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Department of **Biodiversity, Conservation and Attractions**

Understanding sources of feral cats in Dryandra Woodland through DNA analysis

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Biodiversity and Conservation Science

Understanding sources of feral cats in Dryandra Woodland through DNA analysis

Robyn Shaw and Kym Ottewell

Final report to Peel Harvey Catchment Council

February 2023

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Summary

This report presents findings from genetic analysis of feral cat populations located in Dryandra Woodland and surrounds, in the wheatbelt of Western Australia.

Key results include:

- Of 176 tissue samples sent for genomic sequencing (DArTseq), high quality genotypes were obtained for 135 individuals.
- Overall, there was low genetic structure across the study area suggesting feral cats have a high capacity for dispersal in this landscape.
- High relatedness was detected within cat populations to the north and east of the study area suggesting a stable population structure in these areas, representing putative source populations.
- Conversely, individuals within the southern forest blocks were largely unrelated and there was evidence of movement of related individuals between other areas and this population, suggestive of sink dynamics.
- Positive relatedness amongst individual cats was detected at up to 20 km suggesting this is the scale of the genetic neighborhood of cats at Dryandra.
- Of years with sufficient numbers of samples for comparison (2016, 2017, 2018), genetic diversity showed a declining trend in 2018, compared to 2016 and 2017. Effective population size also declined in 2017 and 2018 compared to 2016.
- Trends in genetic diversity and effective population size suggest that feral cat control has resulted in population contraction in the study area.

1 Background

Feral cats (*Felis catus*) are recognised as one of the most harmful invasive species globally (Doherty et al., 2016). Termed ‘feral’ when living independently of humans in the wild, feral cats have a substantial negative impact on native wildlife through predation, competition, and disease transmission (Doherty et al., 2017). They have been implicated in the severe population decline and even extinction of many species (Doherty et al., 2016). Feral cats are also a major driver of mammal declines in Australia, particularly those species that fall within the size range of their preferred prey, known as the ‘critical weight range’ (35 g to 5.5 kg) (Burbidge & McKenzie, 1989). Estimates suggest that there are between 2.1 to 6.3 million feral cats in total across 99.8% of Australia's land area, including both natural and human modified environments (Legge et al., 2017). Thus, effective conservation management strategies are needed to mitigate the impact of feral cats on native wildlife, particularly to maintain viable populations of ‘critical weight range’ threatened Australian mammals.

Genetic data can provide valuable information about the history and ecology of invasive species (Le Roux & Wieczorek, 2009). For example, previous studies in Australia reveal that feral cats likely originated from Europe following human colonisation (Koch et al., 2015; Spencer et al., 2016). Feral cats show high levels of genetic diversity and gene flow across Australia, with limited population genetic structure, suggesting they have a high capacity for dispersal (Spencer et al., 2016). To date, studies using genetics to understand feral cat populations have mainly used mitochondrial and microsatellite genetic markers. However, Single Nucleotide Polymorphisms (SNPs) obtained via genomic sequencing can provide increased resolution to detect subtle patterns of population genetic structure and relatedness (Fischer et al., 2017; Telfer et al., 2015), and therefore may yield additional insights into the dynamics of feral cat populations.

Genetic analyses can help guide the design of management and eradication programs through identifying patterns of genetic structure, gene flow, diversity and relatedness (Koch et al., 2020). This information can highlight areas of the landscape with high connectivity, indicating possible migration routes, as well as potential source populations when new areas are colonised. For example, a study on Christmas Island found that feral cats were able to disperse and breed successfully over the entire island (Koch et al., 2020). Efforts targeting the population as a whole reduced the effective population size over a five-year period. Another study on Dirk Hartog Island, Western Australia, found that the feral cat population on the island was likely reproductively isolated from the mainland, making it feasible to eradicate the feral cat population long-term (Koch et al., 2014).

1.1 Dryandra Woylie/Numbat Predator Control Project

The Dryandra Woodland National Park (comprising of Lol Gray, Highbury and Montague State Forests) is located 160 kilometres south-east of Perth in the Department of Biodiversity, Conservation and Attraction’s (DBCA) Wheatbelt Region. It comprises 17 discrete blocks scattered across a north-south distance of about 50 kilometres, separated and contained by large areas of agricultural land, where edge effects and fragmentation exacerbate impacts of introduced species. Dryandra Woodland represents the largest and most diverse remnants of native vegetation in

the central Western Australian wheatbelt. It is home to over 800 native plant species and 24 mammal, 41 reptile, 8 frog and 98 bird species (DEC, 2011).

Woylies and numbats are listed as critically endangered and endangered, respectively, under Western Australia's Biodiversity Conservation Act 2016. They are also listed as endangered under the Commonwealth's Environmental Protection and Biodiversity Act 1999. Dryandra Woodland is one of only two sites containing wild populations of these species and the localised extinction of either species would result in an irreparable loss of genetic diversity of the species. Prior to feral cat control (detailed below) there was strong evidence that feral cats represented the primary predator of both woylies and numbats in the Woodland, particularly given fox numbers had been reduced by management (Marlow et al., 2015).

The Dryandra Woylie/Numbat Predator Control Project commenced in April 2015 to conserve populations of numbat and woylie in the Dryandra Woodlands through on-ground feral predator management. The Project is an integrated feral cat-fox control program at Dryandra Woodland and adjoining private land. This project aims to recover the wild populations of the woylie and numbat that occur there.

The Project builds upon existing operational management and associated research projects within DBCA estate, including the Western Shield program, protecting highly susceptible fauna from fox and cat predation. This project acknowledges that baiting on conservation estate alone may not be effective in mitigating feral cat numbers and subsequent predation, and therefore combines multiple efforts and tools to control introduced predators. The active participation of neighbours to control feral cats and foxes on their property is needed to increase the effectiveness of the Department's Western Shield program and is critical for the long-term viability of these and other fauna species in Dryandra Woodland. The project targeted cats and foxes through an integrated approach of baiting on reserve, trapping and shooting programs (DBCA, 2017).

The Peel-Harvey Catchment Council (PHCC) partnered with the DBCA Wheatbelt Region and Project Numbat in April 2017 through the Farmers for Fauna project to assist landholders adjacent to Dryandra Woodland to achieve effective off-reserve feral cat and fox control to help prevent invasion and re-invasion into the woodland. The involvement and support of the community, particularly adjacent landowners and managers are important to the conservation of Dryandra Woodland's biodiversity. Their involvement in off-reserve activities increases the effectiveness of integrated feral cat and fox management within Dryandra Woodland. As part of these activities, DNA samples are collected from humanely euthanized animals to contribute to research on their population dynamics.

1.2 Project aims

This project involves undertaking a SNP-based genetic analysis of samples collected following cat control activities, with the aim to:

- Investigate potential source populations of feral cats in Dryandra.
- Explore patterns of relatedness between Dryandra and potential source populations.
- Explore genetic evidence for effectiveness of cat control.
- Provide information that can help adapt and focus control efforts to manage the threat of feral cats in this landscape.

2 Materials and Methods

2.1 Sampling location and material

Feral cat control was undertaken by DBCA Wheatbelt Regional staff within Dryandra Woodlands. In addition, feral cat control off-reserve activities were undertaken by Dryandra Woodland's neighbouring landholders and managers, with support from PHCC. Ear notches were obtained from humanely euthanised individual cats and stored in 70-100% ethanol or 20% DMSO prior to laboratory analyses. Tissue samples were also collected from feral cat carcasses killed by car strikes or suspected baiting deaths found within Dryandra Woodlands and surrounding neighbouring properties (see Appendix 1 for sample information). A total of 176 tissue samples were collected from animals in Dryandra woodland and surrounding townships and agricultural properties (Figure 1) between December 2015 and November 2021.

Samples were grouped spatially based on natural breaks in the landscape and major townsites for visualisation purposes and to enable the comparison of relatedness and parentage estimates among Dryandra and surrounds and potential source populations (Figure 1). Individuals for which coordinate information was missing were removed, as analyses relied on accurate spatial data.

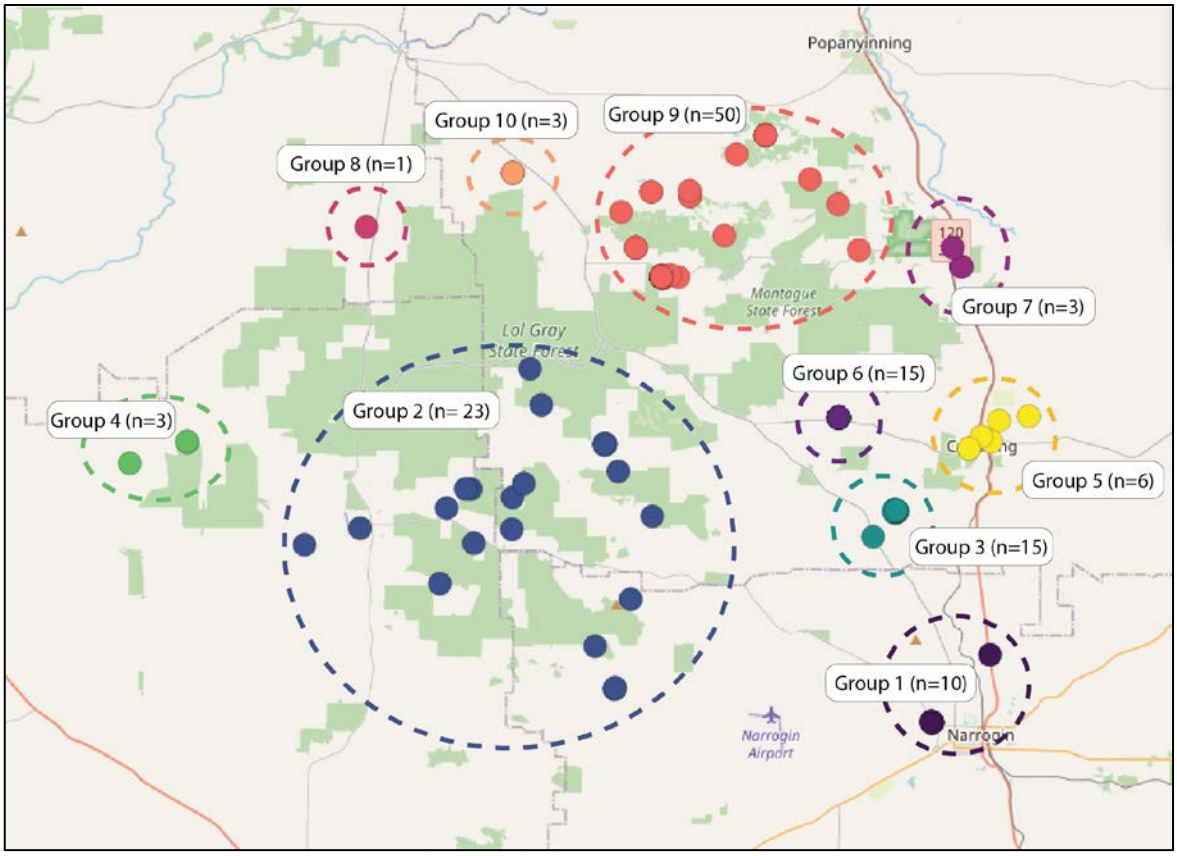


Figure 1 Map of the study area, including spatial groupings and the number of individuals (n) within each group.

