**Parental telomere length predicts offspring sex ratio 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

**Correspondence**: Lewis Spurgin - [lewisspurgin@gmail.com](mailto:lewisspurgin@gmail.com); David Richardson - [david.richardson@uea.ac.uk](mailto:david.richardson@uea.ac.uk)

**Key words**: Sex allocation, sex ratio,

**Abstract**

A major puzzle in evolutionary biology concerns when and why mothers should modify the sex of their offspring to maximise their own fitness. Theory suggests that mothers show bias sex ratios towards the sex with the highest fitness gains when in good condition; however, empirical support for this hypothesis is equivocal. Cooperatively breeding Seychelles warblers (*Acrocephalus sechellensis*) adaptively modify the sex of their offspring, producing an excess of females in good conditions in order to maximise the fitness gains from having related helpers in their territories. Here we show that sex ratio modification in the Seychelles warbler is mediated by parental telomere length in this species. Telomeres are protective caps on the ends of chromosomes that shorten in response to stress, and are excellent biomarkers of individual condition. Mothers and fathers with long telomeres produced 77% daughters, while parents with short telomeres produced 69% sons. Furthermore, we found a positive association between parental and offspring telomere length, suggesting that condition is passed on from parents to offspring. Finally, we found that longer telomeres in juvenile females, but not males, was strongly associated with increased survival. This suggests that female offspring are disproportionately affected by parental investment, and that parents produce female offspring, and that birds produce female offspring when in good condition to maximise their own fitness.

**Introduction**

In many organisms females produce offspring with skewed sex ratios, despite the fact natural selection operates against deviations from unity in a frequncy-dependent manner (Fisher 1930). 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 theoretical work has shown that natural selection may favour either sons or daughters, depending on the quality of the rearing environment (Charnov et al. 1981), paternal attractiveness (Burley 1981), and depending on the life-history and sex-specific demography of the populaiton being studied (Leimar 1996; Schindler et al. 2015).

Given that theory predicts a range of relationships between maternal condition and offspring sex ratio, it is perhaps not surprising that results from empirical studies in natural populations have been highly varied (reviewed in West 2009). When in good condition or a high quality environment, mothers can produce an excess of sons (e.g. Ellegren et al. 1996; Nager and Monaghan 1999; Pryke and Griffith 2009), an excess of daughters (e.g. Komdeur et al. 1997; Hewison et al. 2005), or neither (e.g. Brown and Silk 2002; Postma et al. 2011; MacLeod and Clutton-Brock 2013). However, it has proved difficult to tell whether the patterns observed in natural populations match theoretical expectations (Komdeur and Pen 2002; Abe et al. 2014). A major reason for this is that 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 (Leimar 1996; Schindler et al. 2015).

A second problem with sex allocation studies is that of measuring parental condition. The majority of studies of parental condition and offspring sex ratio have used a measure of body condition based on body fat (e.g. Whittingham and Dunn 2000; Hewison et al. 2005), or a measure of social rank (e.g. Clutton-Brock et al. 1984; MacLeod and Clutton-Brock 2013). However, morphological measures of condition may be related to resource availability in variety of ways, making them a poor measure of condition (discussed in Sheldon and West 2004). Social rank appears to be a better measure of resource availability (Sheldon and West 2004), but its efficacy as a measure of condition is will to vary among species according to social structure, and in many cases (i.e. in species with no obvious distinctions between dominant and subordinate individuals) is unmeasurable. Thus a robust, widely applicable measure of condition is needed to understand sex allocation, but such a measure is currently lacking.

Telomeres are being increasingly recognised as excellent biomarkers of individual condition, reflecting acccumulating costs experienced over an individuals' lifetime (Monaghan and Haussmann 2006; Asghar et al. 2015). These protective caps on the ends of chromosomes shorten with age, and in response to oxidative stress caused by poor environmental and/or social conditions (Von Zglinicki 2002; Epel et al. 2004). 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 (Cawthon et al. 2003; Heidinger et al. 2012; Barrett et al. 2013).Telomeres therefore present an ideal biomarker to test the hypothesis that parental condition is linked to offpsring sex ratio. However, to our knowledge no studies have looked at parental telomere length in relation to offspring sex ratio.

