Conserv Genet
DOI 10.1007/s10592-016-0856-z

RESEARCH ARTICLE

Rainfall and topography predict gene flow among populations of the declining northern quoll (*Dasyurus hallucatus*)

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Received: 22 October 2015 / Accepted: 6 June 2016

Abstract Landscape attributes often shape the spatial genetic structure of species. As the maintenance of genetic connectivity is increasingly a conservation priority, the identification of landscape features that influence connectivity can inform targeted management strategies. The northern quoll (*Dasyurus hallucatus*) is a carnivorous marsupial that has experienced dramatic population declines in recent decades. To inform management of surviving *D. hallucatus* populations across north-western Australia we examined the genetic structure of populations, and identified landscape features that influence gene flow within the Kimberley region. We sampled 249 individuals from 28 populations in three regions of north-western Australia, including the Kimberley, Pilbara and Kakadu. Genetic structuring was evident between the three regions and to a lesser extent between the north and central Kimberley. Landscape genetic analysis of Kimberley populations suggest this structuring may be due in part to the indirect effects of differences in rainfall between these two areas. Also, *D. hallucatus* populations with large areas of open habitat between them tended to be more genetically similar. Managing threats such as the occurrence of intense and frequent fires, and the density of introduced herbivores, could support the persistence of *D. hallucatus* populations, particularly in areas with high rainfall and flat terrain, where greater genetic connectivity confers a better chance of long-term population survival.

Keywords Landscape genetics · Microsatellite · mtDNA · Conservation · Connectivity · *Dasyurus hallucatus*

Electronic supplementary material The online version of this article (doi:10.1007/s10592-016-0856-z) contains supplementary material, which is available to authorized users.

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Introduction

Understanding the environmental factors that facilitate or constrain gene flow across landscapes is a fundamental aim of landscape genetics (Manel et al. 2003; Storfer et al. 2007). Constrained gene flow can lead to decreased genetic diversity through processes such as inbreeding and genetic drift in isolated populations (Frankham 1995a). Loss of genetic diversity can increase extinction risk as it decreases reproductive fitness (Frankham 1995b) and limits potential to adapt to changing conditions (Dlugosch and Parker 2008). Thus, maintaining gene flow and genetic diversity is a goal of many species conservation strategies (Moritz 1994). Landscape genetic studies have been used to identify landscape features that influence gene flow, informing the placement of habitat corridors (Braunisch et al. 2010; Epps et al. 2007), and protected areas (Neel 2008).

Published online: 25 June 2016

Maintaining genetic connectivity between native high-order predator populations is often a conservation priority (Gittleman et al. 2001). Predator communities are important as they can structure the diversity and abundance of animal and plant communities across multiple trophic levels (Sergio et al. 2008). Typically, predators occur at low densities, are cryptic, and have large home ranges. Consequently, understanding how landscape features influence movement of predators has until recently been a logistic and economic challenge, requiring the fates of individuals to be tracked over several generations across large spatial scales (Simcharoen et al. 2008; Wikramanayake et al. 2004). Advances in landscape genetic methods have facilitated the identification of landscape features that enable or inhibit gene flow of high-order predators (Litvaitis et al. 2015; Manel et al. 2003; Schwalm et al. 2014).

The carnivorous northern quoll (*Dasyurus hallucatus*) is one of several native mammals that have recently declined across northern Australia (Woinarski et al. 2014; Woinarski et al. 2011). The species is currently listed as endangered under the Australian Environment Protection and Biodiversity Conservation Act (EPBC 1999). A number of factors are thought to have caused its decline, including the impacts of introduced herbivores (Legge et al. 2011a), changed fire regimes (Andersen et al. 1998; Fisher et al. 2003; Legge et al. 2011b; Vigilante 2001), exotic cane toads (*Rhinella marina*) (O'Donnell et al. 2010), and predation by feral cats (Fisher et al. 2014; Frank et al. 2014; Oakwood 2000). The invasive cane toad is thought to pose the largest threat to *D. hallucatus*, as individuals die after ingesting toxins in the toad's tissues (Hill and Ward 2008). The decline and local extinction of *D. hallucatus* populations has accompanied the spread of the cane toad across northern Australia over the last 50 years (Rankmore et al. 2008). While historically the Kimberley region (Fig. 1) has been a stronghold for *D. hallucatus*, the arrival of the cane toad is likely to cause population collapse within the coming decade (Phillips et al. 2008).

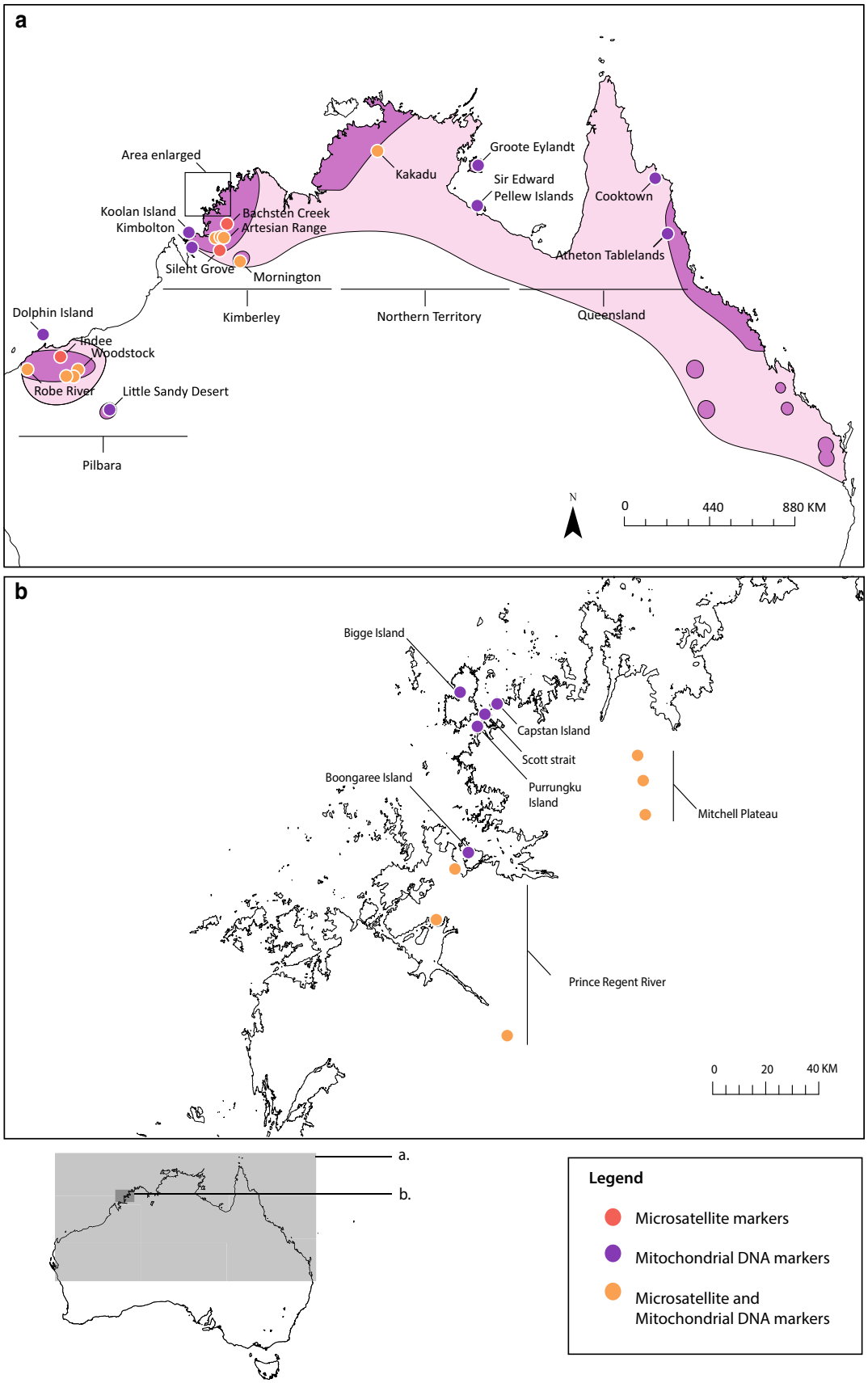
Knowledge of population genetic structure and landscape features that influence gene flow can inform future management strategies that aim to support and restore populations by reintroducing individuals and designating protected or intervention areas. *Dasyurus hallucatus* is an opportunistic forager, and in north-western Australia males have an average home range size of 64.3 ha, and females 6.8 ha (Cook 2010). Males tend to disperse further than females (Oakwood 2000), with consecutive den sites found sometimes over 4 km apart (Cook 2010). A number of landscape features could influence gene flow among *D. hallucatus* populations. Terrain ruggedness appears to be an important landscape characteristic in contemporary

