

Hybridisation rates, population structure, and dispersal of sambar deer (*Cervus unicolor*) and rusa deer (*Cervus timorensis*) in south-eastern Australia

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ABSTRACT

Context. Introduced populations of sambar deer (*Cervus unicolor*) and rusa deer (*Cervus timorensis*) are present across south-eastern Australia and are subject to local population control to alleviate their negative impacts. For management to be effective, identification of dispersal capability and management units is necessary. These species also readily hybridise, so additional investigation of hybridisation rates across their distributions is necessary to understand the interactions between the two species. **Aims.** Measure the hybridisation rate of sambar and rusa deer, assess broad-scale population structure present within both species and identify distinct management units for future population control, and measure the likely dispersal capability of both species. **Methods.** In total, 198 sambar deer, 189 rusa deer, and three suspected hybrid samples were collected across Victoria and New South Wales (NSW). After sequencing and filtering, 14 099 polymorphic single-nucleotide polymorphism (SNP) markers were retained for analysis. Hybridisation rates were assessed before the data were split by species to identify population structure, diversity indices, and dispersal distances. **Key results.** Across the entire dataset, 17 hybrids were detected. Broad-scale population structure was evident in sambar deer, but not among the sites where rusa deer were sampled. Analysis of dispersal ability showed that a majority of deer movement occurred within 20 km in both species, suggesting limited dispersal. **Conclusions.** Distinct management units of sambar deer can be identified from the dataset, allowing independent population control. Although broad-scale population structure was not evident in the rusa deer populations, dispersal limits identified suggest that rusa deer sites sampled in this study could be managed separately. Sambar × rusa deer hybrids are present in both Victoria and NSW and can be difficult to detect on the basis of morphology alone. **Implications.** Genetic analysis can identify broad-scale management units necessary for population control, and estimates of dispersal capability can assist in delineating management units where broad-scale population structure may not be apparent. The negative impacts associated with hybridisation require further investigation to determine whether removal of hybrids should be considered a priority management aim.

Keywords: connectivity, dispersal, hybridisation, invasive species, management units, non-native deer, pest species, wildlife management.

Introduction

The global desirability of deer as a hunting resource has prompted both historical introductions outside of their native range and contemporary anthropogenic movements of deer across landscapes (Dolman and Wäber 2008). As the abundance and distribution of non-native deer populations increase, their negative environmental, economic, and social impacts also increase (Davis *et al.* 2016; Honda *et al.* 2018; Cripps *et al.* 2019;

Hampton and Davis 2020). In Australia, deer were first released in the 19th century, predominantly by Acclimatisation Societies, with some of these introductions establishing self-sustaining wild populations (Bentley 1998; Moriarty 2004). Today, the following six deer species are present in Australia: sambar deer (*Cervus unicolor*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*), rusa deer (*Cervus timorensis*), chital deer (*Axis axis*), and hog deer (*Axis porcinus*). Deer populations have not been considered high priorities for control in Australia until the past decade (Bomford and Hart 2002; Davis et al. 2016; Forsyth et al. 2017). Now, most States and Territories list deer as a pest species (Davis et al. 2016), with localised management undertaken for all six deer species to reduce their abundances and limit range expansions. Despite these efforts, the abundance and distribution of many of these species and their undesirable impacts continue to increase (Bennett and Coulson 2010; Davis et al. 2010; Forsyth et al. 2015; Roberts et al. 2015; Forsyth et al. 2016).

Sambar deer are one of the most successful deer introductions to Australia (Bentley 1978; Long 2003). Introduced by the Victorian Acclimatisation Society to several sites in Victoria during the 1860s and 1870s, the population has expanded to occupy ~29% of Victoria's land area (Forsyth et al. 2015), with the population continuing to expand further north into New South Wales (NSW; Crittle and Millyn 2020). Isolated populations of sambar deer are also present in western Victoria and the Northern Territory (Moriarty 2004; Davies et al. 2022). The closely related rusa deer was also introduced to Australia by Acclimatisation Societies in the 19th century, but has a much smaller range than does sambar deer. Rusa deer were released in Victoria, NSW, Torres Strait, and Western Australia (WA); however, the WA population is thought to have failed to establish, and the population in Victoria was extirpated in the 1940s (Long 2003). Self-sustaining populations of rusa deer have also established in Queensland, likely from deer farm escapees and releases (Dryden 2000; Bengsen et al. 2022a). The current distribution of rusa deer in NSW appears to be patchy; however, large populations are present in Wollongong, Forster, and Port Macquarie (Fig. 1). The expansion of distributions for both sambar and rusa deer appear to have been additionally aided by translocations of animals to new sites, and releases or escapes from deer farms during the 1990s (Moriarty 2004). Both species are farmed in Australia; however, sambar and rusa deer farms comprise only a small proportion of all deer farming in the country (Shapiro 2010).

The abundance and distribution of sambar deer and rusa deer are increasing across south-eastern Australia. In Victoria, annual recreational harvesting of sambar deer has escalated over the past decade, with an estimated 131 258 sambar deer being harvested in 2019 alone (Moloney and Hampton 2020; Moloney et al. 2022). Population growth of the species in Victoria is currently estimated at 15%

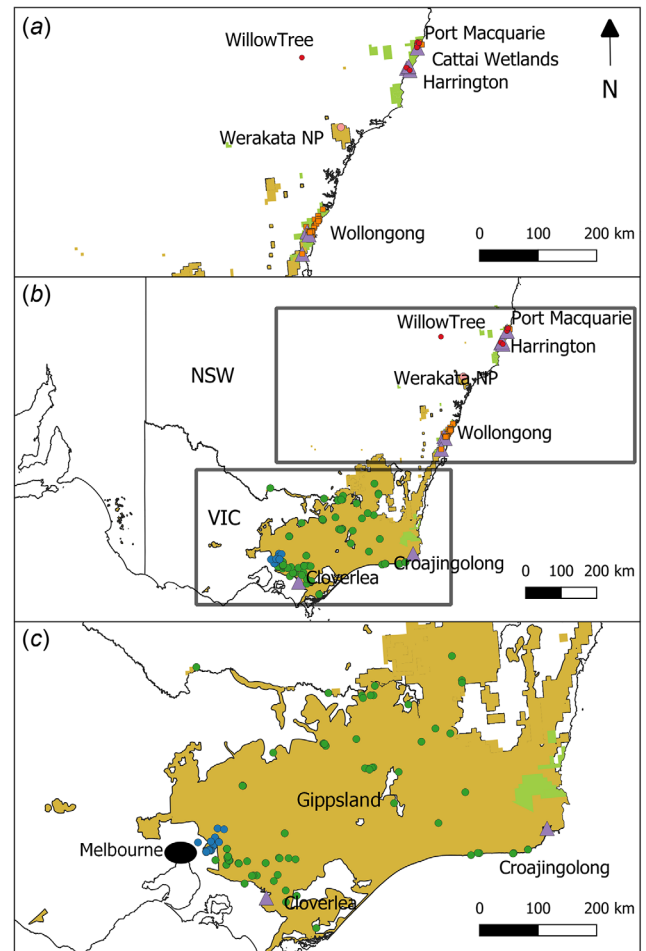


Fig. 1. Map of final samples of sambar (circles) and rusa (squares) deer genotyped for this study. (b) All sites sampled, with (a) and (c) providing further detail of northern NSW and southern NSW/Gippsland/Melbourne sampling sites respectively. Samples that were either morphologically or genotypically identified as hybrids are shown as purple triangles. Colours of circles/squares indicate major geographic regions identified during population genetic analysis: sambar deer Melbourne (blue), sambar deer southern NSW/Gippsland (green), rusa deer NSW (orange), Werakata National Park (pink), and sambar deer northern NSW (red). Distributions of both species are shown in yellow (sambar) and green (rusa).

annually (Watter et al. 2020). Rusa deer distribution continues to expand in NSW (Crittle and Millyn 2020). As the abundance and distribution of both species rises, the negative impacts associated with deer over-abundance become apparent, including destruction of native flora through bark-stripping and trampling, soil erosion, competition with native herbivores for food sources, and wallowing (Keith and Pellow 2005; McDowell 2007; Davis et al. 2008; Bennett and Coulson 2010; Davis et al. 2016). Deer in Australia have also been shown to carry diseases that could affect livestock (Huaman et al. 2020) and deer can additionally pose risk to humans through deer-vehicle collisions, which are likely to

become more commonplace as deer encroach peri-urban environments (Burgin *et al.* 2015; Davies *et al.* 2020). Rusa and sambar deer are also known to hybridise (Martins *et al.* 2018), with suspected hybrids thought to be present in the wild in Australia (Forsyth *et al.* 2015). These hybrids may pose additional negative impacts as increased genetic fitness may be associated with hybrids (heterosis), which can promote changes to life-history traits or phenotypes that may be even more detrimental to native environments than is either parent species (Iacolina *et al.* 2019). Sambar and rusa hybrids grow rapidly and changes in body mass can promote further damage to native environments or increase the severity of deer–vehicle collisions where larger animals are involved (Tuckwell 1998; Pacioni *et al.* 2021). Currently, the extent of hybridisation within sambar and rusa deer populations in Australia is unknown.

