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Conservation Arks: Genomic Erosion and Inbreeding in an Abundant Island Population of Koalas

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ABSTRACT

The persistence of many threatened species depends on isolated habitat patches such as conservation parks, fenced reserves, and islands. While these 'conservation arks' provide refuge from many contemporary threats, they can also pose risks of genetic diversity loss and inbreeding depression, further exacerbating extinction risk. A pertinent example is the Kangaroo Island koala population in South Australia that originated from a few translocated founding individuals in the 1920s but now sustains a large population with a low prevalence of infectious disease. We investigated the extent and consequences of founder effects on genomic diversity, inbreeding, and adaptive potential in Kangaroo Island koalas by comparing them with mainland Australian populations using high-coverage whole genomes. Our findings support sharp, recent declines in effective population sizes (N_e) in both mainland and Kangaroo Island populations. However, Kangaroo Island koalas had much lower individual and population-level diversity. Together with longer and more numerous runs of homozygosity and an increased proportion of homozygous genetic load, these results support the hypothesis that a severe bottleneck has contributed to inbreeding and maladaptation in Kangaroo Island koalas. While Kangaroo Island has the potential to conserve a viable population of koalas, we recommend genetic rescue to restore diversity and mitigate inbreeding depression in this isolated population. Our results emphasise the need for longitudinal genomic monitoring and genetic management to maintain long-term viability and resilience in potential conservation arks. Understanding the demographic history of such populations will help inform future conservation aimed at preventing genetic erosion and preserving biodiversity.

1 | Introduction

Populations of threatened species are increasingly being managed in 'conservation arks' such as fenced reserves, remnant habitat patches, and islands. Not only is endemism often higher in isolated island habitats (Kier et al. 2009), but such locations now also provide refuges for species with previously extensive mainland distributions compromised by introduced pests and rapid land-use changes mainly over the last few centuries (Gallardo et al. 2017). This holds true in Australia,

where contemporary mammal extinctions exceed those of any other continent, and islands in particular harbour a disproportionately large number of threatened animal species (Woinarski et al. 2015; Gibson et al. 2017; Legge et al. 2018; Ball et al. 2022). Unfortunately, the protection afforded by isolation can also pose risks. Indeed, island populations are more vulnerable to demographic and environmental stochasticity (Frankham 1998), and genomic erosion and inbreeding, common in small and isolated populations, can increase vulnerability (Spencer et al. 2017; Groombridge et al. 2018). Identifying genomic risk factors and implementing appropriate genetic management for such populations could improve persistence probability.

When dispersal and opportunities for natural admixture are limited, a closed population must, in general be pre-equipped to cope with current and potentially changing conditions, relying heavily on existing genomic variation (Lande 1995; Willi et al. 2006). However, diversity is often already low in threatened species, and this is compounded when an ark population is established from a few individuals. When the founding population contains only a small subset of the source population's original genetic variation, a founder effect occurs, leading to lower overall diversity and producing allele frequencies that are unrepresentative of the original population (Mayr and Huxley 1954). Similarly, small populations are more susceptible to a loss of diversity via genetic drift, whereby random subsampling of alleles across generations leads to stochastic fluctuations in frequencies and ultimately the fixation of some alleles (Frankham et al. 2004). The smaller the population, the more likely it is that random drift will outweigh the effects of selection, and the loss of adaptive diversity is generally highest when small founding populations remain small for many generations. This means that both demographic bottlenecks and reserves with low carrying capacity can reduce the adaptive potential of populations.

Small populations also tend to have fewer available mates, which increases the likelihood of mating between genetically related individuals (inbreeding). This leads to higher homozygosity, where individuals inherit identical alleles from a common ancestor. When these alleles are deleterious, homozygosity reduces fitness and survival in a phenomenon known as inbreeding depression, which can reduce population growth rates and elevate the risk of extinction (O'Grady et al. 2006). Many studies have documented the harmful effects of population fragmentation and isolation on genetic diversity, fitness, and extinction risk across a range of species (O'Grady et al. 2006; Frankham et al., 2017). While low genetic diversity and inbreeding can result in the fixation of harmful alleles in a population, they can sometimes lead to the beneficial outcome of purging, where natural selection removes deleterious alleles exposed in homozygous form. However, genetic drift can also overwhelm selection, especially in small populations, allowing mildly or even moderately deleterious alleles to rise in frequency or become fixed in a process known as 'drift load' (Dussex et al. 2023). Once fixed, such variants can only be removed through gene flow, underscoring the potential value of connectivity or genetic rescue. The population's demographic history and the length and severity of the bottleneck influence the balance between purging and drift (Mathur et al. 2023; Olazcuaga et al. 2023). Although strong evidence links bottlenecks to lower probabilities of population persistence, the underlying genomic mechanisms remain poorly understood in many cases. Despite the ongoing debate about the best management strategies such as mixing populations to enhance diversity, the benefits of increased genetic diversity generally outweigh the risk of introducing deleterious variation (Ralls et al. 2020). Documenting and addressing genetic issues in conservation arks can therefore improve long-term population sustainability and guide integrated conservation strategies, including metapopulation management and connectivity.

Of the many threatened species in Australia, the koala (*Phascolarctos cinereus*) is a recognisable representative of Australia's unique wildlife. However, it also has a complex history of conservation, particularly in island refuges. The species is distributed across five of the seven mainland states and territories, with populations in all regions substantially impacted by European settlement. Habitat loss and the fur trade brought the southern population(s) (Victoria, South Australia, and southern New South Wales) (Lott et al. 2022) close to extinction by the early 1900s, and koalas were considered absent from South Australia by the 1930s (Robinson 1978). This crisis led to some of the earliest koala conservation efforts, including translocations from the Victorian mainland to several offshore islands during the late 1800s (Herald 1929; Warneke 1978; Kirkwood and Johnston 2006), followed by subsequent restocking in both the Victorian mainland and South Australia (Robinson 1978; Menkhorst 2004). Farther north, koala populations have continued to decline due to habitat loss and diseases such as koala retrovirus and chlamydia, leading to the species' listing as Vulnerable on the IUCN Red List in 2014 (Woinarski and Burbidge 2020). The catastrophic 'Black Summer' bushfires exacerbated their conservation status, resulting in classification as Endangered in three states (Queensland, New South Wales, Australian Capital Territory) in 2022 under the federal *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act).

South Australia's Kangaroo Island (*Karta Pintingga*, or more simply *Karta*) is 4405 km^2 in total area and its koala population is one of those resulting from early southern translocations between 1923 and 1925, and has subsequently grown rapidly since establishment. Despite reportedly being founded from only 18 to 24 individuals, which themselves were translocated from an ark population on Victoria's French Island, the census population size is thought to have reached the hundreds by the 1940s and > 48,000 by 2015 (Robinson 1978; Molsher 2017). Unlike mainland populations, Kangaroo Island koalas have also maintained a low prevalence of communicable diseases. For example, neither targeted studies nor reanalyses of extensive historical data have found evidence of *Chlamydia pecorum* in this population (Patterson et al. 2015; Fabijan et al. 2019), suggesting that the pathogen was never introduced. While southern koalas generally show somewhat less severe symptoms than northern conspecifics, chlamydia remains widespread on the mainland (Fabijan et al. 2019), reinforcing the role of isolation in protecting the Kangaroo Island population. Koala retrovirus is also present at much lower prevalence on Kangaroo Island and is primarily found as the less pathogenic KoRV-B subtype (Simmons et al. 2012).

While current disease risks appear low, the population's long-term ability to respond to new or emerging threats could be limited. This vulnerability, coupled with the population's unique founding history, raises broader concerns about its genetic health and resilience.

The small founding population is hypothesised to have caused genomic erosion and inbreeding that could compromise long-term persistence. Several suspected genetic disorders, including testicular aplasia, skeletal abnormalities, and kidney disease associated with oxalate nephrosis, have been documented on Kangaroo Island (Tarlinton et al. 2021). Earlier work using microsatellites (Cristescu et al. 2009; Buchanan et al. 2022) and reduced representation single-nucleotide polymorphism (SNP) data (Kjeldsen et al. 2019; Tarlinton et al. 2021) and immune-related loci from whole-genome data (Silver et al. 2024) has consistently shown reduced genetic diversity in this population. Although explicit measures of fitness are lacking, this erosion of functional genetic diversity might have already lowered the population's adaptive capacity and individual fitness relative to more genetically diverse populations.

