23

Regional genetic differentiation in the spectacled flying fox (*Pteropus conspicillatus* Gould)

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Introduction

Climatic excursions in the late Pleistocene dramatically reduced habitat available to organisms dependent on forested landscapes (Hopkins et al. 1993; Kershaw 1994; Kershaw et al. 2007; VanDerWal et al. 2009). Pollen analysis and bioclimatic modelling of rainforest in northeastern Queensland indicate the region was subject to massive change during Quaternary glaciations. The consequences for rainforest-dependent species were severe, especially for organisms with limited mobility or adaptability (Schneider et al. 1998). We report here on present-day regional-scale genetic structure in the spectacled flying fox (*Pteropus conspicillatus*), generally assumed to be a rainforest specialist, and on the insights modern-day processes may provide for understanding responses of an extremely mobile animal to Pleistocene habitat contraction and fragmentation.

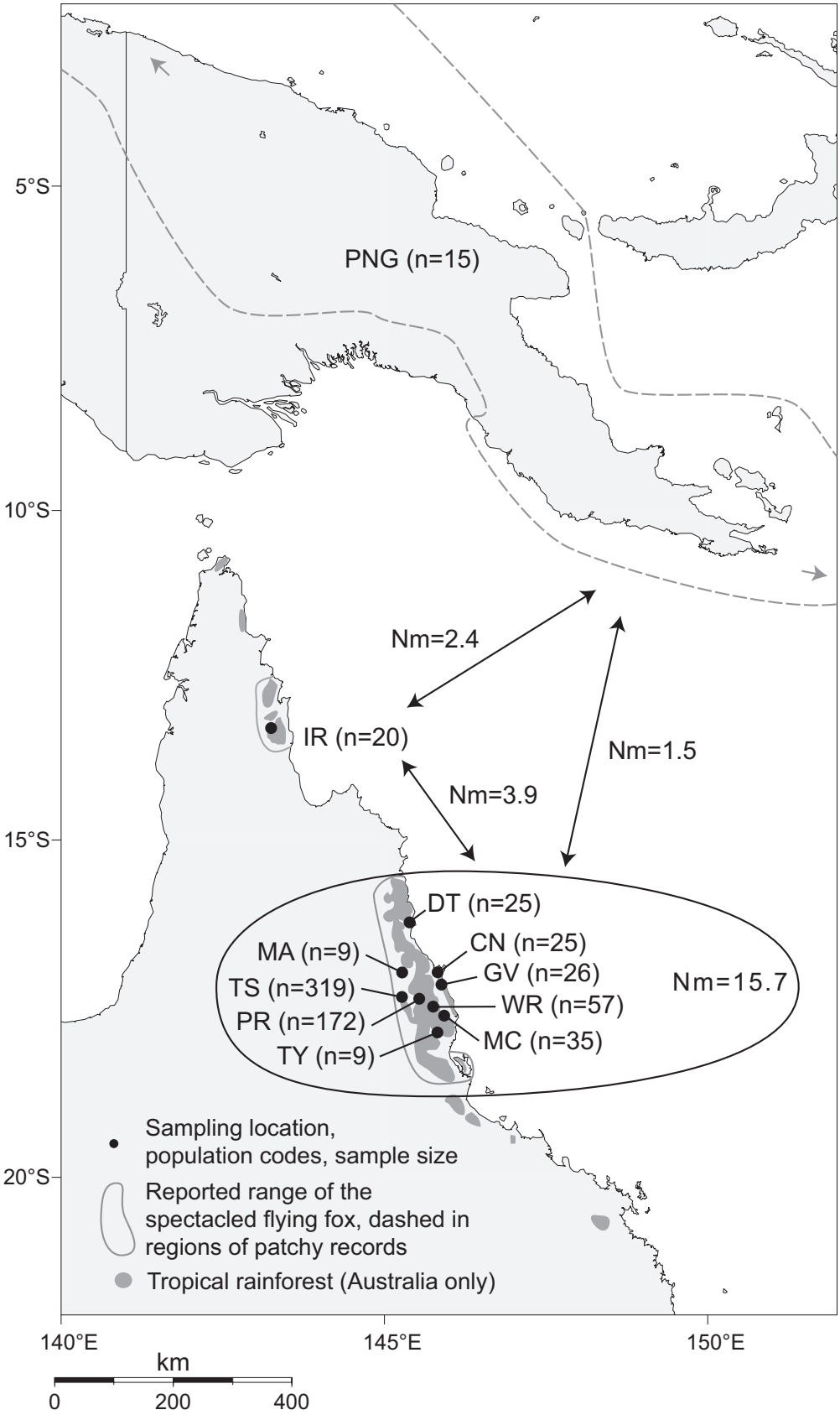

Figure 1. Sampling locations of Pteropus conspicillatus, indicating sample sizes and gene flow estimates (Nm) among regions and within the Wet Tropics region. Locality codes: PNG = Papua New Guinea, IR = Iron Range, DT = Daintree, CN = Cairns, GV = Gordonvale, WR = Whiteing Road, MC = Mena Creek, TY = Tully, PR = Powley Road, TS = Tolga Scrub, MA = Mareeba.