To mitigate the negative impacts of sambar and rusa deer across south-eastern Australia, population control through ground- and helicopter-based shooting is often undertaken. Such control can reduce deer densities locally (Bengsen *et al.* 2022b); however, understanding larger-scale population structure, connectivity and dispersal capability is important for identifying larger-scale and longer-term priorities, as well as for estimating the time needed for reinvasion. (Semiadi *et al.* 1994; Leslie 2011; Comte *et al.* 2022). Whereas determining movements of sambar and rusa deer can be difficult by using direct methods such as telemetry because of their elusive nature, genetic analysis of populations of interest can assist in determining likely dispersal rates from population structure. This in turn can identify distinct management units where control can take place without being compromised by immigration. These methods have been widely implemented for a range of invasive species globally to identify management units for population control or eradication (Cowled *et al.* 2008; Fraser *et al.* 2013; Mora *et al.* 2018; Sjodin *et al.* 2020). Whereas previous genetic studies have examined sambar deer connectivity in Victoria and southern NSW (Davies *et al.* 2022; Rollins *et al.* 2023), and fine-scale structure of rusa deer in Royal National Park, and Wollongong in NSW (Webley *et al.* 2004; Li-Williams *et al.* 2023), the present study aims to expand the sampling range of both species to include additional sites across Victoria and NSW to assess broad-scale population structure in both species across south-eastern Australia, and use 1000s of highly resolving single-nucleotide polymorphism (SNP) markers that will additionally aid in the identification of potential hybrids.

Specifically, we aim to (1) determine the interactions between sambar and rusa deer where the two species co-occur, through hybrid analysis, (2) assess any broad-scale population structure present within sambar deer and rusa deer and identify any distinct management units for each species, and (3) measure the likely dispersal capability of each species.

Methods

Sample collection

Samples of free-ranging sambar deer and rusa deer were collected over 2013–2021, predominantly during culling operations conducted by local government agencies in New South Wales and Victoria (Fig. 1, Table 1). These samples primarily consisted of tissue collected from ear tips by using sterile surgical scissors, scalpels and tweezers and stored in 5 mL vials containing 100% ethanol, or via biopsy punches using Allflex tissue-sampling applicators and tissue-sampling units. In NSW, samples were collected from three known sambar deer populations: Albury in southern NSW (possible natural range expansion), Werakata National Park in the Hunter Valley (likely to have originated from a failed sambar and rusa deer farm), and Harrington (unknown source). Additional incidental sambar deer samples were collected from a combination of culling operations and animals shot by commercial harvesters. Blood samples of sambar and rusa deer were also collected from a cull conducted at Port Macquarie in northern NSW in 2018. The sex of each animal, an indication of whether each animal was an adult or juvenile, and the sampling location were recorded. Deer that showed intermediate characteristics that may indicate that the animal was a hybrid were also noted, including atypical body size and ear morphology.

DNA isolation and sequencing

Blood and a subset of the tissue samples were extracted using a DNeasy Blood and Tissue Kit (Qiagen), following the manufacturers' instructions. Approximately 150 μ L of blood was used as starting material for blood extractions, and all samples were eluted using 100 μ L of deionised H₂O. DNA was quantified using a Qubit 2.0 Fluorometer (Invitrogen), and samples were then either diluted with deionised H₂O or concentrated using an RVC 2–18 Rotational Vacuum Concentrator (John Morris Scientific) to obtain final concentrations between 5 and 15 ng/ μ L.

DNA extracts and the remaining tissue samples were sent to Diversity Arrays Technology (DArT) in Canberra, Australia, for DNA extraction and genotyping by sequencing. Sequencing was conducted using an Illumina HiSeq 2500, using 1.2 million reads per sample, and reads were aligned to a red deer (*Cervus elaphus*) genome available on GenBank (BioProject PRJNA324173). Rusa deer samples sequenced by Li-Williams *et al.* (2023) were additionally combined with the dataset. A total of five samples did not provide sufficient data following sequencing, leaving 404 samples for further analysis.

SNP filtering

Sequencing returned 53 946 single-nucleotide polymorphisms (SNPs), of which 41 492 were successfully aligned to the red

Table 1. Final sample sizes for sambar deer, rusa deer, and morphologically identified hybrids genotyped for this study.

Geographic region	Sambar deer		Rusa deer		Suspected hybrids		Total
	Sex M/F/U	Age A/J/U	Sex M/F/U	Age A/J/U	Sex M/F/U	Age A/J/U	
Melbourne	15/34/4	46/0/7	–	–	–	–	53
Gippsland/southern NSW	34/42/33	48/2/59	–	–	2/0/0	0/0/2	111
*Wollongong	–	–	86/88/1	138/36/1	–	–	175
Werakata National Park	2/2/2	3/0/3	–	–	–	–	6
*Harrington	0/0/6	0/0/6	0/0/1	0/0/1	–	–	7
*Cattai Wetlands	7/3/0	10/0/0	1/1/0	2/0/0	1/0/0	1/0/0	13
*Willow Tree	1/0/0	1/0/0	–	–	–	–	1
*Port Macquarie	4/9/0	13/0/0	3/8/0	11/0/0	–	–	24
Total	63/90/45	121/2/75	90/97/2	151/36/2	3/0/0	1/0/2	390

Sites with an asterisk (*) were grouped into the geographic regions 'sambar northern NSW' or 'rusa NSW' for analysis. M/F/U indicates male/female/unknown sex, A/J/U indicates adult/juvenile/unknown age. Samples collected from Wollongong were provided by Li-Williams et al. (2023).

deer genome. Of these SNPs, 1373 were aligned to either the X or Y red deer chromosome, and to avoid any potential sex-linked markers appearing in the dataset, these SNPs were removed from further analysis. Genotypes were analysed with *dartR 2.0* (Mijangos et al. 2022) except when otherwise specified. Loci were filtered with a minimum call rate of 0.95, a minimum read depth of five for each allele (i.e. the reference and alternative allele), and a minimum reproducibility of 0.99. Individuals were removed if they had less than 80% of loci genotyped. Only one SNP was kept where multiple SNPs within the same read were present, with the SNP comprising the highest repeatability and information content chosen. Monomorphic loci and loci with the minor allele observed only once (i.e. singleton) or twice in the whole dataset were discarded. At the end of these steps, the dataset comprised 390 individuals and 14 333 loci.

This dataset was initially inspected using a Pearson principal-component analysis (PCA) and analysed within a Bayesian framework by using the program *fastStructure 1.0* (Raj et al. 2014), with output files being processed using *StructureSelector* (Li and Liu 2018). We considered the profile of the marginal likelihood and the minimum number of the model components needed to explain the structure in the data to select the optimal number of clusters (K ; Raj et al. 2014). In the former approach, we consider the K value that generates the highest log-likelihood (or where further increases in K cause negligible changes in the log-likelihood). In the latter, for a model with a given K , the clusters needed to explain 99.99% of the ancestry of the sampled individuals were considered the optimum number of genetic clusters. Both beta and logistic distributions were used as suitable priors for the allele frequencies. With the beta prior, we tested a wide range of K values (1–9); however, with the logistic prior, we limited the analysis to a range of $K = 1–8$ as guided by the previous results with the beta prior. Up to five possible genetic clusters were subsequently identified.

Partitioning the data based on these five clusters, we evaluated possible departure from Hardy–Weinberg equilibrium (HWE) for each locus, following recommendations of Waples (2015) and De Meeûs (2018). Briefly, the exact tests (Wigginton et al. 2005) for departure from HWE were performed within each population for each locus with $\alpha = 0.05$. The results were compared with the null expectations of the possible number of significant tests if these were obtained by chance alone (both overall and across multiple populations for the same locus). The Fisher's global test (Fisher 1970) was also used to evaluate whether there is at least one test that is truly significant in the series of tests conducted (De Meeûs et al. 2009). Furthermore, we assessed the correlation between F_{IS} and F_{ST} (Nei 1978) to investigate potential causes of departure from HWE to determine whether loci not in HWE should be removed (e.g. because of possible genotyping errors or loci under selection), or retained (e.g. small population size) because the latter can provide important biological insights. Following these filtering steps, 14 099 polymorphic SNP loci and 390 samples, in total, were retained for the final analysis, comprising 198 sambar deer samples, 189 rusa deer samples, and three suspected hybrids on the basis of morphological identification.

Data analysis

From this final dataset, we conducted several analyses, some of which required subsets of samples as summarised in the flowchart (Fig. 2) and described in more detail below.

Hybridisation

A PCA was undertaken on the final dataset by using the *gl.pcoa* function in *dartR* and plotted by using *ggplot2 3.3.6* (Wickham 2016). Analyses of fixed-locus differences between rusa and sambar deer were calculated using the *gl.fixed.diff* and *gl.collapse* functions in *dartR*, with all suspected hybrid

