CHRONIC INFECTION

Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds

M. Asghar,[1,2] D. Hasselquist,[1]*† B. Hansson,[1] P. Zehtindjiev,[3] H. Westerdahl,[1] S. Bensch[1]†

Recovery from infection is not always complete, and mild chronic infection may persist. Although the direct costs of such infections are apparently small, the potential for any long-term effects on Darwinian fitness is poorly understood. In a wild population of great reed warblers, we found that low-level chronic malaria infection reduced life span as well as the lifetime number and quality of offspring. These delayed fitness effects of malaria appear to be mediated by telomere degradation, a result supported by controlled infection experiments on birds in captivity. The results of this study imply that chronic infection may be causing a series of small adverse effects that accumulate and eventually impair phenotypic quality and Darwinian fitness.

The harmful consequences of infections are well understood in humans and wild and domesticated animals (*1*, *2*), and disease is known to be a key factor in sexual selection (*3*) and life-history evolution (*4*). In wild animals, the cost of disease is most apparent during acute infection, when the host shows sickness behaviors and becomes inefficient in avoiding predators, finding food, and defending territory (*5*). After surviving the acute phase, either the infection is cleared or it may become chronic, often without apparent clinical signs. To study the ultimate long-term effects of infection in natural populations, and the potential proximate mechanisms underlying such effects, detailed data are required from individuals measured repeatedly over their entire lives. Unfortunately, such data are scarce for animals.

We have investigated the ultimate (Darwinian fitness) costs of avian malaria caused by *Plasmodium* and *Haemoproteus* spp. in great reed warblers (*Acrocephalus arundinaceus*), using detailed life-history data collected during 25 years in a wild population breeding at Lake Kvismaren, Sweden (*6*–*8*). The great reed warbler breeds in Eurasia and winters in tropical Africa. Previous studies based on parasite mitochondrial cytochrome b genes have identified 17 different malaria parasite lineages in the great reed warbler study population, all transmitted in Africa (*9*, *10*). This host-parasite system is well suited for investigating long-term effects of infection, because avian malaria disease is characterized by a severe acute phase when individuals first contract the infection, after which most of the survivors retain a persistent, low-level chronic infection (*11*). In wild bird populations, the fitness costs to hosts of being infected are typically not detectable (*12*), probably because individuals with acute infections are difficult to find and study. Moreover, the short-term effects of chronic infection are often reported to be benign over the time scales that infected individuals have been studied (*10*, *12*). On blood samples from great reed warblers, we have used nested polymerase chain reaction (PCR) for DNA-based detection and identification of malaria parasites [*Haemoproteus* and *Plasmodium* (*13*, *14*)] and quantitative PCR (qPCR) for determining infection intensity (*9*, *14*, *15*). In our study population, uninfected birds and birds with low-level nonsymptomatic infections are equally common, and we have detailed knowledge of infection history and reproduction throughout the individuals' lives (*9*, *10*, *14*). Low-level chronic malaria appears to have no direct short-term costs to great reed warblers in our study population. There is no difference in activity patterns between infected and uninfected birds, either in song rate in males [mean ± SEM song rate (percent of time singing per 15 min): infected = 59.3 ± 1.47 (n = 14 birds), uninfected = 58.7 ± 0.83 (n = 12); $t_{1,24}$ = 0.32, P = 0.76; fig. S1A (*14*)] or in nestling feeding rate in females [mean ± SEM feedings per hour and nestling: infected = 2.07 ± 0.91 (n = 21), uninfected = 1.96 ± 0.92 (n = 28); $t_{1,47}$ = 0.41, P = 0.68; fig. S1B (*14*)].

