Extensive genetic differentiation detected within a model marsupial, the tammar wallaby (*Notamacropus eugenii*)

Mark D. B. Eldridge[1,2]*, **Emily J. Miller**[3¤a], **Linda E. Neaves**[1,4], **Kyall R. Zenger**[5], **Catherine A. Herbert**[3¤b]

1 Australian Museum Research Institute, Sydney, New South Wales, Australia, **2** Department of Biological Sciences, Macquarie University, New South Wales, Australia, **3** School of Biological, Earth and Environmental Sciences, University of New South Wales, Kensington, New South Wales, Australia, **4** Royal Botanic Garden Edinburgh, Edinburgh, United Kingdom, **5** College of Science and Engineering and Centre of Sustainable Tropical Fisheries and Aquaculture, James Cook University, Townsville, Queensland, Australia

¤a Current address: Sydney Medical School, University of Sydney, Camperdown, New South Wales, Australia
¤b Current address: Faculty of Veterinary Science, University of Sydney, Camperdown, New South Wales, Australia
* mark.eldridge@austmus.gov.au

Abstract

The tammar wallaby (*Notamacropus eugenii*) is one of the most intensively studied of all macropodids and was the first Australasian marsupial to have its genome sequenced. However, comparatively little is known about genetic diversity and differentiation amongst the morphologically distinct allopatric populations of tammar wallabies found in Western (WA) and South Australia (SA). Here we compare autosomal and Y-linked microsatellite genotypes, as well as sequence data (~600 bp) from the mitochondrial DNA (mtDNA) control region (*CR*) in tammar wallabies from across its distribution. Levels of diversity at autosomal microsatellite loci were typically high in the WA mainland and Kangaroo Island (SA) populations (A = 8.9–10.6; He = 0.77–0.78) but significantly reduced in other endemic island populations (A = 3.8–4.1; He = 0.41–0.48). Autosomal and Y-linked microsatellite loci revealed a pattern of significant differentiation amongst populations, especially between SA and WA. The Kangaroo Island and introduced New Zealand population showed limited differentiation. Multiple divergent mtDNA *CR* haplotypes were identified within both SA and WA populations. The *CR* haplotypes of tammar wallabies from SA and WA show reciprocal monophyly and are highly divergent (14.5%), with levels of sequence divergence more typical of different species. Within WA tammar wallabies, island populations each have unique clusters of highly related *CR* haplotypes and each is most closely related to different WA mainland haplotypes. Y-linked microsatellite haplotypes show a similar pattern of divergence although levels of diversity are lower. In light of these differences, we suggest that two subspecies of tammar wallaby be recognized; *Notamacropus eugenii eugenii* in SA and *N. eugenii derbianus* in WA. The extensive neutral genetic diversity and inter-population differentiation identified within tammar wallabies should further increase the species value and usefulness as a model organism.

Introduction

The study of Australia's unique marsupial fauna, continues to offer valuable insights into multiple fields including evolutionary genetics [1] and conservation biology [2]. The tammar wallaby (*Notamacropus eugenii*) (see [3,4] regarding the change of genus from *Macropus*) is one of the most intensively studied marsupials, and has become a significant model species for reproductive, developmental, physiological, immunological, ecological and genetic research (see [1,5,6–12]. The tammar wallaby was therefore the obvious candidate to be the first Australasian marsupial, and only the second marsupial species, to have its genome sequenced [1,13]. Despite this new found wealth of genomic knowledge, comparatively little is currently known about the distribution and abundance of genetic diversity within and amongst allopatric tammar wallaby populations.

The tammar wallaby is a medium-sized macropodid (4–10 kg) with a naturally disjunct distribution across semi-arid southern Australia (Fig 1) [14]. At the time of European settlement, the tammar wallaby was distributed on the South Australian (SA) mainland and in south-western Western Australia (WA), as well as on five SA and five WA continental islands (Fig 1) [15].
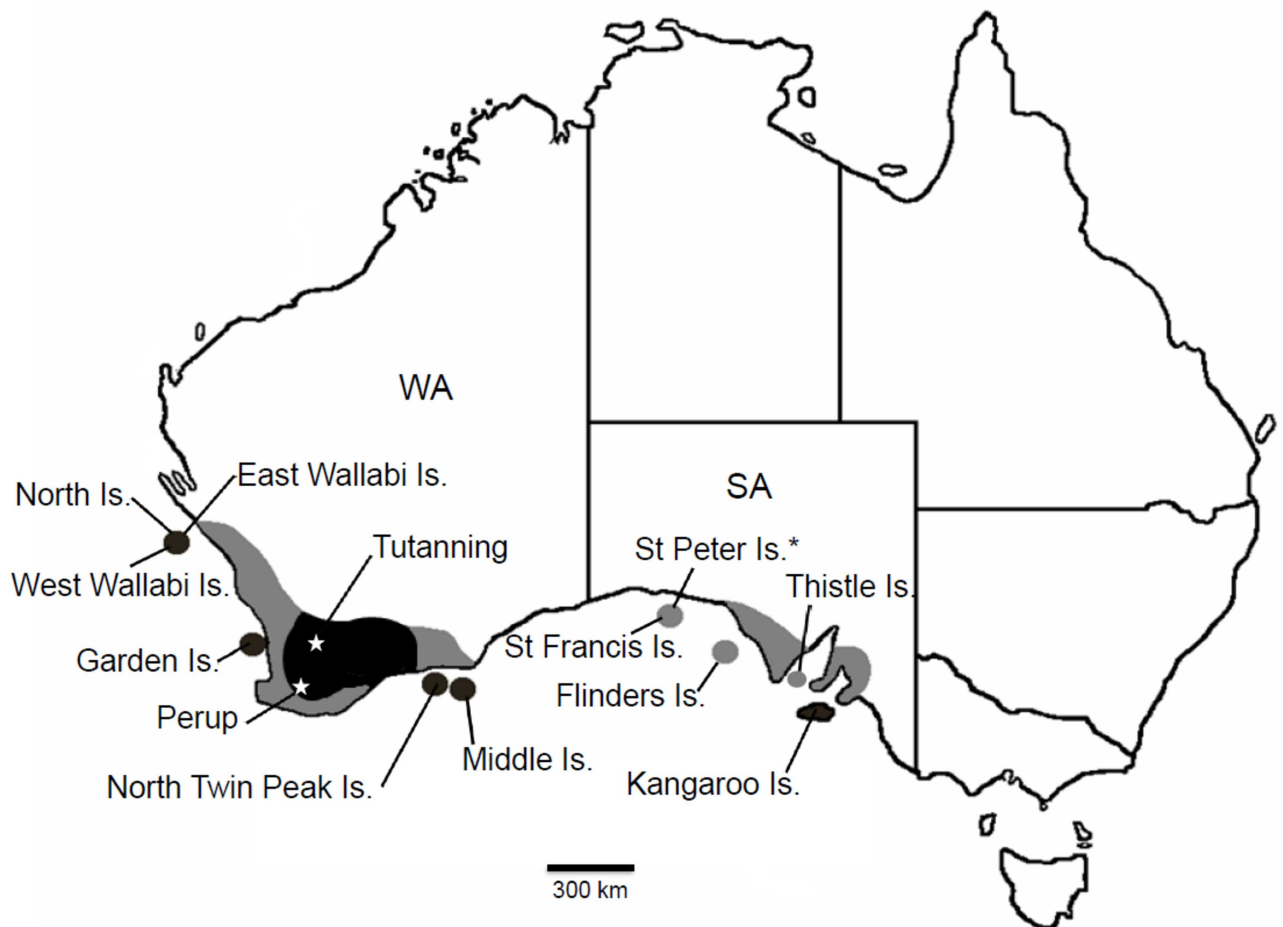
Fig 1. Former and current distribution of the tammar wallaby (*Notamacropus eugenii*) in Southern Australia (after [14]). Collection localities and sites mentioned in the text are indicated. Dark shading represents extant distribution; light shading represents areas where now extinct. SA = South Australia; WA = Western Australia. * = type locality. East Wallabi, West Wallabi and North Islands are part of the Houtman Abrolhos Archipelago; Middle and North Twin Peak Islands are in the Recherché Archipelago; St Francis and St Peter Islands are in the Nuyts Archipelago; Flinders Island is in the Investigator Group.

doi:10.1371/journal.pone.0172777.g001

Table 1. Details of sampled island tammar wallaby (*Notamacropus eugenii*) populations [19–22].

	Island	Separation from mainland (yrs)	Area (ha)
Endemic			
	Kangaroo, SA	9 500	450 000
	Garden, WA	7 000	1 054
	Middle, WA	9 500	1 036
	West Wallabi, WA	11 500	587
	East Wallabi, WA	11 500	307
	North Twin Peak, WA	8 000	272
Introduced			
1983–5	North, WA		176
1870	Kawau, New Zealand		2 058

doi:10.1371/journal.pone.0172777.t001

In SA they have subsequently become extinct on the mainland and on four islands (Flinders, Thistle, St Francis, St Peter Islands, the latter being the type locality), and now only remain on Kangaroo Island (Fig 1), where they are abundant [15,16]. In WA, tammar wallabies have also declined on the mainland but remnant populations survive at several sites with dense native vegetation and are now recovering under ongoing fox control [17]. Tammar wallabies are still extant on all five WA islands; East and West Wallabi Islands in the Houtman Abrolhos Archipelago, Middle and North Twin Peak Islands in the Recherché Archipelago and Garden Island (Fig 1, Table 1) [15]. In SA, tammar wallabies have been introduced to four islands (Boston, Greenly, Granite, Wardang Islands) and in WA to North Island, Houtman Abrolhos Archipelago [15,16]. In the 1870s they were also liberated on Kawau Island, New Zealand and more recently around Rotorua on the North Island of New Zealand [18].

During the last 200 years, observed morphological differences amongst tammar wallaby populations has led to the description of 10 distinct species or subspecies (Table 2), although all are currently regarded as junior synonyms of *N. eugenii* [3,23]. However, the significant morphological differences, confirmed by subsequent analyses [15,24], are suggestive of genetic differentiation and divergence amongst allopatric populations although no comprehensive molecular genetic assessment has yet been made.

Previous genetic comparisons of the Kangaroo Island (SA) and Garden Island (WA) populations, using several techniques, revealed substantial differentiation (reviewed in [5], see also

Table 2. Published scientific names applicable to the tammar wallaby (*Notamacropus eugenii*) [3,23].

Published name	Author and Year	Type Locality
eugenii	(Desmarest 1817)	St Peters Island, Nuyts, Archipelago, SA
derbianus	(Gray 1837)	WA
*derbianus obscurior**	(Gray 1841)	Garden Island, WA
*emiliae**	(Gray 1843)	Houtman Abrolhos, WA
houtmanni	(Gould 1844)	Houtman Abrolhos, WA
dama	(Gould 1844)	?
gracilis	(Gould 1844)	Lake Walyormouring, WA
bedfordi	(Thomas 1900)	?
flindersi	(Wood Jones 1924)	Flinders Island, Investigator Group, SA
eugenii decres	(Troughton 1941)	Kangaroo Island, SA

* nomen nudum

doi:10.1371/journal.pone.0172777.t002

[25,26,27]), although captive mating trials demonstrated the complete fertility of F1 and back-cross hybrids of both sexes [28]. This combination of attributes was subsequently exploited [29] to enable the construction of the first comprehensive physical linkage map for a marsupial [30,31].

