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Communications

Genetic monogamy in two long-lived New Zealand passerines

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High rates of extra-pair paternity (EPP) can be relatively common in passerines whereas low rates or absence of EPP are often associated with taxa that are long-lived and exhibit obligatory paternal care. We examined EPP in an under-represented category: passerine species with relatively long life spans (or low annual mortality rates). Specifically, we studied EPP in New Zealand saddlebacks *Philesturnus carunculatus* and robins *Petroica australis*, two species with unusually low annual mortality rates (6.5–11% and 10–20% respectively). No EPP (0%) was detected in saddlebacks (39 pairs, 202 offspring) and only one case of EPP (1.9%) was detected in robins (54 pairs, 198 offspring). Genetic monogamy in these passerine species supports the hypothesis that low annual mortality rates play an important role in explaining variation in rates of EPP across species.

The detection and frequency of extra-pair paternity (EPP) in avian species is so widespread (e.g. >86% of all socially monogamous passerine species are not genetically monogamous; Griffith et al. 2002) and its benefits so varied (e.g. Petrie and Kempenaers 1998, Tregenza and Wedell 2000, Griffith et al. 2002, Foerster et al. 2003), that recent research has tried to identify factors explaining its absence in some species (Arnold and Owens 2002, Griffith et al. 2002). More than 50% of inter-specific variation in EPP can be explained by phylogeny, making the identification of particular traits difficult (Owens and Bennett 1995, Arnold and Owens 2002, Griffith et al. 2002). Nevertheless, the degree of paternal care required and low annual mortality rates (i.e. long life spans) appear to be key predictors of genetic monogamy (Arnold and Owens 2002, Griffith et al. 2002). The need for paternal care to successfully raise offspring affects whether a female will seek EPP and risk reduced effort or even desertion by her social mate. Females that can rear offspring with little or no paternal care (e.g. many passerines), should be likely to seek EPP (Griffith et al. 2002). If paternal care is essential (e.g. seabirds), rates of EPP should be low. These theoretical predictions are borne out by comparative analyses that have controlled for the effect of phylogeny (Arnold and Owens 2002).

Similarly, phylogeny-based methods using evolutionarily independent contrasts show that longevity explains 25% of the variation in rates of EPP across species (Arnold and Owens 2002, Griffith et al. 2002). Males of short-lived species should stay with their mate, even when paternity is uncertain, because the probability of finding a new mate and breeding again is low. In contrast, males of long-lived species should never tolerate EPP because they have many future opportunities in which to find new mates. Indeed, inter-specific analyses demonstrate that species with annual mortality rates below 30% usually have EPP rates below 20% (Mauck et al. 1999).

Phylogeny-based analyses have been constrained by limited data for species with low annual mortality rates. Most documented EPP for long-lived species exists for non-passerines, especially species in which longevity and obligatory paternal care co-occur (Appendix A in Arnold and Owens 2002). Therefore correlations between longevity and genetic monogamy are biased by certain taxonomic groups and relatively little information is available for passerines, a group that is usually short-lived. In their dataset, Arnold and Owens (Appendix A, 2002) listed only three passerine species with mortality rates below 20%, while the remaining 39 species had values well above this level.

In New Zealand, saddlebacks *Philesturnus carunculatus* and robins *Petroica australis* are relatively long-lived passerines when introduced predators are absent. Saddlebacks are known to live for 17–21 years (Higgins et al. 2006) with estimated annual adult mortality ranging between 6.5–11% (Armstrong et al. 2005, I. Jamieson unpubl. data). Robins can live up to 14 years (D. Armstrong unpubl. data) with annual adult mortality rates between 10–20% (Dimond and Armstrong 2007, I. Jamieson unpubl. data). We examined the frequency of EPP in saddlebacks and robins over several seasons and for robins, across several populations. We predict that low annual mortality rates in both passerine species should result in genetic monogamy.

Methods

Study species

Saddlebacks (80 g; Callaeidae), and robins (30 g; Petroicidae) are non-migratory, remain resident on their breeding territories all year and become sexually mature in their first year, although saddlebacks do not normally breed until their second year. Each has a modal clutch size of two eggs (range 1–3) and normally lays 1–2 clutches per season (Higgins and Peter 2002, Higgins et al. 2006, I. Jamieson unpubl. data). Both are socially monogamous with high mate retention from one season to the next. Males provide care by feeding females during incubation as well as the nestlings and fledglings after hatch (Higgins and Peter 2002, Higgins et al. 2006). It is unknown whether paternal care is required to successfully raise young in these species.

