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Preliminary genetic survey of the Mount Etna ghost bat (*Macroderma gigas*) population, January 2022

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Preliminary genetic survey of the Mount Etna ghost bat (*Macroderma gigas*) population, January 2022

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Final report prepared for Queensland Department of Environment and Science

January 2023

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Summary

This report represents a preliminary survey of the ghost bat population located at Johansens Cave in the Mount Etna Caves National Park, Queensland, based on genetic analysis of non-invasive samples.

Key results include:

- 73 scat samples and 5 tissue samples were collected during a single sampling occasion on 28 January 2022.
- Successful genotypes were obtained for 64 of 78 scat and tissue samples genotyped (82%).
- From 64 samples, we identified a total of 23 ghost bat individuals (10 females and 13 males).
- Of the five ‘known’ individuals (from tissue samples), only three were detected in scats.
- Based on scat abundance, females appeared to be using the cave more frequently than males.
- Thirteen pairs of bats showed genetic relationships representative of parent-offspring or full-sib, with five individuals showing more than one such relationship.
- Further refinement to the SNP panel could improve the effectiveness of the panel for monitoring Queensland ghost bat populations.

1 Background

The ghost bat (*Macroderma gigas*) is a monotypic bat species persisting in disjunct populations across northern Australia, with sub-populations present in the Pilbara and Kimberley regions of Western Australia (WA), the Northern Territory (NT) and in Queensland (Qld), from Rockhampton to Cape York and inland from Cloncurry to Camooweal. It is the largest carnivorous bat in Australia and is a predator of small mammals, reptiles, frogs, birds and insects (Richards et al. 2008). The ghost bat was listed as Vulnerable under the Australian Government's *Environment Protection and Biodiversity Conservation Act* 1999 (EPBC Act) in May 2016, owing to declines of >30% in numbers across its range (Threatened Species Scientific Committee 2016), and is listed as Vulnerable on the IUCN red list. It is listed as Endangered under the Qld *Nature Conservation Act 1992.* Ghost bat survival is critically dependent on finding natural roosts in caves, crevices, deep overhangs and artificial roosts such as abandoned mines (Hall et al. 1997). Ghost bats appear to have regionally centralised permanent roosts that are genetically isolated from each other and are characterised by a pattern of maternal philopatry and male-biased dispersal (Worthington Wilmer et al. 1994).

In Queensland, the ghost bat is currently distributed in only 4-5 highly disjunct populations along the coast and inland from the McIlwraith Range in Cape York to Rockhampton (Worthington Wilmer et al. 1999). Until recently, the population of ghost bats in the Mount Etna Caves National Park and surrounds was regarded as one of the largest in Australia (Nelson 1989), however, mining and anthropogenic land use in the surrounding area may have contributed to its decline with population estimates now in the range of 40 bats (Augusteyn et al. 2017), down from 450 individuals in the 1960's (McKean & Price 1967).

In November 2021, the Queensland Government committed $125,000 over 2 years towards recovery actions for ghost bats in Central Queensland. In order to provide a baseline estimate of the numbers of bats present in the most significant roost within Mount Etna National Park, Johansens Cave, the Qld Department of Environment and Science conducted a preliminary survey of the site over two nights in January 2021.

The primary aim of the survey was to collect non-invasive samples (scats) to enable genetic analysis to identify the numbers of individuals present and their sex. Tissue samples from a small number of captured bats were also obtained.

Genetic monitoring of ghost bat populations in the Pilbara is routinely undertaken using a non-invasive sampling methodology based on Single Nucleotide Polymorphism (SNP) markers developed by the WA Department of Biodiversity, Conservation and Attractions (Thavornkanlapachai et al. in prep; Sun et al. 2021). Whilst the initial panel of SNP markers used in these surveys was originally targeted specifically to the Pilbara population of ghost bats, recent funding obtained from the Threatened Species Initiative (https://threatenedspeciesinitiative.com/) has enabled the development of a panel of markers putatively applicable across WA, NT and Qld

ghost bat populations (‘national’ panel; R. Sun, pers. comm.). We apply these markers for the first time in this report.

Here, we undertake genetic analysis of scat and tissue samples obtained from Johansens Cave in January 2022 to:

- Evaluate the performance of the recently developed ‘national’ ghost bat SNP panel on Queensland non-invasively collected samples;
- Confirm the successful amplification of genetic markers in tissue and scat samples;
- Identify the number of individuals represented in scat samples from Johansens Cave and their sex;
- Estimate genetic relationships amongst the sampled individuals and the effective population size.

2 Materials and Methods

2.1 Sampling location and material

Johansens Cave was visited on the 28 January 2022 (Figure 1) and scat and tissue samples were collected.

Seventy-three fresh ghost bat scats were collected from beneath an active aven within Johansens Cave, after the ghost bats had departed for the evening, placed in individual envelopes and frozen for transport (on dry ice) to the DBCA laboratory, Kensington, Western Australia, for DNA extraction and SNP genotyping.

Tissue samples (wing punches) were obtained from five bats trapped on the same night as scat collection using mist nets under animal ethics permit SA 2019/08/700.