More recently, two microsatellite-based population genetic studies have compared tammar wallabies from Garden Island [32] and the Abrolhos Islands [22] with WA mainland populations, documenting significant genetic differentiation and reduced diversity within the island populations. In addition, the source of the introduced North Island population was identified as West Wallabi Island [29].

Microsatellite loci have also been used to compare the introduced New Zealand tammar wallaby populations (Kawau Island and Rotorua) with Kangaroo Island [21]. This study confirmed that, within New Zealand, the Rotorua population was sourced from Kawau Island. In addition, the Kawau Island population was considered unlikely to have been derived from Kangaroo Island, being thought more likely to represent the 'extinct' SA mainland population [21]. As a consequence, the trial reintroduction of tammar wallabies, sourced from Kawau Island, to Innes National Park on the SA mainland is underway [33].

The publication of the tammar wallaby genome [13] has reinforced this species' role as a key model marsupial and it is increasingly used in genetic, genomic and other studies [e.g., 1,27,34,35–38]. However, to maximise its usefulness as a model organism there is also a need to assess the overall distribution of genetic diversity within the tammar wallaby and the degree of differentiation amongst populations. Since previous genetic studies have focused on different population subsets and used largely non-overlapping panels of neutral genetic markers, we have sampled all surviving endemic tammar wallaby populations and screened them for neutral diversity with a consistent set of autosomal and Y-linked microsatellite loci, as well as examining DNA sequence variation in the mitochondrial DNA (mtDNA) control region (CR). This will, for the first time, enable a comprehensive picture of the distribution of diversity within and amongst extant tammar wallaby populations to be compiled.

Methods

Sample collection and DNA extraction

In 2009 animals from Middle (n = 12) and North Twin Peak (n = 2) Islands were captured in cage traps as previously described [22]. A 2–5 mm diameter ear biopsy was collected from each and stored in 80% ethanol prior to DNA extraction using a standard high salt protocol [39]. Sample collection was undertaken in accordance with WA Department of Conservation permit 03/2009, and University of NSW Animal Ethics approval 06/103A. DNA samples from previous studies [21,22,32,40] were already available from seven populations—East Wallabi, West Wallabi and North Islands (n = 101) sampled 2006–2008; Kangaroo Island (n = 40) sampled 2003; Garden Island (n = 30) sampled 2000–2001; Tutanning (n = 63) sampled 2000–2001; Kawau Island (n = 30) sampled 1996. In addition, DNA samples collected between 1992 and 1996 from Perup (n = 6) and Middle Island (n = 5) Island (n = 3) were donated by other researchers.

Microsatellite amplification and screening

Individuals were genotyped at 16 polymorphic autosomal microsatellite loci derived from the tammar wallaby (T3.1T, T15.1, T31.1, T32.1, T46.5, Me1, Me2, Me14, Me15, Me16, Me17 and Me28) and eastern grey kangaroo (*Macropus giganteus*) (G16.1, G20.2, G26.4, G31.1) [41–43], as previously described [44]. The genotypes for 252 individual *N. eugenii* are given in S1 Table. Males were also genotyped at four tammar wallaby derived Y-linked microsatellite loci

(MeY01, MeY28, MeY37A, MeY37B) [45] as previously described [22]. PCR products were analysed on a AB 3730 DNA Analyser (Applied Biosystems, USA) at the Ramaciotti Centre, UNSW (9 loci) or at AGRF Melbourne (7 loci), with the resultant DNA fragments sized using GeneMapper v3.7 (Applied Biosystems, USA).

Mitochondrial DNA amplification and screening

DNA sequence variation in the hypervariable Domain I of the mtDNA *CR* was determined using marsupial-specific primers [46] and single strand conformation polymorphism (SSCP [47]) as previously described [48]. All unique haplotypes were sequenced including multiple representations (up to four where available) using BigDye termination (Perkin-Elmer Applied Biosystems, Norwalk, CT, USA) and resolved in an AB 3730 DNA Analyser (Applied Biosystems, USA) at the Ramaciotti Centre, UNSW or on an ABI 377 Sequencer at Macquarie University. Sequences were edited using Sequencher v4.8 (Gene Codes Corporation, Ann Arbor, USA) and aligned using Clustal X [49].

Estimates of microsatellite diversity

For the autosomal loci, conformance to Hardy-Weinberg equilibrium was conducted using GenePop v7 [50] via a Markov chain method (5000 iterations). The statistical significance levels were corrected for multiple comparisons using sequential Bonferroni adjustments [51]. Observed heterozygosity (H_o), expected heterozygosity (H_E), allelic diversity (A) and effective allelic diversity (Ae: corrected for sample size $n = 15$) were estimated using Fstat v2.9.3 [52]. The mean number of rare alleles (rA) (allele frequency ≤ 0.05) and unique (private) alleles (uA) per locus was also calculated. Differences in diversity indices amongst sampled populations were assessed via a Wilcoxon rank sign test using SYSTAT9. The effective inbreeding coefficient (F_e, Wright's fixation index) was calculated from the equation: $F_e = 1 - H_{IS} / H_M$ where H_{IS} represents heterozygosity for island populations and H_M represents heterozygosity for mainland populations [53].

For Y-linked microsatellites the average number of alleles per locus (A) and haplotypic diversity (h) were calculated using GenAlEx v6.5 [54,55]. Allelic richness (A_R) was calculated in FSTAT v2.9.3.

Population differentiation

For the autosomal microsatellite data, several methods were used to infer population structure. Firstly, differentiation amongst populations was assessed by calculating pairwise F_{ST} [56], using FSTAT v.2.9.3, with significance tested via 900 permutations. Secondly, population genetic structure was also inferred using a Bayesian model-based clustering analysis in the program Structure v2.3.4 [57]. Structure was run with no *a priori* information on population assignment, under the admixture model with alpha inferred from the data, allele frequencies uncorrelated and lambda set to 1.0. After a burn-in of 200 000, 1 000 000 iterations were performed. For the whole data set, we tested the number of genetic clusters (populations, K) present using values of K between 1 and 12 with 10 replicates of each. The inferred number of populations within the sample was deduced using both maximum posterior probability (L(K) [57]), and maximum delta log likelihood (ΔK [58]) implemented in Structure Harvester 0.6.93 [59]. The resulting barplots were created in Distruct v1.1 [60]. Thirdly, principal component analysis (PCA) was used to assess the degree of genetic similarity amongst individuals, based on allele frequencies, using GenAlEx v6.5.

For the Y-linked microsatellite loci pairwise ϕ_{PT} values amongst populations were calculated using GenAlEx v6.5, with significance estimated using 999 permutations. A Y-haplotype

network was also constructed in NETWORK v4.6.1.3 [61], using the TCS algorithm and the MP options to identify and remove unnecessary median vectors and links [62]. Loci with lower expected mutation rates were assigned higher weights, following [23], with perfect dinucleotide repeats (MeY01) weighted $w = 2$ and imperfect repeats (MeY28, MeY37A and MeY37B) weighted $w = 5$. For the mtDNA CR data pairwise differentiation (Φ_{ST}) amongst populations was estimated and tested for significance in ARLEQUIN v3.5 [63] using 5000 permutations.

Phylogenetic analysis

Phylogenetic relationships amongst identified *N. eugenii CR* haplotypes were analysed using three methods (maximum parsimony (MP), maximum likelihood (ML), neighbour-joining (NJ)) in PAUP* v4b10 [64]. MP analysis was undertaken using a Branch and Bound search, with furthest addition, gaps treated as a fifth state and 1 000 bootstrap replicates; for ML analysis a heuristic search was conducted under the HKY85+G+I model (selected using MODELTEST v3.7 [65]), with random addition, TBR branch-swapping and 500 bootstrap replicates; NJ analysis was conducted using Kimura two-parameter model distances and 10 000 bootstrap replicates. Homologous *CR* sequences from an eastern grey kangaroo (*Macropus giganteus*) and western grey kangaroo (*M. fuliginosus*) (GenBank Accession numbers: AF443160 and AF443174, respectively), were used as outgroup taxa. For complete sequences of all new *CR* haplotypes see GenBank accession numbers KY623685-KY623712.

Results

Sample collection

Samples were obtained from 287 tammar wallabies from ten populations throughout the current range, including from Kangaroo Island, SA ($n = 40$) and Kawau Island, New Zealand ($n = 30$). From WA two mainland (Tutanning, $n = 63$; Perup $n = 6$) and six island (Garden Island, $n = 30$; Middle Island $n = 17$; North Twin Peak Island, $n = 2$; East Wallabi Island, $n = 35$; West Wallabi Island, $n = 30$; North Island, $n = 36$) populations were sampled.

Microsatellite diversity

All of the 16 autosomal microsatellite loci were polymorphic in most populations (S2 Table). Exceptions were for nine loci on North Twin Peak Island (G20.2, G26.4, Me 2, Me14, Me1, Me16, Me17, Me28, T32.1), and three loci on each of Garden Island (G20.2, Me14, T15.1), Middle Island (Me16, Me17, T32.1) and North Island (G20.2, Me14, Me15). In all populations autosomal microsatellite genotype frequencies at all loci conformed to Hard-Weinberg expectations, except for Me16 in East Wallabi Island, G26.4 in Garden Island, T31.1 in North Twin Peak Island and G16.1, G20.2 and Me28 in Tutanning, where significant ($p < 0.05$) deficiencies of heterozygotes were identified.

A total of 270 autosomal microsatellite alleles were identified; 174 in SA (33% unique) and 209 in WA (46% unique) (S2 Table). Of the unique SA alleles, 69% were larger than any of the WA alleles found at the same locus. Populations contained between 1–29 unique alleles, except for North and North Twin Peak Islands which shared all alleles with other populations. The sampled Kangaroo Island population contained 169 alleles (17% unique), while 85 alleles (2% unique) were detected in the Kawau Island (NZ) population. Most Kawau Island alleles (95%) were also found in the Kangaroo Island population. In WA, the combined island populations contained a similar total number of alleles (159 vs 161) and unique alleles (15% vs 14%) to the mainland. The two WA mainland sites (Tutanning and Perup) shared 40% of their alleles.

Table 3. Genetic diversity estimates (mean ± SE) from 16 microsatellite loci in ten sampled tammar wallaby (*Notamacropus eugenii*) populations.