The main aim of our study is to assess the genomic consequences of conservation management in an island ark, using Kangaroo Island koalas as a case study. Through whole-genome sequencing, we aim to (i) establish the timing and severity of founder effects during the establishment of the Kangaroo Island population using demographic modelling based on genomic data; (ii) evaluate the effects of this bottleneck on genetic diversity, inbreeding, and potential adaptive consequences by comparing Kangaroo Island koalas to mainland populations, including analyses of standing genomic variation, runs of homozygosity, and genetic load; and (iii) discuss the implications of our findings in the broader context of managing isolated populations in conservation arks, including the potential need for genetic management such as genetic rescue to improve long-term viability. If the Kangaroo Island koala population has experienced severe founder effects and long-term isolation, then we expect it to exhibit reduced genetic diversity, increased inbreeding (evidenced by longer runs of homozygosity), and a higher homozygous genetic load compared to mainland populations. We also expect a sharp recent reduction in effective population size (N_e) if the demographic bottleneck has resulted from contemporary translocation to the island ark.

2 | Methods

2.1 | Study Design

To investigate the diversity and inbreeding in the koalas of Kangaroo Island in South Australia, we used a comparative approach based on genomic data from 103 individuals, encompassing Kangaroo Island and mainland populations from the states of Victoria and Queensland (Figure 1A; Table S1). We selected the mainland populations to represent southern (Victoria) and northern (Queensland) koala lineages (Lott et al. 2022; McLennan et al. 2024), providing perspective on genetic variation across different environments and population histories. Throughout the Methods, we use 'populations' to refer to Kangaroo Island, Victoria, and Queensland, and 'localities' to refer to specific sampling sites within those regions.

We sampled two localities on Kangaroo Island: Parndana (genomic data for 26 individuals sampled in 2021–2022) and Newland (genomic data for 48 individuals sampled in 2023) in accordance with Flinders University Animal Ethical Approval AEC BIOL5591-15. We and others captured animals using the flag-and-noose method (Madani et al. 2020) followed by chemical immobilisation, skin tissue biopsy, and clinical health assessments. The health assessments included body condition scoring, weight measurement, reproductive and musculoskeletal abnormality screenings, and tooth wear class determination for age estimation. We also did health assessments for an additional 61 individuals from Kangaroo Island captured during the same study period (health assessments for a total of 135 Kangaroo Island individuals).

The Victoria and Queensland data were from samples previously collected as part of the Koala Genome Survey (Hogg et al. 2023). We chose the Victoria localities due to a likely similar recent ancestry as Kangaroo Island koalas, indicated by population-structure analyses (McLennan et al. 2024). The chosen localities spanned a larger area than the other datasets, with a maximum distance between sites of approximately 430 km (compared to ~25 km in Kangaroo Island and ~15 km in Queensland). However, likely due to translocations during the past century, koala populations in Victoria do not conform well to isolation by distance, and all Victoria samples we included form part of a single genetic cluster (McLennan et al. 2024).

The Queensland dataset represents a northern koala lineage from the Moreton Bay region and is unlikely to be admixed with central or southern populations. We included Queensland to provide a genetically diverse reference population, offering context for interpreting genomic variation and demographic history. Its inclusion strengthens comparisons by allowing robust inferences about processes such as population bottlenecks and inbreeding patterns, particularly considering the suspected shared history between Kangaroo Island and Victoria, because historical processes affecting both populations could account for some of the genetic patterns observed on Kangaroo Island.

For the whole-genome data, we sequenced 20 Kangaroo Island individuals from Parndana, 17 Victorian, and 16 Queensland individuals at an average of $>30\times$ coverage depth as part of the Koala Genome Survey (Hogg et al. 2023). We extracted DNA from ear biopsy or whole-blood samples using either the MagAttract HMW DNA Kit (Qiagen, Hilden, Germany; cat: 67563) or a modified salting-out protocol (Aljanabi and Martinez 1997). We prepared libraries with the TruSeq DNA PCR-Free Kit (Illumina, San Diego, CA, USA), with 48 samples pooled per lane. We sequenced samples on an Illumina NovaSeq 6000 platform at the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, Australia). We aligned the resulting Fastq files to the koala reference genome (phaCin_unsw_v4.1 'Bilbo', Johnson et al. 2018) using the Dragen Platform (v3.8.4, Illumina, San Diego, CA, USA); we did not realign indels because it is not recommended for Dragen, but marked duplicates and excluded them for genotyping calculations; for more detail, see Hogg et al. (2023).

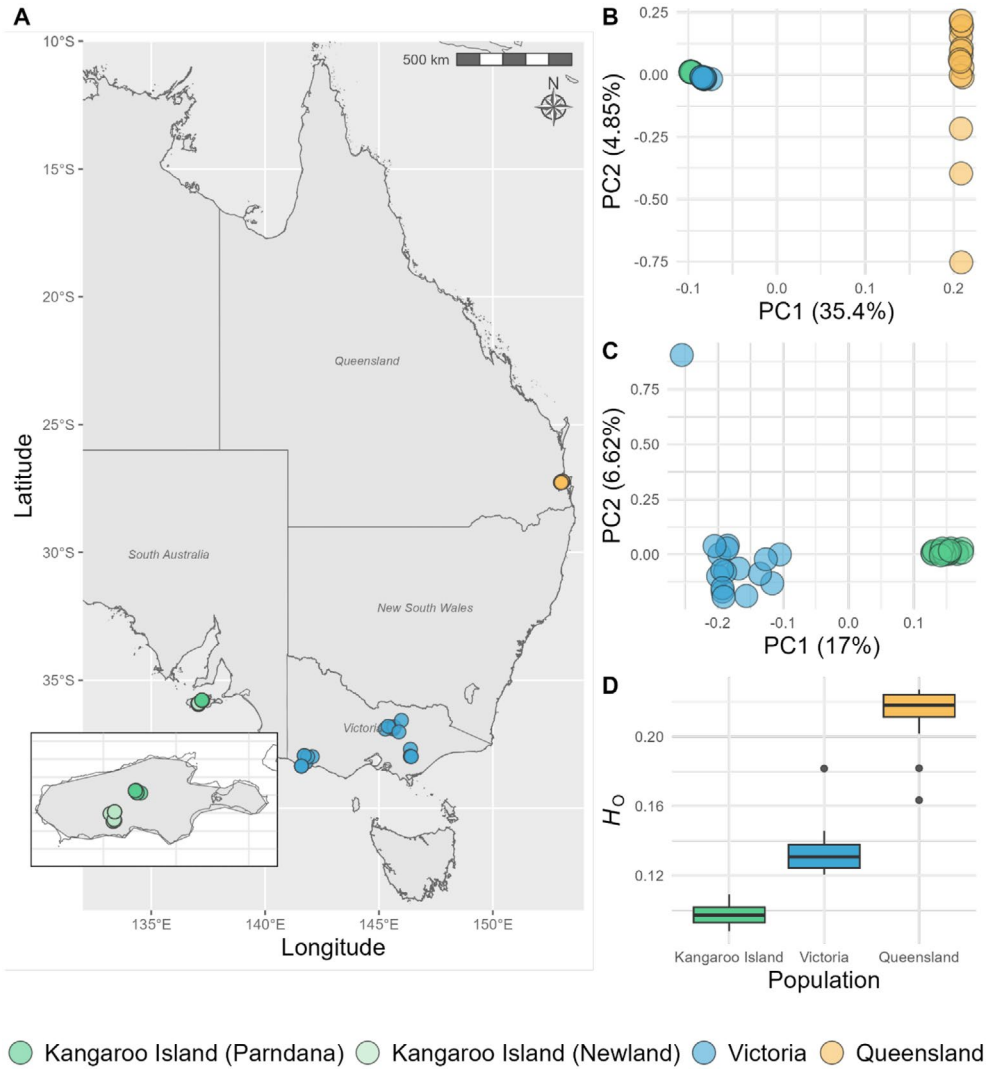

FIGURE 1 | Sampling locations of koalas for whole-genome sequencing from Kangaroo Island, Victoria, and Queensland, and their resulting genomic differentiation based on 3,483,166 single-nucleotide polymorphisms (SNPs). (A) sampling map (inset shows Kangaroo Island); (B) first two axes of variation in a principal components analysis using all individuals; (C) first two principal components axes using only individuals from southern populations (Kangaroo Island and Victoria); (D) Observed heterozygosity (H_O) based on SNPs, with boxplots showing the median (line), interquartile range (box), and whiskers extending to 1.5 × interquartile range, with points beyond this range plotted as outliers.