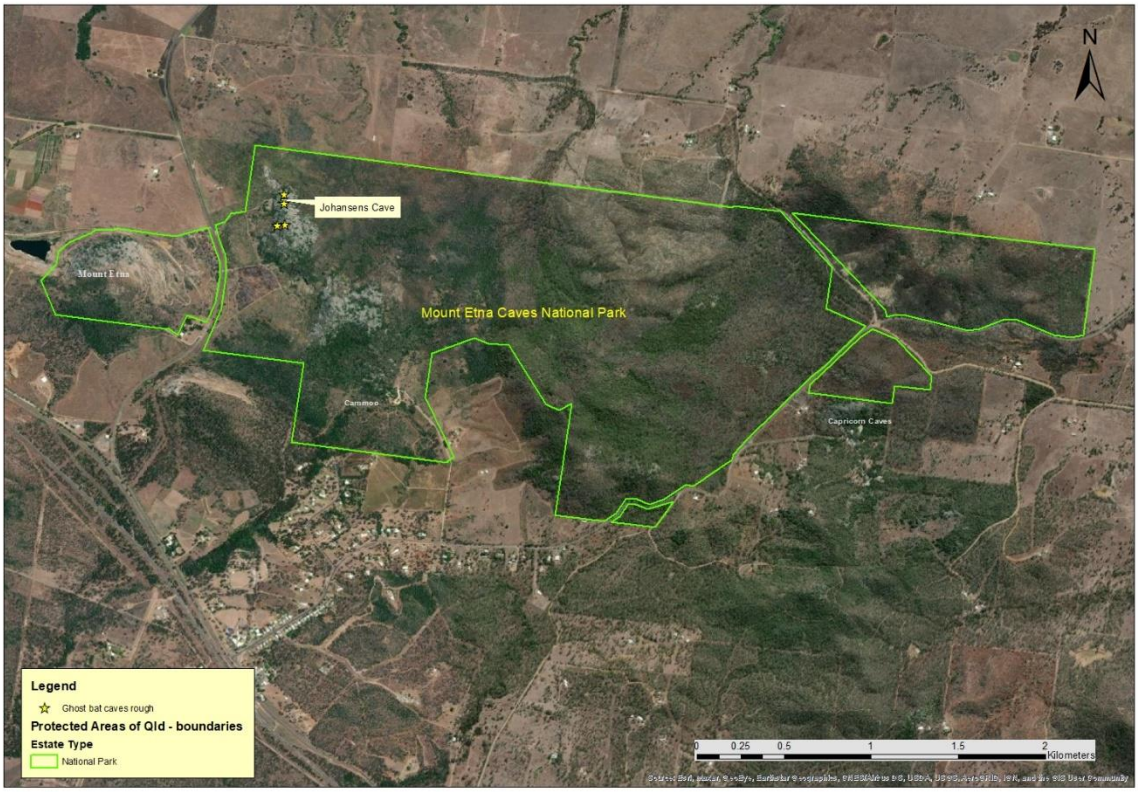

Figure 1 Location of Johansens Cave in Mount Etna Caves National Park, Queensland. Map courtesy of Leanne Henry.

2.2 DNA extraction and SNP genotyping

DNA extraction of scat samples was carried out following methods in Ottewell et al. (2021) with DNA extraction of tissue samples undertaken using a standard ‘salting out’ procedure as described in Ottewell et al. (2020). All samples were given internal DBCA sample codes with scat samples identified with the prefix ‘Sc’ and tissue samples identified with the prefix ‘D’.

All 78 samples were genotyped using a custom-designed multiplexed panel of single nucleotide polymorphism (SNP) markers (n = 46 autosomal SNP loci) specifically designed to target polymorphic SNP loci across WA, NT and Qld populations (R. Sun, pers. comm.). Markers were selected based on properties enabling robust identification of individuals from non-invasive samples Thavornkanlapachai et al. in prep. In addition to autosomal markers, we included four sex-linked SNP markers in the panel, two each from the SRY and Zfx/Zfy genes. 60 µl of each faecal DNA sample was sub-sampled from 100 µl of DNA extract and concentrated down to 20 µl for genotyping. PCR amplification and extension reactions carried out by AGRF were performed using the iPLEX Gold Reagent Kit with 1 µl of the concentrated DNA extract. Resultant SNP genotypes were identified by mass spectrometry and called using MassARRAY TYPERVIEWER 4.0 software (Agena Bioscience). Eight samples were re-genotyped to allow calculation of allelic dropout and false allele error rates. Genotyping errors are frequently observed in studies using scat DNA due to the low quality and quantity of DNA sourced from these samples (Taberlet et al. 1999).

2.3 SNP panel success and data quality

Due to the low number of samples previously available for Queensland ghost bats, the SNP panel has not been extensively evaluated for performance in the Mount Etna population. We evaluated the number of autosomal and sex-linked markers that amplified in the Mount Etna samples and their level of polymorphism. We further assessed the relative performance of tissue and scat samples.

We cleaned the SNP dataset for further analysis by removing poor quality samples with a genotyping success rate of ≤ 80% (i.e. successful genotyping of a minimum of 37/46 loci). Following removal of samples, we filtered SNP loci that (1) failed to amplify, (2) amplified poorly (i.e. in ≤ 80% of samples) or (3) were monomorphic. Probability of identity (P_{ID}) analyses of SNP genotypes indicated that genotyping at a minimum of 25 loci is required to discriminate between related individuals at a high threshold of certainty (PIDsibs < 0.00001).