Study areas

Data on EPP were collected for saddlebacks from Ulva Island and for robins from Ulva Island, the Doubtful Islands, and the Eglinton Valley (a mainland site). Ulva Island is a 270 ha reserve located near Stewart Island, where saddlebacks (n = 30) and robins (n = 20) were reintroduced in 2000 and 2001 after Norway rats *Rattus norvegicus* had been eradicated in 1996. The three Doubtful Islands (130 ha, 25 ha and 137 ha) are located in Lake Te Anau and similarly had 36 reintroduced robins in 2002 and 2003. The Eglinton Valley is a mainland site located in Fiordland National Park with one of the largest populations of robins on the South Island (Heather and Robertson 1996).

Field methods

With few exceptions, all saddlebacks on Ulva Island and all robins on Ulva and the Doubtful Islands were banded and sampled for blood (Table 1). In the Eglinton Valley, a subset of 30 pairs was captured for sampling (Table 1). Each bird was banded with a numbered metal band and a unique combination of colour bands for individual identification. Blood samples (100 µl) were taken from the brachial vein and stored in lysis buffer (Seutin et al. 1991). Birds were captured in mist-nets (saddlebacks and robins), handnets, cage traps, and claptraps (robins only) or at the nest (nestlings). All nests were monitored every 2–4 d and nestlings were banded and sampled approximately 4 d before fledging. Where nests were too high to access, offspring were caught once they had fledged but were still being fed by their parents.

Lab methods

DNA was extracted using proteinase K (10 mg/ml) in a Chelex 100 Resin solution (50 mg/ml). PCR reactions were 10 µl and contained 1 µl DNA, 0.5 µM of each primer, 0.8 µM dNTP, 1 µl buffer, 0.5 U *Taq* DNA polymerase (AB Gene), an optimized concentration of $MgCl_2$, and for primers that produced shadow bands, 2.2 µl betaine (5.0 M) and 0.2 µl DMSO. The PCR profile consisted of denaturation at 92° C for 3 min, followed by 35 cycles at annealing temperature for 30 s, 72° C for 1 min, and 92° C for 1 min followed by one final annealing step for 30 s and extension at 72° C for 4 min. DNA fragments were examined on 6–10% vertical, non-denaturing or 6% denaturing polyacrylamide gels. For denaturing gels, 10 pmols of reverse primers were radioactively end-labelled in 10 µl reaction volumes containing 5 µCi of [γ^{33}P-ATP], 2.5 units T4 polynucleotide kinase (Bioline), and 1X kinase buffer (Bioline). Individuals expressing all known alleles were run on every gel as size standards and on non-denaturing gels, molecular rulers (10 or 20 bp ladders) were used as additional size standards. Saddlebacks and robins were genotyped at 12 and 10 microsatellite loci respectively, each with 2 to 9 alleles (Table 2). Further details on genetic analyses are in Taylor et al. (2007) and Boessenkool et al. (2007).

Table 1. The occurrence of EPP in saddlebacks and robins across years and populations.

Species	Population	Year	No. of pairs	No. of offspring	EPP
Saddlebacks	Ulva	2001/2002	6	9	0
		2002/2003	11	23	0
		2003/2004	17	31	0
		2004/2005	27	59	0
		2005/2006	25	44	0
		2006/2007	23	36	0
		All years	39*	202	0
Robins	Ulva	2001/2002	1	4	0
		2002/2003	8	21	0
		2003/2004	11	22	0
		2004/2005	19	41	0
		All years	26*	88	0
	Eglinton	2004/2005	22	84	1
	Doubtful	2002/2003	2	5	0
		2003/2004	3	9	0
		2004/2005	4	12	0
		All years	6*	26	0
		Total (for robins)	54	198	1

*Some pairs bred in multiple years.

Table 2. Microsatellite locus information for saddlebacks and robins.

