Population genetics of the Ghost Bat (*Macroderma gigas*) in the Pilbara bioregion

Final Report

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Executive Summary

- 1103 *Macroderma gigas* faecal DNA and tissue samples were analysed from 66 cave sites across the Hamersley and Chichester subregions of the Pilbara bioregion.

- Useable genotypes were obtained from 997 samples and we identified 270 unique individuals from these.

- Analyses indicated the effective population size (N_e) of Ghost Bats sampled was ~140 individuals (95% CI 118 – 170), which can be tentatively extrapolated to a census population size of 1200-1700.

- Only a small proportion of individuals detected appeared to be resident in caves, i.e. detected at >1 survey point (16/140 individuals). Individuals were detected in caves in surveys up to 13 months apart. However, as sampling effort varied amongst caves, the sampling design was insufficient to provide robust estimates of temporal patterns of cave use.

- In instances where we detected individuals using multiple caves, dispersal primarily occurred amongst closely-located caves (mean dispersal distance = 4 ± 0.9 km). A putative single long-distance dispersal event between a cave in the western Hamersley and one in the east (268 km) was detected.

- The observed dispersal patterns are consistent with spatial autocorrelation analyses showing high relatedness amongst bats within ~10km, with low levels of relatedness extending to ~300km.

- Spatially-explicit Bayesian clustering analysis resolved genetic clusters in each of the subregions (Chichester, East Hamersley and West Hamersley) with additional sub-structuring within the East Hamersley population group. Overall though, genetic structure amongst the Hamersley and Chichester Ranges Ghost Bat populations was weak (F_{ST} = 0.015-0.023) and there was evidence of admixture. This is consistent with high levels of dispersal providing genetic connectivity within and between subregions.

- Genetic diversity was consistently high amongst subregions (Chichester, and East and West Hamersley Ranges), though analyses at the cave level were limited by low sample sizes.

- We found no evidence of recent genetic bottlenecks for either the Hamersley or Chichester subregions, or across the Pilbara in total. However, observed heterozygosity was lower in this study than a previous genetic study conducted in 1999 which may be indicative of population declines over this time.

Introduction

The Ghost Bat (*Macroderma gigas*) is a monotypic bat species native to the Pilbara and Kimberley regions of Western Australia (WA), the Northern Territory (NT) and Queensland. Throughout northern Australia (QLD, NT, northern WA), they are coastal and occur up to 400 km inland, generally north of the Tropic of Capricorn. They occupy a wide range of habitats from rainforest, monsoon and vine thicket in the tropics to open woodlands and semi-arid savannah areas, such as the Pilbara, which is geographically isolated from extant northern Australian populations and the historical central Australian populations by extensive sandy deserts. The Ghost Bat is an obligate troglodyte, and survival is critically dependent on finding natural roosts in caves, crevices, deep overhangs and artificial roosts, such as abandoned mines (Hall et al., 1997). Populations of this species appear to have regionally centralised maternity roosts that are genetically isolated from each other (Worthington-Wilmer et al., 1994). The species is characterised by high maternal philopatry and male-biased dispersal (Worthington-Wilmer et al., 1994). Populations are known to disperse in the non-breeding (dry) season (Toop, 1979, 1985).

In Western Australia, the range of *M. gigas* appears to have contracted northwards since the Holocene with the onset of more arid conditions (Churchill & Helman, 1990). Abandoned mines and spoil piles of the eastern Pilbara provide thermally-buffered microhabitat for ghost bats and this area has become a stronghold for the species, though populations are also found in natural cave formations elsewhere, mostly in the Hamersley Ranges (Armstrong & Anstee, 2000). Little is known of the genetic relationships amongst regional populations of *M. gigas* in the Pilbara. Preliminary results indicate high genetic connectivity amongst roost sites in the Hamersley Range (Spencer & Tedeschi, 2016) but further research is required on the patterns of connectivity with roost sites found in the northern Pilbara (Chichester region).

Study aims

This project is a continuation and extension of a pilot study developed by Dr P. Spencer (Murdoch University) that successfully demonstrated a proof of concept in using faecal DNA sampling and applying population genetics to understand the spatial and population structure of ghost bats in the Pilbara region (Spencer & Tedeschi, 2016).

The current study aims to:

1. **Collate existing and new ghost bat samples:** Including faecal (scat) and tissue samples provided by Biologic and existing DNA extracts provided by Murdoch University.
2. **Extract DNA from new faecal and tissue samples and provide genetic profiles using existing microsatellite markers**
3. **Develop a new set of polymorphic microsatellite markers and integrate these markers with the existing genetic data set:** This entails re-genotyping existing DNA extracts from Spencer & Tedeschi (2016) to ensure consistency amongst datasets.
4. **Determine the number of unique individual genotypes represented in the sample**
5. **Population genetic analyses**: once individual genotypes have been identified:
    - Estimate the spatial and temporal movement of individuals within and between roost sites and populations. Several methods can be applied:
        - genotype matching will be used to detect residents vs migrants over temporal samples (where sampling is available);
        - genotype matching will also be used to detect the presence of individuals in different caves to determine the spatial scale of cave use;
        - spatial autocorrelation analysis will be used to estimate the genetic neighbourhood-size and infer the spatial scale of dispersal;

    - at the landscape scale, estimates of genetic differentiation amongst populations (F_{ST}) will be used to infer the amount of gene flow.
- Estimate the genetic effective population size, and minimum number of bats genotyped.
- Estimate the genetic structure of ghost bats across the Pilbara and whether the Hamersley and Chichester represent different genetic units.
- Estimate the genetic diversity and 'genetic health' of ghost bat populations in the Pilbara.

Materials and Methods

Sampling locations and material

Ghost bat faecal and tissue samples were collected from 66 locations across the Pilbara bioregion (Figure 1, Appendix 1). The majority of the sampling focussed on roost sites in the eastern Hamersley Ranges, with additional sampling of roost sites near Yarrie in the Gorge Range and Oakover Valley area in the northeast Pilbara and several sites to the west of Karijini National Park, in the Stuart Hill region. For genetic analyses we grouped roost sites into three geographic subregions, including the Chichester, East Hamersley (including FMG sites) and West Hamersley. At each sampling site *Macroderma gigas* scats were collected into envelopes and kept frozen until DNA extraction. Tissue samples were collected from captured bats and stored in 100% ethanol.

DNA extraction

DNA was obtained from ghost bat faecal samples by scraping the outer surface of frozen scats with a blade using the same methodology as Spencer & Tedeschi (2016). The scraped material was processed using the QIAamp Fast DNA Stool mini kit (Qiagen Cat No: 51604). Tissue samples were processed using a standard 'salting out' DNA extraction protocol (Sunnucks & Hales, 1996). DNA was resuspended and stored in TE buffer prior to PCR amplification.

Additional DNA extracts originally analysed by Murdoch University (n = 276; Spencer & Tedeschi, 2016) were consolidated and integrated with 1103 new samples analysed by Department of Biodiversity, Conservation and Attractions, DBCA (Appendix 1). All analyses in this report focus on the consolidated data set.

Microsatellite genotyping

Ghost bat DNA samples were analysed at 17 hyper-variable microsatellite loci, including four (gigas01, gigas06, gigas10, gigas11) sourced from Worthington Wilmer et al. (1999), four unpublished microsatellites developed by J. Hughes (GB18, GB20, GB33, GB42; cited in Spencer & Tedeschi, 2016) and nine novel microsatellites developed specifically for this project (MG03, MG05, MG09, MG21, MG24, MG26, MG28, MG32). Microsatellites were arranged in four PCR multiplexes and amplified using the Qiagen Mulitplex PCR kit (Cat No: 206143) as per the manufacturer's instructions. Microsatellite allele sizes were determined by co-running microsatellite PCR products with the Genescan500 size standard (Applied Biosystems, Melbourne). Fragment analysis was carried out on a 3730xl DNA Analyser (ABI systems, Melbourne) using a commercial service (State Agricultural Biotechnology Centre, SABC) and scored using the Genemapper v5 software (Applied Biosystems, Melbourne). Details of microsatellite loci, allele size ranges and multiplexes are provided in Appendix 2.

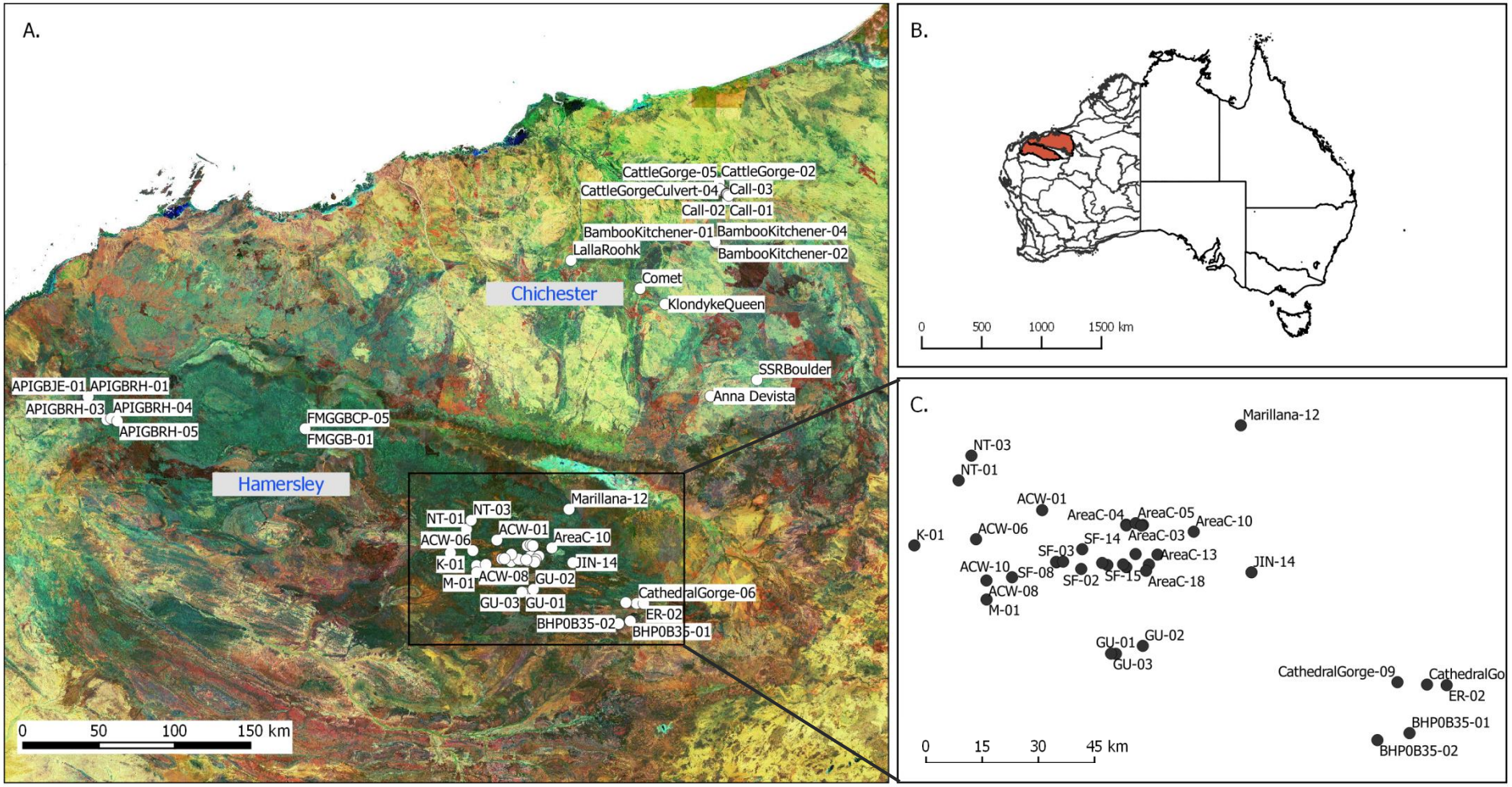


Figure 1: Map of sampling localities of Ghost Bat populations in the Pilbara bioregion. A. Sixty-six roost sites were sampled across the Chichester and Hamersley subregions of the Pilbara. B. Map of Australia showing the location of Chichester and Hamersley IBRA subregions in red. C. Inset map of Eastern Pilbara populations.

DNA and microsatellite genotypes from samples previously analysed by Murdoch University were integrated into the new dataset. This entailed re-genotyping the existing samples for the same microsatellite loci as used in Spencer & Tedeschi (2016) in order to align the existing data with new data created in the DBCA lab. Variation in microsatellite allele sizes can occur between labs and when analysed at different time points due to differences in amplification conditions, or the fluorescent dyes used to detect microsatellite variation and profiling errors, though can vary in predictable ways allowing alignment between datasets if a large enough subset of samples are repeated (Haaland et al., 2011, Ellis et al., 2011). Murdoch University samples were also genotyped at the additional nine new microsatellite loci developed for this project. Due to the low volume of DNA available and potentially some degradation of DNA samples since initial analyses, we encountered a low genotyping success rate for the existing Murdoch University samples (66%, Appendix 1). For this reason there may be some variation in the results reported in Spencer & Tedeschi (2016) and in this report.

Analysis showed that the combined probability of identity (PID) of six markers was sufficient to discriminate between related individuals (PID_{sibs} = 0.002); consequently, we included samples with partial genotypes where six or more loci were successfully genotyped. Overall, the genotyping rate for new samples was high, with 997 of 1103 samples tested producing useable genotypes (i.e. >6 loci) (90%, Appendix 1).

