

Supplementary Methods

Microsatellite Development and Cross-species utility

Methods

We screened 23 primer sets developed from eight bird species, including five species from the Petroicidae and three from other families (19 novel primers, plus Pau2 *Petroica australis* (Townsend et al. 2012), TG02088 *Taeniopygia guttata* (Dawson et al. 2010), Ase18 *Acrocephalus sechellensis* (Richardson et al. 2000), Ppi2 *Pica pica* (Martinez et al. 1999)

To identify novel microsatellite loci, DNA was extracted from frozen tissue of each robin species using the DNeasy blood and tissue DNA extraction kit (QIAGEN). A total of 16.3 µg of RNase-treated genomic DNA was used in 1/8 of a plate for pyrosequencing by an external service provider, the Australian Genome Research Facility (www.agrf.com.au), on a Roche GL FLX (454) system. We used the program QDD (Meglécz et al. 2009), to detect microsatellites and subsequently to design primers. We identified 1,412 sequences that contained putative microsatellite motifs with a minimum of 5 repeats and which had sufficiently-long flanking regions free of nanosatellites for which primers could be designed. We selected 19 loci (based on repeat length, repeat motif and PCR product size) for genotyping, and optimized them into three multiplex panels (Supp Table 2). Eight loci were chosen from the Flame Robin (*Petroica phoenicea*) (our species of special interest), four each from the congeneric Scarlet Robin (*P. boodang*) and Red-capped Robin (*P. goodenovii*), and three from the confamilial Eastern Yellow Robin (*Eopsaltria australis*).

To evaluate cross-amplification amongst different species of *Petroicidae*, blood samples were collected from the brachial vein (Owen 2011) of 25 Flame Robins, 20 Red-capped Robins, 18 Scarlet Robins and 31 Eastern Yellow Robins (including both mitochondrial

clades (Pavlova et al. 2013). A further 45 samples of *P. phoenicea* were collected from four locations spanning 690 km of the species range (see Table 1. for locations and sample sizes). Samples were stored at -20 °C prior to DNA extraction. All samples were collected under approval of Deakin University Animal Ethics Committee A58-2011, Victoria Department of Environment, Land, Water and Planning Scientific Permit 10005964, Australian Museum Animal Ethics Approval 12-03 and New South Wales Scientific Licence SL100886.

DNA samples were sent to the Australian Genome Research Facility, Melbourne, for genotyping. All forward primers incorporated a fluorescent label (FAM, VIC, NED or PET) to allow multiplexed PCR products to be run on an AB 3730xl Sequencer (Applied Biosystems). Genotypes were scored independently by two individuals using GeneMapper version 1.4 (Applied Biosystems). MICROCHECKER 2.2 (Van Oosterhout et al. 2004) was used to check for the presence of null alleles. In addition, significant departures from Hardy-Weinberg and linkage disequilibrium (LD) for each locus site combination were calculated in *Arlequin* version 3.5.1.2 (Excoffier and Lischer 2010). Significance of tests for each locus or site were assessed using sequential Bonferroni correction for multiple tests (Rice, 1989).

Results

Not surprisingly, markers amplified best for the species for which they were designed (Supp table 4), with all except one marker (Ppho7) amplifying for its target species. Interestingly, Ppho7 amplified and was variable in two conspecifics, *P. goodenovii* and *P. boodang*. The three markers designed for the confamilial *Eopsaltria australis* did not amplify in any of the *Petroica* species, but at least one marker designed for each *Petroica* species amplified in *E. australis*.

Of the 23 markers we tested for cross amplification, 16 markers amplified for *Petroica phoenicea*, 14 for *P. boodang*, 13 for *P. goodenovii*, and 8 for *Eopsaltria australis* (Supp table 4). The three markers designed for species in other avian families amplified for at least one robin species but tended to have low variability, especially TGO2088. Ase18 amplified in all robin species, and while Ppi2 did not amplify in three species, it amplified and was highly variable in *P. goodenovii*, having 28 alleles. These results are not surprising, given the impact of the divergence times of these species. *Eopsaltria* is very divergent from the rest of *Petroica* (Loynes et al 2009), and Red-capped robins and Scarlet/Flame robins are quite deeply divergent (around 5.3-7.29 mya based on 2 mtDNA loci and 5 nuclear introns, Kearns et al 2019).