All flying foxes are potentially extremely mobile and some species make seasonal migrations across hundreds of kilometres, following cycles of fruiting and flowering of favoured food trees (Eby 1991; Tidemann and Nelson 2004). Species such as the little red flying fox (*Pteropus scapulatus*) and grey-headed flying fox (*P. poliocephalus*) traverse many hundreds of kilometres in the course of a year and are apparently panmictic (Sinclair et al. 1996; Webb and Tidemann 1996: Luly et al. 2010). In contrast, the spectacled flying fox is considered to be closely associated with rainforest (Richards 1990a, b) and currently has a discontinuous distribution in northeastern Queensland, New Guinea and adjacent islands. By far the largest known populations are associated with coastal and upland rainforest in the Wet Tropics World Heritage Area (hereafter the Wet Tropics) between about Townsville and Cooktown in northeast Queensland (Westcott et al. 2001). A small colony – a few hundred strong at most (Fox 2006) – is found approximately 400 km north of the Wet Tropics in the Iron Range National Park on Cape York Peninsula, where isolated pockets of wet tropical rainforest provide habitat. The species occurs widely and patchily outside Australia (Figure 1), but little is known about population sizes. It is found in the Molucca Islands in Indonesia, in lowland New Guinea from approximately Agats in Irian Jaya, around the north coast to the Port Moresby district in the east and offshore in the D'Entrecasteaux Islands and the Louisiade Archipelago. There is a break in the range on the drier southern coast of New Guinea closest to the tip of Cape York Peninsula (Bonaccorso 1998). The New Guinea distribution is, at its closest, approximately 600 km from the northernmost permanent Australian colony at Iron Range. Radio telemetry and satellite tracking have shown that individual spectacled flying foxes in the Wet Tropics region undertake regular movements between colonies (Shilton pers. comm.). It is not known whether any movement occurs between the Wet Tropics, Iron Range (IR) and Papua New Guinea (PNG), or what movements occur within New Guinea and Indonesia.

Given the assumed habitat specialisation of the spectacled flying fox, the species might be expected to exhibit stronger genetic differentiation across its disjunct range than apparently less specialised congeners. Examining the extent of differentiation, if any, in the modern range of the spectacled flying fox may help inform thinking on the capacity of flying fox species to maintain genetic integrity in the even more severely fragmented habitats that prevailed during glacial episodes. To investigate this, we set out to determine the extent of gene flow between colonies in the Wet Tropics, and between the Wet Tropics as a whole and other regions (IR, PNG) where spectacled flying fox populations occur.

Materials and methods

Samples

Samples were collected from 718 individual spectacled flying foxes distributed widely across the range of the species (see Figure 1). Sampling effort across the species' range was very uneven because of the difficulties of gaining access to elusive animals living in remote areas. Many of our samples were obtained from tick-paralysed bats rescued from camps on the Atherton Tableland (location abbreviations as in Figure 1). Other samples came from bats taken for veterinary attention or to be raised by wildlife care groups, the location assigned to such animals being the camp nearest to where they were found. Samples from IR were obtained from bats captured in mist nets. Wing tissue was obtained from living bats following Worthington-Wilmer and Barratt (1996). A small piece of wing membrane was removed from dead bats with scissors. Tissue samples were stored in 5 M NaCl-saturated 20% DMSO (Dimethylsulphoxide) and refrigerated at 4°C until processed.

Samples from PNG were small pieces of wing membrane obtained from skins held in the Australian Museum. Most (seven) were from Hull Island, Milne Bay Province (museum

accession numbers EBU23156, 23157, 23159, 23162, 23164, 23171, 25578). Two came from elsewhere in Milne Bay Province (EBU23179, 26345) and one from West Sepik Province (EBU25020). The remaining five PNG samples were collected in Madang Province courtesy of the Wildlife Conservation Society. The unevenness of sampling from across the species' range constrained the level of analyses attempted.

DNA extraction and amplification

DNA extraction was carried out using a QIAGEN DNeasy™ tissue extraction kit according to the manufacturer's instructions. DNA was used at a final concentration of 3 ng/μL for amplification by polymerase chain reaction (PCR).