In this study we use telomeres to test the hypothesis that parental 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 1996). Females on high quality territories produce an excess of daughters in order to retain co-breeders and maximise inclusive fitness (Komdeur 1996; Komdeur et al. 1997). Follow up work has shown that there are both direct and indirect fitness benefits of producing daughters on high quality territories (Komdeur 1998; Richardson et al. 2002), suggesting that this is an adaptive trait. However, until now we have never tested whether adaptive sex ratio allocation in the Seychelles warbler is mediated by maternal or paternal condition.

We first test the hypothesis that parents with longer telomeres produce more females. We then then test the hypothesis that parental condition is passed onto offspring by testing for a positive relationship between telomere length in parents and offspring. Finally, we analyse sex-specificrelationships between telomere length and offspring survival to test the hypothesis that survival of daughters, but not sons, is condition dependent. We discuss our results in the context of realised direct and inclusive fitness benefits to parents.

**Methods**

##### Study species and sampling

We utilised a long-term study of Seychelles warblers on Cousin Island, which has been monitored since 1986, intensively so since 1997 (Richardson et al. 2003; Spurgin et al. 2014). Individuals are usually ringed at less than one year old, and then followed throughout their lives. As they do not disperse to or from Cousin (Komdeur et al. 2004), a biannual census of birds on Cousin during each breeding season gives accurate measures of social status, age and survival (Crommenacker et al. 2011; Barrett et al. 2013). Seychelles warblers are highly territorial and all territories are mapped during the breeding seasons using detailed observational data of foraging and territorial defence behaviour, and surveyed for territory quality (Komdeur 1992; Richardson et al. 2003). Full details of catching and monitoring methods can be found in Brouwer *et al.* (2012).

Each time a bird is caught on Cousin body mass and tarsus length are measured (to the nearest 0.1g and 0.1mm, respectively), and age is confirmed on the basis of eye colour (Komdeur 1991) and previous captures (Richardson et al. 2003). A blood sample (*ca* 25 l) is taken via brachial venipuncture, and stored in 1 ml of absolute ethanol in a 1.5 ml screw-cap microfuge tube, at room temperature.

##### Molecular methods and parentage

For each sample, genomic DNA was extracted from a ~2 mm2 flake of preserved blood using the DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's protocol, with the modification of overnight lysis at 37oC and a final DNA elution volume of 80 l. DNA concentration and purity were quantified using a NanoDrop 8000 Spectrophotometer (ThermoScientific), and DNA integrity was validated by visualization with ethidium bromide after electrophoresis on a 1.2% agarose gel. Sex was determined using the molecular method outlined by 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). Using these data, parentage was assigned using MasterBayes (HANNAH TO DO).

Telomeres were measured from a total of 1395 samples, using a qPCR method with absolute standards (full details provided in Barrett et al. 2012). Of these, 276 were from juvenile birds between 1 and 12 months old for which we had telomere length measurements available from at least one parent. We excluded nestlings less than one month old from this analysis as telomere length at this stage is expected to feflect inheritance, whereas telomere length in fledgling birds is expected to reflect early life experiences.Because adult birds are not caught every year, we did not have samples available from parents at the year each juvenile was born. As an overall measure of parental condition, therefore, we calculated an average telomere length for each parent by calculating the mean of all telomere measurements for each adult sample.

*Statistical analyses*

All statistical analyses were carried out using R version 3.2.2 (R Development Core Team 2011). Mixed models were carried out using the lme4 package [Pinheiro2012].

We first calulated sex ratio variation over our 16 year dataset using all available catch data from Cousin. All juveniles caught in their first year of life sexed and sex ratio was calulated separately for each year. We calculated 95% confidence limits for each year using a bootstrapping approach; 1000 populations of males and females were simulated for each year based on observed sample sizes and a 50% chance each offpring being female, and from this 5% and 95% quantiles were extracted.

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, 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 senescence 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 telomere length. Juvenile telomere 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, 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 offspring telomere length and offspring sex were included as covariates. Random effects were specified as above.

**Results**

Overall, sex ratio in our Seychelles warbler dataset did not differ from 50:50 (53% of all juveniles were male; binomial test, P = 0.40). There was variation in sex ratio among years (Fig. 1); of the 17 years we observed an excess of males in two years, and an excess of females in one year.