Supplementary Literature Cited

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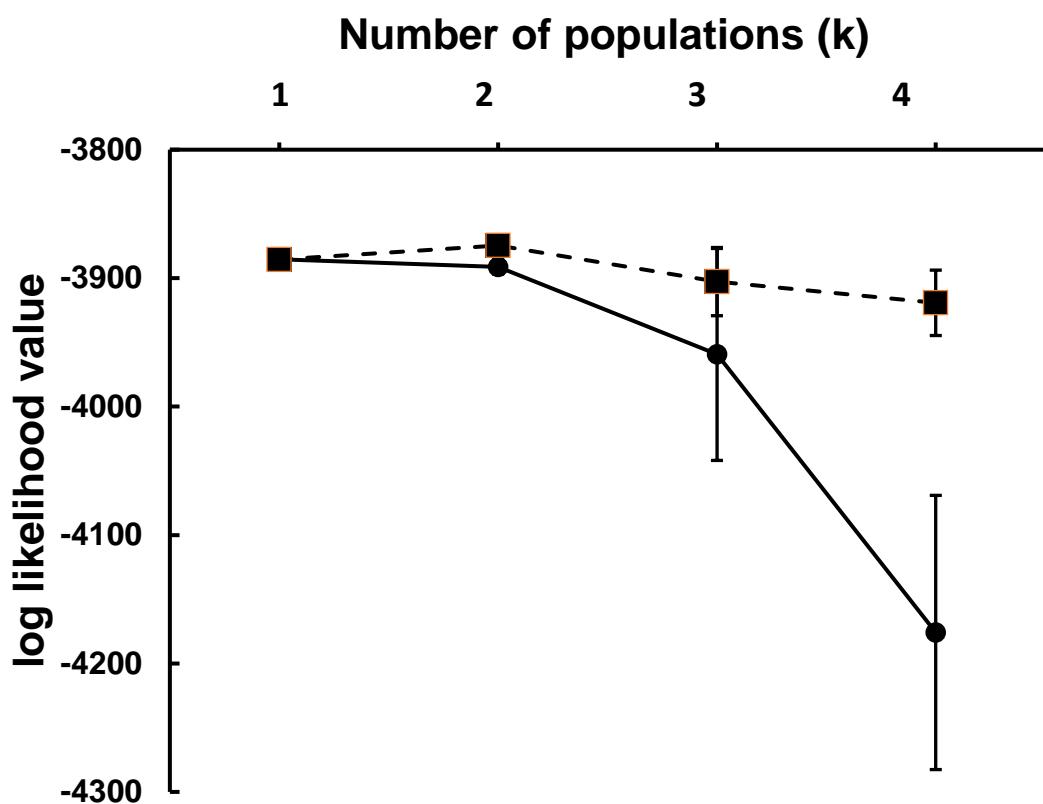
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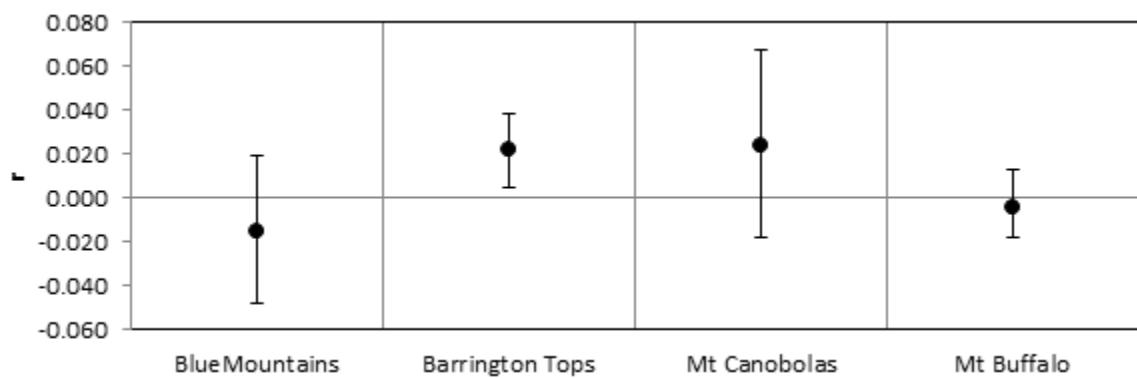
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Supplementary Figure 1. Log likelihood values (mean \pm s.d.) of ten simulations of two model population genetic structures ranging between one and four genetic clusters (K). Models using non-informative locality priors are indicated by filled circles and solid lines. Models using informative locality priors are indicated by filled squares and dashed lines.



Supplementary Figure 2. Mean within site pairwise relatedness values. Dots denote mean within site relatedness, with 95% confidence intervals.

1 **Supplementary Table 1.** Study site, latitude and longitude, distance to closest nearest site, sampling dates, sample size by sex and age and
2 total sample size for the four study sites for *Petroica phoenicea* (Flame Robin).