2.2 DNA extraction and SNP genotyping

Genomic DNA was extracted from tissue samples using a standard 'salting out' protocol (Sunnucks & Hales, 1996) with the addition of 3 µL 10 mg/mL RNase to the TNES buffer to remove RNA contamination. A total of 176 tissue samples were sent to Diversity Arrays Technology Pty Ltd (DArT), for library preparation, sequencing, quality control and SNP genotyping (DArTseq). DArTseq™ uses enzyme digestion to prepare samples for reduced representation sequencing on an Illumina Hiseq2500, followed by read assembly, quality control and SNP calling through DArT's proprietary software (Cruz et al., 2013; Kilian et al., 2012; Melville et al., 2017).

2.3 SNP filtering

SNP datasets contain a large volume of genetic markers, for which individual testing and evaluation is not feasible. Therefore, filtering these data based on sensible quality control thresholds is essential to reduce noise, artefacts and errors that can be introduced in the laboratory or through genotyping error (O'Leary et al., 2018). We followed filtering protocols outlined in Shaw et al., (2022) to rigorously filter the raw SNP dataset provided by DArT, with the goal to obtain a set of genetic markers that accurately represented independent loci. Thresholds were determined by visualising the raw data in R version 4.1.2 (R Core Team, 2022). Full details about the SNP filtering and data cleaning protocol can be found in Table 1 and Appendix 2.

Table 1 Filters and sample sizes for each dataset, where all datasets underwent base line filters and additional filters are listed for specific analyses.

Dataset	Analyses	Filters	Sample sizes
Total (baseline)	All (total, baseline dataset)	Call rate (individual ≥0.55, locus ≥0.9) Read count (≥20 and ≤ 150) Repeatability average (≥0.95) Minor allele frequency (≥0.025) Secondaries (1 SNP per sequence) Sex-linked SNPs (removed) Linkage disequilibrium (≥ 0.5) Missing coordinate data (individuals removed)	Individuals = 135 SNPs = 3,241
A	Relatedness	No additional filters	Individuals = 135 SNPs = 3,241
B	Parentage	Locus call rate (increased to ≥0.95) Minor allele frequency (increased to ≥0.3) Hardy Weinberg Equilibrium (loci that do not conform to HWE removed)	Individuals = 135 SNPs = 387
C	Population genetic structure	Highly related individuals (removed) Spatial trimming (1 individual/500 m radius)	Individuals = 32 SNPs = 3,241

Dataset	Analyses	Filters	Sample sizes
D	Genetic diversity statistics Internal heterozygosity Fine-scale genetic structure	Highly related individuals (removed) Hardy Weinberg Equilibrium (loci that do not conform to HWE removed)	Individuals = 72 SNPs = 3,105
E	Temporal genetic diversity statistics Effective population size	Highly related individuals (removed) Hardy Weinberg Equilibrium (loci that do not conform to HWE removed) Subset to years with large enough sample sizes (ten or more individuals = 2016, 2017 and 2018)	Individuals = 59 SNPs = 3,105

2.4 Relatedness and parentage analysis

We calculated pairwise relatedness (Wang, 2002) in the R package related (Pew et al., 2015), using dataset A (Table 1). Pairs of individuals with a relatedness estimate of ≥0.2 were identified as 'highly related', as these represent plausible values for half-siblings through to parent-offspring relationships. We then summarised these findings spatially, by visualising the number of 'highly related pairs' within and between spatial groups, to explore potential source populations into Dryandra.

To go beyond broad estimates of relatedness, the R package SEQUOIA (Huisman, 2017) was used to assign parentage and identify full sibling clusters within dataset B (Table 1). SEQUOIA is a maximum likelihood method that combines genetic data with demographic information (priors). Here, the sex of individuals (if known) and the minimum and maximum potential year of birth were used as priors in the analysis. The maximum year of birth was taken as the year of collection. We assumed feral cats live an average of five years, so the minimum birth year was taken to be five years before the collection year. We reduced the dataset down to a more informative set of SNPs (387), by filtering for Hardy Weinberg Equilibrium, increasing the call rate to 95% and increasing the minor allele frequency to 0.3, as recommended by Huisman (2017). Again, parentage and full-sibling assignment was summarised in relation to spatial groupings to determine whether parents of Dryandra individuals could be identified in surrounding source locations.

After summarising relatedness and parentage results, we removed one individual from each of the 'highly related pairs', as close relatives can bias population genetic analyses (Wang, 2018). This resulted in 63 individuals being removed from the dataset (Table 1).

2.5 Population genetic structure and genetic diversity

Population genetic structure was investigated to identify any landscape elements that may act as barriers or limit cat movement and to evaluate the potential for assigning migrant individuals in Dryandra to original source populations. To achieve this, the dataset was first trimmed spatially to include just one sample per 1 km^2 (dataset C, Table 1), creating an even distribution across the landscape to reduce bias associated with heavily sampled locations. Next, a Principal Coordinate

Analysis (PCoA) was performed in the R package dartr (Gruber et al., 2018), to determine if there were natural genetic clusters (representing ‘populations’) in the data. We then used the TESS3 algorithm in the R package tess3r (Caye & Francois, 2016) to estimate individual ancestry coefficients, while also taking geographic information into account. We tested for one through to six potential populations (K) with 50 repetitions for each value and a maximum of 200 optimisation iterations. We used the default settings for the remaining parameters and masked 10% of the data to use for the cross-validation. If population genetic structure is present, individuals will fall within K population clusters, as indicated by a plateau, or change in slope in the cross-entropy criterion (based on the best performing run with the lowest root mean squared error).

Genetic diversity summary statistics were then calculated within any population/s detected in the above tess3r analysis, with the HWE filtered dataset D (Table 1, Appendix 2). Observed heterozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient (F) were calculated with the R package adegenet (Jombart, 2008), while allelic richness (AR) was calculated in PopGenReport (Adamack & Gruber, 2014). Summary statistics were also calculated temporally for years with ten or more individuals (considered here as an adequate sample size for such analyses), which included 2016, 2017 and 2018 (dataset E, Table 1), to investigate whether there were substantial changes in genetic diversity with ongoing cat control. Effective population size (Ne) was also estimated using the Linkage Disequilibrium method for each of these years (dataset E, Table 1), using NeEstimator V2.1 (Do et al., 2014), implemented in the dartr package (Gruber et al., 2018). Ne estimates were reported alongside parametric confidence intervals, estimated with chi-square approximation (Waples, 2006).

Additionally, genetic diversity was estimated at the individual level with the R function GENHET (Coulon, 2010), which calculates the proportion of heterozygous loci for each individual (PHt) (Aparicio et al., 2006). We calculated this for all individuals in dataset D and took the average value within spatial groups, interpolating PHt across the landscape, using an inverse distance weighting scheme, to show areas of high or low genetic diversity.

2.6 Fine-scale genetic structure

Fine-scale patterns of genetic structure (at the individual level) were explored to understand any limitations to cat movement using spatial autocorrelation analysis of multilocus SNP genotypes for dataset D (Table 1), following methods outlined in Banks & Peakall, (2012), Double et al., (2005) and Smouse & Peakall (1999). The autocorrelation coefficient, r, describes the genetic similarity between all individuals within specified geographic distance classes and was calculated using the R package dartr (Gruber et al., 2018). The r coefficient was calculated for distance classes of increasing size, for all individuals and separately for females and males, to test for differences in dispersal between the sexes. The distance at which r is no longer significantly positive (where confidence intervals overlap zero) approximates the extent of positive genetic structure (Double et al., 2005). Statistical significance was tested by generating 95% bootstrap confidence intervals using 1000 bootstrap samples.

3 Results

3.1 SNP genotyping and filtering

Of the 176 samples sent to DArT, 20 samples failed to sequence. Library construction was challenging and required multiple attempts for many samples. Additionally, the composition of several samples was dominated by microbial contamination, likely where tissue samples were obtained from animals that had experienced field decomposition and degradation prior to sampling. The raw DArT dataset contained 12,098 SNPs for 156 individuals, although a further 15 individuals were removed during filtering due to a large amount of missing data and a further six individuals were removed due to missing coordinate information. After filtering, the total (baseline) dataset included 3,241 independent SNP loci for 135 individuals (Table 1). This dataset was filtered further for specific analyses, as described in Table 1 (with further detail about each specific filter provided in Appendix 2).

3.2 Relatedness and parentage analysis

There were 316 pairs of ‘highly related individuals’ ($r \geq 0.2$) in the dataset (Figure 2, Appendix 3), with most of these occurring in the northeast of the study area. Of the two Groups with the highest sample sizes, 21% of pairwise individual comparisons in Group 9 were ‘highly related’, compared to only 0.4% in Group 2. For all remaining Groups, the percentage varied between 0 to 33.3% (although some of these Groups had sample sizes as low as three individuals). Thus, it is important to note that these results do not account for sampling effort.