Six microsatellite loci were amplified and scored: one dinucleotide (Ph9) and one trinucleotide (C6) locus characterised from *Pteropus rodricensis* (O'Brien et al. 2007); three dinucleotide loci (PC25b6, PC26a7, PC31h4) and one with a compound repeat (PC36c2) characterised from *P. conspicillatus* by Fox et al. 2007. Primers were labelled with 5' fluorophores HEX, FAM or TET (Geneworks Ltd). Concentrations of reagents in the PCR reaction mix varied by locus (see Table 1). PCR products were purified by centrifugation through 300 μL of Sephadex (G-50), before being analysed on a MegaBace 1000 Genetic Analyser (Amersham BioSciences™) at the Advanced Analytical Centre, James Cook University. Allele sizes were estimated using the ET 400-Rox (Amersham BioSciences ™) internal size standard and the program Fragment Profiler v 1.2 (Amersham BioSciences™).

Table 1. Final concentration of reagents used in amplification of each microsatellite locus for 15 μL PCR reactions (including 1.5 mM MgCl2 and 0.4 units Taq DNA polymerase).
1. 94ºC 3 mins; 4 cycles (94°C 30 secs, 58°C 40 secs, 72°C 1 min), repeated four times with a reduction in temperature of 2°C each step down; 25 cycles (94°C 30 secs, 50°C 40 secs, 72°C 1 min); 72ºC 10 mins.
2. 95ºC 5 mins; 35 cycles (95°C 45 secs, 55°C 30 secs, 72°C 45 secs); 72ºC 10 mins.
3. 94ºC 3 mins; 4 cycles (94°C 30 secs, 50°C 40 secs, 72°C 1 min), repeated three times with a reduction in temperature of 2ºC each step down; 25 cycles (94°C 30 secs, 44°C 40 secs, 72°C 1 min); 72ºC 10 mins.

Locus	$MgCl_2$ (mM)	dNTPs (mM)	Primer (μM)	DNA (ng)	Thermocycler profile
PC25b6	1.5	0.1	0.3	7.5	1
PC26a7	1.5	0.1	0.6	7.5	2
PC36c2	-	0.2	0.4	7.5	1
PC31h4	1.5	0.2	0.2	4.5	2
c6	1.5	0.2	0.2	7.5	3
ph9	-	0.1	0.2	3.0	2

Data analyses

All PNG samples were treated as a single 'colony'. Hardy Weinberg equilibrium (HWE) expectations were tested for each locality by locus using GenAlEx v6.1 (Peakall and Smouse 2005). Micro-Checker (van Oosterhout et al. 2006) was used to detect the presence of null alleles, large allele dropout and stuttering. GenAlEx v 6.1 was used to calculate mean and effective numbers of alleles and expected and observed heterozygosities. Allelic richness, calculated in FSTAT (Goudet 1995), was averaged across loci to give mean allelic richness by locality. Numbers of private alleles, and the number of migrants (N_m) between regions per generation using the private alleles method (Slatkin 1985), were calculated using GenePop (Raymond and Rousset 1995).

Two approaches were used to measure genetic differentiation among localities. Firstly, an analysis of molecular variance (AMOVA), which accounts for gene frequencies and number of mutations, was calculated for F_{ST} as well as R_{ST} using GenAlEx. Pairwise F_{ST} (following Peakall et al. 1995), and R_{ST} (following Michalakis and Excoffier 1996) values were

also calculated, and a principal coordinates analysis (PCA) was performed using a distance matrix. All significance tests were based on 999 random permutations. Secondly, an exact test based on an unbiased estimate of the p-value of a log-likelihood (G) was performed using a Markov Chain method in GenePop. This is a more powerful way of testing for panmixia using multiple loci and unbalanced sample sizes (Ryman and Jorde 2001; Waples and Gaggiotti 2006) Genotypic differentiation across population pairs was calculated using a Markov Chain of 1000 dememorisations, 1000 batches and 10,000 iterations per batch. F_{IS} (*f*) by locality and locus was calculated following Weir and Cockerham (1984) in Fstat.

An analysis of isolation-by-distance (IBD) was performed using the program isolde in GenePop. This uses a regression of $F_{ST}/(1- F_{ST})$ against the shortest distance (in kilometres) between any two localities. A Bayesian population assignment protocol implemented in structure v2.1 (Pritchard et al. 2000) was used to infer the number of populations represented in the data and to assign individuals to those populations. Models were run for 1-15 putative populations. Conditions for running structure included a model run burn-in procedure of 100,000 replicates, followed by 100,000 Markov Chain Monte Carlo (MCMC) simulations, using the admixture model with allele frequencies correlated between populations. Longer burn-in and MCMC trials were performed but likelihood values were not improved up to 250,000 replicates for each. Ten iterations were performed for each putative number of populations (K).

