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Aline Gibson Vega

Michelle L. Hall

Amanda Ridley

Saul J. Cowen

Amy L. Slender

See next page for additional authors

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Authors

Aline Gibson Vega, Michelle L. Hall, Amanda Ridley, Saul J. Cowen, Amy L. Slender, Allan H. Burbidge, Marina Louter, and W. Jason Kennington



Population genetic structure associated with a landscape barrier in the Western Grasswren (*Amytornis textilis textilis*)

ALINE GIBSON VEGA,*¹  MICHELLE L. HALL,^{1,2} AMANDA RIDLEY,¹ SAUL J. COWEN,^{1,3} AMY L. SLENDER,⁴  ALLAN H. BURBIDGE,^{3,5} MARINA LOUTER⁴ & W. JASON KENNINGTON¹

¹School of Biological Sciences, The University of Western Australia, Crawley, WA, Australia

²Bush Heritage Australia, Melbourne, Victoria, Australia

³Department of Biodiversity, Conservation and Attractions, Biodiversity and Conservation Science, Bentley, WA, Australia

⁴College of Science and Engineering, Flinders University, Adelaide, SA, Australia

⁵School of Science, Edith Cowan University, Joondalup, WA, Australia

Dispersal patterns can dictate genetic population structure and, ultimately, population resilience, through maintaining gene flow and genetic diversity. However, geographical landforms, such as peninsulas, can impact dispersal patterns and thus be a barrier to gene flow. Here, we use 13 375 genome-wide single-nucleotide polymorphisms (SNPs) to evaluate genetic population structure and infer dispersal patterns of the Western Grasswren *Amytornis textilis textilis* (WGW, $n = 140$) in the Shark Bay region of Western Australia. We found high levels of genetic divergence between subpopulations on the mainland (Hamelin) and narrow peninsula (Peron). In addition, we found evidence of further genetic sub-structuring within the Hamelin subpopulation, with individuals collected from the western and eastern regions of a conservation reserve forming separate genetic clusters. Spatial autocorrelation analysis within each subpopulation revealed significant local-scale genetic structure up to 35 km at Hamelin and 20 km at Peron. In addition, there was evidence of male philopatry in both subpopulations. Our results suggest a narrow strip of land may be acting as a geographical barrier in the WGW, limiting dispersal between a peninsula and mainland subpopulation. In addition, heterogeneous habitat within Hamelin may be restricting dispersal at the local scale. Furthermore, there is evidence to suggest that the limited gene flow is asymmetrical, with directional dispersal occurring from the bounded peninsula subpopulation to the mainland. This study highlights the genetic structure existing within and between some of the few remaining WGW subpopulations, and shows a need to place equal importance on conservation efforts to maintain them in the future.

Keywords: admixture, dispersal, landscape barrier, peninsula, population genetics, substructure.

Dispersal patterns and subsequent gene flow influence genetic population structure, and hence population resilience through its influence on population-level genetic diversity (Frankham 1996,

Frankham *et al.* 2002). Dispersal is a common behaviour that can be influenced by biotic and abiotic factors (Clarke *et al.* 1997). Some species tend to exhibit short-distance dispersal behaviours, causing strong genetic population structure in an otherwise homogeneous landscape (Aguillon *et al.* 2017). Geographical features may also restrict an individual's capacity to disperse. Landscape features such as highly heterogeneous or fragmented habitat (e.g. islands, peninsulas, roads, artificial waterways and habitat clearing) can be

*Corresponding author.

Email: aline.gibsonvega@research.uwa.edu.au

Twitter: @AGibsonVega

[Correction added on 16 May 2023, after first online publication: The ORCID ID for Amy L. Slender has been included in this version.]

geographical barriers that restrict gene flow over relatively short spatial scales. For example, populations of Dibbler *Parantechinus apicalis*, a small marsupial, separated by small cliffs and unsuitable vegetation have been found to become genetically subdivided over relatively short spatial scales (< 19 km; Thavornkanlapachai et al. 2019a). In the arboreal Western Ringtail Possum *Pseudocheirus occidentalis*, a narrow artificial waterway (30 m wide) was found to be a greater dispersal barrier than a major road an equal distance away (200 m; Yokochi et al. 2016). In White-fronted Chats *Epthianura albifrons*, unsuitable habitat was found to be less of a barrier to gene flow than urbanization, despite the urban populations being substantially geographically closer (20 km) than those separated by unsuitable continuous forest (500 km; Major et al. 2014). In areas with complex coastlines, such as peninsulas, some species may experience restricted dispersal if individuals are unwilling or unable to traverse particular habitat types or open water, as seen in the California Quail *Callipepla californica* (Zink et al. 1987) and several small Californian passerines (Wiggins 1999, Tubelis et al. 2007, Vázquez-Miranda et al. 2022). These few examples highlight how some landscape features can represent significant dispersal barriers.

Understanding genetic structure (and hence landscape barriers), genetic diversity and dispersal can greatly benefit conservation efforts because it can assist in identifying populations of conservation priority (e.g. populations of relative greater genetic importance; von Takach et al. 2021). In addition, genetic studies can guide decision-making for conservation strategies such as translocations (e.g. Onley et al. 2022) to make evidence-based decisions around genetic priorities for the founder population (e.g. maximizing genetic diversity). Conservation science is increasingly putting emphasis on the usefulness of knowing the genetic population structure and genetic diversity of species to improve conservation decision-making (Frankham 2005, IUCN/SSC 2013, DeWoody et al. 2021).

The Western Grasswren *Amytornis textilis* is a small, ground-dwelling passerine endemic to Western and South Australia (Brooker 2000, Black et al. 2009). There are five recognized subspecies of grasswren: *A. t. textilis* is found in the Shark Bay region of Western Australia and *A. t. myall* is restricted to the Gawler Ranges in South Australia. Formerly, *A. t. macrourus* and *A. t. giganturus* were found in southwestern Australia, and *A. t. carteri*

on Dirk Hartog Island in Shark Bay; however, these three subspecies are all now believed to be extinct (Black 2011, Austin et al. 2013). While there has been considerable focus on resolving and redefining the taxonomy of *Amytornis* (e.g. Black et al. 2010, Christidis et al. 2013), little is known about grasswren ecology, with only one grasswren species having been studied in depth (Thick-billed Grasswren *Amytornis modestus*; Louter 2016, Slender 2018). In particular, dispersal distances and subsequent population structuring are not well understood in grasswrens, limiting our ability to make informed conservation management decisions. This information is especially important for the Shark Bay subspecies of the Western Grasswren, which is a Priority 4 taxon (Rare, Near Threatened or in need of monitoring) in Western Australia and is the subject of a conservation translocation to nearby Dirk Hartog Island (Algar et al. 2020).