Population	N	P	nA	A	Ae	Ho	He	uA	rA	Fe
Kangaroo Is.	36	1.0	169	10.6±0.9	8.4±0.6	0.74±0.03	0.78±0.02	1.8±0.36	4.8±0.6	-
Kawau Is.	30	1.0	85	5.3±0.4	4.9±0.3	0.64±0.03	0.64±0.03	0.13±0.06	1.3±0.3	0.17
Tutanning	30	1.0	142	8.9±0.6	7.8±0.5	0.70±0.04	0.77±0.03	1.1±0.24	3.3±0.5	-
Perup	6	1.0	85	5.3±0.7	-	0.77±0.09	0.71±0.04	0.37±0.22	0.0	-
Garden Is.	30	0.81	65	4.1±0.5	3.5±0.4	0.37±0.05	0.41±0.06	0.38±0.10	1.5±0.3	0.47
East Wallabi Is.	35	1.0	65	4.1±0.4	3.6±0.4	0.41±0.05	0.43±0.05	0.25±0.09	1.1±0.1	0.44
West Wallabi Is.	30	1.0	60	3.8±0.3	3.4±0.2	0.43±0.04	0.46±0.04	0.19±0.07	1.1±0.2	0.40
North Is.	36	0.88	42	2.6±0.2	2.4±0.2	0.33±0.04	0.35±0.04	0.00	0.7±0.2	0.55
Middle Is.	17	0.81	65	4.1±0.7	4.0±0.5	0.44±0.08	0.48±0.08	0.56±0.26	0.5±0.3	0.37
North Twin Peak Is.	2	0.44	25	1.6±0.6	-	0.22±0.22	0.20±0.17	0.00	0.0	0.77

N, sample size; P, proportion of polymorphic loci; nA, total number of alleles; A, allelic diversity; Ae, effective number of alleles (n = 15); Ho, observed heterozygosity; He, expected heterozygosity; uA, number of unique alleles; rA, number of rare alleles; Fe effective inbreeding. Values for the Perup and North Twin Peak Island populations should be treated with caution due to small sample size.

The Tutanning and Kangaroo Island populations had similar levels of autosomal microsatellite diversity for all parameters, except that the frequency of *rA* was significantly ($p<0.05$) higher in the Kangaroo Island population (Table 3). Values of *A*, *Ae*, *Ho*, *He*, *uA*, *rA* were significantly ($p<0.05$) lower in all island populations compared to the Tutanning and Kangaroo Island populations (except for values of *uA* between Tutanning and both Garden and Middle Island populations). The introduced North Island population had significantly ($p<0.05$) lower values of *A* and *Ae* than all other populations (Table 3). The Kawau Island population had significantly ($p<0.05$) lower diversity values for all parameters than the Kangaroo Island population. The Perup population had significantly lower *A*, *He* and *rA* than Tutanning, although this most likely reflects the large difference in sample size (Table 3).

Diversity at Y-linked loci was lower than autosomal loci, as expected (Tables 3 and 4). Between two and 12 alleles per locus were identified over the four Y-linked loci genotyped in 178 male tammar wallabies from nine populations (S3 Table). A total of 27 alleles were identified (4–16 per population), 16 in the SA (81% unique) and 14 (86% unique) in the WA

Table 4. Genetic diversity estimates (mean ± SE) from four Y-linked microsatellite loci in nine sampled tammar wallaby (*Notamacropus eugenii*) populations.

Population	N	A	Ae	nA	uA	nH	uH	h
Kangaroo Is.	33	4.00 (±1.59)	2.14 (±0.67)	16	8	16	13	0.38 (±0.19)
Kawau Is.	18	2.00 (±0.41)	1.61 (±0.36)	8	0	5	2	0.39 (±0.13)
Tutanning	39	2.00 (±0.71)	1.46 (±0.49)	8	1	6	6	0.23 (±0.24)
Perup	3	1.00	1.00	4	0	1	1	-
Garden Is.	19	1.00	1.00	4	0	1	1	-
East Wallabi Is.	20	1.00	1.00	4	0	1	1	-
West Wallabi Is.	16	1.25 (±0.25)	1.19 (±0.19)	5	1	2	1	0.08 (±0.08)
North Is.	19	1.00	1.00	4	1	1	0	-
Middle Is.	11	1.50 (±0.29)	1.24 (±0.14)	6	2	3	3	0.08 (±0.05)

N, sample size; A, average alleles per locus; Ae, allelic richness (n = 3); nA, total alleles; uA, total unique alleles; nH, total haplotypes; uH, total unique haplotypes; h = haplotypic diversity.

Table 5. Distribution and frequency of Y chromosome haplotypes identified in nine sampled tammar wallaby (*Notamacropus eugenii*) populations.

Y Haplotype	Population								
	KI	KwI	Tut	Per	GI	EWI	WWI	NI	MI
1	6	3							
2	4	8							
3	3	5							
4	5								
5	3								
6	2								
7	1								
8	1								
9	1								
10	1								
11	1								
12	1								
13	1								
14	1								
15	1								
16	1								
17		2							
18		1							
19			14						
20			14						
21			4						
22			3						
23			2						
24			2						
25					3				
26					19				
27						20			
28							13	19	
29							3		
30									9
31									1
32									1

KI = Kangaroo Island; KwI = Kawau Island, New Zealand; Tut = Tutanning; Per = Perup; GI = Garden Island; EWI = East Wallabi Island; WWI = West Wallabi Island; NI = North Island; MI = Middle Island.

doi:10.1371/journal.pone.0172777.t005

population (Table 4). Overall 70% of alleles were shared amongst populations, although unique alleles were present in the Kangaroo Island, Middle Island and Tutanning populations (Table 4, S3 Table). These alleles formed 32 Y-haplotypes, 88% of which were population specific (Tables 4 and 5, S3 Table). Haplotypes were shared only between North and West Wallabi Islands (YH28), as well as between Kangaroo and Kawau Islands (YH1, YH2, YH3) (Table 5). While most (6/9) populations contained ≤3 Y-haplotypes, 16 were identified within the Kangaroo Island population alone (Tables 4 and 5), most other island populations showed no or limited diversity (Table 4).

Table 6. Distribution and frequency of the 28 mitochondrial DNA control region haplotypes identified in ten sampled tammar wallaby (*Notamacropus eugenii*) populations.

MtDNA Haplotype	Population									
	KI	KwI	Tut	Per	GI	EWI	WWI	NI	MI	NTP
1	12									
2	7	22								
3	6									
4	1									
5		1								
6			9							
7			6							
8			3							
9			1							
10			1							
11			1							
12				4						
13				2						
14					17					
15					3					
16						29				
17						3				
18						1				
19							10			
20							10	12		
21							5			
22							4			
23								18		
24									12	
25									2	
26									1	
27									1	
28										2

KI = Kangaroo Island; KwI = Kawau Island, New Zealand; Tut = Tutanning; Per = Perup; GI = Garden Island; EWI = East Wallabi Island; WWI = West Wallabi Island; NI = North Island; MI = Middle Island; NTP = North Twin Peak Island.

doi:10.1371/journal.pone.0172777.t006

MtDNA control region diversity

A total of 28 *CR* haplotypes were identified amongst the 206 tammar wallabies sampled from ten populations (Table 6). Within the aligned block of 595 bp, 122 variable sites were identified, 102 of which were phylogenetically informative. All populations contained multiple haplotypes (up to six), except for North Twin Peak Island (Table 6). Almost all (93%) identified *CR* haplotypes were population specific. Haplotypes were shared only between North and West Wallabi Islands (H20), as well as between Kangaroo and Kawau Islands (H2) (Table 6).

There was substantial sequence divergence between *CR* haplotypes from SA and WA (14.54 ± 0.7%; mean ± sd); although more modest divergence amongst haplotypes within each region: range of 0.17–3.5% within SA and 0.17–6.4% within WA. Mean divergence within the Kangaroo Island population (1.4 ± 1.4%) was greater than that found between the Kangaroo and Kawau Island populations (0.89 ± 1.2%). Within WA, mean sequence divergence amongst haplotypes within endemic island populations was low (range 0.4–1.0%), but higher mean

Table 7. Genetic differentiation amongst ten sampled tammar wallaby (*Notamacropus eugenii*) populations.

	KI	KwI	Tut	Per	GI	EWI	WWI	NI	MI	NTP
Kangaroo Is.	-	**0.182**	**0.907**	**0.945**	**0.976**	**0.985**	**0.972**	**0.980**	**0.973**	**0.971**
Kawau Is.	**0.097**	-	**0.918**	**0.969**	**0.992**	**0.998**	**0.986**	**0.993**	**0.992**	**1.000**
Tutanning	**0.167**	**0.244**	-	**0.246**	**0.726**	**0.799**	**0.743**	**0.771**	**0.541**	**0.171**
Perup	**0.164**	**0.257**	**0.074**	-	**0.816**	**0.923**	**0.853**	**0.892**	**0.698**	**0.362**
Garden Is.	**0.358**	**0.445**	**0.310**	**0.388**	-	**0.973**	**0.934**	**0.958**	**0.942**	**0.948**
East Wallabi Is.	**0.356**	**0.418**	**0.331**	**0.374**	**0.517**	-	**0.664**	**0.784**	**0.974**	**0.999**
West Wallabi Is.	**0.328**	**0.395**	**0.313**	**0.357**	**0.489**	**0.495**	-	**0.079**	**0.932**	**0.928**
North Is.	**0.400**	**0.462**	**0.387**	**0.453**	**0.542**	**0.551**	**0.150**	-	**0.959**	**0.967**
Middle Is.	**0.298**	**0.388**	**0.218**	**0.274**	**0.452**	**0.498**	**0.433**	**0.512**	-	**0.852**
North Twin Peak Is.	**0.307**	**0.401**	0.214	0.255	0.523	**0.557**	**0.507**	**0.597**	0.283	-

Pairwise Φ_{ST} values for mtDNA data above the diagonal and pairwise F_{ST} for microsatellite data below the diagonal. KI = Kangaroo Island; KwI = Kawau Island, New Zealand; Tut = Tutanning; Per = Perup; GI = Garden Island; EWI = East Wallabi Island; WWI = West Wallabi Island; NI = North Island; MI = Middle Island; NTP = North Twin Peak Island. Values in bold are significant (*P*<0.05). Values for the Perup and North Twin Peak Island populations should be treated with caution due to small sample size.

doi:10.1371/journal.pone.0172777.t007

divergence was evident between island and WA mainland haplotypes (range 3.3–4.9%). WA mainland haplotypes differed by 0.2–5.5%, with a mean of 3.8 ± 1.0% separating the two sampled populations.

Population differentiation

Significant genetic differentiation (F_{ST} and Φ_{ST}) was detected amongst all adequately sampled populations (Table 7). Values were lowest between the two mainland WA populations (F_{ST} = 0.074; Φ_{ST} = 0.25), North and West Wallabi Island populations (F_{ST} = 0.15; Φ_{ST} = 0.097), as well as between Kangaroo and Kawau Islands (F_{ST} = 0.097; Φ_{ST} = 0.18) (Table 7). The WA island populations were highly differentiated from the WA mainland populations (mean F_{ST} = 0.32; Φ_{ST} = 0.69) and each other (mean F_{ST} = 0.47; Φ_{ST} = 0.86) (Table 7). The SA and WA populations were also highly differentiated (mean F_{ST} = 0.34; Φ_{ST} = 0.97).