To infer movement of cats between patches, we identified between 1 – 41 ‘highly related pairs’ shared among spatial Groups. Related individuals were found between Group 2 and five different potential source populations. The number of other groups sharing related pairs was lower, ranging from 0 – 3.

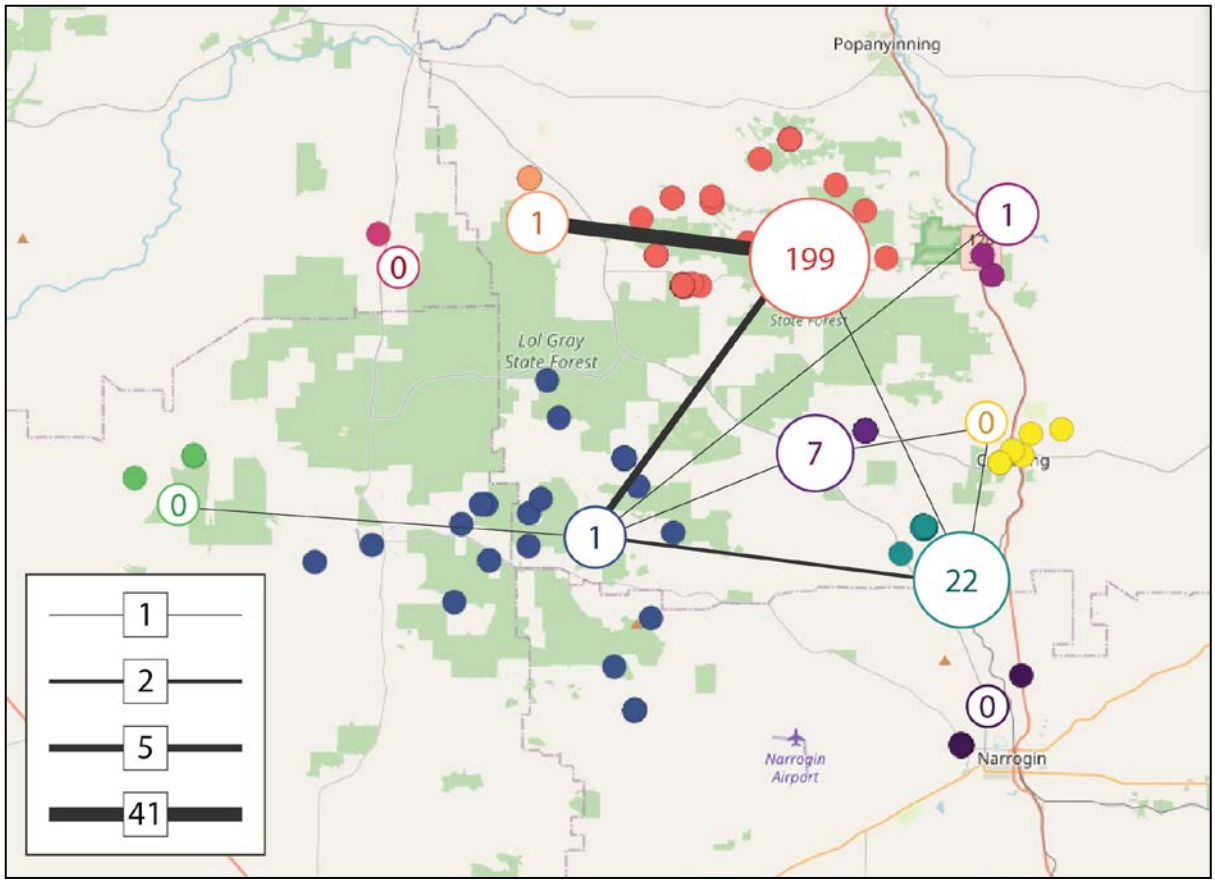


Figure 2 Pairwise relatedness patterns across the study landscape, with circles representing the number of 'highly related pairs' of individuals within each group, and line weight representing the number of 'highly related pairs' among groups.

Parentage analysis revealed a similar trend, with more parent-offspring relationships and full-sibling clusters assigned in the northeast (Group 9) and east (Group 3) of the study area compared to within Group 2 (Tables 1 – 2). Of the 11 full-sibling clusters (representing potential litters, Table 1), eight were from Group 9. The remaining sibling clusters were made up of individuals from Group 3 and 10. Two sibling clusters suggest dispersal of individuals within litters between Group 9 and 10, and between Group 9 and Group 2. Parents (mothers and/or fathers) were assigned for 12 individuals in Group 3 and 9 (Table 2). All parents were from the same location (Group) as their offspring, with the exception of one father-offspring pair from Group 10 and 9, respectively, suggesting Group 10 was the potential source of juvenile recruitment into Group 9.

Table 2 Individuals identified as full-siblings (i.e., same mother and father), potentially from the same litter, with the spatial group the individual was sampled from in parentheses.

Cluster	Offspring 1	Offspring 2	Offspring 3	Offspring 4	Offspring 5
1	T14328 (9)	T14331 (9)	T14422 (9)	NA	NA
2	T14374 (9)	T14373 (9)	NA	NA	NA
3	T14268 (9)	T14376 (9)	T14375 (9)	T14409 (9)	T14336 (9)
4	T14278 (9)	T14387 (9)	T14424 (9)	NA	NA
5	T14400 (3)	T14402 (3)	T14406 (3)	T14399 (3)	NA
6	T14300 (10)	T14301 (10)	T14329 (9)	T14341 (9)	T14389 (9)
7	T14374 (9)	T14380 (9)	T14373 (9)	NA	NA
8	T14376 (9)	T14409 (9)	NA	NA	NA
9	T14279 (9)	T14424 (9)	NA	NA	NA
10	T14278 (9)	T14387 (9)	NA	NA	NA
11	T14314 (2)	T14336 (9)	NA	NA	NA

Table 3 Parentage assignment, with the spatial group the individual was sampled from in parentheses.

id	Mother	Father
T14387 (9)	NA	T14302 (10)
T14328 (9)	NA	T14326 (9)
T14424 (9)	NA	T14326 (9)
T14326 (9)	NA	T14331 (9)
T14380 (9)	T14325 (9)	NA
T14382 (9)	T14325 (9)	T14383 (9)
T14280 (9)	T14390 (9)	T14330 (9)
T14398 (3)	T14395 (3)	NA
T14400 (3)	T14396 (3)	NA
T14402 (3)	T14396 (3)	NA
T14406 (3)	T14396 (3)	NA
T14399 (3)	T14396 (3)	NA

3.3 Population genetic structure and genetic diversity

The first two axes of the PCoA cumulatively explained 6.2% or 9.5% of the variation in the dataset, depending on whether the full or the spatially trimmed dataset was used (Figure 3). In both cases, it did not reveal any genetic clustering of samples indicative of population genetic structure resulting from barriers to animal movement. Likewise, the cross-entropy score for the TESS3 analysis of both datasets decreased linearly for increasing values of K (numbers of populations), suggesting that the samples in this study form one genetic population (Figure 4).

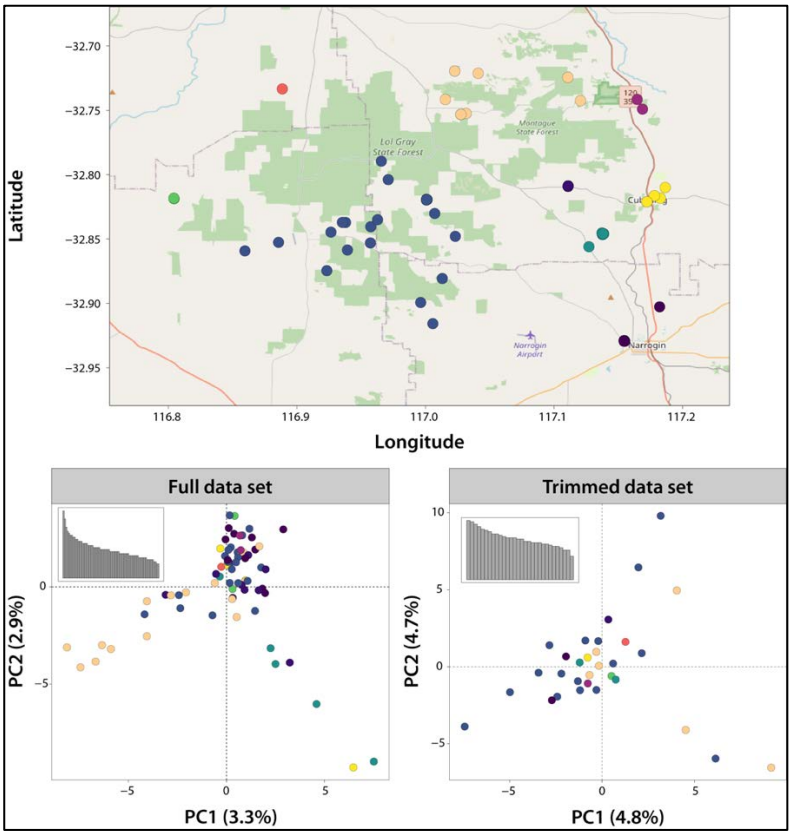


Figure 3 Map of the study region with sample locations and the first two axes of the Principal Coordinate Analysis (for the full dataset and the spatially trimmed dataset), with colours representing the spatial groupings of individuals.