Species	Locus name	Alleles per locus	n	Ho	He	Reference
Saddlebacks	*Ase18*	2	280	0.2500	0.2799	Richardson et al. 2000
	CK5A4B	2	280	0.5036	0.4357	Tarr and Fleischer 1998
	Hru6	4	280	0.6750	0.6972	Primmer et al. 1996
	K13/14	3	280	0.1679	0.1799	Hudson 1999
	Pca08	2	279	0.4050	0.3819	Lambert et al. 2005
	Pca15	2	275	0.6145	0.5009	Lambert et al. 2005
	Pgm1	6	280	0.6107	0.5825	Dowling et al. 2003
	Pcc07	2	278	0.4281	0.4348	King et al. unpubl. data
	Pcc26	2	279	0.3692	0.4113	King et al. unpubl. data
	Pcc40	4	279	0.4839	0.4410	King et al. unpubl. data
	Pcc42	2	279	0.2616	0.2637	King et al. unpubl. data
	Pcc45	2	279	0.1935	0.1751	King et al. unpubl. data
Robins	*2F9*	8	306	0.6111	0.7144	T. King pers. comm.
	Ase18	2	306	0.3268	0.3740	Richardson et al. 2000
	Ase64	9	298	0.7114	0.8068	Richardson et al. 2000
	Escu6	6	306	0.6961	0.7402	Hanotte et al. 1994
	GgaMu128	2	306	0.2810	0.3118	Crooijmans et al. 1997
	Indigo28	3	306	0.4183	0.4996	Sefc et al. 2001
	Pca12	4	306	0.5490	0.5445	Lambert et al. 2005
	Pca13	2	303	0.0330	0.0389	Lambert et al. 2005
	Pgm3	2	306	0.3725	0.4288	Dowling et al. 2003
	Pocc6	4	306	0.5621	0.6457	Bensch et al. 1997

Analysis

All loci were assessed for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GENEPOP v. 3.4 (Raymond and Rousset 1995). Total exclusion probabilities for combined loci were calculated using CERVUS v. 2.0 (Marshall et al. 1998) based on genotypes of the breeding adults. To examine rates of EPP, individuals were organized into family groups consisting of parents and all offspring produced by the pair across all years. EPP was considered to have occurred if offspring had alleles not present in their parents at two or more loci. To account for null alleles/mutations and prevent overestimation of EPP, EPP was not counted if discrepancies between parent and offspring genotypes occurred at a single locus (Ulva Island robins: $n = 4$; saddlebacks: $n = 1$; Pemberton et al. 1995). All mismatches were checked by re-amplifying DNA from the parents and chick(s) and running the samples in adjacent lanes. For each species, EPP rates were calculated by dividing the number of pairs in which EPP occurred by all pairs.

Results

There were no significant deviations from HWE ($P < 0.05$) for either saddlebacks or robins. Significant LD occurred for one saddleback locus pair (CK5A4B and Pcc26; $P = 0.001$). Total exclusionary power for saddlebacks was 0.934 (12 loci) and 0.921 (11 loci; CK5A4B excluded), for robins it was 0.980 (10 loci).

In saddlebacks, no EPP was detected using 1-6 years of data for 39 pairs and a total of 202 offspring (Table 1). In robins, there were two instances of EPP in one brood (rate = 1.9%) across three populations, 54 pairs, 198 offspring and 1–4 years of data (Table 1). EPP occurred in the Eglinton population (2004/05) for both chicks in the same clutch with mismatches at five loci (none of the sampled neighbouring males were more compatible than the social male). No EPP was detected for robins on Ulva or the Doubtful Islands.

Low levels of genetic variation in saddlebacks (Taylor et al. 2007, Taylor and Jamieson 2008) have resulted in microsatellite markers with low polymorphism. Consequently, saddleback exclusionary power is lower than optimal and the one offspring with a single locus mismatch could be an extra-pair young. Robin microsatellite markers had much higher exclusionary power than saddleback markers, nevertheless, the four single locus mismatch robin young could be extra-pair young. Nonetheless, our results indicate that EPP is very rare in saddlebacks and robins regardless of whether single locus mismatch young are included or not.

Discussion

Although both polyandry and polygyny have been reported on rare occasions in New Zealand robins and in one instance a male robin paired with and fed the offspring of a female that had lost her mate (Higgins and Peters 2002), social monogamy is the norm and, according to our results, strict genetic monogamy appears to be the rule for both saddlebacks and robins. A previous study on robins using minisatellite DNA also found no evidence of EPP (Ardern et al. 1997), although sample sizes were small and no data on adult mortality rates were given.

Information on EPP is important for on-going field studies of saddlebacks and robins. Both species are threatened endemics in New Zealand and part of long-term studies examining translocation success and inbreeding depression in wild populations. With known genetic monogamy, field observations of breeding pairs at the nest site can be used to accurately construct pedigrees and estimate reproductive success.