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Study Site	Latitude / Longitude	Distance to next nearest site	Sampling dates	Sex and age	Total N
Mount Buffalo, VIC	-37.73 S, 146.80 E	530 km	Nov. 2011	Adult female, N = 3	28
			Oct. 2012 – Jan. 2013	Adult male, N = 8	
			Oct. 2013 – Jan. 2014	*Chick in the nest (sex unknown), N = 17	
Barrington Tops, NSW	-31.94 S, 151.44 E	240 km	Nov. 2012	Adult female, N = 2	22
Blue Mountains, NSW	-33.75 S, 150.04 E	108 km	Sept. 2012 Oct. 2013 Nov. 2014	Adult female, N = 3 Adult male, N = 8	11
Mount Canobolas, NSW	-33.35 S, 148.98 E	108 km	Oct. 2014	Adult female, N = 2 Adult male, N = 8	10

4 *Only one chick was sampled per nest. No chicks of sampled adults were included.

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16 **Supplementary Table 2.** Nineteen novel microsatellite isolated from four Australian robin species (*Eopsaltria australis* (EYR), *Petroica phoenicea*
 17 (FR), *Petroica goodenovii* (RCR), *Petroica boodang* (SR)). NA: No amplification or non-specific amplification. Size range refers to allele size in base
 18 pairs.
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Marker Name	Source species	Primer Sequences (5' to 3') (F=Forward, R=Reverse)		Motif	Size range			
		EYR	FR		RCR	SR		
Eaus1	<i>Eopsaltria australis</i> - Eastern Yellow Robin	F – PET-GGTCTTTATGAGCCTGCCAC R – CCAGACTTGCATTGCTTTCA	(AGAT) ₁₃	224-252	NA	NA	NA	NA
Eaus2	<i>Eopsaltria australis</i> - Eastern Yellow Robin	F – PET-CTCAACTGCTGCTTGTCAG R – GTCCATCCATCCTCCATCC	(TGGA) ₁₃	109-201	NA	NA	NA	NA
Eaus3	<i>Eopsaltria australis</i> - Eastern Yellow Robin	F – VIC-TGCTGTAGGTCCCTTCCAAC R – CAGGCTGGGTTTCTAGCTG	(TCTATT) ₁₉	184-274	NA	NA	NA	NA
Pboo1	<i>Petroica boodang</i> - Scarlet Robin	F – NED-AGCATTGAGAATCCACCAGG R – AGCATTGAGAATCCACCAGG	(AAAC) ₉	NA	117-212	109-113	113-129	
Pboo2	<i>Petroica boodang</i> - Scarlet Robin	F – PET-AGCATTGAGAATCCACCAGG R – AGCATTGAGAATCCACCAGG	(AT) ₁₃	NA	115-131	113-143	117-133	
Pboo3	<i>Petroica boodang</i> - Scarlet Robin	F – NED-AGCATTGAGAATCCACCAGG R – AGCATTGAGAATCCACCAGG	(AC) ₁₁	111-149	111-135	127-149	109-125	
Pboo4	<i>Petroica boodang</i> - Scarlet Robin	F – FAM-GCTGCTGGGTTGATTGTT R – ACACCCCTAGAACCTGGTCA	(GCCTT) ₁₄	NA	NA	NA	248-283	
Pgood1	<i>Petroica goodenovii</i> – Red-capped Robin	F – PET-AGCATTGAGAATCCACCAGG R – AGCATTGAGAATCCACCAGG	(AGT) ₁₆	160-316	196-264	208-253	205-287	
Pgood2	<i>Petroica goodenovii</i> – Red-capped Robin	F – FAM-TGTTTGTGAAAGCTGCAAG R – TCCTCTGTAGATCAAGTGTGTT	(TA) ₁₄	NA	NA	169-199	NA	
Pgood3	<i>Petroica goodenovii</i> – Red-capped Robin	F – PET-TACGAACTGCAGACTTGCG R – TCTGGCTGTAGCATGACAAT	(AC) ₂₁	NA	126-176	148-184	144-180	
Pgood4	<i>Petroica goodenovii</i> – Red-capped Robin	F – VIC-ATACCTGCTGCAGACCGTG R – GCATACGGATGATGTCCAAA	(GGAT) ₁₀	NA	145-173	145-173	NA	