Maternal and paternal telomere length were positively, but non-signficantly, related (Pearson correlation, estimate = 0.17; CIs, = -0.04, 0.36; Fig. 1A). Therefore while it is possible that offspring with high quality mothers also had high quality fathers, this relationship is very weak. Generalised linear mixed models controlling for maternal age (Table S1) showed that offspring sex ratio was significantly related to maternal telomere length (estimate = -0.67, CIs = -1.19, -0.16; Fig. 1B). There was also 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. 1C). Interestingly, we found that the 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). 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 relationships between parental and offspring telomere length (Table S2). Offspring telomere length not related to maternal telomere length (estimate = 0.06, CIs = -0.28, 0.41; Fig. 3A), but was positively related to paternal telomere length (estimate = 0.46, CIs = 0.08, 0.84; Fig. 3B). Again, however, the strongest relationship was found with mean parental telomere length (estimate = 0.70, CIs = 0.14, 1.27; Fig. 3C). The relationship between parental and offpring telomere length was stronger for females (Fig. 3C; R2 from linear regression = 0.11) than for males (R2 < 0.01); however, the interaction between parental telomere length and sex was not significant (estimate = -0.60, CIs = -1.56, 0.35; Fig. 3C).

Finally, we tested how juvenile telomere length in each was related to survival (Table S3). In a model not including telomere length, we found no difference between sexes in probability of survival to adulthood (est = 0.37; CI = -0.38, 1.12). However, when telomere length was included in the model we found that 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**

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.

Maternal vs paternal effects

Differential effect of parental investment on females

**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.

**References**

Abe, J., Y. Kamimura, and S. a. West. 2014. Inexplicably female-biased sex ratios in melittobia wasps. Evolution 2709–2717.

Asghar, M., D. Hasselquist, B. Hansson, P. Zehtindjiev, H. Westerdahl, and S. Bensch. 2015. Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. Science 347:436–438.

Barrett, E. L. B., W. Boner, E. Mulder, P. Monaghan, S. Verhulst, and D. S. Richardson. 2012. Absolute standards as a useful addition to the avian quantitative PCR telomere assay. Journal of Avian Biology 43:571–576.

Barrett, E. L. B., T. Burke, M. Hammers, J. Komdeur, and D. S. Richardson. 2013. Telomere length and dynamics predict mortality in a wild longitudinal study. Molecular Ecology 22:249–259.

Brouwer, L., D. Richardson, and J. Komdeur. 2012. Helpers at the nest improve late-life offspring performance: evidence from a long-term study and a cross-foster experiment. PLoS ONE 7:e33167.

Brown, G. R., and J. B. Silk. 2002. Reconsidering the null hypothesis: Is maternal rank associated with birth sex ratios in primate groups? Proceedings of the National Academy of Sciences of the United States of America 99:11252–11255.

Burley, N. 1981. Sex Ratio manipulation and selection for attractiveness. Science 211:722–725.

Cawthon, R. M., K. R. Smith, E. O’Brien, A. Sivatchenko, and R. A. Kerber. 2003. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 361:393–395.

Charnov, E. L., R. L. Los-den Hartogh, W. T. Jones, and J. van den Assem. 1981. Sex ratio evolution in a variable environment. Nature 289:27–33.

Clutton-Brock, T. H., S. D. Albon, and F. E. Guinness. 1984. Maternal dominance, breeding success and birth sex ratios in red deer. Nature 308:358–360.

Crommenacker, J. van de, J. Komdeur, and D. S. Richardson. 2011. Assessing the cost of helping: the roles of body condition and oxidative balance in the Seychelles warbler (Acrocephalus sechellensis).

Ellegren, H., L. Gustafsson, and B. C. Sheldon. 1996. Sex ratio adjustment in relation to paternal attractiveness in a wild bird population. Proceedings of the National Academy of Sciences of the United States of America 93:11723–11728.

Epel, E. S., E. H. Blackburn, J. Lin, F. S. Dhabhar, N. E. Adler, J. D. Morrow, and R. M. Cawthon. 2004. Accelerated telomere shortening in response to life stress. Proceedings of the National Academy of Sciences of the United States of America 101:17312–17315.

Fisher, R. A. 1930. The Genetical Thoery of Natural Selection. Oxford University Press.

Frank, S. 1998. Foundations of social evolution. Princeton University Press.