2.4 Identification of unique individuals

MassARRAY SNP results were processed in a custom R package ‘ScatMatch’ Huntley 2021 designed to group scats based on genotype similarity, i.e. by the number of allelic mismatches between samples. The identification of a suitable threshold mismatch number (h) by which scats are clustered into groups (putative individuals) was based on multiple analyses, and at the same time evaluating the impact of different sample and locus amplification rate filters on data thresholds. Firstly, we evaluated the number of groups identified for each h value across different filtering thresholds (Figure 2). The elbow graph (Figure 2, left panel) should show a clear flattened line when the mismatch threshold is reached which indicates that scats were consistently assigned to the same individuals. Using h values past this point typically results in reduced numbers of groups as closely-related individuals are merged. Secondly, we visualise the histogram of genotype mismatches (h) amongst samples across different filtering thresholds (Figure 2, right panel) which should show a clear bimodal distribution with separation between the

SNP differences caused by scats from the same individual (a smaller distribution on the left) and SNP differences caused by scats from different individuals (a larger distribution on the right). The greater the separation between the two distributions, the lower the chance of assigning scats to the wrong individuals. Thirdly, we visualise the number of pairwise mismatches amongst clustered samples using a heatmap (Figure 3). In this figure we wish to see a clear clustering of samples along the centre line in dark blue and we assess the number of mismatches within clusters (dark blue) versus between clusters (lighter colours).

For this dataset we identified h = 5 as the appropriate genotype clustering threshold since the elbow graph stabilised at this point (Figure 2, left panel). H = 6 may also have been appropriate but the same number of individuals are identified as h = 5 when using a sample and locus filtering threshold of 0.8.

