ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

Extrapair mating between relatives in the barn swallow: a role for kin selection?

by

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Supplementary data

Table 1. PCR conditions and polymorphism among microsatellite loci used for genetic analyses in the North American barn swallow (*n* = 375 adults)

			Heterozygosity			
Locus (reference)	PCR conditions (temperatures in degrees Celcius)	n_a	H_{obs}	H_{exp}	P_e	NA_{obs}
Aar4 (Hansson *et al.* 2000)	94(5min), [94(30s)-48(30s)-72(30s)] 29cycles, 72(7min), 4(5min)	13	0.56	0.53	0.34	0.003
Escμ6 (Hanotte *et al.* 1994)	94(5min), [94(30s)-50(30s)-72(30s)] 29cycles, 72(7min), 4(5min)	20	0.86	0.89	0.77	0.005
HrU5 (Primmer *et al.* 1995)	94(5min), 58(1min), 72(1min), [94(30s)-58(30s)-72(40s)] 32cycles, 72(7min), 4(5min)	21	0.92	0.90	0.81	0.001
HrU6 (Primmer *et al.* 1995)	94(5min), 63(1min), 72(1min), [94(30s)-63(30s)-72(40s)] 29cycles, 72(7min), 4(5min)	99	0.95	0.96	0.93	0.003
HrU7 (Primmer *et al.* 1995)	94(5min), 63(1min), 72(1min), [94(30s)-63(30s)-72(40s)] 29cycles, 72(7min), 4(5min)	5	0.50	0.50	0.26	0.005
HrU10 (Primmer *et al.* 1996)	94(5min), 65(1min), 72(1min), [94(30s)-60(30s)-72(40s)] 35cycles, 72(7min), 4(5min)	127	0.96	0.98	0.96	0.009
Ltr6 (McDonald & Potts 1994)	94(5min), [94(30s)-55(30s)-72(30s)] 29cycles, 72(7min), 4(5min)	8	0.64	0.67	0.44	0.003
Phtr2 (Fridolfsson *et al.* 1997)	95 (5min), [95(30s), 50(30s), 72(30s)] 35cycles 72(7min), 4(5min)	14	0.75	0.76	0.57	0.017
POCC6 (Bensch *et al.* 1997)	94(5min), [94(30s)-52(30s)-72(30s)] 29cycles, 72(7min), 4(5min)	16	0.86	0.86	0.73	0.000
				Total	>0.999	

Methods

DNA was extracted from blood and tissue samples using the QIAamp® DNA Blood Kit and DNeasy® Tissue Kit (QIAGEN, Venlo, The Netherlands). The microsatellite markers were amplified by optimised polymerase chain reaction (PCR). PCR was performed in a GeneAmp 9700 Thermocycler (Applied Biosystems, Foster City, U.S.A.). We used fluorescently labelled primers and the PCR products were run on an ABI 3100 sequencer (Applied Biosystems). Allele sizes were determined with GeneMapper v3.0 (Applied Biosystems), and marker polymorphism (n_a = number of alleles; H_{obs} = observed heterozygosity; H_{exp} = expected heterozygosity; P_e = probability of exclusion with one known parent) was calculated by using CERVUS (Marshall *et al.* 1998). Non-amplifying (i.e. null-) alleles (NA_{obs} = observed frequency of null-alleles) were detected on eight loci at low levels. Significant deviations from Hardy-Weinberg expectation (Chakraborty *et al.* 1992) were found only for two loci (HrU6 and Phtr2), and most individuals (17/18 and 79/94) that were homozygous at these two loci had common (i.e. among the 15% most frequent) alleles. This suggests that the number of potentially undetected null-alleles is insignificant and should not be a confounding factor in our genetic analyses.

Initially, we used CERVUS (Marshall *et al.* 1998) to obtain a list of candidate parents for each young. Nestlings were defined as withinpair young if they matched their parents completely (*n* = 603) or had one mismatching allele (*n* = 50). In four cases, a nestling had a genotype that made a complete match with two different males in the colony (i.e. probably first-order relatives). As the social father was involved in all four cases he was conservatively assigned as the genetic father. We defined nestlings with two or more allelic mismatches with the social male as extrapair young (*n* = 264). Eight offspring had one mismatch with their assigned extra-pair father. In seven of these cases, the males sired other

offspring in the nest and so were assigned as genetic fathers, while in a single case a male was assigned genetic father due to the low probability of false inclusion (Jeffreys *et al.* 1992) (= 5.4×10^{-08}, excluding the mismatched locus). Mismatches were maternally biased and appeared at only two loci, for which high mutation rates have previously been reported (Brohede *et al.* 2002). All offspring (n = 917) were the true offspring of the female at the nest, whereas extrapair paternity encompassed 101 of 210 broods and 264 of 917 young.

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