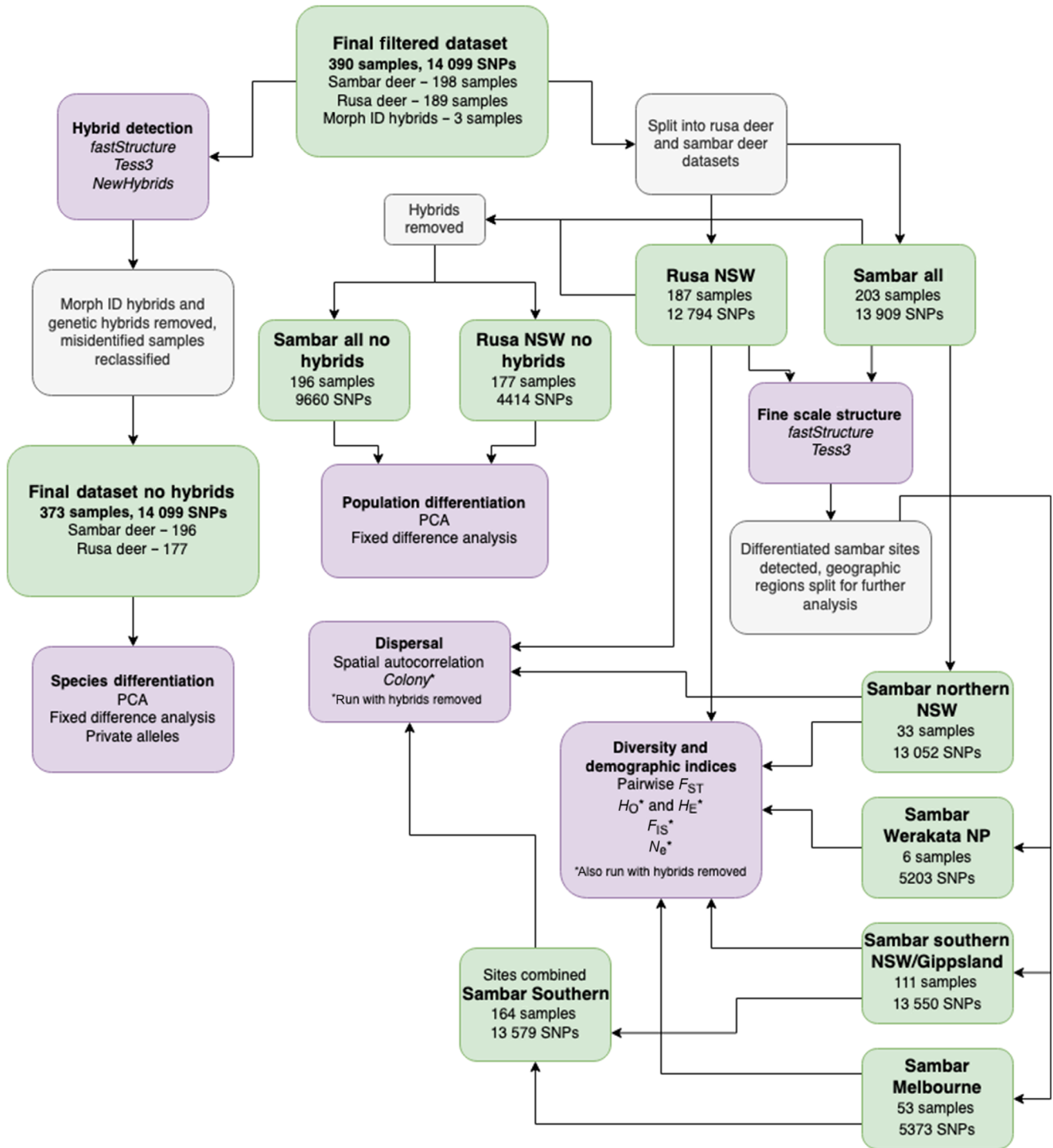


Fig. 2. Flowchart summarising the datasets (green) and analyses (purple) undertaken post-filtering.

samples removed for this analysis. Private alleles between rusa and sambar deer were calculated using the `gl.report.pa` function in `dartR`.

FastStructure was used for the final dataset by using the methods described above and limiting the number of possible clusters to within a range of 1–6. We additionally tested for

structure by using the R package *tess3r 1.1.0* (Caye *et al.* 2016), which provides a spatially explicit analysis of population structure. For the entire dataset, *K*-values 1–9 were tested with 20 repetitions per *K*, 100 000 iterations, and a tolerance level of e^{-7} by using the ‘projected.ls’ method. Best fit for *K* was determined via evaluation of the cross-validation

criterion for each K . The program *NewHybrids 1.1* (Anderson and Thompson 2002), implemented in the R package *parallelnewhybrid 0.0.0.9002* (Wringe et al. 2017), was used to assess hybrid classes within the dataset. *NewHybrids* assigns samples to categories of either parental species, F_1 and F_2 hybrids, or backcrosses to either of the parental species. Because this software has a 200 loci limit, loci with missing data were removed using the `gl.filter.callrate` function in *dartR*, by using a threshold of 1, and the dataset was converted to *NewHybrids* format by using the `gl.nhybrids` function, retaining the first 200 loci ranked on information content by using the 'AvgPIC' method. Thirty samples of sambar deer and 15 samples of rusa deer were randomly assigned as parental species, on the basis of samples identified in *fastStructure* with a Q value above 0.9999 when analysed with $K = 2$. A burn-in of 100 000 and 500 000 sweeps were used, with five replicate runs completed.

Intraspecies population structure and diversity

Following the hybridisation and species-differentiation analysis, the dataset was further subdivided by species and geographic region to detect weaker structure possibly present in the data. When subsets of data were analysed, loci that were monomorphic within each dataset were also removed (Fig. 2). *FastStructure*, *Tess3*, PCA, and the fixed-differences analysis were repeated for each species separately (i.e. rusa and sambar deer), by using the same methods as described above. For the PCA and fixed-differences analysis, hybrid samples were removed.

Pairwise F_{ST} values, diversity indices, inbreeding coefficient F_{IS} , and effective population size (N_e) were all calculated for the distinct geographic regions identified in both the rusa and sambar deer datasets. Pairwise F_{ST} was calculated using `gl.fst.pop`, whereas observed and expected heterozygosity (H_O and H_E), and F_{IS} were calculated using the `gl.report.heterozygosity` function in *dartR*. Effective population size (N_e) was calculated using *NeEstimator V2.1* implemented through the `gl.LDNe` function in *dartR* (Do et al. 2014), by using the linkage disequilibrium method. We used a 0.05 allele frequency cut-off and reported the 95% confidence intervals calculated via jack-knifing of samples. Analyses of H_O , H_E , F_{IS} , and N_e were also run with hybrid samples removed.

Dispersal distance

We investigated whether the genetic data would provide any indication of the dispersal distances in sambar and rusa deer by using a spatial autocorrelation analysis (Smouse and Peakall 1999; Peakall et al. 2003; Double et al. 2005), and analyses of kinship. Here, we consider dispersal the reciprocal distance between two related individuals, with the subsequent dispersal distances observed being reflective of how far individuals disperse from each other in a lifetime. Spatial autocorrelation aims to evaluate whether

samples collected within a distance class are genetically more similar (i.e. related) than would be expected at random. A pairwise genetic distance was constructed as 1 – number of mismatches between pairs of samples, whereas a geographical distance matrix included the linear distance between sampling points (in km) computed using the R package *raster* (Hijmans 2022). Custom distance classes were used, with endpoints being 5, 10, 15, 20, 25, 50, 81, 243, and 729 km. Following other studies (Smouse and Peakall 1999; Peakall et al. 2003; Double et al. 2005), the null hypothesis of no spatial autocorrelation was tested using 1000 permutations and these data were used in a one-tailed test, whereas 1000 bootstraps were conducted to estimate the 95% confidence intervals around the spatial correlation coefficients. The general code used for computing these statistics (`gl.spatial.autoCorr`) was made publicly available in the development version of the R package *dartR* (<https://github.com/green-striped-gecko/dartR/tree/dev>). These analyses were conducted after removing all juveniles and individuals of unknown age, separately for the rusa deer samples, and the southern NSW/Gippsland and Melbourne, and northern NSW sambar deer clusters. The samples from Werakata National Park were not included in this analysis because of small sample sizes. For each dataset, these analyses were also repeated separately for males and females.

Analysis of kinship was undertaken using *Colony 2.0.6.8* to assess the dispersal of closely related kin throughout the landscape (Jones and Wang 2010). *Colony* was run using the same datasets as described above for the spatial autocorrelation analysis, with hybrid samples being removed, and assuming polygamy in both males and females, inbreeding, and no sibship prior or candidate parents. The pairwise full likelihood combined method for analysis was chosen with high precision and updating allele frequencies, and a 0.05 allele dropout rate and false allele rate per locus were also assumed. *Colony* was run three times to ensure convergence of results. Distances between each sibling pair identified by *Colony* were measured, and histograms of dispersal distance classes were generated for the rusa deer samples, the southern NSW/Gippsland and Melbourne sambar deer samples, and the northern NSW sambar deer group by using *ggplot2*. Parameters for a set of candidate distributions (negative binomial, Poisson, log-normal, half-normal and pareto) were then estimated by maximum likelihood by using the R package *fitdistrplus* for each dataset (Delignette-Muller and Dutang 2015). Distances were expressed in metres (m) and, where pairwise distances were 0, 1 m was added to allow the fitting of a log-normal distribution. The fit was compared using the Akaike information criterion and the most supported distribution was used to plot the expected frequency distribution (Burnham and Anderson 1998).

Results

Hybridisation

Principal-component analysis identified three distinct genetic clusters within the entire dataset, corresponding to one rusa deer group, and two groups of sambar deer broadly split into a northern NSW and southern NSW/Victoria group (Fig. 3). A number of samples from NSW fell between the rusa deer and sambar clusters, in addition to two samples from Victoria that were flagged as potential hybrids on the basis of morphology. A third sample that was identified as a potential hybrid on the basis of morphology is clustered with the NSW sambar deer group. After removing suspected hybrid samples and reclassifying two samples that had been misidentified (the morphologically identified hybrid sample clustering in the sambar NSW group, and a sample morphologically identified as rusa deer but genetically assigned as sambar deer), fixed difference analysis found that 2798 loci were fixed between the rusa and sambar

deer samples (false positive expectation 0.4, $P < 0.0001$), with all sambar clusters identified in the PCA collapsing into a single sambar deer population (Supplementary material Fig. S1). In total, 4439 private alleles were present within the rusa deer cluster, and 9685 private alleles were present in the sambar deer cluster.

FastStructure analysis of the entire dataset indicated that $K = 2$ clusters were present, corresponding to the two species (Fig. 4a). Similar to the PCA, a number of samples were intermediate between the two species clusters, including seven samples from northern NSW, eight samples from Wollongong and two samples from southern NSW/Victoria. Results from *Tess3* were largely congruent with the *fastStructure* results, with the optimal K value at $K = 2$ and clusters corresponding to the two species (Fig. 4b). However, additional mixing of the two clusters was evident in the *Tess3* results in the northern NSW group, whereas the samples that showed intermediate genotypes between the two clusters in Wollongong from the *fastStructure* analysis were less evident in the *Tess3* plots.