The Shark Bay subspecies *A. t. textilis* (hereafter referred to as WGW) occupies two disjunct areas in Shark Bay, Peron Peninsula and the adjacent mainland near Hamelin Pool (Fig. 1). We hypothesized that WGW subpopulations in these areas would show strong genetic structuring due to their narrow connectivity via the isthmus, which may restrict gene flow. In addition to geographical features, habitat fragmentation may also influence dispersal in WGW. Western Grasswrens require low recumbent shrub habitats, such as *Acacia* shrubland, which provides low-to-the-ground cover for foraging, shelter and nesting (Brooker 1998, Gibson Vega 2022). The distribution of this species encompasses areas of variable suitable plant communities mixed with patchy unsuitable habitat. Habitat within Peron Peninsula is mostly continuous *Acacia* shrubland with birridas (clay pans) of various sizes scattered throughout the peninsula (Payne et al. 1987, Beard et al. 2013). In contrast, the adjacent mainland subpopulation at Hamelin occupies discontinuous *Acacia* shrubland, with areas containing unsuitable taller *Acacia* species and *Eucalyptus* woodland as well as areas of land degradation by stock (Payne et al. 1987, Beard et al. 2013). In this study, we use genome-wide single nucleotide polymorphisms (SNPs) to evaluate population genetic structure, infer dispersal capabilities and identify any landscape barriers to gene flow in the WGW. First, we examine how genetic variation is partitioned within and between subpopulations in the Shark Bay region. Secondly, we explore fine-scale genetic structure within the

peninsula and adjacent mainland subpopulation and test for evidence of sex-biased dispersal, a common phenomenon in many avian species (Greenwood 1980, Mabry *et al.* 2013, Payevsky 2016). Last, we investigate morphological variation and test whether genetic divergences coincide with any detected morphological differences.

METHODS

Study area and sampling

Two large conservation properties are strongholds for the remaining population of the WGW in Shark Bay. Francois Peron National Park extends over half of Peron Peninsula and is managed by the Department of Biodiversity, Conservation

and Attractions. Hamelin Station Reserve is owned and managed by Bush Heritage Australia as a conservation reserve and is located in the mainland landscape south-east of the peninsula (Fig. 1). WGW were captured from both areas during the breeding season (June–October) between 2019 and 2021 using mist-nets. Blood samples were collected from the brachial vein and stored in 100% ethanol. Samples were obtained throughout the peninsula and on parts of the mainland within the known WGW distribution (Fig. 1). A total of 86 and 54 samples from adult individuals were obtained from Peron and Hamelin, respectively. Juvenile grasswrens, identified by morphological features in-hand, were not included in the dataset, as they have not had the opportunity to disperse and thus would bias the data.

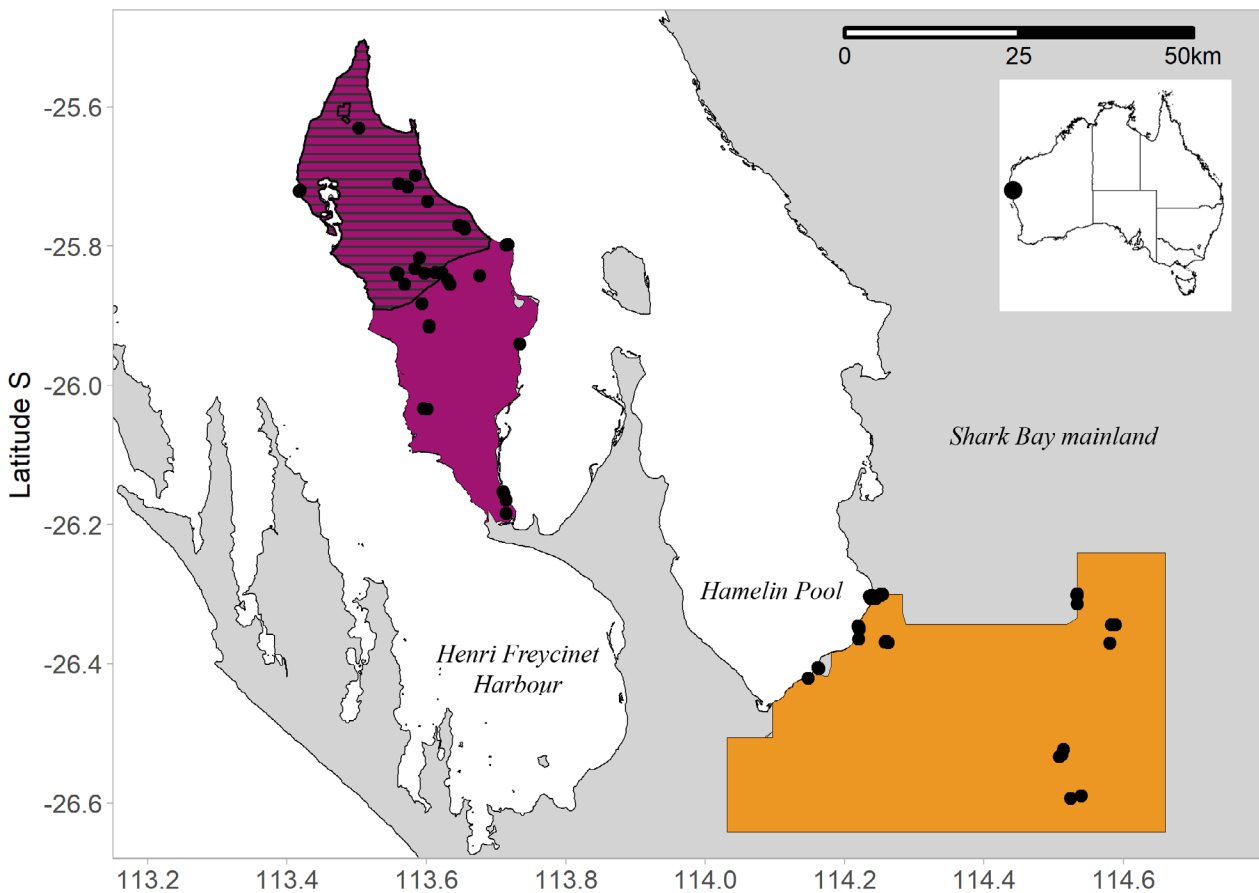


Figure 1. Location of Western Grasswren *Amytornis textilis textilis* samples. Purple- and orange-shaded areas indicate areas of interest where blood samples were collected from Peron Peninsula and Hamelin Station Reserve, respectively, with the hatched area in Peron Peninsula denoting Francois Peron National Park. Black dots indicate from where each sample was taken. White areas denote water.