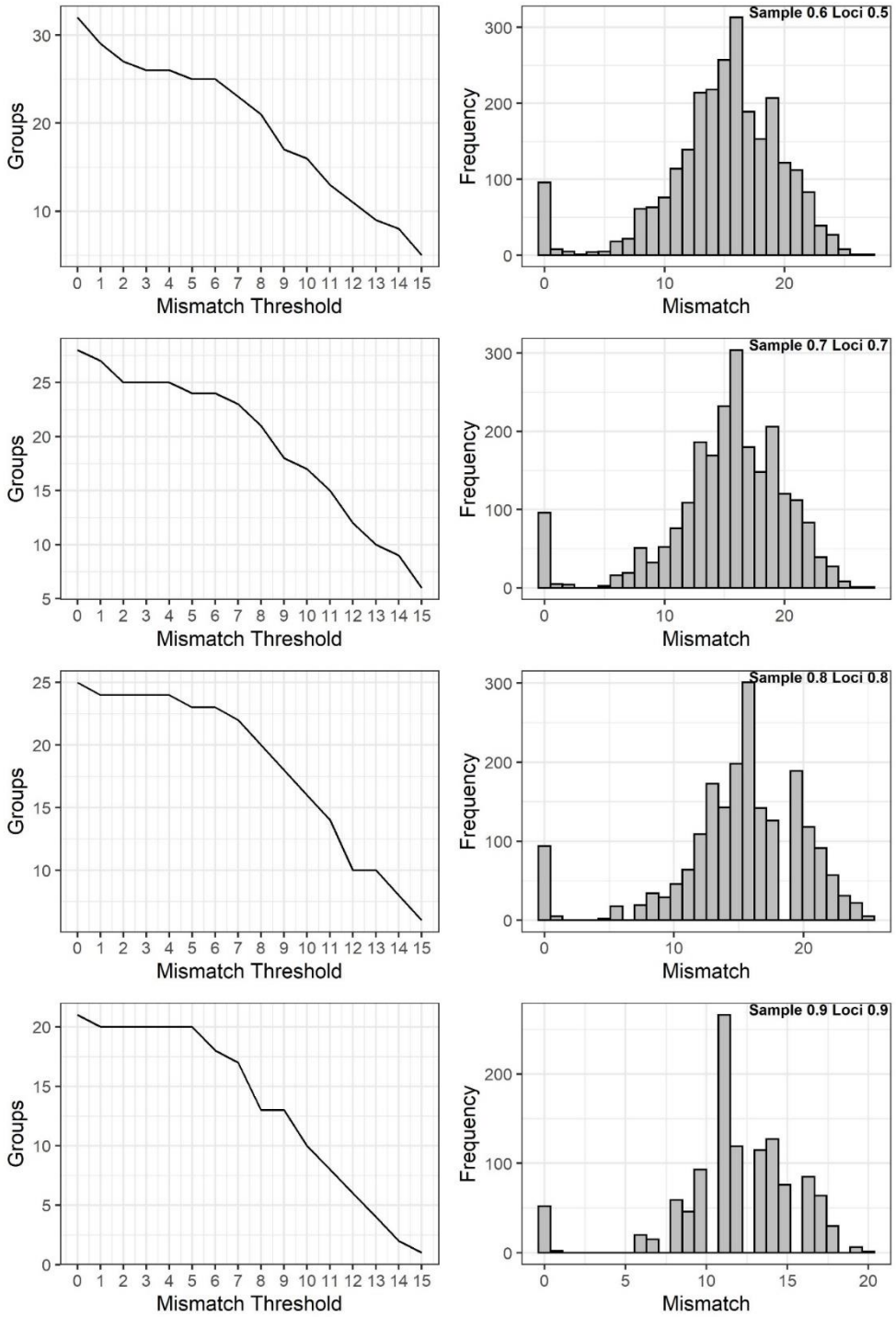

Figure 2 Assessment of the distribution of genotype mismatches and numbers of individuals (Groups) identified amongst Macroderma gigas samples with different sample and locus amplification rate filters (60%, 70%, 80% and 90%) and different mismatch thresholds (h). Left: Elbow graph showing the number of putative individuals (Groups) identified with increasing number of allelic mismatches. Right: Frequency distribution of pairwise allelic mismatches where both samples have genotypes. The bimodal distribution represents mismatches between scats from the same individual (genotyping error) and mismatches between different individuals (biological differences). See text for further detail on choice of final filters and mismatch threshold.

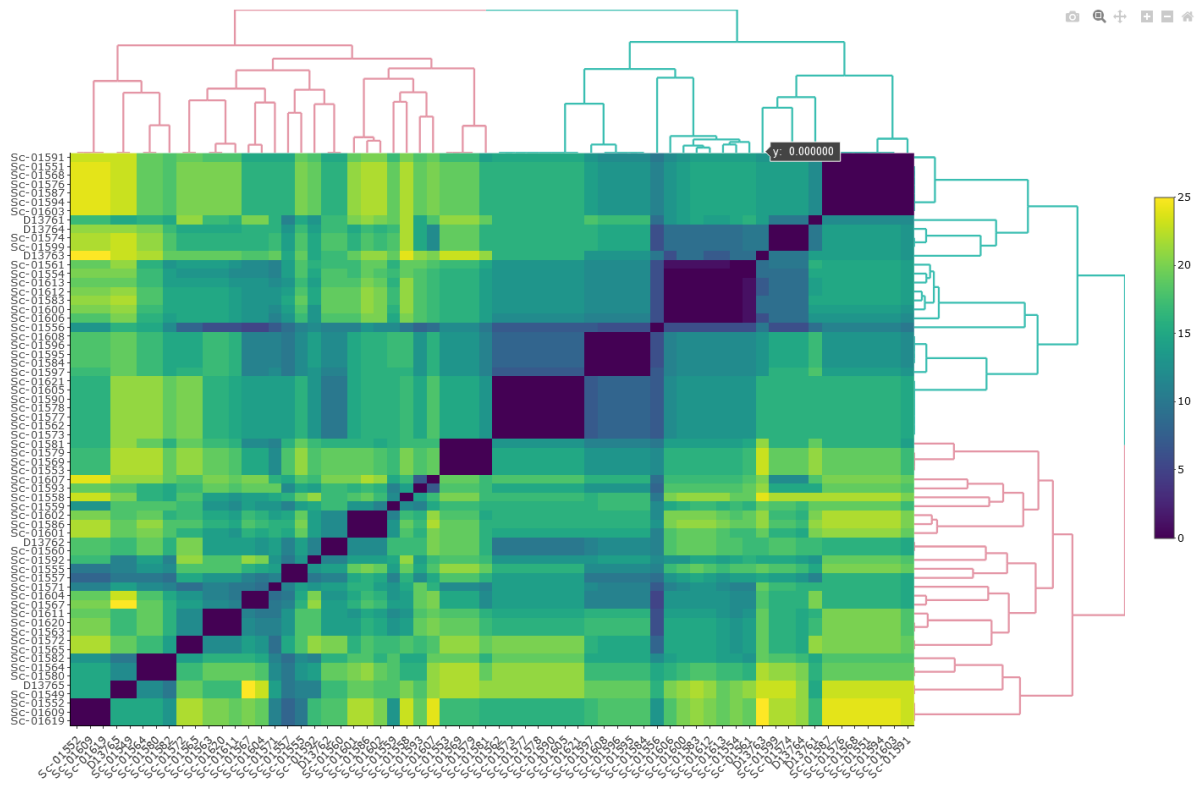

Figure 3 Hierarchical clustering of ghost bat scat and tissue genotypes using a pairwise mismatch threshold of h = 5. The heatmap shows clear clustering of genotypes along the centre line (dark blue).

2.5 Molecular sexing

In addition to sexing markers incorporated within the SNP panel, as a secondary method of sex identification we undertook molecular sexing of scat samples using four custom-designed ghost bat sex-linked customised primers and probes (DDX3Y, SRY, Zfy, and Zfx) as described in Ottewell et al. (2020) arranged in a real-time PCR multiplex. Primers were amplified in 10 µl reactions using the PrimeTime™ Gene Expression Master Mix (Cat No: 1055772) following the manufacturer's instructions with an annealing temperature of 60°C, 40 amplification cycles and 4 µl of unconcentrated DNA. The reactions were run on the CFX96™ Real-Time System C1000 Touch Thermal Cycle (BIO-RAD, Singapore) and analysed in BioRad CFX Maestro software (BIO-RAD, Singapore). Sex is confidently allocated if all three Y-linked markers produced relative fluorescent units (RFUs) ≥ 50, all markers produced the same result and are consistent across multiple scats from the same individual, and Y-linked marker RFU to the total RFU ratios are above 0.1 (DDX3Y, SRY) and 0.3 (Zfy) for males. Sexing results from this method were collated with sexing results from the SNP panel. Where inconsistencies were present (failed marker, mismatch between different scats) sex is indicated as "most likely" if the majority of sex-linked markers amplify or "undetermined" if ≤ 1 marker amplified.