The Bayesian model-based clustering analysis implemented in STRUCTURE indicated that either seven (maximum *L(K)*) or eight (maximum Δ*K*) populations were present in the data (S1 Fig). With K = 7, the inferred populations largely corresponded to sampling locations except for Perup and Tutanning, West Wallabi and North Islands, as well as Middle and North Twin Peak Islands where each pair was combined into single inferred populations (Fig 2a). With K = 8, the groupings were similar to K = 7 but with an additional cluster comprising some Perup individuals (Fig 2b).
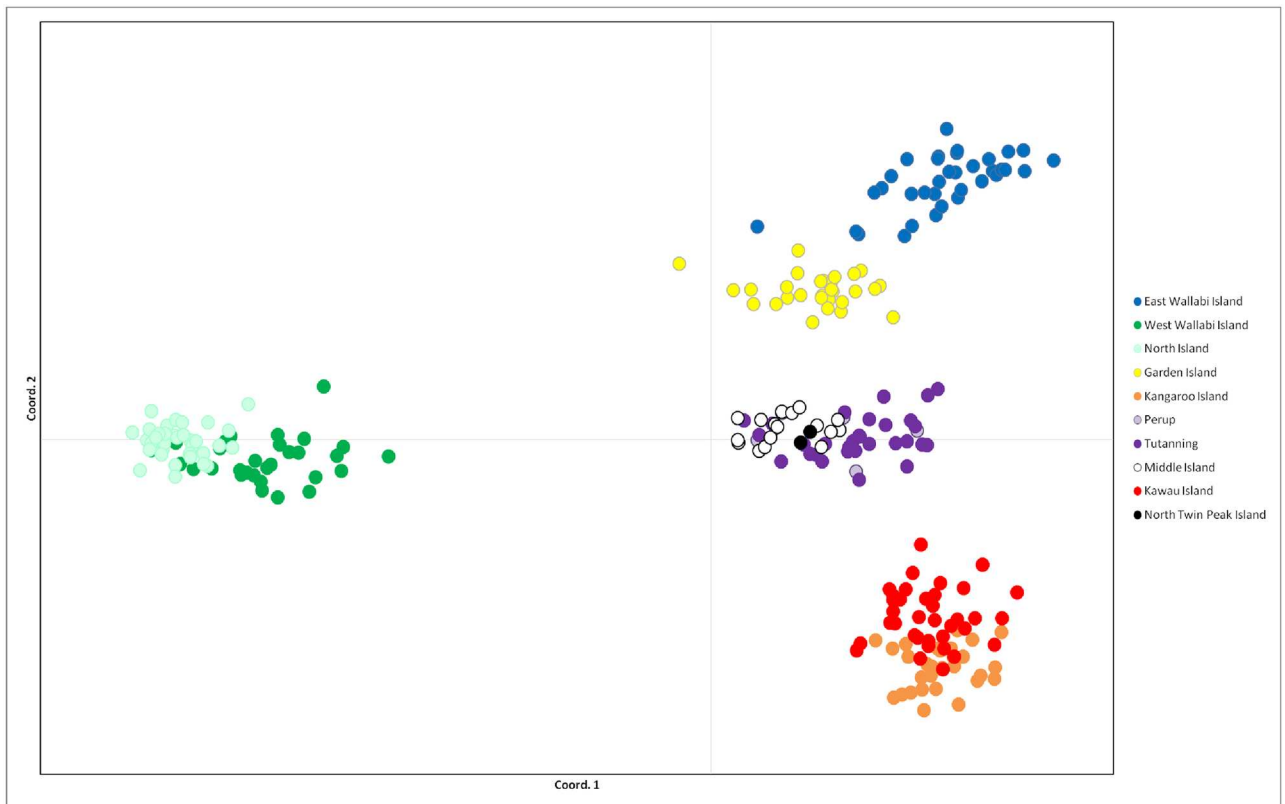
The PCA of autosomal loci was plotted on two axes which cumulatively explained 55.32% of the variation (33.59 and 21.73% respectively) (Fig 3). The PCA plot revealed four main genetic clusters which represented samples from East Wallabi and Garden Islands, West Wallabi and North Islands, Kangaroo and Kawau Islands, and finally samples from Perup, Tutanning, Middle Island and North Twin Peak Island (Fig 3).

The Y-linked microsatellite loci revealed a pattern of significant differentiation amongst all populations, except for North and West Wallabi Islands (Φ_{PT} = 0.15), and between Kangaroo and Kawau Islands (Φ_{PT} = 0.04) (Table 8). The WA island populations were highly differentiated from each other (mean Φ_{PT} = 0.86) and the WA mainland (mean Φ_{PT} = 0.82). The SA and WA populations were also well differentiated (mean Φ_{PT} = 0.72) (Table 8). An AMOVA revealed that genetic diversity was significantly partitioned between SA and WA populations

(a) K=7

(b) K=8

East Wallabi Island

West Wallabi Island

North Island

Garden Island

Perup

Tutanning

Middle Island

North Twin Peak Island

Kangaroo Island

Kawau Island
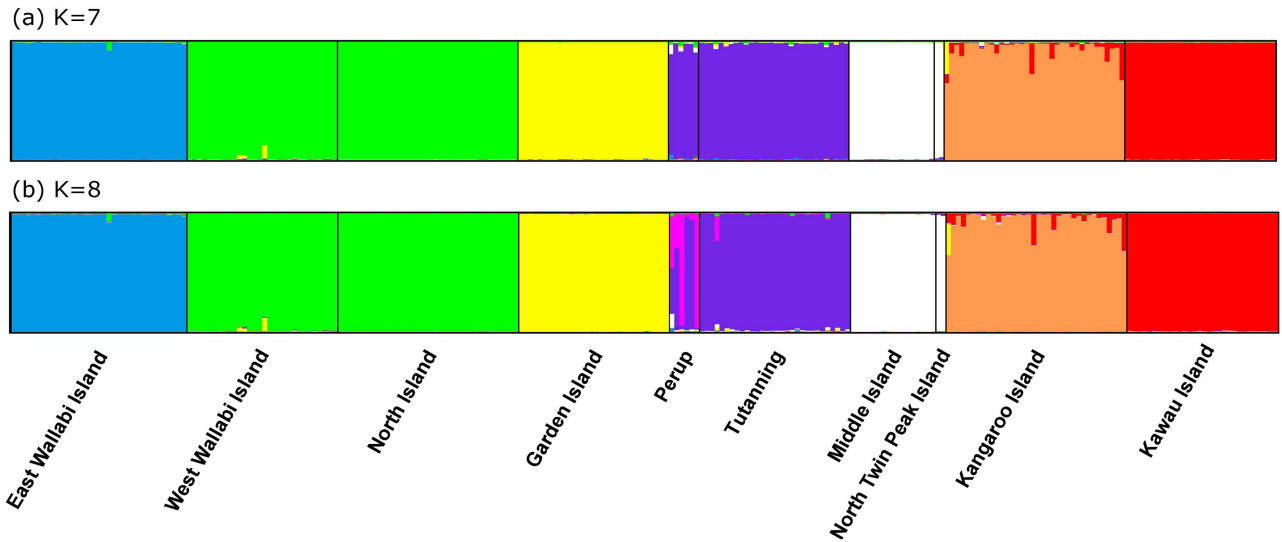
Fig 2. STRUCTURE **plot (autosomal microsatellite loci) showing proportions of inferred ancestry (Q) in the K = 7 (a) and K = 8 (b) genetic clusters identified within tammar wallabies (*Notamacropus eugenii*) sampled from 10 sites.**

doi:10.1371/journal.pone.0172777.g002

- East Wallabi Island
- West Wallabi Island
- North Island
- Garden Island
- Kangaroo Island
- Perup
- Tutanning
- Middle Island
- Kawau Island
- North Twin Peak Island

Fig 3. PCA plot (autosomal microsatellite data) showing four distinct genetic clusters of tammar wallabies (*Notamacropus eugenii*), corresponding to individuals sampled from East Wallabi and Garden Islands; West Wallabi and North Islands; Kangaroo and Kawau Islands; Perup, Tutanning, Middle Island and North Twin Peak Island.

doi:10.1371/journal.pone.0172777.g003

Table 8. Genetic differentiation, for Y-linked microsatellite data, amongst nine sampled tammar wallaby (*Notamacropus eugenii*) populations.

	KI	KwI	Tut	Per	GI	EWI	WWI	NI	MI
Kangaroo Is.	-	0.107	**0.001**	**0.012**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
Kawau Is.	0.036	-	**0.001**	**0.021**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
Tutanning	0.726	0.779	-	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
Perup	0.275	0.406	0.769	-	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
Garden Is.	0.780	0.865	0.579	1.000	-	**0.001**	**0.001**	**0.001**	**0.001**
East Wallabi Is.	0.784	0.869	0.781	1.000	1.000	-	**0.001**	**0.001**	**0.001**
West Wallabi Is.	0.742	0.816	0.659	0.930	0.926	0.928	-	0.085	**0.001**
North Is.	0.780	0.865	0.725	1.000	1.000	1.000	0.153	-	
Middle Is.	0.685	0.765	0.646	0.904	0.930	0.929	0.829	0.934	-

Pairwise Φ_{PT} values below diagonal and significance (based on 999 permutations) above diagonal. KI = Kangaroo Island; KwI = Kawau Island, New Zealand; Tut = Tutanning; Per = Perup; GI = Garden Island; EWI = East Wallabi Island; WWI = West Wallabi Island; NI = North Island; MI = Middle Island. Values in bold are significant ($P<0.05$).

($F_{CT} = 0.41$, $P = 0.03$). Distinct eastern (SA) and western (WA) clusters were also apparent in the Y haplotype network (Fig 4).

Phylogenetic analysis

Phylogenetic analyses of the tammar wallaby mtDNA *CR* revealed two well supported clusters of highly divergent haplotypes, showing complete reciprocal monophyly and corresponding to the sampled SA and WA populations (Fig 5). Within the WA clade, each island population tended to have monophyletic clusters of unique haplotypes. However, the relationship amongst these island lineages and between them and the WA mainland haplotypes was largely unresolved. Within the SA clade there was no phylogenetic separation between the identified Kangaroo and Kawau Island haplotypes (Fig 5).

