

Supplemental Information for:

Phenotypic and environmental correlates of natal dispersal movements in fragmented landscapes. Hugo Robles, Carlos Ciudad, Zeno Porro, Julien Fattebert, Gilberto Pasinelli, Matthias Tschumi, Marta Vila and Martin U. Grüebler

Appendix S1: Protocol for molecular sexing of middle spotted woodpecker's samples

Genomic DNA was using the Qiagen's DNeasy® Tissue Kit. We used two Polymerase-Chain-Reaction (PCR)-based molecular protocols. Firstly, all samples were screened for one intron of the *CHD* gene, the one amplified using primers 3007 and 3112 (Ellegren & Fridolfsson 1997). The size difference of this intron in females and males of *Dendrocoptes medius* is around 15 bp, so the PCR products amplified with primers 3007 and 3112 were separated and visualised with a Bioanalyzer 2100 with DNA 1000 Assay chip (Agilent Technologies). Females were unambiguously identified by the presence of two bands at, approximately, 400 and 385 bp. These bands corresponded with the Z and W-chromosome amplified fragments, respectively. We double-checked the resulting males by amplifying a different intron of the same gene, as suggested by Robertson & Gemmell (2006). For this, we used primers 2550F and 2718R (Fridolfsson & Ellegren 1999) that produced two unambiguous bands in females (ca. 620 and 450 bp). All males identified by the 3007/3112 protocol produced a single band around 620 bp when amplified with the 2550F/2718R one.

Hans Ellegren & Anna-Karin Fridolfsson (1997) Male-driven evolution of DNA sequences in birds. *Nature Genetics* 17:182-185.

Bruce C. Robertson & Neil J. Gemmel (2006) PCR-based sexing in conservation biology: Wrong answers from an accurate methodology? *Conservation Genetics* 7:267-271.

Anna-Karin Fridolfsson & Hans Ellegren (1999) A simple and universal method for molecular sexing of non-ratite birds. *Journal of Avian Biology* 30:166-121.