SNP genotyping

Blood and DNA samples were sent to Diversity Arrays Technology (DArTseq, <http://www.diversityarrays.com/>) for genome-wide SNP sequencing using a genotype-by-sequencing approach. This approach involves a combination of DArT complexity reduction methods and next-generation sequencing platforms (Kilian *et al.* 2012). Several enzyme systems for complexity reduction were tested and the *Pst*I–*Sph*I method was chosen. The *Pst*I-compatible adaptor comprised an Illumina flowcell attachment sequence, a sequencing primer and a staggered barcode region of varying lengths. The reverse adapter (*Sph*I-compatible) contained the Illumina flowcell attachment sequence and an *Sph*I overhang sequence. Only fragments with both *Pst*I and *Sph*I were amplified by polymerase chain reaction (PCR) with an initial denaturing step at 94 °C for 1 min, followed by 30 cycles of temperature changes as follows: denaturation at 94 °C for 20 s, annealing at 58 °C for 30 s and extension at 72 °C for 45 s, with an additional final extension at 72 °C for 7 min (Melville *et al.* 2017).

Following PCR amplification, the products from each sample were bulked and applied to c-Bot (Illumina) bridge PCR followed by sequencing on an Illumina HiSeq2500. The sequencing (single read) was run for 77 cycles. Sequences were processed using proprietary DArT analytical pipelines. In the primary pipeline the 'fastq' files were first processed to filter away poor-quality sequences, applying more stringent selection criteria to the barcode region compared with the rest of the sequence. Identical sequences were collapsed into 'fastqcoll files' and groomed using the DArT proprietary algorithm to correct low-quality bases from singleton reads, using collapsed tags with multiple members as a template. The 'groomed' fastqcoll files were used in the secondary pipeline for SNP calling (Kilian *et al.* 2012).

Following the generation of 71 255 SNP loci, multiple SNP loci on the same contig, loci that were genotyped in fewer than 95% of samples, and loci that had a minor allele frequency of < 0.05 or were monomorphic were removed prior to analysis. Potential outlier loci were screened using three detection methods: BAYESCAN 2.1 using default settings (Foll & Gaggiotti 2008), outflank (Whitlock & Lotterhos 2015) and

PCADAPT (Luu *et al.* 2017). Multiple detection methods were utilized as each method has a different threshold for identifying outliers. Outlier loci were identified by having *q*-values < 0.05 and having been detected across at least two outlier detection methods. These loci were removed from the global dataset prior to assessments of population structure and genetic diversity. Relatedness was determined through GenAlEx 6.503 (Peakall & Smouse 2006, 2012) using the Ritland (1996) *r* estimate. Prior to all subsequent genetic analysis and morphological analysis, one individual from each pair of individuals having a relatedness coefficient of 0.2 or more was removed from the data to avoid potential bias incurred by including highly related family members.

Morphology

Morphological measurements were obtained for all grasswrens at their time of capture. Head–bill (back of head to tip of bill) and tarsus (intertarsal joint to base of toes) were recorded using dial callipers (± 0.05 mm). Wing length (carpal joint to the tip of the longest primaries, flattened against the ruler) and tail length (base to tip of the longest tail feather) were recorded using a ruler (± 0.1 mm). Because grasswrens spend a lot of time in the undergrowth, tail feathers can often be shortened due to wear and damage. Thus, measurement of damaged tails which had obviously lost length were not included in the analysis. Most grasswren weights were recorded using a 100-g Pesola spring scale (± 0.5 g), with the exception of 25 WGW individuals, which were weighed on a digital scale (± 0.1 g). Measurements were recorded by multiple people, with A. Gibson Vega measuring > 60% of the WGW individuals.

Data analysis

Population structure and genetic diversity

Population structure was assessed using Bayesian clustering analysis, a discriminant analysis of principal components (DAPC) and calculation of Nei's (1987) F_{ST} values. The Bayesian clustering analysis was carried out using the software package STRUCTURE 2.3.4 (Pritchard *et al.* 2000). STRUCTURE constructs models using a Bayesian clustering method, which estimates the proportion of each individual's genome having ancestry to

each cluster for any given number of clusters (K ; Pritchard *et al.* 2000). This is achieved through Markov chain Monte Carlo (MCMC) methods for sampling from a probability distribution. The number of genetic clusters we tested ranged from one to six, with 10 replicates for each value of K . A burn-in of 50 000 followed by 100 000 MCMC replicates was set. We determined the most likely value of K using the Delta K method (Evanno *et al.* 2005). As this method cannot determine $K = 1$, we also considered the likelihood probability ($\ln(P)$), where the most likely K is at the 'plateau' of the curve. STRUCTURE results were reorganized and visualized using the R package 'pophelper' (Francis 2017). The DAPC analysis was conducted using the R package 'adegenet' (Jombart *et al.* 2010). DAPC is preferred over the traditional principal components analysis (PCA), as PCA looks for the largest overall variance, ignoring prior knowledge on group assignment. In contrast, DAPC maximizes variance among groups, while minimizing within-group variance. This method achieves the best discrimination of individuals into predefined groups (Jombart *et al.* 2010). The optimal number of principal components to retain was determined by maximizing the highest mean success of successful assignment. The optimal number of clusters was determined using the Bayesian Information Criterion (BIC), where the lowest BIC values are the best model fit.