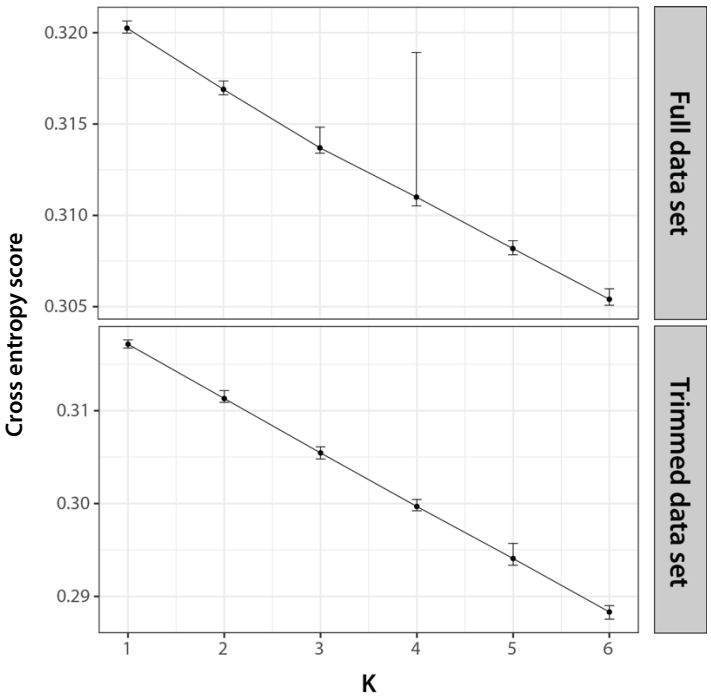


Figure 4 Cross entropy plot from the TESS3 analysis, showing K values one through to six.

The mean allelic richness for the population was 1.997 ± 0.006, with lower observed (0.199 ± 0.002) than expected heterozygosity (0.236 ± 0.003), potentially indicating the presence of some inbreeding (F = 0.144 ± 0.003, Table 3). These values were fairly consistent across 2016 to 2018, although with a slight decrease in 2018 (Figure 5). Effective population size was higher in 2016 (Ne = 244, CIs = 227 – 263.7) than in later years where confidence intervals overlapped (2017: Ne = 153.2, CIs = 148.6 – 158.1; 2018: Ne = 133.6, CIs = 117.6 – 154.5; Figure 5). Sample sizes were lower in years 2019 – 2021 but indicated that Ne also remained low in 2021 (Appendix 4). Spatial interpolation of mean internal heterozygosity (Figure 6) showed that genetic diversity was highest in the northeast, and generally decreased towards the west of the study area.

Table 4 Genetic summary statistics for the total population across all years.

Metric	Mean	Standard error
Observed heterozygosity (Ho)	0.199	2.16×10^3
Expected heterozygosity (He)	0.236	2.49×10^3
Inbreeding coefficient (F)	0.144	3.28×10^3
Allelic richness (AR)	1.997	0.59×10^3

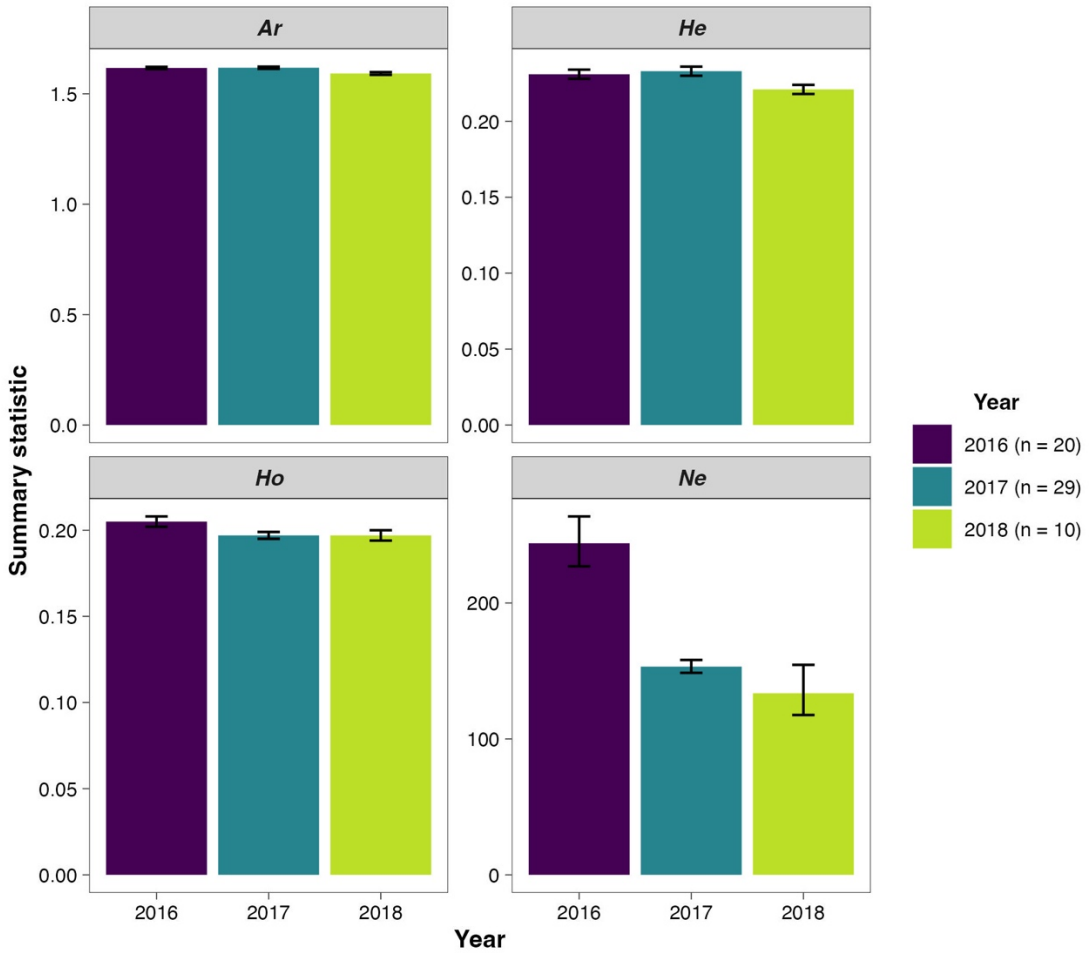


Figure 5 Genetic summary statistics and standard error (Ar, He and Ho), or parametric confidence intervals (Ne) across each year for which sampling sizes were greater than or equal to 10 individuals.

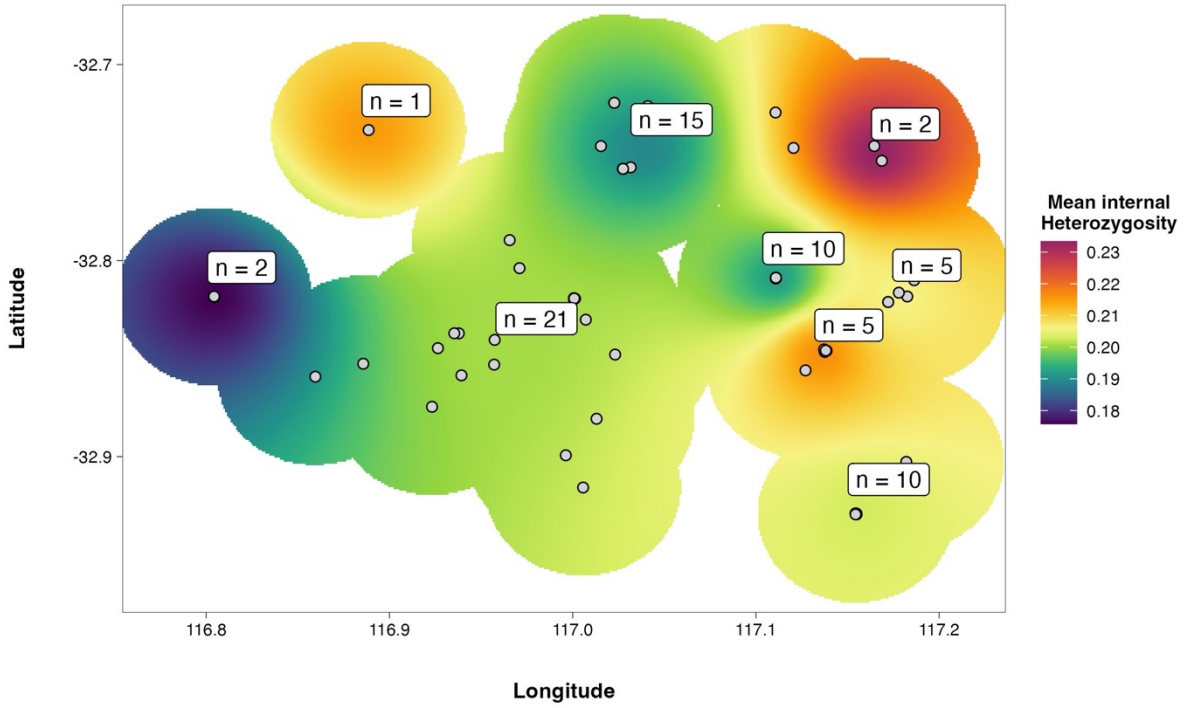


Figure 6 Mean genetic diversity (internal heterozygosity, PHt) for each spatial grouping, interpolated across the study area, with grey points and labels representing the sample sizes used in each group average.

3.4 Fine-scale genetic structure

Multi-locus genetic spatial autocorrelation detected significant fine-scale spatial genetic structure up to 20 km, suggesting that this is the scale of the genetic neighbourhood of individuals in the study area (Figure 7).

There was no significant difference in spatial genetic structure between the sexes, meaning that we did not detect any signature of sex-biased dispersal. However, this could potentially be due to the low female sample sizes resulting in large confidence intervals in this analysis.