Evidence of population expansion/contraction was tested using the program Bottleneck v 1.2.02 (Piry et al. 1999). This program evaluates deviation from a theoretical mutation-drift equilibrium and is expressed as the difference between the measured heterozygosity (H_e, defined and discussed further in Piry et al. 1999), and the heterozygosity expected at mutation-drift equilibrium (H_{eq}). Luikart and Cornuet (1998) suggested that the Wilcoxon's test is appropriate for data sets with fewer than 20 loci. Although only six loci were used in this analysis, the highly polymorphic nature of all loci will increase the power of the test. The one-tailed Wilcoxon's test for heterozygosity excess and the sign test were used for both mutation models (infinite alleles model IAM, and step-wise mutation model SMM). Estimations were made over 1000 replicates. Each locality was tested individually and then all Wet Tropics localities were combined and treated as a single population.

Results

Genetic diversity, Hardy Weinberg equilibrium and linkage disequilibrium

All microsatellite loci exhibited high levels of polymorphism, with the number of alleles recorded ranging from 14 (locus C6) to 22 (locus Ph9). Twenty-three private alleles were detected across all localities and loci. Significant departures from Hardy Weinberg expectations were recorded in the PR, TS and WR localities for one, four and two loci respectively. There was no pattern to the loci that were out of Hardy Weinberg equilibrium. Loci were known not to be linked (Fox et al. 2007).

Evidence of locus stutter, which was attributed to a possible single base mutation but not further verified, was detected by Micro-Checker. No loci showed evidence of large allele drop-out. Several loci showed a homozygous excess. However it was not possible to discriminate between normal population processes and the presence of null alleles. We have assumed the former in our analyses.

Table 2 presents allele frequency statistics by locality. The mean number of alleles per population (across all loci) ranged from 10.0 to 16.2, except for three localities (PNG, MA and TY), two of which had small sample sizes, where the mean number of alleles per population ranged from 6.7 to 7.5 (Table 2). Mean allelic richness by locality over all loci ranged from

5.7 to 7.2 (Table 2). There was no significant difference across localities, but mean allelic richness was significantly lower, at loci PC36c2, C6 and PC31h4 (data not shown). Overall, mean expected and observed heterozygosity was high (H_e 0.79; H_o 0.78). Across localities, H_e ranged from 0.83 in DT to 0.75 in PNG, while H_o ranged from 0.82 in TY to 0.74 in PNG (Table 2). Weir and Cockerham's f (F_{IS}) by locality and locus indicated that some populations exhibit excess heterozygosity or a deficit at some loci, although no single population or locus consistently deviated from zero. Averaged over all localities and loci (0.051) f was low and not significant.

Table 2. Summary of mean standard allele frequency statistics by locality. Na = mean number of alleles across loci; Ne = effective number of alleles; Ho = observed heterozygosity; He = expected heterozygosity; A. Rich = allelic richness across loci. Refer to Figure 1 for locality codes.

	n	Na	Ne	Ho	He	A. Rich.
PNG	15	7.5	4.6	0.74	0.75	5.74
IR	20	10.0	6.3	0.79	0.79	6.91
CN	25	10.3	6.0	0.81	0.81	6.31
DT	25	11.5	7.4	0.78	0.83	7.21
GV	26	11.0	6.6	0.78	0.82	6.81
MA	9	6.7	4.5	0.80	0.75	6.02
MC	35	10.8	6.7	0.78	0.82	6.65
PR	172	14.0	6.6	0.78	0.82	6.59
TS	319	16.2	6.8	0.78	0.82	6.61
TY	9	7.5	5.1	0.82	0.77	6.68
WR	57	12.3	6.2	0.76	0.81	6.48

Genetic differentiation

When the three regions (PNG, IR, Wet Tropics) were compared, AMOVA generated a low but significant F_{ST} value of 0.041 (p=0.001) (Table 3), with most variability found among individuals within populations (96%). Four percent of the total variation occurred among regions (p=0.001). Using R_{ST} values, a larger proportion (17%) and a significant amount of the variability was found among regions (p=0.01). Neither F_{ST} nor R_{ST} pair-wise comparisons of populations were significant after Bonferroni correction, but many were significant before this correction was applied (Table 4). It is worth noting that all between-locality R_{ST} comparisons, including IR, are high, supporting and possibly driving the greater regional R_{ST} result. In the PCA, 90% of the variation could be explained by the first axis and a further 6% was described by the second axis (Figure 2). The PCA highlights the close relationship between localities within the Wet Tropics region, and a distinct difference between Iron Range and the Wet Tropics. The PNG samples are separated from all other localities, mainly on the second axis.

calculated using Fisher's method of combining probabilities across independent tests, revealed 20 locality pairs (out of 55 possible comparisons) as significantly different (before correction for multiple tests), with the majority of those 20 pairs including either PNG or IR. Once the level of significance was adjusted, seven pairs remained significant, six containing PNG and one of the Wet Tropics populations, the last pair containing IR and the Cairns (Wet Tropics) population.