We found that birds infected with malaria had significantly shorter life spans [mean ± SEM years: infected = 1.69 ± 0.15; uninfected = 2.53 ± 0.26; P = 0.006 (Fig. 1A)] and fewer fledglings during their lifetimes [mean ± SEM fledglings: infected = 4.03 ± 0.74; uninfected = 8.63 ± 1.52; P = 0.009 (Fig. 1B)] than uninfected birds. When controlling for life span [general linear model (GLM): $F_{1,75}$ = 78.17, P < 0.0001], the negative effect of malaria infection on lifetime fledging success was not significant [$F_{1,75}$ = 0.89, P = 0.35 (table S1)], which is consistent with a previous study in which we found no difference in annual reproductive success or survival to the next year between infected and uninfected great reed

[1]Department of Biology, Lund University, Ecology Building, 223 62 Lund, Sweden. [2]Infectious Disease Unit, Department of Medicine Solna, Karolinska Institute, 17176 Stockholm, Sweden. [3]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria.
*Corresponding author. E-mail: dennis.hasselquist@biol.lu.se
†These authors contributed equally to this work.

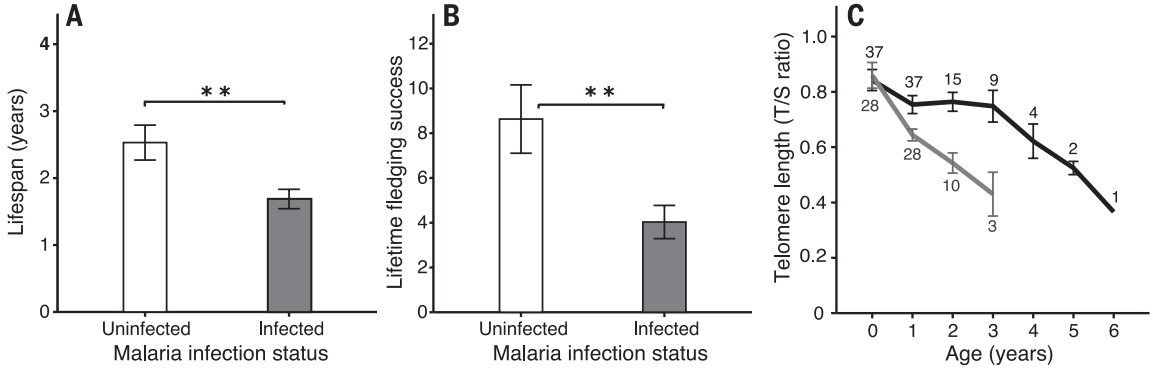

Fig. 1. Relationships between malaria infection status, life span, lifetime fledging success, and telomere length in wild great reed warblers. (**A**) Uninfected birds lived longer than birds with chronic malaria infection (t test: $t_{1,71.6}$ = 2.82, P = 0.006). (**B**) Uninfected birds produced more lifetime fledglings than chronically infected birds ($t_{1,67.5}$ = 2.71, P = 0.009). (**C**) Relationships between telomere length and age in uninfected (black line) and infected (gray line) great reed warblers (over life). Telomere length decreased with age (LME model: age, $F_{1,106}$ = 91.78, P < 0.0001) but at a steeper rate in infected birds (age × malaria status, $F_{1,106}$ = 27.09, P < 0.0001; table S2). Error bars represent mean ± SEM. n values are given for each age group (uninfected above and infected below error bars).

warblers (*10*). Taken together, these data show that a disease (chronic avian malaria) with seemingly negligible short-term costs over the long term induces physiological effects that shorten life span and reduce the number of lifetime offspring.

Recent research has linked telomere shortening to aging (*16*, *17*) and some degenerative diseases (*18*, *19*), and a few observational studies in humans (infected with, e.g., hepatitis C virus or HIV) and mice (infected with *Salmonella*) have shown that infected individuals have shorter telomeres (*20*, *21*). Telomeres are nucleoprotein structures that cap the ends of chromosomes to maintain chromosome integrity (*22*). Telomeres shorten at each cell division and if exposed to oxidative stress. When telomeres become too short, cell death occurs, which can accumulate in tissues to cause organ dysfunction and aging effects (*23*, *24*). Hence, telomere shortening is a possible mechanism for mediating long-term fitness costs of chronic or recurrent mild infections. We used qPCR (*14*, *25*, *26*) to quantify telomere length in blood samples of great reed warblers taken when 8 to 10 days old and then each year throughout their lives when breeding at our study site. We estimated telomere shortening as the between-year change in telomere length (*14*). These data allowed us to estimate early-life telomere length as well as the annual rate of telomere shortening. We found that telomere length in great reed warblers decreased with age ($P < 0.0001$) and more steeply so in infected than in uninfected birds [as shown by the significant interaction age × malaria infection status, $P < 0.0001$ (Fig. 1C and table S2]. Individuals infected with malaria experienced a significantly greater rate of telomere shortening than uninfected individuals [linear mixed-effect (LME) model: malaria infection status, $F_{1,61} = 24.2$, $P < 0.0001$ (table S3)]. The difference between infected and uninfected individuals was also present when we analyzed malaria infection status and telomere shortening over the birds' first year of life, for all parasite lineages combined ($P = 0.0001$) and for the three most common parasite lineages separately (Fig. 2A). The degree of telomere loss seemed to be related to the severity of the infection, because we found a marginally positive correlation between the rate of telomere loss over the birds' first year of life