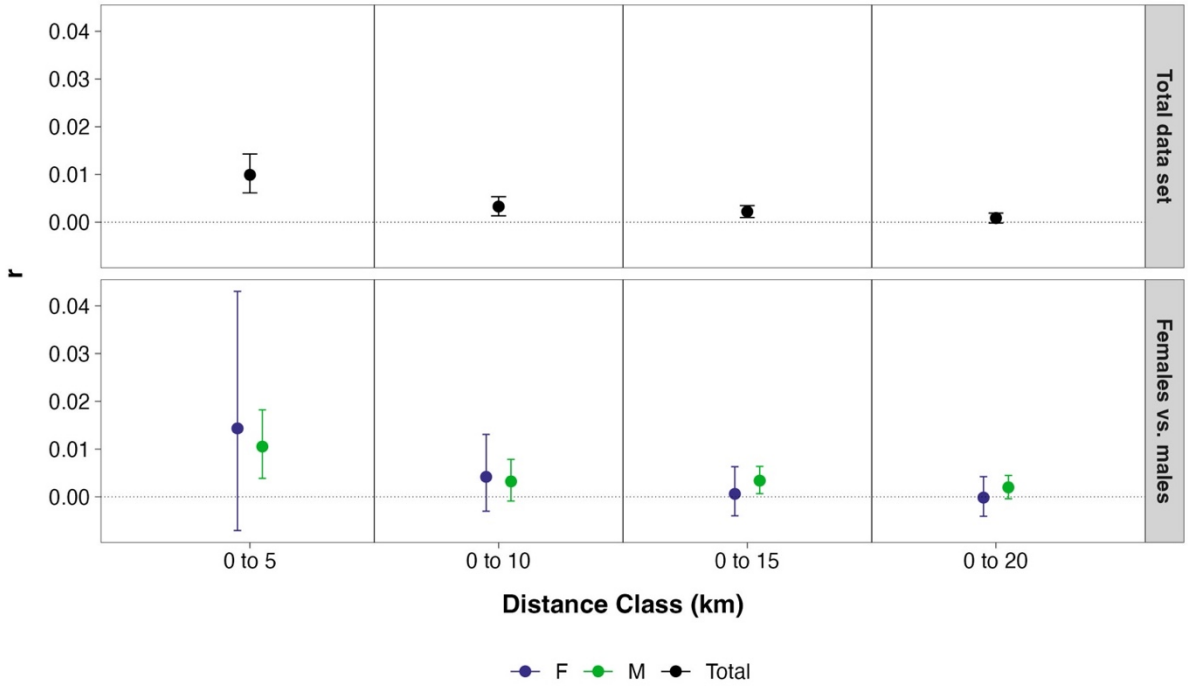


Figure 7 Correlograms for the total dataset, and for females and males, showing fine-scale spatial genetic structure (r) for increasing distance classes. Error bars represent 95% bootstrap confidence intervals and significant fine-scale spatial genetic structure is detected when confidence intervals do not overlap zero.

4 Discussion

Genetic analysis can provide insight into population dynamics of invasive species, in particular by inferring population genetic structure and the spatial scale of dispersal, as well as source/sink dynamics (Burgess et al., 2022; Koch et al., 2020; Watson et al., 2021). Insight into these aspects of invasive species biology can assist managers to design and effectively apply control methods. Here, we used high resolution SNP genotyping to assess patterns of relatedness and dispersal in the feral cat population captured in the Dryandra woodland and surrounding areas, in the wheatbelt region of Western Australia.

Overall, we found there was no detectable population genetic structure in the feral cat population in the studied landscape, suggesting that cats are moving freely and there are no quantifiable barriers to dispersal. This is in line with previous genetic studies on feral cats in Australia, which have also failed to find genetic structure at the scale of islands (Koch et al., 2020), marshes and surrounding uplands (Cowen et al., 2019), to much of the Australian continent (Spencer et al., 2016; Koch et al., 2015). Taken together, these genetic results indicate feral cats have a very high capacity for dispersal which does not appear to be influenced by the spatial configuration of agricultural versus woodland areas, which is likely a limiting factor for dispersal in other native species.

Whilst we found no strong structure at the landscape-scale, analysis of patterns of genetic relatedness amongst individual cats indicated the presence of low, but significant, fine-scale spatial genetic structure up to 20 km (i.e., cats are positively related over this distance). This result can be interpreted as defining the genetic neighbourhood of *Felis catus* in the study region. The genetic neighbourhood area (Na) spans the area where animals could mate randomly and is related to the effective size of a population (Wright, 1969). Male-biased dispersal has been reported for feral cats in the Fortescue Marsh (Cowen et al., 2019), however, sample sizes in our study were insufficient to detect a significant effect of dispersal differences between male and female cats, with spatial autocorrelation results being similar among the sexes.

As was observed in Cowen et al., (2019), the high dispersal capacity of feral cats (i.e., leading to low genetic structure) prohibits the use of population assignment methods to explicitly detect source-sink population dynamics in the current study. Nevertheless, qualitative assessment of patterns of genetic relatedness, particularly the distribution of siblings and parent-offspring pairs amongst spatial groups, and genetic diversity allows some inference on putative source populations in the region. Here, we found that feral cats located towards the north and east of the study area (especially Group 9, located in the outlying blocks of the Montague State forest) comprised high numbers of related individuals suggestive of a stable population structure and a putative source of cats moving into the surrounding landscape. Multiple sibling clusters (families) were detected in Groups 9 and 10 and small family groups dominated Group 3 located on agricultural properties southwest of Cuballing. There was evidence of dispersal of one half of a sibling pair dispersing between Group 9 and Group 2, to the south of the area. Group 2 located in the southern forest blocks had few internally-related individuals, but many related individuals between groups. This pattern is suggestive of a disrupted population structure and potentially represents a population sink. Even though we cannot be confident in the

direction of movement of animals, the lower overall relatedness and lower genetic diversity of this southern population of cats supports this observation. Higher genetic diversity in the north and eastern populations suggest a larger population size in this region and, given the proximity to human settlements, that these areas may be sources. Further sampling of cat populations closer to town infrastructure (e.g. rubbish tips) may also help illuminate the role of ‘urbanised’ populations of cats and whether they are contributing as sources.

Coordinated feral cat control in the Dryandra woodland has been ongoing since April 2017. Although sample sizes varied between years, we found that genetic diversity was similar across years between 2016 and 2018, although with a declining trend observed in 2018. Effective population size (Ne) also showed a declining trend and was significantly lower in both 2017 and 2018, compared to 2016. While sample sizes precluded analysis for some later years, we found that Ne remained low in 2021. We can infer from these results that the control program has reduced the overall feral cat population in the area, and the decrease in effective population size is indicative of this bottleneck (Hedrick, 2011). Additional information on changes in cat abundance, e.g. from camera traps or track counts following an appropriate statistical design, would be beneficial in corroborating these results and confirming an overall reduction in cat numbers due to control methods, rather than a disruption in population dynamics due to other factors that may be unrelated to cat control.

As indicated in Cowen et al., (2019), the use of genetic monitoring to measure the impact and effectiveness of invasive species control programs has potential but ensuring adequate sample sizes over a long enough time-frame, and a large enough scale is critical to the success of such studies (Cowen et al., 2019). For this reason, it is recommended that feral cat samples continue to be collected and stored appropriately for future genetic analysis. Genetic monitoring can complement other methodologies estimating abundance and/or occupancy in demonstrating the success of control programs.

Given the challenge of working with high dispersal species such as the feral cat, we nevertheless were able to find qualitative evidence of potential source populations, and an estimate of the distance individuals are dispersing when establishing new territories across the study area (20 km). Feral cat and fox control seems to have been most effective in the southern forest blocks and future efforts may need to specifically target cat populations in the north and eastern blocks to reduce overall pressure in the area (while maintaining southern control efforts). Additional sampling around townships may assist in determining these as a source of cats moving into woodland areas. Nonetheless, information on spatial genetic patterns in the feral cat population in Dryandra Woodland and surrounding areas demonstrates the importance of coordinated efforts amongst conservation agencies, local Natural Resource Management groups and private landholders in implementing landscape-scale cat management in this system.

Appendices

Appendix 1 Sample information

Collection details for *Felis catus* samples for which DArTSeq SNP genotypes were able to be generated (i.e., excluding information for 20 samples that failed to sequence).

Tissue ID	Location	Date	Sex	Latitude	Longitude	ID Tag
T14258	West Cuballing	1/1/2021	NA	-32.809	117.111	010121U1
T14259	West Cuballing	1/1/2021	NA	-32.809	117.111	010121U2
T14260	West Cuballing	1/1/2021	NA	-32.809	117.111	010121U3
T14261	North of Montague	1/3/2016	F	-32.724	117.111	010316F1
T14262	South of Lol Gray	1/5/2018	NA	-32.819	117.001	010518U1
T14263	West Yornanning	1/6/2017	M	-32.753	117.028	010617M1
T14264	South of Yornaning	1/10/2020	F	-32.749	117.169	011020F1
T14265	South of Yornaning	1/10/2020	F	-32.749	117.169	011020F2
T14266	West Cuballing	2/10/2017	M	-32.809	117.111	021017M1
T14267	Narrogin	3/2/2020	M	-32.929	117.155	030220M1
T14268	West Yornanning	4/6/2017	M	-32.753	117.028	040617M1
T14269	West Cuballing	4/11/2018	NA	-32.809	117.111	041118U1
T14270	West Cuballing	4/11/2018	NA	-32.809	117.111	041118U2
T14273	West Yornanning	5/6/2017	M	-32.753	117.028	050617M1
T14274	West of Contine	5/9/2017	NA	-32.853	116.886	050917U1
T14275	Narrogin	5/10/2016	M	-32.929	117.155	051016M1
T14276	West Cuballing	6/4/2018	NA	-32.809	117.111	060418U1
T14278	West Yornanning	6/6/2017	F	-32.753	117.028	060617F1
T14279	West Yornanning	6/6/2017	M	-32.753	117.028	060617M1
T14280	West Yornanning	6/6/2017	M	-32.753	117.028	060617M2
T14281	West Yornanning	6/6/2017	M	-32.753	117.028	060617M3
T14283	Forestry Road	6/7/2017	M	-32.719	117.041	060717M1
T14284	Narrogin	7/7/2016	M	-32.929	117.155	070716M1