The number of migrants per generation (Nm), estimated using the conservative method of private alleles, was low between the Wet Tropics and PNG (Nm = 1.5), greater between IR and PNG (Nm = 2.4), and greater again between IR and the Wet Tropics (Nm = 3.9). These figures suggest low levels of movement between regions. A relatively high gene flow was inferred

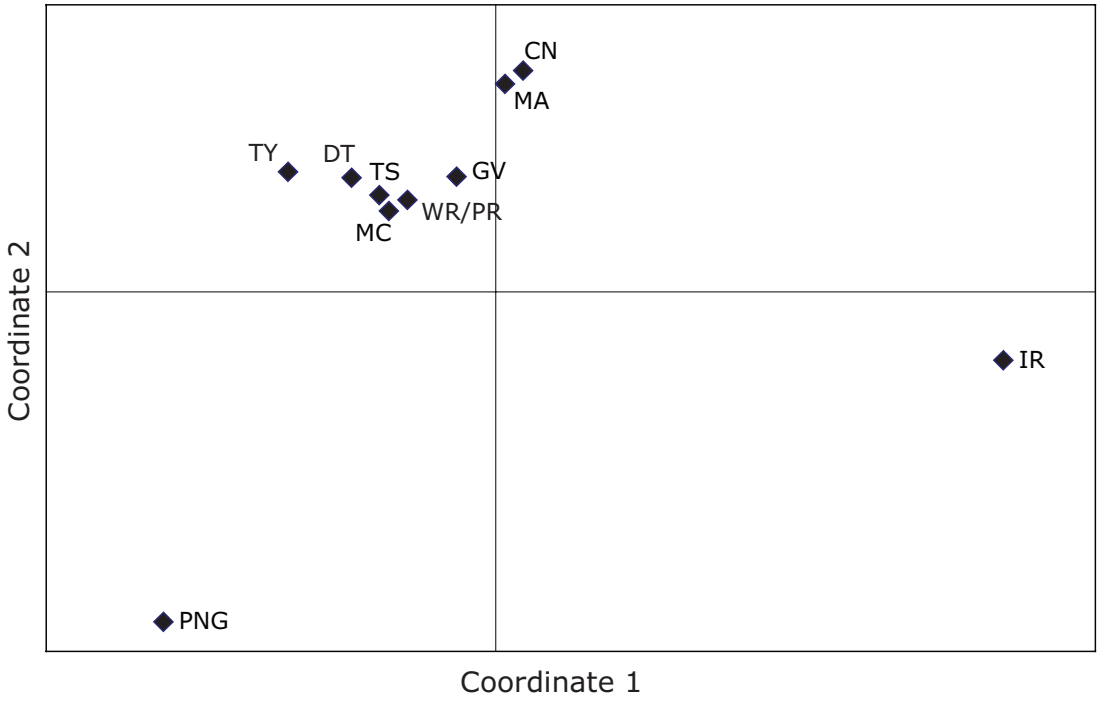

Figure 2. Two-dimensional plot of principal co-ordinates analysis (PCA) based on population pairwise Fst values for sampled locations of *Pteropus conspicillatus*, using a standardised distance analysis. The first two axes explain 96% of the variation of the data set (axis 1 = 90% and axis 2 = 6%). Refer to Figure 1 for locality codes. Note that points for PR and WR are superimposed.

Table 3. Amova estimates of genetic variation among three regions (WT, IR and PNG), among localities and among individuals in the spectacled flying fox.

Source of variation	d.f.	Sum of squares	Variance	Fixation indices	P value	Percentage of variation
F-statistics						
Among regions	2	19.57	0.106	0.041	0.001	4.0
Among populations within regions[1]	8	27.66	0.008	0.003	0.001	0.0
Among individuals within pops	1423	3558.94	2.501	0.044	0.001	96.0
R-statistics						
Among regions	2	3986.94	27.92	0.170	0.01	17.0
Among populations within regions[1]	8	1755.59	0.73	0.006	0.04	0.0
Among individuals within pops	1423	187176.63	131.54	0.18	0.01	82.0

[1] Note that regions PNG and IR are each assumed to consist of one population for the purposes of this analysis.

Table 4. Pairwise FST values (below diagonal) and RST (above diagonal). None was significant after Bonferroni correction for multiple comparisons (Dunn Sidak method – [Sokal and Rohlf 1995]). * significant before Bonferroni correction. Pairwise RST values with IR as one of the localities are shown in bold. Refer to Figure 1 for locality codes.