Discussion

Differentiation between WA and SA

Both our mtDNA and microsatellite analyses have revealed significant differentiation amongst *N. eugenii* populations throughout the species' range. Substantial divergence was identified between eastern (SA) and western (WA) populations, with each region being characterised by a high proportion of unique and divergent alleles/haplotypes. This pattern of divergence is consistent with the long-term isolation of SA and WA *N. eugenii* populations and the lack of recent gene flow between them, allowing each to evolve independently. A similar pattern of genetic divergence is also present between eastern and western populations (or species pairs) in many taxa across southern Australia, including grey kangaroos [66], dasyurids [67,68], bandicoots [69], rodents [70], birds [71–73] and reptiles [74]. This widespread pattern of east/west divergence is believed to reflect the separation of mesic fauna by the arid Nullarbor Barrier, as a consequence of the increasing aridity of Australia during the Pleistocene [75,76]. Although [77] suggested tammar wallabies in WA and SA were separated over 30 000–50 000 years ago, the substantial genetic differentiation detected in our study, especially for mtDNA, indicates a much older divergence ~1MYA [78].

The divergence in *CR* sequence (14.5%) detected here between eastern and western tammar wallabies is similar to that reported between eastern and western grey kangaroos (14%) [66], and amongst six species of rock-wallabies (*Petrogale* spp. 10–17% [79,80]). In addition,
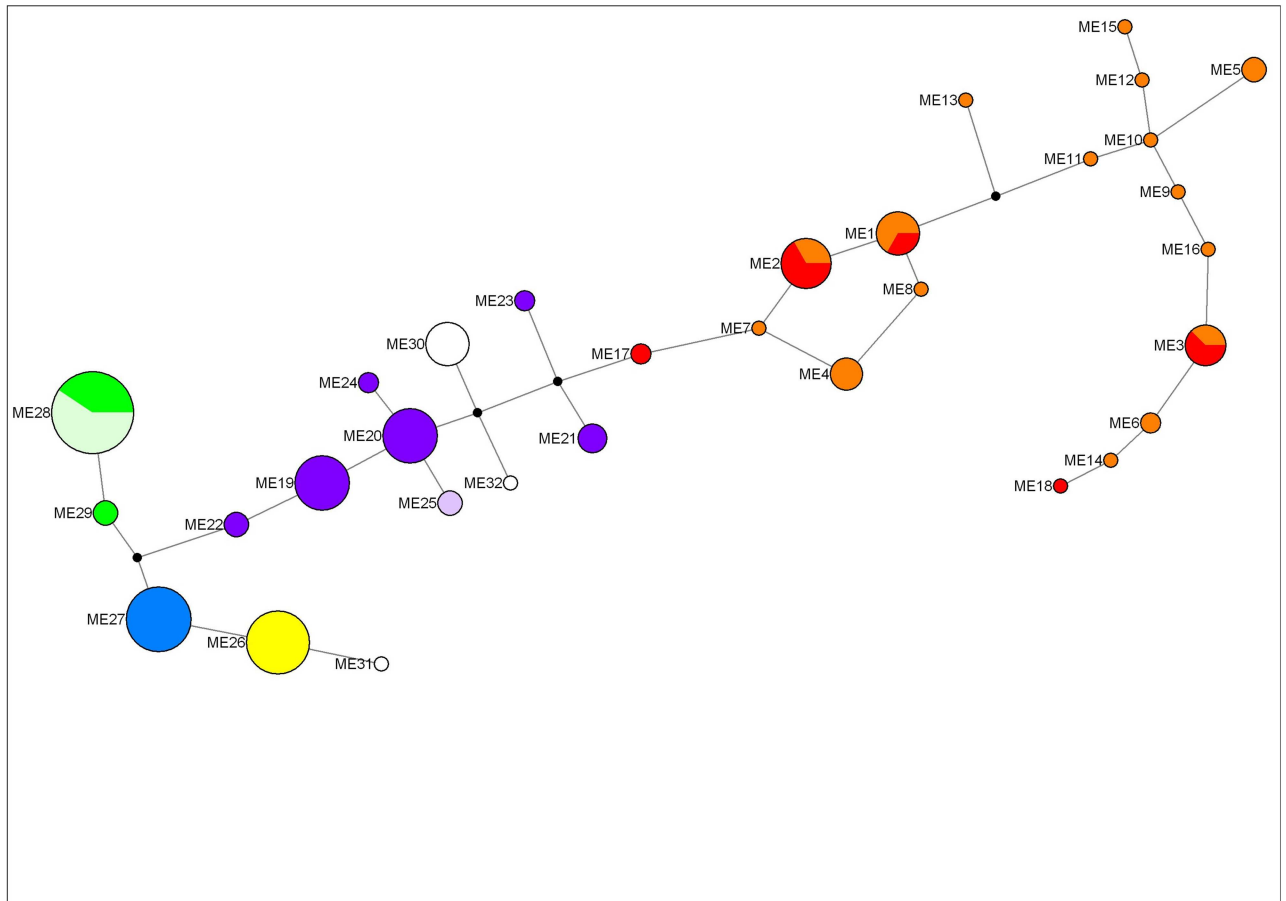
Fig 4. TCS network of Y-linked microsatellite haplotypes identified in nine tammar wallaby (*Notamacropus eugenii*) populations.
Node size is proportional to haplotype frequency (Table 5). Black nodes are inferred intermediate haplotypes. Orange = Kangaroo Island;
red = Kawau Island, New Zealand; dark purple = Tutanning; light purple = Perup; yellow = Garden Island; blue = East Wallabi Island; dark
green = West Wallabi Island; light green = North Island; white = Middle Island.

doi:10.1371/journal.pone.0172777.g004

differences in allozymes between the Kangaroo Is. (SA) and Garden Is. (WA) populations are
similar to that typically found between species [5,15]. Morphological analysis has also revealed
two main clusters within *N. eugenii*, largely reflecting distinct eastern and western groupings,
although with alterative clustering for some southern island populations [15,24].

Since the allopatric populations of SA and WA tammar wallabies are genetically highly dif-
ferentiated, with levels of divergence typical of different species, there would be some justifica-
tion in recognising each as a separate species, for consistency with data from other
macropodid species. However, despite their genetic divergence at neutral loci, SA and WA
tammar wallabies are known to be fully inter-fertile (in captivity), with F1 and back-cross
hybrids of both sexes showing normal fertility [28]. This is quite unlike the similarly divergent
eastern and western grey kangaroos, where both pre- and post-mating reproductive isolation
is more developed, including male hybrid sterility [81,82]. In addition, eastern and western
grey kangaroos occur in sympatry across large areas of eastern Australia with introgression
being only occasionally detected [66]. In contrast, SA and WA tammar wallabies are naturally
allopatric, preventing a direct test of reproductive isolation under field conditions. Since SA
and WA tammar wallabies have been shown to be potentially interbreeding, at least in
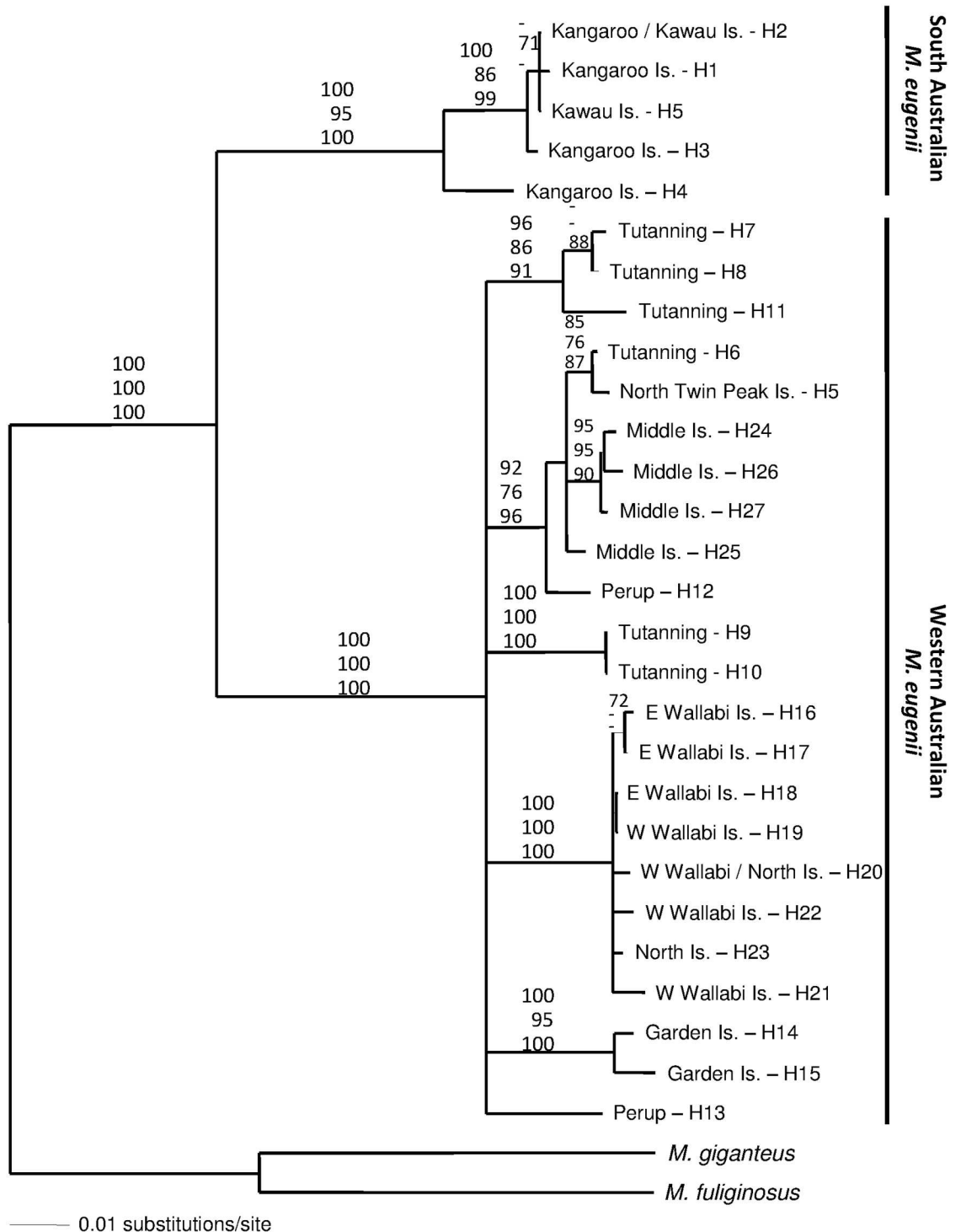
Fig 5. Phylogenetic relationships amongst mtDNA *CR* haplotypes identified from ten tammar wallaby (*Notamacropus eugenii*) populations from South Australia, Western Australia and New Zealand. Haplotypes from *Macropus giganteus* and *M. fuliginosus* were used as outgroups. Numbers on branches indicate percent of bootstrap replicates when ≥ 70% (maximum parsimony, maximum likelihood, neighbour-joining).

doi:10.1371/journal.pone.0172777.g005

captivity, the available evidence is consistent with them constituting a single species as defined by the Biological Species Concept [83]. Therefore, we recommend that a single species of tammar wallaby (*N. eugenii*) continue to be recognised. There is however, the need for further research, since a reduced breeding efficiency when producing Garden Island by Kangaroo Island F1 hybrids in captivity has been reported [24]. It is therefore possible that some incipient pre- or post-mating reproductive isolation is present. This needs to be more thoroughly assessed, since the reported reduced breeding efficiency [24] may be related to the lower reproductive rate observed in Garden Island tammar wallabies under captive conditions (in eastern Australia). Additional experiments should therefore be conducted to assess behavioural interactions and simultaneous mate-choice under more natural conditions, as well as the capacity of Kangaroo Island tammar wallabies to hybridise with individuals from other WA populations. These observations also have implications for conservation biology and taxonomy as they demonstrate that allopatric populations differentiated at neutral loci are not necessarily reproductively isolated, since reproductive isolation appears more associated with differential environmental adaptation rather than geographic isolation and drift [84,85].

