**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,.

1. School of Biological Sciences, University of East Anglia, Norwich Research Park, NR4 7TJ, United Kingdom
2. Department of Zoology, Edward Grey Institute, University of Oxford, Oxford, UK
3. Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands
4. Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK
5. Nature Seychelles, Roche Caiman, Mahé, Republic of Seychelles

**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.

**Introduction**

Evolutionary theory suggests that females should modify the sex ratio of their offspring according to their condition or the quality of their environment (**???**; Trivers and Willard 1973). In a seminal paper, Trivers and Willard proposed that when maternal condition affects offspring fitness, and when fitness of males is affected more than females, mothers in good condition should produce more sons in order to maximise their own fitness (Trivers and Willard 1973).

Despite years of empirical studies, the relationship between maternal condition and offspring sex ratio is unclear (**???**).

There are likely a number of reasons for the mixed results among studies of maternal condition and offspring sex ratio. Recent theoretical developments have shown that the strength of a Trivers-Willard effect is expected to vary according to sex differences in life history traits, and that explains some of the disparity between theoretical and empirical sex ratio studies (**???**).

There is also a problem with measuring maternal condition. The majority of studies of maternal condition and offspring sex ratio have used a measure of body condition based on body fat, or a measure of social rank. However, body fat may be related to resource availability, making it a poor measure of condition. Social rank appears to be a better measure of resource availability, but its efficacy as a measure of condition is also expected to vary among species, and in many systems is unmeasurable.

Telomeres as biomarkers of cost/condition

In this study we test the hypothesis that parental telomere length is related to offspring sex ratio in the Seychelles warbler(*Acrocephalus sechellensis*). This species constitutes a textbook example of adaptive sex ratio modification (Komdeur et al. 1997; Frank 1998). The Seychelles warbler is a facultative cooperative breeder - in good environmental conditions, daughters often remain on their natal territory to help rear their siblings.

We first test the hypothesis that mothers with longer telomeres produce more females. We then then test whether longer telomeres in mothers is related to offspring telomere length and survival.

**Results**

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 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; Table S1). 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 = 1393.93, CIs = 3.39, 2784.46; Fig. 2A). A linear regression showed that maternal telomere length explains a modest amount of variation in offspring telomere length (R2 = 0.08), with a heritability of 0.86. Paternal telomere length was not related to offspring telomere length (estimate = 767.12, CIs = -494.06, 2028.31).

Finally, we tested how maternal telomere length

**Discussion**

Whether telomeres play a causal role in senescence remains debated (Simons 2015).

**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.

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