Fig. 1 *Dasyurus hallucatus* populations sampled across **a** northern Australia, and **b** with detail in the Kimberley. Mitochondrial data was collected from sites indicated by *purple dots*, microsatellite data was collected from sites indicated by *red dots*, and *orange dots* indicate sites where both mitochondrial and microsatellite data was collected. *Dark purple* areas indicate the current distribution of *D. hallucatus* and *light purple* areas indicate the past distribution (adapted from Woinarski et al. (2014)). (Color figure online)

times as *D. hallucatus* has largely disappeared from open savanna habitats (Bradley et al. 1987; Kitchener et al. 1981), and is now most common in complex rocky habitats (Hill and Ward 2008; Oakwood 2002). This is thought to be related to the availability of water, microhabitats, and shelter from predators (Hill and Ward 2008). Access to permanent water is another factor that appears related to the persistence of *D. hallucatus* and therefore may also influence gene flow (Hill and Ward 2008; Woinarski et al. 2008). During the dry season (May until October) water is a limiting factor for many species in northern Australia, and populations with intervening permanent water sources may exhibit greater genetic connectivity. Similarly, populations in high rainfall areas may be more connected, as higher productivity and prey availability of these areas may confer greater ease of movement between local populations. Rainfall can also effect when a species breeds, sometimes leading to genetic structure across rainfall gradients (Danley et al. 2007; Thomassen et al. 2013; Yamamoto et al. 2016).

A number of studies suggest that historical processes such as secondary contact can influence contemporary genetic structure, and that landscape genetic studies should consider phylogeographic relationships between populations before making inferences regarding the impacts of environmental variables on gene flow (Garrick et al. 2009). Previous studies have indicated phylogenetic structuring between *D. hallucatus* populations from nearshore Kimberley islands, but not across the Kimberley mainland (How et al. 2009; Woolley et al. 2015). As a number of our sites were previously unsampled these samples sequenced for mitochondrial DNA (mtDNA) and were combined with existing data to test for historical legacies in contemporary genetic structuring across the Kimberley mainland.

While broad genetic structure of *D. hallucatus* populations has been examined across parts of northern Australia (How et al. 2009; Woolley et al. 2015), no studies have examined the genetic structure among Kimberley populations in detail, and particularly in relation to landscape features. Therefore we examined the genetic structure of *D. hallucatus* populations across north-western Australia and then tested the relationship between landscape features and genetic structure of *D. hallucatus* populations within the Kimberley region. We expected populations to be more


a
Area enlarged
Kakadu
Groote Eylandt
Sir Edward
Pellew Islands
Cooktown
Koolan Island
Kimbolton
Bachsten Creek
Artesian Range
Atheton Tablelands
Silent Grove
Mornington
Dolphin Island
Kimberley
Northern Territory
Queensland
Indee
Woodstock
Robe River
Little Sandy Desert
Pilbara
N
0
440
880 KM
b
Bigge Island
Capstan Island
Scott strait
Purrungku
Island
Boongaree Island
Mitchell Plateau
Prince Regent River
0
20
40 KM
a.
b.
Legend
Microsatellite markers
Mitochondrial DNA markers
Microsatellite and
Mitochondrial DNA markers

genetically similar when connected by areas of high rainfall, high terrain ruggedness and with shorter distances to permanent water.

Methods

Sampling

For population and landscape analyses using microsatellite markers, 249 individuals were sampled from 10 populations within three regions of north-western Australia, comprising of the Kimberley (n = 147), Pilbara (n = 88), and Kakadu (n = 14) (Fig. 1). Sites greater than 40 km apart were considered different populations, except where a substantial marine barrier existed (>500 m of open water). The number of individuals sampled per population varied from 2 to 89, and the number of sites sampled per population varied between 1 and 10 (Table 1, Online Supplementary material 1). In total we had data from 60 females, 80 males and for 109 samples the gender was unknown. Of the 249 samples, 140 were collected during biodiversity surveys run by Australian Wildlife Conservancy and the Department of Parks and Wildlife, Western Australia between 2011 and 2014. In these surveys, sites usually consisting of four cage and twenty Elliot traps, were trapped continuously for three nights. Individuals were captured in treadle-operated wire cage traps (Sheffield Wire Products, Welshpool, Western Australia) and Elliot traps (Elliot Scientific Co., Upwey, Victoria, Australia) baited with a mixture of peanut butter, oats, honey and apple. On first capture, each animal was weighed and sexed, and a tissue sample was obtained from the ear using a biopsy punch (2 mm diameter) and preserved in DMSO preservative solution (Seutin et al. 1991). The remaining 109 samples are from a previously published data set (How et al. 2009) for which trapping intensity is unknown. Only two sites (the Prince Regent and the Mitchell Plateau) included individuals from both data sets, and allele frequencies of these samples did not differ between sampling years (Online Supplementary material 2).

For phylogeographic analyses, 18 individuals from four Kimberley populations were sequenced for the control region of the mtDNA (Online Supplementary material 1). This data was combined with data available on Genbank for 70 individuals from 17 populations within four regions of northern Australia (with the addition of North Queensland as a region; Online Supplementary material 1). Only 18 new individuals were sequenced as these individuals were from previously unsampled sites.