Pop	PNG	IR	CN	DT	GV	MA	MC	PR	TS	TY	WR
PNG		0.366*	0.086*	0.010	0.050*	0.156*	0.085*	0.036*	0.006	0.113*	0.044*
IR	0.090*		0.152*	0.340*	0.222*	0.112*	0.332*	0.290*	0.219*	0.287*	0.256*
CN	0.056*	0.042*		0.069*	0.006	0.000	0.065*	0.020*	0.017	0.062*	0.014
DT	0.033*	0.062*	0.012*		0.047*	0.107*	0.041*	0.020*	0.005	0.079*	0.027*
GV	0.036*	0.041*	0.005	0.009		0.016	0.029*	0.004	0.002	0.008	0.000
MA	0.054*	0.043*	0.021*	0.015	0.011		0.075*	0.015	0.004	0.074	0.019
MC	0.027*	0.053*	0.012*	0.007	0.000	0.010		0.013*	0.007	0.000	0.033
PR	0.029*	0.049*	0.012*	0.008*	0.003	0.005	0.001		0.000	0.014	0.000
TS	0.028*	0.054*	0.012*	0.004	0.005	0.006	0.001	0.001		0.000	0.001
TY	0.031*	0.071*	0.018*	0.012	0.000	0.013	0.000	0.000	0.003		0.034
WR	0.029*	0.047*	0.010*	0.005	0.001	0.006	0.002	0.000	0.001	0.004	

among localities within the Wet Tropics region (Nm=15.7).

A significant IBD effect was found throughout the range of *P. conspicillatus* (R^2=0.47, p=0.003) (Figure 3a). To ensure that this pattern was not solely created by differences between Australia and PNG, the data from PNG samples were removed and the test repeated using only Australian samples. A non-significant result was obtained R^2=0.17, p=0.10) (Figure 3b), indicating that there is weak differentiation between the Wet Tropics localities and Iron Range. STRUCTURE results (not shown) did not support the occurrence of multiple populations, as all individuals shared proportional assignment to multiple populations when K>1.

Statistical analysis of allele frequencies using the program BOTTLENECK indicated that the majority of localities conformed to the Infinite Alleles Model (IAM). A significant excess of heterozygotes relative to that expected under mutation-drift equilibrium was exhibited by TS, PR and WR. A significant result under the IAM for the sign test and the one-tailed Wilcoxon test was detected in PR and WR (Table 5). One locality, PR, was only significant for the one-tailed Wilcoxon test under the IAM model. When combined, all Wet Tropics populations showed a significant result for a bottleneck under the Wilcoxon test.

Table 5. Significance values (*p*-values) for the sign and Wilcoxon's tests for a heterozygosity excess relative to that expected under mutation-drift equilibrium in BOTTLENECK. * Marginally significant (p = 0.05). **Significant (p <0.05) *** Highly significant (p <0.01). Refer to Figure 1 for locality codes. Shaded locality codes are from the Wet Tropics region.

Locality	Sign test	Wilcoxon test (One-tailed for H excess)
PNG	0.204	0.945
IR	0.502	0.922
CN	0.203	0.922
DT	0.196	0.922
GV	0.209	0.922
MA	0.514	0.578
MC	0.481	0.656
PR	0.047*	0.008***
TS	0.249	0.023**
TY	0.412	0.945
WR	0.244	0.039**
All Wet Tropics	0.229	0.016**

