**Adaptive sex ratio allocation is linked to maternal telomere length in the Seychelles warbler**

**Abstract**

**Introduction**

Sex ratio adjustment and adaptive evolution

Sex ratio adjustment in birds

Telomeres as biomarkers of cost/condition

The Seychelles warbler (*Acrocephalus sechellensis*) constitutes a textbook example of adaptive sex ratio modification (Komdeur 1996; Komdeur et al. 1997). The Seychelles warbler is a facultative cooperative breeder - in good environmental conditions, daughters often remain on their natal territory to help rear their siblings.

In this study we combine telomere data with the long-term Seychelles warbler data set to test the hypothesis parental condition is related to offspring sex ratio. Specifically, we test the hypothesis that mothers and fathers with longer telomeres produce more females.

**Methods**

*Study species and sampling* They Seychelles warbler is blah blah blah

*Molecular methods*

DNA is extracted from all blood samples using an ammonium-acetate-based protocol (**???**), and sex is determined using by PCR (Griffiths et al. 1998). All samples were genotyped at 30 polymorphic microsatellite loci arranged into four multiplex reactions (see Spurgin et al. 2014 for details). Telomeres were measured from a total of 1357 samples. Of these, 158 were from juvenile birds for which we had parentage data with telomere length measurements available from at least one parent. We measured absolute telomere length using a qPCR method with absolute standards (full details provided in Barrett et al. 2012).

*Statistical analyses*

All statitical analyses were carried out using R version 3.2.2 (R Development Core Team 2011). Parentage was assigned using MasterBayes (HANNAH TO DO).

We used linear mixed effects models (LMMs) to test for a relationship between parental and offspring TL. Juvenile TL was entered as the response variable, age class as a fixed factor, and as covariates we included maternal and paternal TL, seasonal food availability, territory quality and the number of helpers present in the natal territory. As our dataset spanned many years, and contained multiple juveniles from the same parents, we included birth year, maternal ID and paternal ID as random factors. In order to differentiate between parental quality and parental condition, we partitioned parental TL into 'juvenile parental TL' and 'adult parental TL', and a separate model for each. Juvenile TL is expected to reflect inheritance, whereas adult TL is expected to reflect the stresses encountered over their adult life, and is thus a marker of condition. Because juvenile and adult parental TL are correlated, we ran separate models for these two variables, keep all other variables the same.

We next tested whether parental age at hatching and parental lifespan were related to offspring TL, again using LMMs. While parental lifespan is likely to be an indicator of individual quality, parental age may reflect condition. These models were constructed as above, but with parental age and parental lifespan added as covariates in place of parental TL. We ran these models separately from the models including parental TL due to collinearity issues between TL, age and lifespan [Barrett et al. (2012); Spurgin *et al.* Submitted]; thus, in total we ran four separate LMMs (parental juvenile TL, parental adult TL, parental age and parental lifespan).

Finally, we used generalized linear mixed effects models (GLMMs) with a binomial error structure to test how parental TL is related to offspring sex ratio. Offspring sex was included as a binary response variable, offspring age class as a fixed factor, and territory quality and the number of helpers were included as covariates, as both of these variables have been previously demonstrated to predict offspring sex ratio (Komdeur et al. 1997). Random effects were specified as with the LMMs, above. As with the LMMs, we ran four separate GLMMs, separately testing how parental juvenile TL, parental adult TL, parental age and parental lifespan were related to offspring sex.

**Results**

Overall, sex ratio in Seychelles warblers did not

Linear regressions showed that maternal juvenile TL was not related to offsrping TL (R2 = 0.01; t < 0.01; P = 0.70), but that maternal adult TL was positively related to offspring TL (R2 0.08; t = 2.31; P 0.02; Fig. 1A ). Paternal juvenile TL was positively related to offspring TL (R2 = 0.16; t = 2.47; P = 0.02; Fig. 1B), but paternal adult TL was not (R2 = 0.02; t = 1.30; P = 0.20). Mixed models including potentially confounding variables confirmed the results of the linear regressions: offspring TL was not related to maternal juvenile TL (estimate = 0.02, CIs = -0.98, 1.01), but was positively related to maternal adult TL (estimate = 0.42, CIs = -0.02, 0.86). Offspring TL was positively related to paternal juvenile TL (estimate = 0.91, CIs = 0.30, 1.52), but was not related to paternal adult TL (estimate = 0.37, CIs = -0.20, 0.95).

Offspring TL was not related to maternal age (estimate = -0.03, CIs = -0.66, 0.60), maternal lifespan (estimate = 0.05, CIs = -0.39, 0.49) paternal age (estimate = 0.32, CIs = -0.27, 0.91) or paternal lifespan (estimate = 0.43, CIs = -0.08, 0.94).

Offspring sex ratio was not related to maternal juvenile TL (estimate = 1.06, CIs = -1.71, 3.84), but was significantly related to maternal adult TL (estimate = -1.76, CIs = -2.95, -0.58); mothers with longer telomeres as adults had more female offspring compared to mothers with shorter telomeres (Fig. 2A). Offspring sex ratio was not related to paternal juvunile TL (estimate = -0.64, CIs = -2.22, 0.94), or paternal adult TL (estimate = -0.83, CIs = -1.88, 0.22). Offspring sex ratio was not related to either maternal age (estimate = 0.10, CIs = -0.72, 0.92) or maternal lifespan (estimate = 0.34, CIs = -0.54, 1.22). However, older fathers had significantly more female offspring (estimate = -1.19, CIs = -2.26, -0.12; Fig. 2B). Finally, there was no relationship between paternal lifespan and offspring sex ratio (estimate = -0.17, CIs = -1.27, 0.92).

**Discussion**

It is difficult to disentangle the effects of paternal juvenile telomere length and maternal adult telomere length on offspring telomere length, as these two predictor variables are correlated (R = 0.38, P = 0.06). Need to think about about why this is the case - seems interesting, and likely environment driven.

The effects of paternal age and maternal adult telomere length on offspring sex ratio are almost certainly independent, as these two variables are notcorrelated with one another (R = -0.04, P = 0.78).

**Acknowledgements**

**References**

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