The genetic divergence detected between WA and SA tammar populations is sufficient for them to be recognised as separate Evolutionarily Significant Units (ESUs) (*sensu* [86]). We would suggest that the divergence recognised by ESUs is often equivalent to the concept of subspecies, although accepted criteria to define subspecies remain elusive and controversial [87,90]. Nevertheless, we believe they can play a useful role in identifying major geographically, genetically and/or morphologically distinct subpopulations within species and so we suggest that eastern *N. eugenii* populations (SA) be known as *N. eugenii eugenii*, and western (WA) populations as *N. eugenii derbianus* (Table 2) as recently proposed [3]. This arrangement assumes that the tammar wallabies from the type locality (St Peter Island, Nuyts Archipelago, SA), which are extinct and were not examined in this study, group with the sampled SA populations. Material from the type locality was also not included in two studies of tammar wallaby cranial morphometrics [15,24], although skulls from Flinders Island, Investigator Group, SA (located south-east of St Peter Is.) were examined. While one study [15] concluded that the Flinders Island tammar wallabies were most similar to those from the southwest WA mainland, another [20] concluded they grouped with the SA mainland and Kangaroo Island populations. Therefore, until the relationship of the St Peter Island tammar wallabies can be directly clarified, perhaps using ancient DNA from the very limited skeletal material present in museums, it will remain somewhat uncertain whether the name *eugenii* correctly applies to the eastern or western tammar wallaby populations.

The western affinity of some SA animal populations is not unprecedented; for example, the Pearson Island rock-wallaby (*Petrogale lateralis pearsoni*) also found in the Investigator Group, SA is most closely related to the black-footed rock-wallaby (*P. l. lateralis*) from southwest WA [79]. Similarly, a number of largely south-western WA bird, reptile and mammal species reach their eastern limit on the Eyre Peninsula of SA (eg, little long-tailed dunnart, western yellow robin, rufous tree-creeper [88,89]). However, recent molecular studies of Australian tiger snakes (*Notechis scutatus*) [74] and southern brown bandicoots (*Isoodon obesulus*) [69] from the Nuyts Archipelago have shown their affinities lie with south-eastern rather than southwestern populations.

Differentiation within WA

Although we recommend that a single subspecies be recognised in WA we note that substantial differentiation in microsatellite loci also occurs among many of the sampled WA populations, some of which have historically been proposed as separate taxa (Table 2). However,

the level of mtDNA divergence amongst WA populations appears insufficient to warrant the recognition of further subspecies. For example, the mean *CR* sequence divergence between WA island and mainland populations ranged from 3.3–4.9%, but up to 5.5% divergence was found between haplotypes within the Tutanning population alone. Similarly, although the East and West Wallabi Islands populations appear highly divergent based on autosomal microsatellite data (Table 7, Fig 4) they have almost identical CR haplotypes (Fig 5) indicating very recent common ancestry. Thus the genetically (Fig 4) and morphologically distinct WA island populations [15,24] are most likely the consequence of relatively recent divergence under the influence of small population size, genetic drift and adaptation to an island environment, following their isolation 7 000–11 500 years ago by rising sea levels (Table 1). These recent and relatively rapid evolutionary processes are also reflected in their genetic profiles, with each island having significantly reduced diversity and thus show exaggerated genetic differentiation from each other and the WA mainland populations [90]. While individually each island population is genetically depauperate and inbred (Table 3), together they preserve considerable diversity and also retain unique alleles and haplotypes. As such, these WA island populations represent a valuable genetic resource and have high conservation value, a situation similar to that reported for WA populations of the northern quoll (*Dasyurus hallucatus*) [91].

An exception is the North Twin Peak Island tammar wallaby population, which was found to share all of its autosomal microsatellite alleles with the nearby (31 km west) Middle Island population. Whether this similarity reflects recent gene flow or the preferential retention in both populations of the higher frequency alleles present in the common ancestral population, must await more comprehensive sampling of the North Twin Peak population (currently $n = 2$). However, since the populations do not share *CR* haplotypes and are morphologically distinct (unpublished data) the later hypothesis seems more likely.

On the WA mainland some genetic differentiation is also apparent between the two sampled southwest WA populations (Tutanning and Perup). Although only a limited sample was available from Perup ($n = 6$), unique *CR* and Y haplotypes were detected and 55% of autosomal microsatellite alleles were not shared with the much better sampled Tutanning ($n = 50$) population located 200 km to the northeast. These preliminary data suggest that mainland WA populations are also structured with limited gene flow by both sexes between sites. More comprehensive sampling of remaining tammar wallaby populations throughout southwest WA is required to confirm these findings. However, if these data are typical then considerable unique diversity may exist within each remaining mainland WA population. In this context, it would also be important to examine the pattern of male and female mediated gene flow and extent of population genetic structure throughout the abundant Kangaroo Island tammar population. Since Kangaroo Island is over 150 km long, tammar wallaby populations may show significant genetic structure across the island.

Differentiation and diversity within SA populations

Although the Kangaroo Island and Kawau Island populations showed some differentiation in several analyses they were more similar than expected, given the latter is thought to represent the SA mainland population and they have been considered distinct subspecies [21,33,92]. The Kawau Island population shared most microsatellite alleles, as well as Y and *CR* haplotypes with the Kangaroo Island population. This lack of substantial differentiation was in contrast to all other island-mainland population comparisons and was more similar to the West Wallabi / North Island comparison. However, the shallow divergence between the Kangaroo Island and Kawau Island populations does not necessarily undermine the case

for a SA mainland origin of the Kawau Island population [21]. Since Kangaroo Island is a large island (450 000 ha), supporting a substantial tammar population (up to 106 [93]) that became isolated relatively recently (~9500 ybp), the impact of genetic drift in promoting genetic divergence from the mainland is likely to be much slower than for the considerably smaller WA islands (all < ~1000 ha; Table 1) examined. There is also the possibility that allele and haplotype frequencies in Kawau Island were distorted during the establishment of this population in New Zealand, from a small number of founders, so that unique and rare alleles were preferentially lost. Although two unique Y haplotypes and one unique mtDNA *CR* haplotype were detected in the Kawau Island population these were all very similar (1 mutational step) to haplotypes recorded in Kangaroo Island and so may represent recent mutations in the Kawau Island population or be as yet unsampled in the Kangaroo Island population. In light of this uncertainty, definitive conclusions as to the origins of the Kawau Island population and the distinction of the SA mainland and Kangaroo Island populations remain elusive. To further resolve this matter would require not only genetic data from definitive historic SA mainland tammar wallabies (derived from museum material), but also a better understanding of the distribution of genetic diversity throughout Kangaroo Island, since the population we sampled is from the western end of the island and the degree of population structure across the island remains unknown. The similarity between the Kangaroo Island and Kawau Island tammar populations detected in this study should not impact the ongoing re-introduction of Kawau-derived tammar wallabies to the Yorke Peninsula on the mainland SA, since returning SA tammar wallabies to the mainland is a worthy endeavour for biodiversity conservation and restoring ecosystem function. However the recent use of *eugenii eugenii* to refer to the extinct SA mainland tammar wallaby population as distinct from *eugenii decres* on Kangaroo Island (e.g. [33,92]) is inappropriate; for if distinct island subspecies were to be recognised, *eugenii eugenii* would be most accurately associated with the extinct St Peters Island population (type locality) and no scientific name has yet been specifically associated with the SA mainland population (Table 2).

Although island populations typically have reduced diversity compared to mainland populations [32,94], a remarkable feature of these data is the high genetic diversity detected in the Kangaroo Island tammar wallaby population. For autosomal microsatellites the levels of diversity (*A*, *He*) are amongst the highest yet reported in marsupials [2]. A remarkably high number of Y haplotypes were also detected in the Kangaroo Island population (Table 4, [27]), compared to other tammar populations, a more widespread and abundant macropodid (i.e. western grey kangaroo [95]) and many other species which typically show low variation at sex chromosome loci [96]. These high levels of diversity may be a consequence of Kangaroo Island's large size (Table 1), which has enabled the tammar wallabies to retain a large *Ne* since isolation from the mainland population and so reduce the impact of genetic drift [94,97]. Some macropodid populations on other large Australian islands, for example King and Flinders Islands, also show high diversity [98,99], although not the sympatric western grey kangaroo population on Kangaroo Island [78,100]. The now extinct SA mainland tammar wallaby population is therefore likely to have also been highly diverse, maybe even more so than surviving mainland populations in WA. Reduced diversity in WA populations is hypothesised from biogeography, since tammar wallabies are thought to have spread from eastern to western Australia across the arid Nullabor Barrier [77]. Similarly, in the western grey kangaroo, an expansion across southern Australia (although in the opposite direction) resulted in reduced genetic diversity in the more recently colonised population [78,95,100]. However, determining the original levels of diversity in SA and WA mainland tammar wallaby populations prior to their recent decline, and in SA extinction, is now almost impossible due to poor historic sampling.

Conclusions

To date, most studies of tammar wallaby physiology, reproduction, genetics and development have utilised the Kangaroo Island population, and they are now amongst the best known of marsupials [4,11]. We hope that the significant genetic divergence between SA and WA tammar wallabies revealed in this study will now encourage similar detailed investigations of the diverse WA populations, as their relatively long isolation from the well-researched SA population, their larger latitudinal range and greater diversity in body size, salt and 1080 tolerance and habitat [4] is likely to have resulted in the development of alternate strategies and metabolic pathways. For example, the Kangaroo Island tammar wallaby is well known for its highly synchronised breeding linked to the summer solstice [10]. This is one of only two macropodid species that employ both strict seasonal and lactational control of reproductive quiescence. However, the other species, red-necked wallaby (*Notamacropus rufogriseus*), employs different strategies at different latitudes [10]. The extent to which the control of reproduction varies in tammar wallaby populations across their latitudinal range should also be investigated. Access to the tammar genome [13] and advances in Next Generation sequencing technologies will greatly facilitate the identification, characterisation and utility of variant traits in this model organism, which in turn will add significantly to our understanding of macropodid and marsupial evolutionary biology.