For DArTseq (diversity arrays technology sequencing; reduced representation, enzyme combination PstI and SphI), we sequenced all 74 individuals from Kangaroo Island localities (Parndana $n = 24$; Newland $n = 50$) to estimate pairwise relatedness for the conservation ark population.

2.2 | Preprocessing Genomic Datasets

For the whole-genome data, we merged all individual files into a single variant-call format (VCF) dataset using BCFTOOLS 1.9 + htslib-1.9 merge (Danecek et al. 2021). We chose the '—missing-to-ref' option to maximise dataset completeness, because per-sample missingness was extremely low (< 0.5%), and high average coverage (> 37.7× per sample) made it reasonable to assume that loci absent from individual VCFs were likely invariant rather than truly missing. We filtered high-quality biallelic SNPs for population-level analysis using VCFTOOLS 0.1.16 (Danecek et al. 2011), using a minor allele count > 2, mapping quality > 50, QUAL/DP > 0.05, average depth > 8, QUAL > 19, DP > 19, maximum average depth = 70. We filtered putatively sex-linked regions by calculating the proportion of heterozygous SNPs within sliding windows of 50 kb for each individual using SWhet. For each window, we averaged heterozygosity values across females and males separately and identified regions where female heterozygosity was at least twice that of males, with male heterozygosity thresholds set to account for potential sex misreporting or minor genotyping errors (≤ 0.017 for males, corresponding to a scenario with minimal heterozygosity in nearly all males). We removed variants meeting these criteria (e.g., female heterozygosity > 0.035 and male heterozygosity ≤ 0.017, or females > 0.008 and males = 0). We also filtered loci from any contigs (scaffolded sequences) < 50 kb because these regions are more likely to contain spurious calls. For all downstream applications except for those reliant on linkage disequilibrium, we used a further filtering step for linkage disequilibrium using PLINK 1.9 (Purcell et al. 2007) '--indep-pairwise', with a 5-kb window size, step size = 1, and pairwise R^2 threshold = 0.6.

For the DArTseq data, we trimmed 'high-coverage' sequencing raw data (SNPcallPipe) before aligning against the same reference genome using the Burrows-Wheeler Aligner (BWA) (Li and Durbin 2009). We then merged, deduplicated, and did local realignment around indels. We filtered SNPs using VCFtools to retain only biallelic sites present in at least 80% of individuals, with a minor allele frequency ≥ 0.01 and read depth between 2.5 and 93. We also removed sites with mapping quality < 30, replicability between technical replicates < 97%, and those deviating from Hardy–Weinberg equilibrium in two of the three populations ($p < 0.05$). For population structure analyses, we further thinned the data for linkage disequilibrium by retaining one SNP every 10,000 bp.

2.3 | Diversity and Population Structure

Using the whole-genome dataset, we used VCFTOOLS '--het' to calculate observed heterozygosity (H_O) per individual, and calculated the average for each population. To calculate expected heterozygosity (H_E) based on all called SNPs while obtaining independent values for each population, we first used PLINK 1.9 '--within' '--freq' to generate allele frequencies within populations using the combined dataset. From the within-population minor allele frequency (MAF) given for each SNP, we then calculated H_E as 2MAF(1—MAF), and took the average for each population. We calculated the inbreeding coefficient F_{IS} as $1—H_O/H_E$. Since heterozygosity estimates based only on variable sites can be biased (Schmidt et al. 2024), we also calculated observed autosomal heterozygosity (here abbreviated as H_{Oa}) using both variable and invariable sites. We used VCFTOOLS result of the number of heterozygous sites per individual, dividing this by the total number of genotyped autosomal sites (excluding missing data) in each gVCF.

We calculated the fixation index F_{ST} globally and among each pair of populations using PLINK 1.9 '--fst'. We plotted pairwise F_{ST} in R using the `ggplot2` library (Wickham 2016). We generated a principal components analysis summarising major axes of variation in allele frequencies across all individuals using PLINK 1.9 '--pca', also plotted with `ggplot2`.

2.4 | Demographic History

Using the whole-genome SNP data, we used the linkage disequilibrium method GONE (Santiago et al. 2020) to estimate changes in population size in the recent history of each lineage. Input data were those filtered for high-quality autosomal SNPs, but that we did not prune for linkage disequilibrium. For this analysis, we used a recombination rate of 0.3 cM/Mb (Rossi and Pigozzi 2025), set a maximum c of 0.05 as recommended by the authors (Santiago et al. 2020) and ran for 2000 generations. GONE estimates effective population size (N_e) at multiple time intervals, and its reliability is highest for recent demographic events within ~50–200 generations. Accordingly, we only report results for the last 200 generations, transforming generation estimates to years assuming a 6-year generation time (Phillips 2000). To assess variability within GONE estimates, we calculated the average and 95% confidence interval of 100 runs, each time subsampling ~17,000 SNPs for each of the 60 biggest scaffolds (due to software limitations), resulting in a total of > 1 million subsampled SNPs per run. To inform expectations about the software's performance under different potential demographic scenarios, we also ran GONE using simulated data. We created the simulated data using the R function *runMacs2* from the package `AlphaSimR` (Faux et al. 2016), a wrapper for the MaCS software (Markovian Coalescent Simulator; Chen et al. 2009). For each scenario, we simulated 20 individuals and 15 chromosomes with the default parameters. Simulations included constant population size, bottlenecks 50 or 150 generations ago, and population growth from 50 generations ago, enabling us to assess GONE's sensitivity to various demographic histories.

2.5 | Runs of Homozygosity

Runs of homozygosity are formed when both parents contribute the same segment of DNA to the offspring, leading to segments of the genome being identical by descent. This is most likely to occur if the parents share a common ancestor; therefore, the length and number of runs of homozygosity can provide insights into an individual's ancestry and population history. We used PLINK 1.9 '--homozyg' to assess long runs of homozygosity (> 1 Mb). The input dataset had all filters applied except minor allele frequency (because this could be manipulated by the option '--max hets'), and linkage disequilibrium, because this can bias detection, especially in inbred populations (Meyermans et al. 2020). We initially explored a range of parameters, including scanning window sizes (5, 10, 20, 30, 50, and 80 SNPs), maximum heterozygous SNPs allowed per window (1–6), minimum segment lengths (10 kb to 5000 kb), and minimum variant counts per segment (10 to 5000 SNPs). We selected a scanning window of 20 SNPs, allowing a maximum of 2 heterozygous SNPs per window, a minimum segment length of 1000 kb, and default SNP density parameters. This approach balances sensitivity and specificity for detecting long ROH while minimising false positives in short or ambiguous segments.

We then calculated F_{ROH}, the proportion of the autosomal genome contained within runs of homozygosity > 1 Mb, as a measure of individual inbreeding (McQuillan et al. 2008). We also estimated the number of generations since the origin of runs of homozygosity (time since inbreeding F_{ROH}) following Kardos et al. (2018) using the formula ($g = 100/2rL$) proposed by Thompson (2013), where g is the number of generations (or twice the number of meioses), r is the recombination rate in cM/Mb, and L is the length of the run in megabases (Mb). We binned runs of homozygosity into 1 Mb-length intervals (e.g., 1 – < 2 Mb, 2 – < 3 Mb, etc.). We considered a range of recombination rates from 0.3 cM/Mb (koala recombination rate) to 1.2 cM/Mb (maximum observed in mammals; Rossi and Pigozzi 2025). We converted the number of generations into years by assuming a generation time of 6 years (Phillips 2000).