Ppho1	<i>Petroica phoenicea</i> - Flame Robin	F – FAM-AGCATTGAGAATCCACCAGG R – AGCATTGAGAATCCACCAGG	(AAAG) ₁₈	NA	252- 442	212	210- 458
Ppho2	<i>Petroica phoenicea</i> - Flame Robin	F – NED-AGCATTGAGAATCCACCAGG R – AGCATTGAGAATCCACCAGG	(AAT) ₁₃	NA	164- 188	149- 200	NA
Ppho3	<i>Petroica phoenicea</i> - Flame Robin	F – FAM-AGCATTGAGAATCCACCAGG R – AGCATTGAGAATCCACCAGG	(AC) ₁₅	NA	187- 209	183- 189	193- 221
Ppho4	<i>Petroica phoenicea</i> - Flame Robin	F – VIC-AGCATTGAGAATCCACCAGG R – AGCATTGAGAATCCACCAGG	(AC) ₁₄	209	197- 219	209	203- 209
Ppho5	<i>Petroica phoenicea</i> - Flame Robin	F – FAM-GATGCCTGTAGTCTTCCCCA R – GAAGTCAAAATTGCAGGGCT	(AGT) ₁₅	NA	113- 152	NA	116- 149
Ppho6	<i>Petroica phoenicea</i> - Flame Robin	F – NED-CAGCTGAGGAAGGATCTTGG R – TCATGTCCCTCTTCAAACCA	(AGGT) ₁₁	214	249- 281	257	269- 285
Ppho7	<i>Petroica phoenicea</i> - Flame Robin	F – NED-CTCTGGGAGCACTGGGTCT R – TAGCACCGGCAGGAATAGTT	(AGGT) ₁₀	NA	NA	154- 182	154- 186
Ppho8	<i>Petroica phoenicea</i> - Flame Robin	F – FAM-CTGCTGAAATGGAGTGGTCT R – AGAGATGGATAGCTGGACGG	(AGGT) ₁₀	NA	112- 228	192	NA

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24 **Supplementary Table 3.** Pairwise F_{ST} comparisons of the four study sites for the Flame Robin, calculated using *Arlequin*. Larger values indicate
25 greater distinctiveness. * denotes significantly different from zero at $p<0.001$.

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	Barrington Tops	Blue Mountains	Mt Buffalo
Barrington Tops	-		
Blue Mountains	0.0047	-	
Mt Buffalo	0.0223*	0.0001	-
Mt Canobolas	0.0255	0.0114	0.0063

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28 **Supplementary Table 4.** Microsatellite markers characterised for four Australian robin species (*Eopsaltria australis*, *Petroica phoenicea*, *Petroica*
 29 *goodenovii*, *Petroica boodang*). The number of individuals genotyped (N), number of alleles (N_A), observed heterozygosity (H_O), expected
 30 heterozygosity (H_E), inbreeding coefficient (F_{IS}) and the Polymorphic Information Content (PIC) for each locus are given. *denotes significance at
 31 5% level following Bonferroni correction.

Marker Name	<i>Eopsaltria australis</i>						<i>Petroica phoenicea</i>					
	N	N _A	H _O	H _E	F _{IS}	PIC	N	N _A	H _O	H _E	F _{IS}	PIC
Eaus1	31	9	0.81	0.82	0.03	0.79	-	-	-	-	-	-
Eaus2	31	17	0.94	0.89	-0.04	0.88	-	-	-	-	-	-
Eaus3	31	17	0.68	0.92	0.28*	0.91	-	-	-	-	-	-
Pboo1	-	-	-	-	-	-	24	6	0.29	0.57	0.51*	0.53
Pboo2	-	-	-	-	-	-	24	8	0.67	0.80	0.19	0.78
Pboo3	29	7	0.86	0.73	-0.17	0.68	25	11	0.76	0.86	0.13	0.84
Pboo4	-	-	-	-	-	-	-	-	-	-	-	-
Pgood1	29	19	0.72	0.90	0.21*	0.89	24	13	0.58	0.77	0.27	0.75
Pgood2	-	-	-	-	-	-	-	-	-	-	-	-
Pgood3	-	-	-	-	-	-	16	15	0.69	0.86	0.23	0.85
Pgood4	-	-	-	-	-	-	16	8	0.13	0.82	0.86*	0.80
Ppho1	-	-	-	-	-	-	25	30	0.84	0.93	0.12	0.93
Ppho2	-	-	-	-	-	-	25	10	0.40	0.83	0.53*	0.81
Ppho3	-	-	-	-	-	-	25	14	0.84	0.85	0.03	0.83
Ppho4	31	1	-	-	-	-	25	9	0.72	0.82	0.14	0.80
Ppho5	-	-	-	-	-	-	25	9	0.76	0.81	0.16	0.78
Ppho6	29	1	-	-	-	-	21	9	0.67	0.77	0.08	0.74
Ppho7	-	-	-	-	-	-	-	-	-	-	-	-
Ppho8	-	-	-	-	-	-	23	12	0.35	0.89	0.62*	0.88
Ase18	31	5	0.65	0.77	0.17	0.73	25	6	0.48	0.57	0.17	0.53
PAU2	29	5	0.17	0.42	0.60*	0.39	24	14	0.92	0.89	-0.01	0.88
Ppi2	-	-	-	-	-	-	-	-	-	-	-	-
TG02088	30	6	0.37	0.51	0.29*	0.48	10	3	0.40	0.34	-0.14	0.30