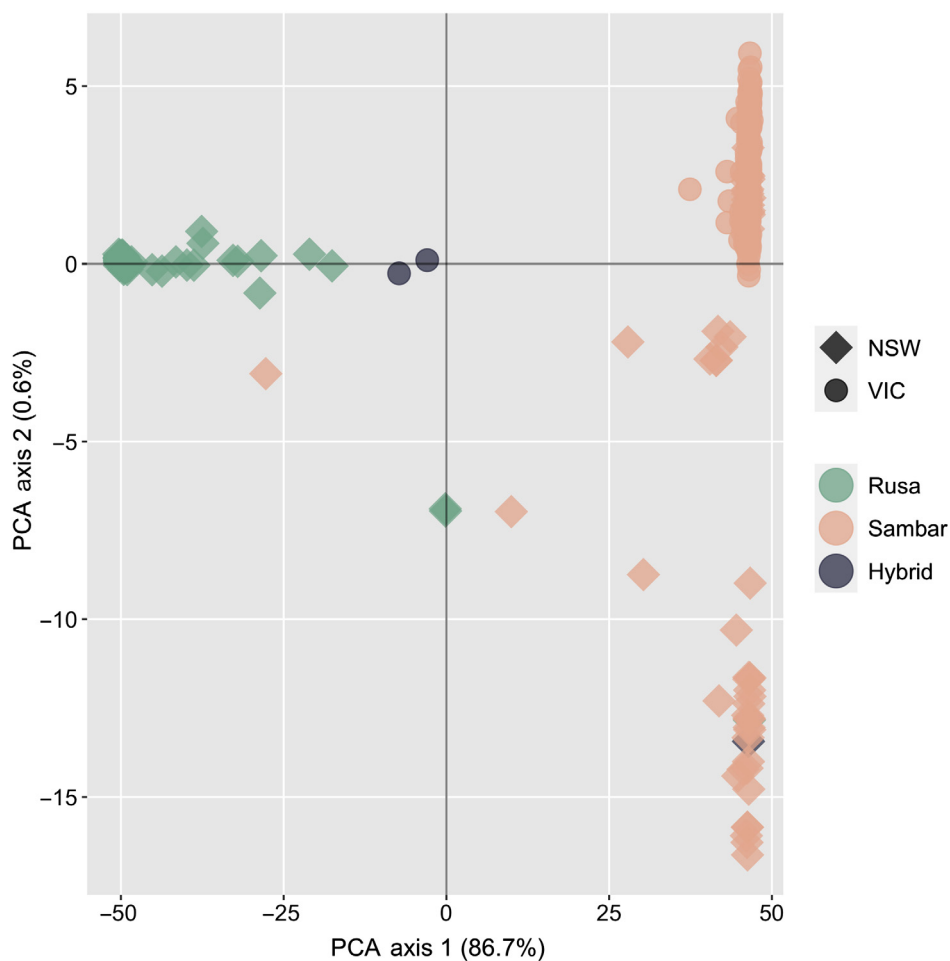


Fig. 3. Principal-component analysis plot of rusa and sambar deer, based on 390 samples and 14 099 polymorphic SNP loci. Samples identified as hybrids in this plot are based on morphological identification prior to genotyping.

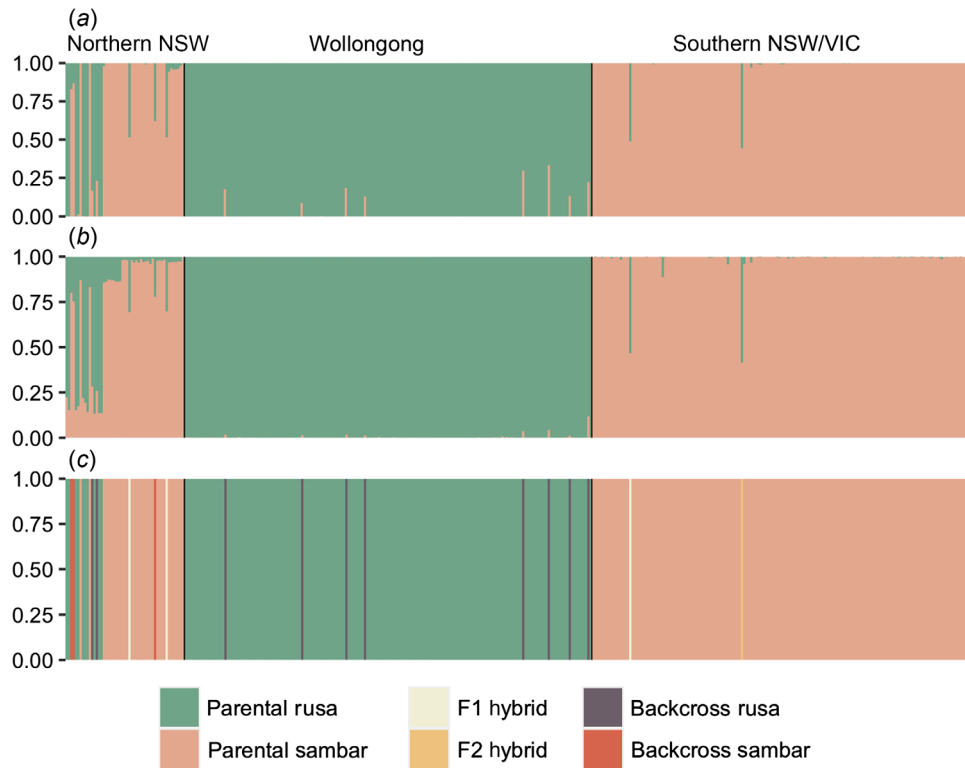


Fig. 4. (a) Best-fit $K = 2$ plot generated from *fastStructure* analysis. (b) Best-fit $K = 2$ plot generated from *Tess3* analysis. (c) *NewHybrids* plot showing individual assignment to parental or hybrid classes. Samples appear in the same order in all plots, with broad geographic range highlighted at the top of the figure.

NewHybrids analysis showed that intermediate samples identified in *fastStructure* and *Tess3* comprised a range of hybrid classes (Fig. 4c). In northern NSW, F_1 hybrids, as well as hybrids backcrossed to either rusa or sambar deer, were detected in this region. Samples from Port Macquarie comprised two individuals that had backcrossed to rusa deer, with one sample each having been morphologically identified as a sambar and rusa deer, and two individuals backcrossed to sambar deer, both morphologically identified as sambar deer during sample collection. Samples obtained from Cattai Wetlands comprised one F_1 hybrid that had been morphologically identified as a rusa deer, and one individual backcrossed to sambar deer, identified during sample collection as a sambar deer. A single sample from Harrington was identified as an F_1 hybrid, previously classified as a rusa deer. In Wollongong, all eight samples showing intermediate genotypes from *fastStructure* analysis were assigned as backcrosses to rusa deer. The two suspected hybrids collected in Victoria were confirmed to be hybrid animals, with the Croajingolong sample identified as an F_1 hybrid, and the Cloverlea sample assigned as an F_2 hybrid. The third suspected hybrid sample collected from Cattai wetlands (northern NSW) was assigned to the parental sambar deer class and comprised a Q value of 0.999 for the sambar deer cluster in *fastStructure*. Finally,

a second individual from Cattai Wetlands morphologically assigned as a rusa deer was in fact a sambar deer on the basis of *fastStructure* and *NewHybrids* results.

Intraspecies population structure and diversity

The dataset was subsequently split into a rusa deer group and a sambar deer group to further elucidate any fine-scale structure present within each species. Both *fastStructure* and *Tess3* identified $K = 2$ genetic clusters in the rusa deer dataset; however, these clusters separated the parental rusa deer from the hybrid samples and were not reflective of population structure detected between sites (Supplementary material Fig. S2). Within the sambar deer samples, *fastStructure* indicated $K = 2$ genetic clusters, again separating parental sambar deer from hybrid samples (Supplementary material Fig. S3); however, *Tess3* identified $K = 5$ genetic clusters within the sambar deer samples (Fig. 5). Whereas one of these clusters comprised only hybrid samples, the remaining four clusters were associated with geographic region, with a distinct cluster being identified in northern NSW, a second cluster specific to Werakata National Park, and the final two clusters mixed between southern NSW/Gippsland and Melbourne. Interestingly, a single sample from northern NSW collected from Willow Tree appeared

to cluster with the southern NSW/Gippsland and Melbourne samples.

Principal-component analysis plots for each species with hybrid samples removed conformed with the sambar deer results from *Tess3*, with four groups broadly corresponding to northern NSW, Werakata National Park, southern NSW/Gippsland, and Melbourne, and intermixing between southern NSW/Gippsland and Melbourne, and the single Willow Tree sample clustering with the Victorian samples (Fig. 6). Fixed-differences analysis between these sambar deer populations showed fixed differences only between Werakata National Park and northern NSW, with only four fixed differences observed. No sambar deer populations were shown to be significantly different, and all collapsed into a single population. When the single Willow Tree sample was removed, and fixed-difference analysis rerun, the number of fixed differences between Werakata National Park and northern NSW rose to five; however, all populations were still not significantly different and again collapsed into a single population. Within the rusa deer samples, some slight clustering could be observed between Port Macquarie and Wollongong, which was not detected by *fastStructure* or *Tess3* (Fig. 6). No fixed differences were detected between these two sites, with both populations collapsing into a single rusa deer group.