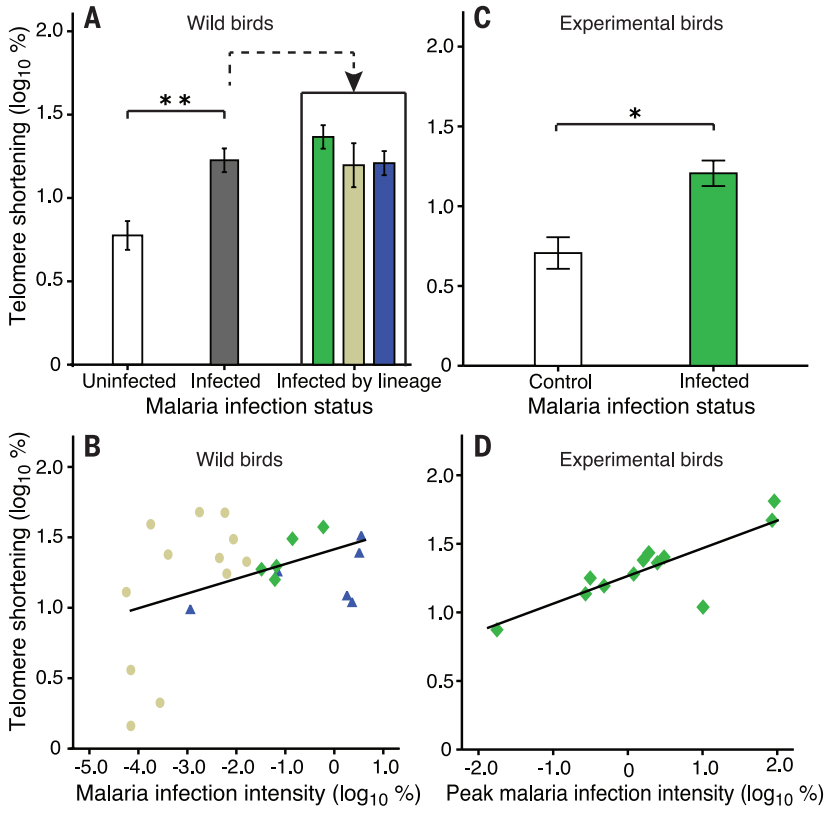

Fig. 2. Relationships between telomere shortening and malaria infection status in great reed warblers. (**A**) In wild birds, uninfected birds (white) experienced a lower degree of telomere shortening over their first year than infected birds (gray) for the common avian malaria lineages combined (*t* test: $t_{1,62.9} = 3.96$, $P = 0.0001$; $n_{uninfected} = 37$ birds, $n_{infected} = 28$), and separately for *P. ashfordi* (green; $t_{1,40} = 2.47$, $P = 0.018$), *P. relictum* (yellow; $t_{1,49} = 2.59$, $P = 0.013$), and marginally so for *Haemoproteus nucleocondensus* (blue; $t_{1,41} = 1.95$, $P = 0.058$). (**B**) In 1-year-old wild birds, there was a marginally positive correlation between malaria infection intensity and telomere shortening for the common malaria parasite lineages combined ($r = 0.41$, $P = 0.051$, $n = 23$), a relationship that was significant for *P. ashfordi* ($r = 0.89$, $P = 0.043$, $n = 5$) and *P. relictum* ($r = 0.60$, $P = 0.038$, $n = 12$) but not for *H. nucleocondensus* ($r = 0.52$, $P = 0.28$, $n = 6$). (**C**) In captive great reed warblers, there was a higher rate of telomere shortening over 9 to 10 weeks in birds experimentally infected with *P. ashfordi* than in uninfected controls (GLM: $F_{1,12} = 19.59$, $P = 0.0008$, $n_{infected} = 12$, $n_{control} = 4$), and (**D**) in the infected captive birds, there was a positive correlation between peak malaria infection intensity and telomere shortening ($r = 0.82$, $P = 0.001$, $n = 12$).