Genotyping quality

Genotyping errors are frequently observed in studies using scat DNA due to the low quality and quantity of DNA sourced from these samples (Knapp et al., 2009, Taberlet et al., 1999). Genotyping error rates were assessed for ghost bat microsatellite loci by re-analysing a subset of samples and comparing the resultant genotypes. Two specific types of errors were assessed: (1) allele dropout and (2) false alleles. Type 1 errors occur more frequently than Type 2 errors with scat DNA (Sethi et al., 2016). Two loci with exceptionally high error rates and that were difficult to consistently score were removed from the analysis (gigas11 and Mg24).

We also tested for cross-amplification of microsatellite loci in two other bat species that co-occur with *M. gigas* and which are sometimes predated by *M. gigas* (and may be co-extracted in *M. gigas* scats). Cross-amplification of microsatellite loci was tested in seven *Taphozous georgianus* and *Vespadelus finlaysoni* samples. Locus gigas06 amplified products in both *T. georgianus* and *V. finlaysoni* with alleles that overlapped in size with *M. gigas*. Locus GB20 amplified weakly but inconsistently in *T. georgianus* and *V. finlaysoni*. These loci were removed from the analysis to prevent potential contamination of genotype profiles from non-target species.

A further two loci (MG03, MG09) were not included in the analyses as they had low levels of polymorphism and were not as informative as the remaining loci (Appendix 2). A final set of 11 microsatellite loci were chosen that were highly variable and could be consistently scored. The combined probability of identity (PID_{sibs}) for the 11 loci was 0.00003. Genotyping error rates were taken into account in the subsequent analyses.

Genotype matching - estimation of the number of unique individuals

To determine the number of unique individuals present in each sample locality we used the software COLONY (Jones & Wang, 2010) to cluster identical scat and, where available, tissue genotypes. COLONY uses an error-tolerant likelihood-based sample matching protocol, combining the probability of obtaining a pair of true genotypes given population allele frequencies and hypotheses about the relationship between the two samples (e.g. samples from full siblings or unrelated individuals), coupled with the probability of observing the sample genotypes given a genotyping error model and genotyping error rates. Both known allele frequencies and locus-specific error rates were input into the sibship models in COLONY. Genotype clusters produced by the software were checked by eye and some minor adjustments were made. In a few cases COLONY identified similar genotypes as

being sibs where these genotypes only differed by 1-2 loci that were homozygous for the same alleles present in a heterozygote in the reference genotype (the pattern observed for Type 1 genotyping errors). Without appropriate reference samples to assess the sib-ship structure of ghost bat colonies, we thought it more conservative to consider these types of genotypes as duplicates rather than sibs. This may have the effect of underestimating the total number of individuals present in a cave, but should only mean that highly related individuals have not been properly detected.

Assessment of sampling effort

The rate of accumulation of new individuals with increasing sample size was assessed using rarefaction analysis. A single, sample-based rarefaction curve was calculated in the software EstimateS v9.1.0 (Colwell, 2013). We used non-parametric extrapolation to explore the trajectory of the rarefaction curve for an additional 1000 samples (n=2178 total). The census population size can be estimated from the rarefaction curve at the point where the curve reaches an asymptote (Eggert et al., 2003).

Population genetic analyses

Summary population genetic diversity statistics, such as observed (H_o) and expected heterozygosity (H_e), number of alleles (N_a) and the inbreeding coefficient (F_{is}) were calculated in GENALEX v6.5 (Peakall & Smouse, 2012). Allelic richness (A_r) was calculated in HP-RARE using rarefaction to equalise sampling size amongst populations (rarefied to n = 5, the smallest population size). Sample sizes (number of individuals) per cave were low so diversity statistics could not be calculated for all caves, and in some instances, we pooled genotypes for caves that were closely located to increase sample sizes. Diversity statistics were only calculated for caves with >5 individuals; however, it should be noted that sample sizes this low do not provide robust estimates of diversity parameters so should not be relied upon. In population genetics, sample sizes of 25-30 individuals are typically required for accurate estimation of diversity statistics (Sinclair & Hobbs, 2009, Hale et al., 2012). Summary statistics are provided at the population (cave) and regional levels (East/West Hamersley and Chichester), as well as the total observed for the Pilbara bioregion.

Evidence of recent population bottlenecks was investigated by testing for an excess of heterozygosity using BOTTLENECK (Piry et al., 1999). In populations where N_e has remained relatively constant in the past there is approximately an equal probability of a locus displaying an excess and a deficiency of heterozygosity. However, in recently bottlenecked populations the majority of loci will display an excess of heterozygosity. Due to the relatively small number of polymorphic loci analysed (n=11), a Wilcoxon sign-rank test was estimated. A mixed model of microsatellite mutation was assumed with a single step mutation assumed at 90%, variance of 12, as suggested by Piry et al. (1998).

Spatial autocorrelation of pairwise genetic relatedness amongst individuals was used to infer the spatial genetic neighbourhood for ghost bats in the study region. The spatial autocorrelation correlogram plots the autocorrelation coefficient (*r*) as a function of distance class, as well as the 95% confidence interval about the null hypothesis of no spatial genetic structure (random) as determined by permutation. We firstly replicated the distance classes used in Spencer & Tedeschi (2016) in a single, fine-scale spatial autocorrelation analysis (0-50 km). Secondly, we performed a larger scale analysis covering the maximum distance amongst sample localities (~450 km) and where individuals are pooled within distance classes of increasing size (Multiple DClass option) to detect the scale of the spatial genetic neighbourhood for ghost bats. In this analysis, when significant positive genetic structure is present, the estimated value of *r* will decrease with increasing size of the distance class. The distance class size at which the estimate of *r* is no longer significant provides an approximation of the extent of detectable positive spatial genetic structure (Peakall et al., 2003). Spatial autocorrelation analyses were performed in GENALEX.

Two methods were used to investigate the regional genetic structure of ghost bats across the Pilbara. Firstly, a non-spatial Bayesian cluster analysis was performed in STRUCTURE (Pritchard et al., 2000) using microsatellite allele frequencies. STRUCTURE was run 10 independent times for the potential number of genetic clusters (K) ranging from K = 1 to 12. The runs assumed an admixture model with correlated allele frequencies, a burn-in length of 100 000 MCMC steps, followed by simulation set at 1 000 000 repetitions. To estimate the number of clusters (K), an ad hoc approach was taken by obtaining the mean posterior probability of the data ΔK using STRUCTURE HARVESTER (Earl & vonHoldt, 2012). To visualize clustering across runs of K, STRUCTURE outputs were collated using CLUMPP (Jakobsson & Rosenberg, 2007).

The second approach used was TESS 2.3.1, which also uses a Bayesian clustering algorithm to determine population genetic structure (Chen et al., 2007). However, TESS incorporates a spatially-explicit model by including the exact geographical co-ordinate for each individual as informed priors (Francois & Durand, 2010). TESS was run using the conditional autoregressive (CAR) Gaussian model of admixture with an interaction parameter of 0.6 as described in Chen et al. (2007). The model was run for 100,000 iterations following a burn-in period of 20,000 MCMC iterations for K = 2 - 12, with 20 replicates for each K. The optimal number of clusters was determined by identifying the K at which DIC values plateaued and by inspecting barplots of cluster assignments for K = 2 to the K at which DIC values plateaued across replicates to confirm that assignments had stabilized. A hierarchical analysis of population structure was performed, firstly investigating genetic structure across the Pilbara, then within each of the two main subregions, the Chichester and Hamersley Ranges.

The program LDNE is used to estimate the contemporary effective population size (N_e) based on genotypic linkage disequilibrium data (Waples & Do, 2008). The program calculates separate estimates using different criteria for excluding rare alleles (suitable for microsatellite data). Simulations presented in Waples & Do (2010) suggest using allele frequencies >0.02 represents the best precision-bias trade-off for the LD method. The program also implements a jack-knife technique to calculate the 95% confidence intervals of the N_e estimate.

Results

Number of unique individuals

Based on genotype matching across sampled scat and tissue samples, we detected 270 unique genotypes (i.e. unique individuals) from across the Pilbara region. At the sub-regional level, 63 unique individuals were detected in the Chichester from 186 genotyped samples, 175 were detected in East Hamersley populations from 918 genotyped and 32 were detected in the West Hamersley populations from 132 genotyped.

The genetic effective population size (N_e) is estimated to be 140 individuals (95% CI's 118 – 170) (Table 1). Given that the effective population size is typically around 10% of the census population size (Frankham, 1995), we can tentatively extrapolate that the population of ghost bats in the Pilbara is between 1200 – 1700 individuals.

Table 1: Estimates of effective population size (N_e) of Ghost Bats sampled in the Pilbara calculated from linkage disequilibrium amongst genotypes excluding alleles with decreasing frequencies. [a] Using alleles with frequency >0.02 is expected to give the most robust estimate of effective population size (Waples & Do, 2010).

	Lowest allele frequency		
	0.05	**0.02** [a]	**0.01**
# comparisons	1564	2988	4228
Estimated Ne	111	140	147
95% CI (jackknife)	92 - 137	118 - 170	122 - 180

The number of unique individuals detected per cave ranged from one to 21, though the sampling effort (number of samples analysed) and genotyping success varied greatly amongst caves (Figure 3). Call-01 cave in the Chichester, K-01 in the eastern Hamersley and APIGBRH-04 in the western Hamersley appear to have the largest number of unique individuals detected with a reasonable sampling effort (>10 samples genotyped; detection ratios of 0.88-0.94, Figure 2). Applying mark-recapture principles, these populations could be expected to have large population sizes and further sampling would detect new individuals. Conversely, caves such as GU-01, FMGGB-01, SF-01 and SF-08 in the eastern Hamersley had high sampling effort but only small numbers of individuals detected (detection ratios 0.04-0.07, Figure 2), suggesting potentially small population sizes for these locations.

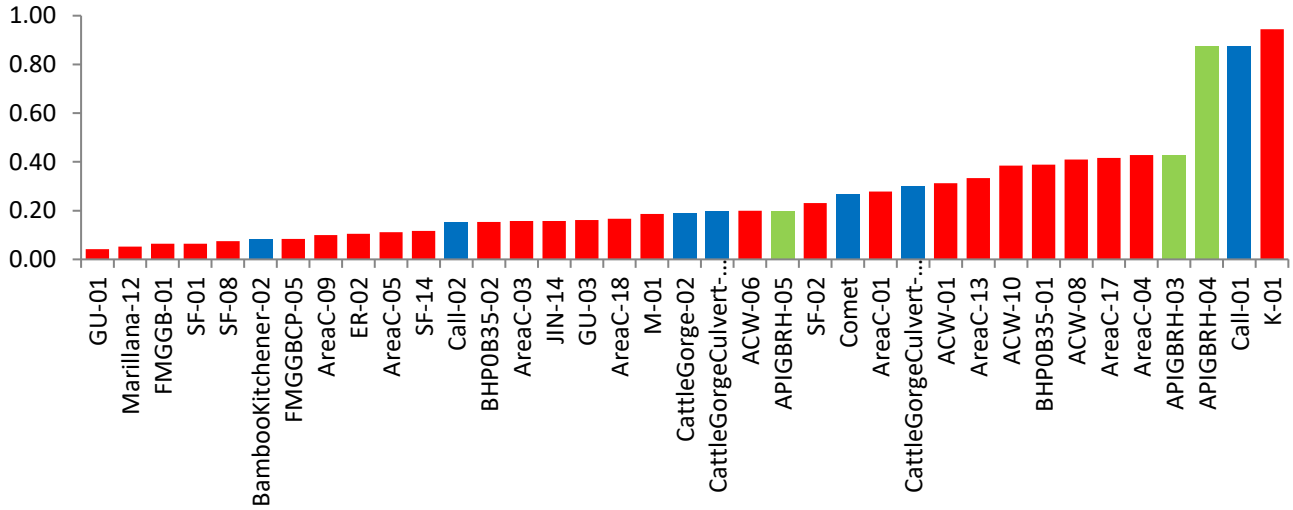


Figure 2: Rate of detection of new individuals, i.e. ratio of the number of unique individuals detected per number of samples genotyped, for caves where >10 samples (scats and/or tissues) were genotyped. Blue = Chichester, Red = East Hamersley, Green = West Hamersley populations