Table 1 Genetic diversity measures of eight microsatellite loci for *D. hallucatus* populations

Population	*N*	*N*a	*P*a	*A*r	*H*e	*H*o	*F*is
Kimberley							
Ar	89	11.50	12	3.09	0.82	0.82	0.01
Bc	11	7.50	0	3.12	0.83	0.77	0.07
Mi	8	6.00	2	3.04	0.81	0.78	0.05
Pr	7	5.38	1	2.95	0.79	0.71	0.10
Si	2	2.88	1	2.88	0.72	0.81	−0.13
Mo	30	5.75	0	2.47	0.66	0.64	0.02
Pilbara							
Rr	4	4.13	0	2.84	0.77	0.68	0.11
Wo	46	7.14	0	2.77	0.74	0.76	−0.03
In	38	6.29	0	2.03	0.75	0.74	0.01
Northern Territory							
Ka	14	6.14	10	2.80	0.75	0.83	−0.10

Sample locations include: Artesian Range (Ar), Bachsten Creek (Bc), Mitchell Plateau (Mi), Prince Regent (Pr), Silent Grove (Sg), Mornington Wildlife Sanctuary (Mo), Robe River (Rr), Woodstock (Wo), Indee (In), and Kakadu (Ka), sample size (*N*), mean number of alleles per locus (*N*a), private alleles (*P*a), allelic richness (*A*r), expected heterozygosity (*H*e), and observed heterozygosity (*H*o)

Genotyping

Eleven of the 12 microsatellite loci published by Spencer et al. (2007) were genotyped specifically: pDG1A1, pDG1H3, pDg5G4, pDG6D5, pDG7F3, 3.1.2, 3.3.1, 3.3.2, 4.4.2, Sh3o, Sh6e. For details on microsatellite amplification see Online Supplementary material 3. Sequencing of the control region of the mitochondrial DNA (479 bp) was conducted following How et al. (2009) and Woolley et al. (2015).

Analysis

Phylogenetic analysis

We used the mtDNA data from our samples (n = 18) and Genbank (n = 70) to conduct Bayesian phylogenetic analyses using MrBayes 3.2.2 (Huelsenbeck and Ronquist 2001). Data were analysed using the GTR + I + G substitution model suggested by the Akaike Information Criterion from jModelTest2 (Darriba et al. 2012; Guindon and Gascuel 2003). Analysis using MrBayes consisted of duplicate MCMC runs where each run had a random starting value, consisted of four chains of 5,500,000 generations, sampled every 500 generations, with burn-in of 2,750 sampled trees (25 %). Three chains were heated with a temperature parameter of 0.1. Chain mixing and the attainment of asymptotes by LnL and model parameters was assessed using Tracer v1.5 (Rambaut et al. 2007b). Convergence of duplicate runs was determined by the standard deviation of the split frequencies being <0.01. Consensus trees were presented using the program FigTree

v1.4.2 (Rambaut 2007a). Posterior probabilities of branch splits were considered informative if they were >0.95.

Genetic diversity and differentiation

We tested if microsatellite genotype frequencies were consistent with Hardy–Weinberg equilibrium using Genepop version 3.4 (Raymond and Rousset 1995) using a level of alpha modified by the false discovery rate for multiple tests ($p < 0.016$) (Narum 2006). Exact H-W tests were performed and p values estimated using 1000 Markov chain batches. We tested for the presence of null alleles and allele-drop out using Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004). Selection at loci was tested using Bayescan version 2.01 (Foll and Gaggiotti 2008).

Genetic diversity measures comprising of the number of alleles (*N*a), observed heterozygosity (*H*o), expected heterozygosity (*H*e), and inbreeding coefficient (*F*is) were calculated for each putative population using Genodive version 2.0 (Meirmans and Van Tienderen 2004). Allelic richness (*A*r) and number of private alleles (*P*a) were calculated using the PopGenReport package (Adamack and Gruber 2014) run in the program R version 3.0.3 (R Development Core Team 2005).

We measured differentiation between populations using the fixation indexes Fst, Fst' and Gst'' calculated by Genodive. An analysis of molecular variance (AMOVA) was also conducted in Genodive; variation between populations and regions was tested using different sample hierarchies. We tested for correlations between pairwise estimates of Fst and the distance between populations using a Mantel test with 999 permutations, in the R package adegenet (Jombart 2008). A plot comparing geographic distance and Fst was made using a ranged major axis regression approach in the R package lmodel2 (Legendre 2014).

We assessed genetic structure using two methods that are not dependent on an a priori allocation of individuals to populations. Bayesian clustering was performed using the program STRUCTURE version 2.2.3 (Pritchard et al. 2000). Bayesian clustering methods apportion an individual's co-ancestry to putative populations based on allele frequencies. We ran the program for 40,000 Markov chain batches, with a burn-in period of 2000 under a population admixture model with correlated allele frequencies, and potential values of K (number of populations) between 1 and 15, with 10 replicates of each run. We selected the optimum number of K using the ΔK method (Evanno et al. 2005) implemented with STRUCTURE HARVESTER (Earl and vonHoldt 2012).

Spatial Principal Component analysis (sPCA) was also conducted, which incorporates Moran's I measure of spatial autocorrelation and genetic variance between individuals into its estimation of patterns of genetic structure represented by Eigen values (Jombart et al. 2008; Moran 1948). Unlike other spatial assignment methods, sPCA can identify spatial genetic clines. Positive Eigen values represent global variance in the form of large-scale genetic structure or clines in allele frequencies, whereas negative Eigen values represent local variance between neighbouring individuals, which is expected when allele frequencies are negatively correlated among neighbours. We generated a connection network between individuals using Delaunay triangulation, thought appropriate for data spread over large spatial scales (Jombart et al. 2008). We tested for global and local spatial structures using 9999 permutations and decided on the number of axes to retain by scanning for visual breaks in the scree and bar plot output (Jombart et al. 2008). Of those axes, we identified spatial structuring by examining the geographic distribution of each individual's Eigen value scores.

Landscape genetics

We examined how both pairwise individual and population genetic distance were predicted by the following landscape variables: terrain ruggedness, distance to water, and rainfall. Terrain ruggedness was measured using the terrain ruggedness index developed by Riley et al. (1999) from the GEODATA 9 Second Digital Elevation Model Version 3.0 (Geosciences Australia 2015). The index describes the difference in elevation between adjacent cells of a digital elevation grid. To derive the terrain ruggedness index for a cell, the difference in elevation between the cell and the eight cells immediately surrounding it was calculated; these eight difference values were then squared, averaged and finally the square root of this value was taken. These values were divided into two groups—low complexity (0–3) and high complexity (>3),—determined by calculating the number of cells within the study area that fell between each terrain ruggedness score (0–1, 1–2, 2–3, etc.) and then dividing the data equally between the categories of low and high. Distance to water was computed by first creating 1 km buffers around major river systems, then all cells intersecting that buffer were counted as being close to permanent water, and cells that did not intersect the buffer were counted as far from water (Geosciences Australia 2014). Average annual rainfall data (from between 1951 and 2016) available in raster format from the Australian Bureau of Meteorology were partitioned into the categories high (1300–900 mm per year), medium (900–700 mm per year), and low rainfall (<700 mm per year) (Bureau of Meteorology 2015). These categories were determined by calculating the number of raster cells within each 100 mm rainfall group, and dividing the data equally between the three groups. We also computed an isolation-by-distance resistance map where every cell was equal to one. This

represented a situation where distance between populations was the best predictor of genetic distance. Vegetation was not included as a predictor variable as *D. hallucatus* is thought to be a generalist predator with no strong preferences for particular vegetation types (Hill and Ward 2008). Cell size of the raster layers was 3 km, as male *D. hallucatus* in the breeding season were found to have home ranges of up to 421 ha (Cook 2010), and a 3 km cell would be sufficient to enclose an individual's home range. Cells with large water bodies such as inlets and estuaries were defined in all layers as inaccessible.

