

JCU ePrints

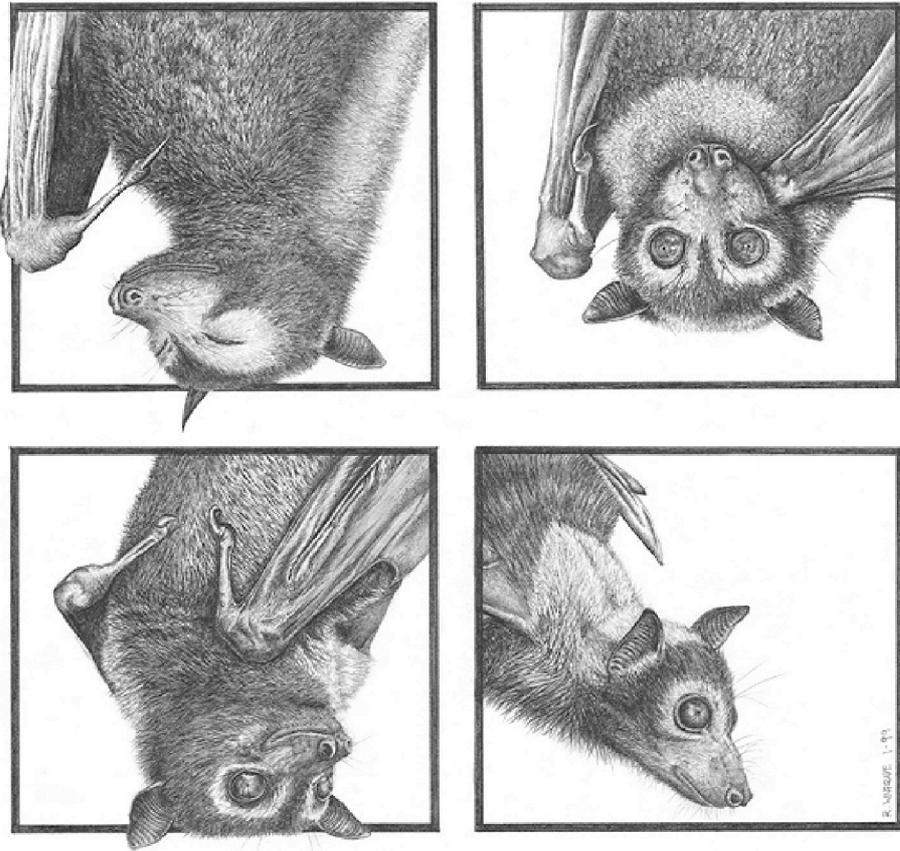
This file is part of the following reference:

Fox, Samantha (2006) *Population structure in the spectacled flying fox, *Pteropus conspicillatus* : a study of genetic and demographic factors.* PhD thesis, James Cook University.

Access to this file is available from:

<http://eprints.jcu.edu.au/8053>

Population structure in the spectacled flying fox,
Pteropus conspicillatus:
A study of genetic and demographic factors



Artist Robin Wingrave. Used with permission

Thesis submitted by
Samantha J Fox B. Sc. Hons. (JCU)
January 2006
For the degree of Doctor of Philosophy
In the Schools of Tropical Biology, and Tropical Environment Studies and
Geography, James Cook University, Townsville

In nature's infinite book of secrecy

A little I am read

- William Shakespeare, 'Anthony and Cleopatra'

Statement of sources

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any University or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.

(Signature)

(Date)

Electronic copy

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to James Cook University, is an accurate copy of the print thesis submitted, within the limits of the technology available.

(Signature)

(Date)

Statement of access

I, the undersigned, author of this work, understand that James Cook University, of North Queensland will make this thesis available for use within the University library and, via the Australian Digital Theses network, for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act.

I wish this work to be embargoed until 28 June 2007, after which I do not wish to place any further restriction on access to this work.