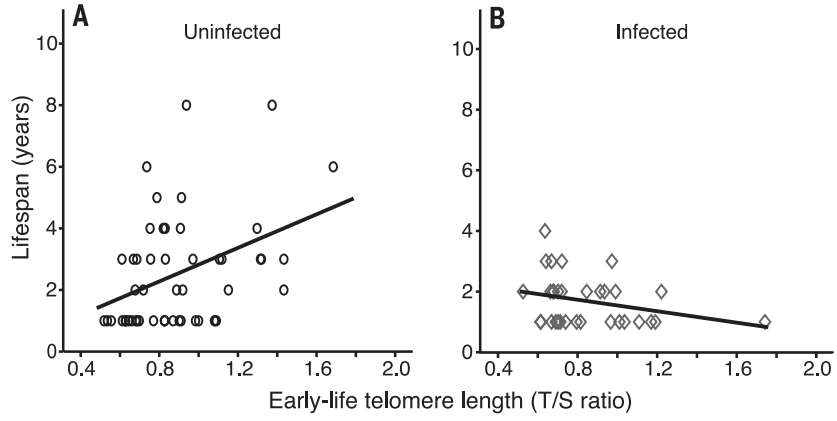

Fig. 3. Relationships between life span and early-life telomere length (TL; at 8 to 10 days of age) in uninfected and infected wild great reed warblers. (**A**) In uninfected birds (circles, black line), there was a significant positive relationship between early-life TL and life span ($r = 0.40$, $P = 0.005$, $n = 49$), whereas (**B**) in malaria-infected birds (diamonds, gray line), there was no such relationship ($r = -0.29$, $P = 0.11$, $n = 32$). The difference in slopes is supported by the significant interaction early-life TL × malaria status ($P = 0.003$).

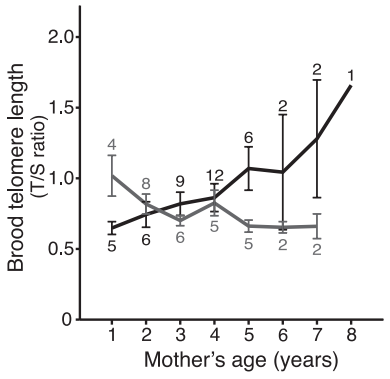

Fig. 4. Relationships between mother's age and the mean early-life telomere length of her offspring in relation to mother's malaria infection status in wild great reed warblers. Uninfected (black line) and malaria-infected (gray line) mothers differed in the relationship between the mother's age and the brood mean telomere length of her offspring. A LME model showed effects of mother's age × mother's malaria status ($F_{1,142}$ = 71.53, P < 0.0001), mother's age ($F_{1,142}$ = 6.96, P = 0.011), and brood year ($F_{1,142}$ = 26.76, P < 0.0001; table S6). Uninfected mothers (n = 20), as they became older, produced broods in which nestlings had longer mean telomeres (P < 0.0001), whereas in malaria-infected mothers (n = 19), this relationship was reversed (P = 0.0004). n values (broods) are given for each age group, and error bars represent mean ± SE of brood means (*14*). The statistical analysis was conducted using a LME model with the early-life telomere length of individual offspring as a dependent variable, the mother's identity as a random factor, and broods fitted into mother as a nested random factor.

and malaria infection intensity for the three parasite species combined (P = 0.051), a pattern that was significant in each of the two *Plasmodium* species (Fig. 2B).