Data analyses were performed using R v4.0.3 unless specified otherwise (R Core Team 2020). Estimates of observed and expected heterozygosity, inbreeding coefficient (F_{IS}) and pairwise fixation index (F_{ST}) were obtained using the R package 'hierfstat' (Goudet 2005), following Nei (1987) for the F_{ST} values. The significance of F_{IS} and F_{ST} values was determined by bootstrapping 1000 replicates with a 95% confidence interval (95% CI). To test for significant differences in observed and expected heterozygosity values between subpopulations, a Friedman test was carried out, each locus representing a block.

Isolation-by-distance and fine-scale genetic structure

Genetic and geographical distance matrices needed for isolation-by-distance (IBD) analysis were obtained through the 'dartR' R package (Gruber *et al.* 2018). A Mantel test determined if there were significant patterns of IBD, facilitated through the 'ecodist' R package (Goslee & Urban 2007). The 95% CIs of Mantel r

coefficients were determined by bootstrapping 1000 replicates. The IBD slope was determined by linear regression and the 95% CI was determined using the *confint* function on the linear regression in R. IBD analysis was conducted across both subpopulations and then separately for each subpopulation.

We investigated fine-scale population structure using multiple distance class (MDC) spatial autocorrelation analysis within each WGWS subpopulation (Peron and Hamelin) for each sex separately with GenAlEx 6.503 software (Peakall & Smouse 2006, 2012). Small and uneven sample sizes warrant using MDC over standard spatial autocorrelation (SA) analysis with discrete distance class sizes because that type of SA is more sensitive to the associated number of samples per distance class and the distance class size chosen. In contrast, MDC spatial autocorrelation analysis is equivalent to restarting a single spatial analysis repeatedly with differing distance classes (Peakall *et al.* 2003). MDC analyses were performed using a subset of 8000 loci, as GenAlEx cannot accommodate larger datasets. Related individuals previously removed for prior analysis were retained in the MDC analysis because we are interested in determining whether there are levels of philopatry. Sub-adults (non-breeding adults) accompanying a breeding pair were removed from the WGWS dataset, as they have not yet dispersed and do not remain on their natal territories for more than a year as adults (Gibson Vega 2022). The mean spatial genetic autocorrelation value (r) was calculated for a series of increasing distance class sizes. To test for statistical significance, we used random permutations and bootstrapped r using 999 replicates to determine the 95% CI around the null hypothesis (no genetic structure) and error bars, respectively. Significant autocorrelation was inferred when two conditions were met: r exceeds the 95% CI around the null hypothesis of no spatial genetic structure, and the 95% error about r does not intercept the x -axis at $r = 0$. This allows for the most conservative approach to test for spatial genetic structure (Peakall *et al.* 2003). When there is significant positive structure, the estimated value of r will decrease as the size of the distance class increases. The distance class size where the value of r is no longer significant provides an estimate of the extent of detectable positive spatial genetic structure (Peakall *et al.* 2003).

Morphological analysis

Morphometric data were first analysed using generalized linear mixed models in the R package 'lme4' (Bates *et al.* 2014) to test for sexual dimorphism while including observer as a random effect to control for any interobserver variation. Sexual dimorphism has been observed in other grasswren species (including the closely related Thick-billed Grasswren *Amytornis modestus*; Slender *et al.* 2017) and hence was tested for the WGW. A separate linear mixed-effect model was created for each morphological trait (body mass, head–bill length, tarsus length, tail length and wing length), where the person conducting the measurement was included as a random effect. Once it was determined that there was evidence for sexual dimorphism, female and male morphometric traits were analysed separately. Morphometric measurements for 40 females and 48 males were analysed using PCA to identify correlations among morphometric traits. Data were normalized so that each variable had a mean of zero and a standard deviation of one. The first three principal components (PC) were retained for each PCA. We used a non-parametric Kruskal–Wallis test to compare PC scores between Hamelin and Peron subpopulations, with *P*-values adjusted with the Bonferroni adjustment method to account for multiple tests.

RESULTS

SNP and sample filtering

Following filtering of the initial 71 255 loci generated by DArTseq, 26 585 were removed because they were identified on the same contig as another locus. Other loci that were removed included 17 888 loci with a low call rate, 108 monomorphic loci and 13 317 loci with a low minor allele frequency. No outlier loci were identified. The resulting neutral dataset contained 13 375 loci, with 1.22% missing data (no SNP was called for that locus in a particular individual).

Fifty-six pairwise comparisons of WGW individuals (0.6% of 9730 pairwise comparisons) were deemed to be closely related ($r \geq 0.2$), with one from each pair removed prior to further analysis. The geographical distance between most close relatives ranged from 0 to 4.9 km and were within the same subpopulation. An exception was a pair of individuals, one from Hamelin (male) and one

from Peron (female), that were captured 83.8 km (straight line distance) apart ($r = 0.332$). Most individuals from the cluster of related birds containing this related pair were caught in Peron, except for the one male caught at Hamelin. After filtering related individuals, the number of individuals retained for subsequent genetic analyses was 62 from Hamelin and 48 from Peron.

Population structure

The DAPC analysis revealed three distinct genetic clusters (Fig. 2a). Individuals collected from Peron formed one cluster. The remaining two clusters consisted of individuals collected from the western and eastern regions of Hamelin (hereinafter referred to as Hamelin W and Hamelin E, respectively). The first two principal components from the DAPC explained 9.56% of the observed variance across all samples. Similar results were found with the STRUCTURE analysis, which separated individuals collected at Peron, Hamelin W and Hamelin E when $K = 3$ (Fig. 2b). However, the distinction between regions within Hamelin was not evident when $K = 2$. Under this model, individuals collected at Peron and Hamelin formed separate groups, indicating the genetic divergence between these two regions was greater than the divergence between Hamelin W and Hamelin E. The STRUCTURE plots also revealed several individuals with mixed ancestry at Hamelin, but not Peron. This suggests there has been asymmetrical gene flow from Peron to the Hamelin subpopulations. Similar STRUCTURE plots were obtained when all adult individuals were included in the analysis (Supporting Information Fig. S1). The Delta K and BIC analyses indicated that the optimal number of genetic clusters was three (Delta K; Fig. S2) or two to three (BIC; Fig. S3). Significant divergence was found between Hamelin and Peron ($F_{ST} = 0.072$, 95% CI 0.069–0.074).