In order to examine how a landscape feature might constrain or facilitate gene flow, we initially tested six cost layers for each landscape variable. Each layer was a different representation of how difficult it would be for an individual to move across that landscape feature (Blair et al. 2013). For example, for the variable "terrain ruggedness" the first three landscape layers described an environment where it was costly to move over areas of low complexity, but easy to move through areas of high terrain ruggedness. These layers differed in the size of their cost ratio (i.e.: 1:10, 1:100, 1:1000). In contrast, the third, forth and fifth terrain ruggedness landscape layers described an environment where the cost of moving through topographically simple areas was low, but cost associated with moving through areas of rugged terrain was high. The size of the cost ratio also differed between these layers (see Online Supplementary material 4 for more detail).

Circuitscape version 4.0 was used to compute resistances between each pair of individuals (McRae et al. 2008), and average resistance values between populations were computed from these pairwise individual values. Each calculation used focal points in pairwise mode and an eight neighbour connection scheme. To determine the optimal cost ratio (i.e.: 1:10, 1:100, 1:1000) of the raster layers, we explored relationships between the proportion of shared alleles and resistance values generated using the prospective cost ratios, using Pearson's correlation conducted in the R package ecodist (Goslee and Urban 2007).

There has been some discussion as to how to deal with non-independence of values in distance matrices when analysing the relationship between landscape variables and genetic distance. We used a linear mixed effects modelling approach and selected between models using the R^2_β statistic, as this method accounts for the issue of non-independence (Edwards et al. 2008; Van Strien et al. 2012). Specifically, maximum likelihood population effects models (MLPE) were run using the package LME4 (Bates et al. 2014) in R. Models were initially fitted to a dataset comprised of pairwise comparisons between individuals, with proportion of shared alleles as the response variable and landscape resistances (terrain ruggedness, distance to water, rainfall, and isolation by distance) as predictors. All models consisted of only a single predictor. Models were also fitted to data describing pairwise comparisons between populations, with Fst as the response variable, the same predictor variables and the same total number of models. As the Prince Regent and Silent Grove populations had low sample sizes (7 and 2 respectively) the population based models were rerun without these two populations, to test if their inclusion changed the results. To test if the results are consisted between different measures of genetic differentiation the models were also run using the same predictor variables but with Jost's *D* (Jost 2008) as the response variable. This measure of genetic differentiation is particularly suited for describing differences in allelic frequencies (Meirmans and Hedrick 2011).

Prior to analysis, all predictors were centred on their mean and both predictors and response variables were rank transformed. Parameter estimation was conducted using a restricted maximum likelihood (REML) method and significance of fixed effects was calculated using the R package MixMod (Beirnacki et al. 2006). To select among competing models and assess model fit we calculated the R^2_β statistic for each model. This statistic is based on the Kenward-Roger F, with degrees of freedom calculated using the R package PBKTEST (Halekoh and Højsgaard 2014). A high R^2_β value (>0.5) suggests a strong association between a given variable and the genetic structure of the species.

Results

Mitochondrial DNA sequence variation and phylogeographic relationships

A total of 67 distinct haplotypes were identified from the 88 individuals analysed. Individuals from the Northern Territory and Queensland grouped into a single clade (Fig. 2). Pilbara samples formed two clades, one with individuals from Robe River (topological support of 0.76), and another with individuals from Woodstock, Dolphin Island and one individual from Robe River. The Kimberley samples formed a number of clades, with little phylogeographic structuring evident between populations (Fig. 2).

Genetic diversity and differentiation

All microsatellite loci were polymorphic and the number of alleles per locus ranged from 7 to 23, with the mean of 14 per locus. Genotype frequencies of three loci (pDG7F3, 3.3.2 and 4.4.2) were inconsistent with Hardy–Weinberg equilibrium. For the remaining eight loci there was no evidence for selection, scoring problems, null alleles or

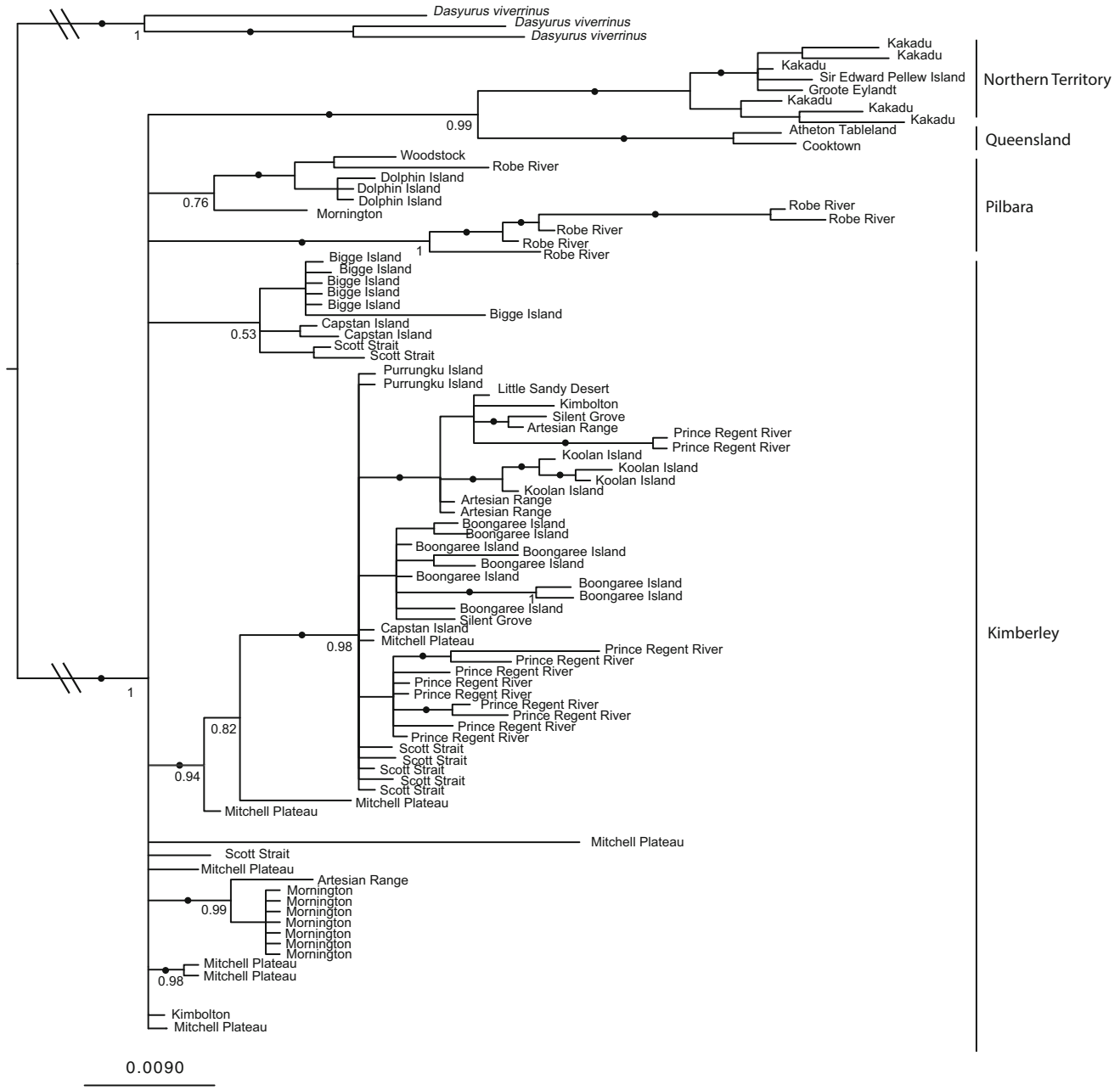


Fig. 2 Phylogenetic tree of *D. hallucatus* based on the control region of the mitochondrial DNA sequence. Branch lengths are scaled relative to the *scale bar*, except where indicated by a *scale break*. *Numbers* at nodes represent posterior probabilities, and dots indicate where probabilities are >0.9

allele drop-out; only these loci were retained for subsequent analyses.