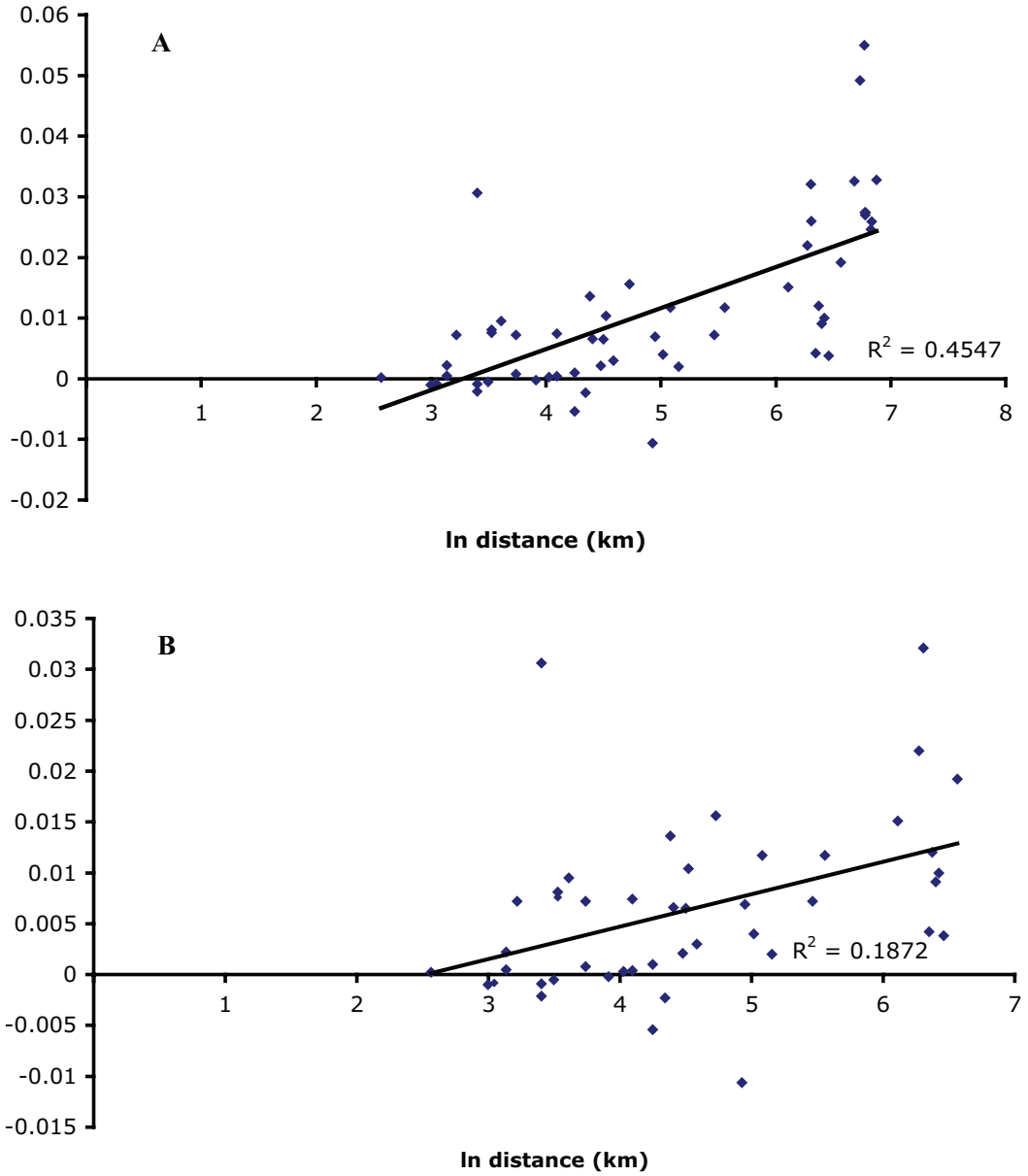

Figure 3. Relationship between genetic distance, calculated using $F_{ST}/(1-F_{ST})$, and the natural logarithm of geographical distance (km). **A.** Test of isolation-by-distance between localities throughout the entire distribution of the spectacled flying fox (*Pteropus conspicillatus*) ($R^2 = 0.47$, $p = 0.003$) (including two separate PNG sampling locations). **B.** Test of isolation-by-distance for colonies of spectacled flying foxes within Australia ($R^2 = 0.17$, $p = 0.104$).

Discussion

In the Wet Tropics region, the spectacled flying fox is panmictic, with no impediments to gene flow detected among colonies throughout the region. Little genetic differentiation was observed between Iron Range and the Wet Tropics region. At the very broadest scale, there is weak genetic structuring across the range of the species. Bats in PNG differ from those in Australia, reflecting the effects of IBD across spatial and topographic barriers, but differences are slight, suggestive either of continuing but rare exchange of individuals between the regions or of relatively recent fragmentation of a previously continuous range.

The Wet Tropics World Heritage Area is repository for the greatest genetic diversity in the spectacled flying fox, suggesting that colonies in this area are part of a large, long-established metapopulation. This finding contrasts with suggestions that the species is a relatively recent

immigrant from PNG or southeast Asia (McKean 1970; Schodde and Calaby 1972). Our conclusion of panmixia is consistent with radio and satellite tracking of individual bats moving throughout the Wet Tropics region (Shilton pers. comm.) and suggests that these movements may underlie the high rate of gene flow within this region. No bats have yet been detected moving between the Wet Tropics and Iron Range, but the low genetic differentiation between these regions suggests either that occasional reproductively effective movements occur, or that there have been few generations since colonisation and isolation of IR.

Application of Bottleneck analysis to samples from the Wet Tropics region suggests a marked reduction in spectacled flying fox numbers at some time in the past. The effect is discernable when Bottleneck is applied individually to the largest sample groups (PR, TS and WR) and when all Wet Tropics samples are pooled. Sample sizes from PNG and IR were inadequate for a meaningful test. The timing of the bottleneck in the Wet Tropics cannot be determined as we are unable to estimate effective population size. However, palaeoenvironmental reconstructions (Hopkins et al. 1993; Graham et al. 2006; Hilbert et al. 2007; Kershaw et al. 2007; VanDerWal et al. 2009) show that rainforest in the Wet Tropics Region was severely reduced through the last glacial cycle, and recovered to approach its pre-European extent in the early Holocene. Habitat contraction provides a potential cause for the decreased populations suggested by Bottleneck. Taken at face value, the genetic data appear to indicate that habitat reductions, and the climate changes that caused them, must have been modest rather than severe in amplitude and that a substantial amount of suitable habitat persisted through the Last Glacial Maximum. This interpretation is at variance with palaeoenvironmental reconstructions of LGM climate and vegetation and illustrates the need for multi-disciplinary perspectives on interpretation of the past, no matter what proxies are employed.