2.6 | Genetic Load

Genetic load is classically defined as the reduction in mean fitness of a population due to the presence of deleterious genetic

variants. While direct measures of fitness are often unavailable, variant annotations can serve as proxies for potentially harmful mutations. To assess putative genetic load, we used SnpEff v5.2 (Cingolani et al. 2012) to annotate the effects of each SNP. We polarised alleles as ancestral or derived for each variant using the common wombat (*Vombatus ursinus*) genome, the closest extant relative of the koala. We downloaded reads from the wombat genomes (SRR8616867) and mapped them to the koala reference genome using the same pipeline described above. We call both variant and invariant sites, retaining genotypes with a minimum of 5× coverage, average quality > 20, and mapped quality > 20. We defined the ancestral allele as the allele shared between the bare-nosed wombat and the koala genomes, ignoring sites that were heterozygotes for the same two alleles in both genomes (we cannot determine which will be ancestral). This allowed us to polarise 8,070,258 variants with ancestral and derived alleles. We calculated three measures of genetic load for each individual: (i) total load (total proportion of putatively deleterious alleles), (ii) homozygous load (proportion in homozygosity, approximating realised load), and (iii) heterozygous load (proportion in heterozygosity, approximating masked load). To account for sample size differences and biases in detecting derived alleles, we normalised our derived deleterious allelic counts by dividing them by the count of derived synonymous alleles in each population.

To compare genetic load across the three koala populations, we calculated the Rxy ratio (Xu et al. 2015), which estimates the relative frequency of derived alleles between population pairs. For each functional category (LoF, Missense, and Synonymous) and each population pair (x, y), we estimated the derived allele frequency per site in the population (Freqsitex and Freqsitey), and per population ($\text{FreqPopx} = \sum \text{Freqpopx}(1 - \text{Freqpopy})$ and $\text{FreqPopx} = \sum \text{Freqpopy}(1 - \text{Freqpopx})$). Then we calculated the ratio $\text{Rxy} = \text{FreqPopx}/\text{FreqPopy}$, where values > 1 indicate a higher frequency of derived alleles in population x, values close to 1 indicate no differences, and values < 1 indicate a lower frequency of derived alleles in population x. We estimated the average and 95% confidence interval using 100 replicate subsets of 500 randomly selected sites per category, and considered Rxy different from 1 if the 95% confidence interval did not include 1.

2.7 | Relatedness

We analysed relatedness specifically for Kangaroo Island individuals to distinguish the influence of historical demographic events from contemporary kin dynamics on observed inbreeding. We based relatedness analyses on filtered DArTseq SNPs generated for all Kangaroo Island individuals, which provided a larger sample size ($n = 74$) than whole-genome sequencing and enabled robust evaluation of relatedness across the two Kangaroo Island localities (Parndana and Newland). We calculated pairwise kinship coefficients using PLINK 2.0 (Chang et al. 2015) '--make-king', which computes the KING (Kinship-based Inference for Genome-wide association studies) kinship coefficient (Manichaikul et al. 2010). This coefficient estimates the probability that a randomly chosen allele from one individual is identical by descent to an allele from another. Estimated kinship coefficient ranges correspond to specific relationship categories: > 0.354 for duplicates or monozygotic twins, 0.177–0.354 for first-degree relatives, 0.0884–0.177 for second-degree relatives, and 0.0442–0.0884 for third-degree relatives. While this kinship coefficient is typically measured from 0 (unrelated) to 0.5 (identical to oneself), the KING coefficient can be negative when individuals share fewer alleles than expected under random mating, and is also interpreted as unrelated. The method is well-suited to non-homogeneous population structures and varying sample sizes. We did not extend this analysis to mainland populations due to logistical constraints and because understanding relatedness on Kangaroo Island was most relevant to our study's focus on the drivers of inbreeding in an island ark.

3 | Results

3.1 | Diversity and Population Structure

The variant calling of the whole-genome sequencing data against the koala reference genome produced 33,538,007 polymorphic sites across all populations (Kangaroo Island, Victoria, Queensland). From this total, we retained 16,370,050 biallelic autosomal single nucleotide polymorphisms (SNPs) after stringent quality filtering (Table S2). Mean depth of coverage was 38.3; Kangaroo Island mean = 31.0 (29.5–32.2); Victoria mean = 29.1 (21.9–35.4); Queensland mean = 46.7 (29.3–62.5). Average coverage of the reference genome was > 99.81% (99.56%–99.9%). From this set, further filtering for linkage disequilibrium produced 3,483,166 putatively unlinked SNPs. The variant calling for the DArTseq data for the two Kangaroo Island localities produced 3266 putatively unlinked SNPs (Table S3).

Genetic diversity in the Kangaroo Island population was lower than in mainland populations. For the whole-genome sequencing dataset, only ~1.1 million SNPs were polymorphic within the Kangaroo Island population, in contrast to ~1.6 million in the closely related Victoria population, and nearly ~2.7 million in the Queensland population. This trend was also reflected in heterozygosity measures, with both the SNP-based observed (H_O) and expected heterozygosity (H_E) considerably lower in the Kangaroo Island compared to Victoria and Queensland populations (Figure 1d, Table S4). Autosomal heterozygosity (H_{Oa}) mirrored this pattern, but with lower absolute values (Figure S1, Table S4).

Analyses of population structure including principal component analyses (Figure 1b,c) and F_{ST} (Figure S2) revealed substantial genetic differentiation between the Queensland koalas and the southern populations (Kangaroo Island and Victoria). When we included all populations in the principal components analysis (Figure 1b), the main axis of variation (PC1; 35.4%) was between the northern and the two southern populations. The second axis corresponded to inter-individual variation within Queensland, overwhelming differences between southern populations in the principal components plot, further reflecting the higher diversity in the Queensland population. When we assessed southern populations separately (Figure 1c), Kangaroo Island and Victoria separated

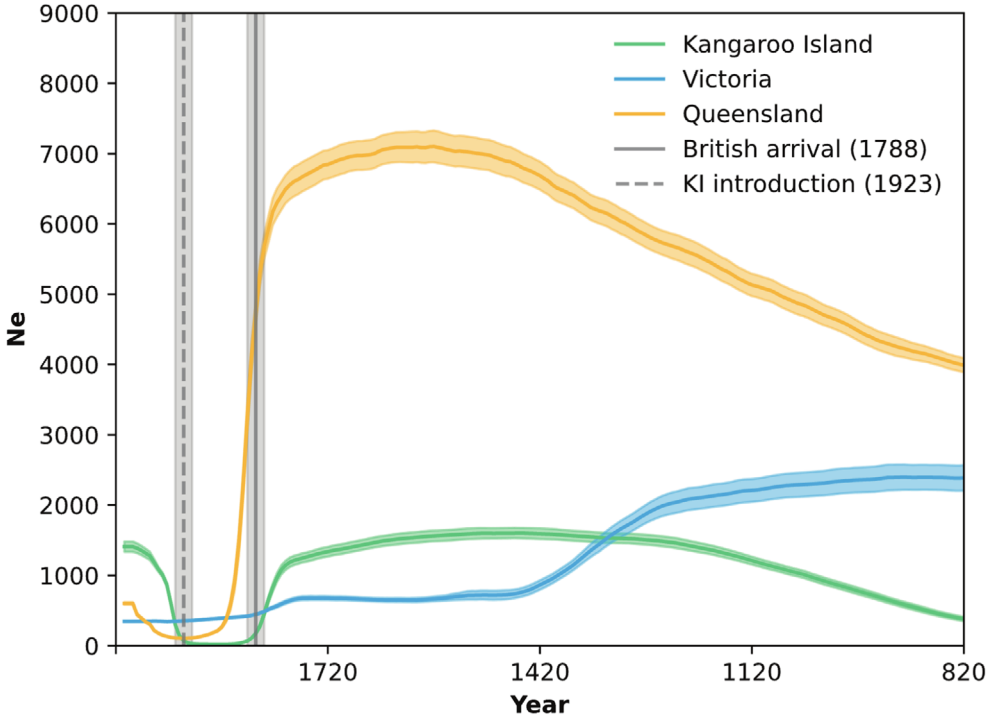

FIGURE 2 | Recent fluctuations in effective population size (N_e) based on GONE for three koala populations: Kangaroo Island (KI; $n = 20$), Victoria ($n = 18$), and Queensland ($n = 17$). Dark lines indicate the average N_e across 100 replicates, each subsampling approximately 1,020,000 SNPs derived from whole genomes (recombination rate = 0.3 *c*M/Mb). Shaded areas represent 95% confidence intervals. Vertical solid line indicates the approximate beginning of the British 'colonial period' in Australia (~33 generations ago), while the vertical dashed line indicates the approximate timing of koala translocations to Kangaroo Island (~13 generations).

into distinct clusters, with greater inter-individual variation in Victoria.