Supporting information

S1 Table. Microsatellite genotypes at 16 autosomal loci in ten tammar wallaby (*Notamacropus eugenii*) populations.
(XLSX)

S2 Table. Allele frequencies for 16 autosomal microsatellite loci in ten tammar wallaby (*Notamacropus eugenii*) populations. KI = Kangaroo Island; KwI = Kawau Island, New Zealand; Tut = Tutanning; Per = Perup; GI = Garden Island; EWI = East Wallabi Island; WWI = West Wallabi Island; NI = North Island; MI = Middle Island; NTP = North Twin Peak Island.
(DOCX)

S3 Table. Allelic combinations of the 32 Y haplotypes identified in nine tammar wallaby (*Notamacropus eugenii*) populations. KI = Kangaroo Island; KwI = Kawau Island, New Zealand; Tut = Tutanning; Per = Perup; GI = Garden Island; EWI = East Wallabi Island; WWI = West Wallabi Island; NI = North Island; MI = Middle Island.
(DOCX)

S1 Fig. STRUCTURE output showing a) maximum *L(K)* at K = 7 and b) maximum Δ*K* at K = 8 (b).
(DOCX)

Acknowledgments

Author Contributions

Conceptualization: ME KZ CH.

Data curation: ME EM LN KZ.

Formal analysis: ME EM LN KZ.

Funding acquisition: ME CH.

Investigation: ME EM LN KZ CH.

Project administration: ME EM CH.

Resources: ME KZ CH.

Supervision: ME KZ CH.

Validation: ME EM LN KZ.

Visualization: ME EM LN.

Writing – original draft: ME.

Writing – review & editing: ME EM LN KZ CH.

References

1. Graves JAM, Renfree MB (2013) Marsupials in the age of genomics. Annual Review of Genomics and Human Genetics 14: 393–420. doi: 10.1146/annurev-genom-091212-153452 PMID: 23834319

2. Eldridge MDB (2010) Population and conservation genetics of marsupials. In: Deakin JE, Waters PD, Graves JAM, editors. Marsupial genetics and genomics. Dordrecht: Springer. pp. 461–497.

3. Jackson SM, Groves CP (2015) Taxonomy of Australian mammals. Melbourne: CSIRO Publishing.

4. Eldridge MDB, Coulson GM (2015) Family Macropodidae (kangaroos and wallabies). In: Wilson DE, Mittermeier RA, editors. Handbook of the mammals of the world Volume 5 Marsupials and monotremes. Barcelona: Lynx Edicions. pp. 630–735.

5. Hinds LA, Poole WE, Tyndale-Biscoe CH, van Oorschot RAH, Cooper DW (1990) Reproductive biology and potential for genetic studies in the tammar wallaby, *Macropus eugenii*. Australian Journal of Zoology 37: 223–234.

6. McKenzie LM, Chesson C, Hope RM, Duckworth JA, Meikle LM (1995) Australian marsupials. Tammar wallaby, fat-tailed dunnart and brush-tailed possum. ANZCCART News 8: 1–8.

7. Tyndale-Biscoe CH (2005) Life of marsupials. Melbourne: CSIRO Publishing.

8. Coulson GM, Eldridge MDB, editors (2010) Macropods: the biology of kangaroos, wallabies and rat-kangaroos. Melbourne: CSIRO Publishing.

9. Tyndale-Biscoe CH, Janssens PA, editors (1988) The developing marsupial: models for biomedical research. Berlin: Springer-Verlag.

10. Tyndale-Biscoe CH, Renfree MB (1987) Reproductive physiology of marsupials. Cambridge, UK: Cambridge University Press.

11. Hickford D, Frankenberg S, Renfree MB (2009) The tammar wallaby, *Macropus eugenii*: a model kangaroo for the study of developmental and reproductive biology. Emerging model organisms. Cold Spring Harbor, USA: Cold Spring Harbor Laboratory Press. pp. 449–494.

12. Deakin JE, Waters PD, Marshall Graves JA, editors (2010) Marsupial genetics and genomics. Berlin: Springer.

13. Renfree MB, Papenfuss AT, Deakin JE, Lindsay J, Heider T, et al. (2011) Genome sequence of an Australian kangaroo, *Macropus eugenii*, provides insight into the evolution of mammalian reproduction and development. Genome Biology 12: R81. doi: 10.1186/gb-2011-12-8-r81 PMID: 21854559

14. Hinds LA (2008) Tammar wallaby *Macropus eugenii*. In: Van Dyck S, Strahan R, editors. The mammals of Australia, 3rd edition. Sydney: New Holland. pp. 330–332.

15. Poole WE, Wood JT, Simms NG (1991) Distribution of the tammar, *Macropus eugenii*, and the relationships of populations as determined by cranial morphometrics. Wildlife Research 18: 625–639.

16. Robinson AC, Canty PD, Mooney T, Rudduck P (1996) South Australia's offshore islands. Adelaide: Department of Environment and Natural Resources, South Australia.

17. Kinnear JE, Sumner NR, Onus ML (2002) The red fox in Australia—an exotic predator turned biocontrol agent. Biological Conservation 108: 335–359.

18. King CM, editor (2005) The handbook of New Zealand mammals. Second Edition. Melbourne: Oxford University Press.

19. Abbott I, Burbidge AA (1995) The occurrence of mammal species on the islands of Australia: a summary of existing knowledge. CALMScience 1: 259–324.

20. Main AR (1961) The occurrence of Macropodidae on islands and its climatic and ecological implications. Journal of the Royal Society of Western Australia 44: 84–89.

21. Taylor AC, Cooper DW (1999) Microsatellites identify introduced New Zealand tammar wallabies (*Macropus eugenii*) as an "extinct" taxon. Animal Conservation 2: 41–49.

22. Miller EJ, Eldridge MDB, Morris KD, Zenger KR, Herbert CA (2011) Genetic consequences of isolation: island tammar wallaby (*Macropus eugenii*) populations and the conservation of threatened species. Conservation Genetics 12: 1619–1631.

23. Calaby JH, Richardson BJ (1988) Macropodidae. In: Walton DW, editor. Zoological Catalogue of Australia 5 Mammalia. Canberra: Australian Government Publishing Service. pp. 60–80.

24. Hadley C, Milne N, Schmitt LH (2009) A three-dimensional geometric morphological analysis in cranial size and shape in tammar wallaby (*Macropus eugenii*) populations. Australian Journal of Zoology 57: 337–345.

25. Cooper DW, Holland EA, Rudman K, Donald JA, Zehavi-Feferman R, et al. (1994) Phosphoglycerate kinase pseudogenes in the tammar wallaby and other macopodid marsupials. Mammalian Genome 5: 531–537. PMID: 8000135

26. Taylor AC, Sunnucks P, Cooper DW (1999) Retention of reproductive barriers and ecological differences between two introduced sympatric *Macropus* spp. in New Zealand. Animal Conservation 2: 195–202.

27. MacDonald AJ, Fitzsimmons NN, Chambers B, Renfree MB, Sarre SD (2014) Sex-linked and autosomal microsatellites provide new insights into island populations of the tammar wallaby. Heredity 112: 333–342. doi: 10.1038/hdy.2013.109 PMID: 24169646

28. McKenzie LM, Cooper DW (1997) Hybridization between tammar wallaby (*Macropus eugenii*) populations from Western and South Australia. Journal of Heredity 88: 398–400. PMID: 9378916

29. McKenzie LM, Collet C, Cooper DW (1993) Use of a subspecies cross for efficient development of a linkage map for a marsupial mammal, the tammar wallaby (*Macropus eugenii*). Cytogenetics and Cell Genetics 64: 264–267. PMID: 8404052

30. Zenger KR, McKenzie LM, Cooper DW (2002) The first comprehensive genetic linkage map for a marsupial: the tammar wallaby (*Macropus eugenii*). Genetics 162: 321–330. PMID: 12242243

31. Wang C, Webley L, Wei K, Wakefield MJ, Patel HR, et al. (2011) A second-generation anchored genetic linkage map of the tammar wallaby (*Macropus eugenii*). BMC Genetics 12: 72. doi: 10.1186/1471-2156-12-72 PMID: 21854616

32. Eldridge MDB, Kinnear JE, Zenger KR, McKenzie LM, Spencer PBS (2004) Genetic diversity in remnant mainland and 'pristine' island populations of three endemic Australian macropodids (Marsupialia): *Macropus eugenii*, *Lagorchestes hirsutus* and *Petrogale lateralis*. Conservation Genetics 5: 325–338.

33. DEH (2004) Re-introduction of the mainland SA tammar wallaby to Innes National Park: Translocation Proposal. Sydney: Department of Environment and Heritage, Adelaide, South Australia.

34. Wang C, Deakin JE, Rens W, Zenger KR, Belov K, et al. (2011) A first-generation integrated tammar wallaby map and its use in creating a tammar wallaby first-generation virtual genome map. BMC Genomics 12: 422. doi: 10.1186/1471-2164-12-422 PMID: 21854555

35. Alsemgeest J, Old JM, Young LJ (2013) Molecular characterisation and expression of Interleukin-6 and Interleukin-6Δ2 in the tammar wallaby (*Macropus eugenii*). Veterinary Immunology and Immunopathology 155: 139–145. doi: 10.1016/j.vetimm.2013.06.009 PMID: 23830893

36. Modepalli V, Kumar A, Hinds LA, Sharp JA, Nicholas KR, et al. (2014) Differential temporal expression of milk miRNA during the lactation cycle of the marsupial tammar wallaby (*Macropus eugenii*). BMC Genomics 15: 1–18.

37. Gamat M, Chew KY, Shaw G, Renfree MB (2015) FOXA1 and SOX9 expression in the developing urogenital sinus of the tammar wallaby (*Macropus eugenii*). Sexual Development 9: 216–228. doi: 10.1159/000439499 PMID: 26406875

38. Hetz JA, Menzies BR, Shaw G, Stefanidis A, Cowley MA, et al. (2016) Effects of nutritional manipulation on body composition in the developing marsupial, *Macropus eugenii*. Molecular and Cellular Endocrinology 428: 148–160. doi: 10.1016/j.mce.2016.03.030 PMID: 27032712

39. Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). Molecular Biology and Evolution 13: 510–524. PMID: 8742640

40. Miller EJ, Eldridge MDB, Herbert CA (2010) Dominance and paternity in the tammar wallaby. In: Coulson GM, Eldridge MDB, editors. Macropods: the biology of kangaroos, wallabies and rat-kangaroos. Melbourne: CSIRO Publishing. pp. 77–86.

41. Taylor AC, Cooper DW (1998) A set of tammar wallaby (*Macropus eugenii*) microsatellites tested for genetic linkage. Molecular Ecology 7: 925–926. PMID: 9691494

42. Zenger KR, Cooper DW (2001) A set of highly polymorphic microsatellite markers developed for the eastern grey kangaroo (*Macropus giganteus*). Molecular Ecology Notes 1: 98–100.

43. Zenger KR, Cooper DW (2001) Characterization of 14 macropod microsatellite genetic markers. Animal Genetics 32: 160–167.