In a combined analysis of malaria infection status and early-life telomere length on life span, we found a significant relationship between life span and malaria infection status (GLM: $F_{1,75}$ = 5.10, P = 0.027; table S4) and a significant positive relationship between life span and early-life telomere length (8- to 10-day-old nestlings; $F_{1,75}$ = 14.51, P = 0.0003). The interaction malaria infection status × early-life telomere length was also significant ($F_{1,75}$ = 9.15, P = 0.003). Thus, in birds remaining uninfected throughout their lives, there was a positive relationship between life span and early-life telomere length (r = 0.40, P = 0.005; Fig. 3A). In contrast, there was no such relationship in birds infected in their first year of life and onward (r = −0.29, P = 0.11; Fig. 3B).

Our study shows a positive relationship between early-life telomere length and life span in a species living under natural conditions. Such relationships have previously been found in comparisons across species and generally interpreted as reflecting the pace of life, where species with high energy turnover rates show fast telomere loss rates and short life spans (*16*, *27*). Within species, there is very little information on the relationship between early-life telomere length and life span apart from one study on captive birds (*16*).

To confirm the relationship between malaria infection and telomere shortening, we analyzed data from an infection experiment (*14, 28*) with *Plasmodium ashfordi*, one of the three common malaria parasites in great reed warblers. Experimentally infected great reed warblers experienced a higher rate of telomere loss than uninfected control birds over 9 to 10 weeks after inoculation (GLM: $F_{1,12}$ = 19.59, P = 0.0008; table S5 and Fig. 2C). In the experimentally infected birds, we also found a significant positive correlation between peak acute phase malaria infection intensity and rate of telomere loss (r = 0.82, P = 0.001; Fig. 2D).

Inspired by recent research suggesting that telomere length in offspring is influenced by parental effects (*25*, *29*), we investigated whether malaria infection in parent great reed warblers affected telomere length in their offspring. We found that malaria-infected mothers produced offspring with, on average, shorter telomeres in comparison to the offspring of uninfected mothers (t test: $t_{1,170.0}$ = 3.45, P = 0.0007), but no such effect was found in fathers ($t_{1,131.7}$ = −1.43, P = 0.16). Early-life telomere length in offspring was positively correlated with their mother's age when the eggs were laid (LME model: P = 0.011, Fig. 4), and there was also a significant interaction mother's age × mother malaria infection status (P < 0.0001; Fig. 4 and table S6). Hence, in uninfected mothers, there was a significant positive relationship between the mother's age (at the breeding event) and offspring early-life telomere length (LME model: $F_{1,75}$ = 29.42, P < 0.0001), whereas in malaria-infected mothers, this relationship was significantly negative ($F_{1,61}$ = 14.08, P = 0.0004; Fig. 4 and fig. S2). Thus, as uninfected mothers become older, they produce chicks with (over the years) successively longer early-life telomeres. In contrast, as infected mothers become older, they produce chicks with successively shorter early-life telomeres. These results suggest that telomere length in offspring, a proxy for phenotypic quality, can be influenced by environmental effects mediated by the mother (*29*, *30*), a finding that may have implications for our understanding of telomere dynamics and aging.

Our study combines longitudinal data from a wild population and experiments to show that individuals with chronic infections suffer long-term costs that act via telomere shortening to have consequences for survival, lifetime reproductive success, and offspring quality.