(Signature)

(Date)

Statement of the contribution of others

Funding

This project was funded by an ARC linkage grant with Industry partners Queensland Parks and Wildlife Service, Queensland Fruit and Vegetable Growers Association, and the Tolga Bat Rescue and Research. Funding was also received from the Rainforest Co-operative Research Centre (CRC), Bat Conservation International (BCI), and from the Queensland Government under their Growing the Smart State PhD initiative.

Stipend Support

I was supported by an APA(I) scholarship, as well as a completion scholarship from James Cook University.

Supervision

Associate Professor David Blair, Dr. Michelle Waycott, Dr. Jon Luly and Dr. David Westcott provided supervision, editorial assistance and statistical support.

Sample collection

Assistance with sample collection was received from the following people; Jenny Maclean, Hume Field, Scott Sullivan, Adam McKeown, Pam Tully, Hugh Spencer, Robin Wingrave, Barbara Mazlin, Heather, Annabelle Olsen, Olivia Whybird, Andrew Mack, Dave Wilson, Cynthia and Pius Sabag, Tim Kerlin, Louise Shilton, Greg Richards, Darryl Dickson, Alan Curtis, Peter Mansfield. Samples were also obtained from the Australian Museum, and the South Australian Museum.

Lab Help

Sue Reilly and Caitlin Mitchel carried out histological processing of teeth. Chrystal Mantyka provided help with DNA extractions. Glenn Dunshea and Michelle Waycott provided assistance with microsatellite loci isolation. Kevin Olival and Andrew Breed supplied Mitochondrial DNA primers.

Maps

Help with creating maps was provided by Adam McKeown and Ainsley Calladine

Editorial Assistance

Tim Harvey, Carolyn Smith-Keune, Dean Jerry and Michelle Boyle provided proof reading and editing.

Acknowledgments

I am an exceptionally lucky person!! I have received so much love, friendship and support during my PhD, that it has been an enriching and rewarding experience. I hope most people can think that at the end of their journey. My journey has taught me a valuable lesson about myself—that I find interaction with people incredibly satisfying and enjoyable. I have never had any doubt that the majority of interactions I am going to have with animals are going to be rewarding, but for me to gain so much from interactions with people, many of whom were new to me at the beginning of my journey, has been a revelation. So thank you to all the various souls that I met along my path, who enriched my life and my PhD experience.

And so on to the more usual form that this section of the PhD takes—thanking all the people who have made my PhD experience easier, memorable and in some cases, possible. Thanks first and foremost to my supervisors, David Blair, Michelle Waycott, Jon Luly and David Westcott. Many people frowned at my “Panel” of supervisors but I have been very lucky in that between them they have managed to cover the majority of skills and knowledge base that a PhD student comes to rely on at certain stages in their tenure. With today's academics also being so insanely busy, having a few supervisors means that you can usually find one that can spare you some time when you absolutely need it! Thanks to David for his incessant “Keep smiling!” And thanks especially to Michelle for being so incredibly patient with me when I required something from her NOW!!! And a big thanks to David and Michelle for working through final drafts over Christmas!

Thanks to my industry partners for their contributions to the project and to all my funding bodies, especially the Rainforest CRC, for supporting me over the past 4 years.

I have been lucky to meet and work with people from both the CSIRO in Atherton and QPWS and count those people as friends as well as colleagues.

Adam McKeown has to be the most enthusiastic field person I have come across, to watch nets for “incoming” bats until 2am and then go out spotlighting for more! He has the most infectious smile that no matter how tired you are never fails to lighten your mood. Thanks heaps for your help Adam—and for sitting in the back of the troopy for the majority of the time!

Scott Sullivan—what can I say about you? I have never had anyone laugh so hard at my expense before, but laugh so long and so hard that I ended up with tears pouring down my face from laughing so hard too! You are the ‘ultimate’ field person and I can’t imagine ever going in the field again without wishing you were with me. Thanks for both Iron Range trips and for looking out for me when you thought I needed it. As I think I may have remarked before—you are a treasure!!