Pairwise F_{ST} values between species and geographic regions for sambar deer were all statistically significant, with comparisons between rusa and sambar deer comprising the highest pairwise values of 0.884–0.912 (Table 2). Within the sambar deer geographic regions, pairwise F_{ST} values ranged from 0.035 to 0.231, with the lowest value between Werakata National Park and Gippsland, and the highest pairwise value between Melbourne and Werakata National Park.

Observed and expected heterozygosity within the major geographic regions identified for rusa and sambar deer showed that the rusa deer NSW population comprised the lowest values, with a H_O of 0.056 and a H_E of 0.057 with hybrid samples retained, and 0.043 for H_O and H_E with hybrid samples removed (Table 3). Sambar deer present in northern NSW comprised the highest observed and expected heterozygosity with hybrid samples included, with a value of 0.153 for each. However, when hybrid samples were removed from this analysis, the sambar deer population present in Werakata National Park comprised the highest H_O and H_E values, followed by southern NSW/Gippsland. F_{IS} values were similar when hybrids were present and absent in the dataset and ranged from -0.069 in the sambar deer Werakata National Park population to $0.083/0.068$ in the sambar deer southern NSW/Gippsland region. Estimates of effective population size were highest for sambar deer populations present in Victoria and southern NSW, with an estimated N_e of 126.9 across southern NSW/Gippsland, and 101.1 within the sambar deer Melbourne samples (Table 3). The rusa deer populations in NSW had an N_e of 63 with hybrids retained and 61 with hybrids removed, with the lowest estimates being observed in the sambar deer populations in northern NSW, with values of 2.8 in Werakata National Park and only 0.7 in northern NSW; however, this value increased to 11.2 when hybrids were removed (Table 3).

Dispersal distances

The Distance class 5–10 km was marginally significant ($P = 0.047$) for the rusa deer NSW dataset ($r = 0.0001$) (Supplementary material Fig. S4). For the same dataset, the Distance classes 20–25 and 25–50 km were also significant

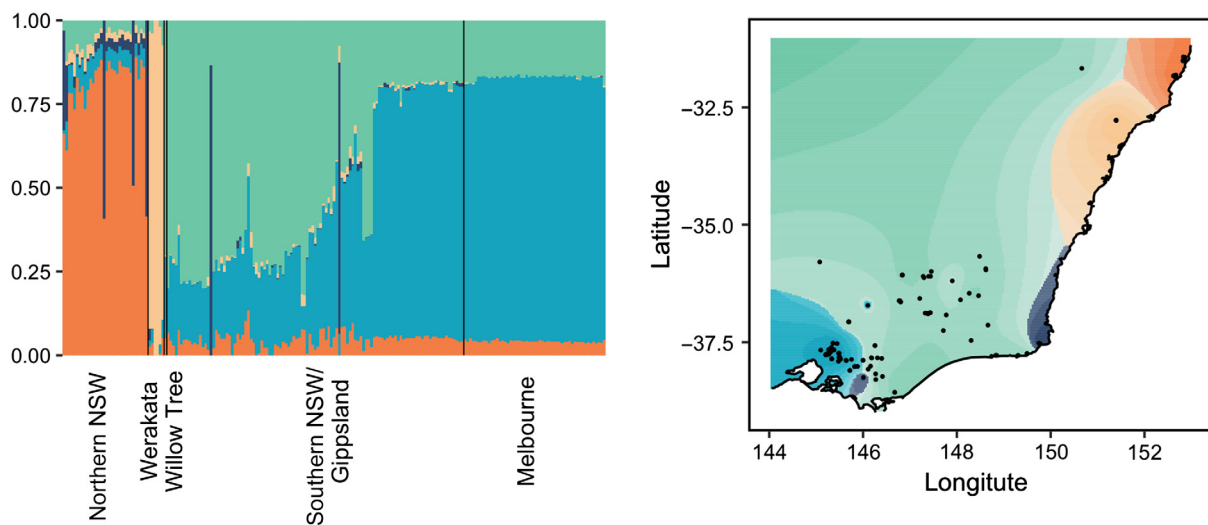


Fig. 5. Best-fit $K = 5$ plot and map generated from *Tess3* analysis for all sambar deer samples. Broad geographic range is identified at the bottom of the bar chart, along with the Werakata National Park site and Willow Tree samples, which form part of northern NSW.

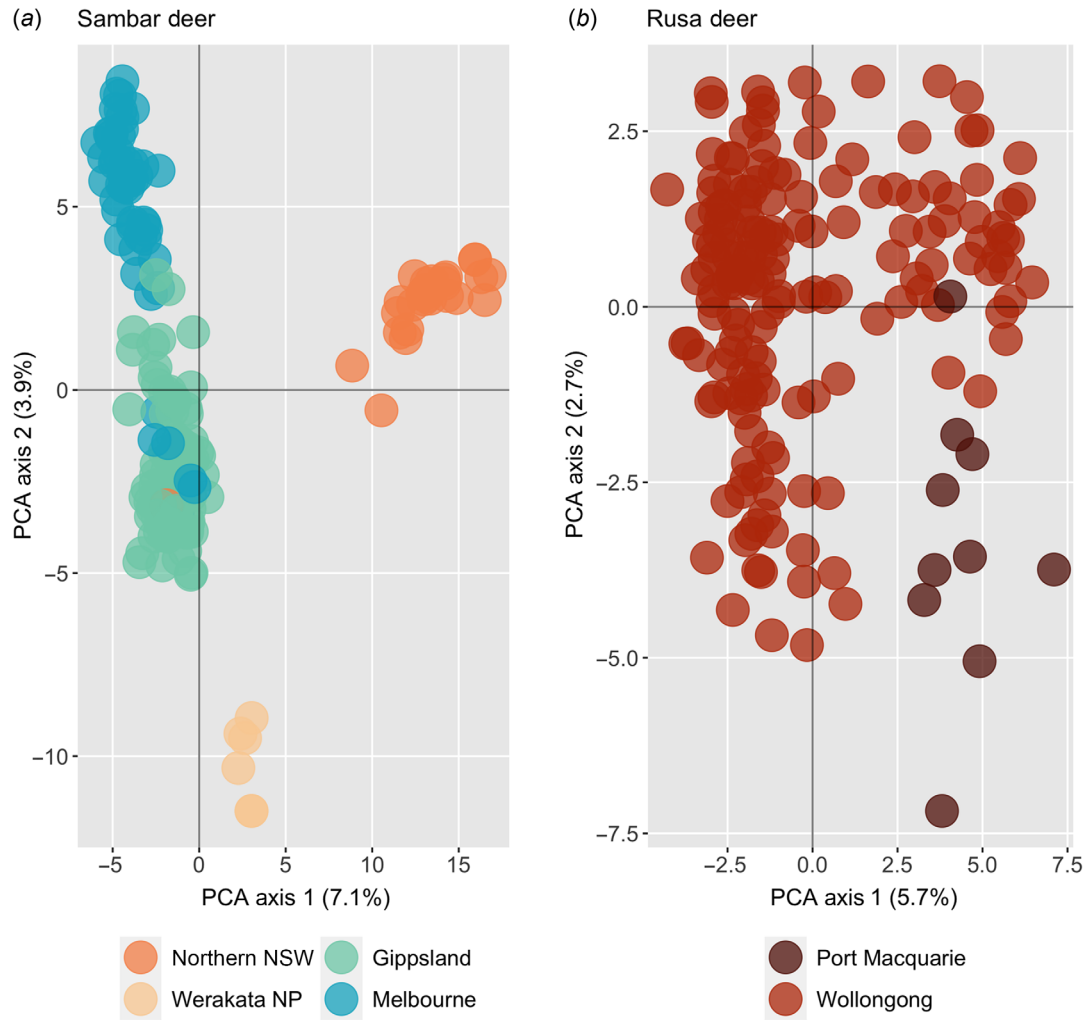


Fig. 6. Principal-component analysis plot for major geographic regions of (a) sambar deer, and (b) rusa deer, with all hybrid samples removed.

Table 2. Pairwise F_{ST} between the major geographic regions identified in the rusa and sambar deer samples.

Item	Rusa NSW	Sambar North NSW	Sambar Werakata	Sambar Gippsland
Sambar northern NSW	0.892			
Sambar Werakata	0.906	0.134		
Sambar Gippsland	0.884	0.097	0.035	
Sambar Melbourne	0.912	0.166	0.231	0.160

All pairwise comparisons were significant with $P < 0.001$.

($P \leq 0.028$) with negative coefficients ($r = -0.0002$ and -0.0001 respectively). The last distance class (>243 km) was also significant ($P = 0.037$) and positive ($r = 0.00015$). No distance classes were significant when rusa deer males were analysed alone and only the Distance classes >15 km had a significant ($P \leq 0.04$) and negative correlation coefficient ($r \leq 0.0003$) when females were analysed alone. Kinship analysis showed 89 full sibling pairs with probabilities above 0.9 in the rusa deer NSW dataset, with no

half-sibling pairs being identified. Of these sibling pairs, five were from Port Macquarie, and the remaining 84 from Wollongong, with no siblings being identified between the two sites. Within Port Macquarie, four sibling pairs were sampled less than 1 km apart, with one pair being separated by 2.6 km (Fig. 7). At Wollongong, 39 sibling pairs were sampled less than 1 km apart, 29 sibling pairs were identified between 1 and 4.9 km, four pairs were separated by a distance of 5–9.9 km, and 10 pairs sampled greater than 10 km apart,

Table 3. Diversity indices for rusa and sambar deer based on distinct geographic regions observed.