Kakadu and the Pilbara populations almost always exhibited significant genetic differentiation from populations in other regions (Table 2, Online Supplementary material 5) ($p < 0.016$). Within the Pilbara, populations were mostly significantly different from one another. Differentiation between the north Kimberley populations and Mornington in the central Kimberley was also mostly significant (Table 2). AMOVA indicated that over 91 % of the genetic variation was found among individuals. Populations within regions (Kimberley, Pilbara, Kakadu) were significantly different from one another ($F_{sc} = 0.035$, $p < 0.001$), as were populations when considered irrespective of their region ($F_{st} = 0.067$, $p < 0.001$). Differences were also detected between regions ($F_{ct} = 0.046, p < 0.004$, Table 3). The Mantel test indicated a significant association between the geographic and genetic distance of populations across the study range ($r^2 = 0.623$, $p = 0.003$) and 62 % of the variation in genetic data was accounted for by geographic distance (Fig. 3).

Table 2 Pairwise Fst of *D. hallucatus* populations for eight microsatellite loci

Fst											
Regions		Ar	Bc	Mi	Pr	Si	Mo	Rr	Wo	In	Ka
Kimberley	Ar										
	Bc	0.010									
	Mi	0.009	−0.006								
	Pr	0.046*	0.025	0.005							
	Si	0.039	0.020	−0.002	−0.006						
	Mo	0.075*	0.087*	0.070*	0.102*	0.079					
Pilbara	Rr	0.069*	0.053	0.061*	0.055	0.132	0.152*				
	Wo	0.073*	0.065*	0.057*	0.068*	0.091*	0.108*	0.054*			
	In	0.065*	0.051*	0.063*	0.080*	0.111*	0.130*	0.037	0.018*		
Northern Territory	Ka	0.124*	0.104*	0.138*	0.129*	0.170*	0.210*	0.145*	0.151*	0.134*	

Sample locations include: Artesian Range (Ar), Bachsten Creek (Bc), Mitchell Plateau (Mi), Prince Regent (Pr), Silent Grove (Sg), Mornington Wildlife Sanctuary (Mo), Robe River (Rr), Woodstock (Wo), Indee (In), and Kakadu (Ka)

* $p < 0.016$ (p adjusted using the false discovery rate approach)

Table 3 Analysis of molecular variance (AMOVA) between populations (Table 2) and regions (Kimberley, Pilbara and Northern Territory) for *D. hallucatus*

	% variation	F statistic	F value	*p*
Within individuals	0.916	F_it	0.084	–
Among individuals within populations	0.005	F_is	0.005	0.309
Among sites	0.067	F_st	0.067	0.001
Among populations within regions	0.033	F_sc	0.035	0.001
Among regions	0.046	F_ct	0.046	0.004

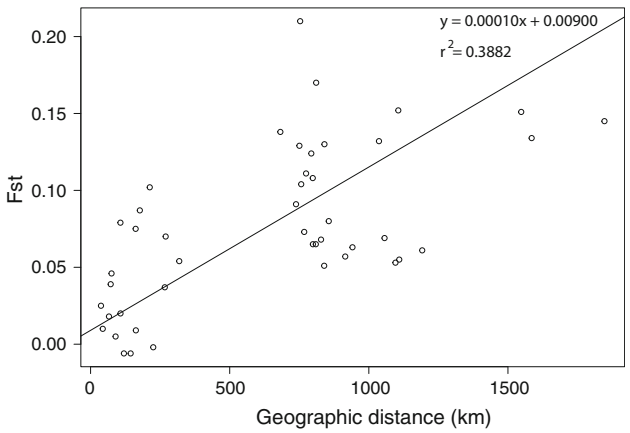


Fig. 3 The relationship between pairwise Fst of populations and geographic distance across the entire study range using a ranged major axis regression approach

Using the ΔK method we found that the K = 2 population structure had the most support. One group included all populations from the Pilbara (Robe River, Indee, Woodstock), while the next included all other populations from the Kimberley and Kakadu (Fig. 4a, Online Supplementary material 6,7). To identify the extent of finer scale population structuring, Pilbara populations were removed and STRUCTURE was re-run. The population grouping with the most support was K = 4, which separated Mornington, Kakadu, and the combined north Kimberley populations, the latter containing gradation between two groups (Fig. 4b, Online Supplementary material 6,7). The next most likely population grouping was K = 2, in which Mornington was grouped as one population, and Kakadu and the north Kimberley populations were grouped together as the other. The K = 3 population grouping displayed Mornington, Kakadu and the north Kimberley populations separately.

The sPCA permutation test indicated the dataset had significant structure at a global scale (nper = 9999, max(t) = 0.032, $p = < 0.0001$). Local sPCA axes were weakly distinguished from one another (Fig. 5), but some evidence of local structuring was found (nper = 9999, max(t) = 0.011, $p = 0.008$). The scree and bar plots indicated that the first three positive Eigen values were discontinuous relative to the others and thus these were retained for further exploration (Fig. 5). sPC 1 indicated genetic differentiation between the Pilbara (strongly negative), Kakadu (weakly negative) and the Kimberley (strongly positive) (Fig. 5, Online Supplementary material 8). Genetic structuring within the Kimberley was indicated by sPC 2 and 3 (Fig. 5).