Thanks Peter Latch for forever being my “Mentor”—I will always look up to you! I have so enjoyed our little ‘chats’ that always turned out to be so informative at the same time. You have always had an ear for me to bend and I have appreciated that with your busy schedule. I will always have enormous respect for the enthusiasm you maintain in your position given the restrictions you are constantly under from bureaucracy.

A ton of thanks and love to Jenny and Claire at the bat hospital for all the ‘experiences’ we have had together—they have been truly unforgettable. Going through a tick paralysis and baby bat season at the bat hospital will certainly make you appreciate the unsung heroes in the world of wildlife carers and admire their dedication in going weeks without a break for the love of an animal. Thanks for being a grumpy bum with me Claire after many sleepless nights together!

Many thanks go to all the wildlife carers and ‘batty’ people who collected ‘bits’ of bat for me at various times. Thanks especially to Pam Tully and Hugh Spencer, two more inspiring people in their constant dedication to a cause!

Doesn’t ‘thanks’ become an awfully repetitive word at this point?? Need a new one to replace it with. Much appreciation (nah, boring!) goes to Andrew Dennis

and Louise Shilton at CSIRO for editing of written work and interesting conversations re-everything batty.

There are many people to thank in the genetics lab as along the way many people have added their input especially when I went through hell and back when nothing would work (but then who doesn't go through hell in the lab at some stage?). Thanks to Jamie for being so concerned and fielding enquiries from other concerned lab-mates, to Selma for advice and trinkets from Taiwan, to Brad for constantly topping up my 10x buffer supplies and everybody else for just making time in the lab bearable. Very special thanks go to Glenn for being my 'bantering' partner in the lab, and someone I still miss when I am in there. And a huge thanks to Carolyn Smith-Keune, who really told me what it was all about when she said, "you don't learn anything in genetics unless it goes wrong". There was an element of truth in that, no matter how unhappy I was about it! Thanks so much for all your help Carolyn, you have been an angel!

Special thanks to my pseudo-supervisor, Ainsley, for everything—not only for all the times he fixed my computer and got the printer working, or showed me how to use Illustrator, helped me create maps, explained genetics to me in baby language, and put up with my constant nagging about when my genotyping would be done, but for always being able to do it with a smile—most of the time! You deserve a medal my friend!

Tons of thanks to Andy Mack who persevered in getting samples for me from PNG when I had no other way of getting them, and for bolstering my flagging motivation during times of trouble. To Andy Lowe for advice on genetics analysis, to Sue Reilly and Caitlin Mitchel for histology help, and Chrystal Mantyka for help in the lab.

Thanks to Hume Field for organising our combined field trip to Iron Range and making it such a memorable experience with all the 'extra hands' that came along.

Thanks heaps to Line Bay for helping me with some of the statistics packages, and Ross Crozier for discussion on Relatedness.

And so to all the people who gave me a different type of support—emotional support. Thanks to Michelle Boyle for being an understanding office mate when I have had people traipsing in and out of our office and for turning a blind eye when I just wanted to cry quietly at my desk. But also for pushing so hard to get our new office just at the right time when we really needed it. It's been wonderful to have you there from the word 'GO' to weather all the highs and lows with, to laugh at our mutual homeless status with, and to head for the finish line with.

Damn! Thought I would get it into 2 pages! Thanks to Will R. for helping me to understand life tables and for lots of fabulous hugs when I really needed them.

Thanks to Leonie Valentine, Basil Byrne, Jen Martin and Euan Ritchie for Friday night bludgers, for lots of positive thoughts and pep talks when the going got tough. Thanks to Rob Gegg for always being there when I was going through a rough patch and giving me a shoulder to cry on and a sickly sweet caramel slice that would choke those not hardy enough!! And of course, thanks and love to Yvette Williams for always having a smile even though the problems in her life made mine look like piffle! Thanks for all the hand holding through my many relationship woes, nursing me after a hospital visit and for all the dinners when I couldn't be bothered (or couldn't afford!!) to cook for myself. You deserve more gorgeous!