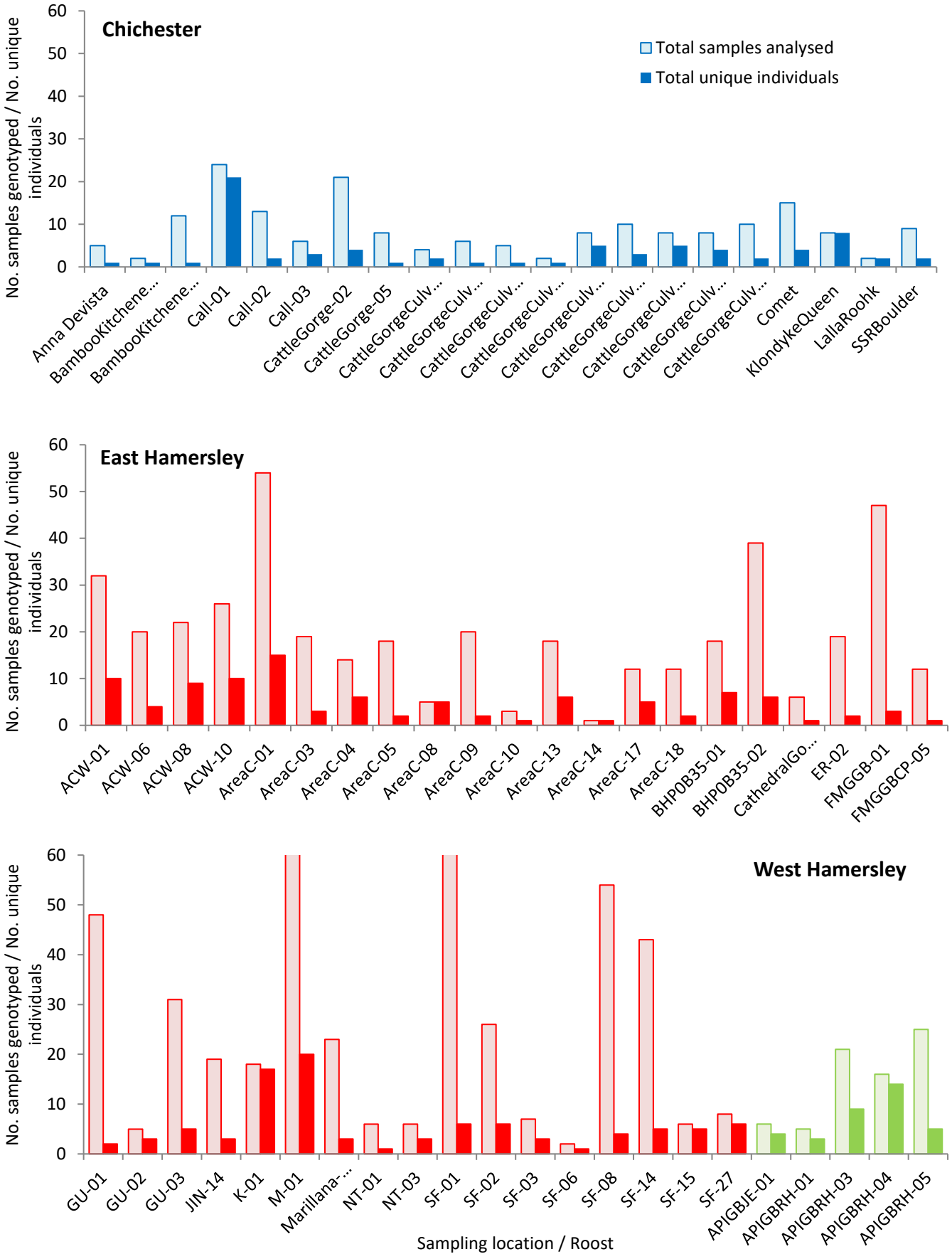


Figure 3: Summary of number of unique individuals detected per ghost bat roost, along with sampling effort (number of samples genotyped). Note that the number of samples genotyped was truncated for M-01 and SF-01 to enable better display; see Appendix 1 for correct totals.

Sampling effort and population size

There was a slow, approximately linear increase in the number of unique individuals detected with increasing numbers of samples analysed (blue line, Figure 4). Rarefaction analysis indicated a slow rate of accumulation but the curve did not appear to reach an asymptote, even with extrapolation to an additional 1000 samples. This suggests that further sampling is required to fully sample the existing Ghost Bat population in the Pilbara bioregion. Rarefaction curves for each of the subregions (Appendix 3) showed that only the West Hamersley population reached an asymptote, suggesting a smaller population size at this location and that only a modest increase in sampling effort would be required to fully sample the population.

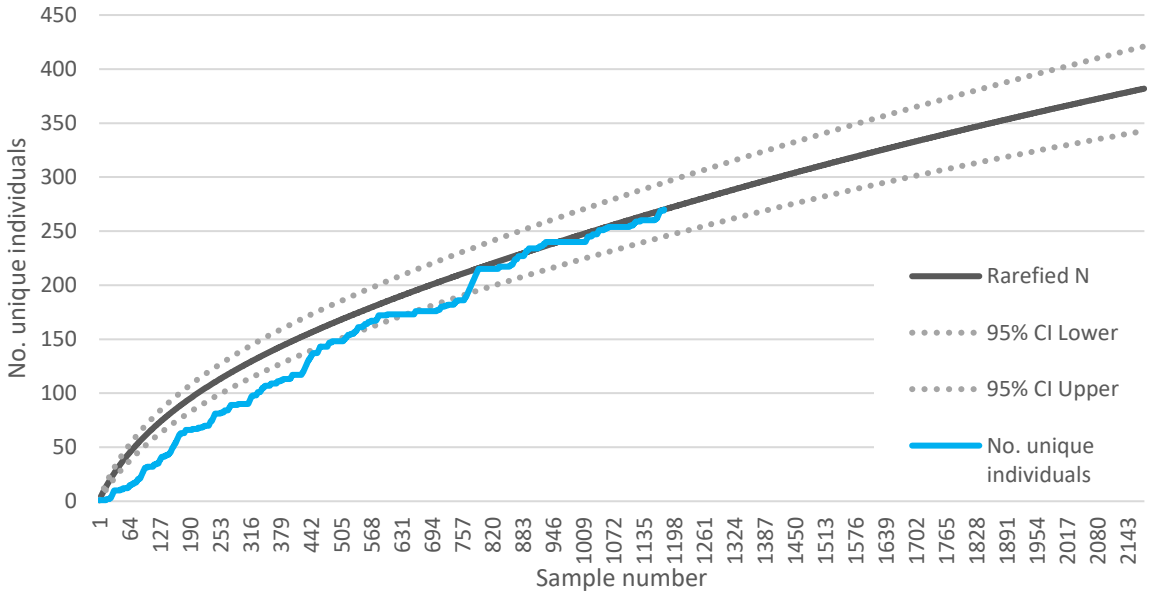


Figure 4: The number of unique individuals detected with increasing number of samples analysed and the estimated rarefaction curve for the Pilbara bioregion. The number of individuals detected per number of samples analysed per cave is plotted in Appendix 3 also.

Cave use with time

Eighteen of the 66 caves in this study were monitored across multiple time periods (Figure 5). Within these, we detected 17 incidences involving 16 individuals that were present at more than one sampling time (individual 174 was detected in two caves, Table 2) but overall, in each cave only a small proportion of the individuals present were detected on multiple occasions. Most individuals were present in adjacent surveys. Individuals 174 and 308 were detected in surveys 12 and 13 months apart, potentially demonstrating high cave fidelity for these individuals (Table 2). For the majority of individuals that were present at multiple sampling times there was a high detection rate (i.e. the individual was present in a high proportion of sampling times), indicating these individuals are likely residents of these caves. This contrasts with the 124 individuals that were only detected at one of the multiple sampling times within these caves (Appendix 4). As survey times were temporally and spatially inconsistent, it is not possible to indicate the residency times of these individuals. Further systematic survey (e.g. monthly surveys) may provide better data on temporal patterns of cave use by individuals.

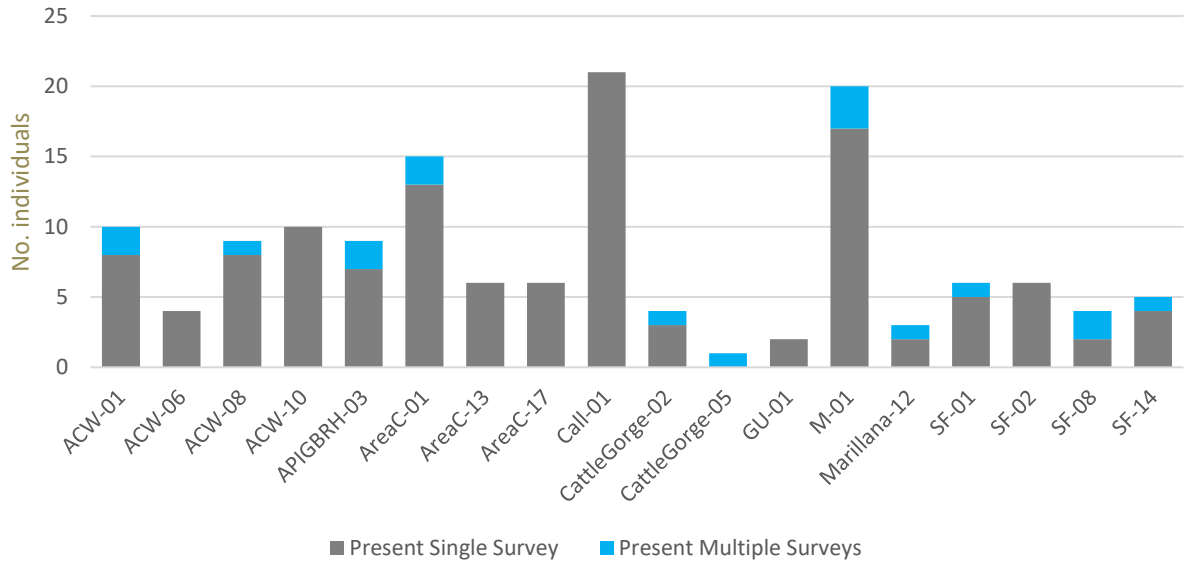


Figure 5: Number of individual ghost bats that were detected in caves at single versus multiple time points when caves were monitored in more than one time period. More detail on cave use is provided in Table 2.

Table 2: Summary of individual ghost bats detected on multiple sampling occasions, the proportion of sampling times in which the individual was detected and the maximum time between sampling times in which the individual was detected. Shaded cells indicate sampling time and solid dot indicates presence of individual at that sampling time.

Individual ID	Cave	2015-11	2015-12	2016-04	2016-05	2016-06	2016-10	2016-12	2017-02	2017-05	Detected (%)	Max time
1	ACW-01	●	●	●	●						100	7 mo
3	ACW-01			●	●						50	2 mo
21	ACW-08			●	●						66	2 mo
49	APIGBRH-03				●		●				100	5 mo
50	APIGBRH-03				●		●				100	5 mo
79	AreaC-01			●	●						100	2 mo
88	AreaC-01			●	●						100	2 mo
174	CattleGorge-02				●				●	●	100	12 mo
174	CattleGorge-05						●		●		100	5 mo
269	M-01	●	●	●	●						100	7 mo
282	M-01			●	●						50	2 mo
283	M-01	●	●	●							75	6 mo
300	Marillana-12		●				●				100	11 mo
308	SF-01	●		●	●	●		●			100	13 mo
343	SF-08			●	●						100	2 mo
344	SF-08			●	●						100	2 mo
349	SF-14			●	●						100	2 mo

Spatial scale of cave use

We detected 27 individuals using more than one cave (Table 3: Summary of individuals detected in more than one cave location, the distance between sampling locations, the sampling time in which they were detected and whether individuals were detected in different caves during the same or different sampling times. [a] Where multiple caves were used by the same individual, the mean and maximum distances amongst caves is presented. Note that Cattle Gorge Culvert sites are feeding sites and represent day roosts only.
). In the majority of cases, individuals were detected in two caves but several individuals were detected using multiple caves (Individual 161 - 4 caves, 189 - 3 caves, 192 - 3 caves, 330 - 3 caves and 340 - 3 caves). In 19 cases, scats from individuals were detected in different caves during the same survey period indicating they were using these caves concurrently. Some individuals were detected in different caves at different time points, however, the temporal sampling was inconsistent across the survey so it is difficult to know whether these time points were distinct dispersal events or whether bats were using multiple caves concurrently but not detected.

Some cave systems appeared to have high levels of shared use (e.g. APIGBRH caves, Area C caves, Cattle Gorge caves and SF caves) though this observation should be balanced against differences in the sampling intensity at these caves which impacts detection rates (Figure 3). Further systematic survey (e.g. monthly surveys) may provide better data on the spatio-temporal patterns of cave use by individuals.

We found a putative single incidence of shared cave use across geographic subregions. A single scat of individual 14 was found in cave ACW01 (eastern Hamersley), though individual 14 was frequently detected in APIGBRH-05 (western Hamersley) (Table 3: Summary of individuals detected in more than one cave location, the distance between sampling locations, the sampling time in which they were detected and whether individuals were detected in different caves during the same or different sampling times. [a] Where multiple caves were used by the same individual, the mean and maximum distances amongst caves is presented. Note that Cattle Gorge Culvert sites are feeding sites and represent day roosts only.
). The individual was detected in both caves during the same sampling time period: samples were collected from APIGBRH-05 on 26/5/16 and from ACW01 on 30/5/16, encompassing the sampling period 21/4/16 - 30/5/16. It is possible that long-distance dispersal occurred over this time period, though seems unlikely given the expected dispersal capacity of *M. gigas*. Further investigation would be required to confirm this result and to discount errors from mislabelling or sample mix-up.

We primarily detected individuals using caves at highly localised scales; most bats were detected in immediately neighbouring caves (Table 3: Summary of individuals detected in more than one cave location, the distance between sampling locations, the sampling time in which they were detected and whether individuals were detected in different caves during the same or different sampling times. [a] Where multiple caves were used by the same individual, the mean and maximum distances amongst caves is presented. Note that Cattle Gorge Culvert sites are feeding sites and represent day roosts only.
). Excluding the putative long-distance dispersal event (above), the spatial scale of dispersal between caves ranged from 30 m up to 21 km (Figure 6), with a mean dispersal distance of 3.99 ± 0.87 km.