REFERENCES AND NOTES

1. T. M. Uyeki, *Clin. Infect. Dis.* **49**, 279–290 (2009).
2. S. I. Hay *et al.*, *PLOS Med.* **7**, e1000290 (2010).
3. W. D. Hamilton, M. Zuk, *Science* **218**, 384–387 (1982).
4. B. C. Sheldon, S. Verhulst, *Trends Ecol. Evol.* **11**, 317–321 (1996).
5. N. T. Ashley, J. C. Wingfield, in *Eco-Immunology*, R. M. Nelson, G. Demas, Eds. (Oxford Univ. Press, Oxford, 2012), pp. 45–91.
6. S. Bensch, *J. Anim. Ecol.* **65**, 283–296 (1996).
7. B. Hansson *et al.*, *Mol. Ecol.* **9**, 1529–1538 (2000).
8. D. Hasselquist, *Ecology* **79**, 2376–2390 (1998).
9. M. Asghar, D. Hasselquist, S. Bensch, *J. Avian Biol.* **42**, 530–537 (2011).
10. S. Bensch *et al.*, *J. Anim. Ecol.* **76**, 112–122 (2007).
11. M. Asghar *et al.*, *Parasitology* **139**, 1246–1252 (2012).
12. D. A. LaPointe, C. T. Atkinson, M. D. Samuel, *Ann. N.Y. Acad. Sci.* **1249**, 211–226 (2012).
13. J. Waldenström, S. Bensch, D. Hasselquist, O. Ostman, *J. Parasitol.* **90**, 191–194 (2004).
14. Materials and methods are available as supplementary materials on *Science* Online.
15. H. Westerdahl, M. Asghar, D. Hasselquist, S. Bensch, *Proc. Biol. Sci.* **279**, 577–584 (2012).
16. B. J. Heidinger *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 1743–1748 (2012).
17. R. M. Cawthon, K. R. Smith, E. O'Brien, A. Sivatchenko, R. A. Kerber, *Lancet* **361**, 393–395 (2003).
18. M. Jaskelioff *et al.*, *Nature* **469**, 102–106 (2011).
19. E. Sahin, R. A. Depinho, *Nature* **464**, 520–528 (2010).
20. P. Ilmonen *et al.*, *J. Evol. Biol.* **21**, 834–841 (2008).
21. C. M. Kong, X. W. Lee, X. Wang, *FEBS J.* **280**, 3180–3193 (2013).
22. T. de Lange, *Genes Dev.* **19**, 2100–2110 (2005).
23. J. H. J. Hoeijmakers, *N. Engl. J. Med.* **361**, 1475–1485 (2009).
24. E. Sahin *et al.*, *Nature* **470**, 359–365 (2011).
25. M. Asghar, S. Bensch, M. Tarka, B. Hansson, D. Hasselquist, *Proc. Biol. Sci.* **282**, 20142263 (2015).
26. F. Criscuolo *et al.*, *J. Avian Biol.* **40**, 342–347 (2009).
27. E. L. Barrett, T. A. Burke, M. Hammers, J. Komdeur, D. S. Richardson, *Mol. Ecol.* **22**, 249–259 (2013).
28. P. Zehtindjiev *et al.*, *Exp. Parasitol.* **119**, 99–110 (2008).
29. E. S. Epel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 17312–17315 (2004).
30. D. Hasselquist, M. Tobler, J.-Å. Nilsson, in *Eco-Immunology*, R. M. Nelson, G. Demas, Eds. (Oxford Univ. Press, Oxford, 2012), pp. 165–224.

ACKNOWLEDGMENTS

This work was supported by the Swedish Research Council (grants to D.H., B.H., H.W., and S.B.), the Higher Education Commission of Pakistan (to M.A.), and CAnMove (a Linnaeus research excellence environment financed by the Swedish Research Council and Lund University). The field work was conducted in collaboration with Kvismare Bird Observatory (report 174), and the experiment was conducted at Kalimok Field Station (Bulgarian Academy of Sciences, report 56). We thank C. Cornwallis, J.-Å. Nilsson, T. Alerstam, M. Strandh, and A. Marzal for manuscript comments and discussions. M.A., S.B., and D.H. designed the study. Field data and blood samples were collected by S.B., B.H., D.H., H.W., and many field assistants over 25 years. M.A. and S.B. developed the qPCR methods to quantify parasite intensity and telomere length. S.B. and P.Z. did the experimental infections. M.A. did the molecular analyses and analyzed the data together with S.B., D.H., and B.H. All authors interpreted the data. M.A., D.H., and S.B. wrote the manuscript, with extensive contributions from B.H., H.W., and P.Z. Data are available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.d04h0. We declare no competing financial interests.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/347/6220/436/suppl/DC1
Material and Methods
Figs. S1 to S2
Tables S1 to S7
References (*31–53*)

11 September 2014; accepted 16 December 2014
10.1126/science.1261121

Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds

M. Asghar *et al.*

Science **347**, 436 (2015);
DOI: 10.1126/science.1261121

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.