least one, three, eight or 12 populations to be conserved.

that also address broader threats, including habitat restoration (e.g. through fire and feral herbivore management), predator exclusion, and translocations (including targeted gene flow), should be considered and evaluated (Kelly and Phillips, 2016; von Takach et al., 2022a). Our results further suggest that these management actions should not be limited to one or two populations, but should be implemented across multiple populations and localities to maximise the conservation of genomic diversity and adaptive potential of the species.

4.2. Metrics for conservation prioritisation and monitoring

Some highly differentiated populations in our study include island populations that show large F_{ST} values due to a lack of genomic diversity, rather than the evolution of unique/novel diversity. This likely results from small founder population sizes combined with long-term isolation, genetic drift, and/or chronic population bottlenecks (von Takach et al., 2022b). We found that such populations contributed positively to among-population gene diversity, but contributed negatively to within-population gene diversity and within- and among-population allelic diversity. The formal prioritisation did not tend to rank these populations highly, and this likely aligns with core conservation planning concepts such as irreplaceability (Kukkala and Moilanen, 2013; Pressey et al., 1993), suggesting that using F_{ST} as a proxy for irreplaceability is not always appropriate (Weeks et al., 2016).

It is easy to use RAD-seq datasets to identify putatively adaptive loci across one or more environmental gradients, and apply this information to conservation management. However, while a small proportion of loci under selection across the genome can be identified by this process, we consider it broadly inadvisable and inappropriate to manage species based on a small number of allelic variants that are identified in a fraction of a species genome via associations with a small number of spatially-varying environmental variables (Hoban et al., 2021; Hohenlohe et al., 2021; Wadgymar et al., 2017). A range of other factors also need to be considered before interpreting such results, including that populations are often exposed to multi-dimensional selection pressures (Dauphin et al., 2023), small-effect loci involved in polygenic traits are difficult to detect (Lasky et al., 2023), and different genes may underlie local adaptation to a single trait in different populations (Lasky et al., 2023).

We used a metric of allelic diversity for our prioritisation analysis, and investigated the change in heterozygosity for all possible conservation planning population network conservation options, but several other population genomic metrics can also be used to inform management, including the effective population size (N_e). Maintaining large N_e values for regional populations is a key conservation concern. This metric was recently proposed as a headline indicator for genomic diversity in the Convention on Biological Diversity Kunming-Montreal Global Biodiversity Framework (CBD, 2022), which recommends that the "proportion of populations within species with an effective population size $>$ 500" be monitored. Previous analysis has shown that most northern quoll populations have N_e values below 1000, and many have N_e values $<$500, particularly once cane toads have invaded a locality (von Takach et al., 2022b). We suggest that priorities for future monitoring of threatened species should include quantification of both effective and census population sizes at as many localities as possible. This could be achieved through a combination of genomic data and mark-recapture data, and would help to provide longitudinal data on population trends and properly evaluate the efficacy of management actions.

4.3. Inbreeding and population isolation

Our analysis of inbreeding coefficients showed that naturally occurring populations of northern quolls on islands (e.g. Marchinbar Island in the Top End), as well as mainland populations with higher levels of geographic isolation (e.g. Mornington Sanctuary in the Kimberley), have relatively higher levels of inbreeding than large and well-connected mainland populations. Our results for Groote Eylandt showed a high level of inbreeding relative to mainland populations and a low level of inbreeding relative to itself. We suspect this is due to individuals avoiding mating with close relatives within the population, and such a pattern is not unexpected because the relatedness metrics are calculated relative to the set of samples being analysed. Importantly, populations with high inbreeding coefficients were less likely to be ranked highly in the formal prioritisation analysis and had lower contributions to overall genomic diversity.