2.6 Genetic diversity, relatedness and effective population size

Summary population genetic diversity statistics, including observed (Ho) and expected heterozygosity (He), number of alleles (Na) and the inbreeding coefficient (Fis) were calculated in GENALEX v6.5 (Peakall & Smouse 2012). Pairwise genetic relatedness was calculated in the R package “related” (Pew et al. 2015) using the Lynch & Ritland method (Lynch & Ritland 1999).

Effective population size (Ne) is estimated from the genetic data using the NeEstimator software (Do et al. 2014). We used the Linkage Disequilibrium method (Waples & Do 2010) using loci with allele frequencies >0.02. The program also implements a parametric bootstrapping method to estimate the 95% confidence intervals of the Ne estimate (Jones et al. 2016).

3 Results

3.1 Genotyping success and SNP panel performance

Of the 46 autosomal markers included in the 'national' ghost bat SNP panel, four markers failed to amplify in the Mount Etna population. All tissue and scat samples produced genotypes, however, we removed 14 samples with SNP genotyping rates less than 80% (range 36 – 79%). Following removal of poor-quality samples, we identified a further three loci with low amplification rates (≤ 80%) and nine loci that were monomorphic (invariant) and removed these from the dataset. Our final SNP genotyping dataset therefore consisted of 64 scat and tissue samples genotyped at 30 autosomal loci, a success rate of 82%. Comparison of replicate samples indicated high confidence in the genetic data with complete matches between replicate samples resulting in error rates: allelic dropout = 0.0 ± 0.0, false alleles = 0.0 ± 0.0.

Overall, we found that tissue samples had a higher amplification rate on average (97 ± 1.1%) compared to scat samples (87 ± 1.6%).

3.2 Sexing marker success

Molecular sexing of scats was successful for both SNP array and qPCR-based methods, however we identified mismatches between methods for two scat samples (Appendix 2). Final sex determinations were made by consensus across scats identified from the same individual.

3.3 Number of unique individuals and sex

Genetic clustering analyses identified 23 ghost bat individuals from the 64 genetic samples (Table 1). Ten individuals were identified as female and 13 individuals were identified as male/likely male (Figure 4), representing a sex ratio of 0.56 M:0.44 F.

Only three of the five captured bats were detected in scat samples (Table 1). No scats were identified from Male BNT-OCT15-053 or Female BNT-OCT17-020 despite being captured at the same location as scats were sampled.

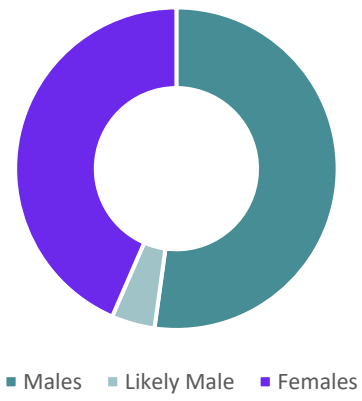

Figure 4 Ratio of male and female bats identified from genetic samples from Johansens Cave, January 2022.

Table 1 Ghost bat individuals identified through genetic clustering analysis of SNP genotyping data, their sex identified from sex-linked markers, and number of scats attributed to each individual.

Individual	Sex	Tissue	Scats	Total 'detections'
1	Male	BNT-OCT15-053 (D13761)	-	1
2	Male	BNT-OCT17-016 (D13762)	1	2
3	Female	BNT-OCT17-020 (D13763)	-	1
4	Male	BNT-OCT17-069 (D13764)	2	3
5	Male	DNA-582 (D13765)	1	2
6	Female	-	7	7
7	Female	-	3	3
8	Male	-	4	4
9	Female	-	7	7
10	Male	-	2	2
11	Male	-	3	3
12	Male	-	1	1
13	Likely Male	-	1	1
14	Female	-	7	7
15	Female	-	3	3
16	Male	-	3	3
17	Female	-	2	2
18	Male	-	1	1
19	Female	-	5	5
20	Female	-	3	3
21	Male	-	1	1
22	Male	-	1	1
23	Female	-	1	1

3.4 Ghost bat 'activity'

Based on the number of genetic detections of individuals from either scats or tissues, it appears females are more likely to be considered resident bats in Johansens Cave, with four individuals having higher numbers of detections than male bats (Females #6, #9, #14 and #19; Figure 5). Six of the 13 male bats were only detected once (46%).