Genetic diversity

All WGW population regions had lower observed (H_o) than expected heterozygosity (H_e ; Table 1). There was a significant difference between Hamelin and Peron subpopulations in both observed and expected heterozygosity (Friedman's test; H_o ; $\chi^2 = 33.20$, $df = 1$, $P < 0.001$, H_e ; $\chi^2 = 53.52$, $df = 1$, $P < 0.001$), with the Hamelin subpopulation having greater observed heterozygosity than the Peron subpopulation (Table 1).

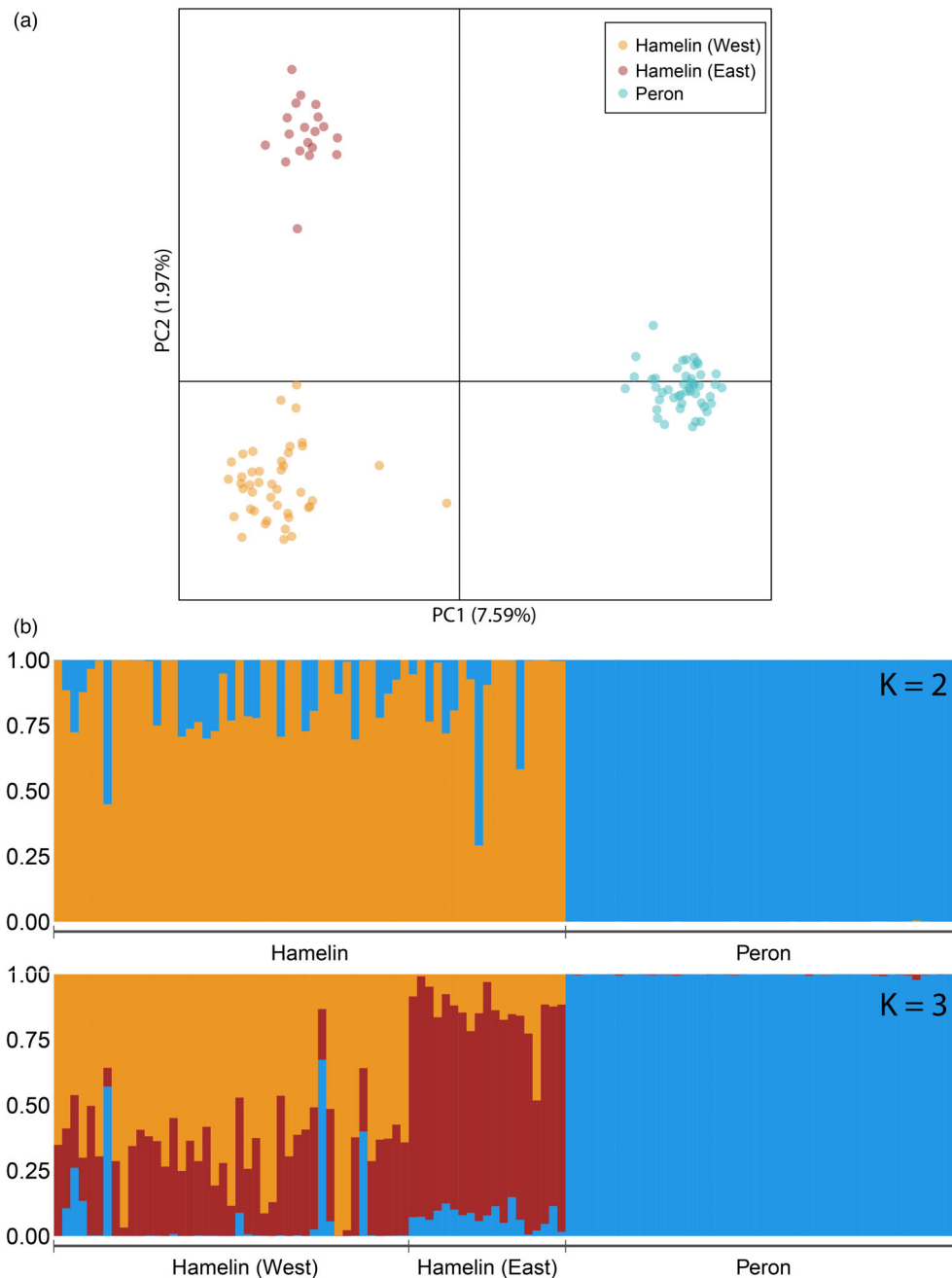


Figure 2. (a) Summary of the discriminant analysis of principal components on a population of Western Grasswren *Amytornis textilis textilis* (Hamelin and Peron) with each region represented by different colours. The proportion of variance explained by each principal component is given in parentheses. (b) Summary of the STRUCTURE analysis of the Western Grasswren *Amytornis textilis textilis* with genetic clusters represented by different colours. Each column represents an individual's estimated allocation to a genetic cluster.

Isolation-by-distance and spatial autocorrelation analysis

There was evidence of IBD across the whole sampled Western Grasswren population (Mantel test:

$r = 0.627$, $P = 0.001$, bootstrapping around the 95% CI 0.608–0.650). The correlation between genetic dissimilarity and geographical distance was weaker within subpopulations than overall, but IBD nevertheless also found within Hamelin

Table 1. Summary of descriptive population genetic statistics, observed heterozygosity (H_o), expected heterozygosity (H_e) and inbreeding coefficient (F_{IS}), for two subpopulations of Western Grasswren *Amytornis textilis textilis*.

Region	n	$H_o (\pm se)$	$H_e (\pm se)$	$F_{IS} (\pm se)$
Peron	48	0.201 \pm 0.001	0.233 \pm 0.001	0.128 \pm 0.002*
Hamelin (All)	62	0.204 \pm 0.001	0.243 \pm 0.001	0.152 \pm 0.002*
Hamelin (West)	43	0.215 \pm 0.001	0.248 \pm 0.001	0.121 \pm 0.002*
Hamelin (East)	19	0.178 \pm 0.001	0.224 \pm 0.001	0.178 \pm 0.003*

Descriptive genetic statistics were also given for specific regions within the Hamelin subpopulations, Hamelin (West) and Hamelin (East). Asterisks indicate significant deviation from zero at $P = 0.05$ level.