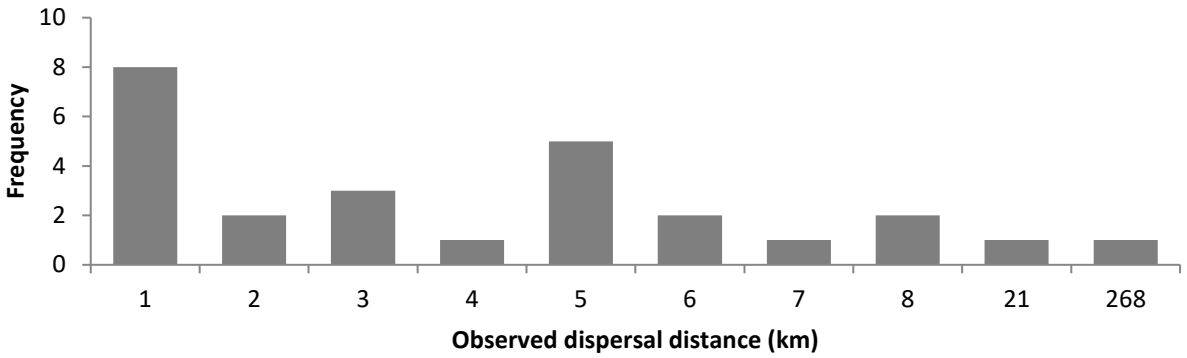


Figure 6: Frequency histogram of the distance of detected dispersal events of individual ghost bats amongst caves. More detail on dispersal events is provided in Table 3**.**

Table 3: Summary of individuals detected in more than one cave location, the distance between sampling locations, the sampling time in which they were detected and whether individuals were detected in different caves during the same or different sampling times. [a] Where multiple caves were used by the same individual, the mean and maximum distances amongst caves is presented. Note that Cattle Gorge Culvert sites are feeding sites and represent day roosts only.

Individual ID	Cave	Distance [a] (km)	2015-12	2016-04	2016-05	2016-08	2016-10	2016-12	2017-02	2017-05	2017-08	Same (S) / different (D) times
14	ACW-01	268			●							S
	APIGBRH-05				●							
29	ACW-08	5			●							D
	M-01			●								
43	APIGBJE-01	21			●							S
	APIGBRH-01				●							
49	APIGBRH-03	3			●		●					S / D
	APIGBRH-04				●							
52	APIGBRH-03	3					●					D
	APIGBRH-04				●							
80	AreaC-01	5			●							D
	AreaC-13						●					
83	AreaC-01	5			●							D
	AreaC-18							●				
84	AreaC-01	5			●							D
	AreaC-13						●					
98	AreaC-03	0.2					●					S
	AreaC-04						●					
107	AreaC-05	1.5					●					S
	AreaC-09						●					
122	AreaC-13	8					●					S
	AreaC-17						●					
135	BHP0B35-01	8			●							S
	BHP0B35-02				●							
161	Call-01	2.3 (mean)					●					S / D
	CattleGorgeCulvert-06	4.2 (max)				●						
	CattleGorgeCulvert-07					●						

	CattleGorgeCulvert-08					●						
167	Call-02	0.1							●			S
	Call-03								●			
174	CattleGorge-02	0.6			●				●	●		S / D
	CattleGorge-05						●		●			
183	CattleGorgeCulvert-03	2				●						S
	CattleGorgeCulvert-05					●						
189	CattleGorgeCulvert-06	0.5 (mean)				●						S
	CattleGorgeCulvert-07	0.7 (max)				●						
	CattleGorgeCulvert-08					●						
190	CattleGorgeCulvert-06	0.5				●						S
	CattleGorgeCulvert-07					●						
192	CattleGorgeCulvert-07	1 (mean)				●						S / D
	CattleGorgeCulvert-08	1.5 (max)				●						
	CattleGorgeCulvert-09										●	
206	FMGGB-01	0.03						●				D
	FMGGBCP-05				●							
226	GU-01	7		●								S
	GU-02			●								
330	SF-01	4 (mean)		●								S / D
	SF-06	6 (max)						●				
	SF-15			●								
340	SF-03	5 (mean)		●								S / D
	SF-14	7 (max)		●								
	SF-27				●							
343	SF-03	0.1		●								S
	SF-08			●	●							
349	SF-14	6		●	●							S
	SF-27				●							
351	SF-14	6		●								D
	SF-27				●							

Genetic diversity

Overall, the genetic diversity of Ghost Bats across the Pilbara region is high. Mean expected heterozygosity is 0.78, observed heterozygosity is 0.68 and the mean number of alleles observed is 12 (Table 4, Appendix 5).

There was little variation in genetic diversity statistics across populations of *M. gigas* at the individual cave level (Figure 7, Appendix 5), nor across regions; for example, observed heterozygosity ranged from 0.67 – 0.69 and expected heterozygosity from 0.74 – 0.78 across individuals sampled in the Chichester, East and West Hamersley (Table 4). Inbreeding coefficients (F) varied considerably amongst populations (partially due to low sample sizes) but was positive overall (F = 0.12), suggesting some level of inbreeding across the Pilbara.

Overall, the Chichester subregion had the highest observed heterozygosity and lowest inbreeding values, suggesting populations here may be in marginally better genetic health than those in the Hamersley.

Table 4: Summary of the range and mean of genetic diversity statistics from individual Ghost Bat populations in three Pilbara subregions; Chichester, East and West Hamersley. n = number of populations, N = number of samples analysed per population, N_a = Number of alleles, A_r = Rarefied allelic richness, H_o = Observed heterozygosity, uH_e = unbiased expected heterozygosity, F = inbreeding coefficient. Further detail on population-level statistics is in Appendix 5.

Region	N	N_a	A_r	H_o	uH_e	F
Chichester						
Range (n = 6)	5 – 20	3 - 7	2.9 - 5.0	0.38 - 0.81	0.55 - 0.78	-0.33 - 0.15
Mean	61.5 ± 0.4	9.9 ± 1.1	4.0 ± 0.3	0.70 ± 0.03	0.75 ± 0.04	0.05 ± 0.03
East Hamersley						
Range (n = 13)	5 – 19	3.7 - 6.5	3.6 - 4.9	0.55 - 0.78	0.63 - 0.78	-0.16 - 0.24
Mean	164.3 ± 1.1	9.9 ± 0.8	4.2 ± 0.1	0.68 ± 0.02	0.78 ± 0.03	0.13 ± 0.02
West Hamersley						
Range (n = 4)	5 – 14	4.5 - 5.1	4.1 - 4.5	0.64 - 0.70	0.69 - 0.76	-0.03 - 0.067
Mean	32.9 ± 0.4	8.2 ± 0.7	4.4 ± 0.1	0.66 ± 0.05	0.76 ± 0.03	0.12 ± 0.05
Pilbara Total	**259 ± 1**	**11.8 ± 1.2**	**4.2 ± 0.1**	**0.68 ± 0.02**	**0.78 ± 0.03**	**0.12 ± 0.01**

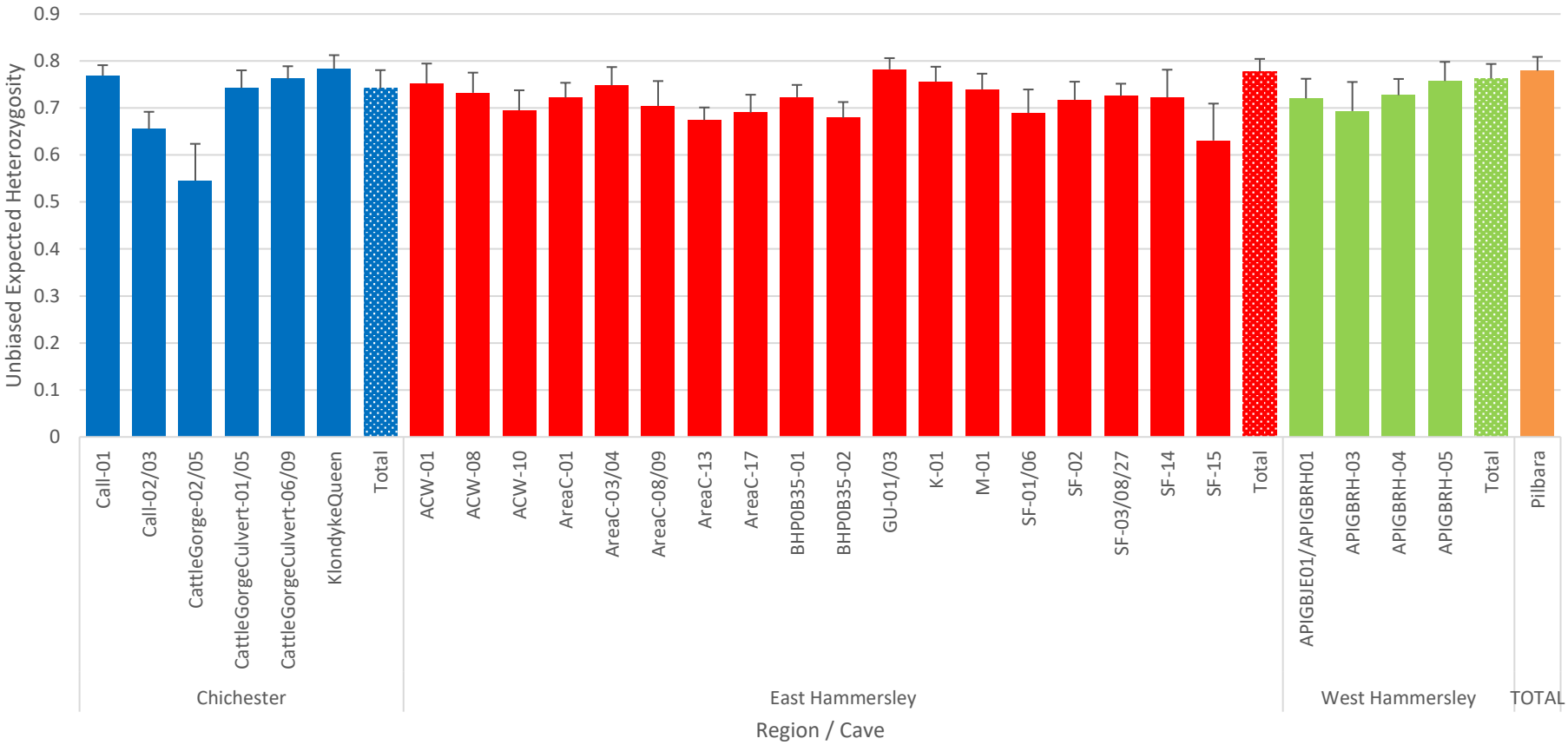


Figure 7: Expected heterozygosity, a measure of genetic diversity, across populations in each of three Pilbara subregions, Chichester, East and West Hamersley. Expected heterozygosity is presented, rather than observed heterozygosity, as it is less dependent on sample size. Full details on population genetic diversity is in Appendix 5.

Genetic bottlenecks

One-tailed Wilcoxon tests were used to test for excess heterozygosity in microsatellite loci indicative of population bottlenecks in the Hamersley and Chichester subregions and across the Pilbara in total. There was no evidence of population bottlenecks in any of the Pilbara populations or in total (all P-values non-significant; Table 5).

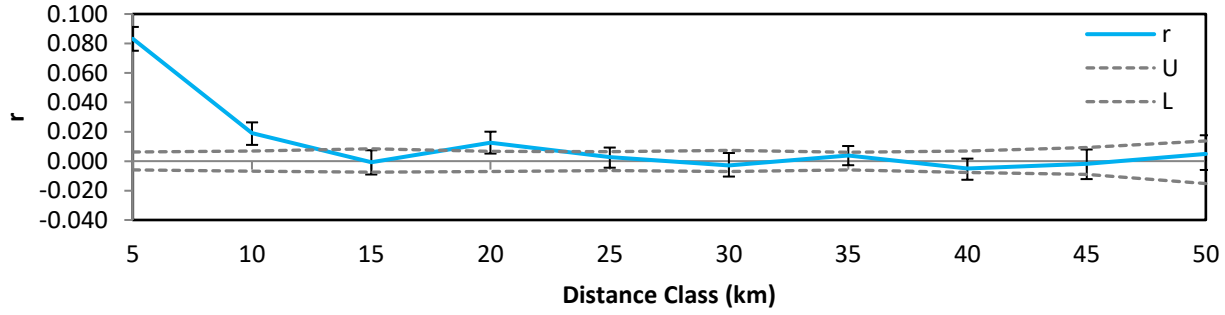
Table 5: Results of one-tailed Wilcoxon tests for genetic bottlenecks in Ghost Bat regional populations and in total. All p-values were non-significant.

Population	No. loci heterozygosity deficiency	No. loci heterozygosity excess	P-value
Hamersley	6	5	0.612
Chichester	7	4	0.926
Pilbara Total	6	5	0.817

Spatial autocorrelation

We visualised the genetic relatedness of individuals over geographic distance in order to define the spatial genetic neighbourhood for *M. gigas*. At finer scales (0-50 km), we detected a significant positive genetic relatedness amongst individuals up to 10 km (Figure 8a). Using the multiple distance class method (Figure 8b) which combines relatedness scores of individuals within increasing distance classes, we again find a high positive relatedness of individuals within 10 km that declines with increasing distance. Low but positive spatial genetic structure amongst individuals was detected up to 300 km. This analysis infers that the majority of Ghost Bat dispersal occurs within shorter distances, but that low frequency dispersal may be occurring up to ~300 km.

a.



b.