Tissue ID	Location	Date	Sex	Latitude	Longitude	ID Tag
T14285	West Cuballing	7/9/2021	NA	-32.809	117.111	070921U1
T14286	NA	7/11/2017	M	NA	NA	071117M1
T14287	NA	7/11/2017	M	NA	NA	071117M2
T14288	NA	7/11/2017	M	NA	NA	071117M3
T14289	NA	7/11/2017	M	NA	NA	071117M4
T14290	NA	7/11/2017	M	NA	NA	071117M5
T14291	NA	7/11/2017	M	NA	NA	071117M6
T14292	West Cuballing	8/5/2020	NA	-32.809	117.111	080520U1
T14294	South of Lol Gray	8/8/2016	F	-32.819	117.001	080816F1
T14295	East of forest blocks	8/8/2016	M	-32.818	116.804	080816M1
T14296	West Yornanning	8/8/2019	F	-32.753	117.028	080819F1
T14297	West Yornanning	8/8/2019	NA	-32.753	117.028	080819U1
T14298	Narrogin	8/10/2018	M	-32.929	117.155	081018M1
T14300	North of Lol Gray	9/6/2017	F	-32.712	116.958	090617F1
T14301	North of Lol Gray	9/6/2017	F	-32.712	116.958	090617F2
T14302	North of Lol Gray	9/6/2017	M	-32.712	116.958	090617M2
T14303	South of Lol Gray	9/8/2016	M	-32.820	117.001	090816M1
T14304	NA	9/8/2018	M	NA	NA	090818M1
T14305	NA	9/8/2018	M	NA	NA	090818M2
T14306	Dryandra	9/12/2017	M	-32.790	116.966	091217M1
T14307	Dryandra	10/12/2017	M	-32.804	116.971	101217M1
T14308	North of Contine	11/4/2016	M	-32.881	117.013	110416M1
T14310	East of Yornaning	11/6/2020	M	-32.743	117.121	110620M1
T14311	Forestry Road	11/6/2021	NA	-32.721	117.041	110621U1
T14312	Forestry Road	11/6/2021	NA	-32.721	117.041	110621U2
T14313	South of Lol Gray	11/7/2016	F	-32.819	117.001	110716F1
T14314	North of Contine	11/10/2016	M	-32.837	116.938	111016M1
T14315	Cuballing townsite	11/10/2018	M	-32.821	117.172	111018M1
T14317	West Cuballing	14/5/2020	NA	-32.809	117.111	140520U1
T14318	Cuballing	14/8/2017	F	-32.810	117.186	140817F1

Tissue ID	Location	Date	Sex	Latitude	Longitude	ID Tag
T14319	Cuballing	14/8/2018	M	-32.818	117.183	140818M1
T14320	Cuballing	14/10/2018	NA	-32.808	117.200	141018U1
T14321	NA	14/11/2017	F	NA	NA	141117F1
T14322	West Cuballing	14/11/2017	M	-32.809	117.111	141117M1
T14323	Cuballing	14/12/2015	F	-32.816	117.178	141215F1
T14324	Contine	15/3/2019	F	-32.837	116.935	150319F1
T14325	South of Yornaning Road	15/4/2017	F	-32.753	117.036	150417F1
T14326	North of Montague	15/4/2017	M	-32.715	117.097	150417M1
T14328	Forestry Road	15/6/2017	F	-32.705	117.063	150617F2
T14329	North of Montague	15/6/2017	F	-32.697	117.076	150617F3
T14330	North of Montague	15/6/2017	M	-32.697	117.076	150617M2
T14331	North of Montague	15/6/2017	M	-32.697	117.076	150617M3
T14332	North of Contine	15/10/2016	M	-32.840	116.957	151016M1
T14335	Cuballing townsite	16/10/2018	NA	-32.821	117.172	161018U1
T14336	North of Yornaning Road	17/2/2017	M	-32.742	117.015	170217M1
T14338	Narrogin	17/5/2021	M	-32.929	117.155	170521M1
T14339	Narrogin	17/6/2017	M	-32.929	117.155	170617M1
T14340	West Yornanning	18/6/2017	F	-32.753	117.028	180617F1
T14341	West Yornanning	18/6/2017	F	-32.753	117.028	180617F2
T14342	West Yornanning	18/6/2017	F	-32.753	117.028	180617F3
T14343	West Yornanning	18/6/2017	F	-32.753	117.028	180617F4
T14344	North of Contine	18/10/2016	M	-32.853	116.957	181016M1
T14345	South of Lol Gray	18/11/2016	M	-32.848	117.023	181116M1
T14346	North of Yornaning Road	19/2/2017	M	-32.742	117.015	190217M1
T14347	Contine	19/3/2019	M	-32.899	116.996	190319M1
T14348	North of Contine	19/5/2016	NA	-32.845	116.926	190516U1
T14349	West Yornanning	19/7/2017	F	-32.753	117.028	190717F1
T14350	North of Yornaning Road	19/7/2017	F	-32.720	117.023	190717F2
T14351	West Yornanning	19/7/2017	F	-32.753	117.028	190717F3
T14353	North of Yornaning Road	19/7/2017	M	-32.720	117.023	190717M2

Tissue ID	Location	Date	Sex	Latitude	Longitude	ID Tag
T14354	North of Montague	19/7/2017	M	-32.697	117.076	190717M3
T14355	East of forest blocks	19/8/2016	M	-32.819	116.804	190816M1
T14356	North of Contine	19/10/2016	M	-32.848	116.990	191016M1
T14357	Cuballing townsite	19/10/2018	NA	-32.821	117.172	191018U1
T14358	NA	19/10/2020	F	NA	NA	191020F1
T14359	NA	19/10/2020	M	NA	NA	191020M1
T14360	Narrogin	19/12/2015	M	-32.835	116.963	191215M1
T14363	West Yornanning	20/5/2017	F	-32.753	117.028	200517F1
T14364	Narrogin	20/7/2016	M	-32.929	117.154	200716M1
T14365	Cuballing	20/8/2018	NA	-32.816	117.178	200818U1
T14366	West Cuballing	20/8/2021	M	-32.809	117.111	200821M1
T14368	NA	20/10/2017	M	NA	NA	201017M1
T14369	North of Contine	20/11/2016	F	-32.830	117.007	201116F1
T14371	West Cuballing	21/6/2018	NA	-32.809	117.111	210618U1
T14372	East of Cuballing	21/12/2016	F	-32.809	117.111	211216F1
T14373	North of Yornaning Road	22/2/2017	M	-32.727	117.009	220217M1
T14374	South of Yornaning Road	22/4/2017	F	-32.752	117.027	220417F1
T14375	South of Yornaning Road	22/4/2017	F	-32.753	117.028	220417F2
T14376	South of Yornaning Road	22/4/2017	M	-32.753	117.028	220417M1
T14377	South of Contine	22/9/2019	NA	-32.916	117.006	220919U1
T14378	Yornaning	22/11/2017	M	-32.742	117.165	221117M1
T14379	North of Yornaning Road	23/2/2017	M	-32.742	117.015	230217M1
T14380	South of Yornaning Road	23/4/2016	M	-32.752	117.032	230416M1
T14381	York Williams Road	23/4/2019	NA	-32.733	116.889	230419F1
T14382	West Yornanning	23/5/2017	F	-32.753	117.028	230517F1
T14383	West Yornanning	23/5/2017	M	-32.753	117.028	230517M1
T14384	West Yornanning	23/5/2017	M	-32.753	117.028	230517M2
T14385	West Cuballing	23/5/2021	F	-32.809	117.111	230521F1
T14386	West Yornanning	23/6/2021	NA	-32.753	117.028	230621U1
T14387	North of Montague	23/8/2017	F	-32.697	117.076	230817F1

Tissue ID	Location	Date	Sex	Latitude	Longitude	ID Tag
T14388	West Yornanning	23/8/2017	F	-32.753	117.028	230817F2
T14389	North of Montague	23/8/2017	F	-32.697	117.076	230817F3
T14390	North of Montague	23/8/2017	F	-32.697	117.076	230817F4
T14391	West Yornanning	23/8/2017	M	-32.753	117.028	230817M1
T14392	West Yornanning	23/8/2017	M	-32.753	117.028	230817M2
T14393	West of Contine	23/8/2017	NA	-32.859	116.860	230817U3
T14394	Narrogin	23/11/2021	M	-32.929	117.155	231121M1
T14395	Southwest of Cuballing	25/4/2017	F	-32.845	117.138	250417F1
T14396	North of Yornaning Road	25/4/2017	F	-32.846	117.137	250417F2
T14397	Southwest of Cuballing	25/4/2017	F	-32.845	117.137	250417F3
T14398	Southwest of Cuballing	25/4/2017	F	-32.846	117.138	250417F4
T14399	Southwest of Cuballing	25/4/2017	F	-32.846	117.137	250417F5
T14400	Southwest of Cuballing	25/4/2017	F	-32.846	117.137	250417F6
T14401	Southwest of Cuballing	25/4/2017	F	-32.847	117.138	250417F7
T14402	Southwest of Cuballing	25/4/2017	F	-32.846	117.138	250417F8
T14403	West Yornanning	25/4/2017	F	-32.754	117.028	250417F9
T14404	Southwest of Cuballing	25/4/2017	M	-32.846	117.138	250417M1
T14405	North of Yornaning Road	25/4/2017	M	-32.846	117.138	250417M2
T14406	Southwest of Cuballing	25/4/2017	M	-32.846	117.138	250417M3
T14407	Southwest of Cuballing	25/4/2017	M	-32.845	117.138	250417M4
T14409	South of Yornaning Road	25/4/2017	M	-32.753	117.028	250417M6
T14410	Contine	25/5/2017	F	-32.875	116.923	250517F1
T14411	West Yornanning	25/5/2017	M	-32.753	117.028	250517M3
T14414	Contine	26/5/2017	M	-32.859	116.939	260517M1
T14415	West Cuballing	27/3/2018	M	-32.809	117.111	270318M1
T14416	Contine	27/5/2017	M	-32.866	116.942	270517M1
T14417	Dryandra	28/2/2016	M	-32.845	117.137	280216M1
T14419	Southwest of Cuballing	28/4/2017	M	-32.846	117.139	280417M1
T14422	Forestry Road	28/6/2017	M	-32.719	117.041	280617M1
T14423	West Yornanning	28/9/2020	NA	-32.753	117.028	280920U1

Tissue ID	Location	Date	Sex	Latitude	Longitude	ID Tag
T14424	North of Yornaning Road	29/1/2017	F	-32.737	117.057	290117F1
T14425	East of forest blocks	29/6/2016	M	-32.827	116.777	290616M1
T14426	Southwest of Cuballing	29/6/2017	F	-32.856	117.127	290617F1
T14427	West Cuballing	29/10/2018	F	-32.809	117.111	291018F1
T14428	Narrogin	30/6/2016	F	-32.929	117.154	300616F1
T14429	West Yornanning	30/7/2017	M	-32.753	117.028	300717M1
T14431	South of Contine	30/11/2016	F	-32.916	117.006	301116F1
T14432	South of Contine	30/11/2016	M	-32.916	117.005	301116M1
T14433	South of Turners Road	31/7/2018	NA	-32.825	117.032	310718U1
T14434	Narrogin	31/7/2018	NA	-32.903	117.182	310718U2
T14435	NA	15/6/2017	M	NA	NA	150617M1
T14436	NA	9/6/2017	M	NA	NA	090617M1
T14437	NA	NA	M	NA	NA	NA

Appendix 2 SNP Filtering

SNP filtering and data cleaning protocol, including filtering steps and the thresholds chosen.