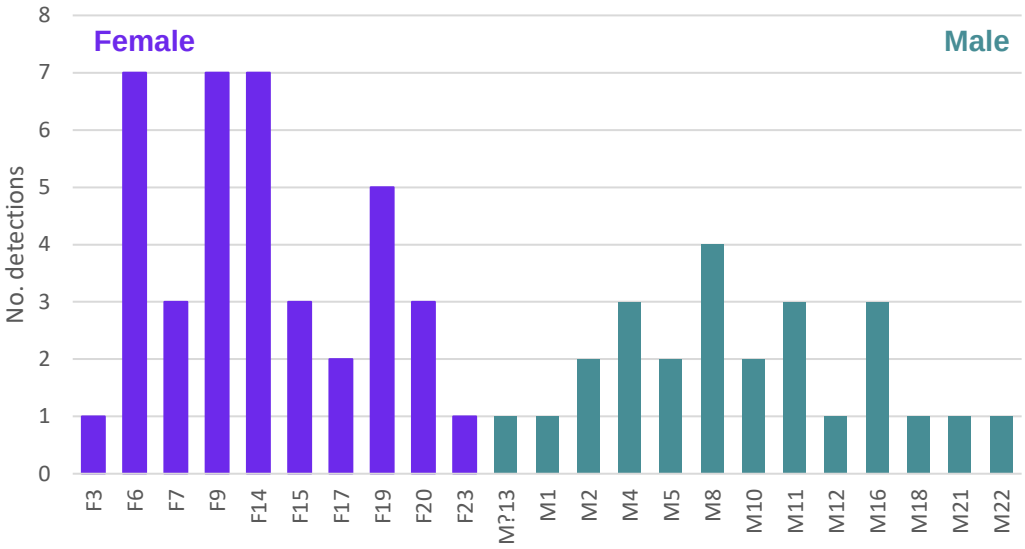

Figure 5 Number of genetic 'detections' of ghost bat individuals sampled at Johansens Cave based on either tissue or scat samples.

3.5 Relatedness

Pairwise genetic relatedness estimates indicated several individuals (M1, M2, M5, F9, M10) had 1st order pedigree relationships (parent-offspring or full-sib) with more than one other individual (Figure 6). Only four individuals (F19, F20, M18, & M21) did not have higher order pedigree relationships with other sampled bats in the cave. All other bats were related at the level of cousins or above. Overall, however, mean pairwise relatedness was -0.047 ± 0.014.

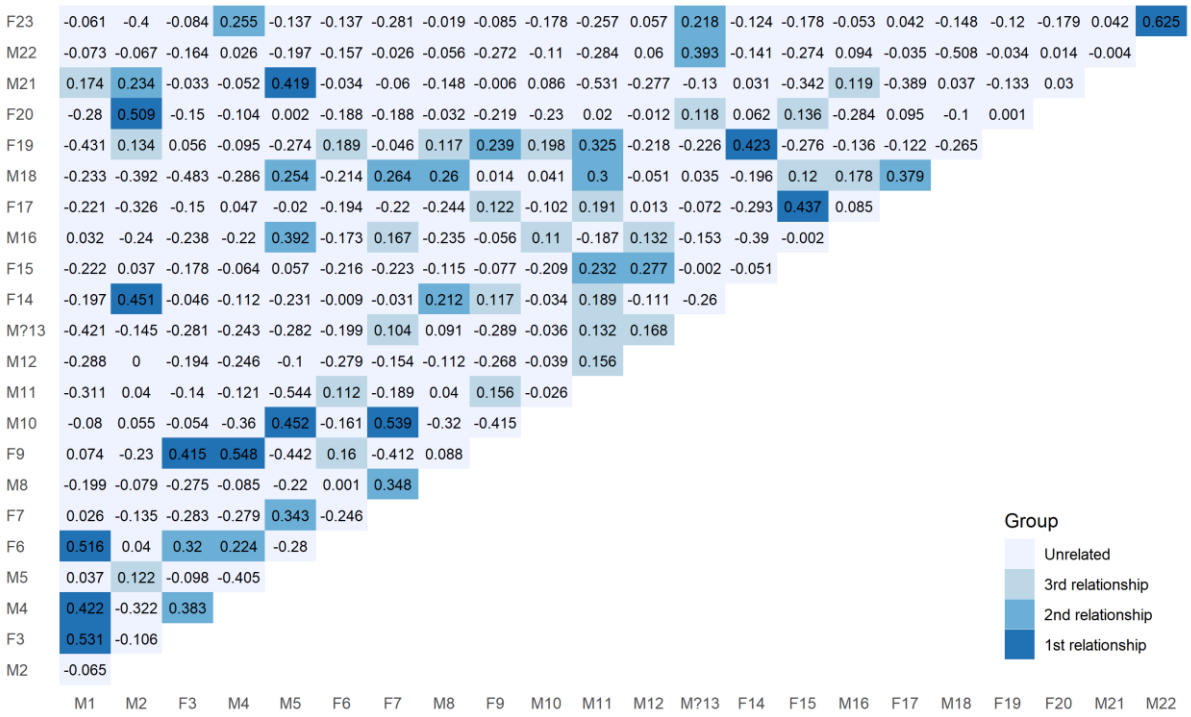

Figure 6 Pairwise relatedness of individuals detected in Johansens Cave January 2022. Pairwise genetic relatedness (R) was calculated using the Lynch & Ritland method Lynch & Ritland 1999. Colour intensity indicates genetic similarity in categories of first-order relationship (R = 0.5; parent-offspring, full-siblings), second-order relationship (R = 0.25; half-sibling, uncle/aunt – nephew/niece, grandparent – grandoffspring) and third-order relationship (R = 0.125; full cousin, great-grandparent – great-grandoffspring, great-uncle/aunt – great-nephew/niece, half-uncle/aunt – nephew/niece) Ritland 1996.