Species/region	Hybrid samples retained					Hybrid samples removed				
	<i>N</i>	<i>H_O</i>	<i>H_E</i>	<i>F_{IS}</i>	<i>N_e</i>	<i>n</i>	<i>H_O</i>	<i>H_E</i>	<i>F_{IS}</i>	<i>N_e</i>
Rusa NSW	187	0.056 (0.124)	0.057 (0.117)	0.021	63 (56–70.2)	177	0.043 (0.127)	0.043 (0.120)	0.010	61 (53.5–69.9)
Sambar northern NSW	33	0.153 (0.146)	0.153 (0.138)	0.014	0.7 (0.1–2.3)	28	0.090 (0.168)	0.089 (0.161)	0.012	11.2 (6.9–18.6)
Sambar Werakata	6	0.143 (0.231)	0.123 (0.177)	–0.069	2.8 (0.9–infinite)	6	0.143 (0.231)	0.123 (0.177)	–0.069	2.8 (0.9–infinite)
Sambar southern NSW/Gippsland	111	0.117 (0.162)	0.127 (0.170)	0.083	126.9 (96.1–171.3)	109	0.107 (0.168)	0.114 (0.176)	0.068	126.9 (96.4–177.6)
Sambar Melbourne	53	0.101 (0.172)	0.103 (0.171)	0.028	101.1 (92.8–204.6)	53	0.101 (0.172)	0.103 (0.171)	0.028	101.1 (92.8–204.6)

n, sample number; *H_O*, observed heterozygosity (s.d.); *H_E*, expected heterozygosity (s.d.); *F_{IS}*, inbreeding coefficient; *N_e*, effective population size (95% CI).

with the largest distance between two siblings recorded at 25.8 km (Fig. 7). Two sibling pairs were identified with a sample where no location information was taken, so kin dispersal could not be measured.

The overall spatial autocorrelation analyses were not significant for the northern NSW sambar deer group (Supplementary material Fig. S5) nor with males only. When females were analysed separately, only the first distance class (0–5 km) returned a significant ($P = 0.007$) correlation coefficient ($r = 0.0057$). Kinship analysis identified seven full-sibling pairs and 17 half-sibling pairs among the northern NSW sites, with 11 pairs collected from the same sites, and 13 pairs collected from different sites. Pairs sampled from the same site showed dispersal distances <2 km, whereas pairs sampled from Cattai Wetlands and Harrington showed dispersal distances of 6.6–8.7 km, accounting for five sibling pairs (Fig. 7). Surprisingly, kin pairs were identified between Cattai Wetlands and Harrington to Port Macquarie, with dispersal distances of 38.1–40 km; two individuals from Cattai Wetlands, and one individual from Harrington, are responsible for the kin pairings to Port Macquarie, with the two samples from Cattai Wetlands also relatives (Fig. 7). In total, six sibling pairs were identified between Cattai Wetlands and Port Macquarie, and two sibling pairs between Harrington and Port Macquarie.

The first three distance classes (<15 km) for sambar deer from southern NSW/Gippsland and Melbourne were significant ($P < 0.0001$), with correlation coefficients between 0.001 and 0.008 (Supplementary material Fig. S6), and the last five (distance classes >25 km) had significant ($P \leq 0.016$) negative correlation coefficients (range –0.002 to –0.001), except for the 50–81 km distance class, which had a positive correlation coefficient ($r = 0.001$). When the two sexes were analysed separately, this pattern was consistent in females, with all distance classes being significant except for the Distance class 50–81 km. Only the first three distance classes (<15 km) and the last two (>243 km) were significant ($P < 0.0001$) in males, with positive correlation coefficients in the first three and negative in the last two distance classes. When comparing the autocorrelation plots for the two sexes with this dataset, although

the correlation coefficients had the tendency of being higher for females, these were not greatly different, and the 95% confidence intervals generally encompassed the same distance classes for each sex (Fig. 8). Kinship analysis identified 35 full-sibling pairs with values above 0.9. Sixteen pairs had generic location coordinates for both samples, so movement could not be measured; however, all pairs were sampled from the same sites. For the other pairs identified, 10 were sampled <1 km apart, four sibling pairs were observed 1–4.9 km apart, one pair was detected 5–9.9 km apart, and four sibling pairs were sampled at a distance >10 km apart (Fig. 7). Of the sibling pairs identified at a distance greater than 10 km, two of these were between the Melbourne and southern NSW/Gippsland regions. The largest dispersal distance of 61.2 km was observed between two sambar deer in Kosciuszko National Park. The most supported distribution (Supplementary material, Table S1) was a negative binomial, with parameter size of 0.3 and μ of 3171 for the rusa deer dataset and size of 0.365 and μ of 1492 for sambar deer in northern NSW. The log-normal distribution was the most supported for the sambar deer in southern NSW/Gippsland and Melbourne (with mean of 6.76 and s.d. of 2.06 on the log scale).

Discussion

Hybridisation

In total, 17 hybrids were identified in the dataset, representing multiple hybrid classes in both NSW and Victoria. These samples comprised <1% missing data, except two samples from Port Macquarie that had between 14% and 16% missing data. Of the three samples flagged as hybrids prior to genetic analysis, only two were confirmed to be hybrids, and comprised an F_1 and an F_2 hybrid. On the contrary, 11 individuals identified as rusa deer and four identified as sambar deer were in fact F_1 , F_2 hybrids or backcrosses. These results demonstrated the difficulties in distinguishing hybrids on the basis of morphology alone, and although some intermediate characteristics can be observed in sambar deer \times rusa deer hybrids, particularly

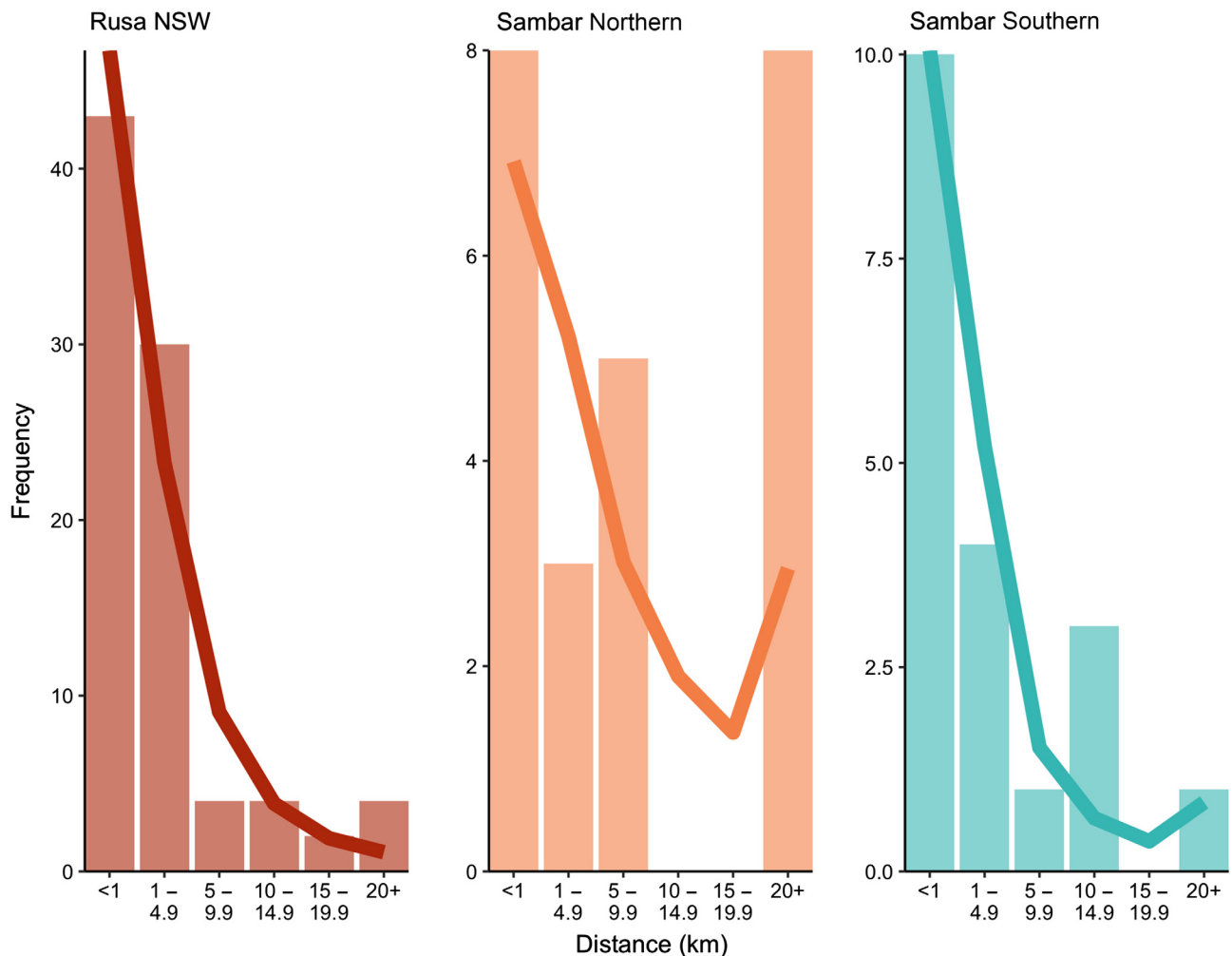


Fig. 7. Bar plots showing the frequency of kinship pairs observed at a range of dispersal distances for both sambar deer and rusa deer. Lines depict the expected frequency for each distance class on the basis of the most supported distribution for each population (negative binomial for rusa NSW and sambar northern, and log-normal for sambar southern). All kinship pairs comprised a probability value of >0.9 .

