Journal of Avian Biology

JAV-00589
Ceresa, F., Belda, E. J., Kvist, L., Rguibi-Idrissi, H. and Monrós, J. S. 2015. Does fragmentation of wetlands affect gene flow in sympatric *Acrocephalus* warblers with different migration strategies? – J. Avian Biol. doi: 10.1111/jav.00589

Supplementary material

Appendix 1

PCR procedures

Microsatellites

For both reed and moustached warblers, we performed the Polymerase Chain Reaction (PCR) in 10-µl volumes using 2 µl of DNA template, 1 µl of 10x PCR-buffer, 0.9 µl of dNTPs (2 mM), 0.8 of primers (10 µM) (or 0.4 of 20 µM primer), 0.4 of $MgCl_2$ (2 mM) and 0.1 µl of DNA-polymerase (Biotools). The PCR procedure consisted of an initial denaturation for 5 min (94°C), 35 cycles of denaturation for 45 s (94°C), annealing for 45 s (from 48° to 63°C) and synthesis for 1 min (72°C), and a final synthesis for 7 min (72°C). Annealing temperatures for the primers were 50°C for Gf05 and Pdo5, 52°C for Ppi2, 54°C for Cuµ28 and ZL54, a touchdown from 60° to 50°C for Pocc2, from 63° to 53°C for Aar5, FhU2, Pca3 and Pdoµ1, from 54° to 48°C for Aar4 and Ase34, and from 60° to 54°C for Ase18, Ase25, Ase37, Ase48 and Ase58.

Mitochondrial DNA

For reed warblers, we performed the PCR in 10-µl volumes using 2 µl of DNA template, 1 µl of 10x PCR-buffer, 0.5 µl of MgCl2 (2 mM), 1 µl of dNTPs (2 mM), 0.4 µl primer CO1F (20 µM), 0.4 µl of primer CO1R (20 µM) and 0.06 µl of DNA-polymerase (Biotools). We used the following PCR profile: denaturation for 1 min (95°C), 35 cycles of denaturation for 30 s (94°C), annealing for 45 s (49°C) and synthesis for 45 s (72°C), and a final synthesis for 10 min (72°C).

For moustached warblers, we carried out the PCR in 10-µl volumes using 5 µl of DNA template, 1 µl of 10x PCR-buffer, 0.4 µl of MgCl2 (2 mM), 1 µl of dNTPs (2mM), 0.4 µl primer CO1F (20 µM), 0.4 µl of primer CO1R2 (20 µM) and 0.06 µl of DNA-polymerase (Biotools). We used the same PCR profile employed for reed warblers, but with 50°C as annealing temperature.