The effect of the unavoidably uneven sampling effort among localities limits the extent to which some relationships can be analysed and conclusions drawn. In particular, interpretations of weak differences in genetic structure at the broadest sampling scale should be made cautiously. The geographical gap between PNG and Australian bats is reflected in a measurable IBD effect. However, analyses in Structure did not differentiate between regions but instead suggested that all *P. conspicillatus* samples, including those from PNG and IR, came from a single genetic population. Small sample sizes from some localities (Waples and Gaggiotti 2006) and the frequency of common alleles (data not shown) among the PNG samples would have reduced the ability of Structure to resolve what may be subtle differences in population genetic structure. Thus, population similarity between PNG and Australia might reflect a type II error rather than a meaningful biological finding, and further sampling in PNG and elsewhere is needed to confirm these results.

The weak genetic distinction between bats in Australia and PNG suggests that there is at least some interchange between regions. Estimated numbers of migrants between Australia (especially the Wet Tropics) and PNG are low. Although one migrant per generation is theoretically sufficient to offset genetic drift between populations (Mills and Allendorf 1996; Whitlock and McCauley 1999), in 'real world' scenarios, especially where population sizes fluctuate (Vucetich and Waite 2000), 10-20 migrants per generation may be needed to slow drift-induced divergence. Movement of spectacled flying foxes between Australia and PNG has not yet been recorded but, based on the apparent interchange between the Wet Tropics and Iron Range, the distance is not insurmountable. Return flights do occur to PNG by the black flying fox (*Pteropus alecto*), an apparent habitat generalist, via Torres Strait (Breed et al. 2010). The limited genetic differentiation between Australia and PNG would be consistent with occasional cross-Torres Strait flights of spectacled flying foxes. It would also be consistent with a geologically recent disjunction between Australian and PNG bats. A much more intensive sampling effort in PNG will be required to draw further conclusions with confidence.

In Australia, weak inter-regional differentiation is readily understandable in the light of the capacity of the spectacled flying fox for strong and sustained flight and its apparent plasticity of habitat use at times of environmental stress. Although the spectacled flying fox rarely roosts more than 6 km from wet tropical rainforest, and has long been assumed to feed primarily on rainforest species (Richards 1990a, b), in reality individuals regularly feed on a wide variety of non-rainforest species, including eucalypts (*Eucalyptus* spp., *Corymbia* spp.) in tall open forests adjoining rainforest communities and in tropical woodland and savanna ecosystems (Parsons 2005; Parsons et al. 2006). When circumstances require it, the species is able to cross, or survive within, substantial tracts of sclerophyllous vegetation, as illustrated by the dispersal response elicited by severe tropical cyclone Larry (Shilton et al. 2008). This event occurred in March 2006, caused massive damage to rainforest across a broad swathe of the Wet Tropics, and was followed by near total evacuation of known haunts of the spectacled flying fox in the region (Shilton et al. 2008). A year after the cyclone, the majority of animals had returned to the Wet Tropics from wherever it was that they had found refuge.

Such catastrophic events might be the trigger for colonisation of distant patches of suitable habitat, such as IR. There is no evidence that the Wet Tropics animals moved *en masse* to IR after Cyclone Larry, as no field surveys were conducted. However, any such dispersal could lead to levels of gene flow between Australian regions, and conceivably beyond, sufficient to offset divergence through genetic drift. Iron Range and PNG are themselves prone to natural disasters (cyclones, perhaps also volcanic eruptions in PNG) which could disperse populations. Given that severe tropical cyclones are frequent on a micro-evolutionary time-scale, these could be potent drivers of dispersal and population mixing in a volant species such as the spectacled flying fox. Fleeing a disaster is not an option open to an overwhelming majority of rainforest inhabitants of the Wet Tropics region: localised extinction is a more likely event (Schneider et al. 1998).

We have established that there is substantial gene flow between colonies in the Wet Tropics, but some, albeit rather weak, genetic structure when the three regions are considered (Wet Tropics, IR and PNG). Does current gene flow between widely separated habitats provide a model for survival of the species during glacial periods? It appears that under present-day conditions, the spectacled flying fox is able to maintain functional gene flow across distances of several hundred kilometres of apparently unsuitable habitat. When considered against the modelled extent of potential Pleistocene habitat in Australia (VanDerWal et al. 2009), we conclude that the mobility of this species, in combination with its habitat plasticity, evident under both stressed and normal circumstances, would allow gene flow to occur reasonably freely throughout the region and between isolated rainforest patches at that time.

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