in antler growth patterns, ear morphology, and increased body size in the smaller rusa deer (Bentley 1978), these characteristics are likely to become less apparent as F_1 and F_2 hybrids backcross to parental species. Similar patterns have been observed in white-tailed deer (*Odocoileus virginianus*) \times mule deer (*Odocoileus hemionus*) hybrids and hog deer (*Axis porcinus*) \times chital deer (*Axis axis*) hybrids, where hybrid individuals phenotypically resemble a parental species rather than intermediate characteristics of both parents (Hill et al. 2019; Combe et al. 2022), and in sika deer (*Cervus nippon*) \times red deer (*Cervus elaphus*) hybrids where hunters misidentified 21% of deer at sites where hybrids were known to occur (Smith et al. 2014). It is therefore not surprising that a majority of the hybrids detected through genetic analysis in this study were not flagged as suspected hybrids prior to analysis and this highlights the need for genetic testing of sambar and rusa

deer to accurately determine hybridisation rates between the species. Deer hybrids can present as larger in body size than their parental species, and this phenomenon is often encouraged in the deer farming industry to promote larger meat yields (Pearse 1992; Tate et al. 1997; Tuckwell 1998). Hybridisation in ungulates has also been shown to increase disease resistance (Grossen et al. 2014; Barbato et al. 2017), growth rate and bodyweight (Asher et al. 1996; Ismail and Saidi 2009; Senn et al. 2010), and is suggested to aid in population growth and dispersal (Goedbloed et al. 2013; Manunza et al. 2016; Iacolina et al. 2019). These characteristics may lead to an increase in negative impacts associated with deer in areas where sambar deer, rusa deer, and their hybrids co-occur, with further research being necessary to understand the full scale of the negative impacts that are likely to be associated with the presence of rusa deer \times sambar deer hybrids in the Australian landscape.

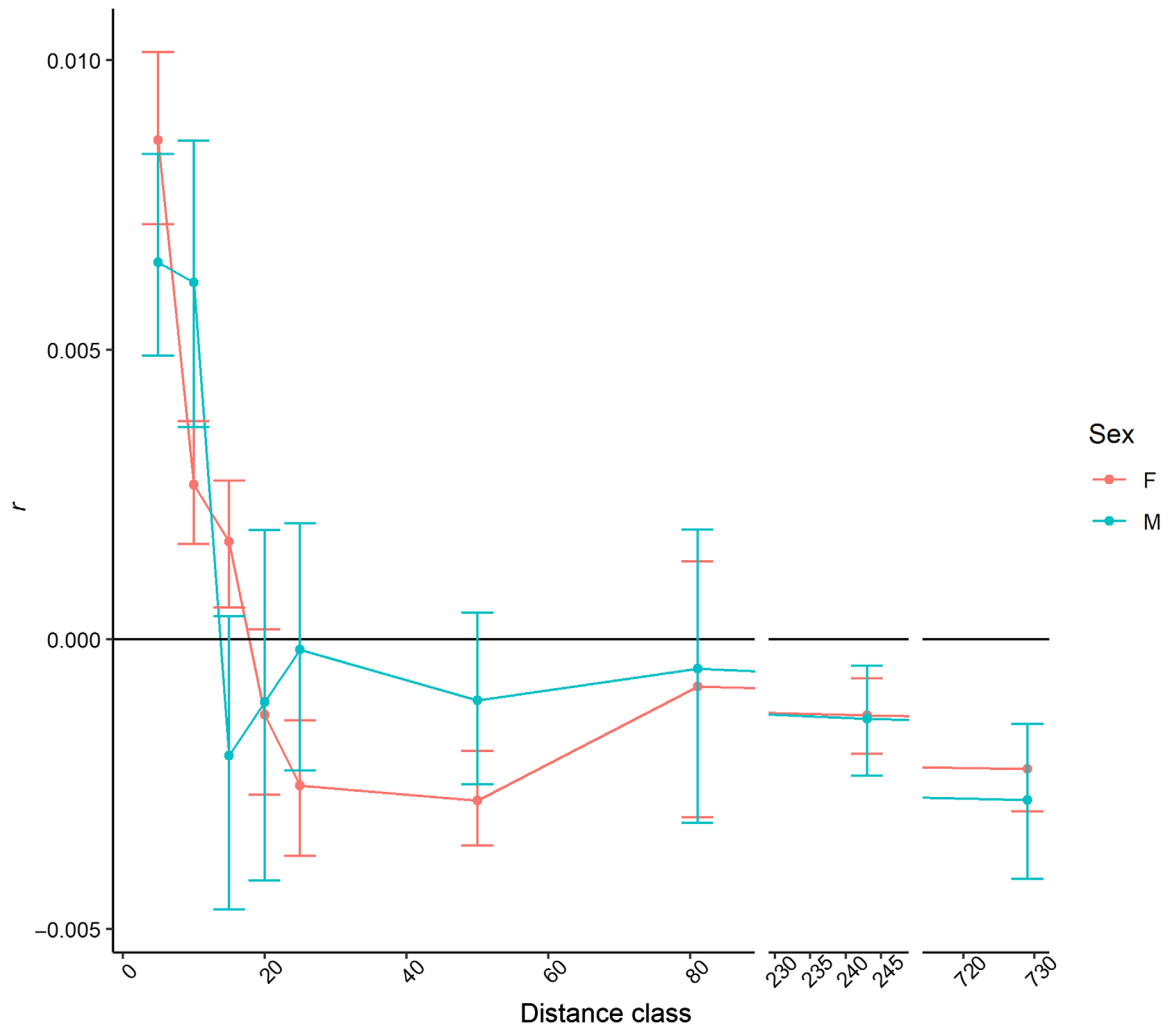


Fig. 8. Auto-correlogram with distance classes in kilometres on the x-axis and spatial correlation coefficient (r) on the y-axis for males and females from the southern sambar deer cluster. Error bars are estimates of the 95% confidence intervals obtained via bootstrap analysis. Note that the x-axis is broken to improve visibility.

Whereas many of the hybrids identified in this study were collected from sites where both sambar and rusa deer occur (Port Macquarie, Cattai Wetlands, Harrington, Wollongong), hybrids were additionally detected at two sites in Victoria (Croajingolong and Cloverlea), where rusa deer are not believed to be established (Bentley 1978; Forsyth *et al.* 2015). Dispersing male rusa deer have been observed in Victoria close to the NSW border (Forsyth *et al.* 2015), and so it is possible that a dispersing rusa individual has encountered the Victorian sambar deer population, which has given rise to the hybrid sample collected from Croajingolong. At Cloverlea, where natural dispersal of rusa deer is highly unlikely, an escapee from a nearby local farm managed for recreational hunting is the most likely explanation for the hybrid observed at this site; however, it is unclear whether a hybrid or a pure rusa deer individual has escaped, with the parental rusa deer subsequently interbreeding with the

local sambar deer population. The hybrid detected in Cloverlea also highlights the ongoing issues of deer escapees.

Population structure

Genetic structure was observed between the major geographic areas sampled for sambar deer, particularly between northern NSW and southern NSW/Gippsland and Melbourne, whereas populations of rusa deer did not appear to be genetically distinct. Fine-scale genetic analysis of rusa deer in Wollongong does show evidence of population structure (Li-Williams *et al.* 2023); however, the different spatial scales between the work of Li-Williams *et al.* (2023) and the present study, coupled with differences in SNP filtering, has likely led to the differences observed between the two studies. Also, it is important to note that Li-Williams *et al.* (2023) removed ‘outliers’ from their datasets, which were

identified as rusa deer backcrosses in our study and the presence of these individuals in our analysis may have masked more subtle population structure within rusa deer that is present in the data. These two studies highlighted the importance of assessing genetic structure at multiple spatial scales to understand the connectivity among multiple populations and how populations may have arisen in the landscape, and additionally investigating genetic structure within more localised areas to identify fine-scale structure that can assist with local management of the species.

The genetic structure evident between Victoria and southern NSW and the northern NSW sambar deer populations is likely to be due to different founding events between the two regions and a subsequent genetic drift. Genetic structure is evident in invasive species where multiple introductions have occurred (Zalewski *et al.* 2010; Johansson *et al.* 2018; Mora *et al.* 2018) and can additionally be observed as invasive species continue to expand their distributions (Short and Petren 2011; Kajita *et al.* 2012). The lack of genetic structure observed in sambar deer in Victoria and southern NSW suggests a relatively high level of gene flow across Gippsland and southern NSW and is consistent with the findings of other studies assessing population structure of sambar deer across this region with microsatellites (Davies *et al.* 2022; Rollins *et al.* 2023). Sambar deer were released at several sites in the Gippsland region in Victoria in the 19th century by the Victorian Acclimatisation Society, with this population believed to have naturally dispersed as far as the Blue Mountains region near Sydney (Forsyth *et al.* 2015; Crittle and Millyn 2020). Northern NSW sambar deer are believed to have established relatively recently, with deer in Port Macquarie first being observed in the 1980s, and deer in Werakata National Park released/escaped from farms in the 1990s. The analyses of genetic diversity seem to support this, with low estimates of heterozygosity and N_e compared with the southern populations. Deer releases and escapees from farms are estimated to account for 35% of the deer populations present in Australia, second only to translocations as the source of new deer populations (Moriarty 2004). Genetic structure attributed to multiple founding events and subsequent genetic drift has been observed in sambar deer previously (Davies *et al.* 2022), and combined with the results from the present study, suggests that these factors are the major contributors to genetic structure observed in the species in Australia.

