

Effects of early-life competition and maternal nutrition on telomere lengths in wild meerkats

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Supplementary methods

DNA extraction and qPCR determination of telomere lengths

For DNA extraction, 25mg of skin was excised from each tail tip, mixed with 180 μ l MACHEREY-NAGEL Lysis Buffer T1 and 25 μ l Proteinase K (20mg/ml) (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany), and incubated at 56°C for 1-3 hours until the tissue was completely lysed. DNA was then purified from lysate using MACHEREY-NAGEL NucleoMag® Blood 200 μ L kits (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) in tandem with KingFisher™ Flex Purification System (Thermo Scientific, Wilmington DE, USA), following kit protocols. DNA was eluted in kit elution buffer MBL5 (5 mM Tris, pH 8.5) and stored at -20°C until further use. DNA concentration and purity were assessed using a Nanodrop-8000 Spectrophotometer (Thermo Scientific, Wilmington DE, USA). Average DNA concentration was 135.2 ± 83.8 ng/ μ l (mean \pm standard deviation (S.D.)) and average 260/280 ratio was 1.98 ± 0.08 . DNA integrity was assessed by running 10ng of DNA in a 0.8% agarose gel and was deemed to be acceptable for telomere measurement.

We used quantitative PCR (qPCR) analysis to measure telomere length, based on published protocols, with some modifications [1, 2]. This measure represents the average telomere length across cells in a sample, and is reported as the level of telomeric sequence abundance relative to a reference non-variable copy number gene. For qPCR measurement of relative telomere lengths, we used RAG1 as a reference gene due to its known status as a single copy gene in vertebrates [3], and used a primer pair designed from Accession JQ073171.1 selected for good performance, lack of non-specific binding, and lack of primer-dimer (confirmed by melt curve analysis and gel electrophoresis) during optimization. HPLC purified primers were synthesized by IDT® (Integrated DNA Technologies, Leuven, Belgium) resuspended, diluted and stored at -20°C until assays were run. DNA samples (1.25ng) were assayed in triplicate and on separate plates for telomere and single-copy targets. Reactions were conducted using 1X Absolute blue qPCR SYBR green Low Rox master mix (Thermo Scientific, Wilmington DE, USA) with RAG1 forward (5'-CAT TGA GAC AGT CCC TTC CAT AG-3') and reverse (5'-

GGA GGC ATT GGG ATT CTT GTA-3') primers at 500nM and telomere primers Tel1b (5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3') and Tel2b (5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3') [4] at 900nM, bringing reaction volumes up to 25 μ l with water. Mx3000P 96-well skirted plates (Agilent, Santa Clara, United States) were manually loaded, sealed with 8x strip optical caps (Agilent, Santa Clara, United States) and run in an Agilent Technologies Stratagene Mx3005P real-time PCR machine. RAG1 thermal profile was 15 min at 95°C, followed by 40 cycles of 15 s at 95°C, 30 s at 60°C, 30 s at 72°C. Telomere thermal profile was 15 min at 95°C, followed by 30 cycles of 15 s at 95°C and 30 s at 58°C. Both assays were followed by melt curve analysis of (58–95°C 1°C/5 s ramp). Dissociation curves showed a single peak for both assays in all reactions. The telomere assay had occasional very late amplification in the no template control (NTC) ($C_q < 28$; with the exception of one reaction amplifying at $C_q > 25$). This was deemed to be late formation of primer-dimer and acceptable considering the highest C_q of our DNA samples (16.18). Following optimization, the RAG1 assay showed late amplification ($C_q > 35$) in some reactions for the NTC, deemed to be primer-dimer formation. Comparing dissociation curves NTC with experimental samples showed much lower melting temperature and fluorescence in the NTC. With no sign of this peak profile in experimental samples and with a C_q much later than the highest of our samples (27.91), this was deemed to be acceptable.

A pooled aliquot of 33 DNA samples was serially diluted (5ng to 0.31ng) to generate a 5-point standard curve for each plate, which was used to calculate plate efficiencies (1.947-2.033 for RAG1 plates; 1.964-2.137 for telomere plates). The R^2 for all plates was > 0.985 . Quantification cycles (C_q s) were imported into qBase + V3.0 (Biogazelle, Zwijnaarde, Belgium) for further calculation. Based on the $\Delta\Delta C_q$ method, qBase+ calculates relative quantities of a sample (RQ) for each target by comparing C_q s of a sample with the average C_q s of all samples for that gene, controlling for reaction efficiency of that particular plate. RQ is then normalized by dividing by the geometric mean C_q of the reference gene, giving normalized relative quantity (NRQ). We used an inter-run calibration step with three samples run on every plate as inter-run calibrators (2.5ng and 0.625ng standard points and one "golden" sample run on every plate). This calculates calibrated normalized relative quantities (CNRQ) of telomeric DNA, equivalent to T/S ratios [5]. Technical replicates falling outside 0.5 C_q s were excluded or repeated, and samples were assigned to plates randomly. The average intraplate coefficient of variation of the C_q values for RAG1 and telomere plates were 0.18% and 0.68%, respectively. Intraplate variation was calculated as the coefficient of variation of the 1.25ng standard curve sample

within a given plate. Interplate variation, calculated as the coefficient of variation of the ΔC_q for the 1.25ng standard curve sample between plates was 3.37%, and coefficient of variation of the CNRQ (T/S ratio) of the 1.25ng standard curve sample across plates was 11.24%.

References

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