3.6 Genetic diversity and effective population size

Genetic diversity statistics for the ghost bat population sampled at Johansens Cave are provided in Table 2. Expected and observed heterozygosity are similar, and the inbreeding coefficient is close to zero. The estimated effective population size for the Johansens Cave ghost bat population is 19 bats (95% CIs 10 – 43).

Table 2 Mean genetic diversity statistics (and standard error) for ghost bats identified at Johansens Cave. Note that genetic diversity statistics were calculated using only polymorphic loci (n = 30). Genetic diversity statistics include N_a = Number of alleles, H_o = Observed heterozygosity, H_e = Expected heterozygosity, uH_e = unbiased expected heterozygosity, F = inbreeding coefficient, Ne = effective population size and 95% confidence intervals determined by parametric bootstrapping.

	N	***N_a***	***H_o***	***H_e***	***uH_e***	***F***	***Ne***
Mean	22.500	2.000	0.408	0.381	0.389	-0.067	18.7
SE	0.125	0.000	0.033	0.026	0.026	0.040	(CI 10.4 – 42.9)

4 Discussion

The Mount Etna caves sustain the southernmost population of ghost bats in Queensland. The population has declined substantially since the mid-1960's (estimated ~450 bats; McKean & Price 1967) to 150–180 bats in the mid-1970s and early 1990s (Toop 1985; Toop & Davie unpubl.). Hoyle et al. (2001) concluded, on the basis of capture–mark–recapture data obtained between 1975 and 1980, that the population was stable at ~150 bats. However, the most recent surveys conducted by Augusteyn et al. (2017) indicated a contemporary population size of ~40 individuals, representing a 79% decline in population size since 2001.

Live capture of bats and genetic analysis of scat material collected from the most substantial maternity cave within the Mount Etna National Park, Johansens Cave, over a single night in January 2022 indicated a minimum population size estimate of 25 individuals. A slight male bias was identified with the sampled population consisting of 10 females and 15 males. We noted that only three of the five captured bats (60%) were represented in the scat sampling despite being captured in the same location. This suggests that the true population size is larger than the estimate obtained through scat sampling alone. A rough extrapolation, assuming that scat sampling only detected 60% of the individuals present, suggests a population size of around 38 bats, very close to the 2017 estimate of Augusteyn et al. (2017).

Effective population size (Ne) estimated from the genetic data is a theoretical measure of the number of breeding pairs contributing to the observed population and is almost always lower than the observed census size (N). We estimated Ne of the Johansens Cave population to be 19 individuals (CI 10 – 43 individuals). Although not directly comparable due to the differences in genetic markers used, this estimate is very close to the previous data provided in Augusteyn et al. (2017) with an Ne estimate of 25 (CI 14 – 49) individuals. Repeated monitoring of the Johansens Cave population could help to establish the trend in Ne and enable detection of population size changes.

Analysis of genetic relatedness patterns amongst the sampled bats showed a high proportion of bats within Johansens Cave are related at the level of parent-offspring or full-sibs ($R \approx 0.5$). This is not unexpected given the time of sampling, which was conducted in January when young may still be spending significant time with their mothers. Further, it was noted that sample collection occurred from beneath several avens that were located close together, increasing the probability of sampling scats from mothers and their offspring (J. Lowry, pers. comm.).

Three of the four ghost bats identified to have more than one first-order pedigree relationship within the population were males. In most cases this appeared to represent a triad, i.e. mother-father-offspring, but in the case of M1, the relationship included two males and two females. It is not possible to distinguish parent-offspring pedigree relationships from full-sib relationships based on the genetic data alone, so it is unclear whether this pattern represents a male siring multiple offspring or that the relationship includes a sibling pair as well as a triad. Access to additional

information on the relative ages of bats or their recapture histories following repeated surveys might further illuminate the dynamics of these relationships.

We provide estimates of genetic diversity of the ghost bat population in Johansens Cave, however, at this stage it is not possible to compare the relative 'genetic health' of this population to others. The genetic diversity estimates presented here are specific to the SNP array used - the 'national' panel - which has not yet been applied to other ghost bat populations. It is anticipated that estimates of genetic diversity using this SNP panel will become available for Northern Territory populations in the very near future and sampling of additional Queensland populations would help to put the Mount Etna population in regional context. Of note, we found nine of the 46 autosomal markers in the 'national' panel were invariant in the Mount Etna population. Whilst this might reflect a technical failure of the SNP panel (only a small number of Queensland samples were available during the panel design process meaning panel performance could not be properly evaluated prior to use), the lack of variation at selected markers may also be a result of genetic erosion that has occurred in the Mount Etna population due to declining population size. Further sampling of additional Queensland populations will help to indicate whether this an accurate characterization of the Mount Etna population or an artefact of the SNP markers selected for the 'national' panel.