44. Miller EJ, Eldridge MDB, Cooper DW, Herbert CA (2010) Dominance, body size and internal relatedness influence male reproductive success in eastern grey kangaroos (*Macropus giganteus*). Reproduction, Fertility, and Development 22: 539–549. doi: 10.1071/RD09061 PMID: 20188027

45. MacDonald AJ, Sankovic N, Sarre SD, Fitzsimmons NN, Wakefield MJ, et al. (2006) Y chromosome microsatellite markers identified from the tammar wallaby (*Macropus eugenii*) and their amplification in three other macropod species. Molecular Ecology Notes 6: 1202–1204.

46. Fumagalli L, Pope LC, Taberlet P, Moritz C (1997) Versatile primers for the amplification of the mitochondrial DNA control region in marsupials. Molecular Ecology 6: 1199–1201. PMID: 9421920

47. Sunnucks P, Wilson AC, Beheregaray LB, Zenger K, French J, et al. (2000) SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. Molecular Ecology 9: 1699–1710. PMID: 11091307

48. Browning TL, Taggart DA, Rummery C, Close RL, Eldridge MDB (2001) Multifaceted genetic analysis of the "Critically Endangered" brush-tailed rock-wallaby *Petrogale penicillata* in Victoria, Australia: Implications for management. Conservation Genetics 2: 145–156.

49. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24: 4876–4882.

50. Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8: 103–106. doi: 10.1111/j.1471-8286.2007.01931.x PMID: 21585727

51. Rice W (1989) Analysing tables of statistical tests. Evolution 43: 223–225.

52. Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86: 485–486.

53. Frankham R (1998) Inbreeding and extinction: Island populations. Conservation Biology 12: 665–675.

54. Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288–295.

55. Peakall R, Smouse PE (2012) GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28: 2537–2539. doi: 10.1093/bioinformatics/bts460 PMID: 22820204

56. Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358–1370.

57. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–959. PMID: 10835412

58. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. Molecular Ecology 14: 2611–2620. doi: 10.1111/j.1365-294X.2005.02553.x PMID: 15969739

59. Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4: 359–361.

60. Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Molecular Ecology Notes 4: 137–138.

61. Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16: 37–48. PMID: 10331250

62. Polzin T, Daneshmand SV (2003) On Steiner trees and minimum spanning trees in hypergraphs. Operations Research Letters 31: 12–20.

63. Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564–567. doi: 10.1111/j.1755-0998.2010.02847.x PMID: 21565059

64. Swofford DL (2000) PAUP*. Phylogenetic analysis using parsimony (*and other methods). 4.0 (beta 8) ed. Sunderland, Massachusetts: Sinauer Associates Inc.

65. Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics Applications 14: 817–818.

66. Neaves LE, Zenger KR, Cooper DW, Eldridge MDB (2010) Molecular detection of hybridization between sympatric kangaroo species in south-eastern Australia. Heredity 104: 502–512. doi: 10.1038/hdy.2009.137 PMID: 19812615

67. Spencer PBS, Rhind SG, Eldridge MDB (2001) Phylogeographic structure within *Phascogale* (Marsupialia: Dasyuridae) based on partial cytochrome *b* sequence. Australian Journal of Zoology 49: 369–377.

68. Crowther MS, Spencer PBS, Alpers D, Dickman CR (2002) Taxonomic status of the mardo, *Antechinus flavipes leucogaster* (Marsupialia: Dasyuridae): morphological, molecular, reproductive and bioclimatic approach. Australian Journal of Zoology 50: 627–647.

69. Zenger KR, Eldridge MDB, Johnston PG (2005) Phylogenetics, population structure and genetic diversity of the endangered southern brown bandicoot (*Isoodon obesulus*) in south-eastern Australia. Conservation Genetics 6: 193–204.

70. Salinas M, Bunce M, Cancilla D, Alpers DL, Spencer PBS (2009) Divergent lineages in the heath mouse (*Pseudomys shortridgei*) are indicative of major contraction to geographically isolated refugia on the eastern and western sides of Australia during the early Pleistocene. Australian Journal of Zoology 57: 41–47.

71. Donnellan SC, Armstrong J, Pickett M, Milne T, Baulderstone J, et al. (2009) Systematic and conservation implications of mitochondrial DNA diversity in emu-wrens, *Stipiturus* (Aves: Maluridae). Emu 109: 143–152.

72. Toon A, Mather PB, Baker AM, Durrant KL, Hughes JM (2007) Pleistocene refugia in an arid landscape: analysis of a widely distributed Australian passerine. Molecular Ecology 16: 2525–2541. doi: 10.1111/j.1365-294X.2007.03289.x PMID: 17561911

73. Toon A, Hughes JM, Joseph L (2010) Multilocus analysis of honeyeaters (Aves: Meliphagidae) highlights spatio-temporal heterogeneity in the influence of biogeographic barriers in the Australian monsoonal zone. Molecular Ecology 19: 2980–2994. doi: 10.1111/j.1365-294X.2010.04730.x PMID: 20609078

74. Keogh JS, Scott IAW, Hayes C (2005) Rapid and repeated origin of insular gigantism and dwarfism in Australian tiger snakes. Evolution 59: 226–233. PMID: 15792242

75. Ford J (1987) Hybrid zones in Australian birds. Emu 87: 158–178.

76. Keast JA (1961) Bird speciation on the Australian continent. Bulletin of the Museum of Comparative Zoology 123: 303–495.

77. Oliver AJ, King DR, Mead RJ (1979) Fluoroacetate tolerance, a genetic marker in some Australian mammals. Australian Journal of Zoology 27: 363–372.

78. Neaves LE, Zenger KR, Prince RIT, Eldridge MDB (2012) Impact of Pleistocene aridity oscillations on the population history of a widespread, vagile Australian mammal, *Macropus fuliginosus*. Journal of Biogeography 39: 1545–1563.

79. Eldridge MDB, Wilson AC, Metcalfe CJ, Dollin AE, Bell JN, et al. (2001) Taxonomy of rock-wallabies, *Petrogale* (Marsupialia: Macropodidae). III. Molecular data confirms the species status of the purple-necked rock-wallaby *Petrogale purpureicollis* Le Souef 1924. Australian Journal of Zoology 49: 323–343.

80. Telfer WR, Eldridge MDB (2010) High levels of mitochondrial DNA divergence within short-eared rock-wallaby (*Petrogale brachyotis*) populations in northern Australia. Australian Journal of Zoology 58: 104–112.

81. Kirsch JAW, Poole WE (1972) Taxonomy and distribution of the grey kangaroos *Macropus giganteus* Shaw and *Macropus fuliginosus* (Desmarest), and their subspecies (Marsupialia: Macropodidae). Australian Journal of Zoology 20: 315–339.

82. Poole WE, Catling PC (1974) Reproduction in the two species of grey kangaroos, *Macropus giganteus* Shaw and *M. fuliginosus* (Desmarest). I. Sexual maturity and oestrus. Australian Journal of Zoology 22: 277–302. PMID: 4475649

83. Mayr E (1963) Animal species and evolution. Cambridge, MA: Harvard University Press.

84. Frankham R, Ballou JD, Eldridge MDB, Lacy RC, Ralls K, et al. (2011) Predicting the probability of out-breeding depression. Conservation Biology 25: 465–475. doi: 10.1111/j.1523-1739.2011.01662.x PMID: 21486369

85. Frankham R, Ballou JD, Dudash MR, Eldridge MDB, Fenster CB, et al. (2012) Implications of different species concepts for conserving biodiversity. Biological Conservation 153: 25–31.

86. Moritz C (1994) Defining "Evolutionary Significant Units" for conservation. Trends in Ecology and Evolution 9: 373–375. doi: 10.1016/0169-5347(94)90057-4 PMID: 21236896

87. Aplin KP, Rhind SG, Ten Have J, Chesser RT (2015) Taxonomic revision of *Phascogale tapoatafa* (Meyer, 1793) (Dasyuridae; Marsupialia), including descriptions of two new subspecies and confirmation of *P. pirata* Thomas, 1904 as a 'Top End' endemic. Zootaxa 4055: 1–73. doi: 10.11646/zootaxa.4055.1.1 PMID: 26701461

88. Schodde R, Mason IJ (1999) The directory of Australian birds. Collingwood, Victoria.: CSIRO Publishing.

89. Menkhorst PW, Knight F (2011) A field guide to the mammals of Australia. Third Edition. Melbourne: Oxford University Press.

90. Frankham R, Ballou JD, Ralls K, Eldridge MDB, Dudash MR, et al. (2017) Genetic management of fragmented animal and plant populations Oxford, UK: Oxford University Press.

91. How RA, Spencer PBS, Schmitt LH (2009) Island populations have high conservation value for northern Australia's top marsupial predator ahead of a threatening process. Journal of Zoology, London 278: 206–217.

92. Maxwell S, Burbidge AA, Morris K, editors (1996) The 1996 Action Plan for Australian Marsupials and Monotremes. Canberra: Wildlife Australia.

93. Wright M, Stott P (1999) The Kangaroo Island tammar wallaby: assessing ecologically sustainable commercial harvesting. Canberra: Rural Industries Research and Development Corporation.

94. Frankham R (1997) Do island populations have less genetic variation than mainland populations? Heredity 78: 311–327. PMID: 9119706

95. Neaves LE, Zenger KR, Prince RIT, Eldridge MDB (2013) Paternally inherited genetic markers reveal new insights into genetic structuring within *Macropus fuliginosus* and hybridisation with sympatric *Macropus giganteus.* Australian Journal of Zoology 61: 58–68.

96. Frankham R (2012) How closely does genetic diversity in finite populations conform to predictions of neutral theory? Large deficits in regions of low recombination. Heredity 108: 167–178. doi: 10.1038/hdy.2011.66 PMID: 21878983

97. Frankham R, Ballou JD, Briscoe DA (2010) Introduction to conservation genetics. Second edition. Cambridge, UK: Cambridge University Press.

98. Le Page SL, Livermore RA, Cooper DW, Taylor AC (2000) Genetic analysis of a documented population bottleneck: introduced Bennett's wallabies (*Macropus rufogriseus rufogriseus*) in New Zealand. Molecular Ecology 9: 753–763. PMID: 10849291

99. Macqueen P, Goldizen AW, Seddon JM (2009) Response of a southern temperate marsupial, the Tasmanian pademelon (*Thylogale billardierii*), to historical and contemporary forest fragmentation. Molecular Ecology 18: 3291–3306. doi: 10.1111/j.1365-294X.2009.04262.x PMID: 19573029

100. Neaves LE, Zenger KR, Prince RIT, Eldridge MDB, Cooper DW (2009) Landscape discontinuities influence gene flow and genetic structure in a large, vagile Australian mammal, *Macropus fuliginosus.* Molecular Ecology 18: 3363–3378. doi: 10.1111/j.1365-294X.2009.04293.x PMID: 19659477