Almost lastly, I want to thank Miki for putting up with me coming home late, not giving her as many walks as she would like or as much attention as she is used to and for always welcoming me home with a huge smile and a howl! My love and thanks to my parents for all of their encouraging words from the other side of the world and being understanding to my time limitations for communication. I can't wait for you guys to be here and to have a normal relationship with you again!!

And finally, and most importantly, I want to thank Tim.

Abstract

The spectacled flying fox (*Pteropus conspicillatus*) is a difficult species to manage due to its dual status as an agricultural pest and a native species under threat. Like other flying fox species, the spectacled flying fox is very mobile, roosts in colonies during the day in largely inaccessible places, and is active at night. These factors make the spectacled flying fox a difficult species to study and are in part responsible for the lack of knowledge on the biology and ecology of this species. The population structure of the spectacled flying fox was examined using two genetic markers (highly polymorphic microsatellites, and a portion of the mtDNA D-loop) and cementum layers around the root of canine teeth to determine age structure. Incorporating both genetic and demographic factors, as well as examining population structure across several temporal and spatial scales, provided a more comprehensive understanding of this species.

A new hypothesis for the origin and evolution of flying foxes in Australia is presented: that flying foxes are an old lineage in Australia and that they colonised PNG from Australia rather than the other way around. The spectacled flying fox has experienced a tumultuous history, including population expansion and contraction as a result of climatic and geographic events. These events have aided in shaping the contemporary structure of highly connected colonies within a single panmictic population in the Wet Tropics region, along with an isolated population at Iron Range and populations of unknown status in Papua New Guinea. High allelic and haplotypic diversity suggest an old lineage within Australia, and the patterns of diversity suggest colonisation from Australia to PNG. Introgression between black flying foxes and spectacled flying foxes suggests a close association of these two species. The possibility of incomplete lineage sorting also suggests that *Pteropus alecto* and *Pteropus conspicillatus* might still be in the process of diverging. *Pteropus poliocephalus* might also belong to such a species complex, although no relevant data are yet available. Although high levels of gene flow occur among colonies within the Wet Tropics region, some sub-structuring in the form of kin groups within colonies is indicated, with several cohorts of

young remaining with their mothers before the young reach sexual maturity. High rates of mortality and low reproductive output may be putting this species at risk of decline, especially as their average longevity is considerably less than expected.

This study highlights the need for spectacled flying foxes to be managed on a regional scale. In addition, mortality rates need to be investigated throughout the Wet Tropics to determine the applicability of mortality rates estimated in this study, across the range of the species.

Table of contents

Statement of sources	iii
Electronic copy	iv
Statement of access	v
Statement of the contribution of others	vi
Acknowledgments	viii
Abstract	xii
Table of contents	xiv
List of tables	xvii
List of figures	xviii
Chapter One – studying population structure in the spectacled flying fox	1
Megachiroptera	2
Australian flying foxes	4
The spectacled flying fox— <i>Pteropus conspicillatus</i> Gould, 1850	5
Understanding population structure in <i>Pteropus conspicillatus</i>	11
Coloniality in flying foxes.....	11
Definition of a ‘Population’	14
Studying colony/population structure	14
Demographic factors	16
Genetic factors	18
Mitochondrial DNA.....	19
Microsatellites.....	20
Population structure, conservation and management	22
Impetus for this study and aims of the thesis.....	22
Chapter Two – Isolation and characterisation of polymorphic microsatellite markers in the spectacled flying fox	24
Chapter Three – Phylogeography, historical population expansion and introgression inferred from mtDNA	28
<i>Introduction</i>	28
<i>Methods</i>	32
Laboratory protocols	32
Data analysis.....	34
<i>Results</i>	35
Sequence analysis.....	35