3.2 | Recent Demographic History

GONE estimates of effective population size (N_e) inferred declines in all three populations from around 45 generations ago, or within the last 275 years given a generation time of 6 years (coinciding closely with the beginning of the British colonial period; Figure 2). We inferred the smallest recent N_e for the Kangaroo Island population ($N_e = 21$) between 22 and 26 generations ago (~1869–1893). However, this was also the population demonstrating the most obvious recovery of N_e, with the most rapid increase indicated from ~13 generations ago (or from approximately mid-1920s, coinciding with their introduction to Kangaroo Island). Results indicated that historically, both the Kangaroo Island and Victoria populations maintained relatively low, long-term N_e compared to the Queensland population. In contrast, the Queensland population exhibited a larger beginning population, but also a much larger relative decline, with N_e decreasing from ~7000 individuals approximately 45 generations ago to < 100 individuals by around 30 generations ago, followed by a slight increase. Despite GONE's ability to detect bottlenecks, there was variability in the inferred timing of demographic changes depending on parameter settings. When we ran GONE using the simulated coalescence data (Figure S3), GONE's estimated timing of bottleneck events was approximately 50 generations earlier than simulated.

3.3 | Inbreeding and Relatedness

To investigate the history of consanguineous (close-relative) mating, we assessed long runs of homozygosity (> 1 Mb) in each population. In Kangaroo Island koalas, we detected between 557 and 695 runs of homozygosity per genome, averaging almost twice the total length of coverage of any other population (mean sum of segment lengths = 1202 ± 84 Mb; mean genomic inbreeding $F_{ROH} = 0.387$; Figure 3a). In comparison, we detected 236-507 runs of homozygosity in Victoria genomes (mean sum of lengths = 625 ± 168 Mb; mean $F_{ROH} = 0.201$), and 98–382 runs in Queensland genomes (mean sum of lengths = 336 ± 165 Mb; mean $F_{ROH} = 0.108$; Figure 3a).

Across all ROH length intervals, Kangaroo Island individuals exhibited the highest F_{ROH} (range: 0.1891 for runs ≥ 1 Mb to 0.0002 for runs ≥ 11 Mb; Figure 3b, Figure S4). While most F_{ROH} was contributed by shorter segments in all populations (coalescence year potentially predating 1877, Figure S4), runs ≥ 7 Mb were also present, which we inferred to coalesce between 1877 and 1995. Victoria samples had lower F_{ROH} across the same run size classes (range: 0.1386 for runs ≥ 1 Mb to 0.0003 for runs ≥ 8 Mb), while Queensland had the lowest F_{ROH} for runs ≥ 1 and ≥ 2 Mb, but higher F_{ROH} than Victoria in run categories ≥ 5 Mb.

We assessed pairwise relatedness for Kangaroo Island localities based on the DArTseq dataset using the KING kinship coefficient. Across the entire sample set, we found a mean pairwise kinship of −0.015 ± 0.051. Within localities, we also found negative mean pairwise kinship values for both sites (Parndana: −0.013 ± −0.049;

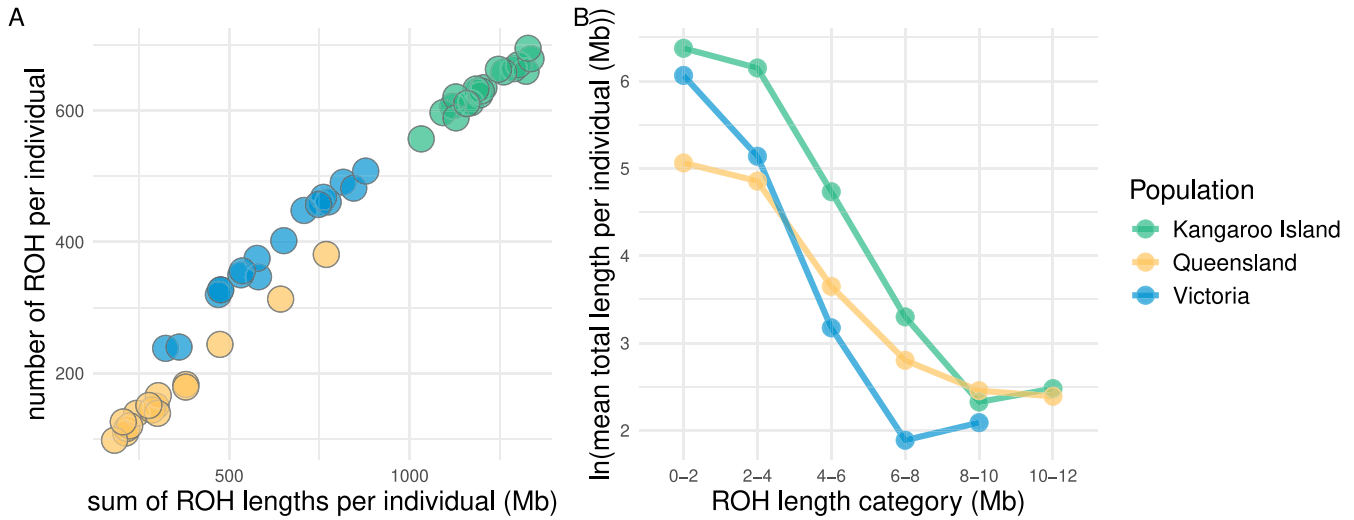

FIGURE 3 | Long runs of homozygosity in koalas. (A) Number of runs > 1 Mb per individual. (B) Total length of runs per length category. The *y*-axis shows the $\log_e$ of the mean summed lengths per individual. ROH = runs of homozygosity.

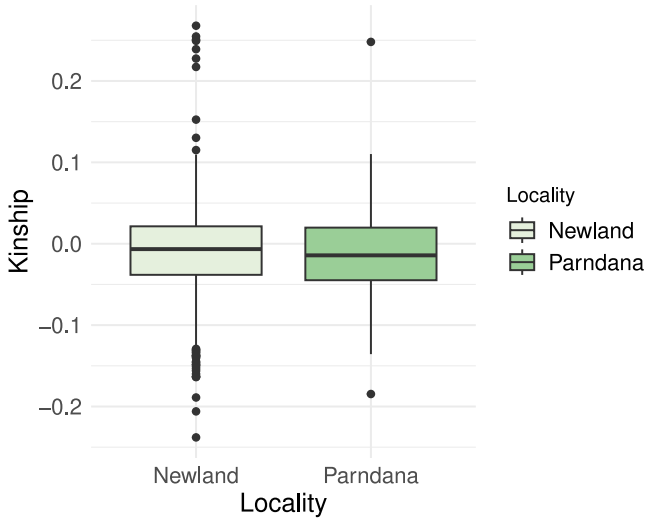

FIGURE 4 | Relatedness within two Kangaroo Island localities using KING kinship coefficient based on 3266 DArTseq single nucleotide polymorphisms. The boxplot shows the median (line), interquartile range (box), and whiskers extending to the largest and smallest values within 1.5× interquartile range, with points beyond this range plotted as outliers.