The total dataset underwent filters 1 to 9. Additional filters were then performed where relevant, depending on the assumptions of the specific analysis.

Filter	Description	Thresholds (no. loci remaining)
1. Call rate (individual)	The threshold for excluding low quality individuals was chosen by balancing the trade-off between number of samples, importance of lower quality samples (i.e., in unique versus well sampled locations) and number of loci.	≥ 0.55 (16 individuals removed, 12,098 loci remaining)
2. Call rate (locus)	To determine the call rate threshold across loci, the relationship between call rate and population genetic summary statistics (heterozygosity estimates and F-statistics) was explored, under the assumption that data quality and biologically meaningful population genetic metrics should not be correlated. The threshold that minimised this correlation, while retaining a large enough dataset for adequate statistical power was chosen.	≥ 0.9 (6,123)
3. Read count	Upper and lower thresholds were chosen for average read count, as lower read counts under call heterozygotes (sample bias), while very high read counts may represent paralogous regions. To determine the average read count upper and lower thresholds, the relationship between read count and population genetic summary statistics was explored (as above). Thresholds that minimised this correlation, while retaining a large enough dataset for adequate statistical power was chosen.	≥20 and ≤ 150 (4,329)
4. Repeatability average	The repeatability filter threshold was chosen to exclude poor quality loci, while not filtering out SNPs with high levels of heterozygosity (as heterozygote genotype calls may vary between replicates due to slight variations in reference/SNP reads).	≥0.95 (4,329)
5. Minor allele frequency	A MAF threshold that balanced the trade-off between including SNPs that represented sequencing error, versus removing true low frequency/private alleles was chosen. A minimum threshold that equated to each allele appearing at least twice in the dataset was used.	≥0.025 (3,874)
6. Secondaries	Non-independent SNPs were removed by randomly retaining one locus per sequence (and excluding other loci found in the same fragment).	1 SNP/ sequence (3,691)

Filter	Description	Thresholds (no. loci remaining)
7. Sex-linked	Loci that BLASTed to the X or Y chromosome in the *Felis catus* genome were removed.	Removed: *"NC_058386.1_chromosome_X"* *"NW_025408526.1_chromosome_X_ chrX_random_Un_scaffold_68"* (3,593)
8. Linkage disequilibrium	The LD filter was performed last, as many non-independent SNPs were removed by the earlier filters. This means that performing the LD filter last maximises the number of quality loci that are retained. *SNPRelate* version 1.20.1 (Zheng et al., 2012) was used to perform pairwise genotypic correlations within a sliding window of 500,000 base pairs, removing SNPs with a correlation of ≥0.5.	≥ 0.5 (3,241)
9. Missing data	Individuals with missing coordinate information were removed, as all analyses required spatial information.	Missing coordinate information (6 individuals removed, 3,241 loci remaining)
10. Relatedness	Wang's pairwise relatedness (Wang, 2002) was calculated, using a relatedness threshold that likely represented half-siblings and above. One individual from each highly related pair was removed, to avoid biasing genetic analyses.	≥ 0.2 (63 individuals removed, 3,241 loci remaining)
11. Spatial trimming	To avoid biasing genetic clustering analyses by including uneven sampling of locations, samples were thinned to only include one randomly chosen individual within a 500 m radius. Note that spatial thinning was used for PCoA and genetic clustering analyses only (and these results were compared to the full dataset).	1 individual/500 m radius (40 individuals removed, 3,241 loci remaining)
12. Hardy Weinberg Equilibrium	For analyses that assume HWE, the R package dartr (Gruber et al., 2018) was used to detect loci that significantly departed from HWE assumptions, which were then removed from the dataset.	Significantly out of HWE (3,105)

Appendix 3 Pairwise relatedness

Pairwise relatedness for 'highly related pairs' (where relatedness ≥0.2).

Individual 1	Individual 2	Wang's relatedness
T14326	T14424	0.7143
T14325	T14380	0.6976
T14340	T14342	0.6468
T14328	T14422	0.6466
T14377	T14378	0.6461
T14341	T14389	0.6457
T14340	T14279	0.6312
T14300	T14389	0.6271
T14300	T14341	0.6225
T14330	T14280	0.6149
T14326	T14436	0.609
T14328	T14331	0.6049
T14301	T14389	0.602
T14329	T14436	0.5867
T14300	T14301	0.5822
T14302	T14387	0.5815
T14263	T14411	0.5775
T14331	T14422	0.5766
T14374	T14375	0.5753
T14395	T14398	0.5734
T14301	T14436	0.5722
T14280	T14390	0.5674
T14389	T14436	0.5665
T14424	T14436	0.5621
T14326	T14331	0.5551
T14301	T14424	0.5526
T14411	T14383	0.5523
T14402	T14396	0.5505
T14325	T14382	0.5478
T14326	T14301	0.547
T14341	T14436	0.5437
T14326	T14422	0.5357
T14396	T14399	0.5319
T14301	T14341	0.5289
T14382	T14383	0.5285
T14396	T14406	0.5282

Individual 1	Individual 2	Wang's relatedness
T14424	T14389	0.5274
T14376	T14409	0.5248
T14341	T14424	0.5223
T14326	T14328	0.5214
T14329	T14389	0.514
T14326	T14389	0.5102
T14374	T14373	0.5099
T14300	T14424	0.5075
T14432	T14431	0.5052
T14329	T14341	0.5049
T14343	T14300	0.5008
T14279	T14436	0.5003
T14279	T14342	0.4986
T14301	T14329	0.4972
T14340	T14330	0.4962
T14400	T14396	0.4926
T14397	T14398	0.4921
T14363	T14383	0.491
T14329	T14424	0.4888
T14300	T14278	0.4873
T14374	T14383	0.4801
T14273	T14336	0.4794
T14313	T14425	0.4728
T14376	T14336	0.4681
T14300	T14329	0.4672
T14402	T14399	0.4661
T14326	T14341	0.4632
T14409	T14336	0.4578
T14268	T14336	0.454
T14326	T14329	0.4517
T14343	T14424	0.4501
T14375	T14377	0.4496
T14429	T14336	0.4496
T14374	T14382	0.4437
T14346	T14379	0.4403
T14343	T14301	0.4395
T14343	T14387	0.438
T14349	T14273	0.4372
T14289	T14378	0.4369
T14424	T14422	0.4355
T14343	T14341	0.4346

Individual 1	Individual 2	Wang's relatedness
T14325	T14263	0.43
T14331	T14424	0.4226
T14419	T14396	0.4198
T14343	T14329	0.4163
T14400	T14406	0.4152
T14374	T14409	0.4116
T14268	T14376	0.4115
T14343	T14342	0.4089
T14332	T14417	0.4059
T14374	T14376	0.4057
T14278	T14387	0.4055
T14330	T14281	0.4052
T14409	T14429	0.4023
T14343	T14389	0.3965
T14263	T14391	0.396
T14326	T14279	0.3953
T14329	T14387	0.3949
T14374	T14411	0.3944
T14376	T14429	0.3938
T14401	T14398	0.3937
T14387	T14424	0.3907
T14302	T14342	0.3902
T14279	T14330	0.3899
T14328	T14436	0.3894
T14397	T14407	0.3888
T14302	T14340	0.3883
T14300	T14326	0.385
T14330	T14390	0.3848
T14279	T14424	0.3831
T14301	T14279	0.3809
T14401	T14395	0.3806
T14269	T14427	0.3791
T14406	T14399	0.3784
T14258	T14259	0.3782
T14278	T14341	0.373
T14302	T14281	0.3659
T14301	T14278	0.3648
T14328	T14279	0.3637
T14373	T14383	0.3637
T14328	T14424	0.3619
T14325	T14374	0.3608

Individual 1	Individual 2	Wang's relatedness
T14278	T14389	0.36
T14343	T14278	0.3595
T14268	T14374	0.3584
T14325	T14391	0.3578
T14268	T14409	0.3569
T14310	T14330	0.3553
T14374	T14429	0.3546
T14328	T14329	0.3545
T14258	T14260	0.3539
T14380	T14382	0.3531
T14382	T14373	0.3501
T14310	T14302	0.3488
T14269	T14270	0.3484
T14259	T14260	0.3482
T14340	T14281	0.3475
T14374	T14336	0.3469
T14375	T14409	0.3454
T14411	T14382	0.3441
T14342	T14424	0.3438
T14402	T14406	0.3432
T14340	T14280	0.3426
T14342	T14387	0.3423
T14368	T14388	0.339
T14329	T14304	0.3384
T14270	T14427	0.337
T14278	T14424	0.3361
T14279	T14422	0.3357
T14268	T14429	0.3344
T14400	T14402	0.3317
T14375	T14336	0.3296
T14343	T14281	0.3295
T14328	T14390	0.329
T14269	T14415	0.3255
T14422	T14436	0.3246
T14409	T14383	0.323
T14263	T14380	0.3214
T14279	T14389	0.3211
T14302	T14330	0.3171
T14310	T14281	0.3165
T14329	T14281	0.3161
T14343	T14326	0.3158