Phylogenetic analysis	36
Molecular diversity	36
Population genetic structure	37
Demographic analyses	39
<i>Discussion</i>	41
The phylogeographic structure of spectacled flying foxes	41
Introgression between <i>P. alecto</i> and <i>P. conspicillatus</i>	41
Support for an historical population expansion.....	44
Time since expansion	46
<i>Conclusion</i>	48
Chapter Four – Genetic diversity across the range of the spectacled flying fox	50
<i>Introduction</i>	50
<i>Methods</i>	54
Sample collection	54
DNA extraction and amplification.....	54
Data analysis.....	56
<i>Results</i>	59
Genetic diversity, Hardy-Weinberg and linkage disequilibrium.....	59
Genetic differentiation	62
<i>Discussion</i>	67
High levels of genetic diversity	67
No population differentiation due to high gene flow	68
Population bottleneck in the Wet Tropics	71
<i>Conclusion</i>	75
Chapter Five – kin group sub-structuring found within spectacled flying fox colonies	76
<i>Introduction</i>	76
<i>Methods</i>	79
Data analysis.....	80
<i>Results</i>	82
<i>Discussion</i>	88
Broad-scale view of relatedness across populations of the spectacled flying fox.....	88
Low average colony relatedness not indicative of colony social structure.....	89
Pairwise relatedness indicates possible kin structuring in the Powley Rd colony.....	90
No evidence of inbreeding in the spectacled flying fox.....	93
<i>Conclusion</i>	93

Chapter Six – Life history data reveal negative growth and short life-span in the spectacled flying fox.....	95
<i>Introduction.....</i>	<i>95</i>
<i>Methods.....</i>	<i>98</i>
Data collection.....	98
Teeth.....	98
Tooth preparation and sectioning.....	99
Counting cementum layers.....	100
Analysis.....	100
Life tables.....	101
<i>Results.....</i>	<i>103</i>
Validation of aging technique.....	103
Age structure and demographic parameters.....	104
Life history tables.....	105
<i>Discussion.....</i>	<i>106</i>
Age at first reproduction.....	108
Longevity shorter than previously thought.....	110
Reproductive rate.....	110
High mortality rate in the year studied.....	111
Negative population growth rate.....	112
<i>Conclusion.....</i>	<i>113</i>
Chapter Seven – genetic and demographic insights into the past, present and future of the spectacled flying fox.....	115
The Past.....	115
The origin of flying foxes in Australia.....	115
The fossil record.....	116
Restricted distribution of the spectacled flying fox.....	117
Low species diversity of Megachiroptera in Australia.....	118
Proposed origin of spectacled flying foxes in Australia.....	118
Introgression/incomplete lineage sorting.....	120
Vicariance and dispersal.....	121
The Present.....	122
Broad scale.....	122
Local scale.....	124
Future.....	126
Conservation and management of the spectacled flying fox.....	126
Future research.....	127
Conclusion.....	129
References.....	130
Appendices.....	158

List of tables

Table 2.1 Attributes of eight microsatellites developed for <i>Pteropus conspicillatus</i> . Ta refers to final annealing temperature. N is the number of samples scored for each locus. Ho & He are the observed and expected heterozygosity calculated by GenAlEx v6.0 (Peakall and Smouse 2005).....	27
Table 2.2 Attributes of six polymorphic microsatellite loci designed for <i>Pteropus</i> species by G. McCracken but tested against individuals of <i>P. conspicillatus</i>	27
Table 2.3 Volumes of reagents used for each primer in the PCR reaction	27
Table 3.1 Molecular diversity indices for all colonies. n = number of individuals sequenced, n_h = number of haplotypes, h = haplotype diversity, π = nucleotide diversity.....	37
Table 3.2 Pairwise F_{ST} values between colonies (lower diagonal) and their correspondent significance values (upper diagonal).....	37
Table 4.1 Private or rare alleles found in each population by locus. Calculated in GENEPOP v3.4 (Raymond and Rousset 1995). n = sample size for each population.	59
Table 4.2 Summary table of average standard allele frequency statistics by population. Na = number of alleles; Ne = effective number of alleles; Ho = observed heterozygosity; He = expected heterozygosity; A. Rich = allelic richness.....	60
Table 4.3 F_{IS} by population (rows) and locus (columns) following Weir and Cockerham (1984). * denotes significant F_{IS} values according to bootstrapping over loci (95% CI)	61
Table 4.4 Pairwise population F_{ST} values (below diagonal) and pairwise population R_{ST} (above diagonal). None were significant after Bonferroni correction for multiple comparisons (Dunn Sidak method - (Sokal and Rohlf 1995)). Population codes: PNG, Papua New Guinea; IR, Iron Range; CN, Cairns; DT, Daintree; GV, Gordonvale; MA, Mareeba; MC, Mena Creek; PR, Powley Rd; TS, Tolga Scrub; TY, Tully; WR, Whiteing Rd.....	65
Table 4.5 Sign and Wilcoxon results from BOTTLENECK. ^a populations conform to the SMM. ^b populations conform to the IAM. ^{a+b} BOTTLENECK retained both models for the population. * Marginally significant (p=0.05). **Significant (p<0.05) *** Highly significant (p<0.01).....	65
Table 6.1 Life history table for female <i>Pteropus conspicillatus</i> . Symbols for columns: x , Age in years; l_x , survivorship (mortality was set at 50% for the first year of life and 40% for the remainder; b_x , natality; $l_x b_x$, age-specific reproductive rate; R_0 , net reproductive rate per generation; G , mean generation time; r , intrinsic rate of increase of population.	106