Our results are also important for inter-specific analyses investigating the prevalence and potential causes of avian EPP. At present, little data exist for rates of EPP in

long-lived passerine species. Previous research indicated that species with annual mortality rates below 30% should also show low levels of EPP (Mauck et al. 1999). Both saddlebacks and robins have mortality rates well below 30% and have correspondingly low levels of EPP (0% and 1.9%). These results expand the range of passerine species with low annual mortality and known rates of EPP and should help to reduce the taxonomic bias evident in earlier inter-specific analyses. Although alternative explanations may exist for the occurrence of genetic monogamy in our study species (e.g. Petrie et al. 1998, Griffith 2000) our data are consistent with the hypothesis that low annual mortality rates play an important role in explaining low rates of EPP across species.

Publication of a large number of studies that could be incorporated into cross-species analyses has done much to clarify the causes of inter-specific variation in rates of EPP (Owens and Bennett 1995, Mauck et al. 1999, Arnold and Owens 2002). Cross-species studies have shown that two of the most important life history traits are longevity and paternal care (Griffith et al. 2002). Unfortunately, these traits appear to be closely correlated in many species (Westneat and Stewart 2003). In addition to seabirds (Arnold and Owens 2002), long life spans occur jointly with obligatory paternal care in owls (Arsenault et al. 2002, Saladin et al. 2007), parrots (Masello et al. 2002), vultures (Decker et al. 1993), and corvids (Henderson et al. 2000). Determining the importance of low mortality rate versus obligatory paternal care will depend on future studies in species where these traits do not co-occur or where their effect can be distinguished.

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References

Ardern, S. L., Ma, W., Ewen, J. G., Armstrong, D. P. and Lambert, D. M. 1997. Social and sexual monogamy in translocated New Zealand robin populations detected using minisatellite DNA. – Auk 114: 120–126.

Armstrong, D. P., Davidson, R. S., Perrott, J. K., Roygard, J. and Buchanan, L. 2005. Density-dependent population growth in a reintroduced population of North Island saddlebacks. – J. Anim. Ecol. 74: 160–170.

Arnold, K. E. and Owens, I. P. F. 2002. Extra-pair paternity and egg dumping in birds: life history, parental care and the risk of retaliation. – Proc. R. Soc. B 269: 1263–1269.

Arsenault, D. P., Stacey, P. B. and Hoelzer, G. A. 2002. No extra-pair fertilization in Flammulated Owls despite aggregated nesting. – Condor 104: 197–202.

Bensch, S., Price, T. and Kohn, J. 1997. Isolation and characterization of microsatellite loci in a Phylloscopus warbler. – Mol. Ecol. 6: 91–92.

Boessenkool, S., Taylor, S. S., Tepolt, C. K., Komdeur, J. and Jamieson, I. G. 2007. Large mainland populations of South Island robins retain greater genetic diversity than offshore island refuges. – Conserv. Genet. 8: 705–714.

Crooijmans, R. P. M. A., Dijkhof, R. J. M., Van Der Poel, J. J. and Groenen, M. A. M. 1997. New microsatellite markers in chicken optimized for automated fluorescent genotyping. – Anim. Genet. 28: 427–437.

Decker, M. D., Parker, P. G., Minchella, D. J. and Rabenold, K. N. 1993. Monogamy in black vultures: genetic evidence from DNA fingerprinting. – Behav. Ecol. 4: 29–35.

Dimond, W. J. and Armstrong, D. P. 2007. Adaptive harvesting of source populations for translocation: a case study with New Zealand robins. – Conserv. Biol. 21: 114–124.

Dowling, D. K., Adcock, G. J. and Mulder, R. A. 2003. Novel polymorphic microsatellite markers for paternity analysis in the red-capped robin (*Petroica goodenovii*: Aves). – Mol. Ecol. Notes 3: 517–519.

Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J. T. and Kempenaers, B. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. – Nature 425: 714–717.

Griffith, S. C. 2000. High fidelity on islands: a comparative study of extrapair paternity in passerine birds. – Behav. Ecol. 11: 265–273.

Griffith, S. C., Owens, I. P. F. and Thuman, K. A. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. – Mol. Ecol. 11: 2195–2212.

Hanotte, O., Zanon, C., Pugh, A., Greig, C., Dixon, A. and Burke, T. 1994. Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting *Emberiza schoeniclus*. – Mol. Ecol. 3: 529–530.

Heather, B. and Robertson, H. 1996. The field guide to the birds of New Zealand. – Viking, Auckland.