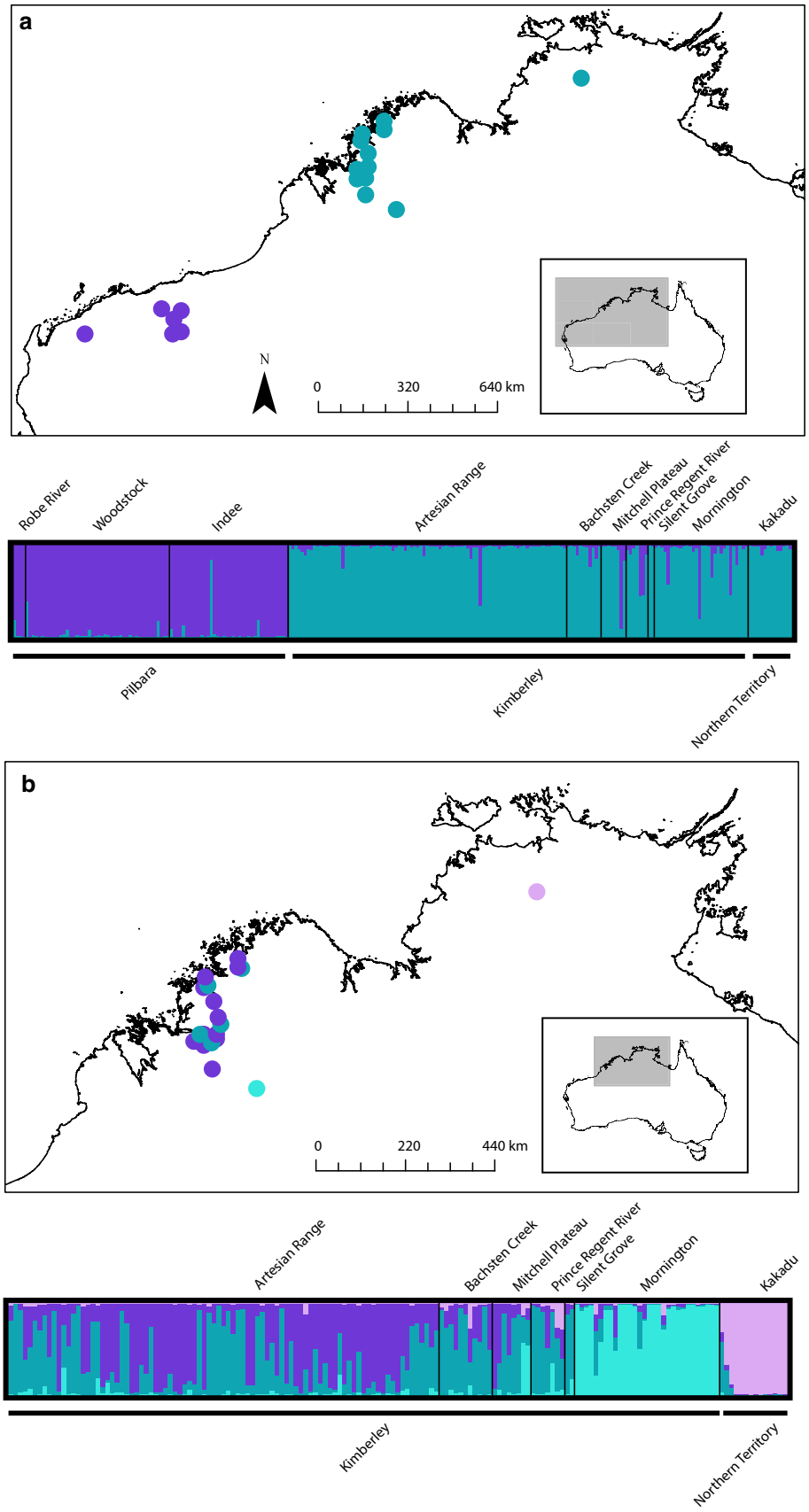


Fig. 4 Results of the genetic clustering algorithm employed by the program STRUCTURE for *D. hallucatus* samples from **a** all populations (K = 2), and **b** excluding populations from the Pilbara region (K = 4). *Vertical bars* represent individuals and the colour indicates the co-ancestry of each individual within each of K groups

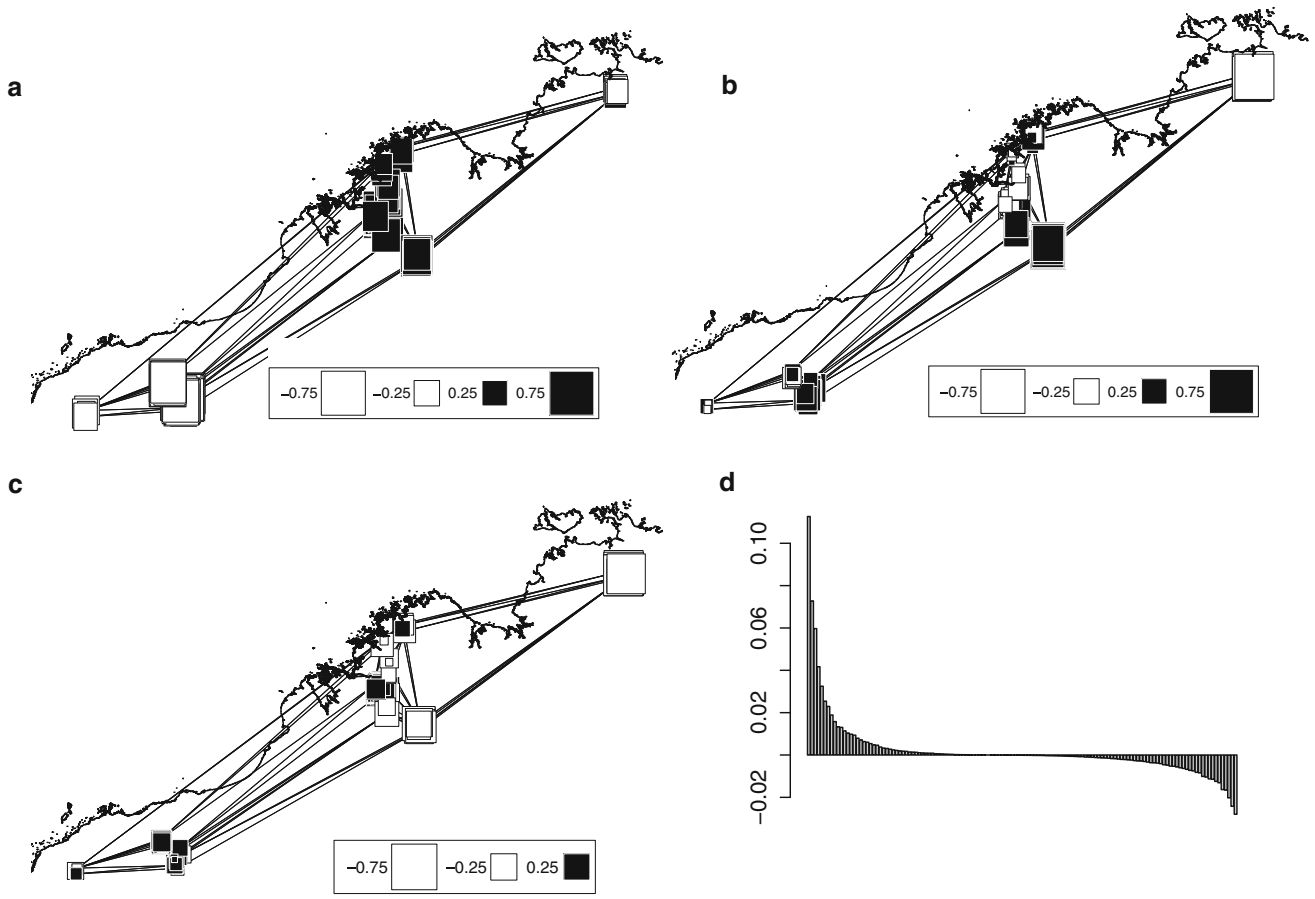


Fig. 5 Analysis of global Eigen value scores from the spatial principal component analysis (sPCA) performed on *D. hallucatus* in north-western Australia. **a**–**c**. display spatially the global axes 1–3 (respectively), and lines on these plots indicate the connection network (Delaunay triangulation) used in the analysis. **d**. displays Eigen values for each axis where the *bars* on the *left* (above the *x* axis) represent global structure, and those on the *right* (below the *x* axis) represent local structure

Landscape genetics

Landscape models built on pairwise individual comparisons

Pearson's correlation coefficents indicated that the ratio of 1:10 was the cost parameterisation (of the options: 1:10, 1:100, 1:1000) that correlated best with proportion of shared alleles, therefore layers with cost values 1:10 were used in subsequent analyses. Some resistance layers were also intercorrelated (Online Supplementary material 9,10). Of the models that analysed the relationship between individual pairwise proportion of shared alleles and landscape resistance values, no models had R^2_β values of >0.01 (Table 4). This suggests that none of the variables were good predictors of the proportion of shared alleles between individuals.