Griffiths, R., M. C. Double, K. Orr, and R. J. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7:1071–5.

Heidinger, B. J., J. D. Blount, W. Boner, K. Griffiths, N. B. Metcalfe, and P. Monaghan. 2012. Telomere length in early life predicts lifespan. Proceedings of the National Academy of Sciences of the United States of America 109:1743–8.

Hewison, J. M., J. M. Gaillard, P. Kjellander, C. Toïgo, O. Liberg, and D. Delorme. 2005. Big mothers invest more in daughters - Reversed sex allocation in a weakly polygynous mammal. Ecology Letters 8:430–437.

Komdeur, J. 1991. Cooperative breeding in the Seychelles warbler. PhD Thesis, Cambridge University.

Komdeur, J. 1996. Facultative Sex Ratio Bias in the Offspring of Seychelles Warblers.

Komdeur, J. 1992. Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler. Nature 358:493–495.

Komdeur, J. 1998. Long-term fitness benefits of egg sex modification by the Seychelles warbler. Ecology Letters 1:56–62.

Komdeur, J., and I. Pen. 2002. Adaptive sex allocation in birds: the complexities of linking theory and practice. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 357:373–380.

Komdeur, J., S. Daan, J. Tinbergen, and C. Mateman. 1997. Extreme adaptive modification in sex ratio of the Seychelles warbler’s eggs. Nature 385:522–525.

Komdeur, J., T. Piersma, K. Kraaijeveld, F. Kraaijeveld-Smit, and D. S. Richardson. 2004. Why Seychelles warblers fail to recolonize nearby islands: unwilling or unable to fly there? Ibis 146:298–302.

Leimar, O. 1996. Life-history analysis of the Trivers and Willard sex-ratio problem. Behavioral Ecology 7:316–325.

MacLeod, K. J., and T. H. Clutton-Brock. 2013. No evidence for adaptive sex ratio variation in the cooperatively breeding meerkat, Suricata suricatta. Animal Behaviour 85:645–653.

Monaghan, P., and M. F. Haussmann. 2006. Do telomere dynamics link lifestyle and lifespan? Trends in Ecology and Evolution 21:47–53.

Nager, R., and P. Monaghan. 1999. Experimental demonstration that offspring sex ratio varies with maternal condition. Proceedings of the ….

Postma, E., F. Heinrich, U. Koller, R. J. Sardell, J. M. Reid, P. Arcese, and L. F. Keller. 2011. Disentangling the effect of genes, the environment and chance on sex ratio variation in a wild bird population. Proceedings. Biological sciences / The Royal Society 278:2996–3002.

Pryke, S. R., and S. C. Griffith. 2009. Genetic incompatibility drives sex allocation and maternal investment in a polymorphic finch. Science 323:1605–1607.

R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; R Foundation for Statistical Computing.

Richardson, D. S., T. Burke, and J. Komdeur. 2002. Direct benefits and the evolution of female-biased cooperative breeding in Seychelles warblers. Evolution 56:2313–2321.

Richardson, D. S., T. Burke, and J. Komdeur. 2003. Sex-specific associative learning cues and inclusive fitness benefits in the Seychelles warbler. Journal of Evolutionary Biology 16:854–861.

Schindler, S., J.-M. Gaillard, A. Grüning, P. Neuhaus, L. W. Traill, S. Tuljapurkar, and T. Coulson. 2015. Sex-specific demography and generalization of the Trivers-Willard theory. Nature 526:249–252. Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.

Sheldon, B. C., and S. A. West. 2004. Maternal dominance, maternal condition, and offspring sex ratio in ungulate mammals. The American Naturalist 163:40–54.

Spurgin, L. G., D. J. Wright, M. van der Velde, N. J. Collar, J. Komdeur, T. Burke, and D. S. Richardson. 2014. Museum DNA reveals the demographic history of the endangered Seychelles warbler. Evolutionary Applications 7:1134–1143.

Trivers, R., and D. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90–92.

Von Zglinicki, T. 2002. Oxidative stress shortens telomeres. Trends in Biochemical Sciences 27:339–344.

West, S. 2009. Sex Allocation. Princeton University Press 482.

Whittingham, L. a, and P. O. Dunn. 2000. Offspring sex ratios in tree swallows: females in better condition produce more sons. Molecular Ecology 9:1123–9.