List of figures

- Figure 1.1 Distribution of the Pteropodidae (amended from Hill and Smith (1984)). Horizontal lines indicate position of the equator (middle), Tropic of Cancer (upper) and Tropic of Capricorn (lower). 3
- Figure 1.2 The spectacled flying fox, *Pteropus conspicillatus*. Photo: Bruce Thomson. Used with permission. 7
- Figure 1.3 Distribution of Australia's flying foxes (after Hall and Richards 2000). *Pteropus conspicillatus* (spectacled flying fox), yellow area; *Pteropus alecto* (black flying fox), black area; *Pteropus scapulatus* (little red flying fox) inland limit, red line; *Pteropus poliocephalus* (grey-headed flying fox), grey area. Black and red dots on map of PNG = records of occurrence of black and little red flying foxes respectively in PNG. Arrow indicates approximate position (on land) of Iron Range. Blue line signifies the Wet Tropics World Heritage Area (WTWHA). 8
- Figure 1.4 Known colony locations of *Pteropus conspicillatus* (spectacled flying fox) in the Wet Tropics region, principal area of its range in Australia. Red dots are camps not sampled in this study, purple dots are camps that were sampled. Atherton Tablelands colonies comprise Mareeba, Tolga Scrub, Powley Rd and Whiteing Rd..... 9
- Figure 1.5 Census results for 1998–2005; counts of spectacled flying fox (SFF) colonies in the Wet Tropics region during December. Data from Threatened Species Network, Queensland Parks and Wildlife Service..... 10
- Figure 1.6 Contribution of demographic and genetic factors affecting population structure. 15
- Figure 3.1 Distribution and sampling strategy map for mtDNA phylogeographic analysis. Purple circles = spectacled flying fox colonies. Yellow boxes contain codes for each colony sampled and sample size. Population/colony codes: PNG, Papua New Guinea; IR, Iron Range; DT, Daintree; TS, Tolga Scrub; TY, Tully. 33
- Figure 3.2 Maximum likelihood tree of D-loop mtDNA sequences from *Pteropus conspicillatus* individuals throughout their range, with a closely related congeneric species (*Pteropus alecto*) as the outgroup. Sample names comprise the unique individual identifier followed by the colony from which the sample was collected. Numbers on branches represent percentage bootstrap replicate support. Locality codes: PNG, Papua New Guinea; TSTRAIT, Torres Strait; TVL, Townsville. 38
- Figure 3.3 Mismatch distribution analysis for clade 1 and 2. Clade 1 fits the expected model of population expansion while clade 2 follows a model of stationarity. Blue points are the distribution of the data for Clade 1 and 2, while the pink points are the expected distribution under that particular model (expansion model for Clade 1 and stationarity model for Clade 2) 40