Landscape models built on pairwise population comparisons

At the population level, the model describing the relationship between Fst and topography was the best fitting model, with an R^2_β of 0.65 (Table 5). The resistance values in this model were generated based on lower cost of movement in the less topographically complex areas, and higher cost in areas with rugged terrain. The positive coefficient estimate of this model indicates that populations with higher resistance between them (connected by areas of rugged terrain) were also more genetically different with higher Fst values. The next best model examined the relationship between Fst and rainfall ($R^2_\beta = 0.52$). The cost surface used to generate the resistance values in this model described a situation where it was less costly to move through high rainfall areas, and more costly to move through low rainfall areas. The positive coefficient

Table 4 Parameter estimates and measures of fit for linear mixed effect models examining the relationship between individual genetic distance (proportion of shared alleles) and measures of resistance for the variables topographic complexity, distance to water, and rainfall

Hypothesis*	Cost ratio	High cost predictor variable	$\hat{\beta}$	SE	R^2_β
H1	10:1	Topography	−0.208	0.011	0.007
H4	10:1	Open plains	−0.209	0.009	0.009
H1	10:1	Large distance to water	−0.231	0.011	0.011
H4	10:1	Short distance to water	−0.221	0.010	0.009
H1	10:5:1	Low rainfall	−0.211	0.010	0.008
H4	10:5:1	High rainfall	−0.206	0.012	0.015
–	1	Isolation by distance	−0.220	0.011	0.013

* See Online Supplementary material 3 for more information on cost ratios of resistance layers

Table 5 Parameter estimates and measures of fit for linear mixed effect models examining the relationship between population genetic distance (Fst) and measures of resistance for the variables topographic complexity, distance to water, and rainfall

Hypothesis*	Cost ratio	High cost predictor variable	$\hat{\beta}$	SE	R^2_β
H1	10:1	Open plains	0.303	0.240	0.052
H4	10:1	Topography	0.868	0.155	0.650
H1	10:1	Large distance to water	0.418	0.254	0.069
H4	10:1	Short distance to water	0.437	0.227	0.120
H1	10:5:1	Low rainfall	0.763	0.178	0.525
H4	10:5:1	High rainfall	0.221	0.259	0.018
–	1	Isolation by distance	0.041	0.235	0.097

* See Online Supplementary material 3 for more information on cost ratios of resistance layers

estimate of this model suggests that populations in high rainfall regions were less genetically distinct with lower levels of Fst. Models that included the variables distance to water and raw distance produced low R^2_β values (<0.09, Table 5).

Models run without the two populations that had low sample sizes produced very similar results to those described above. The best fitting model included a resistance layer that described a situation where it was difficult to move across areas of rugged terrain (R^2_β of 0.69). The next best model included a resistance layer where it was easy to move between populations in high rainfall areas (R^2_β of 0.64). All other models produced R^2_β of <0.35 (Online Supplementary material 11). Models run using Jost's *D* as a response variable (but including all populations) produced higher R^2_β values, but overall the results were also very similar to those described above (Online Supplementary material 12). The best fitting model included the rainfall resistance layer that described a situation where it was costly to move through low rainfall areas ($R^2_\beta = 0.76$). The model that described a situation of lower genetic connectivity between *D. hallucatus* populations connected by rugged terrain, also had a high R^2_β value (0.72).

Discussion

Landscape characteristics such as rainfall and terrain ruggedness may be factors driving variation in genetic structuring of *D. hallucatus* populations across the Kimberley. Low genetic distance was detected between populations separated by open plains, and in high rainfall areas. While few northern quolls now persist in open habitats, these results and historical records suggest that individuals may have commonly moved across open habitats in the recent past. The effects of rainfall on landscape productivity and the timing of breeding in *D. hallucatus* may also influence genetic connectivity, particularly between the north and central Kimberley.

Both the STRUCTURE and sPCA identified genetic differentiation among the Pilbara, Kimberley, and Kakadu. Some degree of genetic structuring was also detected between the north and central Kimberley. Levels of genetic differentiation (Fst) also support these divisions but indicate that the degree of differentiation between these two areas of the Kimberley is less than the degree of differentiation between more distant regions. These results are supported by a previous study that also detected genetic structure between the Kimberley and Pilbara *D. hallucatus* populations using microsatellite markers (How et al. 2009). However, genetic structure between the north and central Kimberley has not previously been detected, because populations such as Mornington in the central Kimberley were not sampled (How et al. 2009). Structure between the north and central Kimberley may reflect the lower genetic diversity of the Mornington population (in terms of allelic richness and heterozygosity). This population may benefit from ongoing monitoring as low genetic diversity may make the population vulnerable to extinction after cane toads arrive.

Overall *D. hallucatus* populations in the north Kimberley that receive high annual rainfall appeared to be more genetically similar to one another than to populations in central Kimberley that receive lower annual rainfall. While there appears to be some inter-annual variation in breeding dates for *D. hallucatus* (Braithwaite and Griffiths 1994), there is a relationship between rainfall and the timing of breeding, which may contribute to this association between genetic relatedness and rainfall. Mornington Sanctuary in the central Kimberley receives on average just over 700 mm of rain each year (Bureau of Meteorology 2015), and pouch young are most frequently found in October (K. Tuft, pers. com). Similarly, the Pilbara receives on average below 500 mm of rain each year and pouch young are most frequently found in September (How et al. 1991). In contrast the Mitchell Plateau, Kakadu, and Groote Island receive over 1000 mm of rain annually (Bureau of Meteorology 2015), and pouch young appear as early as July and are most frequently found in August and September (I. Radford, pers. com, J. Heiniger, pers. com, Braithwaite and Griffiths (1994)). The date of a first significant rainfall event in the wet season may also have strong impacts on the timing of breeding for *D. hallucatus*, but potentially at least in the Kimberley rainfall timing and annual rainfall are interrelated (Online Supplementary material 13). Thus synchronisation between the timing of breeding and rainfall patterns may contribute to the patterns of *D. hallucatus* genetic differentiation detected across rainfall gradients. Rainfall driven shifts in the timing of reproduction are thought to have led to speciation between populations of giraffes (Thomassen et al. 2013), plants (Lamont et al. 2003), crickets (Yamamoto et al. 2016) and moths (Danley et al. 2007), and has also been related to genetic distance between populations of band-rumped storm-petrels (*Oceanodroma castro*) (Smith and Friesen 2007).

