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Supplementary material

Appendix 1: Telomere assay

As a single control gene, we used the chicken zinc finger protein, with primer sequences defined by Primer 3 software as: (ZENK1: 5'-TACATGTGCCATGGTTTTGC-3'; ZENK2: 5'-AAGTGCTGCTCCCAAAGAAG-3'). This gene was previously used and found to be non-variable in copy numbers (thereafter non-VCN; Smith et al. 2011) within our population in king penguins (Geiger et al. 2012). In addition, using a standard electrophoresis on a 3% agarose gel run in standard TBE buffer (50 V for 15 min and 100 V thereafter for 30 min), qPCR amplicon size of control gene was checked for 8 different and randomly chosen individuals per species. One single and similar band was found around 170 bp for each individual. Telomere and non-VCN gene amplification were done on different plates (runs). T/S ratio were evaluated over 5 (great tit) or 3 (coal tit) telomere and (5 or 3) non-VCN gene runs, where elevation and time (chick age) were randomly mixed. To standardize the T/S ratio among runs, we used a T/S ratio of a control sample repeated in all the runs, following (Pfaffl 2001) equation. In all runs (telomere and non-VCN gene) were included a serial dilution (in duplicate, 5 ng, 2.5 ng, 1.25 ng, 0.625 ng, 0.3125 ng, 0.15625 ng) of a standard sample (either great or coal tit) and a negative control. A reference curve was calculated from serial dilutions for each run to calculate the amplification efficiency (and r^2 of the fit curve). Final calculation of telomere length (T/S ratio) was done using the telomere and non-VCN accurate efficiencies of each run (Pfaffl 2001).

Primer concentrations in the final mix were 100 nM for telomere length and 500 nM for the non-VCN gene determination. Telomere and non-VCN gene PCR conditions were 2min at 95°C followed by 40 cycles of 15s at 95°C, 30s at 56°C, 30s at 72°C and 60s at 95°C. We used 2.5ng DNA per reaction and the Power SYBR® Green PCR Master Mix fluorescent probe (Applied Biosystems, USA). A melting curve was calculated for each run (final melt step with

the temperature ramping from 50°C to 95°C in 1°C increments) to control for non-specific / primer-dimer amplification.

For great tits, mean amplification efficiency of the qPCR runs for telomere and non-VCN genes were 99.86 ± 0.22% and 100.16 ± 0.31%. Intra-run mean coefficients of variation (CVs) based on duplicates for Ct values were 1.74 ± 0.08 % for the telomere assay and 0.81 ± 0.03 % for the non-VCN assay. Inter-run CVs for Ct values based on five repeated samples over runs were 2.15 ± 0.11% for the telomere assay and 1.04 ± 0.12% for the non-VCN assay. Coefficients of variation for the final T/S ratio were 12.71 ± 1.03% (intra-run) and 14.53 ± 2.72% (inter-run).

For coal tits, mean amplification efficiency of the qPCR runs for telomere and non-VCN genes were 111.60 ± 0.34% and 90.10 ± 0.35%. Intra-run mean CVs were 1.21 ± 0.08 % for the telomere assay and 2.67 ± 0.13 % for non-VCN assay. Inter-run CVs based on six repeated samples over runs were of 1.68 ± 0.81% for the telomere assay and 0.83 ± 0.24% for the non-VCN assay. Coefficients of variation for the T/S ratio were 8.96 ± 1.67% (intra-run) and 11.57 ± 0.86% (inter-run).