Although genetic structure was consistently identified in analyses between the northern NSW and southern NSW/Gippsland sambar deer groups, the extent of population structure was less apparent between southern NSW/Gippsland and Melbourne. Intermixing between these two regions is evident in the *tess3* analysis and PCA; however, pairwise F_{ST} between these two regions was higher than in the comparison between southern NSW/Gippsland and northern NSW (0.16 and 0.097 respectively). While Rollins *et al.* (2023) sampled

sambar deer from Yarra Valley, a site that was encompassed within the Melbourne population in the present study, they did not identify these samples as potentially belonging to a genetic cluster separate from southern NSW/Gippsland. This may be due to differences in the genetic markers used. Generally, SNPs are considered to have a higher resolution power than do microsatellites when many hundreds of SNPs are used (Helyar *et al.* 2011; Flanagan and Jones 2019). Rollins *et al.* (2023) sampling of Melbourne and surrounding areas was also limited. It is also possible that this site is on the fringe of the two genetic clusters, with possible contributions from both the Melbourne and southern NSW/Gippsland groups. The Melbourne population is likely to represent the range edge of sambar deer from the Gippsland population, and so the pattern observed here may be reflective of range edge effects in the Victorian population. Populations on the edges of a given species' distribution can show reduced genetic diversity and population structure, and higher levels of inbreeding than for core-range populations; however, these findings are not always consistent across species (Arnaud-Haond *et al.* 2006; Eckert *et al.* 2008; Assis *et al.* 2013). Estimates of genetic diversity and inbreeding through F_{IS} in the sambar deer Melbourne population were consistent with those from the other sambar deer regions sampled, potentially owing to the proximity of Melbourne to one of the initial release sites of sambar in Victoria in the 19th century (Kinglake; Forsyth *et al.* 2015). Alternatively, it is possible that landscape and urbanisation may play some role in the genetic structure observed in the Melbourne population. The sambar deer samples collected in Melbourne were predominantly from the peri-urban region of the city, and previous studies of deer have shown that urban habitats can lead to genetic differentiation within urban sites and among rural areas (Blanchong *et al.* 2013; Fraser *et al.* 2019). Genetic analysis of rusa deer in a peri-urban environment has demonstrated that a fine-scale population structure is present across the region (Li-Williams *et al.* 2023) and therefore additional sampling of sambar deer across peri-urban Melbourne and surrounds, and an assessment of landscape features that may facilitate or inhibit movement, are also warranted to further understand the patterns of population structure observed in the present study.

Dispersal distance

Across all datasets, shorter distance classes (<20 km) have generally positive and significant correlation coefficients for both sambar and rusa deer. The correlation coefficients rapidly drop for distances >20 km, becoming often significantly negative. Although this pattern was strongly pronounced in the southern cluster of sambar deer, it was less so in rusa deer and the northern cluster of sambar deer. The point where positive (and significant) spatial autocorrelation coefficients become not significant and close to zero are usually interpreted as the limit of the

dispersal distance (as defined in the Methods) of the species. On the contrary, consistently negative coefficients may reflect sampled distance classes beyond the possible dispersal distance, causing an isolation by distance effect. Conversely, the sample size was much lower in the northern sambar deer dataset. However, given that the two sambar deer datasets have a similar pattern, these results suggest that the natural lifetime dispersal distance in sambar deer within Australia is likely to be within 20 km, and it is unlikely that sambar deer are able to disperse beyond 50 km in a lifetime. Rusa deer seem to have a more homogeneous dispersal pattern within 20 km, but similarly it would appear unlikely that individuals of this species disperse beyond 50 km. These data also suggest that both sexes have similar dispersal capacity, although the majority of females may have a slightly reduced dispersal distance. These results are also supported by the analysis via kinship. In fact, using the estimated distributions of the dispersal distances, 20 km would fall within the 77–97th percentile.

Both sambar and rusa deer are considered sedentary species (Long 2003), with home-range size estimates of rusa deer encompassing roughly 4 km², with a core range of 1 km² in Queensland, Australia, (Amos *et al.* 2023), being consistent with results elsewhere (Spaggiari and de Garine-Wichatitsky 2006; Pairah *et al.* 2015). Estimates of home-range size in sambar deer appear to vary on the basis of habitat, season, and sex, but most recent analyses in Taiwan have suggested average home ranges of 1.43 km² in males and 0.7 km² in females, which is largely consistent with previous work (Leslie 2011; Chatterjee *et al.* 2014; Yen *et al.* 2019). Dispersal via kinship analysis showed that rusa and sambar deer movements were largely within these ranges, with over half of all kin pairs moving 0–5 km for both species; however, some dispersal events were >10 km, with the highest reported distance of 61.2 km between two sambar deer. It is not possible to confirm through these analyses if a single deer has travelled this distance, or if both sibling pairs have equally dispersed at smaller distances; nonetheless, the estimates of lifetime dispersal measured through both spatial autocorrelation and kinship would suggest that movement is largely limited. However, some consideration of the drivers of dispersal and how sambar and rusa deer have come to disperse across much of Victoria and NSW is needed. Larger home ranges in male sambar deer are attributed to the exploratory nature of males during rut to find females (Chatterjee *et al.* 2014), and it is common for males and juveniles to be the predominant dispersers in many mammalian species (Stephen Dobson 1982; Shaw *et al.* 2006). Dispersal can also be observed through attempts to avoid inbreeding with relatives during breeding seasons (Long *et al.* 2008; Bioss *et al.* 2015), and environmental factors such as habitat cover, landscape features, and bushfires may also be important to understand natural deer movement (Long *et al.* 2005; Long *et al.* 2010; Kelly *et al.* 2014; Davis *et al.* 2016). However, given the

distances among samples in some of the genetic clusters identified in both the sambar deer and rusa deer datasets, particularly between Willow Tree and southern NSW/Gippsland for sambar deer (~490 km between the nearest sites), and Wollongong and Port Macquarie for rusa deer, anthropogenic dispersal may also provide some explanation. Releases or escapees of deer from farms have already been discussed as a potential cause of some of the genetic structure and hybrids observed in the dataset and are additionally likely to contribute to increases in deer distributions. Additionally, translocations by hunters keen to expand hunting opportunities in their local areas can lead to increases in range of sambar and rusa deer. Given the large distance between the sample collected from Willow Tree and the other samples assigned to this cluster in southern NSW/Gippsland, and the high degree of genetic similarity, it is likely that this sample represents a translocated animal or a descendent. Translocated deer and pigs have been detected via genetic methods in Australia previously (Spencer and Hampton 2005; Hill *et al.* 2022), and elsewhere in the world (Frantz *et al.* 2006; McDevitt *et al.* 2009; Carden *et al.* 2011), with many of the deer populations present in Australia today believed to have arisen through recent translocations (Moriarty 2004). Additional genetic sampling of Willow Tree and surrounds is necessary to understand the extent of translocations in this area.

Management implications

Measurements of lifetime dispersal capabilities of rusa and sambar deer, and the presence of population structure between sambar deer sites sampled, provide some insights for population control moving forward. Although rusa deer populations in Wollongong and Port Macquarie appear genetically similar, the dispersal capability of this species suggests that they cannot rapidly disperse between these sites and can be managed separately. However, additional sampling in Port Macquarie can confirm that kin do not appear between these two sites. Assessment of genetic structure within a single population of rusa deer (Li-Williams *et al.* 2023) also suggests that localised management units of rusa deer can additionally be identified for further management intervention and highlights the need for localised studies in both species in the future. In sambar deer, population structure is evident, suggesting multiple management units where population control can be undertaken independently. Currently, Port Macquarie is managed separately to Cattai Wetlands and Harrington; however, the results reported here suggest that gene flow exists among these sites, with kinship pairs observed among the three areas. It would therefore be beneficial in future to coordinate population control of these sites to achieve greater reductions in deer density overall and maintain reductions at either site. Further sampling of deer at these sites would also be of value to determine whether the connectivity observed extends to additional deer

species. Rusa deer occur at these sites as well, in addition to fallow deer (*Dama dama*), red deer, and chital deer (*Axis axis*) at Port Macquarie (Pacioni et al. 2021) and understanding dispersal pathways and connectivity early in the invasion process can greatly assist in successfully managing problematic species (LaRue et al. 2011; Adams et al. 2014). The Werakata National Park population appears isolated from all other sambar deer populations; however, additional monitoring is necessary to understand the range of this population.

The southern NSW/Gippsland population of sambar deer will be much more challenging to manage given the scale of gene flow across this region. Management of the species should continue in high-priority sites such as National Parks to reduce their impacts. Results of lifetime dispersal capability of sambar deer can assist in determining local scales of population control to reduce reinvasion. Further research is necessary across Gippsland and southern NSW to determine potential landscape features that may be facilitating or limiting gene flow in sambar deer. Forest cover has already been suggested as important for sambar deer dispersal (Davies et al. 2022), so confirming this in addition to identifying potential barriers can assist in using landscape or human-made features to aid control within this region. However, challenges in ensuring any artificial barriers to deer dispersal do not also negatively affect movements of native species are also likely to arise (Jones et al. 2021).

Last, further research is necessary to understand the impacts rusa × sambar deer hybrids are likely to have on the Australian landscape, and if management at sites where hybrids occur should be considered a high priority to prevent further spread. Measuring phenotypic, behavioural, and fitness changes in hybrids compared with parental species is warranted to understand the scope of this potential threat. Currently, hybridisation appears to be mostly isolated to the northern NSW region; however, further sampling of the contact zone between the two species at the Victorian–NSW border will elucidate the likelihood of hybrids further spreading throughout this region.

Supplementary material

Supplementary material is available [online](#).

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Data availability. Raw data have been uploaded to CloudStor and will become publicly available upon publication of the manuscript.

Conflicts of interest. David Forsyth was a guest Associate Editor for this special issue. Despite this relationship, he did not at any stage have editor-level access to this manuscript while in peer review, as is the standard practice when handling manuscripts submitted by an editor to this journal. *Wildlife Research* encourages its editors to publish in the journal, and they are kept separate from their manuscripts' decision-making process. The other authors have no relevant financial or non-financial interests to disclose.

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