Marker	<i>Petroica goodenovii</i>							<i>Petroica boodang</i>						
	Name	N	N _A	H _O	H _E	F _{IS}	PIC	N	N _A	H _O	H _E	F _{IS}	PIC	
Eaus1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eaus2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eaus3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pboo1	19	3	0.11	0.10	-0.01	0.99		17	5	0.29	0.48	0.41	0.45	
Pboo2	15	11	0.67	0.85	0.25	0.88		13	9	1.00	0.83	-0.17	0.81	
Pboo3	20	9	0.75	0.81	0.10	0.78		17	6	0.65	0.73	0.15	0.70	
Pboo4	-	-	-	-	-	-		15	8	0.27	0.82	0.69*	0.80	
Pgood1	18	13	0.89	0.89	0.03	0.88		18	13	0.83	0.80	-0.01	0.78	
Pgood2	11	12	0.82	0.90	0.14	0.90		-	-	-	-	-	-	
Pgood3	14	12	0.71	0.82	0.17	0.81		16	9	0.56	0.62	0.13	0.60	
Pgood4	12	12	0.42	0.89	0.56*	0.88		-	-	-	-	-	-	
Ppho1	20	1	-	-	-	-		17	19	0.88	0.89	0.04	0.89	
Ppho2	20	14	0.65	0.90	0.30*	0.89		-	-	-	-	-	-	
Ppho3	20	4	0.75	0.59	-0.25	0.53		18	12	0.78	0.89	0.16	0.88	
Ppho4	20	1						18	4	0.33	0.56	0.43	0.47	
Ppho5	-	-	-	-	-	-		18	12	1.00	0.87	-0.12	0.86	
Ppho6	20	1	-	-	-	-		18	5	0.50	0.66	0.27	0.60	
Ppho7	12	9	0.42	0.85	0.54*	0.83		15	8	0.60	0.84	0.32	0.82	
Ppho8	18	1	-	-	-	-		-	-	-	-	-	-	
Ase18	20	6	0.65	0.63	-0.01	0.59		18	16	0.94	0.85	-0.08	0.84	
PAU2	20	7	0.30	0.27	-0.07	0.27		17	9	0.65	0.86	0.28	0.85	
Ppi2	20	28	0.80	0.95	0.19	0.95		-	-	-	-	-	-	
TG02088	-	-	-	-	-	-		-	-	-	-	-	-	

Supplementary Table 5. FST mean values, range and significance estimated under different scenarios (Simulations) of isolation (Generations separated), migration rate and sampling (Sample size).

Simulation	Sample size	Generations separated	Migration rate	FST (mean)	FST (range)	Proportion significant
1	10	30	0	0.0057	0.002-0.01	0.4
2	10	30	0.01	0.0006	-0.0052-0.0054	0
3	10	30	0.1	0.0039	0.0007-0.0091	0
4	10	100	0	0.0101	0.0016-0.0213	0.8
5	10	100	0.01	0.0023	-0.0084-0.011	0.2
6	10	100	0.1	0.0041	-0.0016-0.0084	0.2
7	25	30	0	0.0047	0.0009-0.0077	0.8
8	25	30	0.01	0.0015	-0.0014-0.0044	0.4
9	25	30	0.1	0.0006	-0.0020-0.0025	0
10	25	100	0	0.0121	0.0070-0.0153	1
11	25	100	0.01	0.0039	-0.0018-0.0073	0.6
12	25	100	0.1	0.0016	-0.0006-0.0041	0.2

Supplementary Table 6. Means and 95% confidence intervals of the posterior distributions for migration rates between sites per generation. Source sites are shown on the vertical and recipient on the horizontal. Values on the diagonal represent the proportion of that are not migrants.

	Barrington Tops	Mt Buffalo
Barrington Tops	0.93 (0.88-0.99)	0.06 (0.01-0.12)
Mt Buffalo	0.28 (0.24-0.33)	0.72 (0.67-0.76)