Newland: = −0.010 ± 0.053; Figure 4), indicating overall low pairwise relatedness among animals sampled in proximity. Within localities, we identified 8 pairs with equivalent kinship of first-degree relatives (> 0.177), 7 of which were in Newland (Figure 4) that had the larger sample ($n = 48$ individuals). Pairwise comparisons between localities produced similar kinship values (−0.020 ± 0.050) and revealed no first-degree relatives at this distance (~25 km).

3.4 | Genetic Load

We compared three measures of genetic load (total, homozygous, and heterozygous) between the Kangaroo Island and Victorian koala populations. Homozygous load was higher in Kangaroo Island (mean = 0.2206) than in Victoria (0.2017; $p < 0.0001$), and heterozygous load was also lower in Kangaroo Island (0.0507 vs. 0.0673; $p < 0.0001$). However, there was no statistical evidence for a difference in total load (Kangaroo Island = 0.2713; Victoria = 0.2690; $p = 0.4149$).

Normalising derived deleterious allele counts by synonymous counts revealed similar patterns to raw estimates, although magnitudes differed (Figure 5a–f). Kangaroo Island had the highest load for both LoF (0.0215) and missense alleles (0.4926), but these were not different from Victoria (LoF = 0.0213; missense = 0.4905). Both populations had a higher load than Queensland (LoF = 0.0195; missense = 0.3759; $p < 0.0001$). Missense load was an order of magnitude higher than LoF, suggesting stronger purifying selection or more efficient purging at LoF loci. Total load patterns were mainly driven by homozygous load. In contrast, heterozygous load normalised by synonymous counts showed a different trend. Although raw estimates suggested a minor contribution of heterozygous load, the normalised values were higher than the corresponding homozygous loads, likely reflecting reduced diversity rather than methodological bias.

Queensland had the highest raw heterozygous load (0.1057), followed by Victoria (0.0673) and Kangaroo Island (0.0507; all $p < 0.0001$). After normalisation, these patterns reversed: Kangaroo Island had higher LoF heterozygous load (0.0267) than Queensland (0.0238), and Victoria was higher than Queensland for missense (0.6365 vs. 0.5665). These results suggest that while Queensland retains a higher masked (heterozygous) load, Victoria and Kangaroo Island have lost this potential load along with overall heterozygosity. The elevated total load in the latter two populations likely reflects conversion of heterozygous to homozygous load due to inbreeding and drift.

Kangaroo Island and Victoria had a higher load compared to Queensland based on relative measures (Figure 5g; Kangaroo Island LoF: 1.26–1.33; missense: 1.29–1.89; Victoria LoF: 1.23–1.30; missense: 1.30–1.83, all 95% confidence intervals). Rxy also indicated marginally higher LoF load in Kangaroo Island compared to Victoria (1.004–1.06). We detected no differences in derived synonymous allele counts, suggesting either

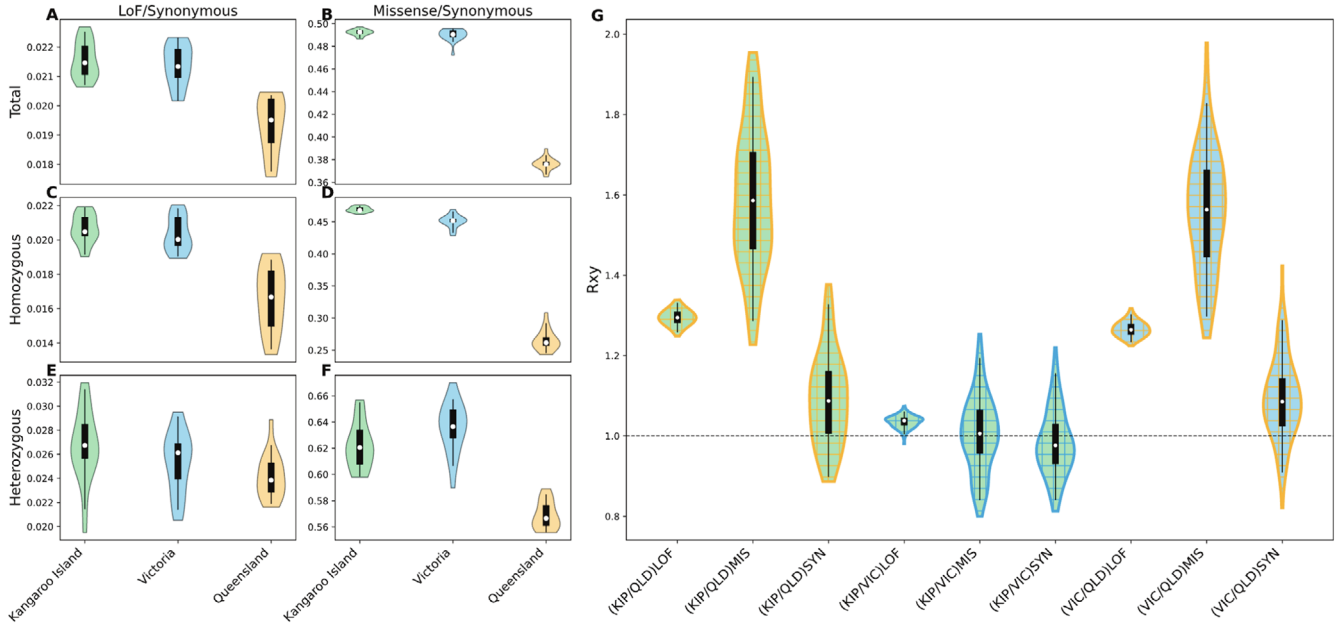

FIGURE 5 | Genetic load in three populations of koalas. (A–B) Total load of deleterious variants. (C–D) Homozygous load of deleterious variants. (E–F) Heterozygous load of deleterious variants. (A, C, E) Load estimate from loss of function (LoF) variants. (B, D, F) Load estimated from missense variants. (A–F) Load normalised by synonymous allele counts. (G) Relative frequency of derived alleles between pairs of populations for three functional categories of variants, loss of function (LoF), missense (MIS), and synonymous (SYN). Dashed line indicates Rxy when allele frequencies are not different between populations. Rxy above the line indicates a higher frequency of the derived allele in population *x*, while Rxy below the line indicates a lower frequency of the derived allele in population *x*. Where population *x*/*y* are KIP/QLD = Kangaroo Island/Queensland, KIP/VIC = Kangaroo Island/Victoria, VIC/QLD = Victoria/Queensland. Differences are indicated when the 95% confidence intervals do not include 1. (A–G) Violin plots show the distribution of the data, with the median (white dot), interquartile range (box), and 95% confidence interval (whiskers).

purging in Queensland or accumulation in southern populations. Higher Rxy for missense than LoF alleles supports stronger purifying selection at LoF loci.

3.5 | Population Health

We clinically assessed 135 koalas (57 males; 78 females) captured on Kangaroo Island between 2021 and 2023 to characterise indicators of health and fitness in this population. This included all 74 individuals we used for genomic analyses (Table S6). Males weighed 10.36 ± 2.52 kg, and females 7.48 ± 1.81 kg. Most individuals were young to mid-aged adults, with fewer sub-adults (3 males, 9 females) and older adults (2 males, 4 females). Body condition rankings ranged from 'emaciated' to 'excellent', with most males ($n = 27$) and females ($n = 47$) classified as being in 'good' condition (modal category for both sexes). We observed reproductive abnormalities in 7 males (testicular hypoplasia or aplasia) and 1 female (pouch hypoplasia). We recorded musculoskeletal abnormalities in three males and one female, including jaw malformations and one case of congenital pedal aplasia in a male. In addition, we sighted two other uncaptured males with limb deficiencies during the study.