High rainfall areas may also have greater productivity, vegetation cover providing protection from predators, and abundance of prey, supporting higher-density and more connected *D. hallucatus* populations than lower rainfall areas. Prior to the arrival of cane toads, *D. hallucatus* populations in more arid parts of the Kimberley including the south-west, south-east, and east, suffered declines to a greater degree than populations in the north Kimberley (Archer 1979; Kitchener 1978; McKenzie 1981; McKenzie et al. 2007; Radford et al. 2014). Similarly in the Northern Territory, *D. hallucatus* disappeared from the arid parts of its range prior to the arrival of the cane toad (Ziembicki et al. 2013). Declines in these populations may have resulted from a number of factors such as the impacts of contemporary fire patterns, the introduction of cattle grazing and other large herbivores, and the impacts of feral cats (Woinarski et al. 2011, 2014). However, these threats are present across the region including the north Kimberley, where many *D. hallucatus* populations persist today (Carwardine et al. 2011). Therefore, factors related to high rainfall such as greater vegetation cover, or greater productivity of the landscape may have supported population persistence and connectivity in the north Kimberley, and led to isolation and drift occurring in remnant populations in more arid areas including the central Kimberley.

Terrain ruggedness also appears to explain genetic distance between *D. hallucatus* populations in the Kimberley. Despite the strong affiliation between current *D. hallucatus* populations and rocky and topographically complex habitats (Hill and Ward 2008), our results indicate that populations connected by more open areas are more genetically similar. For example, the genetic distinction of the central Kimberley population could reflect difficulty of movement through the rocky and complex King Leopold Range. A number of landscape genetics studies have found associations between complex topography and gene flow (Funk et al. 2005; Giordano et al. 2007; Pérez-Espona et al. 2008; Wasserman et al. 2010). Male *D. hallucatus* have home ranges of up to 421 ha (Cook 2010), and historical records indicate that the species was once widespread through most savanna habitats in northern Australia (Bradley et al. 1987; Kitchener 1978). Therefore, while few *D. hallucatus* populations persist in open habitats today, individuals may have once lived and moved more freely across open habitats, prior to population declines in these regions. Persistence in more topographically complex areas is potentially related to the constant availability of shelter from predators, particularly as the savanna habitats in northern Australia are prone to frequent and intense fires that remove vegetation cover within the fire scar (Leahy et al. 2016; Vigilante et al. 2004). However as the terrain ruggedness and rainfall resistance layers were correlated to some degree, discerning the relative contributions of these variables is difficult, and future studies may benefit from also evaluating model uncertainty (Dudaniec et al. 2016).

A number of variables including distance to water source and raw geographic distance were poor predictors of genetic distance in the Kimberley. This suggests that permanent water availability does not limit dispersal or population persistence, at least at the scales tested here. Also, while geographic distance correlated with genetic distance when all regions were considered (including the Pilbara, Kimberley and Northern Territory), there was no relationship when only Kimberley populations were considered. The observed relationship between geographic and genetic distance at larger spatial scales may also be confounded by historical factors (Hutchison and Templeton 1999).

No relationships were detected between genetic distance (proportion of shared alleles) and any of the landscape variables for individual-level comparisons. We suggest that

this may be in part due to the low allelic diversity of populations such as Mornington in the central Kimberley, relative to populations such as the Mitchell Plateau and Artesian Range in the north Kimberley. As Mornington has low genetic diversity, it is unlikely to exhibit a high proportion of alleles shared with the genetically diverse populations of the north Kimberley. Yet, high diversity across the north Kimberley also means that two individuals from different populations in the north are also unlikely to share a high proportion of shared alleles. Ultimately this study would benefit from including samples from other populations in the central Kimberley. These may be difficult to attain as remote camera surveys suggest that the *D. hallucatus* abundance in the ranges surrounding Mornington Sanctuary is low and fluctuating (S. Legge and K. Tuft unpublished data). Although other studies have advocated individual-level analyses (Landguth et al. 2010), population-based approaches should be more robust to regional differences in genetic diversity, and thus be more appropriate for examining genetic variation between isolated populations.

The phylogenetic tree built from mtDNA showed some evidence of deeper genetic structure between regions, but there was little evidence of phylogeographic structuring within the Kimberley. This supports the importance of environmental variables as explanations of spatial genetic variation within the Kimberley. Our results are similar to those of How et al. (2009), that described eastern (Northern Territory and Queensland) populations as distinct from western (Kimberley and Pilbara) populations. These results contrast with a recent study that found a strong division between *D. hallucatus* individuals from northern Australia (the Kimberley, Northern Territory and Queensland) and the Pilbara (Woolley et al. 2015). Variation between these trees may be in part due to differences in the number of genes examined. Both our study and How et al. (2009) used the control region of the mtDNA, but as Woolley et al. (2015) were looking at relationships between multiple quoll species across Australia, their analysis included several other mtDNA markers. Compared to the Woolley et al. (2015), our study included a greater number of sampling sites (21 compared to 16), and a greater number of individuals per site (on average 4.1 compared to 1.6). Also there are only 13 shared samples between our study and Woolley et al. (2015), almost half of which are from a single site in Kakadu.

Northern quoll populations in the Kimberley are on the verge of collapse as the invasive cane toad colonises the region (Phillips et al. 2008). The cane toad occupies 60 % of the former range of *D. hallucatus*, and is likely to spread across the rest of its range, including both high and low rainfall areas, within the next 10–20 years (Hill and Ward 2008). There are small *D. hallucatus* populations in north-eastern Australia that have survived alongside cane toads, and these tend to be in rugged areas that have been less disturbed by fires (Woinarski et al. 2008). Therefore in high rainfall areas of the Kimberley where the high degree of genetic connectivity gives those populations a better chance of surviving cane toad arrival, controlling threats such as the occurrence of intense and frequent fires could support the persistence of *D. hallucatus* populations. Managing threats in open habitats might also contribute to maintaining connectivity between populations, particularly in regions where the cane toad has not yet reached, such as the Pilbara.

In this study we aimed to understand genetic structure of Kimberley populations and test landscape features that might influence connectivity between populations. Low genetic distance between populations separated by less topographically complex habitats suggests that individuals may have commonly moved across more open habitats where few individuals now persist. Rainfall and its effects on landscape productivity and the timing of breeding in *D. hallucatus* may also drive genetic distance, particularly between the north and central Kimberley. Other factors such as land use change may have contributed to isolating *D. hallucatus* populations in the central Kimberley, promoting independent genetic drift.

Acknowledgments This study was funded by supporters of the Australian Wildlife Conservancy, an Australian Research Council Linkage Grant (100100033), and the Department of Parks and Wildlife (Western Australia). The study methods were approved by the University of Tasmania Animal Ethics Committee (permit number A12516). This project would not have been possible without the support of staff at Australian Wildlife Conservancy's Mornington Wildlife Sanctuary. Terry Webb also gave tremendous assistance sourcing and processing GIS layers. Also, thankyou to Alex Hartshorne, Hannah Cliff, Jillian Smith, Joel Murray, David James, Peter Richardson, Kaely Kreger, Davina Bright, Tom Crawford and Iris Bleach for their help with fieldwork.

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