Overall, the 'national' ghost bat panel performed at a level adequate to identify ghost bat individuals within the Johannsen Cave at Mount Etna National Park. Scat genotyping success rates using the SNP panel were high with 81% (59/73) of samples successfully genotyped. This compares to a genotyping rate of 42% (8/19) from the previous methodology using microsatellite markers reported in Augusteyn et al. (2017). Careful handling of the faecal samples during collection and rapid transport of samples on dry ice to lab facilities in WA were essential contributors to the high success rate of scat samples.

Although several SNP markers in the 'national' panel failed to amplify and others were uninformative, we were able to identify individuals based on genotypes present at 30 loci. A minimum of 25 loci are required to distinguish related individuals with sufficient confidence, indicating that the SNP genotyping method is suitable for mark-recapture analyses in the Queensland ghost bat population. However, higher confidence in individual ID's could be achieved by identifying further variable SNP markers in Queensland populations to include in the 'national' panel but would require additional sequencing and panel re-design. The feasibility of this may need to be judged on the frequency with which scat sampling will be undertaken in the future and the availability of tissue samples for sequencing. Understandably there may be difficulty in obtaining new tissue samples from ghost bat populations for sequencing, but the viability of samples from previous genetic studies (Augusteyn et al. 2017, Worthington Wilmer et al. 1999) could be investigated. If scat monitoring is to become routine for surveying ghost bat populations in Queensland, investment in improving the performance of the SNP array would be worthwhile.

Appendices

Appendix 1 Individual ID and molecular sexing results

Individual ID and sex of ghost bat scat and tissue samples. Group ID indicates the genetic cluster (individual) scats and tissues belong to, Sex indicates the consensus sex from qPCR and array-based sexing methods and Final Sex indicates the final determination based on consensus across the identified group.

Group (Individual) ID	Sample ID	Sex	Final Sex
1	D13761 (BNT-OCT15-053)	M	M
2	D13762 (BNT-OCT17-016)	M	M
2	Sc-01560	M	M
3	D13763 (BNT-OCT17-020)	F	F
4	D13764 (BNT-OCT17-069)	M	M
4	Sc-01574	M	M
4	Sc-01599	M	M
5	D13765 (DNA-582)	M	M
5	Sc-01549	M	M
6	Sc-01551	Likely F	F
6	Sc-01568	F	F
6	Sc-01576	F	F
6	Sc-01587	F	F
6	Sc-01591	F	F
6	Sc-01594	F	F
6	Sc-01603	mismatch	F
7	Sc-01552	F	F
7	Sc-01609	F	F
7	Sc-01619	F	F
8	Sc-01553	M	M
8	Sc-01569	M	M
8	Sc-01579	M	M
8	Sc-01581	M	M
9	Sc-01554	Likely F	F
9	Sc-01561	F	F
9	Sc-01583	F	F
9	Sc-01600	F	F
9	Sc-01606	F	F
9	Sc-01612	F	F
9	Sc-01613	F	F
10	Sc-01555	Likely M	M
10	Sc-01557	M	M
11	Sc-01556	M	M
11	Sc-01567	M	M
11	Sc-01604	M	M

12	Sc-01558	M	M
13	Sc-01559	Likely M	Likely M
14	Sc-01562	F	F
14	Sc-01573	F	F
14	Sc-01577	F	F
14	Sc-01578	F	F
14	Sc-01590	F	F
14	Sc-01605	F	F
14	Sc-01621	F	F
15	Sc-01563	F	F
15	Sc-01611	F	F
15	Sc-01620	F	F
16	Sc-01564	M	M
16	Sc-01580	M	M
16	Sc-01582	M	M
17	Sc-01565	F	F
17	Sc-01572	F	F
18	Sc-01571	M	M
19	Sc-01584	F	F
19	Sc-01595	F	F
19	Sc-01596	F	F
19	Sc-01597	F	F
19	Sc-01608	F	F
20	Sc-01586	F	F
20	Sc-01601	F	F
20	Sc-01602	mismatch	F
21	Sc-01592	M	M
22	Sc-01593	M	M
23	Sc-01607	F	F

Glossary

monotypic	having only one type or representative, especially of a genus containing only one species.
obligate	restricted to one particularly characteristic mode of life.
troglobiont	an animal living in or restricted to caves.
maternal philopatry	philopatry is the tendency of an organism to stay in or habitually return to a particular area. Maternal philopatry indicates females habitually return to a particular area to give birth.
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.
autosomal	genetic variation located within the set of chromosomes not related to sex.
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.
allelic dropout	when DNA concentration is very low, one allele may be preferentially amplified over the other. When one allele has insufficient amplification to be measured, this is termed allelic dropout.
polymorphism	the condition of occurring in several different forms, i.e. genetic variation.
monomorphic	having or existing in only one form (invariant).
heterozygosity	the presence of two different alleles at a particular gene location, as opposed to homozygosity (same alleles at a particular gene location).
inbreeding coefficient	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	a theoretical measure of the number of breeding individuals in a randomly mating population that would give rise to the observed genetic diversity.

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