4 | Discussion

Our findings highlight the complex role of islands as conservation arks, reinforcing their potential and limitations in maintaining viable populations. While islands offer refuges for species threatened on the mainland, they also pose risks to genetic diversity and long-term resilience. Our analyses revealed lower genomic diversity in Kangaroo Island koalas compared to mainland populations, with more extensive runs of homozygosity and a higher proportion of realised genetic load. These results indicate that Kangaroo Island koalas have experienced a severe bottleneck and continue to face the risk of inbreeding depression. The reduction in genetic diversity and increased inbreeding in Kangaroo Island (Figure 3; Table S4) reflect the well-established effects of genetic drift and founder effects that occur in isolated populations (Frankham 1998; Groombridge et al. 2018). The sharp reduction in effective population size (Figure 2) is also consistent with founder events and subsequent genetic drift. Although Kangaroo Island koalas are currently abundant, they originate from a small founding group translocated from another similarly constrained population, indicating a severe genetic bottleneck that likely still impacts their genomic health. Indeed, isolated populations, especially those founded by few individuals, tend to experience long-term reductions in standing genetic variation (Mathur et al. 2023).

4.1 | Contemporary Population Declines and Loss of Diversity

The limited dispersal opportunities and lack of genetic inflow to Kangaroo Island koalas have likely contributed to genetic drift from their mainland relatives, indicated in all analyses of population structure and differentiation (Figures 1B,C and

S2). Although the contrast with Queensland is the most pronounced, this could have arisen because of longer-term demographic differences between the populations, also supported by our historical N_e analysis. However, given that the Victoria and Kangaroo Island populations likely shared common ancestry as recently as 100 years ago (Robinson 1978; Warneke 1978), it is plausible that successive founder effects are directly responsible for the difference in genetic diversity. Furthermore, the observed reduction in diversity in Kangaroo Island compared to Victoria (Table S4) might underestimate the extent of the genomic erosion, given that the Victorian population probably also experienced some loss of diversity during this period (Kjeldsen et al. 2019; Tarlinton et al. 2021).

The accumulation of longer runs of homozygosity in Kangaroo Island koalas, along with consistently higher individual values of F_{ROH}, suggests that consanguineous mating has been more prevalent there than in the other two populations, increasing their potential for inbreeding depression through the fixation of deleterious alleles (Spencer et al. 2017). Longer runs of homozygosity can also indicate more recent inbreeding (Meyermans et al. 2020; Schmidt et al. 2024). The greater presence of long runs of homozygosity in Kangaroo Island compared to Victoria ($\geq$ 7 Mb; coalescence inferred between 1877 and 1995) suggests recent inbreeding has been more severe in Kangaroo Island than its source population, consistent with a founder effect during island translocations. Interestingly, the high F_{ROH} in short runs of homozygosity classes (1–2 Mb; coalescence inferred between 1020 and 1870) in both Kangaroo Island and Victorian koalas also suggests relatively small historical N_e in these populations (predating European arrival) compared to that of Queensland. In contrast, Queensland had the lowest F_{ROH} in short run classes, yet had higher F_{ROH} than Victoria for runs $\geq$ 5 Mb. This pattern might be symptomatic of a relatively recent population decline in Queensland, which aligns with their current EPBC classification as endangered.

These genomic patterns of inbreeding are broadly consistent with demographic reconstructions from GONE, which inferred the strongest bottleneck in Kangaroo Island. We found evidence for a precipitous decrease in *Ne* on Kangaroo Island beginning around 40 generations ago (Figure 2). Assuming a generation time of 6 years, this could indicate population declines beginning prior to translocation and as early as the late 1700s, coinciding with European invasion. This is consistent with the near-extirpation of southern koala populations during that period due to hunting, habitat loss, and disease introduction, which ultimately prompted the earliest conservation translocations (Robinson 1978; Warneke 1978; Kirkwood and Johnston 2006). However, it is also relevant to note that our GONE runs using simulated bottlenecks consistently overestimated the number of generations since bottleneck events (Figure S3), and it is plausible that major declines began more recently than we have inferred. It is also important to note that non-random mating can downwardly bias more recent estimates, which we account for using the recommended *c* value but could still have some effect. Despite these factors, it is likely that subsequent demographic recovery did not begin until after translocation to Kangaroo Island in the 1920s. The strong signal of population growth is consistent with reports of rapid expansion observed on the island by the 1940s (Robinson 1978; Whisson and Carlyon 2010).

As noted earlier, the elevated F_{ROH} in short homozygosity tracts in Kangaroo Island and Victoria suggests historically low effective population sizes relative to Queensland, a pattern also supported by GONE analyses. Prior to the recent bottleneck, both Kangaroo Island and Victoria populations maintained stable but low N_e compared to Queensland. These long-term patterns are consistent with early reports of koala rarity in southern regions at the time of European invasion (Warneke 1978). A higher historical N_e in Queensland could partially explain why genetic diversity is so much higher there than in southern populations in general. However, there are few data on population densities in Queensland during the early colonial period (Gordon and McGreevy 1978), making it difficult to align expectations based on demographic data. Broadly consistent with our findings, De Cahsan et al. (2025) also inferred declines in effective population size across multiple regions in the last 200–500 years, although their study did not include South Australian samples. The strong signal of recovery we observed on Kangaroo Island contrasts with the more limited genomic rebound De Cahsan et al. (2025) reported for other southern populations.

Despite these results, the low pairwise relatedness in koalas sampled from both localities of Kangaroo Island (Figure 4) is consistent with a lack of extensive familial clustering. If recent consanguineous mating were common (e.g., due to restricted dispersal), we would expect a skewed distribution with higher individual outliers. In contrast, if elevated homozygosity is mainly the result of historical founder effects and drift, we expect more uniformly elevated inbreeding coefficients but low variance in pairwise kinship as seen in our results. While this does not imply the absence of relatedness, it establishes that sampled individuals do not show excess relatedness relative to the current population baseline, and suggests that inbreeding might be more likely a consequence of bottlenecks during or prior to the population's establishment, rather than more recent kin dynamics on the island.

4.2 | Implications for Conservation and Population Viability

Koalas across Australia are threatened by habitat loss and degradation, disease, and climate change (Beyer et al. 2018). The Kangaroo Island population, although nearly disease-free and abundant compared to most other populations across the species' range, is not expected to be immune to these challenges. Southern koala populations were nearly driven extinct by hunting and habitat destruction in the past (Warneke 1978); subsequent translocations, including the establishment of the Kangaroo Island population, were an important aspect of demographic recovery even though this came at a cost to genetic diversity.

In small or bottlenecked populations, inbreeding and drift exacerbate genetic load because harmful alleles are more likely to become homozygous and expressed (Bertorelle et al. 2022; Dussex et al. 2023). This genetic load arises through at least two mechanisms: inbreeding load refers to the expression of recessive deleterious alleles due to increased homozygosity, while drift load results from the fixation of mildly deleterious variants due to genetic drift (Charlesworth and Willis 2009). These

processes often co-occur in small populations and may be difficult to disentangle without direct measures of fitness, but both can contribute to reduced evolutionary potential. An important caveat is that, although genetic load can be inferred from genome sequences, it remains unclear how well such predictions reflect actual fitness effects in the absence of empirical validation (Kardos et al. 2024), and very few studies have so far tested correlations between genetic load proxies and individual fitness (though see emerging work by Robledo-Ruiz et al. 2025).

Our results showed that Kangaroo Island koalas had significantly higher homozygous load than Victoria and Queensland (Figure 5), indicating that deleterious variants are more often present in homozygous form and potentially contributing to reduced fitness. This shift is consistent with the unmasking of deleterious alleles following increased homozygosity caused by inbreeding. At the same time, Kangaroo Island had lower raw heterozygous load than both Victoria and Queensland, reinforcing the idea that much of the potential (heterozygous) genetic load has been converted to realised (homozygous) load. Despite these shifts in load composition, total load did not significantly differ between Kangaroo Island and Victoria, suggesting that purifying selection has not effectively reduced harmful alleles, likely because genetic drift has overwhelmed selection during and after the founder event. Slightly higher Rxy values in Kangaroo Island than in Victoria further suggest that some harmful variation has failed to be purged since the founder event.

Whether purging of load occurs during a genetic bottleneck depends on various factors, including the severity and length of the bottleneck, the size of the founding population, and the strength of selection (Dussex et al. 2023). Our functional categorisation of genetic load provides further insight into the action of selection in these populations. Missense load was substantially higher than LoF load across all populations, suggesting that purifying selection has been more effective at removing highly deleterious mutations, while mildly deleterious missense variants have persisted. This pattern is consistent with expectations under strong drift, where selection is less able to act efficiently on alleles of small effect. Rxy analyses supported this interpretation, with Kangaroo Island and Victoria showing elevated frequencies of derived deleterious alleles compared to Queensland, especially at missense sites.