(Mantel test: $r = 0.315$, $P = 0.001$) and Peron (Mantel test: $r = 0.213$, $P = 0.005$) subpopulations, with non-overlapping Mantel r coefficients (bootstrapping around the 95% CI: Hamelin, 0.279–0.345; Peron, 0.162–0.264) indicating a stronger relationship at Hamelin than at Peron. The IBD slope was 0.043 (95% CI 0.039–0.047) for Hamelin and 0.021 (95% CI 0.017–0.025) for Peron subpopulations (see Supporting Information Appendix S4 for IBD plots).

Evidence of fine-scale genetic structure was observed at various distance classes in both WGW subpopulations (Fig. 3). Spatial autocorrelation within the Hamelin subpopulation revealed significantly positive r -values for increasing distance classes up to 35 km in males and 30 km in females (Fig. 3a). Similar patterns were observed in the Peron subpopulation, with males showing significant spatial autocorrelation only up to a distance class of 20 km in males and 15 km in females (Fig. 3b).

Morphological differences

Males were significantly larger than females for all morphological traits (body mass, $\chi^2 = 34.0$, $P \leq 0.001$; head–bill, $\chi^2 = 33.6$, $P \leq 0.001$; tail length, $\chi^2 = 16.7$, $P \leq 0.001$; tarsus length, $\chi^2 = 27.9$, $P \leq 0.001$; wing length, $\chi^2 = 20.3$, $P \leq 0.001$). Within females, the first principal component (PC1) explained 32.2% of the variation in morphological traits and was associated with weight and tail length (see Supporting Information Table S1 for PC table). The second principal component (PC2) explained 19.5% of the morphological variation and was associated most with wing length and tarsus width. The third principal component (PC3) explained 16.5% of the variation and was associated most with head–bill and tarsus length. Within males, PC1 explained 26.1% of the

variation and was associated with bird weight and tail length. PC2 explained 21.8% of the variation and was associated with head–bill and tarsus width. PC3 explained 18.9% of the variation and was associated with wing length and tarsus length (see Figure S5 for PC plots). After adjusting P -values for multiple tests, there were no significant principal component differences between the subpopulation regions within females (Kruskal–Wallis test; PC1, $\chi^2 = 0.1$, $P = 1$; PC2, $\chi^2 = 20.6$, $P = 1$; PC3, $\chi^2 = 0.9$, $P = 1$). Similarly, no subpopulation differences were found among males after adjusting P -values (Kruskal–Wallis test; PC1, $\chi^2 = 0.8$, $P = 1$; PC2, $\chi^2 = 4.5$, $P = 0.103$; PC3, $\chi^2 = 3.7$, $P = 0.161$).

DISCUSSION

The WGW exhibits a high level of genetic structure in Shark Bay that aligns closely with the two separate study subpopulations that occur on a narrow peninsula (Peron) and the adjacent mainland (Hamelin), respectively. The Peron subpopulation is connected to the mainland by a narrow (2.5-km-wide) isthmus, much of which may not be suitable habitat for WGW (Brooker 2000). This may greatly restrict dispersal between the peninsula and mainland subpopulations, as grasswrens are not expected to be able to traverse water due to their wing morphology and have a tendency to walk and hop across the ground rather than fly (Rayner 1988). This is consistent with what has been seen in a morphologically similar species, the White-winged Fairywren *Malurus leucopterus*, which shows concordant patterns of genetic structure in the Shark Bay region (Walsh et al. 2021). Although previous records have observed some WGW directly south of the isthmus (Brooker 2000), the narrow corridor of land could still restrict gene flow, as it creates a bottleneck

