**Adaptive sex ratio allocation is linked to maternal telomere length in the Seychelles warbler** Lewis G. Spurgin1,2, Hannah, L. Dugdale3,4, Kat Bebbington1, Eleanor A. Fairfield1, Jan Komdeur3, Terry Burke4 and David S. Richardson1,5,.

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**Abstract**

Previous research has shown that cooperatively breeding Seychelles warblers (*Acrocephalus sechellensis*) adaptively modify the sex ratio of their offspring, producing an excess of females in good conditions in order to maximise the chance of gaining helpers in their territories. Here we show that offspring sex ratio is related to maternal telomere length in this species. Mothers with longer telomeres produced an excess of daughters, while mothers with short telomeres produced an excess of sons. Furthermore, mothers with longer telomeres had offspring with longer telomeres and lower rates of telomere shortening, suggesting that maternal condition is positively related to offspring condition. Finally,longer maternal telomeres were associated with increased survival to adulthood in offspring, and female offspring had lower survival probabilities than males. Our data show that female birds can adaptively modify offspring sex ratio according to their condition, and that telomeres can provide useful insights into sex ratio evolution.

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.

Overall, sex ratio in our Seychelles warbler dataset did not differ from 50:50 (51% of all juveniles were male; binomial test, P = 0.87). There was variation in juvenile sex ratio among years (Fig. 1A); of the 23 years we observed an excess of males in 4 years, and an excess of females in 1 year.Generalised linear mixed models controlling for maternal age, territory quality and availability of helpers showed that offspring sex ratio was significantly related to maternal adult telomere length (estimate = -1.73, CIs = -2.90, -0.56). Mothers with short telomeres (<4kb) as adults produced on 68% males, while mothers with long telomeres (>7kb) produced 71% females (Fig. 1B).We found no evidence that sex ratio was linked to paternal telomere length (estimate = -0.84, CIs = -1.93, 0.25).

We then tested whether maternal condition was passed on to offspring by testing for a relaitonship between maternal and offspring telomere length and dynamics. Offspring TL was positively related to maternal adult TL (estimate = 1382.45, CIs = 52.63, 2712.26; Fig. 2A). Maternal telomere length was also (estimate = -1.19, CIs = -3.55, 1.16; Fig. 2A) Paternal telomere length was not related to offspring telomere length (estimate = 767.12, CIs = -494.06, 2028.31), nor to telomere loss.

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).

**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 1365 samples. Of these, 161 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.

**Acknowledgements** We thank Nature Seychelles for facilitating the long-term Seychelles warbler project. The Seychelles Bureau of Standards and Department of Environment gave permission for sampling and fieldwork. We thank Emma Barrett for her work on this project, everyone who has helped in the field, and the current Seychelles warbler research group for useful discussions. This work was funded by two Natural Environment Research Council (NERC) grants to DSR (NE/F02083X/1 and NE/K005502/1). LGS is supported by an Edward Grey Instiute Fellowship, and HLD was funded by a NERC fellowship.

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