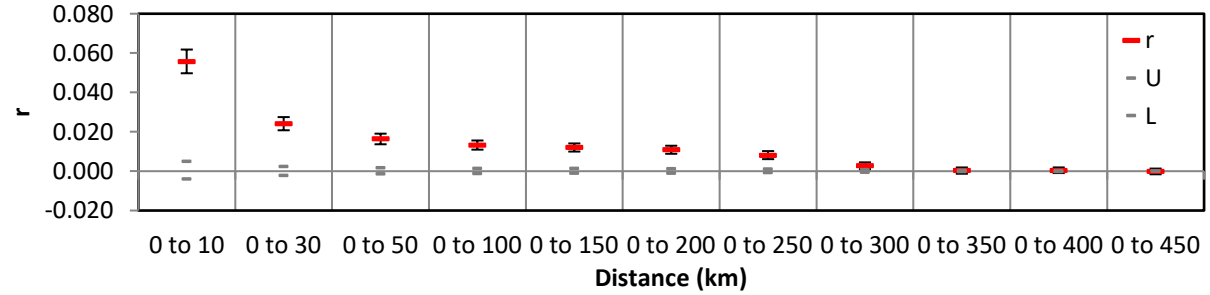


Figure 8: (a) Spatial autocorrelation of genetic and geographic distances at fine spatial scales (up to 50 km) and (b) combined spatial autocorrelation values (*r*) for increasing distance class sizes up to 450km. Spatial autocorrelation values are statistically significant if they sit outside the 95% upper (U) and lower (L) confidence intervals around the null hypothesis of no genetic structure (r = 0).

Genetic structure within the Pilbara

We implemented two Bayesian clustering approaches to investigate the population genetic structure of Ghost Bats across the Pilbara. The STRUCTURE method attempts to group individuals into genetic clusters that are in Hardy-Weinberg equilibrium with no regard to the spatial location of individuals (i.e. the method is aspatial). The deltaK method used to evaluate the optimal number of populations (K) from the STRUCTURE analysis indicated that K=4 best represents the genetic data (Figure 9a), however, the plot of individual cluster membership (Appendix 6) shows that there was little geographic structuring to the identified genetic clusters, with all populations showing high levels of admixture.

(a) (b)

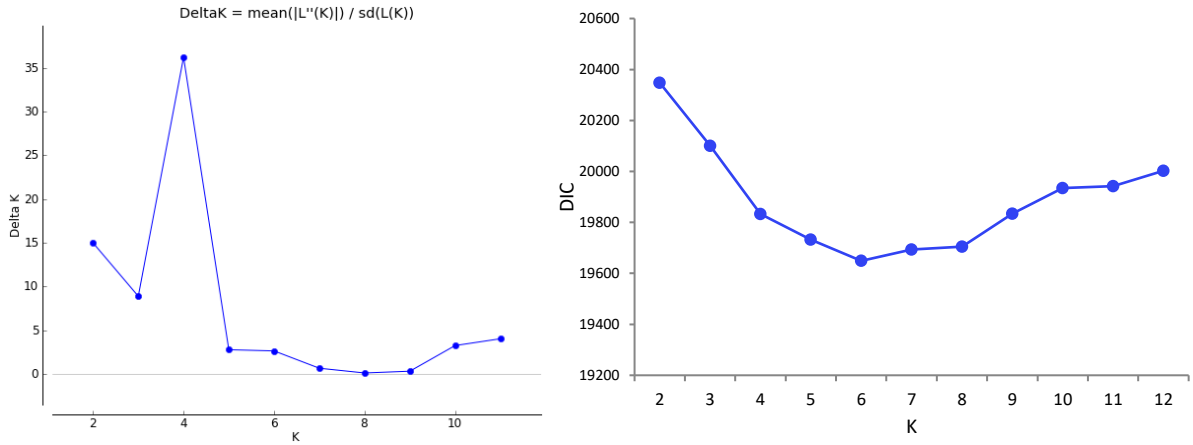


Figure 9: (a) Delta K method to find optimal number of clusters (K) from STRUCTURE analysis indicated four genetic clusters, and (b) plot of DIC values from TESS analysis. Optimal K is selected where the plot begins to plateau, also indicating four genetic clusters.

The second approach, implemented in TESS, detects clusters based on their genetic identity, as well as spatial location. The optimal number of clusters was determined by identifying the K at which DIC values plateaued (Figure 9b). TESS identified K = 4 best represents the genetic data, given the spatial information. The plot of individual cluster membership (Figure 10) shows that TESS resolved genetic groupings that relate to the Pilbara subregions, with the West Hamersley roosts showing some admixture with both the Chichester and East Hamersley. Within the East Hamersley, the AreaC-01 roost seems to be identified as a further genetic cluster (Cluster 3) (Appendix 7).

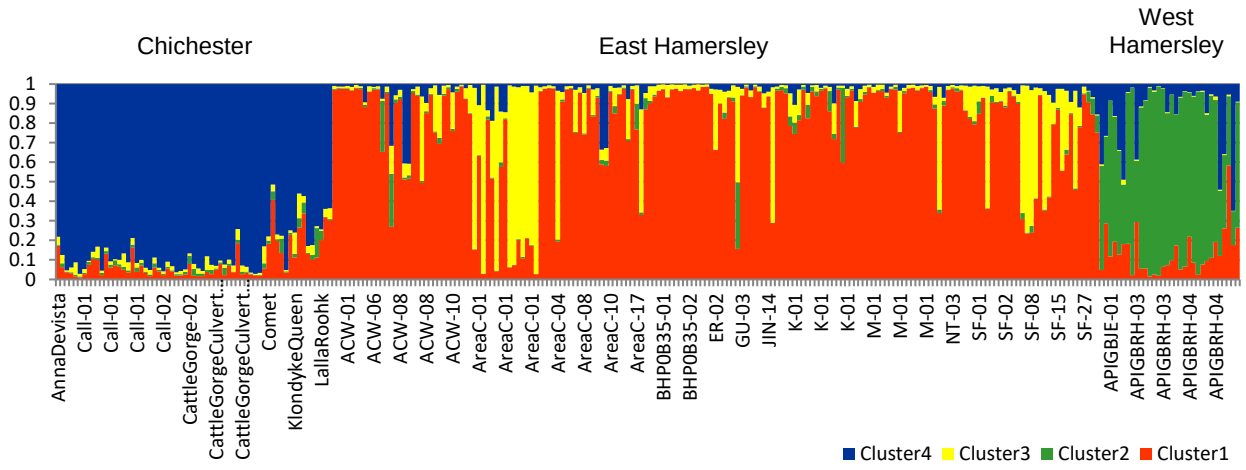


Figure 10: Barplot of proportional membership of individuals to each genetic cluster from spatially-explicit TESS analyses. Each bar represents an individual, with population names across the x-axis.

Analysis of genetic differentiation in allele frequencies (F_{ST}) amongst subregions indicated a low, but statistically significant, level of genetic structure amongst subregions (Table 6). Based on allele frequencies, it appears that there is marginally greater affinity between the Chichester and East Hamersley Range populations (F_{ST} = 0.015), than between East and West Hamersley populations (F_{ST} = 0.023). When the East and West Hamersley populations are pooled, F_{ST} between the Chichester and Hamersley Range subregions was 0.013. In these analyses F_{ST} can range from zero (no genetic differentiation) to one (complete fixation of alternative alleles).

Table 6: Pairwise genetic differentiation (F_{ST}) amongst subregions and statistical significance determined by permutation testing (n = 999). F_{ST} values below the diagonal and p-values above. F_{ST} between the Chichester and Hamersley subregions was 0.013 (p<0.001).

	Chichester	East Hamersley	West Hamersley
Chichester	-	0.001	0.001
East Hamersley	0.015	-	0.001
West Hamersley	0.023	0.020	-

Despite low genetic structuring there was some unique genetic diversity present in each region, with the mean number of private alleles (alleles unique to each region) higher in the Chichester region than the east/west Hamersley regions (Figure 11).

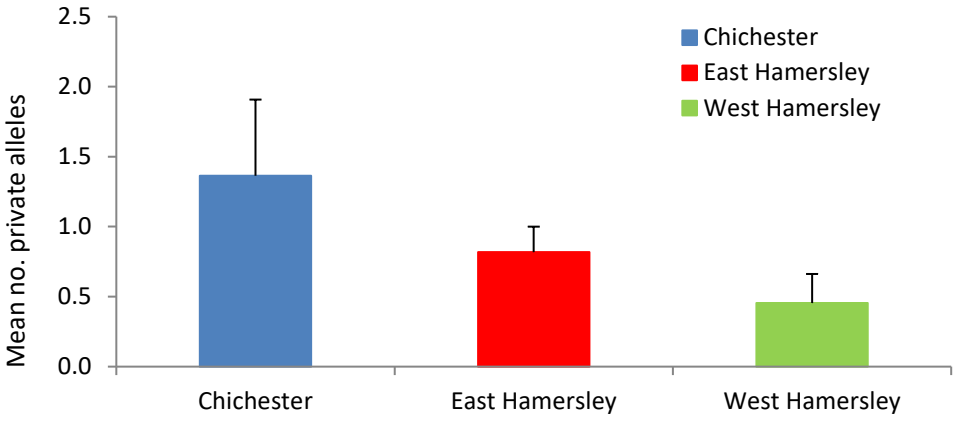


Figure 11: Mean number of 'private' alleles, i.e. alleles unique to each sub-region

Genetic structure within subregions

We ran further clustering analyses on populations within the two main subregions, the Chichester and Hamersley Ranges, to investigate hierarchical patterns of genetic structure. TESS failed to detect any genetic sub-structuring within the Chichester sub-region (Appendix 8a) but identified some weak population structuring within the Hamersley Range. TESS analysis indicated the optimal number of genetic clusters (K) was five (Appendix 8b; Figure 12). Populations in the East Hamersley show relatively high levels of admixture across the region, though populations ACW-01 and AreaC-01appear to form relatively distinct genetic clusters (cluster 1 and 2 respectively). Some SF caves (SF-08, SF-14) appear to have some admixture with AreaC-01 (Appendix 9), which may indicate dispersal between these caves. As detected above, roosts in the West Hamersley form a genetic cluster distinct from the East Hamersley (cluster 3).

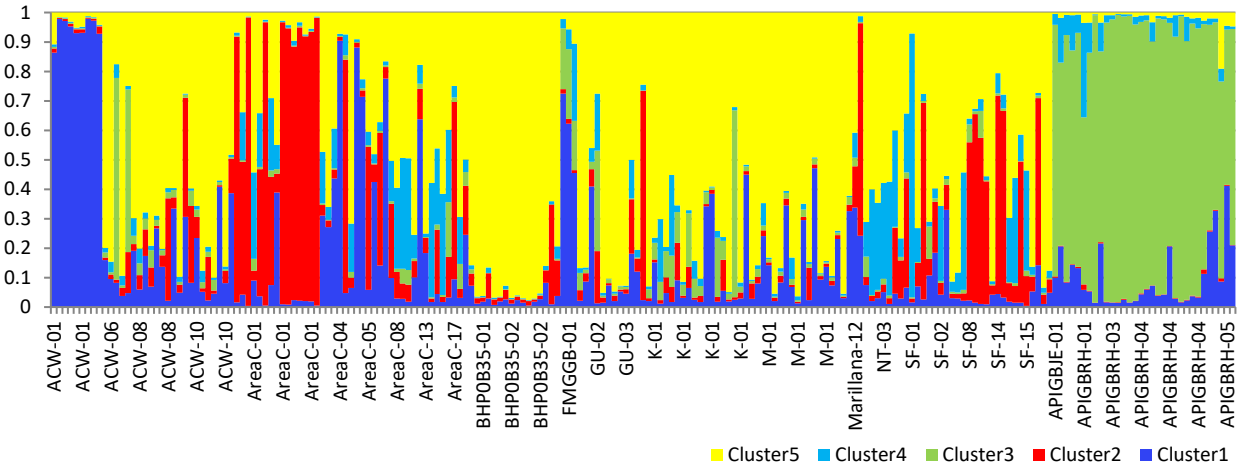


Figure 12: Barplot of individual membership proportions to each genetic cluster for populations within the Hamersley Ranges sub-region.

Discussion

Building on preliminary work by Spencer and Tedeschi (2016), faecal DNA analysis has provided an opportunity to use a non-invasive technique to gain detailed information on the genetic diversity and connectivity of Ghost Bat populations in the Pilbara. This has provided insights into the spatial and temporal cave use by bats, as well as an indication of the genetic structure and genetic health of *M. gigas* across the Hamersley and Chichester regions.

Overall, we found a high genotyping success rate for new scat DNA samples with 90% of samples tested giving a useable genotype. As part of this study we expanded the number of microsatellites available to the study of Ghost Bats by isolating nine novel microsatellites. Refinement of the original genotyping set showed eleven microsatellites produced reliable and consistently scoreable genotypes for further analysis.