Henderson, I. G., Hart, P. J. B. and Burke, T. 2000. Strict monogamy in a semi-colonial passerine: the jackdaw *Corvus monedula*. – J. Avian Biol. 31: 177–182.

Higgins, P. J. and Peter, J. M. (eds). 2002. Handbook of Australian, New Zealand and Antarctic birds, Volume 6: Pardalotes to shrike-thrushes. – Oxford University Press, Melbourne.

Higgins, P. J., Peter, J. M. and Cowling, S. J. (eds.). 2006. Handbook of Australian, New Zealand and Antarctic birds, Volume 7: Boatbill to starlings. – Oxford University Press, Melbourne.

Hudson, Q. J. 1999. Genetic variation within and among populations of kokako (*Callaeas cinerea*). – University of Waikato, Hamilton.

Lambert, D. M., King, T., Shepherd, L. D., Livingston, A., Anderson, S. and Craig, J. L. 2005. Serial population bottlenecks and genetic variation: translocated populations of the New Zealand saddleback (*Philesturnus carunculatus rufusater*). – Conserv. Genet. 6: 1–14.

Marshall, T. C., Slate, J., Kruuk, L. E. B. and Pemberton, J. M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. – Mol. Ecol. 7: 639–655.

Masello, J. F., Sramkova, A., Quillfeldt, P., Epplen, J. T. and Lubjuhn, T. 2002. Genetic monogamy in burrowing parrots *Cyanoliseus patagonus*? – J. Avian Biol. 33: 99–103.

Mauck, R. A., Marschall, E. A. and Parker, P. G. 1999. Adult survival and imperfect assessment of parentage: effects on male parenting decisions. – Am. Nat. 154: 99–109.

Owens, I. P. F. and Bennett, P. M. 1995. Ancient ecological diversification explains life-history variation among living birds. – Proc. R. Soc. B 261: 227–232.
Pemberton, J. M., Slate, J., Bancroft, D. R. and Barrett, J. A. 1995. Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. – Mol. Ecol. 4: 249–252.
Petrie, M., Doums, C. and Møller, A. P. 1998. The degree of extra-pair paternity increases with genetic variability. – PNAS 95: 9390–9395.
Petrie, M. and Kempenaers, B. 1998. Extra-pair paternity in birds: explaining variation between species and populations. – Trends Ecol. Evol. 13: 52–58.
Primmer, C. R., Møller, A. P. and Ellegren, H. 1996. A wide-range survey of cross-species microsatellite amplification in birds. – Mol. Ecol. 5: 365–378.
Raymond, M. and Rousset, F. 1995. Genepop (Version 1.2): population genetics software for exact tests and ecumenicism. – J. Hered. 86: 248–249.
Richardson, D. S., Jury, F. L., Dawson, D. A., Salgueiro, P., Komdeur, J. and Burke, T. 2000. Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-amplification in other passerine birds. – Mol. Ecol. 9: 2226–2231.
Saladin, V., Ritschard, M., Roulin, A., Bize, P. and Richner, H. 2007. Analysis of genetic parentage in the tawny owl (*Strix aluco*) reveals extra-pair paternity is low. – J. Ornithol. 148: 113–116.
Sefc, K. M., Payne, R. B. and Sorenson, D. 2001. Characterization of microsatellite loci in village indigobirds *Vidua chalybeata* and cross-species amplification in estrildid and ploceid finches. – Mol. Ecol. Notes 1: 252–254.
Seutin, G., White, B. N. and Boag, P. T. 1991. Preservation of avian blood and tissue samples for DNA analyses. – Can. J. Zool. 69: 82–90.
Tarr, C. L. and Fleischer, R. 1998. Primers for polymorphic GT microsatellites isolated from the Mariana crow, *Corvus kubaryi*. – Mol. Ecol. 7: 247–255.
Taylor, S. S. and Jamieson, I. G. 2008. No evidence for loss of genetic variation following sequential translocations in extant populations of a genetically depauperate species. – Mol. Ecol. 17: 545–556.
Taylor, S. S., Jamieson, I. G. and Wallis, G. P. 2007. Historic and contemporary levels of genetic variation in two New Zealand passerines with different histories of decline. – J. Evol. Biol. 20: 2035–2047.
Tregenza, T. and Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: Invited review. – Mol. Ecol. 9: 1013–1027.
Westneat, D. F. and Stewart, I. R. K. 2003. Extra-pair paternity in birds: causes, correlates, and conflict. – Ann. Rev. Ecol. Evol. Syst. 34: 365–396.