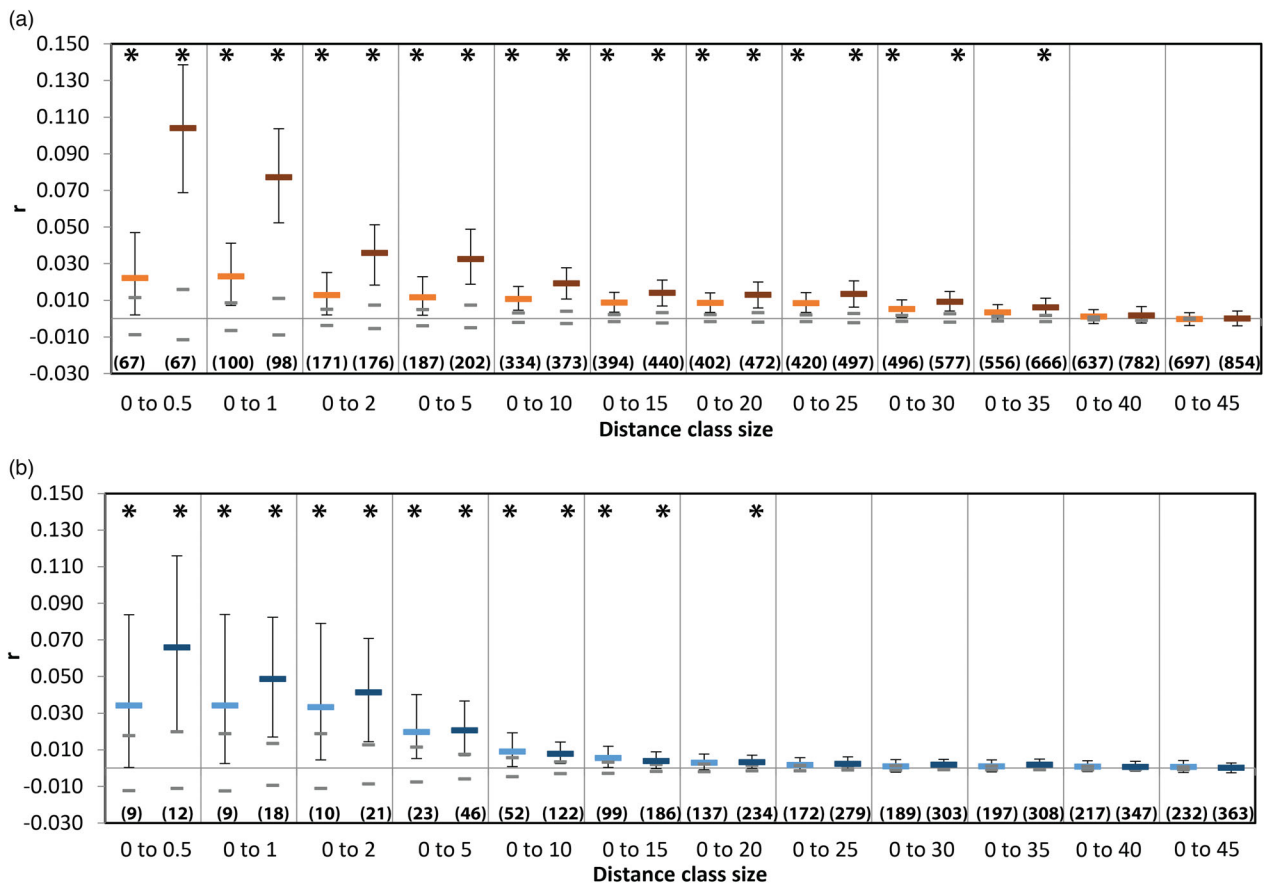


Figure 3. Multiple distance class (MDC) plots of spatial autocorrelation (r) at variable distance classes for the Western Grasswren *Amytornis textilis textilis* at two subpopulations (Hamelin and Peron) for each sex. (a) Hamelin MDC plot, with females denoted by orange and males by brown. (b) Peron MDC plot, with females denoted by light blue and males by dark blue. In both plots, grey marks represent the upper and lower 95% CI about the null hypothesis of no genetic structure. Error bars bound the 95% CI about r as determined by bootstrap sampling. Sample sizes for each distance class for each sex are in parentheses. Asterisks denote significant positive spatial autocorrelation for that sex at that distance class size.

dispersal effect, limiting the amount of gene flow between subpopulations. In addition, although no genetic sub-structuring within Peron was evident, the mainland subpopulation (Hamelin) was divided into west and east regions in our STRUC-TURE and DAPC analysis. This suggests that discontinuous habitat may be an additional barrier to gene flow in WGW, as habitat continuity and type is one of the main differences in landscape between the Hamelin and Peron areas (Payne *et al.* 1987, Beard *et al.* 2013).

The restricted, but present, gene flow between the peninsula and mainland was found to be asymmetrical, with some Hamelin birds showing low levels of mixed ancestry, yet there was no evidence of historical or contemporary mixing in the Peron individuals. Those individuals showing the

highest levels of mixed ancestry between study subpopulations were found in the western part of Hamelin (which was closest to the Peron subpopulation), providing evidence of infrequent directional dispersal between Peron and parts of Hamelin. Sourcing individuals from the landscape between Peron and Hamelin (if there are any WGW present) may provide further evidence of how prevalent dispersal between Peron and Hamelin may be. However, attempting to capture individuals in the landscape between Peron and Hamelin was not attempted, as the habitat visible from the road did not look suitable and Brooker (2000) did not find any WGW in prior surveys. Given the limited time to capture individuals, other areas with known WGW sightings were prioritized.

Given that grasswrens are thought to be poor dispersers, as they are small, have a ground-dwelling nature (Christidis *et al.* 2010) and their short round wings limit flight distances (Rayner 1988), our restricted gene flow results on the WGW could be reflective of innately poor dispersal tendencies. Spatial autocorrelation can better tease out the effects of geographical barriers vs. inherent dispersal capabilities. Our spatial autocorrelation analyses suggest WGW exhibit sex-biased dispersal, with males displaying higher levels of philopatry than females, which is consistent with our field observations of dispersed WGW males found nearer to their natal territories than females (A. Gibson Vega unpubl. data). High levels of philopatry observed in WGW males align with previous studies of closely related species within the family Maluridae (e.g. fairywrens; Cockburn *et al.* 2003, Double *et al.* 2005, Leitão *et al.* 2019). Our spatial autocorrelation analyses also indicate that local population genetic structure extends over larger distances at Hamelin than at Peron for both sexes. Habitat heterogeneity may explain the different spatial autocorrelation profiles we observed among females and males in each population region. Greater habitat heterogeneity at Hamelin may be driving males to disperse further than what might be expected in a generally male-philopatric taxon (Greenwood 1980), as males must cover a greater distance of unsuitable habitat to get to suitable areas compared with males from Peron, which may have more opportunities to find a territory closer to natal areas. Hamelin females may also have to traverse longer distances than Peron females to find suitable habitat and mates due to the contrast in habitat heterogeneity. However, we cannot discount the possibility that differences in spatial autocorrelation profiles between Hamelin and Peron were due to low sample sizes at small distance class sizes, limiting our ability to detect fine-scale genetic structure within the Peron subpopulation. There were fewer opportunities to capture birds at Peron than at Hamelin and hence we prioritized sampling across the peninsula. We documented an apparent long-distance male dispersal event, evident from the one closely related pair of individuals sampled at Hamelin and Peron. The individuals in question (a male sampled at Hamelin and a female sampled at Peron) were closely related to two other individuals at Peron, suggesting that the male at Hamelin had dispersed from Peron. However, the STRUCTURE analysis

identified this male individual as having mixed ancestry under both $k = 2$ and $k = 3$ scenarios, suggesting that it was the descendant of a long-distance disperser or that it had originated from an undetected intermediate subpopulation situated between Hamelin and Peron. Nevertheless, the resighting of a Hamelin colour-banded female grasswren approximately 30 km from the nearest origin that that bird could have come from (band colours were only confirmed for one leg, so the exact individual could not be identified; R. McLellan pers. comm., 2020) confirmed that WGW females can disperse tens of kilometres.

Despite some evidence for gene flow across tens of kilometres, there seem to be landscape barriers present within the Hamelin region because it comprises two genetic clusters (Hamelin W and Hamelin E) rather than one. Sampling within Hamelin was less evenly distributed than at Peron, which may have contributed to the sub-structuring results observed within this region. However, if Peron and Hamelin had similar distance-constrained migration or landscape barriers, the slopes of the IBD relationships should be similar (Coulon *et al.* 2010). Hamelin had a steeper IBD slope than Peron, suggesting that the clustering of west and east Hamelin regions was not driven purely by distance-constrained migration. Hence, our spatial autocorrelation analysis, coupled with the population genetic structuring observed, suggests that the genetic divergence between Peron and Hamelin (both Hamelin W and Hamelin E) was not due to poor dispersal capabilities but rather to geographical landforms restricting dispersal. Similarly, the genetic divergence between Hamelin W and Hamelin E was probably due to discontinuous habitat restricting dispersal opportunities.