All together we detected 270 unique genotypes from 1103 scat and tissue samples taken from across the Pilbara. There was some difficulty in identifying unique genotypes from the microsatellite data due to the prevalence of allelic dropout in scat samples (a common problem in scat DNA studies; Knapp et al., 2009, Taberlet et al., 1999) making it difficult to distinguish between cases of allelic dropout or true homozygous profiles. We could have mis-classed a small proportion of scat samples as being from the same individual when they were from different, but highly related, individuals. As a result, the number of unique individuals detected in this study should be considered a minimum estimate.

Rarefaction analyses can be used to infer the census population size (N_c) from non-invasive genetic sampling (Kohn et al., 1999; Eggert et al., 2003; Luikart et al., 2010), similar to mark-recapture approaches. The 'true' population size can be inferred when the cumulative number of unique individuals detected with increasing sample size reaches an asymptote. Rarefaction analysis showed that additional sampling is required to reach an asymptote so we were unable to effectively estimate the census population size of Ghost Bats in the Pilbara using this method. Genetic estimates of the *effective* population size (N_e), however, suggest our genetic sample represented between 118 -170 individuals (mean = 140). It is a common premise that N_e is 10% of N_c (Frankham, 1995), suggesting our estimates extrapolate to a census population size of 1200-1700 individuals. This is consistent with existing demographic estimates of the total population size of Ghost Bats in the Pilbara (estimated 1300-2000, Threatened Species Scientific Committee, 2016), though the true relationship of N_e:N_c is not known for Ghost Bats.

The number of individual bats detected per cave varied significantly, though is highly dependent on differences in sampling intensity per cave. We detected several caves that appeared to have high numbers of individuals for the number of samples analysed and, applying a mark-recapture approach, could potentially be considered to have large population sizes (Call-01 cave in the Chichester, K-01 in the eastern Hamersley and APIGBRH-04 in the western Hamersley). Conversely, several caves had a high sampling effort where only a small number of individuals were detected and so are likely to have low population sizes (e.g. GU-01, SF-01 and SF-08 in the eastern Hamersley and FMGGB-01 in the central Hamersley). These observations should also be assessed against the length of time collections sheets were deployed in the field, as this varied amongst caves and could impact on individual detection rates.

From repeat surveys of a subset of caves we detected a small number of individuals that appeared to be resident in caves. Most were detected in two adjacent study periods but one individual was detected using a single cave over a 13 month period. However, the greatest proportion of individuals identified was detected only at one survey period. This could suggest a high level of transiency for Ghost Bats in the Pilbara, though a thorough assessment of the sampling regime

used in this study would be needed to determine whether temporal sampling and sample sizes were sufficient to accurately detect resident vs. transient individuals.

Using assignment tests, Spencer and Tedeschi (2016) suggested there was no evidence of dispersal amongst caves in their study of a limited number of caves. With increased sampling we were able to directly detect individuals using multiple caves, primarily involving movement of individuals amongst closely-located caves. Estimates of dispersal distances amongst caves suggested a mean of 4 ± 0.9 kms (excluding a single putative long distance dispersal event). This is consistent with our spatial autocorrelation analyses that suggest a high level of relatedness amongst bats at short distances, and indicating a spatial genetic neighbourhood size of approximately 10 km. The larger-scale spatial autocorrelation analysis using the multiple distance class method additionally indicated low levels of spatial genetic structure amongst Ghost Bats over ~300km, suggesting low levels of long-distance dispersal. We detected a single putative dispersal event between a cave in the East Hamersley Ranges and a cave ~270km to the west, which supports this scale of movement though further work is needed to confirm this result.

At the landscape scale, genetic differentiation amongst the Chichester and Hamersley subregions was low (F_{ST} = 0.013), suggesting relatively high connectivity amongst the subregions that is consistent with the scale of dispersal indicated above. Bayesian genetic clustering analysis implemented in the software STRUCTURE did not resolve any genetic structuring between the Chichester and Hamersley subregions. Previous analyses by Spencer and Tedeschi (2016) also found no genetic structure amongst sampled caves in the Hamersley, suggesting the Hamersley Ranges represents a single genetic population.

However, we used a second approach that clusters individuals based both on their genetic identity and their spatial location using the software TESS. As indicated by the spatial autocorrelation analyses above, the distribution of genetic diversity across the sampled Ghost Bats has a spatial signal, thus the TESS approach has more power to detect and visualise subtle spatial genetic structuring. Accordingly, we resolved four genetic clusters at the landscape level; three representing the Chichester, East Hamersley and West Hamersley subregions and the fourth representing admixture within the East Hamersley sub-region. Hierarchical genetic structure analysis within the East Hamersley indicated some further genetic clustering, with ACW-01 and AreaC-01 populations showing some genetic differentiation from remaining populations within the East Hamersley group. At all levels, there was evidence for admixture amongst the identified genetic clusters suggesting interaction amongst bats at different sampling locations.

Overall though, the level of genetic structuring across the Pilbara was low. Worthington Wilmer et al. (1999) found similarly low levels of genetic structuring amongst Ghost Bat populations at a regional scale (F_{ST} = -0.03 - 0.02) that they attributed to male-mediated gene flow. This contrasts with exceptionally high genetic differentiation observed at maternally-inherited mitochondrial loci that indicates a high level of female philopatry to maternal roost sites (Worthington-Wilmer et al., 1994; Worthington Wilmer et al., 1999). Although sexing information was not available at the time of this study, we presume that high gene flow amongst Pilbara populations may similarly be mediated by male-biased dispersal.

Each of the subregions (Chichester, East Hamersley and West Hamersley) had some unique genetic diversity (private alleles) consistent with this pattern of low, but observable, genetic differentiation. Summary genetic diversity statistics, such as observed and expected heterozygosity and allelic diversity, were remarkably similar across the regions, with the Chichester showing marginally higher diversity levels than the Hamersley populations (though not statistically significant). Overall, the genetic diversity of the *M. gigas* population in the Pilbara had higher observed heterozygosity (H_o = 0.68) than reported for some other northern Australian populations (Rockhampton H_o = 0.39, Camooweal H_o = 0.60) in Worthington Wilmer et al. (1999), but was lower than the value reported for the Pilbara (H_o = 0.81) in this study conducted almost 20 years ago. The decrease in genetic diversity could potentially reflect a pattern of declining population size since that

time, though we did not find any evidence of genetic bottlenecks that would represent a sudden, large reduction in population size.

We provide diversity estimates for individual caves but it should be noted that many are not likely to be accurate due to the low sample sizes for most caves.

Summary

The genetic data provided in this report indicate that the Ghost Bat (*Macroderma gigas*) in the Pilbara currently retains relatively high genetic connectivity and genetic diversity across the Chichester and Hamersley subregions. At local scales, bats are observed using multiple, closely-located roost sites, with some bats observed using the same caves for up to 13 months. Patterns of dispersal inferred from spatial autocorrelation analysis indicate the majority of dispersal within ~10 km, but with low frequency dispersal up to ~300 km. Some regional genetic structuring is observed that is consistent with these spatial patterns of dispersal. Further work is required to ascertain whether patterns of gene flow are mediated by male-biased dispersal as has been reported elsewhere (Worthington-Wilmer et al., 1994; Worthington Wilmer et al., 1999).

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Appendices

Appendix 1

Sample localities, numbers of Ghost Bat samples analysed by either DBCA (new) or Murdoch University (MU, existing samples), number successfully genotyped and the number of unique individuals detected at each location. [a] Subregions EH = East Hamersley, C = Chichester, WH = West Hamersley; [b] Genotyping of a sample was considered successful if genotypes were obtained at six or more microsatellite loci (see text).

Sampling Location	Sub-region [a]	No. tested			No. successful [b]			No. Individuals
		DBCA	MU	Total	DBCA	MU	Total	
ACW-01	EH	29	7 (1)	**37**	28	3 (1)	**32**	10
ACW-06	EH	20		**20**	20		**20**	4
ACW-08	EH	24	(1)	**25**	21	(1)	**22**	9
ACW-10	EH	27	1	**28**	25	1	**26**	10
Anna Devista	C	6		**6**	5		**5**	1
APIGBJE-01	WH	8		**8**	6		**6**	4
APIGBRH-01	WH	4 (1)		**5**	4 (1)		**5**	3
APIGBRH-03	WH	26 (1)		**27**	20 (1)		**21**	9
APIGBRH-04	WH	16		**16**	16		**16**	14
APIGBRH-05	WH	27		**27**	25		**25**	5
AreaC-01	EH	33	24	**57**	32	22	**54**	15
AreaC-03	EH	20		**20**	19		**19**	3
AreaC-04	EH	20		**20**	14		**14**	6
AreaC-05	EH	20		**20**	18		**18**	2
AreaC-08	EH	20		**20**	5		**5**	5
AreaC-09	EH	20		**20**	20		**20**	2
AreaC-10	EH	3		**3**	3		**3**	1
AreaC-13	EH	18		**18**	18		**18**	6
AreaC-14	EH	1		**1**	1		**1**	1
AreaC-17	EH	12	2	**14**	12		**12**	5
AreaC-18	EH	12		**12**	12		**12**	2
BambooKitchener-01	C	5 (1)		**6**	1 (1)		**2**	1
BambooKitchener-02	C	13		**13**	12		**12**	1
BambooKitchener-04	C	7		**7**	0		**0**	0
BHP0B35-01	EH	17 (1)		**18**	17 (1)		**18**	7
BHP0B35-02	EH	45		**45**	39		**39**	6
Call-01	C	31		**31**	24		**24**	21
Call-02	C	13		**13**	13		**13**	2
Call-03	C	9		**9**	6		**6**	3
CathedralGorge-06	EH	7		**7**	6		**6**	1
CathedralGorge-09	EH	7		**7**	0		**0**	0
CattleGorge-02	C	21		**21**	21		**21**	4
CattleGorge-05	C	7 (1)		**8**	7 (1)		**8**	1
CattleGorgeCulvert-01	C	4		**4**	4		**4**	2

CattleGorgeCulvert-02	C	8		**8**	6		**6**	1
CattleGorgeCulvert-03	C	6		**6**	5		**5**	1
CattleGorgeCulvert-04	C	2		**2**	2		**2**	1
CattleGorgeCulvert-05	C	8		**8**	8		**8**	5
CattleGorgeCulvert-06	C	10		**10**	10		**10**	3
CattleGorgeCulvert-07	C	10		**10**	8		**8**	5
CattleGorgeCulvert-08	C	10		**10**	8		**8**	4
CattleGorgeCulvert-09	C	10		**10**	10		**10**	2
Comet	C	15 (1)		**16**	14 (1)		**15**	4
ER-02	EH	21		**21**	19		**19**	2
FMGGB-01	EH	51		**51**	47		**47**	3
FMGGBCP-05	EH	12		**12**	12		**12**	1
GU-01	EH	45	12	**57**	45	3	**48**	2
GU-02	EH	5	6	**11**	4	1	**5**	3
GU-03	EH	31		**31**	31		**31**	5
JIN-14	EH	20		**20**	19		**19**	3
K-01	EH	20		**20**	18		**18**	17
KlondykeQueen	C	(11)		**11**	(8)		**8**	8
LallaRoohk	C	(2)		**2**	(2)		**2**	2
M-01	EH	58	72 (1)	**131**	57	49 (1)	**107**	20
Marillana-12/MARXX1	EH	20	8	**28**	19	4	**23**	3
NT-01	EH	5	4	**9**	4	2	**6**	1
NT-03	EH	5	5	**10**	4	2	**6**	3
SF-01	EH	56	61 (1)	**118**	55	37 (1)	**93**	6
SF-02	EH	15	13	**28**	15	11	**26**	6
SF-03	EH	7		**7**	7		**7**	3
SF-06	EH	2		**2**	2		**2**	1
SF-08	EH	35	27 (1)	**63**	35	18 (1)	**54**	4
SF-14	EH	20	28 (1)	**49**	20	23 (1)	**43**	5
SF-15	EH	8		**8**	6		**6**	5
SF-27	EH	7	(1)	**8**	7	(1)	**8**	6
SSRBoulder	C	9		**9**	9		**9**	2
Grand Total		**1103**	**276**	**1379**	**997**	**181**	**1178**	**270**

Appendix 2

Details of Ghost Bat microsatellite loci, including locus name, locus source, PCR multiplex number, fluorescent label used, allele size range in base pairs, number of alleles (N_a), information index (I), observed heterozygosity (H_o), unbiased expected heterozygosity (uH_e) and inbreeding coefficient (F). Genetic diversity statistics were calculated for the greater data set including all samples analysed.