Figure 4.1 Map of the distribution of the spectacled flying fox (<i>Pteropus conspicillatus</i>) including detail of colonies within the Wet Tropics region (sample sizes from each colony in yellow boxes). Population/colony code: PNG, Papua New Guinea; IR, Iron Range; DT, Daintree; CN, Cairns; GV, Gordonvale; MA, Mareeba; TS, Tolga Scrub; PR, Powley Rd; WR, Whiteing Rd; MC, Mena Creek; TY, Tully.....	55
Figure 4.2 a. Mean allelic richness over all six loci by population (+/- SD). b. Mean allelic richness across all 11 populations by locus (+/- SD) Population codes: CN, Cairns; DT, Daintree; GV, Gordonvale; IR, Iron Range; MA, Mareeba; MC, Mena Creek; PNG, Papua New Guinea; PR, Powley Rd; TS, Tolga Scrub; TY, Tully; WR, Whiteing Rd.....	61
Figure 4.3 Microsatellite allele size distribution by locus for each of three regions of <i>Pteropus conspicillatus</i> . Region codes: PNG, Papua New Guinea; IR, Iron Range; WT, Wet Tropics	64
Figure 4.4 Unrooted neighbour-joining tree of pairwise F_{ST} values among populations for multilocus microsatellite genotypes of the spectacled flying fox.....	66
Figure 5.1 Distribution map of the spectacled flying fox showing sampling regime for this study. Broad scale comparisons use all colonies. Colony Codes: PNG, Papua New Guinea; IR, Iron Range; DT, Daintree; CN, Cairns; GV, Gordonvale; MA, Mareeba; TS, Tolga Scrub; PR, Powley Rd; WR, Whiteing Rd; MC, Mena creek; TY, Tully. Detailed analyses only use Tablelands colonies TS, PR and WR.....	80
Figure 5.2 Frequency distribution of all pairwise relatedness values across 11 colonies of the <i>Pteropus conspicillatus</i> (A) within and (B) between colony comparisons, calculated by MARK. All non-related values (zero or negative) are excluded.....	83
Figure 5.3 Frequency distribution of all pairwise relatedness values between individuals within colonies, for all colonies across the range of <i>Pteropus conspicillatus</i> (unrelated values removed). Calculated using the SSR relatedness calculated using MARK (Ritland 2004).....	84
Figure 5.4 Population-by-population average pairwise relatedness for all within- and between-population pairwise individual relatedness comparisons (D2 estimates from MARK – (Ritland and Travis 2004)).....	85
Figure 5.5 UPGMA tree of all individuals (n=172) sampled from PR colony, clustered relative to their pairwise relatedness values. Highlighted box indicates the sub-group investigated in more detail (see Fig. 5.6). Numbers at the end of each branch indicate the unique identifier for each individual.....	86
Figure 5.6 Sample sub-group from PR colony showing scale of relatedness between individuals (A), with family group highlighted showing age and sex of family members (B). Scale of pairwise relatedness values ≥ 0.50 ; 0.25-0.5; 0-0.25; Not related. Red individuals in (B) are mother/offspring pairs determined both by relatedness values and at the time of collection of samples. (J) = juvenile	87
Figure 6.1 Diagram of tooth sectioning. i. Above gum, exposed tooth, removed before processing. ii. Mid-section of root below gum-line,	

removed to be embedded in wax. iii. Lower apical section of root removed before processing.....	99
Figure 6.2 Photo of cementum layers surrounding root of tooth taken from a spectacled flying fox. There are 6 rings in this photo indicating an individual of 6 years.	101
Figure 6.3 Age distribution for sample population from Tolga Scrub colony on the Atherton Tablelands for 2001-2002 (males and females combined). Young-of-year of collection not included.	104
Figure 6.4 Proportion of females lactating in each age class. Sample sizes for age classes 8 years onward are low (8, n=2; 9, n=4; 11, n=2; 13, n=2).	105
Figure A1. Contents of CD containing appendices.....	158