High homozygous load in Kangaroo Island koalas is consistent with long-term low effective population size (N_e), as indicated by the analyses of ROHs and GONE. A historically small N_e may have already purged strongly deleterious variants before the recent bottleneck, resulting in reduced masked load and, therefore, a lower potential for masked-to-realised load conversion (van Oosterhout et al. 2022). This is supported by simulations and by analogous findings in whooping cranes, where individuals from historically small populations showed higher realised than masked load (Fontsere et al. 2024). While purging may reduce the burden of highly deleterious alleles, it often comes at the cost of functional diversity and adaptive potential, potentially heightening disease vulnerability (Femerling et al. 2023). In benign environments like Kangaroo Island, selection may be relaxed, allowing individuals with higher realised load to persist while hard selection thresholds are not crossed. Simulations by Dussex et al. (2023) further indicate that in pre-bottleneck populations, masked load tends to exceed realised load, but this relationship can reverse following severe bottlenecks.

While Kangaroo Island supports some of the highest koala densities in Australia (Molsher 2017), the prevalence of genetic load raises concerns about the population's long-term health and adaptability, particularly in the face of environmental change or disease introduction, such as chlamydia or koala retrovirus, which have severely impacted northern populations (Woinarski and Burbidge 2020). Although Kangaroo Island has historically provided an ideal refuge, this status is not guaranteed. Recent bushfires, the ongoing clearing of blue gum plantations, and biosecurity risks all underscore future threats. As previously noted, chlamydia has not been detected on the island, reinforcing the protective role of historical isolation. However, reduced genetic diversity may compromise the population's ability to respond to emerging challenges, including novel pathogens and climate-driven habitat change.

There is also a clear precedent for genetic disorders in southern koalas, particularly in Kangaroo Island individuals. Southern populations are known to exhibit elevated rates of testicular aplasia, skeletal malformations, and renal disease, likely associated with inbreeding and genetic erosion (Cristescu et al. 2009; Tarlinton et al. 2021; Buchanan et al. 2022). Our clinical assessments of 135 Kangaroo Island koalas detected multiple cases of reproductive abnormalities (e.g., testicular aplasia and hypoplasia, pouch hypoplasia) and musculoskeletal defects (e.g., jaw malformations, pedal aplasia), consistent with these previous reports (Table S6). These physiological abnormalities occurred despite high population abundance and generally good body condition, suggesting that they reflect a genetic burden. Unfortunately, we have insufficient overlap between whole-genome sequencing and clinical record data to test this assumption, so targeted functional genomic studies will be required to identify genomic regions associated with deleterious phenotypes and to inform potential genetic management interventions.

4.3 | Genomic Data to Guide Conservation Management

Whole-genome data can reveal signatures of inbreeding, load, and demographic history that are otherwise difficult to quantify, and can inform management strategies such as translocations, reintroductions, or genetic rescue (Kardos et al. 2021; Dussex et al. 2023; Hogg 2024). Recent studies using whole-genome sequencing have shown varied outcomes of inbreeding and genetic load in endangered species. For instance, inbreeding depression has been linked to fitness declines in Indian tigers (Khan et al. 2021), orcas (Kardos et al. 2023), and northern elephant seals (Hoelzel et al. 2024), where homozygous load or ROH burden correlated with reduced survival or performance. Meanwhile, studies on vaquitas (Robinson et al. 2022) and the Iberian lynx (Kleinman-Ruiz et al. 2022) have found genomic signatures of load purging following prolonged bottlenecks, with authors suggesting potential for recovery with minimal genetic supplementation. However, the tiger, orca, and elephant seal studies also reported some evidence of purging, indicating that inbreeding depression can occur despite partial purging.

While direct links between genotypes and fitness have yet to be confirmed for Kangaroo Island koalas, our findings are broadly consistent with this pattern, highlighting that genetic rescue could be warranted despite demographic abundance.

Rapid (and complementary) advances in computational power, sequencing technology, and genetically explicit, forward-projection software (e.g., SLiM; Haller and Messer 2023) are facilitating the development and application of predictive models for simulating competing scenarios of genetic rescue (Jackson et al. 2022; van Oosterhout et al. 2022; Cavill et al. 2024; Beaman et al. 2025). Simulation modelling has immense potential to improve the careful planning and implementation of genetic rescue and can help avoid pitfalls while maximising the benefits of increased genetic diversity. Selecting the source population for genetic rescue is beyond our aim here, but the decision to attempt genetic rescue is being considered as part of a larger programme measuring neutral and functional genetic diversity, mapping demographic densities, and developing advanced spatial models to identify the source population(s) for genetic rescue of South Australian koalas and to predict its potential effectiveness (e.g., Beaman et al. 2025).

While a conventional definition of genetic rescue might involve increasing population growth rates via gene flow, more recent literature recognises that rescue can be motivated by the need to reduce inbreeding, improve adaptive potential, or pre-empt risks before demographic collapse occurs (Ralls et al. 2020). This is particularly important in isolated populations where inaction could result in long-term erosion of evolutionary potential. While Kangaroo Island koalas are abundant, they have limited genetic diversity and high homozygous load, which could reduce resilience to future threats such as disease emergence, habitat change, or further environmental disturbance. While concerns about introducing maladaptive or deleterious alleles through genetic rescue are valid, such risks are predictable (Frankham et al. 2011) and generally outweighed by the benefits of increased genetic diversity in isolated populations (Ralls et al. 2018).

Similar patterns could be expected in other species with small founding populations, especially because it is unusual for translocated populations to increase as rapidly as Kangaroo Island koalas have. This suggests that generally, natural processes alone are unlikely to safeguard genetic health in isolated conservation arks (Bertorelle et al. 2022). Our genetic load estimates could even be slightly conservative because our variant-merging approach assumed that sites absent from individual files were identical to the reference. While this is a reasonable assumption given our low missing data, it might slightly under-report deleterious variants by masking rare heterozygous or derived alleles. Our findings could therefore represent a lower bound on the true burden of deleterious variants.

In addition to genetic rescue, ongoing genomic monitoring is required in conservation arks to track changes in inbreeding, genetic diversity, recruitment, and adaptive potential over time (e.g., Marshall et al. 2022). Monitoring metrics such as heterozygosity, inbreeding coefficients, and increasing runs of homozygosity could help identify when interventions are needed and provide data to guide adaptive management decisions, especially as climate change and habitat degradation progress. Integrating genomic data with health and reproductive assessments will provide a more complete understanding of the population's status, enabling more informed and dynamic management.

4.4 | Conclusion

Our study highlights the genomic challenges of conservation in island arks. While these reserves can reduce the probability of immediate demographic decline, they also risk becoming genetic traps if isolation persists without proper genetic management (Frankham 1998; Spencer et al. 2017). The koalas on Kangaroo Island exemplify this paradox because their large population size masks underlying genomic risks stemming from founder effects, inbreeding, and a loss of adaptive potential. To safeguard Kangaroo Island's future as a conservation ark for koalas, genetic management strategies such as genetic rescue and ongoing genomic monitoring are warranted.

Author Contributions

L.B.B. conceived and supervised the study with assistance from C.J.A.B. K.B.S., J.E.B. and K.G. obtained samples. K.G. and J.S.-C. performed data analysis. K.G., J.S.-C. and L.B.B. interpreted results with assistance from co-authors. L.B.B, C.J.A.B., K.B.S, C.J.H. and K.B. contributed resources. K.G. wrote the paper. Co-authors revised the manuscript and approved its final version.

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Disclosure

Benefit-Sharing: Benefits from this research accrue from the sharing of our data and results on public databases as described above.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The whole genome and DArTseq datasets are available in Figshare: https://figshare.com/s/fbe2e2c42f6d77769086.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** mec70097-sup-0001-Supinfo.docx.