Locus	Source [a]	Multiplex	Label	Size Range (bp)	N_a	I	H_o	uH_e	F
gigas01	WW	1	PET	131-159	15	2.204	0.752	0.869	0.135
GB33	JH	1	NED	166-202	13	2.127	0.700	0.855	0.180
gigas10	WW	1	VIC	110-128	9	1.614	0.714	0.784	0.090
GB18	JH	1	FAM	84-110	12	1.382	0.520	0.656	0.207
gigas06	WW	2	FAM	133-165	16	2.093	0.635	0.846	0.249
GB20	JH	2	VIC	96-136	11	1.803	0.646	0.761	0.151
gigas11	WW	2	NED	101-121	11	1.594	0.617	0.755	0.183
GB42	JH	2	PET	182-204	12	1.796	0.704	0.776	0.093
MG24	DBCA	3	FAM	160-180	5	1.054	0.476	0.608	0.216
MG09	DBCA	3	VIC	103-124	8	0.515	0.193	0.207	0.067
MG28	DBCA	3	VIC	157-195	15	2.323	0.681	0.889	0.233
MG03	DBCA	3	NED	84-98	5	0.761	0.389	0.513	0.241
MG21	DBCA	3	NED	137-161	12	1.794	0.750	0.795	0.056
MG32	DBCA	4	FAM	192-238	21	1.936	0.699	0.813	0.140
MG20	DBCA	4	VIC	130-150	10	1.983	0.764	0.841	0.091
MG05	DBCA	4	PET	97-114	7	1.044	0.453	0.536	0.153
MG26	DBCA	4	PET	158-180	8	1.448	0.553	0.699	0.208

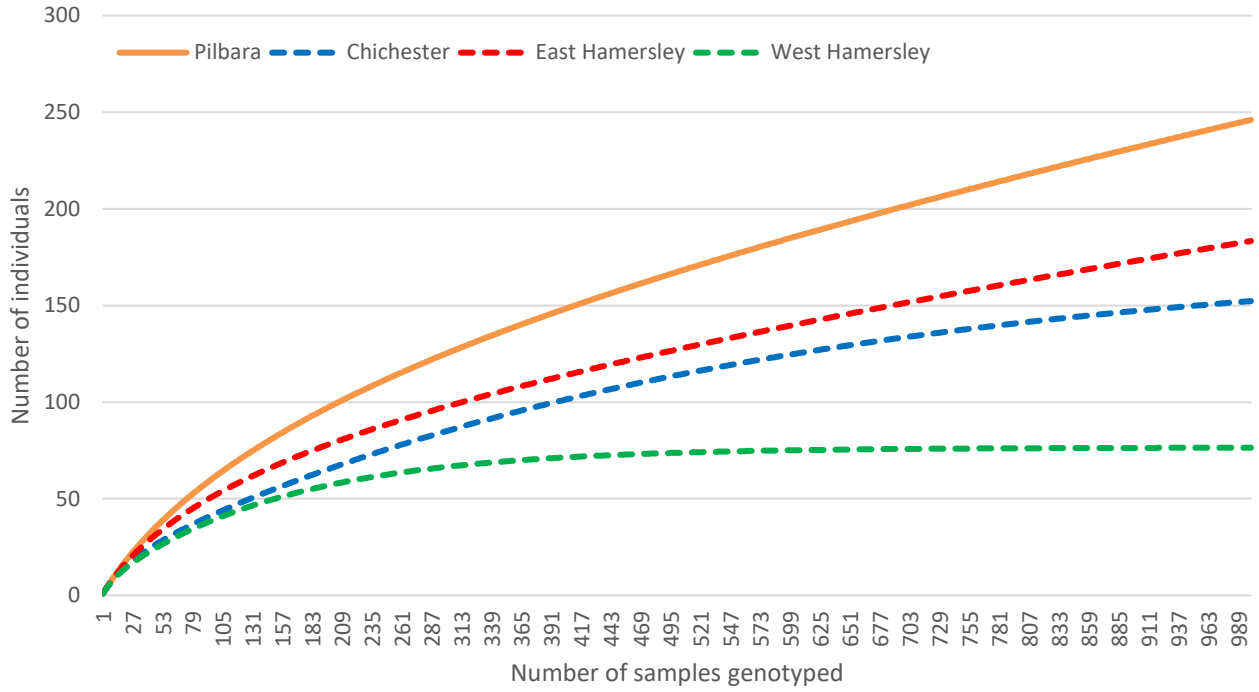
[a] WW = Worthington Wilmer et al., 1999

JH = Jane Hughes, unpublished

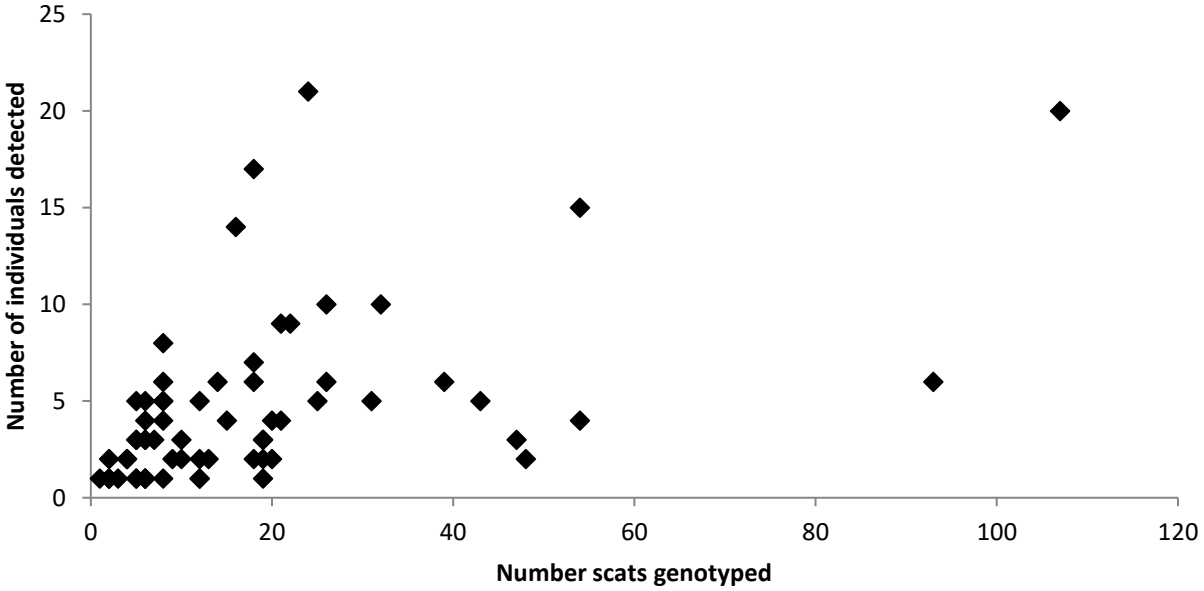
DBCA = Department of Biodiversity, Conservation and Attractions, unpublished

Appendix 3

Rarefaction curves to assess sampling effort for each of the sampled Pilbara subregions and in total. For each of the subregions, rarefaction curves were extrapolated to 1000 samples. The West Hamersley population is the only one to reach an asymptote suggesting a smaller population size in this area compared to East Hamersley and Chichester subregions.



Number of individuals detected vs. number of scats genotyped *per cave*. The plot shows that there was not a straightforward association between the number of scats genotyped and the number of unique individuals detected per cave.



Appendix 4
List of caves that were sampled at multiple time points and the number of individuals detected at only one time period versus multiple time periods.

Pop	Present Multiple Surveys	Present Single Survey	Total
ACW-01	2	8	10
ACW-06	0	4	4
ACW-08	1	8	9
ACW-10	0	10	10
APIGBRH-03	2	7	9
AreaC-01	2	13	15
AreaC-13	0	6	6
AreaC-17	0	6	6
Call-01	0	21	21
CattleGorge-02	1	3	4
CattleGorge-05	1	0	1
GU-01	0	2	2
M-01	3	17	20
Marillana-12	1	2	3
SF-01	1	5	6
SF-02	0	6	6
SF-08	2	2	4
SF-14	1	4	5
Total	**17**	**124**	**141**

Appendix 5

Genetic diversity statistics for populations of *M. gigas* with n>5. N = number of samples analysed, N_a = Number of alleles, A_e = Effective number of alleles, A_r = allelic richness rarefied to the smallest population size, n=5, H_o = Observed heterozygosity, uH_e = unbiased expected heterozygosity, F = inbreeding coefficient.

[a] Regional totals were calculated by pooling individuals into one population, not as means across sub-populations

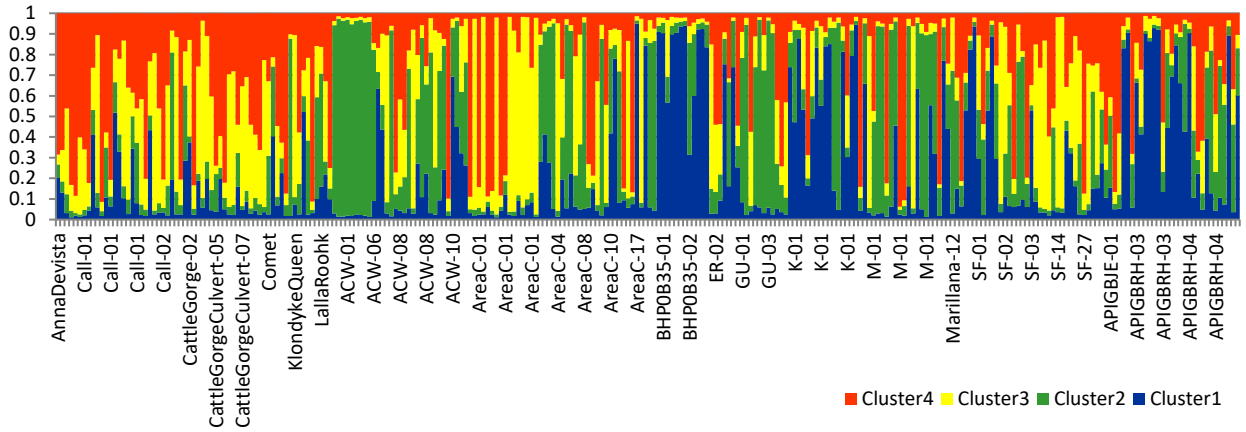
[b] Allelic richness was rarefied to the minimum sample size (n=32) across the three regions

Region / Cave	**N**	**N_a**	**A_e**	**A_r**	**H_o**	**uH_e**	**F**
Chichester							
Call-01	20.3 ± 0.2	7 ± 0.6	4.2 ± 0.3	4.6 ± 0.2	0.64 ± 0.04	0.77 ± 0.02	0.14 ± 0.07
Call-02/03	4.8 ± 0.1	3.1 ± 0.2	2.6 ± 0.2	3.1 ± 0.2	0.78 ± 0.06	0.66 ± 0.04	-0.33 ± 0.07
CattleGorge-02/05	5 ± 0	2.9 ± 0.3	2.4 ± 0.3	2.9 ± 0.3	0.38 ± 0.09	0.55 ± 0.08	0.15 ± 0.13
CattleGorgeCulvert-01/05	9.7 ± 0.1	5.4 ± 0.5	3.8 ± 0.4	4.4 ± 0.3	0.69 ± 0.04	0.74 ± 0.04	0.02 ± 0.04
CattleGorgeCulvert-06/07/08/09	13.8 ± 0.1	5.9 ± 0.5	4.1 ± 0.4	4.5 ± 0.3	0.75 ± 0.04	0.76 ± 0.03	-0.03 ± 0.06
KlondykeQueen	8 ± 0	6.3 ± 0.5	4 ± 0.3	5.0 ± 0.3	0.81 ± 0.05	0.78 ± 0.03	-0.1 ± 0.05
Chichester Total [a]	**61.5 ± 0.4**	**9.9 ± 1.1**	**4.6 ± 0.6**	**8.6 ± 0.9 [b]**	**0.70 ± 0.03**	**0.75 ± 0.04**	**0.05 ± 0.03**
East Hamersley							
ACW-01	8.4 ± 0.2	5.6 ± 0.6	4 ± 0.5	4.6 ± 0.4	0.69 ± 0.05	0.75 ± 0.04	0 ± 0.07
ACW-08	8.6 ± 0.2	5.4 ± 0.5	3.7 ± 0.4	4.4 ± 0.4	0.77 ± 0.05	0.73 ± 0.04	-0.12 ± 0.04
ACW-10	10 ± 0	5.5 ± 0.6	3.4 ± 0.4	4.2 ± 0.3	0.66 ± 0.04	0.7 ± 0.04	-0.02 ± 0.06
AreaC-01	14.7 ± 0.1	5.6 ± 0.6	3.7 ± 0.4	4.1 ± 0.3	0.75 ± 0.05	0.72 ± 0.03	-0.08 ± 0.05
AreaC-13	5.4 ± 0.2	4.3 ± 0.4	2.8 ± 0.3	4.1 ± 0.3	0.65 ± 0.07	0.67 ± 0.04	-0.06 ± 0.09
AreaC-17	4.8 ± 0.1	3.8 ± 0.4	3 ± 0.4	3.8 ± 0.4	0.62 ± 0.06	0.69 ± 0.05	-0.03 ± 0.07
AreaC-03/04	8.7 ± 0.1	5.1 ± 0.5	3.6 ± 0.3	4.3 ± 0.3	0.71 ± 0.03	0.75 ± 0.03	-0.01 ± 0.06
AreaC-08/09	6.4 ± 0.2	3.8 ± 0.4	3.2 ± 0.3	3.7 ± 0.3	0.63 ± 0.08	0.7 ± 0.04	0.03 ± 0.1
BHP0B35-01	6.5 ± 0.2	4.1 ± 0.3	3.1 ± 0.2	3.8 ± 0.2	0.62 ± 0.07	0.72 ± 0.03	0.07 ± 0.1
BHP0B35-02	6 ± 0	3.7 ± 0.2	2.8 ± 0.2	3.6 ± 0.2	0.71 ± 0.05	0.68 ± 0.03	-0.16 ± 0.07
GU-01/03	10 ± 0	5.6 ± 0.5	4.2 ± 0.4	4.6 ± 0.3	0.78 ± 0.05	0.78 ± 0.02	-0.05 ± 0.05
K-01	14.8 ± 0.5	6.3 ± 0.5	4.1 ± 0.4	4.5 ± 0.3	0.55 ± 0.04	0.76 ± 0.03	0.24 ± 0.05
M-01	19 ± 0.3	6.5 ± 0.5	4 ± 0.4	4.4 ± 0.3	0.63 ± 0.05	0.74 ± 0.03	0.14 ± 0.06

