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

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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.

**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). More recent extensions of the Trivers-Willard hypothesis have shown that either sons or daughters can be favoured, depending on the life-history and sex-specific demography of the species or populaiton being studied (**???**).

Given that theory predicts a range of relationships between maternal condition and offspring sex ratio, it is perhaps not surprising that empirical studies in natural populations have found that high maternal condition is associated with an excess of sons [], an excess of daughters, or neither []. However, it has proved difficult to tell whether the patterns observed in natural populations match theoretical expectations, for two main reasons. First, in many species the life-history data required to generate predictions about sex allocation are lacking []. In such cases it is very difficult to tell whether observed relationships (or lack thereof) are consistent with evolutionarily plausible scenarios, or whether they reflect type I and II error. Second, there is the problem of 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. Having an adequate measure of condition has been shown to have a significant effect on the likelihood of detecting adaptive sex allocation (**???**), and is therefore paramount.

Telomeres are protective caps on the ends of chromosomes that shorten with age, and in response to oxidative stress caused by poor environmental and/or social conditions. Telomere shortening is also directly involved in cellular senescence and cell death, and telomere length and dynamics have been linked to survival and lifespan in a number of species. Whether telomeres are causal in organismal senescence is not yet clear (**???**). What is clear, however, is that telomere can act as biomarkers of individual condition, reflecting acccumulating costs experienced over an individuals' lifetime (Asghar et al. 2015). Telomeres therefore present an ideal biomarker to test the hypothesis that maternal condition is linked to offpsring sex ratio. Moreover, by measuring offspring telomeres and relating this to maternal telomere length, we can also test whether maternal condition is passed on to her offspring.

In this study we use telomeres to test the hypothesis that maternal condition 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 []. Komdeur (1997) showed that females on high quality territoried produce an excess of daughters in order to retain co-breeders and maximise inclusive fitness. Follow up work has shown that this effect is not due to the selective disappearance of males, and that there are indeed benefits of producing daughters on high quality territories in terms of inclusive fitness. However, until now we have never tested whether adaptive sex ratio allocation in the Seychelles warbler is mediated by maternal condition.

We first test the hypothesis that mothers with longer telomeres produce more females. We then then test whether longer telomeres in mothers is associated with longer telomeres in offspring. Finally, we analyse sex-specific patterns of offspring survival to test the hypothesis that sex ratio variation in adaptive.

**Results**

Overall, sex ratio in our Seychelles warbler dataset did not differ from 50:50 (51% of all juveniles were male; binomial test, P = 0.76). There was variation in sex ratio among years (Fig. 1); of the 17 years we observed an excess of males in 2 years, and an excess of females in 1 year.

We found no evidence that maternal and paternal quality, measured in terms of telomere length, were related. Generalised linear mixed models controlling for maternal age showed that offspring sex ratio was significantly related to maternal telomere length (Fig. 1B). There was a tendency for fathers with longer telomeres to have more female offspring, although this was not significant (estimate = -0.43, CIs = -0.95, 0.09; Fig. 1B). Interestingly, average telomere length of both mother and father had the strongest effect on offspring sex ratio (estimate = -0.69, CIs = -1.09, -0.29; Fig. 1D). (estimate = -0.67, CIs = -1.19, -0.16). Pairs with short telomeres (<4kb) produced on average 69% males, while pairs with long telomeres (>6kb) produced 77% females We found no effect of territory quality or the number of helpers on offspring sex ratio (Table S1).

We then tested whether parental condition was passed on to offspring by testing for a relationship between parental and offspring telomere length and dynamics. Offspring telomere length not related to maternal telomere length (estimate = 0.07, CIs = -0.26, 0.40; Fig. 3A), but was positively related to paternal telomere length (estimate = 0.50, CIs = 0.13, 0.87; Fig. 3B). Again, the strongest relationship was found with mean parental telomere length (estimate = 0.69, CIs = 0.12, 1.25; Fig. 3C). The relationship between parental and offpring telomere length was strongest for females (Fig. 3C); however, the interactrion between parentla telomere length and sex was not significant (estimate = -0.78, CIs = -1.69, 0.14; Fig. 3C).

Finally, we tested how juvenile telomere length in each was related to survival. Survival was significantly higher in males (est = 3.12; CI = 1.10, 5.14), and positively related to telomere length (est = 0.52; CI = 0.14, 0.89). Importantly, there was a significant interaction effect between sex and telomere length on survival (est = -0.64; CI = -1.08, -0.20). Telomere length in female juveniles was positively related to juvenile survival, while no such relationship was observed in males (Fig. 3D).

**Discussion**

Komdeur (1997) showed that Seychelles warblers modify the sex ratio of their offspring according to their territory quality. Here, by using telomeres, we show that

Telomeres better measure than body condition/social status

One possibility is that the relationship between maternal telomere length and offspring sex ratio is due to selective disappearance of females: as females

Differential effect of maternal investment on 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 (Richardson et al. 2000), 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 1392 samples. Of these, 276 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 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). We also included parental age as a covariate to control for potential confounding effects of senescnece on offspring sex ratio. 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.

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, maternal and paternal age, seasonal food availability, territory quality and the number of helpers present in the natal territory. Random effects were specified as with the GLMMs, above.

Finally, we used GLMMs to test whether parental telomere length was related to survival to adulthood. Survial to adulthood (yes/no) was included as the response variable, and parental telomere length, offspring telomere length and offspring sex were included as covariates. Random effects were specified as above.

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