Interestingly, both subpopulations displayed a deficiency of heterozygotes (significantly positive F_{IS}). As genetic divergence and low genetic diversity were found between the WGW subpopulations, the levels of F_{IS} could be indicative of inbreeding. However, F_{IS} may not be a true reflection of inbreeding, as it can be influenced by sampling design (Wang 2014). Further investigation of sampled breeding pairs would inform whether inbreeding is actively occurring in WGW subpopulations.

Although there is strong evidence for genetic divergence within the population distribution of WGW, we found no evidence of morphological

divergence. Another species of grasswren, the Thick-billed Grasswren *Amytornis modestus*, with strong genetic subdivision, was found to have morphological differences between populations, so the findings in this study provide an interesting contrast (Slender *et al.* 2017). This suggests that there are similar processes of selection throughout the distribution of the WGW (Schlotfeldt & Kleindorfer 2006). This is not too surprising, as although the Hamelin study population has greater discontinuous habitat, the local resource availability may be similar to that found in Peron due to the large overlap in vegetation type, and hence resources between the two study populations (Payne *et al.* 1987, Beard *et al.* 2013, A. Gibson Vega pers. obs.).

Having a better understanding of dispersal and genetic structure within one of the few remaining WGW populations can benefit the development of conservation and management strategies (e.g. von Takach *et al.* 2021), such as translocation to a nearby island (Algar *et al.* 2020). The WGW subspecies *Amytornis textilis carteri* once occurred on Dirk Hartog Island (also in Shark Bay) but is believed to have become extinct due to land degradation from overgrazing by introduced stock and goats, and possibly predation by feral cats (Cale 2003, Black *et al.* 2021). There are plans to utilize the mainland WGW subspecies as an ecological replacement for the extinct island grasswren species (Algar *et al.* 2020), but data on most aspects of Western Grasswren ecology have been lacking, thereby restricting development of an informed translocation plan. The IUCN/SSC *Guidelines for Reintroductions and Other Conservation Translocations* (IUCN/SSC 2013) outline genetic considerations for conservation translocations, including aiming to provide adequate genetic diversity, mixing subpopulations to maximize diversity, while minimizing the risk of genetic incompatibilities. Our results suggest that the WGW translocation may benefit from sourcing from all population regions (Hamelin W, Hamelin E and Peron) to increase genetic diversity and retain adaptive potential of the founding population on Dirk Hartog Island (Binks *et al.* 2007, Kennington *et al.* 2012, Thavornkanlapachai *et al.* 2019b). The latter is of particular importance, as apparently suitable habitat for WGW on Dirk Hartog Island, although similar, is not identical to habitat present in Peron or Hamelin (Brooker 2000, Black 2011, A. Gibson Vega pers. obs.).

CONCLUSION

Our study has shown that two WGW subpopulations in close proximity are genetically distinct and suggest that although grasswrens can and do move relatively large distances, dispersal is constrained by water barriers such as those found in Shark Bay. Our results suggest that grasswrens are able to traverse unsuitable habitat (as seen between Hamelin W and Hamelin E) but complex coastlines, including open water (i.e. between Peron and Hamelin), present a significant barrier. The limited, but directional, gene flow between two Shark Bay WGW subpopulations further suggests that dispersal has been from Peron Peninsula to Hamelin rather than the reverse through the narrow neck leading to the peninsula. Shark Bay is the only known location for WGW in Western Australia, and although there has been no recent observed population decline in the Shark Bay area itself, the distribution of the remaining population of WGW in Western Australia is geographically limited and thus vulnerable to stochastic events. From a management perspective, our results show a need to place equal importance on the peninsula and mainland subpopulations, as they are genetically distinct, with limited contemporary gene flow between them.

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AUTHOR CONTRIBUTIONS

Aline Gibson Vega: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; visualization; writing – original draft; writing – review and editing. **Michelle L. Hall:** Conceptualization; supervision; writing – original draft; writing – review and editing. **Amanda Ridley:**

Investigation; supervision; writing – original draft; writing – review and editing. **Saul J. Cowen**: Conceptualization; funding acquisition; methodology; supervision; writing – original draft; writing – review and editing. **Amy Lee Slender**: Writing – review and editing. **Allan H. Burbidge**: Writing – original draft; writing – review and editing. **Marina Louter**: Writing – review and editing. **W. Jason Kennington**: Conceptualization; formal analysis; methodology; supervision; writing – original draft; writing – review and editing.

ETHICAL NOTE

Samples were collected from Western Australia under Department of Biodiversity, Conservation and Attractions (DBCA) licence FO25000186 (A. Burbidge). Animal ethics approval was granted by the DBCA (2019-05A) for Western Grasswren blood sample collection. No animal was removed from its natural habitat for the purpose of this study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Data Availability Statement

SNP data are available through Dryad: [10.5061/dryad.kh1893278](https://doi.org/10.5061/dryad.kh1893278).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Summary of the STRUCTURE analysis of Western Grasswren *Amytornis textilis textilis* with genetic clusters represented by different colours. Each column represents an individual's estimated allocation to a genetic cluster. This analysis includes all adult individuals, with known non-breeding individuals removed (i.e. a maximum of one female and one male sample per territory).

Figure S2. Delta K estimates for varying values of genetic clusters (K ; solid line) and probability estimates for each K ($\ln(P)$; dotted line), derived from the STRUCTURE analysis. Optimal genetic clusters for this analysis is three genetic clusters.

Figure S3. BIC against different number of clusters for a DAPC analysis of Western Grasswren *Amytornis textilis textilis* populations. Optimal genetic clusters for this analysis is between two and three genetic clusters.

Figure S4. Isolation-by-distance plots of Western Grasswren *Amytornis textilis textilis* (A) and within two populations, Hamelin (B) and Peron (C).

Table S1. Principal component analysis on morphological traits from Western Grasswren *Amytornis textilis textilis*.