Our findings are consistent with previous studies on other species, which have shown that populations with limited gene flow are more prone to inbreeding and the accumulation of deleterious alleles (Frankham, 2015; Frankham, 1997; Keller and Waller, 2002; Lynch et al., 1995). Islands have also previously been shown to harbour less diverse populations of northern quolls (von Takach et al., 2022b), a common feature of island populations that results from the combined effects of isolation, genetic drift, and founder effects (Frankham, 1997). Genomic assessments for various Australian species, including the golden bandicoot (*Isoodon auratus*) (Rick et al., 2023) and the boodie (*Bettongia lesueur*) (Nistelberger et al., 2023), have consistently found substantially higher genomic diversity in mainland Australian populations compared to island refuges, and it appears that only very large islands naturally support populations with comparable levels of genomic diversity to mainland areas (von Takach et al., 2023; von Takach et al., 2021). Notably, northern quoll populations on Astell Island and Pobassoo Island, which were established from multiple mainland source populations, were the only island populations that we found to have a positive contribution to the allelic diversity of the species.

These characteristics of island populations likely explain why island populations of northern quolls were not highlighted as priorities for conservation in our prioritisation analysis. Conserving only island populations would be highly suboptimal for the retention of intraspecific genomic diversity. This highlights a conundrum, as island populations are the easiest to conserve due to their naturally lower probability of being colonised by cane toads (particularly when uninhabited by humans). Our analysis shows that relying on entirely on existing island populations as 'arks' for conservation would result in the loss of much mainland genomic diversity.

To capture representative diversity in areas that can be kept free of cane toads, and to avoid the negative impacts of inbreeding, it may also be necessary to consider using translocations to increase genomic diversity and to reduce the accumulation of deleterious alleles in some populations. Many northern quoll populations will never experience natural connectivity/gene flow with other populations, due to a combination of (1) the scale of anthropogenic impacts, (2) the massive geographic breadth of the species distribution, and (3) the presence of major biogeographic breaks across northern Australia (Fig. 1). However, we suspect that artificial gene flow via translocations will be an increasingly common conservation action in the foreseeable future, as has been the case for the western quoll (*Dasyurus geoffroii*) (Jensen et al., 2021).

Genetic rescue via translocation has proved vital to the conservation of several taxa, including the mountain pygmy-possum (Weeks et al., 2017), Florida panther (*Puma concolor coryi*) (Hedrick, 1995; Hostetler et al., 2013), European adder (*Vipera berus*) (Madsen et al., 1999), and Rocky Mountain bighorn sheep (*Ovis canadensis*) (Hogg et al., 2006). Recent work on northern quolls suggests that mixing of quite distant populations can be done without strong outbreeding depression being apparent (Kelly et al., 2021). Importantly, delaying such actions, particularly in the Kimberley region, where extensive recent declines have occurred, is only likely to result in a longer period of drift and inbreeding, with concomitant loss of diversity and accumulation of deleterious alleles.

5. Conclusions

Our study highlights the importance of conserving multiple genetically distinct populations to effectively conserve species undergoing widespread declines. Our findings show that the simplest conservation option for species (e.g. retention of a naturally occurring island population), is not necessarily an adequate or efficient way to conserve intraspecific genomic diversity, particularly for a widespread species. Similarly, conservation of a single locality or population, or even several, may not be sufficient to conserve substantial amounts of genomic variation and adaptive capacity. Our results also demonstrate the importance of using multiple criteria to prioritise conservation management and monitor population persistence. Metrics such as heterozygosity, inbreeding coefficients, and effective population size can be incorporated into species management and used to monitor changes in genomic diversity and health over time. Critically, for the northern quoll, the window of time that is available to act on conserving intraspecific diversity is disappearing, with rapid action required to manage this diversity in the face of ongoing threats.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw sequence data has been uploaded to the Bioplatforms Australia Oz Mammal Genomics Initiative data portal (https://data.bioplatforms.com/organization/about/bpa-omg) (dataset IDs 102.100.100/52650 and 102.100.100/52623). This data is made available openly under a Creative Commons Attribution license. All other data and scripts have been uploaded to the Dryad Digital Repository (https://doi.org/10.5061/dryad.kwh70rzbf).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2024.110467.

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