SF-01/06	7 ± 0	4.6 ± 0.3	3.1 ± 0.3	4.1 ± 0.3	0.74 ± 0.08	0.69 ± 0.05	-0.13 ± 0.09
SF-02	5.7 ± 0.1	4.1 ± 0.3	3.2 ± 0.3	3.9 ± 0.3	0.69 ± 0.07	0.72 ± 0.04	-0.05 ± 0.1
SF-03/08/27	11.5 ± 0.3	5.9 ± 0.5	3.5 ± 0.3	4.3 ± 0.3	0.6 ± 0.05	0.73 ± 0.03	0.15 ± 0.05
SF-14	4.8 ± 0.1	4.5 ± 0.4	3.3 ± 0.4	4.5 ± 0.4	0.63 ± 0.07	0.72 ± 0.06	0.02 ± 0.07
SF-15	4.9 ± 0.1	3.7 ± 0.4	2.8 ± 0.3	3.7 ± 0.4	0.65 ± 0.1	0.63 ± 0.08	-0.13 ± 0.11
East Hamersley Total [a]	**164.3 ± 1.1**	**9.9 ± 0.8**	**5.1 ± 0.6**	**8.0 ± 0.7 [b]**	**0.68 ± 0.02**	**0.78 ± 0.03**	**0.13 ± 0.02**
West Hamersley							
APIGBJE-01/APIGBRH-01	7 ± 0	5.1 ± 0.5	3.5 ± 0.4	4.4 ± 0.4	0.66 ± 0.05	0.72 ± 0.04	0 ± 0.07
APIGBRH-03	8.8 ± 0.1	4.9 ± 0.4	3.5 ± 0.4	4.1 ± 0.3	0.68 ± 0.08	0.69 ± 0.06	-0.03 ± 0.07
APIGBRH-04	13.8 ± 0.1	6.6 ± 0.6	3.8 ± 0.4	4.4 ± 0.3	0.68 ± 0.05	0.73 ± 0.03	0.04 ± 0.05
APIGBRH-05	5 ± 0	4.5 ± 0.4	3.5 ± 0.3	4.5 ± 0.4	0.64 ± 0.07	0.76 ± 0.04	0.07 ± 0.08
West Hamersley Total [a]	**32.9 ± 0.4**	**8.2 ± 0.7**	**4.5 ± 0.5**	**7.8 ± 0.7 [b]**	**0.66 ± 0.05**	**0.76 ± 0.03**	**0.12 ± 0.05**

Appendix 6

Barplot of individual membership proportions to genetic clusters identified by STRUCTURE.



Appendix 7

Summary of population mean individual membership proportions to each genetic cluster identified by TESS. Membership proportions of >60% to a genetic cluster are highlighted in bold.

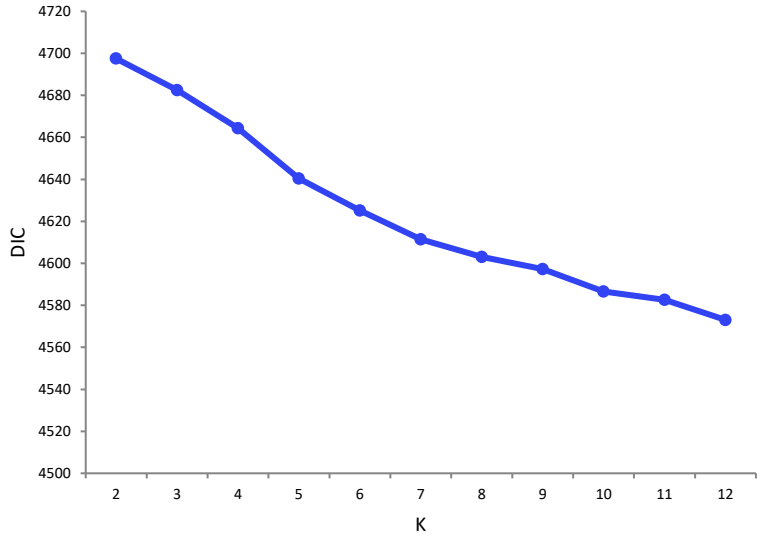
Region / Population	Cluster1	Cluster2	Cluster3	Cluster4
Chichester	*0.089*	*0.018*	*0.029*	***0.865***
AnnaDevista	0.174	0.003	0.042	**0.781**
BambooKitchener-01	0.063	0.020	0.042	**0.875**
BambooKitchener-02	0.037	0.003	0.007	**0.953**
Call-01	0.060	0.012	0.028	**0.901**
Call-02	0.033	0.006	0.013	**0.949**
Call-03	0.049	0.007	0.024	**0.920**
CattleGorge-02	0.036	0.017	0.014	**0.933**
CattleGorgeCulvert-01	0.018	0.024	0.027	**0.931**
CattleGorgeCulvert-02	0.016	0.015	0.013	**0.956**
CattleGorgeCulvert-03	0.039	0.010	0.070	**0.882**
CattleGorgeCulvert-04	0.028	0.020	0.026	**0.926**
CattleGorgeCulvert-05	0.056	0.016	0.015	**0.913**
CattleGorgeCulvert-06	0.111	0.011	0.043	**0.835**
CattleGorgeCulvert-07	0.026	0.014	0.031	**0.928**
CattleGorgeCulvert-08	0.031	0.004	0.014	**0.950**
CattleGorgeCulvert-09	0.020	0.003	0.008	**0.969**
Comet	0.211	0.024	0.042	**0.724**
KlondykeQueen	0.169	0.030	0.048	**0.753**
LallaRoohk	0.157	0.099	0.009	**0.735**
SSRBoulder	0.310	0.004	0.048	**0.637**
East Hamersley	***0.796***	*0.019*	*0.147*	*0.037*
ACW-01	**0.963**	0.005	0.013	0.020
ACW-06	**0.888**	0.069	0.014	0.029
ACW-08	**0.707**	0.041	0.095	0.157
ACW-10	**0.879**	0.009	0.094	0.018
AreaC-01	0.296	0.006	**0.642**	0.057
AreaC-03	**0.972**	0.003	0.014	0.011
AreaC-04	**0.805**	0.007	0.166	0.022

AreaC-05	**0.750**	0.005	0.216	0.029
AreaC-08	**0.888**	0.006	0.083	0.023
AreaC-09	0.585	0.026	0.058	0.331
AreaC-10	**0.960**	0.006	0.024	0.009
AreaC-13	**0.924**	0.016	0.046	0.014
AreaC-14	**0.709**	0.010	0.204	0.077
AreaC-17	**0.768**	0.044	0.147	0.040
AreaC-18	**0.937**	0.009	0.043	0.011
BHP0B35-01	**0.964**	0.001	0.032	0.003
BHP0B35-02	**0.978**	0.001	0.018	0.003
CathedralGorge-06	**0.950**	0.001	0.025	0.024
ER-02	**0.781**	0.002	0.189	0.028
FMGGB-01	**0.834**	0.076	0.005	0.085
GU-01	**0.876**	0.018	0.069	0.037
GU-02	0.534	0.181	0.275	0.010
GU-03	**0.960**	0.004	0.021	0.015
JIN-14	**0.701**	0.002	0.262	0.034
K-01	**0.879**	0.051	0.043	0.026
M-01	**0.942**	0.007	0.035	0.016
Marillana-12	**0.607**	0.019	0.334	0.040
NT-01	**0.890**	0.025	0.014	0.070
NT-03	**0.969**	0.009	0.014	0.008
SF-01	**0.870**	0.017	0.103	0.009
SF-02	**0.820**	0.010	0.145	0.024
SF-03	**0.918**	0.008	0.033	0.041
SF-08	0.299	0.019	0.667	0.016
SF-14	0.572	0.005	0.386	0.037
SF-15	**0.715**	0.010	0.231	0.044
SF-27	**0.758**	0.006	0.172	0.064
West Hamersley	*0.136*	***0.701***	*0.005*	*0.158*
APIGBJE-01	0.162	**0.603**	0.004	0.231
APIGBRH-01	0.154	0.419	0.014	0.413
APIGBRH-03	0.082	**0.835**	0.003	0.080
APIGBRH-04	0.105	**0.816**	0.005	0.074
APIGBRH-05	0.281	0.376	0.004	0.338

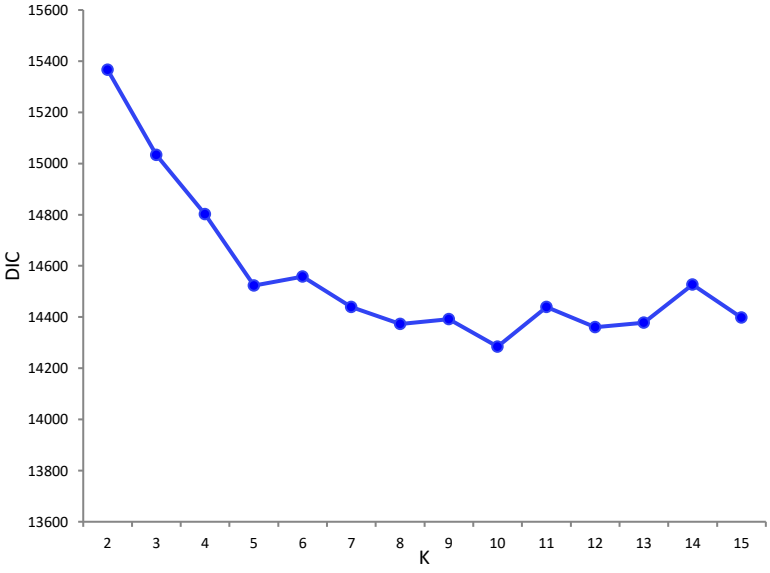
Appendix 8

Plot of DIC values for each K tested in TESS analyses for (a) Chichester and (b) Hamersley subregions. The DIC plot did not plateau for the Chichester analysis and inspection of membership plots showed no genetic structure across the Chichester region. Analysis of the Hamersley sub-region suggested optimal K = 5.

(a)



(b)



Appendix 9
Summary of population mean membership proportions to genetic clusters identified in TESS analyses. Populations with >60% membership to a genetic cluster are highlighted in bold.

Region / Population	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5
Central Hamersley					
FMGGB-01	**0.601**	0.014	0.204	0.120	0.062
East Hamersley					
ACW-01	**0.945**	0.010	0.003	0.004	0.038
ACW-06	0.094	0.015	0.180	0.032	**0.679**
ACW-08	0.144	0.089	0.089	0.022	**0.656**
ACW-10	0.167	0.110	0.019	0.013	**0.691**
AreaC-01	0.051	**0.711**	0.008	0.070	0.161
AreaC-03	0.339	0.030	0.004	0.118	0.509
AreaC-04	0.522	0.176	0.009	0.057	0.236
AreaC-05	0.058	0.486	0.005	0.046	0.405
AreaC-08	0.294	0.178	0.007	0.097	0.423
AreaC-09	0.023	0.055	0.050	0.378	0.495
AreaC-10	0.101	0.055	0.009	0.080	**0.755**
AreaC-13	0.279	0.055	0.009	0.155	0.502
AreaC-14	0.044	0.218	0.018	0.258	0.461
AreaC-17	0.084	0.191	0.067	0.166	0.491
AreaC-18	0.072	0.042	0.014	0.013	**0.858**
BHP0B35-01	0.019	0.030	0.002	0.007	**0.942**
BHP0B35-02	0.017	0.012	0.002	0.003	**0.967**
CathedralGorge-06	0.082	0.042	0.001	0.014	**0.860**
ER-02	0.024	0.229	0.004	0.026	**0.718**
GU-01	0.054	0.032	0.036	0.012	**0.867**
GU-02	0.211	0.118	0.183	0.120	0.367
GU-03	0.042	0.013	0.009	0.005	**0.932**
JIN-14	0.108	0.313	0.002	0.059	0.517
K-01	0.079	0.032	0.103	0.043	**0.742**
M-01	0.166	0.021	0.014	0.015	**0.785**
Marillana-12	0.290	0.431	0.016	0.053	0.210
NT-01	0.075	0.027	0.069	0.117	**0.712**
NT-03	0.034	0.022	0.009	0.327	**0.608**
SF-01	0.040	0.139	0.009	0.332	0.481
SF-02	0.120	0.173	0.027	0.062	**0.618**
SF-03	0.025	0.020	0.009	0.233	**0.713**
SF-08	0.014	0.540	0.042	0.020	0.384
SF-14	0.039	0.447	0.007	0.042	0.466
SF-15	0.013	0.175	0.009	0.251	0.552
SF-27	0.064	0.173	0.007	0.019	**0.737**
West Hamersley					
APIGBJE-01	0.133	0.003	**0.760**	0.094	0.010
APIGBRH-01	0.096	0.011	**0.681**	0.191	0.021
APIGBRH-03	0.043	0.002	**0.910**	0.032	0.014
APIGBRH-04	0.059	0.003	**0.891**	0.031	0.016
APIGBRH-05	0.258	0.005	**0.653**	0.020	0.065