Electronic supplementary material for "*The couple that sings together stays together: duetting, aggression, and extra-pair paternity in a promiscuous bird species*"

Daniel T. Baldassarre, Emma I. Greig, and Michael S. Webster

Simulated territory intrusions

We presented a combination of artificial mounts and song playback to territorial males. Mounts were created using lightweight clay and both artificial and real feathers. Three types of mounts were used: red mounts of the *M. m. cruentatus* subspecies, orange mounts of the *M. m. melanocephalus* subspecies, and heterospecific mounts of the white-winged fairy-wren *M. leucopterus*. Importantly, the red/orange back patch of the red-backed fairy-wren mounts was comprised of real back feathers to ensure accuracy of the color. Songs of both subspecies and the white-winged fairy-wren were recorded during dawn choruses and played back at intervals of approximately 10 s. Each focal male could be tested with up to six experimental treatments: 1) own subspecies song and plumage, 2) own subspecies song and other subspecies plumage, 3) other subspecies song and own subspecies plumage, 4) other subspecies song and plumage, 5) heterospecific song and own subspecies plumage, and 6) own subspecies song and heterospecific plumage. Trial order was balanced across individuals and multiple trials were separated by at least one day. Once we identified a male present on the territory, the experimental trial began. We attached the mount to a natural perch, referred to as the "mount bush", and concealed a speaker underneath. Observers retreated to a distance of 15 m and hid behind vegetation. Playback duration was dynamic and variable, and could last up to five minutes. Once the focal bird came to the mount bush, we continued the playback for three more songs, after which time we stopped the playback and allowed the focal bird to interact with the mount without the song playing. We then observed the male for ten minutes after the playback had stopped. If the male did not respond to the playback after five minutes, we discontinued the trial, meaning a trial could last up to 15 minutes. All trials were video recorded, and observers dictated their observations into the camera. Two observers who were blind to the experimental design transcribed the behavioral responses in the videos. More detailed methods can be found in [1].

Microsatellite loci used for genetic paternity analysis

When combined, the seven microsatellite loci were highly polymorphic and informative for paternity analysis (mean number of alleles per locus = 11.57, mean expected heterozygosity = 0.701, Table S1). Allele frequencies did not deviate from Hardy-Weinberg expectations, but two loci (*Mcy2* and *Smm7*) had an estimated null allele frequency greater than 0.05, which was taken into account during subsequent paternity assignments. The average probability of excluding a randomly chosen male as the father was high, with a combined probability of exclusion of 0.998.

Locus	Annealing Temperature (°C)	# Alleles	Expected Heterozygosity (*He*)	Observed Heterozygosity (*Ho*)	Prob. of exclusion	Null allele frequency
Mcy1	55-58	9	0.731	0.731	0.529	-0.006
Mcy2	61	5	0.137	0.137	0.068	0.135
Mcy4	55-58	11	0.838	0.690	0.682	0.036
Mcy7	61	16	0.806	0.792	0.641	0.006
Msp6	55-58	8	0.691	0.695	0.450	0.009
Ase9	61	14	0.800	0.798	0.630	0.001
Smm7	61	18	0.906	0.805	0.807	0.057

Table S1: Characteristics of microsatellite loci used for paternity analysis. Probability of exclusion is the probability of excluding a randomly selected, unrelated male as the sire given the genotype of the offspring and mother. References for primers are as follows: all *Mcy* loci from [2], *Msp6* from [3], *Ase9* from [4], and *Smm7* from [5].

References

1. Greig, E. I., Baldassarre, D. T. & Webster, M. S. 2015 Differential rates of phenotypic introgression are associated with male behavioral responses to multiple signals. *Evolution* **69**, 2602–2612. (doi:10.1111/evo.12756)

2. Double, M. C., Dawson, D., Burke, T. & Cockburn, A. 1997 Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairy-wren *Malurus cyaneus*. *Molecular Ecology* **6**, 691–693. (doi:10.1046/j.1365-294X.1997.00228.x)

3. Webster, M. S., Tarvin, K. A., Tuttle, E. M. & Pruett-Jones, S. 2004 Reproductive promiscuity in the splendid fairy-wren: effects of group size and auxiliary reproduction. *Behavioral Ecology* **15**, 907–915. (doi:10.1093/beheco/arh093)

4. Richardson, D. S., Jury, F. L., Dawson, D. A., Salgueiro, P., Komdeur, J. & Burke, T. 2000 Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Molecular Ecology* **9**, 2225–2230. (doi:10.1046/j.1365-294X.2000.105338.x)

5. Maguire, G. S., Guay, P. J. & Mulder, R. A. 2006 Isolation and characterization of microsatellite markers in the southern emu-wren (*Stipiturus malachurus*: Aves). *Molecular Ecology Notes* **6**, 422–424. (doi:10.1111/j.1471-8286.2006.01257.x)