Individual 1	Individual 2	Wang's relatedness
T14301	T14422	0.3152
T14340	T14422	0.3147
T14329	T14279	0.3143
T14330	T14342	0.3139
T14330	T14422	0.313
T14281	T14424	0.3123
T14343	T14436	0.3109
T14422	T14390	0.3108
T14342	T14422	0.3087
T14289	T14377	0.3085
T14389	T14422	0.3071
T14340	T14390	0.3068
T14301	T14342	0.3064
T14331	T14436	0.3062
T14342	T14436	0.3061
T14376	T14375	0.3059
T14263	T14382	0.3054
T14384	T14403	0.3054
T14305	T14403	0.305
T14281	T14387	0.3041
T14363	T14382	0.304
T14311	T14312	0.3036
T14273	T14409	0.3036
T14297	T14383	0.3035
T14395	T14407	0.3029
T14263	T14374	0.3021
T14411	T14391	0.3008
T14395	T14397	0.3007
T14310	T14340	0.3
T14329	T14422	0.2983
T14328	T14281	0.298
T14301	T14328	0.2979
T14297	T14374	0.2979
T14328	T14340	0.2941
T14328	T14389	0.2938
T14342	T14281	0.2934
T14314	T14336	0.2926
T14328	T14341	0.2923
T14325	T14373	0.2919
T14300	T14304	0.2913
T14349	T14403	0.2905

Individual 1	Individual 2	Wang's relatedness
T14326	T14387	0.2887
T14302	T14279	0.2881
T14404	T14405	0.2877
T14300	T14342	0.286
T14328	T14342	0.2849
T14325	T14411	0.2836
T14300	T14387	0.2824
T14341	T14387	0.282
T14331	T14389	0.2814
T14279	T14281	0.2795
T14302	T14390	0.2788
T14329	T14331	0.2788
T14343	T14302	0.2769
T14300	T14281	0.2766
T14278	T14329	0.2766
T14387	T14389	0.2753
T14374	T14363	0.2751
T14342	T14389	0.2743
T14301	T14331	0.2741
T14301	T14281	0.2729
T14331	T14281	0.2719
T14353	T14321	0.2712
T14341	T14281	0.2704
T14328	T14330	0.27
T14429	T14383	0.2698
T14387	T14436	0.2691
T14375	T14429	0.2687
T14326	T14342	0.268
T14411	T14373	0.2673
T14310	T14390	0.2665
T14273	T14374	0.2663
T14268	T14375	0.2661
T14279	T14280	0.266
T14326	T14278	0.2659
T14343	T14304	0.2655
T14331	T14342	0.2651
T14341	T14331	0.2642
T14279	T14331	0.2628
T14300	T14422	0.2627
T14280	T14281	0.2617
T14279	T14341	0.2614

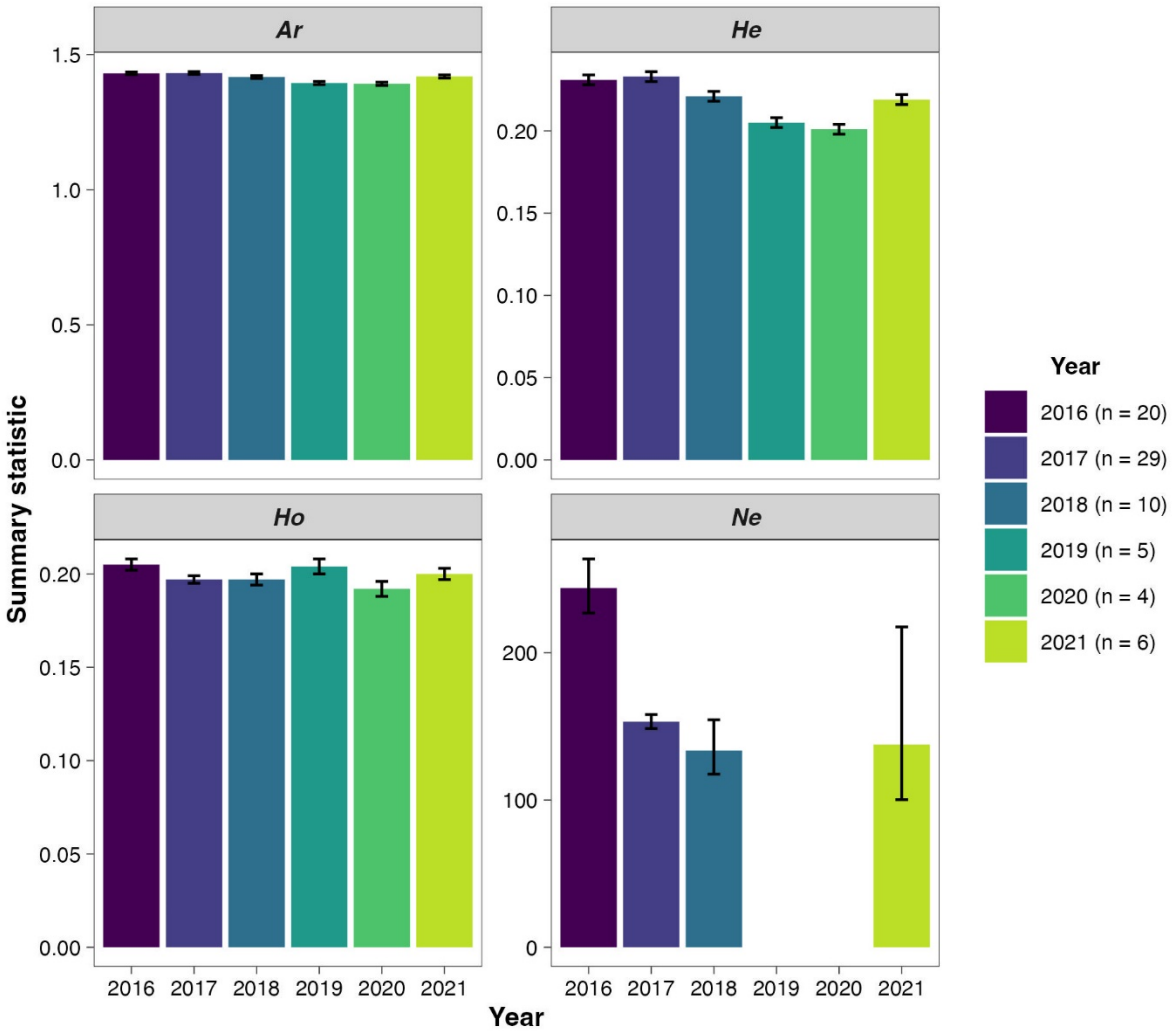
Individual 1	Individual 2	Wang's relatedness
T14300	T14436	0.2608
T14301	T14387	0.2604
T14297	T14409	0.26
T14266	T14320	0.2591
T14279	T14387	0.2591
T14329	T14342	0.2589
T14302	T14424	0.2577
T14380	T14391	0.2562
T14341	T14342	0.2554
T14400	T14399	0.2554
T14341	T14422	0.2553
T14374	T14380	0.2537
T14281	T14422	0.2528
T14278	T14342	0.2519
T14302	T14422	0.2498
T14302	T14278	0.2467
T14375	T14373	0.2467
T14300	T14331	0.2465
T14310	T14422	0.2446
T14273	T14429	0.2445
T14273	T14376	0.2444
T14262	T14371	0.2437
T14310	T14342	0.2421
T14331	T14390	0.242
T14398	T14407	0.2403
T14340	T14424	0.24
T14314	T14417	0.2392
T14281	T14390	0.2392
T14406	T14407	0.2386
T14310	T14424	0.2384
T14297	T14376	0.2363
T14321	T14350	0.2355
T14297	T14411	0.2349
T14304	T14387	0.2346
T14278	T14436	0.2334
T14349	T14384	0.2334
T14374	T14391	0.2333
T14264	T14265	0.2322
T14281	T14389	0.2307
T14280	T14342	0.23
T14303	T14373	0.229

Individual 1	Individual 2	Wang's relatedness
T14326	T14281	0.228
T14332	T14336	0.2273
T14297	T14382	0.2263
T14343	T14279	0.2261
T14321	T14373	0.2256
T14330	T14424	0.2249
T14340	T14387	0.2246
T14409	T14382	0.2242
T14300	T14328	0.2227
T14384	T14363	0.2184
T14304	T14389	0.2174
T14280	T14422	0.2166
T14340	T14331	0.2162
T14273	T14375	0.2154
T14304	T14424	0.2142
T14328	T14280	0.2133
T14305	T14363	0.2119
T14296	T14336	0.2117
T14409	T14411	0.2117
T14343	T14422	0.21
T14323	T14398	0.21
T14302	T14329	0.2094
T14375	T14383	0.2091
T14363	T14373	0.2087
T14304	T14281	0.2084
T14302	T14328	0.208
T14396	T14407	0.2078
T14310	T14328	0.2065
T14296	T14409	0.2061
T14281	T14436	0.205
T14303	T14383	0.2046
T14263	T14373	0.2044
T14417	T14336	0.2039
T14403	T14391	0.2015

Appendix 4 Genetic diversity estimates 2016 - 2021

Estimates of genetic diversity parameters over sampling years.

Allelic richness (*Ar*), expected heterozygosity (*He*), observed heterozygosity (*Ho*) and effective population size (*Ne*). Note that years with sample sizes n < 10 have low confidence. Effective population size could not be calculated for 2019 and 2020.



Glossary

Single Nucleotide Polymorphism	a single nucleotide polymorphism (abbreviated SNP, pronounced 'snip') is a genomic variant at a single nucleotide base position in the DNA where one nucleotide (adenine, thymine, cytosine, or guanine) is replaced with another (e.g. adenine is replaced with cytosine). SNPs are the most common type of genetic variation present between individuals and are ubiquitous through the genome.
alleles	an allele is one of two or more versions of a DNA sequence (a single base or a segment of bases) at a given genomic location.
Heterozygosity (He)	the presence of two different alleles at a particular gene location, as opposed to homozygosity (same alleles at a particular gene location).
inbreeding coefficient (F_{IS})	the probability that two alleles at any locus in an individual are identical by descent from the common ancestor of the two parents. A measure of population-level inbreeding.
effective population size (Ne)	a theoretical measure of the number of breeding individuals in a randomly mating population that would give rise to the observed genetic diversity. Effective population size is often contrasted with the census population size (Nc), with Ne typically being less than Nc.

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